Volume 34 (**Supplement 3**) May 2007

1st Annual Meeting

The Canadian Association for Neuroscience /

Association canadienne de neuroscience

TORONTO, ONTARIO, MAY 23-25, 2007

ABSTRACTS

Abstract Legend

First number - Indicates Abstract number
Letter - Indicates day and time of presentation
A - Thursday, May 24 Morning, **B** - Thursday, May 24 Afternoon
C - Friday, May 25 Morning, **D** - Friday, May 25 Afternoon

Last series of numbers - Indicates room & board number
100 series - Giovanni Room, **200 series** - Terrace Room
300 series - St. Lawrence Room, **400 series** - St. George Room,
Example: 1 A201 - Abstract number 1, being presented on Thursday, May 24 Morning, in Terrace Room on board #1

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

Volume 34 Number 2 (Supplement 3) May 2007

THE CANADIAN JOURNAL OF Neurological Sciences

LE JOURNAL CANADIEN DES Sciences Neurologiques

We acknowledge the assistance of the Government of Canada through the Publications Assistance Program towards our mailing costs. Canada

The official journal of: / La Revue officielle de:

The Canadian Neurological Society
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The Canadian Journal of Neurological Sciences is published quarterly. The annual subscription rate for Individuals are: C$90 (Canada), US$90 (USA), and US$95 (elsewhere). Subscription rates for Institutions are: C$100 (Canada), US$100 (USA), and US$105 (elsewhere). Resident, intern and student rates are available. See www.cjns.org for details. Single copies C$25 each plus postage and handling. All manuscripts and communications should be sent to: Canadian Journal of Neurological Sciences, 709 - 7015 Macleod Trail SW, Calgary, AB Canada T2H 2K6. Telephone (403) 229-9575; Fax (403) 229-1661. E-mail: journal@cjns.org; Website: www.cjns.org

Mailed under Publications Mail Agreement no: 40007777; PAP Registration no: 09824. Postage paid at Calgary, Alberta. This journal is indexed by *Abstracts on Hygiene and Communicable Diseases, Aquatic Sciences and Fisheries Abstracts, ASCA-Automatic Subject Citation Alert, Biological Abstracts, BIOBASE, BIOSIS, Chemical Abstracts Current Awareness in Biological Sciences, Current Contents (Clinical Medicine and Life Sciences), Dental Index, e-psyche, Excerpta Medica, Index Medicus, Index to Scientific Reviews, Journal Watch Neurology, Laboratory Hazards Bulletin, Leisure, Recreation and Tourism Abstracts, MEDLINE, Neurosciences Citation Index, Nutrition Abstracts and Reviews, Nutrition Research Newsletter, Pharmaeconomics and Outcome News, PsycInfo, Psychological Abstracts, Reactions Weekly, Referativnyi Zhurnal, Review of Medical and Veterinary Mycology, Science Citation Index, Weed Abstracts.*

Le Journal Canadien des Sciences Neurologiques est publié trimestriellement. L'abonnement annuel est de 90 $C (non-membres au Canada); 90 $É-U (Etats Unis) et 95 $É-U (ailleurs); l'abonnement annuel for pour les institutions est de 100 $C (non-membres au Canada); 100 $É-U (Etats Unis) et 105 $É-U (ailleurs); Internes, résidents, fellows pré et post doctoral voir www.cjns.org pour détails. Copie simple: 25 $C plus affranchissement et manutention. Toutes les communications et les manuscrits doivent être adressés à Journal Canadien des Sciences Neurologiques, 709 - 7015 Macleod Trail SW, Calgary, AB Canada T2H 2K6. Téléphone (403) 229-9575; Fax (403) 229-1661. E-mail journal@cjns.org; Website: www.cjns.org

Posté sous poste-publications: numéro de convention: 40007777; numéro d'enregistrement PAP 09824. Port payé à Calgary, Alberta. Le Journal est cité et indexé dans *Abstracts on Hygiene and Communicable Diseases, Aquatic Sciences and Fisheries Abstracts, ASCA-Automatic Subject Citation Alert, Biological Abstracts, BIOBASE, BIOSIS, Chemical Abstracts Current Awareness in Biological Sciences, Current Contents (Clinical Medicine and Life Sciences), Dental Index, e-psyche, Excerpta Medica, Index Medicus, Index to Scientific Reviews, Journal Watch Neurology, Laboratory Hazards Bullentin, Leisure, Recreation and Tourism Abstracts, MEDLINE, Neurosciences Citation Index, Nutrition Abstracts and Reviews, Nutrition Research Newsletter, Pharmaeconomics and Outcome News, PsycInfo, Psychological Abstracts, Reactions Weekly, Referativnyi Zhurnal, Review of Medical and Veterinary Mycology, Science Citation Index, Weed Abstracts.*

Printer/Imprimeur:
Sundog Printing Limited, 1311 Ninth Avenue SW
Calgary, Alberta T3C 0H9

ISSN 0317 - 1671

The Canadian Association for Neuroscience / Association canadienne de neuroscience

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Cognition & Behaviour

1 B101
PERCEIVING AN OBJECT'S MATERIAL PROPERTIES THROUGH DYNAMIC SOUND ACTIVATES VENTRAL OCCIPITOTEMPORAL AND INFERIOR PARIETAL CORTICES

Stephen R. Arnott, Jonathan S. Cant, Melvyn A. Goodale. CIHR Group for Action and Perception, Department of Psychology, The University of Western Ontario

Knowledge of an object's material composition (i.e., what it is made of) alters how we interact with that object. Seeing the bright glint or hearing the metallic crinkle of a foil plate for example, confers information about that plate before we have even touched it. In a previous study (Cant & Goodale, 2007) it was shown that visually attending to an object's material properties as opposed to its shape or color, elicited greater hemodynamic activity in ventral occipitotemporal regions. In the present fMRI study, we investigated whether there are comparable brain areas that are sensitive to material properties derived from sound alone. Using a passive visual adaptation paradigm we first localized an area in the right parahippocampal region that was most selective for material as compared to shape or colour properties of objects. In a separate series of runs, participants were presented with two-second normalized sound clips and asked to categorize them as either Material (crumpling styrofoam, plastic, tinfoil or paper), Noise (scrambled versions of each of the material sounds), or Human sounds (coughing, yawning, snoring or throat clearing). Although functional analyses of the auditory data within the visual material region did not reveal any discernable modulation, expanding the region to include all of parahippocampal cortex revealed an area that was selective for Material sounds as compared to Noise or Human sounds. In addition, a voxel-wise analysis of the auditory experiment also revealed greater 'Material' activity in left lateral inferior parietal area, perhaps reflecting the action-related nature (i.e., crumpling) of the sounds. Our findings point to an important multimodal role for the parahippocampal region in the analysis of material properties, and are consistent with the notion that the medial aspect of the ventral pathway is specialized for processing the material properties of objects.

2 B102
THE EFFECTS OF ACUTE ALCOHOL INTOXICATION ON IMPLICIT AND EXPLICIT LEARNING

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The impact of alcohol on implicit, emotional learning is not well understood, partly because factors ranging from family history, drug use and task demands influence these processes. The Conditioned Pattern Preference (CPP) task provides a more ecologically valid means to investigate implicit cognition in the lab because it has low demand awareness and relies on associative learning with nonlinguistic cues that were previously paired with reward. This study examined the effects of acute alcohol intoxication on implicit learning using the CPP task in 89 intoxicated and 69 sober young adults. Information on individual drug use, family history, impulsivity and alcohol expectancies was also collected. Alcohol intoxication affected explicit, but not implicit, learning on the CPP. In addition, participants who reported a positive family history of addiction (FH+) or individual recreational drug use did not exhibit a preference for cues previously paired with reward. Preference formation on the CPP task recruits motivational neurocircuitry, an effect that is unaltered by alcohol. Group differences in implicit emotional learning on this task may represent neurocognitive differences in individuals at risk for addiction.

3 B103
REPRESENTATION OF COMPLEX OBJECTS IN THE MACAQUE TEMPORAL LOBE REVEALED BY FMRI

Bell, Andrew H, Hadj-Bouziane, Fadila, Ungerleider, Leslie G., Tootell, Roger B.H., 1Laboratory of Brain and Cognition, NIMH/NIH, Bethesda, MD, USA, 2 Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts General Hospital, Charlestown, MA, USA

It is increasingly clear that a functional organization of stimulus properties exists in primate inferior temporal (IT) cortex. Early physiological work suggested that this organization is based on variations in physical stimulus properties. However, recent fMRI work has interpreted such differences in terms of object category (i.e., semantic) properties. To explore

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

these two hypotheses and the evolutionary generality of human fMRI results, we measured contrast-agent enhanced fMRI signals in 3 awake fixating macaques, at 3T. The subjects maintained central fixation while blocks of stimuli from the following categories were presented: monkey faces, body parts, familiar objects, familiar scenes, and scrambled images. Face and scene images were presented both upright and inverted, in separate blocks. As in previous studies, these categories activated distinct (though partially overlapping) regions of cortex. Images of faces (relative to all other categories) activated right anterior IT cortex, and a bilateral area farther posterior within the STS. Body part images activated cortex adjacent and medial to face-selective regions. Objects and scenes activated areas in the inferior temporal gyrus, hippocampus, intraparietal sulcus, and specific prefrontal regions. Comparing the responses to upright vs. inverted images revealed an inversion effect for faces but not places; responses to inverted faces were reduced compared to upright faces. These results revealed that stimuli similar to each other (e.g. faces) activated more focal regions of cortex, compared to stimuli that were visually less similar (e.g., scenes, objects). To measure the sensitivity to individual stimulus properties, an additional experiment was performed in which blocks of single exemplars were presented for each category. For each category, we found exemplar-selective voxels, ranging from very few (for faces) to larger proportions (for objects). Thus, macaque IT cortex shows a modular organization similar to that reported in human fMRI, currently interpreted in terms of object category specificity. However, our single-image tests suggest that a functional organization for lower-level stimulus properties also exists, within presumptive category-selective regions.

4 B104

BEHAVIOURAL EFFECTS OF SUBCHRONIC PHENCYCLIDINE IN RATS: TESTS OF AMPHETAMINE-STIMULATED LOCOMOTOR ACTIVITY AND MEMORY

Jonathan Beuk1, Michael van Adel1, James N. Reynolds2 and Richard J. Beninger1,3. Depts. [1]Psychology, [2]Pharmacology and Toxicology and [3]Psychiatry, Queen's University, Kingston

Schizophrenia is a crippling disease that is characterized by positive symptoms represented as an excess in normal functioning (e.g., hallucinations or delusions), negative symptoms represented as a deficiency in normal functioning (e.g., social withdrawal, apathy) and cognitive problems (e.g., disorganized thoughts, memory impairments). Phencyclidine (PCP) administration produces schizophrenia-like psychotic symptoms in humans and exacerbates psychotic symptoms in schizophrenic patients. PCP is an n-methyl-D-aspartate (NMDA) glutamatergic receptor antagonist. These observations have led to the proposal that NMDA receptor hypofunction may contribute to schizophrenic symptoms. This suggested the hypothesis that PCP treatment may lead to memory impairments. To test this hypothesis, male Sprague-Dawley rats were treated subchronically with PCP (4.5 mg/kg, i.p. twice a day for 7 consecutive days) or saline and tested 7 days following the last injection. In the first experiment, rats were tested in a locomotor activity task followed by a watermaze memory task. The subchronic PCP (n = 12) group showed a significant increase in amphetamine (1.5 mg/kg)-stimulated locomotor activity similar to the increased sensitivity to amphetamine seen in schizophrenia; however, they did not significantly differ in watermaze task response latency when compared to controls (n = 12). In experiment 2, rats were extensively trained to make correct arm entries in a double-y maze that consisted of a spatial task followed by an alternation task before receiving drug treatment. Groups were subsequently tested in the double-y maze task and for locomotor activity. After subchronic PCP administration, rats (n = 12) exhibited a significantly greater number of overall arm entry errors when compared to controls (n = 9) in the double-y maze memory task; however, the two groups did not differ significantly in the locomotor activity task. These findings suggest that subchronic blockade of NMDA receptors leads to impaired memory of a previously learned task in rats, similar to the cognitive impairments observed in human schizophrenia patients. Moreover, subchronic PCP produced an enhanced response to amphetamine in rats, reminiscent of the effect of amphetamine in schizophrenic patients; however, rats with extensive memory task training failed to show this effect. Results provided some support for the hypothesis that PCP treatment may lead to memory impairments. (Funded by OMHF)

5 B105

EFFECTS OF FOOTSHOCK STRESS ON THE REINSTATEMENT OF COCAINE SEEKING FOLLOWING EXTENDED POST-STRESS DELAY PERIODS

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Footshock stress robustly reinstates drug seeking in rats when tests for reinstatement are conducted immediately following termination of the stressor. However, it has not previously been determined whether delays in the opportunity for drug seeking following exposure to footshock affects reinstatement of drug seeking. Examining the effects of post-stress delay periods on reinstatement of drug seeking is of interest, since individuals with a history of drug dependence do not always have access to seek out or use drugs immediately following a stressful life event. The objective of the present study was to determine for how long footshock stress remains effective in inducing relapse to cocaine seeking following its termination, and whether the context in which post-stress delay periods are experienced affects the magnitude of reinstatement. Rats were allowed to self-administer cocaine (1.0 mg/kg per infusion) for 8-10 days. Following a 7-day drug-free period, drug-taking behaviour was extinguished and, subsequently, animals were tested for reinstatement in response to intermittent footshock stress (20 min; 0.8 mA). In a first series of experiments, animals were tested for reinstatement following a 0-, 20- or 60-min post-stress delay period. In a second series of experiments, animals were tested for reinstatement following a 40- or 60-min post-stress delay period that was given in either the self-administration (SA) chamber or homecage (HC). Footshock reliably and robustly reinstated cocaine seeking following post-stress delays of up to 40 minutes. No differences in response levels were observed between animals that spent the delay period in the SA chamber versus HC. Thus, within a limited time window, footshock stress is effective in reinstating cocaine seeking when testing is delayed following termination of the stressor. Overall, the findings are consistent with earlier work from this laboratory, in which central injections of the pharmacological stressor, corticotrophin-releasing factor, were effective in inducing reinstatement of cocaine seeking after extended post-injection delays. The findings will be discussed in terms of a contextual conditioning account of reinstatement.

6 B106

COMMON AND DISTINCT FEATURES OF SLOW CORTICAL OSCILLATION DURING NATURAL SLOW-WAVE SLEEP AND KETAMIN-XYLAZINE ANESTHESIA

Chauvette, Sylvain and Timofeev, Igor. Centre de recherche Université Laval Robert-Giffard, Québec*

While sleep and anesthesia are different states, they have common traits and may share common mechanisms. Based on this notion, anesthetized animals have been used to study sleep mechanisms at levels spanning from a single cell to large and distributed neuronal networks. During natural slow-wave sleep cortical neurons display a slow oscillation (< 1 Hz) consisting in an alternation between active and silent states. Ketamine-xylazine anesthesia induces a similar oscillation in cortical neurons and it is often used as a model of slow-wave sleep because of similar electrographic appearance. Here we asked: What is common, and what is different, in the mechanisms of sleep and anesthesia? To answer this question, we used simultaneous multi-site field potentials recordings (16) and simultaneous multi-site intracellular

recordings both, in naturally sleeping and anaesthetized animals. Analysis of dynamics of local field potential recordings revealed that activity started at deeper locations and in some superficial locations, but appeared later in the middle of the cortex. The current-source density analysis revealed that during both slow-wave sleep and ketamine-xylazine anesthesia there are strong sinks in the upper layers during silent states and sources in deeper layers. Upon transition to active states, the picture reverses to the opposite: sinks in the deeper and sources in the upper layers. During active states, the sources and sinks are generally weaker and more variable in both space and time than during silent states. The sinks and sources that are present in upper layers in natural sleep are weaker in anaesthetized animals. Intracellular activities revealed the same tendency in both preparations. Although in a given set of neurons each neuron located at any depth could be leading a particular cycle, most of the cycles were led by deeply lying neurons and the extent of variability of neuronal activities onsets was similar in both preparations. Finally, extracellular unit recordings from the same neurons during sleep and anesthesia demonstrated that both periodicity and firing rates during ketamine-xylazine anesthesia were higher. We conclude that ketamine-xylazine-induced slow oscillation reproduces major patterns of sleep slow oscillation. Exceptions from this rule are stronger rhythmicity and higher firing rates of cortical neurons. Supported by CIHR and NSERC

7 B107

BRAIN AREAS THAT MEDIATE PERCEPTION AND NAME SELECTION DURING OBJECT NAMING

Philippe A Chouinard, Brendan F Morrissey, Stefan Köhler, Melvyn A Goodale. CIHR Group on Action and Perception, Department of Psychology, University of Western Ontario, London

Naming an object requires one to first perceive the object and then select a name. Disruption to any one of these processes will impair a person's ability to name objects. The use of functional neuroimaging to parcellate object-naming areas into areas responsible for perception and name selection has proved challenging. Areas responsible for each of these processes may depend on each other and naming may result in top-down influences in perceptual areas. Our study used a slow event-related design while measuring neural activity in the brain with fMRI. We presented two images in succession that were either of the same object (same exemplar), different objects with the same name (different exemplar), or different objects with different names (control). This was done while participants named and did not name objects. The design enabled us to assume that responses to the first object would be equal in a given naming condition and that any difference in BOLD would be driven by differences in effects induced by the second object. We also measured reaction times during BOLD acquisition. We found repetition suppression induced by shared physical features in the left posterior-fusiform gyrus (pFus), differential contributions of the left ventrolateral-prefrontal cortex (VL-PFC) and areas in the medial-frontal cortex in name selection, and top-down influences in the left pFus induced from naming objects. We demonstrate further relationships between behavioral priming induced from naming objects and differences in BOLD in the left VL-PFC. Our study provides insight into which areas play a role in perception and which play a role in name selection when participants name objects. Moreover, the fact that we found an influence of naming on activity in the left pFus, demonstrates that neural processing in relatively early perceptual areas can be influenced by top-down semantic processing. Relationships between task performance and neural activity were found in areas related to name selection and not in areas related to perception. We speculate that this is because identification involves neural processes in later stages of object naming.

8 B108

LEFT VERSUS RIGHT HEMISPHERE DUAL TASK INTERFERENCE ON FACE PROCESSING

Jesse MacKewn, Paul Brewster and Jennifer Steeves. Department of Psychology and the Centre for Vision Research, York University, Toronto*

A variety of evidence shows that the right hemisphere of cortex is critical for mediating face recognition. Some research also suggests that there are gender differences in face recognition ability (women are better than men) and that men show more asymmetric brain activation in favour of the right hemisphere than women. Here we examined the role of gender in a dual task paradigm during face processing. If faces are processed primarily in the right hemisphere, then face recognition should be adversely affected because of interference with left finger tapping since they are both using resources in the right hemisphere of the brain when compared to right hand tapping. Ninety right handed students were tested on their ability to recognize faces during concurrent unimanual finger tapping. There were 6 tapping conditions compared to a no tapping condition— either left or right hand tapping during a) encoding, b) recognition or c) both encoding and recognition of faces. In each condition, participants were shown three faces once for 3000 ms and were required to learn these faces. Participants were then asked to recognize the three faces they had just learned among 6 distracter faces. Participants made a vocal response and accuracy and latency were measured. Overall, men were slightly faster than women at recognizing faces. In the no tapping control condition, women were somewhat better at recognizing faces than men which is consistent with previous research. Women, however showed a greater reduction in face recognition with left finger tapping during the recognition and encoding/recognition conditions compared to right finger tapping. Men showed the largest reduction in performance with left finger tapping during the encoding/ recognition condition and with right finger tapping during encoding and encoding/recognition. These findings suggest that overall men were more affected by dual task interference in either hemisphere but that women were most affected by the dual task when it required neural resources from the right hemisphere for face recognition.

9 B109

CYCLICAL AND SLEEP-LIKE ALTERNATIONS OF BRAIN STATE UNDER URETHANE ANAESTHESIA

Clayton T. Dickson, Alto S. Lo, Elizabeth A. Clement, Alby Richard. Department of Psychology and Centre for Neuroscience, University of Alberta

Alternations in brain state during unconsciousness are a normal feature of sleep, but are not a characteristic feature of general anaesthesia. Previously, we have shown that rats maintained under urethane anaesthesia demonstrate a spontaneous and cyclical alternation of brain state that is remarkably similar in a variety of ways to the REM/non-REM cycle expressed during natural sleep. In the present study we performed a comparison of the brain state effects under urethane to three other common veterinary anaesthetics (ketamine/xylazine, pentobarbital, and isoflurane) by performing long-term neocortical and hippocampal field recordings in rats. We show that although all anaesthetics promote an increase in slow power at dosages promoting anaesthesia, (perhaps equivalent to deep slow wave sleep or in some cases even coma), only urethane allows the spontaneous alternations between activated and deactivated forebrain EEG patterns that are typical of natural sleep while still providing a uniformly consistent anaesthetic state. Our results suggest that urethane's pharmacological action in the brain may closely overlap the physiological mechanisms for the maintenance of the natural sleeping state in rats.

10 B110

EFFECTS OF INTRACCUMBAL AMPHETAMINE ON EMISSION OF 50-KHZ VOCALIZATIONS IN INBRED LINES OF LONG-EVANS RATS

Shannon E.G. Duffus1, Jeff Burgdorf2, Jaak Panksepp3, and Stefan M. Brudzynski1. 1Department of Psychology and Centre for Neuroscience, Brock University, St. Catharines, 2Falk Center for Molecular Therapeutics, Department of Biomedical Engineering, Northwestern University, Evanston, USA, 3Department of VCAPP, Washington State University*

Emission of 50-kHz vocalizations was observed in a number of appetitive social situations and is regarded as an expression of positive state in rats. Emission of 50-kHz calls is associated with the activity of the mesolimbic dopamine system. Rats were taken from three lines of Long-Evans rats (high, low, and random), which were selectively bred for high and low emission of 50-kHz calls induced in response to heterospecific play. Eighteen rats from the 17th generation of breeding (6 from each line) were implanted with intracerebral cannulae in the shell of the nucleus accumbens. Amphetamine (5-10 μg/0.5μl) was unilaterally injected into the accumbens and ultrasonic vocalizations were recorded for 5 min following a 2 min delay. All rats emitted species-typical 50-kHz calls regardless of the situation, line, and dose of amphetamine; the calls differed neither in the individual duration, frequency nor bandwidth. After injection, a significant increase in the number of 50-kHz calls ($p < 0.01$) was observed in a dose-dependent manner. However, there was no difference in the number of induced calls among the lines. Interestingly, the number of calls emitted by rats of the high line was not significantly different from that of the low line. These preliminary results suggest that the sensitivity of the mesolimbic dopamine system, as tested pharmacologically in the accumbens, is similar across the inbred lines. This study was supported by the NSERC of Canada.

11 B111

VERTICAL SHIFTS IN WORK-RESPONSE FUNCTION SUGGESTS VULENRABILITY DIFFERENCES TO WHEEL RUNNING ADDICTION

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Wheel running has been postulated as a non-drug model of addiction (Eikelboom and Lattanzio, 2003). Exploring changes or differences in drug dose-response functions has been helpful in understanding drug motivation in addiction. Horizontal shifts in the drug dose-response function demonstrate the occurrence of drug sensitization and tolerance. Recently, it has been suggested that vertical differences in drug self-administration dose-response functions are indicative of vulnerability differences to addiction (Piazza, Deroche-Gamonent et al, 2000). The present study attempted to replicate Piazza et al., (2000) by exploring a work-response function in wheel running addiction. Different degrees of friction were applied to running wheels to make them more or less easy to turn. 24 adult male rats were permitted to run with low, medium or high friction, alternated on a daily basis for 36 days, at which point running had plateaued. Although running was decreased when the degree of friction increased, the work exerted by rats (distance run x friction) showed an inverted U function similar to that evident in the drug dose-response function. Rats were separated into high and low runners based on a medium split of the final 24 h running amount on the low friction condition. In comparing high and low running rats, it was observed that their work-response function did not differ horizontally (shifting to the right or left), but rather showed a vertical shift, as high runners acquired their high levels of running over the first part of the study. Therefore, it could be concluded that only some animals have a predisposition to wheel running addiction, and they work harder across all levels of friction. (This work was funded by a NSERC grant to RE.)

12 B112

NEURAL MARKERS OF OBJECT SEGREGATION AND RECOGNITION IN THE HUMAN BRAIN: EVIDENCE FROM MAGNETOENCEPHALOGRAPHY (MEG)

Stephen M. Emrich, Susanne Ferber, Bernhard Ross. University of Toronto - Department of Psychology, Rotman Research Institute - Toronto*

Traditional models of object recognition posit that processing of figure segregation precedes the identification and recognition of an object. However, recent evidence suggests that some recognition processes may occur prior to or simultaneously with segregation. The present research delineates the temporal sequence of processing stages involved in figure-segregation and object-recognition. Neural activity was recorded using magnetoencephalography (MEG) while participants observed moving displays containing scrambled or intact objects, as well as simple coherent motion. An event-related synthetic aperture magnetometry (erSAM) technique was used to identify brain regions of maximal activation in response to the moving objects, and source activity for these regions was estimated for each condition. The results demonstrate sources located in regions corresponding to the motion-sensitive MT+, the object-sensitive lateral occipital cortex (LOC), as well as regions in extra-striate cortex and the inferior temporal lobe. At early latencies, activity in MT+ and the LOC was similar between scrambled and intact objects. This was followed by activity in the the inferior temporal lobe. The activity in the inferior temporal sources in response to recognizable objects was significantly different from the activity in response to scrambled forms. Activity in these regions was followed by additional activity in the LOC which also differentiated between intact and scrambled objects. The results provide evidence that recognizable and scrambled objects are initially processed similarly by extra-striate and lateral occipital cortices. This initial activity is followed by feedback modulation from inferior temporal cortex. Activity in the LOC at later latencies is greater for objects than for scrambled objects. These findings contrast traditional models of object recognition, and suggest that the LOC may initially perform figure-segregation processes.

13 B113

THE GAZE FIXATION ASYMMETRY EXHIBITED BY HUMANS WHEN VIEWING FACES IS INDEPENDENT OF AUDIOVISUAL SPEECH PERCEPTION AND FACIAL ATTRIBUTES

Ian T. Everdell, Heidi Marsh, Micheal D. Yurick, Kevin G. Munhall, and Martin Paré. [1]Centre for Neuroscience Studies, [2]Biological Communication Centre, and Departments of [3]Otolaryngology, [4]Physiology, and [5]Psychology, Queen's University, Kingston, Ontario*

Speech perception under natural conditions requires integration of auditory and visual information. Understanding how a viewer assimilates the information contained in these sensory modalities requires detailed descriptions of the available speech information and the way that information is processed. To better understand how facial information is gathered, we quantified the distribution of gaze fixations of humans performing an audiovisual speech perception task with dynamic talking faces. We examined the degree of gaze fixation asymmetry to determine whether left-right biases were a result of asymmetries in the face stimulus itself or of strategies of the viewer. Most participants preferentially fixated the right side of the faces, suggesting a right visual field bias; this bias was present in the first fixations following stimulus onset and persisted with horizontally mirrored faces, different talkers, and static faces. Participants showed stronger fixation asymmetries when viewing dynamic faces, in comparison to static faces or face-like objects, and especially when their gaze was directed to the talker's eyes. Correlation analysis revealed that the magnitude and direction of an individual's gaze asymmetry during audiovisual speech (i.e., dynamic stimuli) are predicted by the same parameters measured during simple face processing (i.e., static stimuli). Although viewing dynamic faces significantly enhanced speech perception, we did not find any correlation between task performance and fixation asymmetry or between performance and fixation of

specific facial features. Similarly, asymmetry did not appear to be related to other measures of laterality (handedness and eye-dominance), nor was there a difference in performance between original and horizontally mirrored stimuli. Viewing patterns over time were relatively consistent regardless of the talker's face or its orientation: mouth fixation probability increased at speech onset and decreased at speech offset, but in general, fixations were not related to details of the stimulus. These results suggest that asymmetrical distribution of gaze fixations reflects the participants' general face-viewing strategies rather than being a product of asymmetry within the faces themselves. That these strategies did not predict audiovisual speech perception suggests that the process of gathering information from a talker's face involves a large visual span that is independent of the ability to gather that information.

14 B114

ATTENDING TO MIND AND BODY: MINDFULNESS TRAINING REVEALS DISTINCT NEURAL MODES OF SELF-AWARENESS

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Despite of considerable progress specifying the cognitive and neural mechanisms underlying the temporally extended self (e.g., retrieval of personality traits from memory), little is known about the neural mechanisms by which humans monitor momentary selfexperience in the psychological present. To characterize these two aspects of self-awareness, we used fMRI to examine regional brain activity in individuals before or after 8 weeks of training in mindfulness meditation (MT), during 1) "narrative" self-focus (NF), characterized by monitoring the self related to enduring traits and 2) "experiential" self-focus (EF), characterized by monitoring the self related to moment-to-moment experience, while reading personality descriptors. Novice participants engaged cortical midline self-referential regions (medial prefrontal cortex, mPFC) to a lesser degree during EF than NF. Following MT, EF resulted in more pronounced and widespread reductions in midline cortical activity, which were replaced by a right lateralized network, comprised of the ventral and dorsal PFC and posterior cortical foci, including regions associated with viscero-somatic representations (right insula, inferior parietal lobule and secondary somatosensory cortex). Consistent with the importance of mPFC disengagement for EF, functional connectivity analyses demonstrated a decoupling between the right insula and mPFC following but not before MT. These results suggest a fundamental neural dissociation between distinct forms of self-awareness uncovered through mindfulness training—a mental and bodily self. Focused monitoring of moment-to-moment experience is associated with increased access to right lateralized neural representations of the bodily self, potentially representing the underlying older substrates and origins of selfhood.

15 B115

THE ROLE OF THE HIPPOCAMPUS IN RECENT AND REMOTE CONTEXTUAL DISCRIMINATION

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The acquisition of contextual fear conditioning depends on the hippocampus. However, as the contextual fear memories mature, they may become less precise. To examine the role of the hippocampus in the maintenance and precision of contextual fear memory, mice were trained in a context discrimination procedure where one context was paired with shock (i.e., A+), whereas another was not (i.e., B-). One or 21 days later, mice received a sham or excitotoxic hippocampal lesion, and conditioned freezing in contexts A and B were then evaluated one week later. In the 1-day group, the sham animals discriminated between contexts, showing higher levels of freezing in A compared to B. Hippocampal lesions abolished this discrimination and greatly reduced freezing levels. In the 21-day group, the sham animals showed similarly high levels of freezing in context A and B, indicating generalization across contexts. Again, hippocampal lesions greatly reduced freezing levels in this group. Conditioned freezing in either the 1-day and 21-day lesion groups could not be reinstated by a reminder shock and did not show spontaneous recovery when tested one week later. However, both lesion groups could be retrained and showed normal performance in the open field suggesting that the lesioned-mice had no performance deficit related to motor or sensory dysfunction. We further showed that the hippocampal-lesioned mice were impaired in the hidden version of the water maze, therefore validating the effectiveness of the lesion in another behavior task. These results indicate that discrimination between similar contexts always requires the hippocampus. Surprisingly, we found no evidence for a graded effect of these lesions on levels of conditioned freezing, suggesting that the hippocampus may also play an extended role in the expression of conditioned fear.

16 B116

ESTROGEN RECEPTOR α, BUT NOT β, IS INVOLVED IN REGULATING ESTROGEN-INDUCED CONDITIONED TASTE AVOIDANCE IN MALE RATS.

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When estrogen is paired with the consumption of a sucrose solution, rats will subsequently display conditioned taste avoidance as indexed by a significant reduction in sucrose consumption. A variety of studies have shown that estrogen exerts its many effects through activation of at least 2 estrogen receptor subtypes. However, it is unclear as to which estrogen receptor subtype, either estrogen receptor alpha (ERα) or beta (ERβ), is involved in mediating estrogen-induced conditioned taste avoidance. The present study examined the contributions of ERα and ERβ in the production of conditioned taste avoidance in male Long Evans rats. A lickometer apparatus was used to measure changes in sucrose consumption and drinking patterns. In addition, a novel automated open field apparatus was used to investigate possible drug-induced sedation and/or anxiety effects. After habituation to the lickometer and adjustment to a water deprivation schedule, animals were tested on three separate conditioning days and one vehicle test day. On each conditioning day, animals were injected with either propyl pyrazole triol (PPT,an ERα agonist), diarylpropionitrile (DPN, an ERβ agonist), 17β-estradiol (E2) or vehicle (10% ethanol- 90% saline) 20 minutes before drinking a sucrose solution from the lickometer. Immediately following this, animals were placed into the automated open field for 30 minutes. It was found that both E2 and PPT significantly reduced sucrose drinking on Conditioning Days 2 and 3, and produced a robust conditioned taste avoidance on the vehicle test day. The ERβ agonist DPN did not significantly reduce sucrose intake compared to vehicle controls on any experimental day. There were no significant drug effects on either locomotor activity or anxiety measures. Together, these results suggest that E2-induced conditioned taste avoidance is regulated by estrogen's activation of ERα, and not ERβ. This research is supported by NSERC.

17 B117

THE EFFECTS OF REACHABILITY AND TOOL USE ON FMRI ACTIVATION IN HUMAN BRAIN REGIONS INVOLVED IN HAND ACTIONS

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Electrophysiological recordings in macaques and neuropsychological studies in humans have reliably shown that the parietal cortex encodes a

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

unique representation of visual space within reach of the arm (peripersonal space). In particular, previous research from our lab has shown that an area involved in reaching, the superior parieto-occipital cortex (SPOC), is active during the passive viewing of objects placed within reach of the arm. As such, this area appears to encode a potential for action on the object. Here we used functional magnetic resonance imaging (fMRI) to examine how SPOC encodes object distance from the body. We predicted that SPOC would show a preference for objects within reach of the arm, even during passive viewing when no action is required. In order to investigate this hypothesis we presented 8 subjects with graspable objects at six different locations on a platform placed over each subject's hips. Each subject's upper right arm was braced only permitting an arc-like range of movement with their lower arm. Within single trials of an event-related design subjects were required to perform grasping and reaching actions to an object location along the arc of reachability, at the point corresponding to the subject's sagittal midline (H location). Subjects were also instructed to passively view five object locations: two near locations (N1 and N2) positioned in the right and left visual fields, equally eccentric from fixation (such that only the N1 location was within range of the subject's reach); two middle locations on the left and right (M1 and M2), both positioned at a unreachable further distance; and a far location (F) that was positioned in the right visual field far beyond reach. Subjects were required to maintain fixation throughout all trials and keep their hand positioned on the nearest edge of the platform in between action trials and during passive viewing trials.

A voxelwise conjunction analysis between passive viewing conditions for N1 (within reach) vs. each of the non-reachable locations (N2, M1, M2, F) revealed activation within SPOC. This finding suggests that SPOC encodes objects within reach of the hand as our results cannot be attributed to object eccentricity (less activation in N2) or visual field (less activation in M2 and F) effects. Furthermore, we also investigated whether SPOC is sensitive to the enlargement of reachable space with tool use. Subjects in the same experimental design performed grasping and reaching with a set of tongs to objects at a tool (T) location (located in the subject's midline but further than the H location) while passively viewing objects at all other pre-specified locations (N1, N2, M1, M2, F). In this case, the range of space reachable with the tool now encompassed passive viewing locations N1, N2, and M1. Using a region of interest approach, analyses in SPOC indeed suggest that object locations reachable by the tool are newly encoded as being within reach.

18 B118

AN INVESTIGATION OF THE FREQUENCY SELECTIVITY OF ATTENTIONAL MODULATION OF PRIMARY AUDITORY CORTEX

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Neurons in the primary auditory cortex (PAC) are targeted by cholinergic projections from the basal forebrain that modulate synaptic plasticity by making neurons more sensitive to their afferent inputs. This suggests that the basal forebrain system which is corticotopically organized may perform some of the functions of an attention mechanism. Consistent with this hypothesis, we have shown that the amplitude of the 40-Hz auditory steady state response which localizes to the PAC is enhanced by attention. SSR amplitude increased when subjects detected targets in a 1s stream of 40-Hz stimulation compared to conditions in which subjects passively experienced the stimuli.

The present study investigated whether modulation of the PAC by attention can be frequency specific. Subjects (n=16) were presented with two auditory streams differing in carrier frequency (CF) but containing separate targets embedded in each. The two streams (CF either 250 Hz or 4100 Hz) were presented simultaneously at moderate intensities (65 dB SPL) but amplitude modulated at different rates (37 or 41 Hz, counterbalanced) so that the brain response to each stream could be separated by filtering. Targets were AM pulses of enhanced amplitude that occurred randomly in each stream about once per second (combined target rate 0.5 Hz), requiring a sharp focus of attention on the relevant CF. Trials were 120s in duration and delivered in blocks separated by a brief rest. In the first half of the attention task participants were asked to press a button to targets in one auditory stream and, in the second half, in the other stream. Behavioral performance was assessed by relating each button press to the immediately preceding target in each stream. SSR was assessed by DFT applied to each 120s block. Behavioral analyses showed orderly response latency distributions when responses were timed to targets in the attended stream, but random distributions when related to targets in the unattended stream. Subjects therefore complied with the attentional requirements of the task. Distinctive 37 Hz and 41 Hz SSRs were generated by each carrier frequency. However, SSR amplitude did not track the attention requirement. The findings suggest that the spotlight of attention cannot selectively illuminate specific tonotopic regions in PAC. Our ability to attend to different frequency components of a sound may depend on other mechanisms. (Supported by CIHR and NSERC of Canada)

19 B119

DISSOCIABLE ROLES FOR NUCLEUS ACCUMBENS SUBREGIONS IN EFFORT-BASED DECISION MAKING

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Animals are constantly faced with decisions involving cost-benefit analyses, assessing which particular course of action is most appropriate so that the potential reward exceeds the costs. Decisions regarding choosing a larger magnitude of reward that is accompanied by a greater effort are mediated by a distributed neural network of anterior cingulate, basolateral amygdala, and mesoaccumbens dopamine system. Previous studies investigating the neural mechanisms underlying effort-based decision making have employed a T-maze task in which rats have had the option to climb a barrier in one arm to obtain a high reward (HR), or exert no effort by choosing an arm without a barrier, receiving a low reward (LR). Although earlier studies have investigated the role of nucleus accumbens (NAc) dopamine in this form of decision making, surprisingly there have been no studies assessing the effects of inactivation of different subregions of the NAc regions on these tasks. The present study investigated the role of the NAc core and shell using an automated effort discounting decision making procedure in an operant chamber. The task consisted of 4 discrete blocks of 10 trials, whereby rats were given the choice of pressing one lever once to receive 2 reward pellets (LR) or another lever that delivered 4 pellets (HR) after a fixed ratio of presses that increased with each block (2, 5, 10, or 20). In a subsequent experiment, the delay to receive the LR was equalized to the time it took the animal to complete the ratio on the HR lever (0.5-7s), to asses whether these effects were due to differences in delay to reinforcement. In both experiments, inactivation of the NAc core, but not the shell, reduced the preference for the HR lever. These effects were not accompanied by changes in motor or motivational functions. Thus, the NAc core, but not shell, is part of a distributed neural circuit that mediates effort-based decision making. Furthermore, the contributions that NAc core makes to this form of decision making are distinct from those which mediated delay-based decision making.

20 B120

ENCODING OF COMPLEX VISUAL MOTION BY A SINGLE NEURAL PATHWAY

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The lobula giant movement detector (LGMD) and its target neuron, the descending contralateral movement detector (DCMD), constitute one motion sensitive pathway in the locust visual system that is preferentially sensitive to objects approaching on a direct collision course. Previously described LGMD responses to motion stimuli suggest that this pathway should be

sensitive to approaches of individual objects within a complex visual scene. To test responses to complicated object motion, we presented intact locusts with compound looming objects while recording from the DCMD. Presentation of paired objects approaching from different regions of the visual field at nonoverlapping, closely timed and simultaneous approach intervals were designed to study DCMD responses to multiple looming stimuli. We also presented single objects that deviated from a direct collision trajectory to test whether DCMD responses encode visual parameters that emulate stimulation during collision avoidance behaviours. Looming compound objects evoked characteristic DCMD responses that were similar to size-matched simple objects. Specifically, the time of peak firing was consistent with predicted values based on a weighted ratio of the half size of each distinct object edge and the absolute approach velocity. For paired approaches the azimuthal position and approach interval affected DCMD firing properties. Moreover the DCMDs responded to individual objects approaching within 106 ms of each other. Comparisons between individual and paired approaches revealed that overlapping approaches are processed in a strongly sublinear manner. Deviations away from a looming trajectory by single objects evoked a distinct peak in the DCMD firing rate that was independent of object size or time of deviation. These findings suggest that this single, motion sensitive pathway is able to encode complex aspects of visual scenes that emulate conditions during production of natural behaviour.

21 B121

NICOTINE DEPENDENT AND WITHDRAWN MICE DEMONSTRATE CONDITIONED PLACE AVERSIONS THAT ARE ABOLISHED BY THE DOPAMINE ANTAGONIST ALPHA-FLUPENTHIXOL

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Although nicotine is one of the most widely used addictive drugs, the motivational properties that make it so addictive are largely unknown and the current practice for characterizing the nicotine abstinence syndrome and its motivational effects in the mouse is unreliable. The purpose of our work is to determine whether mice will experience nicotine withdrawal, and if so, what neurobiological substrates are mediating the motivational effects of this withdrawal. We implanted mice with osmotic minipumps containing a high dose of nicotine (100 mg/kg/day) for 2 weeks and observed them for somatic and motivational withdrawal upon pump removal. These mice demonstrated a nicotine somatic abstinence syndrome that was strongest 8 hours after pump removal. Furthermore, nicotine dependent mice showed an aversion to the withdrawal-paired side in a conditioned place preference paradigm when conditioned 8 hours after pump removal. Historically, the mesolimbic dopamine (DA) system is believed to play an important role in the rewarding and aversive aspects of many drugs of addiction, including nicotine. Therefore we investigated the role of DA in dependent nicotine aversion. Interestingly, when our nicotine dependent and withdrawn mice were pre-treated with the broad-spectrum DA receptor antagonist alpha-flupenthixol (0.8 mg/kg, i.p.) under conditions where alpha-flupenthixol produces no motivational effects on its own, their conditioned place aversions were abolished. However, naive mice that were administered a single dose of nicotine (5 mg/kg, s.c.) and conditioned 8 hours later showed a significant conditioned place preference for the acute withdrawal paired side that was not blocked by alpha-flupenthixol. These results suggest that dopamine receptor activation mediates the aversive motivational affects of nicotine withdrawal when animals are in a drug dependent and withdrawn state.

22 B122

VISUO-TACTILE BINDING IN MIRROR-INDUCED ILLUSORY SPACE

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The temporal perception of stimuli changes depending on their relative spatial location. We compared the effect of actual and illusory spatial separation between components of a bimodal stimulus pair on perceived timing. Subjects were presented with light/touch stimulus pairs with various onset asynchronies and were asked to make temporal order judgments (TOJs) and simultaneity judgments. Using a mirror, we manipulated the perceived spatial location of the stimuli. The left hand's mirror reflection appeared as an illusory right hand that could be either congruent or incongruent with the right hand's true location. The just noticeable differences of the TOJs were significantly smaller than for simultaneity judgments: paradoxically the TOJs of stimuli could sometimes be accurate even when the stimuli were perceived as simultaneous. While TOJs were affected by the separation of the stimuli in space, simultaneity judgments depended on whether the stimuli were presented within a particular spatial integration window, suggesting at least two temporal coding mechanisms.

23 B123

DIFFERENTIAL RESPONSES TO THE D3 RECEPTOR ANTAGONIST U99194 IN DBA/2 VS. C57/B16 MICE TREATED WITH CHRONIC ETHANOL

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Neural mechanisms that underlie behavioural sensitization, the progressive increase in responding to a drug over time, have been postulated to be similar to those that underlie behavioural pathologies such as substance abuse and relapse. A better understanding of these mechanisms could be helpful in identifying treatment strategies, particularly in the realm of pharmacotherapy. When studying the mechanisms of sensitization, most studies have focused on the respective roles of the D1 and D2 dopamine receptor subtypes. Recently, there has been evidence to suggest that the D3 dopamine receptor subtype also plays a role in behavioural sensitization, although its exact function remains largely unknown. We previously observed in our laboratory that D3 receptor knockout mice (D3 KOs) were resistant to the sensitizing effects of ethanol (EtOH) when compared to their wild type littermates. In an effort to observe whether the lack of D3 function has a true effect on sensitization or whether the observed resistance to EtOH sensitization in D3 KOs was a compensatory mechanism, we hypothesized that temporary blockade of D3 receptors with an antagonist would interfere with the development and expression of sensitization to EtOH in normal mice. In 2 separate strains of mice, one more susceptible to the sensitizing effects of EtOH (DBA/2), and the other more resistant to EtOH sensitization (C57/Bl6), animals received 7 bi-weekly injections of EtOH (2.2 g/kg i.p.) or saline, followed 10 days later by challenge dose of the D3 antagonist U99194 (10 mg/kg s.c.). In a second experiment, the 2 strains of mice received U99194 (10 mg/kg s.c.) co-administered with every EtOH injection (2.2 mg/kg i.p.). In the first experiment, the D3 antagonist challenge had no significant effect on EtOH sensitization in either strain of mice; however, in the second experiment where the co-administration of D3 antagonist significantly blocked the development of sensitization in DBA/2 mice, C57/Bl6 mice developed sensitization to the D3 antagonist itself. Results of these studies suggest that in addition to having differential effects in two genetically different strains of mice, D3 antagonists do not block the expression of sensitization once it has been induced, but may be useful in preventing the development of sensitization in individuals genetically more susceptible to EtOH sensitization. Support Contributed By: NSERC, OGS and CAMH Research Fund.

24 B124

AFFECTIVE CHRONOMETRY OF SECURITY MOTIVATION

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A recent theory (Szechtman & Woody, Psychol Review, 111:111-127, 2004) posits a Security Motivation System (SMS) activated by potential, rather than by imminent, threat to the individual. SMS coordinates species-typical motor activity that probes the environment for danger and includes behaviors such as checking (eg, for the presence of predators) and cleaning (for potential threats from germs, etc). Activation of SMS induces also an affective phenomenological cue of potential danger that is experienced as anxiety. We investigated whether a physiological correlate of an activated SMS would show the expected properties: activation by a relevant stimulus and persistence of activity until performance of the appropriate behavior. We report here on a paradigm that produces the expected results, a paradigm that can be employed in future studies to investigate the disturbance in SMS proposed to characterize obsessive-compulsive disorder. Participants were instructed to contact a high contamination-threat stimulus (diapers appearing soiled). Physiological measures of anxiety—heart rate variability and facial EMG activity—were taken prior to, during, and after exposure. Separate groups were either permitted to engage in the appropriate behavior (washing) or engaged in an irrelevant task. Measures of anxiety were significantly higher after exposure to the diapers as compared to baseline resting recordings. These measures remained significantly elevated in participants not given an opportunity to wash as compared to those permitted to wash. After hand washing, measures of anxiety returned to baseline. Supported by CIHR MOP134450.

25 B125

IMPLICATION OF THE TRANSCRIPTION FACTOR NUR77 AND ENDOGENOUS STRIATAL NEUROPEPTIDES ENKAPHALIN AND DYNORPHIN IN AMPHETAMINE INDUCED BEHAVIORAL SENSITAZATION

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Several studies suggest that striatal enkephalin (ENK) and dynorphin (DYN) are important for neuroadaptation following psychostimulant exposure. Other evidences show that nuclear receptors acting as transcription factors are involved in the regulation of dopaminergic pathways. Among them, Nur77 seems implicated in adaptation and homeostatic regulation of dopaminergic systems. Therefore, by using mice deficient for the gene Nur77 (Nur77 (-/-)), ENK knockout (ENK (-/-)) and DYN knockout mice (DYN (-/-)) versus wild type (WT) mice, we investigated the respective role of this particular transcription factor and these two neuropeptides in behavioural sensitization induced by repeated amphetamine (AMPH) administration. WT, Nur77 (-/-), ENK (-/-) and DYN (-/-) mice received a five-day AMPH pre-treatment (2.5 mg/kg/day) and were challenged five days later with the same AMPH dose. Both basal and AMPH-induced locomotor activity were recorded. Levels of ENK, DYN and Nur77 mRNA were measured by in situ hybridization. Basal activity was lower in ENK (-/-) and DYN (-/-) mice than in WT whereas Nur77 (-/-) mice displayed an enhanced basal activity compared to their WT littermates. In response to repeated AMPH injections, ENK (-/-) and DYN (-/-) mice failed to show behavioural sensitization whereas Nur77 (-/-) mice displayed a higher response to repeated AMPH treatment than WT mice. Biochemical analysis in mice brains showed similar basal expression levels for DYN in WT and ENK (-/-) mice, whereas Nur77 basal expression was higher in ENK (-/-) mice. Following AMPH treatment, ENK (-/-) mice showed an increase of Nur77 and a no change of DYN mRNA expression whereas WT mice displayed a decrease of Nur77 concomitant to a slight increase of DYN expression. Contrary to the ENK (-/-), DYN (-/-) mice demonstrated a decrease of Nur77 expression compared to WT mice following AMPH chronic administration. Interestingly, we observed increased level of ENK expression in Nur77 (-/-) compared to WT type mice in both basal and post-AMPH treatment conditions. Taken together, these results demonstrated that striatal ENK and DYN are both necessary to develop behavioural sensitization. They also suggested that Nur77 involvement in homeostatic regulation of dopaminergic systems probably consist in a complex regulation of ENK and DYN expression. Finally, they supplied strong evidence of an interaction between the direct and indirect dopaminergic pathways.

26 C101

CROSS MODAL IDENTITY RECOGNITION: THE EFFECTS OF FACE AND VOICE INTEGRATION ON RECOGNITION

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Humans have an impressive ability to recognize hundreds of individuals by their face. A similar recognition ability is encountered with individual voices. How often does one pick up the phone and immediately recognize the identity of the caller by just the word, “hello”? Here we asked whether bringing the two modalities together, vision and hearing, will result in enhanced identity recognition. In addition, females have shown better face recognition performance compared to men; will this gender bias be maintained with cross modal stimuli? We trained 60 participants on 5 sets of female and male identities each. Identities consisted of a visual face image and a voice sample pair. Face image contrast was reduced so that latency for recognition of the face alone was consistent with latency for voice alone. The learning phase consisted of three runs of 20s presentations for each of the face images with the matching voice sample. Two practice trials with feedback ensured that participants had learned the faces and voices of their respective identities prior to testing. We tested recognition performance (accuracy and correct latency) in a learned/ novel identity paradigm. There were five conditions in which face and voices were paired: 1) learned congruent-- learned faces and voices paired correctly; 2) learned incongruent– learned faces and voices paired incorrectly; 3) face learned and novel voice -- ; 4) novel face and voice learned --; 5) novel face and novel voice. There was no difference in recognition performance between female and male participants. There were however, significant differences in performance between the conditions. Novel face and voice pairings were most quickly and accurately recognized. In contrast, novel face/ learned voice pairings were better recognized than novel voice/ learned face pairings. This suggests that participants rely more heavily on visual than auditory information when recognizing identity. Finally, unlike identity recognition from a face image alone, women do not show better recognition compared to men when identity is based on both auditory and visual information.

27 C102

MANIPULATING PROBABILITY TO INVESTIGATE THE RELATIONSHIP BETWEEN OVERT AND COVERT ORIENTING

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Previous studies investigating the relationship between overt orienting (saccadic eye movements) and covert orienting (visuospatial attention) have often led to contradictory results. Evidence has suggested that these two processes are subserved by either the same, interdependent or independent neural mechanisms. For the most part, these studies utilized instructive cues in which to allocate attention then asked if motor preparation follows. We examined this issue by taking the opposite approach; that is, saccade preparation was first allocated by manipulating the probability of a saccadic target being presented to either the left or right and we tested whether visuospatial attention followed. During a minority of trials, a probe (a ring

with a gap at the top or the bottom) was presented at different time intervals before the target stimulus. Subjects were required to maintain fixation during these trials and discriminate the orientation of the gap with a keyboard press. Overt orienting- Probability influenced overt orienting; as the probability of the target increased, saccadic reaction time to the target decreased and the proportion of erroneous saccades directed towards a probe stimulus at that location increased. In addition, as the time of probe presentation neared time of target presentation, the proportion of erroneous saccades also increased. Covert orienting- Probability influenced covert orienting in a manner similar to that of overt orienting; as the probability of the target increased, the proportion of correct probe discriminations also increased. The proportion of correct discrimination also increased as the time of the probe presentation neared the time of target presentation. Overall, we also found a positive correlation between behavioural indexes of overt and covert orienting. Together, our results suggest that the neural processes subserving overt and covert orienting are either interdependent or the same. (Supported by CIHR)

28 C103

A NEURAL NETWORK MODEL THAT ILLUSTRATES HOW THE DYNAMIC PROPERTIES OF SPATIAL UPDATING DEPEND ON THE TYPE OF SIGNALS USED TO DRIVE THE UPDATING

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It is currently believed that remembered visual target locations are stored in eye-centered coordinates and updated across eye movements. Neuronal behavior associated with this updating has been observed in brain areas associated with saccade generation, in the form of transient receptive field remapping prior to and during the saccade. The dynamics of this remapping, however, remain largely unexplored. We trained three 3-layer recurrent neural networks to perform the saccade-related target updating associated with the double-saccade task to examine how representations of target position evolve during this updating. Target position during fixations was represented in the output layer as a hill of activation in a 2-D topographic array of units. Network inputs were initial target position, dynamic eye position, and the signal(s) used to drive the updating, which, for the three networks were 1) the initial 'cortical' representation of the saccade target, 2) the dynamic 'brainstem' velocity signal of the saccade, and 3) both. In the first network, predictive updating was observed in which the hill of activity jumped directly from initial to remapped target position in a single time-step. In the second network, a gradual shift in the output hill of activation from initial to remapped target position over the duration of the saccade was observed, the hill's amplitude being suppressed during this movement. In the third network, the evolution of the output activation combined that of a jumping and a moving hill. Only networks 1) and 3) showed remapping latencies, as measured by the onset of activity at the updated target location, that spanned the time immediately before and during the saccade, similar to what has been observed neurophysiologically in the frontal eye fields (Umeno and Goldberg 1997). Networks 1) and 3) also showed an absence of increase of activity at the remapping midpoint during the updating observed in the frontal eye fields (Sommer and Wurtz 2006). Only networks 2) and 3) were able to perform the all components of updating across a full 3-D saccadic eye movement. Thus, only by using a combination of updating signals were all of the properties observed in updating to date replicated.

29 C104

NEUROANATOMICAL AND COGNITIVE PREDICTORS OF SEXUAL ORIENTATION IN RIGHT-HANDED MEN: AN MRI STUDY

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Numerous studies have consistently shown that homosexual men have an increased prevalence of non-right-handedness and atypical patterns of hemispheric functional asymmetry compared to heterosexual men (e.g., Lalumiere et al., 2000). Within the general population, non-right-handedness in men has been associated with increased size of the corpus callosum (CC), particularly of the isthmus, the posterior region of the callosal body interconnecting parietotemporal cortical regions (Witelson, 1989). Since sexual orientation is associated with hand preference and since hand preference is associated with CC anatomy in men, it was hypothesized that sexual orientation in men is associated with callosal anatomy, specifically the isthmus. Our study focussed on the relationship of CC anatomy and sexual orientation in strongly right-handed men, defined as consistently-right-handed (CRH), in order to control the confound caused by the increased prevalence of non-right-handedness among homosexual men. We predicted that CRH homosexual men would have a greater callosal isthmus compared to CRH heterosexual men. To determine whether this sample of CRH homosexual men differed cognitively from heterosexual men as in the literature, we administered a battery of tests which assessed spatial and verbal abilities. Twelve homosexual and ten heterosexual young healthy men underwent MR imaging at Sunnybrook Health Sciences Centre. Area measures of the total midsagittal surface of the hemisphere, the CC and four subdivisions including the isthmus, were made. Independent t-tests revealed that isthmal area was larger in the homosexual group (directional t–test, $p = 0.03$). Binary logistic regression analysis was undertaken to predict group membership (i.e., homosexual vs heterosexual) and all anatomic and psychological variables were included in the stepwise (forward selection) analysis. The best-fit model included Left Hand Performance (a measure of manual asymmetry), isthmal area, the Shipley Abstraction score (a measure of logical reasoning), and Water Level score (a measure of spatial perception). This model yielded 96% correct classification ($p < 0.001$) using 85% confidence limits (one heterosexual man was misclassified) and accounted for 84% of the variance. These results suggest that neuroanatomical structure and cognition are associated with male sexual orientation and add to the evidence of a neurobiological basis in the etiology of sexual orientation.

30 C105

THE CONTRIBUTION OF THE DORSOLATERAL PREFRONTAL CORTEX TO EXECUTIVE FUNCTION

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Introduction: The dorsolateral prefrontal cortex (DL-PFC) is thought to be involved in the monitoring of information held in working memory [1]. This view has been further supported by previous fMRI studies using the Wisconsin card sorting task (WCST) [2]. However, functional imaging studies alone cannot assess the exact contribution of a specific brain structure, e.g., the increased DL-PFC activity detected while the subject received feedback during the WCST may not be essential for monitoring, but may only be an epiphenomenon. Therefore, to confirm the role of this region, we investigated the performance of WCST during repetitive transcranial magnetic stimulation (rTMS) of the DL-PFC. Methods: Ten right-handed healthy subjects were stimulated using rTMS over the right DL-PFC and vertex (control). The positioning of the coil was chosen according to the previous fMRI study [2] and using frameless stereotaxy [3]. While the

subject performed the WCST or the control task, rTMS was given to the subject's right DL-PFC or vertex at three different timing; (a) at the beginning of feedback, (b) at the beginning of matching or (c) every 6 sec during the task (desynchronized stimulation). In each train of stimuli, 20Hz-rTMS was delivered for the duration of 250 msec in 110% of resting motor threshold. The interval between each train depended on the subjects' reaction time, i.e., roughly about 4 to 6 sec. Paired t-tests were completed in order to assess potential differences in the reaction time and the number of errors with rTMS in the DL-PFC versus the vertex. Results and Discussion: Reaction time was significantly longer for DL-PFC stimulation than vertex stimulation (control) when rTMS was given during feedback phase but not during matching or when administering desynchronized stimulation. There was no significant difference observed between DL-PFC and vertex stimulation during the control task that does not require active monitoring of working memory. These findings demonstrated that the DL-PFC is essentially implicated in the monitoring of information held in working memory rather than in the set-shifting process. This is in agreement with the previous fMRI study that showed increased DL-PFC activity while the subjects received feedback during the WCST [2]. [1] Petrides M, (2000) Exp Brain Res.; [2] Monchi et al.,(2001) J. Neurosci.; [3] Paus et al.,(1997) J. Neurosci.; Support Contributed By: CIHR, FRSQ and REPRIC

31 C106

EFFECTS OF REPEATED YOHIMBINE ON THE EXTINCTION AND REINSTATEMENT OF COCAINE SEEKING

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It is well established that acute exposure to some environmental and pharmacological stressors reliably reinstates drug seeking in rats following prolonged extinction training and drug-free periods. To date, however, little has been done to explore the effects of repeated stress exposure on the extinction of drug taking or on subsequent stress-induced reinstatement of drug seeking. Because stress typically recurs, investigation of the effects of repeated stress on drug seeking is warranted. The present study was designed to determine whether repeated exposure to stress during extinction affects extinction learning and subsequent reinstatement of drug seeking, and whether these effects are modulated by the context in which the stressor is experienced. Rats were trained to self-administer cocaine for 8-10 days. Subsequently, they were given five consecutive days of multiple extinction sessions; prior to the first extinction session each day, animals were pretreated with the pharmacological stressor, yohimbine (YOH; 1.25 mg/kg, i.p.), or its vehicle (VEH). One hour after being returned to the home cage (HC), animals were given a second injection of the alternate substance, i.e, VEH or YOH. Thus, for one group of animals, YOH was associated with extinction learning and the self-administration context, and for the other group of animals it was not. An additional group of animals was given VEH at both times. After this period of extinction training, animals were left undisturbed in the HC for nine days. Subsequently, they were given five additional days of extinction, in the absence of any YOH treatment, followed by a test for the reinstatement of cocaine seeking induced by a YOH challenge. (1.25 mg/kg, i.p.) In the first extinction phase, animals given YOH before the daily extinction sessions exhibited increased resistance to extinction. Following the second extinction phase (no YOH pre-treatment), these same animals showed a marked attenuation in the reinstatement of cocaine seeking induced by a YOH challenge. The results demonstrate that repeated exposure to YOH during extinction training induces context-dependent resistance to extinction and attenuation of YOH-induced reinstatement of cocaine seeking. This research has implications for understanding how repeated stress exposure can modulate extinction learning and affect subsequent reinstatement of drug seeking.

32 C107

INFLUENCE OF THE INTRASPECIES RELATIONS RESTORATION ON THE BEHAVIOR AND COGNITION PROCESSES OF THE RATS

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Long-lasting deprivation of the social contacts in the early ontogenesis results in stable disorders of the emotional sphere and behavior. Any stable form of the inadequate behavior is representation of a pathology, because manifests breaching of the relations between organism and its milieu. The purpose of present stude was to determine, whether is it possible to eliminate deprivation-induced disorders following restoration of the normal intraspecies connections. Behavior of rats' were investigated in the following groups: the rats, were isolated since 14th day (I group) and the 30th day (II group) of development. Following the two months of isolation these rats were 're-socialized', i.e. each isolated rat was placed into normal social milieu. During investigation of the rats' behavior following their re-socialization, alterations were found in those animals only, which were isolated since 30 days of age. when testing in 'proconflict' situation number of the punished drinking acts was significantly higher than in the intact animals of the same group. After 10-days stressing of the 're-socialized' rats testing the active avoidance response it was found that an index of the correct responses was 90%, whereas before the 're-socialization', the active avoidance responses were completely deteriorated and the new training was required for their acquisition anew. Considering this observation, we suggest that the state, which develops after 're-socialization' in the rats isolated since one month of age, must be attributed to normalized emotional status in these animals. Therefore, has been determined significance of specific periods of the postnatal development. In our experiments, long-lasting isolation (two months) at early stage (at 14th day) of the postnatal development elicits the stable alterations in the emotional sphere, learning and memory processes, in the mechanisms of adaptation to stressogenic environment. Following recovery of the social contacts anew, the above disorders are irreversible. Meanwhile, when the rats are isolated since 30 days of age 're-socialization' is capable of partial recovery of the normal level of the behavior and cognition processes.

33 C108

INCREASES IN UNREINFORCED RESPONDING DURING LIMITED ACCESS TO INTRAVENOUS SELF-ADMINISTRATION OF HEROIN

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We have conducted a series of experiments aimed at exploring a phenomenon observed in rats self-administering heroin on a continuous schedule of reinforcement. That is, during the acquisition phase of heroin self-administration, the number of unreinforced lever presses show consistent and progressive increases that remain high during maintenance. This is interesting because operant training should result in a decrease, not an increase, in lever presses that do not produce additional drug-infusions. Our studies indicate that this increase is neither related to a tendency to simply emit more operant responding with training, nor to excessive responding to the cue light present during heroin infusions. Rather, our experiments suggest that increases in unreinforced responding reflect a progressive enhancement in the animal's motivation to seek heroin, possibly as a result of a change in drive to self-administer the drug. In fact, we have found that the effects of heroin on locomotion, as well as responding for heroin on a progressive ratio schedule, both show progressive increases during the acquisition of heroin self-administration. In addition, levels of unreinfoced responding in sucrose-trained rats is directly modulated by levels of food-deprivation and, in heroin-trained rats, an injection of heroin (3 mg/kg) administered prior to a self-administration session decreases unreinforced responding. Supported by NSERC, CFI and OIT.

34 C109

DIFFERENCES BETWEEN ADOLESCENT AND ADULT FEMALE AND MALE RATS IN AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY AND CONDITIONED PLACE PREFERENCE

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In rodent models, there are differences between adolescents and adults in response to abused drugs that parallel developmental differences in people, with adolescents typically less vulnerable to aversive effects and more sensitive to their reward value compared to adults (Leslie et al., 2004). However, most of the preclinical research has focused on prepubescence or early adolescence. We investigated whether female and male rats in late adolescence differ from adults in locomotor and reward responses to amphetamine. Reward was assessed using a non-biased conditioned place preference (CPP) test, in which the animal is trained to associate one tactile cue with the drug and another with saline, and is then allowed to choose between the cues in a drug-free state. Locomotor activity was recorded during the conditioning phase of CPP. Long-Evans rats (n = 120) were tested for locomotor activity and conditioned place preference for amphetamine (0.25, 0.5, or 1.0 mg / kg) during late adolescence (45 - 52 days) or adulthood (69 - 76 days). Consistent with studies with prepubescents/early adolescents (Laviola et al., 1999), late-adolescent females had less locomotor activity to the first injection of amphetamine compared to adult females irrespective of dose, whereas late-adolescent males did not differ from adults. Late-adolescent females also had significant increases in locomotor activity to subsequent injections of amphetamine at all three doses, whereas such sensitization was only found at the highest dose for adult females and males of both ages. There was no effect of repeated injection at any dose in late-adolescent males, although they moved significantly more to amphetamine than to saline. There were no age differences in CPP, and for all, CPP increased with higher doses. In addition, females showed greater CPP during diestrous than during proestrous/estrous phases of the cycle. The sex-specific age differences in locomotor responses to amphetamine are not due to gonadal immaturity (i.e., females are cycling in late adolescence), but may reflect continuing maturation of the neural substrates underlying locomotor responses to amphetamine, but perhaps not those more relevant for reward.

35 C110

RESPONSES TO A REFLECTION OF DOMINANT AND SUBORDINATE CRAYFISH DIVERGE WITH TIME OF PAIRING

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Reflection has been shown to modify several aspects of crayfish behaviour, but only in socialized crayfish. Socialization of crayfish leads to a stable dominance hierarchy and pairing results in one dominant and one subordinate crayfish per pair. Dominant crayfish paired for 2 weeks spend more time in a reflective environment than in a non-reflective environment, but subordinate crayfish exhibit no preference. Specific behaviours, such as turning toward reflective corners and crossing toward reflective walls are enhanced in dominant crayfish and are not enhanced in subordinate crayfish. The present study sought to determine how these responses develop with time of social pairing. Crayfish housed in a community tank and pairs housed together for 30 min. and 3 days were observed in an aquarium with mirrors on one half of the tank and a non-reflective plastic on the other side. Pairing crayfish for 30 minutes enhanced most behaviour in response to reflection in both dominant and subordinate crayfish. After 3 days of socialization dominant crayfish continued to prefer reflection, while subordinate crayfish appeared to avoid reflection. Thus, responses of dominant and subordinate crayfish to mirrors diverge with time of pairing. Research supported by NSERC.

36 C111

CHRONIC SOCIAL STRESS IN ADOLESCENCE DECREASES ANXIETY-LIKE BEHAVIOUR, OR INCREASES RISK-TAKING BEHAVIOUR, IN THE ELEVATED-PLUS MAZE IN FEMALES, WHICH LASTS INTO ADULTHOOD

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We have reported previously that chronic social stress (SS) in adolescence leads to lasting increases in behavioural responses to psychostimulants in females, but perhaps not males (McCormick et al., 2004, 2005; McCormick & Ibrahim, 2007). Nevertheless, the effects of SS on neuroendocrine function in adolescence may be greater in males, which showed increased stress-induced CRH mRNA expression in the central nucleus of the amygdala compared to controls (McCormick et al., 2007). Thus, the effects of SS in adolescence in males may manifest for other behavioural endpoints. Here we investigated whether SS in adolescence (16 days of daily 1 h isolation and daily change of cage partner, days 30 to days 45 of age) increased anxiety-like behaviour in the elevated plus maze (EPM; anxiety inversely related to open arm exploration) compared to acute stress (1 h of isolation on day 45 only) and no stress (CTL) groups. We also investigated whether SS effects in the EPM would be evident several weeks after SS exposure in adolescence when the rats were adults. When tested on day 45, SS spent more time on the open arm and had a higher ratio of open arm entries to total arm entries than CTL ($p = 0.005$, $p = 0.01$), and than ACUTE ($p = 0.09$, $p = 0.06$). SS also had higher levels of locomotor activity overall in the EPM than CTL ($p = 0.007$). Estrous Cycle Phase was not a significant factor in the EPM at day 45. When tested as adults (70 days), SS spent more time on the open arms than CTL ($p = 0.001$), but only for estrus, not diestrus rats, and SS had a lower ratio of open arm entries to total arm entries than CTL ($p = 0.05$), and there was no effect of, or interaction with, Cycle Phase (both $p > 0.15$). For males, only the increase in locomotor activity at 70 days of age for SS compared to CTL was significant, although SS males at that age tended to spend less time in the open arms compared to controls ($p = 0.08$). Others report increased anxiety after stress exposure in adult males with the effect of stress on anxiety sometimes delayed (e.g., Vyas & Chatterjie 2004; Matuszewich et al., 2007). Chronic stress effects on EPM behaviour in adult females are inconsistent (e.g., Adamec et al., 2006; Vyas et al., 2005; Renard et al. 2005) perhaps due to estrus-related variation (Mora et al., 1996; Marcodes et al., 2001). The lasting differences in behaviour in the EPM after SS in adolescence in females observed here may reflect increased risk-taking (Macri et al., 2002).

37 C112

NEURAL CORRELATES OF EXPECTED VALUE IN THE SUPERIOR COLLICULUS

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The expected value of an event is an important variable in the decision making process because choosing the option with the highest expected value maximizes the chooser's intake of reward over time. Expected value is computed by multiplying the probability of reward by the magnitude of reward. Whereas the probability and magnitude of reward have previously been shown to influence the production of saccadic eye movements, we hypothesized that expected value would have a greater impact on saccade generation than either of these variables alone. Specifically, we measured how expected value influenced saccadic reaction times while recording the preparatory activity of neurons within the primate superior colliculus. We recorded neurons in the superior colliculus of non-human primate subjects while they performed a simple saccadic task. This task required subjects to look to a visual target that could be presented either to the left or right of a central fixation point. There was a short period with no visible stimuli between fixation offset and target onset to allow subjects time to prepare a

saccadic response. Across blocks of trials, the probability and magnitude of reward associated with the left and right targets varied. We found that as expected value of a saccadic target being presented at a location increased, SRTs decreased. We investigated how both preparatory activity in advance of target presentation and activity time-locked to the presentation of the visual stimulus was affected by manipulations of expected value. We found that both forms of neuronal activity were influenced by the expected value associated with the potential saccadic responses. Moreover, these neuronal signals were negatively correlated with the time to initiate a saccade to these targets. Our findings demonstrate that expected value is a decision variable whose importance is not limited to higher order decision making but is also a critical determinant in preparing simple motor acts such as saccadic eye movements.

38 C113

ELECTRICAL STIMULATION OF THE PRIMATE FRONTAL CORTEX REVEALS EYE-CENTERED AND HEAD-CENTERED REFERENCE FRAMES FOR GAZE COMMANDS

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Electrical microstimulation of gaze control structures as the superior colliculus (SC) produces gaze shifts defined in an eye-centered reference frame (Klier et al. 2001). In contrast, the supplementary eye fields (SEF) appear to encode gaze commands in multiple reference frames (Martinez-Trujillo et al. 2004; Park et al. 2006). Previous single-unit recording experiments in head-restrained monkeys (Russo and Bruce 1996) suggest that the frontal eye field (FEF) encodes the location of visual targets in an eye-centered reference frame. However, there is reason to suspect that the input code (revealed by unit recording) and the output code (probably revealed by stimulation) should not always employ the same reference frame (Smith and Crawford 2005). Currently it is not known if electrical stimulation of the FEF in head-free animals produces gaze shifts toward an eye, head, or space-fixed goal. We implanted two macaques with recording chambers over the FEF and search coils to measure eye and head rotations. Stimulation trains were delivered at 80 μA, 300 Hz, 200 ms, with gaze oriented at a variety of positions. Gaze shifts were evoked from 114 FEF sites in two monkeys (M1 n= 70, M2 n=44). Evoked gaze trajectories were mathematically rotated trajectories into three coordinate systems (eye/head/space). Then we examined gaze convergence in each of these frames. The distribution of gaze end-points had the lowest amount of convergence to a common point when plotted in gaze coordinates (M1 = 4639.82°2, M2 = 2485.60°2). In contrast, gaze end-points had greater convergence when plotted in head (M1 =1277.95°2, M2 = 423.23°2) or eye (M1 = 423.2°2, M2 = 170.21°2) coordinates. This suggest that the FEF stimulation output is characterized by an intermediate trend between an eye-centered reference frame as observed in the SC (Klier et al. 2001) and a multiple reference frame code used in the SEF (Martinez-Trujillo et al. 2004).

39 C114

MEASURING THE PERCEPTUAL UPRIGHT IN GREAT APES: EFFECTIVENESS OF THE OCHART

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We investigated the role of visual background on the Perceptual Upright (PU) in four Sumatran orangutans (Pongo abelii), housed at the Toronto Zoo, using the Oriented CHAracter Recognition Technique (OCHART) (Dyde et al., 2004 Exp Brain Res. 173: 612). Prior to experimental sessions, two training phases were administered for the animals to learn the discrimination between the character 'p' and 'd', which were superimposed on a circular polarized picture. In phase 1 of the training protocol, response windows appeared once the stimulus had been removed, whereas in phase 2, response windows appeared concurrently with the stimulus. The experiment was designed so that once criterion level was reached in phase 2, animals would move to Phase 3 (the experimental phase). In this phase, five experimental probes were randomly incorporated within every 20 Phase 2 trials. Phase 3 consisted of a total of 168 probe trials consisting of eight orientations of the letter probes, three background orientations, and seven repetitions. After an average of 470 trials, none of the animals reached criterion level in phase 1 and were consequently not able to move to phases 2 and 3. Because of this, we attempted a second experiment with human participants. In Experiment 2, we investigated the effectiveness of the OCHART with a nonsense figure ' '. Observers were trained to identify the figure as 'Glib' when orientated at 0 degrees and 'Glob' when orientated at 180 degrees. Ten observers participated and a within-subject design was used. The influence of the background on the PU, using the nonsense figure and the p/d letter probe, was then measured. The two tasks resulted in a similar psychometric function and showed a large effect of the visual background on the PU. However, analysis of the variances of the estimates of the PU indicate that the observers' judgment in the p/d trials were less reliable. These results appear to suggest that measuring the PU using the nonsense figure ' ' is at least as effective as using the letter probes 'p' and 'd.' Furthermore, because the 'p' and 'd' letter probe of the OCHART are nonsense figures for orangutans, it is evident based on experiment 2 that testing the animals with the OCHART may be an appropriate method for measuring the PU, given sufficient training on the discrimination task.

40 C115

THE ROLE OF THE PREFRONTAL CORTEX IN EXTINCTION AND REACQUISITION OF HEROIN SEEKING BEHAVIOUR IN THE RAT

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This study looked at the extinction and reacquisition of heroin-seeking behaviour in conditioned place preference. The purpose of the study was twofold: firstly, to investigate whether the medial prefrontal cortex (mPFC) is necessary for the formation of reacquisition memory; and secondly, whether it is necessary for the expression of extinction and reconditioning memory. Bilateral cannulae were surgically implanted into the ventromedial prefrontal cortex. Using the conditioned place preference task, animals underwent habituation, conditioning (0.3mg/kg), and extinction. Following extinction, rats were reconditioned with saline (equivalent to an additional day of extinction), or heroin (1.0mg/kg). Group one received intra-cranial infusions of either vehicle or GABAA/B agonists (muscimol and baclofen, 0.03 and 0.3 nmol, respectively/0.5μl/side over 2 min) twenty minutes following the reconditioning session. The day after reconditioning, Group two received vehicle or muscimol/baclofen twenty minutes prior to the second, drug-free CPP test. Post-reconditioning infusions of muscimol/baclofen did not disrupt the subsequent re-emergence of a place preference, while pre-testing infusions had no effect on the expression of extinction memory or on drug-seeking in heroin reconditioned rats. Supported by NSERC.

41 C116

THE FACILITATIVE EFFECTS OF D-CYCOSERINE ON EXTINCTION OF A COCAINE-INDUCED CONDITIONED PLACE PREFERENCE CAN BE LONG LASTING AND RESISTANT TO REINSTATEMENT: EFFECTS OF EXTINCTION VARIABLES

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Extinction of conditioned responses based on aversive and appetitive unconditioned stimuli (US) is used as a therapeutic measure to reduce fears or cravings. For example the reduction of conditioned fear involves the repeated presentation of conditioned fear stimulus (CS) in the absence of the

US (an extinction procedure). Similarly, the reduction of conditioned cravings or appetites can be brought about by repeated presentation of the conditioned appetitive stimulus (CS) in the absence of a US, such as food or injection of a drug of abuse. Recently it was shown in a series of experiments that extinction of conditioned fear responses can be facilitated by injection of the partial NMDA glutamatergic receptor agonist, D-cycloserine (D-4-amino-3-isoxazolidone, DCS), a compound that acts at the strychnine-insensitive glycine-recognition site of the NMDA receptor complex. We found previously that DCS significantly accelerates extinction of cocaine-induced conditioned place preference (CPP) when rats are given systemic or basolateral amygdala injections immediately, but not 4 hours, after each extinction trial. Here we report that when extinction trials are spaced, the effect of DCS can be long-lasting and resistant to cocaine-induced reinstatement. Groups of rats were trained on a CPP where they were given 4 cocaine (10 mg/kg, i.p.) and 4 saline pairings with one of two compartments of a 3-compartment test box. Following a CPP test, extinction trials were given on days 4, 7, 10, and 24 and were followed immediately by DCS (15 mg/kg, i.p.) or saline. A final extinction test was given on D38 followed by a reinstatement test 5 days later in which rats were given 5 mg/kg cocaine before the test. When extinction trials were given at 3-day intervals there was little extinction of the CPP in the saline group. However, extinction was greatly facilitated in rats that received DCS after each trial and no reinstatement was seen. In other experiments, we are exploring other parameters of extinction training on the effect of DCS. Support Contributed By: Canadian Institute of Health Research

42 C117

ADOLESCENCE AS A SENSITIVE PERIOD FOR DEVELOPMENT OF ENDOCRINE AND BEHAVIOURAL STRESS RESPONDING IN RATS

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Dynamic processes underlie gene-environment interactions during development, and these have long lasting consequences for the adult phenotype. Relative to the neonatal period, less work has examined development during adolescence, though different components of neural systems are sensitive at this time. In particular, programming might occur in frontal components of stress response circuitry during the adolescent period, since major developmental modifications occur here at this time. We have previously developed an ethologically relevant adolescent stressor paradigm, periadolescent predator odour (PPO) exposure, for use in rats. This manipulation is considered to model a higher risk environmental context. The present series of experiments undertook to examine the temporal specificity of effects, through comparison of PPO effects in juveniles with those in a control group who received stressor exposure in early adulthood. Behavioural assessment involved two open field sessions (OF1 and OF2) and a predator odour stress test (OFPO) administered ~20 days following juvenile or adult manipulations. Though there were effects of treatment age and sex, only a few were specific to cat odour treatment (which was compared with control odour treatment in both age frames). Regardless of treatment age, those who received no prior odour experience groomed more frequently during OF2 (p=0.028), but less frequently during OFPO, relative to those who had received odour priming (p=0.013). Juveniles who received odour priming spent less time in contact with the odour source during OFPO, compared with juveniles who had not (p0.001). This pattern was not present in the adult-treated group. Plasma corticosterone (cort) levels examined during priming revealed a higher normalized cort response in the odour-primed animals (p=0.002). Normalized cort responses to OFPO were higher in juvenile-exposed relative to adult-exposed animals, regardless of odour treatment (p=0.01). Testosterone (T) levels were also examined in males, and, while odour treatment had no effect on circulating T levels, there were significant correlations between T levels and cort responses to OFPO. These effects support the contention that exposure to species-specific stressors plays a role in guiding late (adolescent) developmental processes related to stress response circuitry, resulting in altered adult physiology and behaviour.

43 C118

EFFECT OF MICROSTIMULATION OF MACAQUE ANTERIOR CINGULATE CORTEX ON REACTION TIMES AND PERFORMANCE OF PRO- AND ANTI-SACCADES

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The dorsal anterior cingulate cortex (ACC), Brodmann's area 24c, is a brain region that has been implicated in decision making, error detection, task-switching and other aspects of executive control. The anti-saccade task has become a popular tool in the investigation of the voluntary control of behaviour. This task requires the suppression of the automatic tendency to look towards a flashed stimulus, and a vector-remapping of a voluntary saccade in the opposite direction to its mirror location in the visual field. Our laboratory has recently demonstrated that, before peripheral stimulus presentation, neurons in the monkey ACC exhibit task-selectivity when the monkey prepared to look towards a peripheral stimulus (pro-saccade) compared to when they prepared to look away from the stimulus (anti-saccade). Here, we have further investigated the ACC's role in top-down control by administering electrical microstimulation in the ACC of the monkey while it performed alternating blocks of the pro- and anti-saccade tasks. Microstimulation pulse trains were administered (biphasic, 0.3 ms, 100 Hz, 50-80 mA) 200 ms prior to stimulus presentation and persisted for a duration of 300 ms in total. Microstimulation increased reaction times significantly compared to no stimulation for pro-saccades in both the leftward and rightward direction ($p < 0.0001$). An effect of microstimulation was also evident for anti-saccade reaction times, where contralateral reaction times were decreased compared to no stimulation and ipsilateral reaction times were increased ($p = 0.0014$ for contralateral, $p = 0.0011$ for ipsilateral). Performance as a percentage correct of total trials was also significantly affected by microstimulation for ipsilateral pro-saccades on which microstimulation impaired performance ($p = 0.024$), and contralateral anti-saccades, where microstimulation enhanced performance ($p = 0.0107$).

44 C119

ROLE OF THE HUMAN POSTERIOR PARIETAL CORTEX IN TRANSSACCADIC MEMORY

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We previously reported that transsaccadic memory has a similar capacity for storing simple visual features as basic visual memory (Prime & Crawford, VSS abstracts, 2006). Here, we tested how many object features and locations could be retained across saccades while applying single-pulse transcranial magnetic stimulation (TMS) over the right dorsal posterior parietal cortex (PPC). Five subjects were presented with a random number of targets (1, 3, 4, 5, 6, or 8) with different spatial positions and orientations. Subjects were instructed to fixate and remember the positions and orientations of the targets. Then, subjects made a saccade to a different random location and were presented with a probe at the same location as one of the pre-saccadic targets, but tilted 9 clockwise or counter-clockwise. Subjects made a force-choice response to indicate how the probe's visual feature differed from the original target. In each trial, we randomly delivered a single-pulse at one of seven different time intervals centred around the saccade-go signal (-300ms, -200ms, -100ms, 0ms, +100ms, +200ms, +300ms). Thereby, allowing us to obtain information of the timing of the contribution of the right PPC during task performance (causal chronometry). Our preliminary data shows that performance was

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

disrupted during stimulation of the right PPC, particularly between 100ms to 300ms after the saccade-go signal. Stimulation at the other time intervals showed no statistical differences compared to the baseline (no TMS). The findings suggest that TMS over the right PPC transiently disrupt the putative spatial processing involved in transsaccadic memory.

45 C120

RESPONSES OF MEDIAL PREFRONTAL CORTEX NEURONS TO STIMULATION OF THE THALAMUS AND HIPPOCAMPAL FORMATION: AN *IN VIVO* STUDY IN THE CAT

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Interactions of the hippocampus and prefrontal cortex are known to be critical in supporting higher cognitive functions such as learning and memory. The hippocampo-prefronto-thalamic network is composed of the medial prefrontal cortex (mPFC), the reuniens nucleus of the thalamus (RE) and the hippocampal formation (HF). It has been proposed that the RE may serve as an interface between the mPFC and HF, given that the mPFC does not reciprocate connections with the HF (Vertes 2006). While the hippocampo-cortical pathway of this hippocampo-cortico-thalamic circuit has been well characterized, the thalamo-cortical one has not. Here we describe for the first time mPFC neural responses to RE and HF electrical stimulation. We have performed in vivo intracellular recordings in the mPFC of ketamine/xylazine anesthetized cats. Out of 28 neurons retained for analysis, 18 were identified as regular-spiking, 2 as intrinsically-bursting and 2 as fast-rhythmic-bursting. Eleven out of 20 tested neurons were responsive to RE stimulation, 6 out of 22 to HF stimulation, and 1 out of 16 to both RE and HF stimulation. Thalamic stimulation led to a primarily excitatory response of variable latency throughout the dorso-ventral extent of the mPFC with some responses being followed by a period of disfacilitation and a rebound. Antidromic spikes of 2-4 ms were detected approximately 6 mm below the cortical surface, in what corresponds to the prelimbic area of the cat. On the other hand, mPFC responses to HF stimulation were not always reliable; stimuli delivered at slightly supra-threshold intensities were able to elicit an initial response from 14% to 100% of the time, suggesting a gating mechanism in the HF – mPFC pathway. Responses usually consisted of an initial excitation, with a latency of approximately 10 ms, followed by a silent period of disfacilation which in turn led to a rebound. The initial excitation was not, however, consistently paired with a hyperpolarizing event. We conclude that the RE-mPFC network interacts efficiently and that only limited parts of mPFC receive inputs from both RE and HF. Supported by NSERC and CIHR.

46 C121

ROLE OF THE CAUDATE NUCLEUS IN SELF-ORDERED AND EXTERNALLY TRIGGERED MONITORING TASKS: AN EVENT-RELATED FMRI STUDY

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It has been suggested that the dorsolateral prefrontal cortex (DLPFC) is involved the monitoring of information [2]. Monchi et al. have proposed that the caudate nucleus is significantly involved during the planning of self-generated actions [1]. Here, we attempted to test the hypothesis that the caudate nucleus is required to a greater extent when the monitoring is self-generated as opposed to externally triggered. Twelve right handed healthy subjects (6 males, 18 to 30 years old) were scanned using 3T fMRI while performing the self-initiated and externally triggered monitoring tasks of Petrides [2]. Six faces were presented in two rows of three. There were four different conditions. In the self-ordered condition, the subject had to select a different face on each one of six trials. All six faces were presented on each trial but at a different location. In the externally triggered condition, during the first five trials, a different face was cued by the computer and the subject was required to select these faces and track them regardless of their location. On the sixth trial, the participant had to select the only face that had not previously been cued. In the recognition condition, one of the six faces was cued, before the first trial. During the six trials that followed the subject was asked to select the cued face independently of its location. In the control condition, the same face was cued throughout the six trials and the subject was asked to select it. Following an anatomical T1 sequence, four functional runs were acquired, each containing 204 frames (TR: 2.5s; 36 slices; voxel size: 3.4x3.4x3.4 mm3). Subjects completed four blocks of the four conditions per run. As predicted, we found significant increased activity of the DLPFC in the self-initiated condition and the externally triggered condition as opposed to the recognition and control conditions. Furthermore, we observed a significant increased activity in the right caudate nucleus, but not in the DLPFC when we compared the self-initiated condition to the externally triggered condition. The study provides further evidence that the DLPFC is particularly important for the monitoring of information in working memory regardless of whether it is internally or externally triggered. However, unlike the dorsolateral PFC, the caudate nucleus seems particularly important when monitoring is self-generated as opposed to externally-triggered.

47 C122

EFFECT OF AMYGDALA LESIONS ON LITHIUM-INDUCED CONDITIONED DISGUST AND TASTE AVOIDANCE

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The effect of amygdala lesions on conditioned disgust and taste avoidance were examined in male Sprague-Dawley rats that received bilateral NMDA (12.5 mg/ml) lesions of the basolateral amygdala (BLA) or ibotenic acid (10 µg/µl) lesions of the central nucleus of the amygdala (CeA) compared to sham-lesioned rats in taste reactivity (TR) and consumption (bottle) tests. Following recovery from surgery, the rats received two conditioning trials in which they were intraorally infused with 0.1% saccharin solution (at a rate of 1 ml/min for 5 min) followed by an injection of 0.15 M lithium chloride (130 mg/kg; Group Paired) at a volume of 20 ml/kg or equivolume physiological saline (Group Unpaired). During the TR test, rats were infused with the conditioned stimulus (CS) solution for a period of 2 min and their orofacial and somatic responses were recorded. During the consumption tests, rats received one-bottle (CS) and two-bottle (CS and water) tests. The amount consumed, over several time periods, was recorded. The results suggest that frequency of total aversive responses (e.g., gape, chin rub and paw tread) exhibited by BLA- and CeA- lesioned rats, to intraoral infusion of a lithium-paired taste, are similar to those of sham-paired rats; that is, conditioned disgust may not be affected by BLA or CeA lesions. However, only BLA lesioned rats exhibited a conditioned taste avoidance deficit. The findings support the notion that the BLA (not CeA) plays a role in conditioned taste avoidance learning and the amygdala is not involved in conditioned disgust.

48 C123

ULTRASONIC VOCALIZATIONS IN RESPONSE TO PALATABLE FOOD CUES ARE REDUCED IN M5 KNOCKOUT MICE

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Rats produce high frequency ultrasonic vocalizations (USVs) in appetitive situations. High frequency USVs (50kHz) are related to the

activation of dopaminergic systems. Previous evidence indicates that muscarinic receptors in VTA are involved in activation of dopamine neurons, and in food-related behaviours in rats and mice. The present study investigated whether M5 muscarinic receptors are involved in rewarding aspects of food stimuli in mice. USVs (40-110kHz) were studied in female wild-type and M5 muscarinic receptor gene knockout mice with palatable and unpalatable food cues (Moles & d'Amato, 2000). Mouse pairs were separated for 7 hours, and then one mouse was exposed to 30 min of chow, including either chocolate (palatable) or fennel seeds (unpalatable). Each mouse was then placed in a cage with the other mouse, and social USVs were measured for the pair. In the first minute after placing the mice together, wild-type mice produced significantly more USVs in response to palatable than unpalatable food cues. The difference declined in the second minute, and disappeared in the third minute. USVs in M5 knockout pairs were not different between palatable and unpalatable food, suggesting that the M5 receptor is required for the increase in USVs in response to palatability cues. When the mice were deprived of food during the separation, M5 knockout mice showed fewer USVs than wild-type mice after exposure to either palatable or non-palatable food. These data show that M5 receptors play an important role in mediating USVs in response to rewarding food stimuli. Thank Stephan Steidl for advice and discussion. Supported by CIHR grant to JY.

49 C124

IMPAIRMENT OF FEAR EXTINCTION IN THE GAD65 KNOCK-OUT MOUSE

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Extinction is demonstrated as a reduction in fear that occurs when stimuli once associated with an aversive event are no longer coupled with that event and involves new learning and memory formation rather than erasure of the original memory. Pharmacological studies have implicated GABA in extinction. GABA production is mediated by the enzyme GAD which exists in 2 isoforms: GAD65 and GAD67. GAD67 maintains basal GABA levels whereas GAD65 is rapidly activated in times of high GABA demand and is essential for regulating responses to environmental signals, such as those encountered during learning. Mice lacking GAD65 (GAD65-/-) display elevated anxiety levels. Fear and extinction learning were examined in GAD65-/- mice and wild-type littermates (GAD65+/+). Fear conditioned GAD65+/+ mice froze more to the tone associated with the foot shock than to the neutral tone, whereas GAD65-/- mice showed high freezing to both tones, indicating stimulus generalization. Extinction training consisted of 6 sessions. In GAD65+/+ mice, extinction of fear was apparent in the 4th session and still evident 24hrs later, demonstrating long-term memory for extinction training. Extinction of fear was less pronounced in GAD65-/- mice, not being readily apparent until the final session with no long-term retention. Field potential recordings from the lateral amygdala (LA), CA1 and prefrontal cortex (PFC) were simultaneously obtained in freely behaving mice during extinction training. Before extinction training, GAD65+/+ and -/- mice exhibited high theta phase correlation levels between all 3 brain areas, with the LA-CA1 correlation being the highest, consistent with high fear behaviour. After extinction training, the LA-CA1 correlation decreased in GAD65+/+ and -/- mice. Theta phase correlation levels remained high between the PFC-CA1 and LA-PFC after extinction training in GAD65+/+ mice but decreased in the GAD65-/- mice. Thus, in GAD65+/+ mice the correlation levels were uneven across the 3 brain areas, with LA-CA1 being the lowest, consistent with low fear behaviour. In GAD65-/- mice, the LA-CA1 correlation remained the highest of the 3 even though its value had decreased after extinction training, consistent with persistence of fear behaviour. Taken together, the development of high theta phase correlative activity between the PFC-CA1 and LA-PFC does not occur in GAD65-/- mice during extinction training, indicating that GABA signalling is required for this process.

50 C125

POST-TRAINING EFFECTS OF THE GINKGO BILOBA LEAF EXTRACT, EGB761, ON MEMORY

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Ginkgo biloba extracts are widely used for their pro-mnemonic effects. One such extract, called EGb761, has repeatedly been shown to improve learning when administered chronically prior to and/or during the learning phase. However, the influence of EGb761 on memory per se, assessed by administering the extract during the interval between training, is less clear. In the present study, we examined the acute and chronic effects of post-training administration of EGb761 in laboratory rats using a double Y-maze task. Each trial consisted of two components: a spatial discrimination and a delayed alternation. The non-mnemonic demands of the two components are roughly equivalent; that is, both involve a food-reinforced two-arm discrimination in a Y-maze. However, in trained animals only the delayed alternation component requires the use of working memory. Accordingly, if a treatment selectively influences performance in the delayed alternation task, we can confidently rule out the treatment's influence on non-mnemonic factors, such as motivation, motor function, and sensory alteration. In the first experiment, twelve rats received 24 consecutive training trials per day in the double Y-maze until they reached a training criterion of 21/24 correct trials over 3 consecutive days. Rats then were injected with vehicle, 0.75, 7.5, 41.25, or 75 mg/kg EGb761 (i.p.), 30 min prior to each test session, in a counterbalanced order using a within-subject design. Delay intervals (0, 15 or 60 sec) were introduced between successive trials (8 trials at each delay), serving to vary the task's working memory demands. Rats were re-trained to criterion between drug treatments. Results revealed that acute administration of EGb761 had no significant effect on either the spatial discrimination task, or the delayed alternation (i.e., working memory) task. In a second experiment, the same rats were randomly assigned to two groups. One group received 21 daily injections of vehicle, while the other group received 21 daily injections of 42.25 mg/kg EGb761 (i.p.). Rats were then tested twice in the double Y-maze, once under the influence of the drug and once in a drug-free state. Results revealed no difference in performance between groups, under both drugged and drug-free conditions. Taken together, these data suggest that both acute and chronic post-training administration of EGb761 does not enhance memory.

51 D101

GLYCINE INHIBITION OF STARTLE MEDIATING NEURONS IN THE RAT CAUDAL PONTINE RETICULAR FORMATION

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The mammalian startle response is an excellent model for studying cellular and molecular mechanisms underlying behaviour and learning. The startle response is strongly controlled by glycine inhibition. An impairment of glycinergic inhibition is responsible for the neurological disorder Hyperekplexia. Glycine inhibits startle on the level of the spinal cord, however, there is controversial evidence for additional glycine inhibition on the level of the brainstem, namely in the caudal pontine reticular nucleus (PnC). Giant neurons in the PnC play a crucial role in mediating the mammalian startle response. They receive input from different sensory pathways and project directly to facial, cranial and spinal motoneurons. Furthermore, they integrate modulatory input from different brain regions that either enhance or inhibit startle responses. In the present study we performed patch-clamp recordings of PnC giant neurons in rat brain slices

and we tested the effect of glycine and the glycine antagonist strychnine on active and passive cell parameters as well as on synaptic signals evoked by stimulation of auditory and trigeminal afferent fibres within the startle pathway. While strychnine had no effect on PnC giant neurons, the application of glycine strongly inhibited PnC neuron activity. The membrane resistance dropped from 101 ±16 MΩ to 30 ±4 MΩ (F(3,31) 06.68; p=0.0011) , leading to an altered resting potential, decrease in spontaneous activity and to significant inhibition of both evoked spiking activity and evoked synaptic currents (EPSCs; F(3,31)=7,72; p=0.0005) while the paired pulse ratio of EPSCs was not altered. All effects could be reversed by additional application of 10 µM strychnine. Our results show that exogenous glycine activates functional glycine receptors expressed on PnC giant neurons, and that this is able to mediate a powerful inhibition of the startle mediating neurons over a wide range of glycine concentrations.

52 D102

PARIETAL LOBE ANATOMY AND VISUOSPATIAL ABILITY IN A GIFTED GEOMETRICIAN: CASE STUDY OF HAROLD S.M. COXETER

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The definition and measurement of intelligence and the neurobiological basis of its variation have been the subject of much study. Intellectual ability is related to cerebral anatomy in complicated relationships (Witelson, Beresh & Kigar, 2006; Colom et al., 2006). One component of general intelligence is visuospatial ability which is largely mediated by the superior parietal lobules (SPL). The SPL of both hemispheres are activated in tasks such as mental rotation of 3-D objects and the generation and manipulation of visual imagery. The purpose of this study was to examine brain structure in an individual case of visuospatial excellence, compared to a group of age-matched normal controls. The case subject, Harold Coxeter (HC) was a world-acknowledged gifted geometrician. He volunteered to be part of our research program at the age of 92, upon learning of our study of the brain of Albert Einstein. Intelligence testing was performed on HC using the Wechsler Adult Intelligence Scale and Coxeter scored in the 97th, 93rd, and 97th percentiles for Verbal, Performance and Full Scale IQs respectively. Structural MRI's were acquired at Sunnybrook Health Sciences Centre. Our hypothesis was that the anatomy of his parietal lobes would be different from normals based on our previous findings on Albert Einstein. The MRI scans of twelve cognitively normal control subjects were obtained from Sunnybrook (mean age 81.1 years). All, including HC, had T1-weighted 3-D magnetic resonance imaging scans acquired on a General Electric Signa 1.5 Tesla scanner using standard parameters. All MRIs were processed using the program Semi-Automated Brain Region Extraction (Dade et al., 2004), which parcellates the brain into 26 volumes of interest, using a set of manually defined landmarks and coordinates. We found that HC's left SPL was 4.7 standard deviations larger than the control group. There was a tendency for the right SPL to be greater (SD = 1.6). No differences were observed in other regions. We suggest that his increased parietal region may be part of a neuroanatomical substrate that allowed for extraordinary visuospatial ability. The finding of atypical anatomy and increased volume in the parietal region in two cases with exceptional visuospatial ability supports the notion of an association between ability in a specific cognitive domain and anatomy of the relevant cortical regions. The roles of heredity and experience in this association remain to be addressed.

53 D103

THE NEURAL REPRESENTATION OF STIMULUS SALIENCE PREDICTS BEHAVIORAL CHOICE WHEN SELECTING VISUAL OBJECTS

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Humans can substantially improve the search for a particular visual object among several others by guiding their visual attention to a given feature of that object. We have previously shown how the visual behavior of nonhuman primates is also influenced by scene composition and how their visual search strategies are guided by stimulus salience (Shen and Paré, 2006). In the current study, we investigated the neural mechanisms underlying these contextual effects by recording 39 visuo-motor neurons from the intermediate layers of superior colliculus (SC) while three monkeys performed a visual conjunction (color+form) search task. The search target appeared pseudorandomly with 11 distractors in a concentric array. As color was the most discriminable feature in this task, the number of distractors sharing the color of the target was varied between three distractor ratios (2 same-color: 9 different-color; 6:5; and 9:2). As previously reported, monkeys' search strategies changed flexibly with the display type: saccades were more likely to land on distractors sharing the target color when there were few of them in the display (i.e., when they were salient) and monkeys were less likely to choose those distractors when they were numerous. The initial (first 25 ms) neuronal response to the target or distractors was invariant with distractor ratio. Over time, however, the ideal observer discriminated the target from distractors. The discrimination of the target from a salient distractor was more difficult and occurred later than the discrimination of the target from less salient distractors across all display types, suggesting that neurons are functionally feature selective. At the time when neurons generally discriminated the target from distractors, neuronal activity associated with each distractor type (i.e., functional feature selectivity) was proportional to the monkeys' probability of selecting that stimulus for a saccade. These results extend previous behavioral distractor-ratio studies by showing how superior colliculus activity reflects the flexible guidance of visual attention in different contexts.

55 D105

DOPAMINERGIC MODULATION OF RISKY-DECISION MAKING

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Alterations in decision making about risks and rewards is associated with a variety of clinical disorders, including substance abuse, ADHD, and Parkinson's disease. Animal studies suggest that the mesolimbic dopamine (DA) system is involved in certain types of decision making, but the role of DA in risky-decision making has yet to be fully explored. Lesions to DA terminal regions (e.g. ventral striatum, orbitofrontal cortex) alter patterns of risky-decision making when rats have to choose between small, certain rewards and large rewards delivered on a probabilistic schedule. We examined the role of DA and its specific receptor subtypes in risky-decision making. We used an automated task conducted in an operant chamber in combination with systemic administration of the DA releaser amphetamine, as well as selective DA agonists and antagonists. Rats were initially trained to press retractable levers to receive sugar pellet reward. During the risky-decision making task, a single press on one lever (small/certain) immediately delivered one pellet, whereas a press on the other lever (large/risky) delivered four pellets but with the probability of receiving reward decreasing across the four trial blocks (100%, 50%, 25%, 12.5%). Once choice behavior stabilized, separate groups of rats received injections of saline or the following dopaminergic drugs: amphetamine, SCH23390 (D1 antagonist), eticlopride (D2 antagonist), L745,870 (D4 antagonist), SKF81297 (D1 agonist), PD168,077 (D4 agonist). Administration of amphetamine dose dependently increased preference for the large/risky lever at 3 doses tested (0.25, 0.5, 1.0 mg/kg). Blockade of D1 or D2 receptors with SCH23390 (0.005 mg/kg) or eticlopride (0.01 mg/kg) respectively, decreased preference for the large/risky lever. Furthermore, co-administration of these drugs blocked the amphetamine-induced increase in risky choice. Blockade of D4 receptors also caused a moderate decrease in risky choice, but the effects were not as prominent as those observed with D1 and D2 antagonists. Direct stimulation of D1 receptors with SKF81297 increased choice of the large/risky lever at one dose (0.3 mg/kg) but exerted a biphasic effect at a higher dose (1.0

mg/kg), where rats were risk averse on the 50% probability block but risk prone on the 25% probability block. PD168,077 (1.0 mg/kg) also increased risky choice, but again, the effects were not as prominent as with the D1 receptor agonist. The results of this study suggest that DA activity contributes to risky-decision making. D1 and D2 receptors appear to be strongly mediating this behaviour, with D4 receptors playing a less important role. These results clarify the specific role of DA receptor subtypes in risky-decision making, which may have implications for clinical disorders that are associated with alterations in this form of executive functioning.

56 D106

INDIVIDUAL DIFFERENCES IN VULNERABILITY TO STRESS- AND COCAINE-INDUCED RELAPSE TO COCAINE SEEKING IN RATS

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The aim of the present study was to investigate individual differences in relapse vulnerability as assessed by differences in the reinstatement of operant responding for a cocaine-conditioned stimulus. Thus, rats (n = 98) received three sessions of Pavlovian conditioning whereby a light-buzzer stimulus was paired with intravenous infusions of cocaine (0.5 mg/kg/inf; one 3h session/day). Following withdrawal (5 days), operant responding for the light-buzzer stimulus was assessed on 3 test sessions (3hr/day) in extinction conditions, and on two counterbalanced tests of reinstatement induced by foot shock stress and by cocaine primes (15 mg/kg i.p.). Despite identical regimen of cocaine exposure, 8% of the subjects showed selective reinstatement to stress, 14% selective to cocaine, 36% responded to both stress and cocaine, and 42% showed no reinstatement. These data in rats suggest that individual differences in vulnerability to relapse can be independent from intensity of previous drug exposure. Supported by CIHR, CFI and OIT

57 D107

CAFFEINE-CONDITIONED PLACE AVERSIONS ARE SWITCHED TO CONDITIONED PLACE PREFERENCES BY DOPAMINE RECEPTOR BLOCKADE

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Despite caffeine's status as the most widely consumed psychoactive substance in the world, the precise neurobiological mechanisms underlying its motivational effects remain unclear. The mesolimbic dopaminergic system is important for both the rewarding and aversive motivational aspects of many natural and drug rewards. For this reason, we investigated the role of dopamine in caffeine motivation using a fully counterbalanced conditioned place preference paradigm. Systemic (i.p.) administration of caffeine in wild type C57Bl/6 mice produced dose-dependent conditioned place aversions at 10, 30 and 100 mg/kg. However, pre-treatment with the broad-spectrum dopamine receptor antagonist alpha-flupenthixol (0.8 mg/kg, i.p.), under conditions where alpha-flupenthixol produces no motivational effects of its own, not only abolished these caffeine conditioned place aversions but resulted in robust caffeine conditioned place preferences. In addition, the conditioned place aversion produced by 10 mg/kg caffeine was blocked, and a conditioned place preference was revealed, by pretreatment with both D1- and D2-receptor-selective antagonists, and this effect was replicable in D1- and D2-receptor knockout animals. These results suggest that dopamine neurotransmission via D2 dopamine receptors mediates the aversive effects of caffeine, and that a non-dopaminergic substrate must be responsible for mediating caffeine's rewarding properties.

58 D108

SACCADE PREPARATORY ACTIVITY WITHIN THE PRIMATE SUPERIOR COLLICULUS IS PREDICTIVE OF UPCOMING CHOICES DURING A STRATEGIC GAME.

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Previous research suggests that during perceptual-based decisions, incoming sensory evidence leads to an accumulation of neural activity in brain regions involved in generating the appropriate motor response. Here we investigated whether motor preparatory activity accumulates in a similar manner during strategic decision-making in which there is no immediate sensory information to indicate the correct choice. Monkeys played an oculomotor version of the mixed-strategy game 'odds-evens' while the activity of single neurons with saccade preparatory activity was recorded from intermediate/deep layers of the superior colliculus (SC). Each trial began with fixation of a central visual stimulus which was extinguished for a 600 ms warning period before two targets were presented; one in the center and the other opposite the neuron's response field. If both monkey and the computer opponent chose the same target (i.e., 'evens'), the monkey received a liquid reward; otherwise (i.e., 'odds') the computer opponent received a virtual liquid reward. Statistical analyses showed that monkeys approached the optimal strategy of choosing each target stochastically and in equal proportions. This behavioral equilibrium suggests that the two responses were, on average, of equal desirability. Preparatory activity within the SC became increasingly selective of whether the target in the response field or opposite the response field would be chosen as the saccadic target. Despite an overall equality in desirability for the two responses, the timing of the differential accumulation of saccade preparatory activity leads us to conclude that monkeys commit to one of the responses in advance of target presentation.

59 D109

A MUTATION IN CREB DISRUPTS LONG-TERM MEMORY FOR HABITUATION AND BLOCKS MEMORY ASSOCIATED CHANGES IN GLUTAMATE RECEPTOR SUBUNIT EXPRESSION

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Through behavioural mutant analysis we have shown that the transcription factor CREB (cAMP response element binding protein) is necessary for long-term, but not short-term memory of mechanosensory habituation in the nematode, Caenorhabditis elegans. We tested crh-1 (homologous to the mammalian CREB protein) mutant worms, which have a 979 DNA base pair deletion that eliminates 38 amino acids of the bZIP DNA binding domain (Kimura et al., 2002), for short-term and long-term memory deficits. crh-1 mutant worms showed no significant differences from wild-type worms when tested for short-term habituation, but when tested for long-term memory of habituation training, crh-1 mutant worms showed no evidence of long-term memory 24 hours later. Through confocal imaging of GLR-1 (homologous to non-NMDA type glutamate receptor subunit 1) subunits tagged with GFP our lab has shown that consolidation of memory for habituation in C. elegans is associated with a decrease in the average area of GLR-1 synaptic clusters 24 hours after long-term memory training. This is not observed in naïve worms (Rose et al., 2003). Here we report that crh-1 mutant worms expressing the GLR-1::GFP transgene do not show a decrease in the average area of GLR-1 synaptic clusters 24 hours after long-term memory training. This suggests that the decrease observed in wild-type worms expressing the transgene is caused by an as yet unknown CREB-dependent mechanism. We are currently using a candidate gene approach to identify kinases that are responsible for CREB activation in C. elegans during long-term memory training for habituation, as well as using a CRE (cAMP response element) reporter gene assay to identify which neurons, within the

neural circuit that mediates the reflexive response to mechanosensory stimuli, undergo changes in CREB-mediated synaptic plasticity. So far we have tested the C. elegans homologues to PKCε, PKCβ, and PKA catalytic subunit. All of these kinases tested have shown normal long-term memory for habituation.

This work was supported by operating grants from NSERC to CHR and by Graduate Fellowships from MSFHR and CIHR to TAT. Kimura Y et al. (2002). EMBO reports, 3:962-966.; Rose JK, Kaun KR, Chen SH, Rankin CH (2003). Journal of Neuroscience, 23:9595-9600.

60 D110

NEURONAL ACTIVATION DURING FOOD ANTICIPATORY ACTIVITY IN RAT

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Food anticipatory activity (FAA) is developed in rats when food is restricted to a few hours daily. FAA is characterized by increasing of locomotor activity during 2-3 hours preceding food access. The mechanisms underlying the food-entrained oscillator (FEO) remain elusive. Detection of c-fos expression in the brain during FAA may help to reveal the role of brain structures involved in the FEO. Methods: All rats were maintained under a 12:12h light/dark cycle (lights on between 6h00 and 18h00). During three weeks two groups of rats (AL1 and AL2) were fed ad libitum, while other rats were subjected to restricted scheduled feeding. All rats maintained on scheduled feeding had the access to food between 12h00 and 14h00. On fourth week food-restricted rats were sacrificed during FAA 3 hours (food-anticipated, FA3), 2 hours (FA2), 1 hour (FA1) and 0 hour (FA0) before scheduled feeding or after one hour of feeding (refed, RF1). Ad libitum fed groups, AL1 and AL2 were sacrificed simultaneously with respectively FA3 and FA0 groups. Plasma insulin and corticosterone levels as well as the expression of c-fos mRNA in the brain were analyzed. Results: Plasma corticosterone was significantly increased during FAA. This increase in corticosterone levels was reversed by feeding. In contrast, the levels of insulin were decreased during FAA and raised upon feeding. In the brain we have found particular patterns of c-fos mRNA expression during food anticipation and feeding. Expression of c-fos mRNA in two regions, the nucleus of the solitary tract (NTS) and the magnocellular part of the paraventricular hypothalamic nucleus (PVHm), was increased after feeding but not during FAA. In the dorsomedial hypothalamic nucleus c-fos mRNA was highly expressed in the dorsal part during FAA and in the ventral part after feeding. FAA increased c-fos expression in the lateral hypothalamus, anterodorsal thalamic nucleus and septohippocampal nucleus. During FAA c-fos also expressed in the parvocellular PVH that in concert with significant increase of plasma corticosterone suggests the activation of the hypothalamic pituitary adrenal (HPA) axis. The activation of suprachiasmatic nucleus (SCN) and the anterior paraventricular thalamic (PVTa) nucleus during FAA preceded that of the parvocellular PVH. The present data provide evidence for early induction of expression of c-fos mRNA in the SCN and PVTa and following HPA axis activation during food anticipation.

61 D111

ESTIMATING THE SIZE OF EYE MOVEMENTS FOR UPDATING SPATIAL MEMORY

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To maintain a stable perception of the world, our brain must update our representation of visual space each time our eyes move. But when the brain updates spatial memory during eye movements, how does it estimate the size and direction of those movements? We know that some updating actually precedes the eye movement: neurons in various brain regions code the predicted post-movement retinal locations of objects before the movement begins (e.g., Duhamel et al, 2002). So the brain likely uses a fast internal estimate of the eye's movement. We tested whether the brain relies on the same internal efferent signals when updating spatial memory as it does to guide saccades to their goal. We altered people's eye control using saccadic adaptation, e.g., we trained subjects to saccade only 15° when they saw a target 20° left (i.e., a saccadic amplitude 75% of the seen distance). Within 20 trials, subjects were producing leftward saccades that undershot the 1st target's initial seen location by 73-77%. The visual feedback however, indicated that the eyes had gone the whole distance, e.g. 20° left. The subjects' task was to then saccade towards a second, remembered target (which had been briefly flashed before they made their leftward movement). The 2nd target was always located to the right of the 1st because adapting leftward saccades leaves rightward ones unchanged. We examined error patterns to the 2nd remembered target to determine if updating was also impaired in the same way as adapted saccades. That is, do subjects update the 2nd target by 20°, computing eye position of the 1st target based on its initial seen location, and subsequently overshoot the true location of the 2nd target? Or do they compensate for the shorter adapted saccade based on actual efferent signals for that movement, and land accurately on the 2nd target site? Our results suggest that subjects partly compensated for the induced adapted saccade when updating the location of the 2nd target, landing closer to the actual target site than to the location predicted if the brain had misestimated the 1st saccadic amplitude. This incomplete compensation suggests that the estimate of eye motion being updating was influenced by the seen distance of the 1st target. These findings suggest that the brain relies mostly, but not exclusively, on feedback from signals that guide the actual eye movement when updating spatial memory.

62 D112

TEMPORAL DYNAMICS OF SACCADE COMMANDS IN MONKEY SUPERIOR COLLICULUS DURING IMMEDIATE INSTRUCTION SWITCHING OF PRO AND ANTISACCADES

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Our ability to flexibly respond to a sensory event has been extensively examined by psychophysical experiments: behavioral performance is worse when the instruction switches across consecutive trials than when it repeats. The purpose of this study is to map this phenomenon onto the dynamics of neural circuits by neurophysiological experiments in awake, behaving monkeys performing a psychophysical paradigm. After monkeys maintained their eyes on the central fixation point (FP), a visual stimulus was turned on at 15° left or right from the FP. Based on the color of the FP, the monkeys were required to make a saccade to the stimulus (prosaccade) or to the opposite direction of the stimulus (antisaccade). In 33 or 50 % of trials, the color of the FP switched after stimulus onset and the monkeys were required to follow the second instruction. In switch trials, the monkeys' responses were dominated by error saccades based on the first instruction. However, as reaction times increased, the dominant response switched from error saccades to correct saccades. We quantified the time period from a switch time to the time when the dominant response switched (Switch Signal Reaction Time: SSRT). SSRTs were approximately 220 ms across the monkeys and conditions. To identify the temporal dynamics of neural activity during this paradigm, we recorded the activity of saccade related neurons in the superior colliculus (SC). When the monkeys had to cancel a saccade toward the movement field of neurons after instruction switching, suppression of activity occurred earlier than behaviorally estimated SSRTs + switch times (-46 ms and -24 ms from the behavioral estimates for pro and antisaccade cancellation, respectively). However, when the monkeys reprogrammed a saccade toward the movement field of neurons after instruction switching, activation of the neurons occurred almost at the same time with the behavioral estimates (-8 ms and -1 ms from the behavioral estimates for pro and antisaccade reprogramming, respectively). These results suggest that, at least at the level of the SC, the two processes occur in

serial order: cancellation of a saccade based on the first instruction and reprogramming of a saccade based on the second instruction.

63 D113

DETERMINING THE ROLE OF THE PREFONTAL CORTEX IN SUPPRESSION OF REFLEXIVE SACCADES

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The prefrontal cortex (PFC) has been implicated in the ability to perform complex behaviours requiring the implementation of cognitive control. Theories of PFC function have suggested that this area participates in cognitive processes via a top-down control mechanism. Top-down control has been extensively investigated in both humans and primates using the antisaccade task. In this task, subjects are required to produce a rapid eye movement in a direction opposite to a suddenly appearing visual stimulus. Correct performance of this task requires that the subject inhibits the automatic tendency to look toward the stimulus location (prosaccade). Here, we investigated whether PFC microstimulation impacts behavioural performance and saccadic reaction time. We applied microstimulation at 35 PFC sites while a single monkey performed a paradigm of randomly interleaved prosaccade and antisaccade trials. Stimulation occurred on 50% of the trials and was aligned to stimulus onset. Behavioural performance and saccadic reaction times were recorded. For 43% of the PFC sites, microstimulation significantly affected behavioural performance and/or saccadic reaction time. Across all stimulation sites, we found that PFC microstimulation resulted in significantly shorter reaction times for contralateral prosaccades, and significantly longer reaction times for ipsilateral prosaccades and ipsilateral antisaccades, when the stimulus was presented to the contralateral field. In addition, there were significantly more ipsilateral antisaccade errors during PFC stimulation. These results support a role for PFC in reflexive saccade suppression.

64 D114

DIFFERENTIAL EFFECTS OF CHRONIC AMPHETAMINE TREATMENT ON SET SHIFTING AND REVERSAL LEARNING

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Psychostimulants such as amphetamine (AMPH) promote the release of dopamine, but chronic exposure to these drugs can induce long-term negative effects that disrupt prefrontal cortex function. For example, Fletcher et al. (2005) examined the effects of chronic AMPH treatments on behavioural flexibility using a set shifting task where Sprague Dawley rats were trained to dig for food in one of two bowls that could be discriminated on the basis of either texture or odour. Repeated AMPH treatment impaired extradimensional set shifting and subsequent reversal learning, suggesting that these treatments may alter the functioning of the medial and orbital prefrontal cortex. To explore the issue further, the present study examined the effects of chronic AMPH sensitization on behavioural flexibility using a cross-maze set-shifting task. Long Evans rats were initially habituated to the maze, after which they received 15 AMPH or saline injections (1-5 mg/kg every 2nd day, increasing the dose by1 mg every 3rd injections). Following a 2 week drug wash-out period, rats were trained on a series of discriminations to receive food reward. Rats received 40 trials per day until they achieved criterion performance of 10 correct consecutive choices, followed by a probe trial, where they were released from a different start location on the maze. Rats were initially trained on a visual-cue discrimination. On the strategy shift, rats were trained to use a response strategy (e.g. always turn left, ignore the visual cue). For the reversal, rats were trained to reverse their turn direction. AMPH treatment did not impair learning of the initial cue discrimination, nor did it alter performance during the set shift. In fact, AMPH treated rats tended to require fewer trials to achieve criterion on the shift than controls. Surprisingly, AMPH treated rats learned the reversal faster than controls, but required a greater number of probe trials to complete the task, indicating an impairment in the ability to apply the learned rule to a new context. Viewed collectively, these results suggest that repeated AMPH exposure can enhance behaviour flexibility assessed in this manner, but impairs applying rules to a new situation. These effects may be attributable to deficits in latent inhibition, in that AMPH treatments may have impaired learning to ignore task-irrelevant stimuli, facilitating learning new stimulus-reward associations when those stimuli become relevant.

65 D115

EVIDENCE THAT GRIP SCALING, BUT NOT PERCEPTUAL ESTIMATION, RESISTS THE PONZO ILLUSION

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Compelling evidence for dissociations between vision-for-perception and vision-for-action has come from psychophysical studies in healthy subjects showing that pictorial illusions, which by definition have robust effects on perception, have little or no effect on precision grasping in the right hand. Critics have argued, however, that the task demands for grasping are quite different from those required for perceptual judgments. When a participant reaches out to grasp an object, they have argued, he or she need only attend to one of the targets. When making a perceptual judgment, however, the participant typically attends to both targets in the illusory display. To circumvent this issue, we carried out a study in which only one target was presented at a time, placed in different positions within a Ponzo illusion. When the target is placed where the lines in the display converge, it typically appears larger than when it is placed at the diverging end of the display. Participants were asked to perform two tasks: an Action task, in which they were simply asked to reach out and pick up the target (a small disk that varied in size from trial to trial), and a Perception task, in which they were asked to keep their hand in a stationary position on the start button and to indicate the perceived size of the disk by opening their index finger and thumb a matching amount. The results were clear. Peak grip aperture (PGA) in the Action Task was not influenced by the Ponzo display. In other words, participants maintained a constant PGA for a given target size regardless of where it was placed on the illusory background. In contrast, the Manual Estimation Aperture (MEA) in the Perception task was affected by the Ponzo display; participants estimated the target to be larger when it was placed at the converging as compared the diverging end of the display. This dissociation between the effects of the illusion on Action and Perception tasks remained significant even when the scores were adjusted for differences in the sensitivity (slopes) of the two measures. Furthermore, no differences in within-subject variability were observed between the action and perception tasks. Taken together, the results suggest that the relative insensitivity of the visuomotor system to pictorial illusions cannot be explained by appealing to differences in attention, precision, and/or the relative sensitivity of grip scaling vs. manual estimation to changes in target size.

66 D116

DIFFERENT EFFECTS OF AGE AND OF CHRONIC STRESS IN BEHAVIOUR IN THE FORCED SWIM TEST IN FEMALE AND MALES RATS

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We previously reported that social stress in adolescence (SS; daily 1 h isolation and change of cage partner from day 30 to day 45 of age) increased behavioural responses to psychostimulants in females, but not males (McCormick et al., 2004, 2005). Here we investigated whether SS in adolescence would increase depressive behaviour as measured in the Porsolt

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

forced swim test (FST). The SS group and a comparison group that underwent swimming only (CTL) were tested on day 45 of age. To test the enduring effects of SS, a separate group of SS and CTL were tested on day 70 of age. FST involves a 15 min swim in a cylindrical tank of water (260C) and 24 h later a 5 min swim in the tank. Behaviours measured during the FST were climbing walls of the tank, swimming, and immobility. Time spent climbing or immobile were used for statistical analyses. FEMALES: A Group X Age X Day X Behaviour ANOVA found the factor of AGE to be significant ($F1,54 = 7.63$, $p = 0.008$), whereby adults engaged in less climbing and immobility than adolescents. Age did not interact with any other factor. Post hoc analysis of the interaction of Group by Days by Behaviour ($F1,54 = 5.27$, $p = 0.026$) indicated that on Day 1, SS was more immobile ($p = 0.04$) with less climbing ($p = 0.01$) than CTL, but the groups did not differ on day 2. Whereas CTL had a significant increase in immobility ($p < 0.0001$) and a decrease in climbing ($p = 0.001$) from Day 1 to Day 2, SS did not change from Day 1 to Day 2. MALES: A Group X Age X Day ANOVAs found an interaction of Group by Age ($F1,55 = 5.15$, $p = 0.03$) for climbing, with SS males climbing more than CTL for the adults ($p = 0.09$) but not for the adolescents, and with the adolescents climbing more than adults for the CTL ($p = 0.07$) but not for SS. For immobility, only the Day by Age interaction was significant ($F1,55 = 15.06$, $p < 0.0001$), with adults decreasing immobility ($p = 0.03$) and adolescents increasing immobility from day 1 to day 2 ($p = 0.006$). These results indicate that females exposed to chronic stress in adolescence show increased depressive behaviour in the FST that remains evident several weeks after stress exposure. Age was a greater factor for FST behaviour in males, with adolescent males appearing more prone to depressive behaviour than adults, and a history of chronic stress tending to increase vigorous "non-depressive behaviour" (climbing) in adult males.

67 D117

A COMPARISON OF SACCADE AND POINTING TOPOGRAPHY BETWEEN MEDIAL AND LATERAL AREAS IN THE HUMAN POSTERIOR PARIETAL CORTEX

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Previous functional magnetic resonance imaging (fMRI) studies identified areas in the posterior parietal cortex (PPC) that are active during memory-guided saccades and pointing to visual targets. Both the areas for saccades and pointing are lateralized, showing higher activation for actions directed toward the contralateral hemifield (Medendorp et al., 2003, Journal of Neuroscience; Fernandez-Ruiz et al., in press, Cerebral Cortex). The purpose of this study was to clarify topographical areas of activation in the PPC related to saccading to and pointing to remembered targets as well their activity in an anti-pointing task. fMRI was used to measure the blood oxygenation level-dependent response while right-handed individuals either made saccades or pointed to remembered visual targets in a blocked design. Subjects also performed an anti-pointing task. Contrasts between right- and left-visual targets revealed topographic areas related to each effector. The saccade task produced lateralized activation in the medial intraparietal sulcus (IPS; left Talairach coordinates (TC): -23, -60, 43). The pointing task produced topographic activation in a medial region of the PPC (left TC: -12, -82, 32), near the superior parieto-occipital sulcus, as well as the IPS region activated by saccades. These two regions are consistent with previous studies. In addition, a previously unidentified area in the precuneus also demonstrated topography for pointing (left TC: -8, -59, 57). These results show that a region in the IPS has topographic activity for visually directed saccades and pointing, while a more medial region is topographically activated by pointing alone. Examination of activity in these and other topographically organized areas during an anti-pointing task are expected to provide further insight into their role in visuospatial behavior.

68 D118

INDIVIDUAL DIFFERENCES IN CONTEXTUAL FEAR CONDITIONING ASSOCIATED WITH NATURAL VARIATIONS IN MATERNAL CARE ARE NOT REVERSED BY ENVIRONMENTAL ENRICHMENT IN THE ADOLESCENT PERIOD IN RATS

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In rats, maternal care influences the development of hormonal and physiological responses to stress. The adult male offspring of Low-licking and grooming (LG) mothers show increased anxiety, spend less time in the centre of a novel open field and show impaired spatial learning in the Morris water maze compared to the offspring of High-LG mothers. Environmental enrichment has been reported to reverse the effect of maternal care on spatial learning as well as the effect of maternal separation on the stress response. In this study, we examined whether maternal care influences contextual fear conditioning as well as centre exploration time in an open field and whether environmental enrichment from days 22 to 70 of life can reverse these effects. The results confirmed that maternal LG was correlated with centre time in the open field, and showed that this effect was abolished by environmental enrichment. In addition, maternal LG was highly correlated with contextual fear conditioning measured by time spent freezing in the previously shocked context. Offspring of Low-LG mothers showed enhanced freezing in the conditioned context compared to offspring of High-LG mothers. However, enrichment had no observable effect on contextual fear conditioning. The results suggest that maternal care exerts an important influence on learning and memory processes associated with aversive events, and these effects, unlike spatial learning and anxiety measured in the open field, are not reversed by environmental enrichment in the adolescent period. Supported by CIHR

69 D119

IMPAIRMENT OF HIPPOCAMPAL NEUROGENESIS IN DIABETIC RATS

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Adult neurogenesis in the dentate gyrus is an evolutionarily preserved trait in most mammals examined thus far. Neuronal proliferation and subsequent integration of new neurons into the hippocampal circuit are regulated processes that can have profound effects on an animal's behaviour. A streptozotocin model of type I diabetes, characterized by low insulin and high plasma glucose levels, affects not only the body's overall metabolism but also brain activity. We report here that seven weeks after the onset of uncontrolled diabetes, neuronal production is dramatically reduced. Neurogenesis was measured within the dentate gyrus of the hippocampus using standard immunohistochemical markers Ki67, Doublecortin, Calbindin and BrdU. Cell proliferation was reduced by 46% and neuronal maturation was reduced by 53% for a combined reduction of neuronal production of 75%. Such reduction is expected to cause a significant functional impairment of learning and memory in the diabetic animals. These results may shed light not only on causes of diabetic neuropathy but also provide an explanation for the proposed beneficial therapeutic effects of exogenous insulin on memory functions. Supported by CIHR

70 D120

PREPULSE INHIBITION OF STARTLE: TIMING OF GLYCINE ALPHA1, GABAA AND GABAB MEDIATED INHIBITION IN MICE

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Prepulse inhibition (PPI) involves midbrain pathways for approach responses that inhibit giant neurons in the caudal pons that elicit startle. PPI is mediated largely by muscarinic and GABAB receptors in the pons at interstimulus intervals (ISIs) of 100-500 ms in rats and mice, but little is known about receptors mediating PPI at ISIs of 10-50 ms. We found that the GABAA blocker, bicuculline (1 mg/kg i.p.) reduced PPI at ISIs of 30 and 50 ms, but not at 4-20 ms, ISIs where PPI in B6 mice is especially strong (over 50%). Glycine α1 receptor transgenic mice with reduced glycine function (Becker et al, 2002) showed stronger startle responses than B6 wild-type mice, but much less PPI at a wide range of ISIs from 4-300 ms. Bicuculline blocked most of the remaining PPI in glycine α1 receptor mutant mice at ISIs of 30 and 50 ms. In temporal order, then, PPI in B6 mice depends on glycine α1, GABAA, and finally, both muscarinic and GABAB receptors. These ISI-specific effects of blockers on PPI in mice are related to transmitters (glycine, GABA and acetylcholine) and receptors mediating inhibition of giant neurons in the pontine reticular formation.

71 D121

DEVELOPMENT OF ROBUST INTRAVENOUS NICOTINE SELF-ADMINISTRATION BEHAVIOR IN DRUG-NAIVE SQUIRREL MONKEYS WITH NO EXPERIMENTAL HISTORY UNDER FIXED-RATIO AND PROGRESSIVE-RATIO SCHEDULES OF REINFORCEMENT

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Nicotine, a psychoactive component of tobacco plays a major role in smoking dependence, but reinforcing effects of nicotine that contribute to smoking dependence have been difficult to demonstrate directly in past controlled laboratory studies with both animals and humans as experimental subjects. Although the ability of nicotine to act as a reinforcer in experimentally naïve rodents has been demonstrated under limited conditions, its ability to act as a reinforcer in experimentally naïve non-human primates is still unclear. In the present experiments, intravenous nicotine self-administration behavior developed in drug-naive squirrel monkeys with no history of operant behavior training and no setting conditions such as food deprivation. Once self-administration behavior developed, nicotine sustained robust responding under a Fixed-Ratio schedule of reinforcement where 10 lever presses were required to produce each intravenous injection. The behavior was under the control of nicotine injections since lever pressing extinguished when the nicotine solution was changed to saline solution and varying the injection dose of nicotine resulted in a typical inverted U-shaped dose-response curve. Nicotine self-administration behavior also maintained at high rates under a Progressive-Ratio schedule of reinforcement and varying nicotine dose again resulted in an inverted U-shaped dose-response curve. These results demonstrate that nicotine can function as a prototypic drug of abuse serving as a robust reinforcer in squirrel monkeys. This non-human primate represents a valuable animal model for studying the neurobiological basis of nicotine dependence. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse (NIDA), National Institutes of Health and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003). Research was supported by the Intramural Research Program of the NIDA, NIH, DHHS.

72 D122

THE ROLE OF SEXUAL ORIENTATION IN FACE RECOGNITION

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Previous research has suggested a sex difference in face recognition ability such that women tend to outperform men. Recent evoked potential research has shown gender effects with men displaying more right lateralized activation while women are more bilateral in face processing, suggesting gender differences in face processing at a cortical level. Although gay men display a cross-sex shift in some sexually dimorphic tasks—with their performance resembling that of female participants—the implications of this for face recognition tasks not been measured. The current study examined the performance of homosexual men on a basic old/new face recognition task compared to heterosexual men and women. Twenty four women, 22 heterosexual men and 11 homosexual men were tested. Participants learned five male and five female faces and subsequently were tested for accuracy and latency of recognition for the learned faces among a set of 30 unlearned faces. Overall, latencies of heterosexual men were significantly higher than those of women, and latencies of gay men fell between those of heterosexual male and female participants. The sex of visual face images also produced an interaction such that gay men's latencies for female face images were comparable to those of female participants, while latencies for male face images were comparable to those of heterosexual men. Observed sex differences in overall latency are consistent with previous visual cognitive studies showing participant gender effects on face recognition. The shorter latencies of gay men compared to those of heterosexual men suggest neurobiological correlates of homosexuality that may be evidenced in face processing.

73 D123

SEASONAL VARIATION IN HIPPOCAMPAL NEURON RECRUITMENT IN THE FOOD-STORING BLACK-CAPPED CHICKADEE

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Black-capped chickadees store food in a seasonally-varying fashion with a peak in storing activity in the fall and winter. Previous research has shown that these birds retrieve stored food by remembering the spatial locations of large numbers of spatially-dispersed caches. Memory for cache sites is hippocampus-dependent. We have recently described a mid- to late-winter peak in the recruitment of new neurons into the chickadee hippocampus using the cell-birth marker BrdU. There was no indication of seasonal variation in neuronal recruitment into the anatomically adjacent hyperpallium apicale. Further results show that new neurons are incorporated into the hippocampus of the black-capped chickadee at a much higher rate than into the hippocampus of the non-food-storing house sparrow. Chickadees also had a greater total number of hippocampal neurons in spring than in fall, a seasonal difference not found in house sparrows. Taken together, these results suggest that hippocampal neuronal recruitment may contribute to memory for cache sites or to other seasonally-varying components of food storing behaviour.

74 D124

SEXUAL DIMORPHIC EFFECTS OF NEONATAL SYSTEM IMMUNE ACTIVATION WITH LIPOPOLYSACCHARIDE ON THE BEHAVIOURAL RESPONSE TO A HOMOTYPIC ADULT IMMUNE CHALLENGE

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Research has shown that acute immune activation during the early postnatal period with the gram negative endotoxin, lipopolysaccharide (LPS), alters a variety of physiological and behavioural process in the adult animal. For example, neonatal LPS exposure affects disease susceptibility later in life though these effects appear to be modulated by time of exposure, gender, and immune stimulus. The current study examined sex differences in the effect of neonatal LPS treatment on the hypoactivity response to adult LPS administration. Male and female Long-Evans rats were treated systemically with either LPS (50µg/kg) or saline (0.9%) on postnatal days 3 and 5. Later in adulthood (postnatal day 92), all animals were subjected to an adult LPS challenge and were injected (i.p.) with 200µg/kg LPS. Two hours after injection, animals were placed in a non-novel open-field and locomotor activity was assessed for 30 min. Body weights were determined both at time of injection and 24hr later to examine LPS-induced weight loss. Adult males treated neonatally with LPS exhibited significantly less horizontal and vertical activity in response to the LPS challenge relative to males treated neonatally with saline. This effect was not observed in females. Thus, the current study revealed that neonatal LPS exposure potentiates the adult hypoactivity response to a homotypic immune challenge in rodents. These findings may be related to sexually dimorphic effects of neonatal LPS on the hypothalamic-pituitary-gonadal axis and the immunoenhancing effects of estrogen. These findings have potential clinical significance given that neonatal exposure to pathogens is a fairly common occurrence and gram-negative bacteria are a common cause of neonatal bacterial infections.

DEVELOPMENT

75 B401

A POTENTIAL ROLE FOR MICROGLIA IN NEURODEVELOPMENTAL DISORDERS

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Microglia are resident hematopoietic cells that play a key role in neurodegeneration and neural injury in the adult nervous system. However, apart from its predominant role in regulating developmental cell death, the role of microglia in normal brain development remains an open question. To investigate its role in developmental disorders, we quantified microglia in the hippocampus of newborn Noonan Syndrome mice and found that there was a 4-fold increase in the number of microglia relative to age-matched wild type littermates. In cortical precursor cultures from E12.5 mice, microglia are present at 0.2% and increasing them to 0.8% perturbed cells numbers and differentiation. This effect could be further mimicked by conditioned medium from developing microglia. Further, ablating microglia from our control cortical precursor cultures caused a decrease in total numbers of precursors and neurons, suggesting the role of soluble factors in this process. In order to examine if microglial proliferation and activation comprises a common them in various neurodevelopmental disorders, we are examining the microglial population in the mouse model for Fragile X. Preliminary results suggests that glial development in fmr1 knockout mice is altered. The molecular mechanisms neural cell development and the role of microglia in this regard is being investigated.

76 B402

ROLE OF SENSORY ACTIVITY IN THE FATE SPECIFICATION OF NEWLY GENERATED CELLS IN THE ADULT OLFACTORY BULB

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During development and shortly after birth, neuronal activity contributes to the organization of the nervous system. The adult olfactory bulb (OB), which is the first-order sensory relay for olfactory processing, retains the ability to acquire new interneurons throughout life. It is therefore a particularly appropriate region for studying the role of experience in sculpting a neuronal network postnatally. Interestingly, interneurons arriving to the adult OB differentiate into distinct neuronal types, including GABAergic cells located in the granule cell layer and a diverse set of neurons in the glomerular layer comprising also GABAergic and dopaminergic interneurons, as well as other neuronal subtypes expressing calretinin and calbindin. To investigate the potential role of afferent activity in the fate specification program of OB newborn cells, we performed unilateral nostril occlusion in adult mice. We explored the number, density and proportion of chemospecific newly generated cells in the odor-deprived animals. To understand whether sensory activity plays a role in the acquision and/or maintenance of chemospecific phenotype, we analyzed the cells born 21 days before or 21 days after sensory deprivation. Our analysis demonstrates, in agreement with previously reported results, that sensory deprivation resulted in drastic reduction of the number of dopaminergic periglomerular cells. This effect was specific to the dopaminergic cells, since neither calbindin-positive nor calretinin-containing periglomerular cells were affected. Surprisingly, however, unilateral sensory deprivation also induced a drastic up-regulation of the density of cells expressing Pax6, a transcriptional factor known to be involved in the dopaminergic phenotype specification in the adult OB. Our results suggest that the adult OB is a highly plastic structure, which attempts to compensate for the dopaminergic cell loss by up-regulating the number of Pax6-positive cells.

77 B403

INSULIN-LIKE GROWTH FACTOR 1-STIMULATED PROTEIN SYNTHESIS IN OLIGODENDROCYTE PROGENITORS

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Several lines of investigation have provided evidence that insulin-like growth factor-1 (IGF-1) is essential for oligodendrocyte (OL) development, promoting their proliferation, survival and differentiation. Moreover, IGF-1 null mutant mice have a decreased number of OL progenitors (OLPs) and CNS myelination. IGF-1 interacts with its receptor to activate two main transduction pathways, the PI3K/Akt and the Ras-Raf-MEK/ERK cascades, which mediate survival or proliferation of OLPs. The objective of this study was to elucidate the signalling pathways mediating IGF-I-stimulated protein synthesis, important for growth and differentiation of OLs. In other cellular systems, the PI3K/Akt pathway is involved in protein translation. Furthermore, mTOR and the p70 S6 kinase are downstream effectors that phosphorylate translation initiation factors (e.g. eIF-4E) and their regulators (e.g. 4E-BP1). OLPs, obtained from primary cultures, were treated with IGF-1 with or without pharmacological inhibitors for PI3K (LY294002 or wortmannin), mTOR (rapamycin), Akt (III or IV), ERK (PD09859), PKC (bisindolylmaleimide) and an adenovirus with dominant negative Akt. Protein synthesis was assessed by metabolic labeling with 35S-methionine, and kinase activation by Western blotting. IGF-1-stimulated protein synthesis was dose-dependent and required PI3K, mTOR, Akt, ERK and PKC activation. Concordantly, Western blotting revealed that IGF-1 stimulates phosphorylation of Akt, mTOR, ERK, S6 and 4E-BP1. Activation of S6 and inactivation of 4E-BP1 occur through phosphorylation and are required for protein synthesis to take place. These events are dependent on the upstream activation of PI3K, Akt and mTOR. Project funded by the MS Society of Canada.

78 B404

SKIN-DERIVED PRECURSORS (SKPS): IN VIVO CELL FATE IS LIMITED TO THE NEURAL CREST LINEAGE, AND IS DETERMINED BY TISSUE-SPECIFIC FACTORS

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Skin-derived precursors (SKPs) are a neural crest-related precursor found in both rodent and human postnatal dermis, where they reside within a hair follicle dermal papilla niche. In culture, SKPs are multipotent, generating both mesodermal cells and those with peripheral neural characteristics. However, their differentiation potential in vivo has not been explored. Here, we asked whether SKPs are multipotent in vivo, and whether their tissue environment influences their differentiation potential. To perform these experiments, SKPs were generated from dermis of neonatal (P1-P3) YFP-expressing mice and adult YFP-expressing rats. These YFP-positive SKP spheres were transplanted into either the dermis or the sciatic nerve of adult NOD-SCID or immunosuppressed Shiverer mice (deficient in myelin basic protein gene), respectively. Six weeks following transplantation, SKPs transplanted into the skin had integrated throughout the thickness of the dermis, and were present within the dermal papilla and dermal sheath of hair follicles, suggesting that they were able to re-enter their original niche. SKPs integrating into dermis expressed appropriate dermal cell markers, and did not express markers of inappropriate cell types (ie. neurons or peripheral glia). Moreover, SKPs were never seen to contribute to epidermal components of the skin or hair follicle. In contrast, when SKPs were transplanted into the injured sciatic nerve, they integrated within the nerve where they differentiated into myelinating Schwann cells and cells with characteristics of perineurial fibroblasts. However, they never expressed markers characteristic of hair follicle dermal papilla or sheath cells, nor did they differentiate into cells with characteristics of neurons. Thus, SKPs differentiate in vivo to both neural and mesodermal phenotypes, but their differentiation is apparently restricted to cells of neural crest lineage. The finding that SKPs differentiate into non-overlapping subsets of cells within the dermis versus the sciatic nerve indicates that tissue environment plays a key role in restricting and/or determining their differentiation into tissue-appropriate cell types, much as it does for embryonic neural crest stem cells.

79 B405

THE ONTOGENIC DEVELOPMENT OF MAMMALIAN MOTOR SYSTEMS USING A MARSUPIAL

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By using Monodelphis domestica, an opossum born more immature than eutherians, motor systems development can be largely studied postnatally without constraint of the uterus or marsupium, the species being pouchless. Newborn measure 10mm snout-rump and weigh 100mg. Their forelimbs are just sufficiently developed to perform rhythmic, alternate movements that, together with the trunk that sways from side to side, allow the animals to climb on the mother from the birth canal to a nipple where they attach. The hindlimbs are little more than immobile buds and only start moving in the 2nd week. The limbs of newborn comprisde immature cartilaginous bone models surrounded by loose muscle fibers, except in the foot where no muscle tissue is formed; the connective tissue is mesenchymous. The spinal enlargements, especially at lumbar levels, are very immature: the large central canal is surrounded by thick ventricular and intermediate zones in which cells are small, many having the appearance of migrating cells, and a thin marginal zone. Mitotic figures are seen at lumbar levels, probably of glial rather than neuronal precursors. Motoneurons are recognized by their position and their slightly larger size, but are undifferentiated and form a continuous mass bordering the ventral horn. They express choline acetyltransferase, the synthetic enzyme of acetylcholine. The number of cholinergic neurons in the ventral horn increases until two weeks, suggesting that not all motoneurons had yet reached their final position. Glutamate, GABA and glycine are present in spinal neurons of newborn but increase postnatally. There are very few synapses in the spinal enlargements of newborn; synaptogenesis is mostly a postnatal event that occurs along rostrocaudal and ventrodorsal gradients, in accordance with the mostly or entirely postnatal growth of propriospinal and descending systems into the cord. Therefore, the forelimb movements of newborn are presumably the expression of the spinal central pattern generator. A study of spinal spontaneous activity and of the gap junction protein connexin is underway. The limbs of newborn contain few cholinergic terminals, which have the appearance of growth cones; their motor innervation proceedes mostly postnatally, especially the hindlimbs. Myelinogenesis in the spinal enlargements and the corresponding ventral and dorsal roots only starts in the 2nd week and continues for months, in accordance with the protracted development of locomotion.

80 B406

INSIGHTS INTO OLIGODENDROCYTE AND MYELIN GENERATION GAINED FROM THE ANALYSIS OF HUMAN EMBRYONIC AND ADULT PLATELET-DERIVED GROWTH FACTOR RESPONSIVE NEURAL PRECURSORS

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Understanding human oligodendrocyte precursors and their potential to contribute to remyelination therapies has been limited by an inability to isolate and propagate these cells in culture. We have recently found that the neurosphere culture system can be used to isolate and expand mouse embryonic platelet-derived growth factor (PDGF)-responsive neural precursors (PRPs), which predominantly produce myelinating oligodendrocytes. Thus, we adapted the identical neurosphere culture system for the study of human embryonic and adult PRPs. Human embryonic PRPs generated neurospheres that contained a large proportion of oligodendrocytes, some of which expressed myelin basic protein, neurons and a small number of astrocytes. In the presence of PDGF, fibroblast growth factor 2 (FGF2) promoted human embryonic PRP expansion for several generations. In contrast, embryonic epidermal growth factor- and FGF-responsive neural stem cells rarely generated oligodendrocytes and several lines of evidence suggest that they are phenotypically distinct from embryonic PRPs. In comparison to their embryonic counterparts, adult human PRPs isolated from corpus callosotomies required twice the culture period to generate neurospheres, which contained oligodendrocytes and astrocytes, but not neurons. Strikingly, adult human PRPs did not self-renew even in the presence of FGF2. This study suggests that differences in the self-renewal properties of embryonic and adult human PRPs may contribute to limited, intrinsic remyelination in adult humans. Moreover, expandable human embryonic PRPs may be useful for transplantation therapies aimed at remyelination. Supported by the Neuroscience Canada Foundation and the Multiple Sclerosis Society of Canada.

81 B407

A TRYPTOPHAN HYDROXLYASE 1 TRANSGENIC REPORTER DETECTS ABNORMALITIES IN THE DEVELOPING SEROTONERGIC SYSTEM OF MICE CARRYING THE ANOREXIA (ANX) MUTATION

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The wide spread projections of the serotonergic (5-HT) system modulate emotion-influenced behaviors, such as appetite and aggression. Not surprisingly, serotonergic dysfunction has been implicated in human psychiatric disorders. While much has been learned about early specification

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

of 5-HT neurons from analyses of mouse mutations, the density, pervasiveness, and heterogeneity of 5-HT neuronal processes have complicated the analyses of 5-HT innervation. A transgenic reporter system in the mouse that labels only a subset of 5-HT neuronal processes during a psychiatrically critical developmental window would help uncover the molecular mechanisms that govern the integrity of 5-HT innervation. Here we have genearated a 5-HT-specific developmentally sensitive reporter line labeling axons, which uses the 6.1kb upstream promoter region of Tryptophan Hydroxylase I (Tph1) to drive the expression of placental alkaline phosphatase (PLAP) in 5-HT processes. Rather than labeling all 5-HT processes, the Tph1-PLAP reporter labels only a subset of 5-HT neurons and, thereby, facilitates axonal tracing. A second strength of this reporter is its developmental sensitivity. Tph1 expression peaks at P21 in the mouse and then decreases towards adulthood. We have tested the utility of this reporter by analyzing the onset and region-specific 5-HT anomalies in mice carrying the anorexia (anx) mutation. 16-21 day old anx/anx mice are known to exhibit selective hyper-innervation of 5-HT neurons throughout the brain, which contributes to hyperactivity, appetite suppression, and head shaking behavior. While pan-5-HT immunoreactivity was indistinguishable between anx/anx and +/+ littermates, the Tph1-PLAP reporter uncovered 5-HT developmental deficits as early as P0 in anx/anx mice. Furthermore, while pan-5-HT immunoreactivity had previously revealed global 5-HT hyperinnervation, analyses of Tph1-PLAP reporter revealed region-specific alterations in the 5-HT system of anx/anx mice. Thus, the Tph1-PLAP reporter provides a highly sensitive indicator, which should prove invaluable in future analyses of the 5-HT system in other mouse mutants. Finally, A developmental role and, thus, sensitivity to developmental perturbations have been postulated for Tph1. Our work shows that Tph1 expression is highly sensitive to developmental perturbations, and lends further support to the importance of Tph1 in psychiatric conditions.

82 B408

NSF CONTROLS SYNAPTIC GROWTH BUT NOT SYNAPTIC STRENGTH BY REGULATING DLK/JNK SIGNALING

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N-ethylmaleimide-sensitive factor (NSF) is an ATPase well known for its ability to influence synaptic vesicle traffic by dissociating the SNARE complex of proteins. Recent evidence suggests that NSF also has additional cellular roles. Notably, a dominant-negative version of Drosophila NSF2 induces a remarkable overgrowth of the neuromuscular junction (NMJ), which correlates with disrupted filamentous actin and impaired vesicle mobility. In order to determine which signaling pathways may lead to these novel phenotypes we undertook genetic interaction analysis to identify loss-of-function mutations that could suppress the NSF2-induced NMJ overgrowth. We found that flies bearing heterozygous mutant alleles for genes of the jun N-terminal kinase (JNK) pathway, but not the p38 kinase pathway, were potent suppressors of NMJ overgrowth. Suppression of the overgrowth phenotype by mutants of Dual Leucine zipper-bearing Kinase (DLK) /wallenda indicate that the genetic interaction extends to the top of a conserved MAP kinase pathway. Interestingly, electrophysiological analysis of synapses showing morphological rescue failed to show any rescue of the dominant-negative NSF2 physiological phenotypes. These data therefore indicate firstly, a novel interaction between NSF and MAP kinase signaling pathways that restrains synaptic growth and secondly, that NSF likely controls synaptic physiology and development by two independent pathways.

83 B409

GLUCOSE DEPRIVATION INHIBITS MITOCHONDRIAL PROTEIN IMPORT: THE ROLE OF TOM20

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The majority (99%) of mitochondrial proteins is nuclear-encoded and must be imported into mitochondria. The import process is complex and dependent on an array of translocases and chaperones localized to the inner and outer mitochondrial membranes. Little is known about protein import in neurons but recent studies have shown that at least one progressive neurodegenerative disease (Human Deafness Dystonia) is due to an import deficit. Previous studies using PC12 cells stably transfected with an inducible mitochondrially-targeted GFP (mtGFP) have established that mitochondrial protein import can be inhibited by a variety of sub-lethal stressors, including glucose/glutamine deprivation-reperfusion (GD/R). We hypothesized that overexpression of Tom20, an integral component of the protein import machinery, would increase protein import and ameliorate the GD/R-induced decline in import. Overexpression of Tom20 was achieved by transfecting differentiated PC12 cells with full-length human Tom20 (transfection efficiencies at 24 hrs was 33% ± 6%). Western blot confirmed that under basal conditions overexpression of Tom20 significantly increased Tom20 expression and Tom20 import to mitochondria. In these cells mtGFP import also increased; 24hr post-transfection mtGFP levels in mitochondria were increased by 29% ± 3% and 38% ± 4% by 48hrs. In normal cells mtGFP expression and import were unchanged immediately post-GD but by 24h mtGFP import was reduced by 27% ± 3% (assessed by flow cytometry) and 22% ± 4% (assessed by Western blot). The decrease in mtGFP import was sustained; at 48hrs mtGFP import was reduced by 32% ± 5% . Intramitochondrial turnover of mtGFP was unchanged. In these cells levels of endogenous Tom20 declined significantly. Mitochondrial membrane potential and ATP levels were unchanged but ROS levels increased by 71% ± 8% and 60% ± 14% versus controls at 24h and 48h post-GD/R. Overexpression of Tom20 prior to GD prevented the GD induced decline in Tom20 expression and reduction in mitochondria, and restored mtGFP import to levels above controls. Our findings indicate that in neurons sublethal GD reduces Tom20 expression and causes a sustained decline in Tom20 in mitochondria. This decrease in Tom20 is associated with a sustained decline in the import of mtGFP and both effects can be reversed by overexpression of Tom20. These findings argue that Tom20 is sensitive to GD and is a key loci at which protein import can be modulated

84 B410

INDUCTIBLE EXPRESSION OF MUTANT T1A-BETA 5 SUBUNIT IMPAIRS PROTEASOME FUNCTION

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by intracellular inclusions (Lewy bodies) and loss of neurons in the substantia nigra. While the etiology of PD is poorly understood, α-synuclein and the ubiquitin-proteasome system (UPS), the major non-lysosomal pathway for the removal of unwanted proteins via ubiquitin conjugation, are considered key pathogenetic factors. Defects in the UPS are thought to lead to the build-up of insoluble proteins that disrupt cellular function and ultimately result in cell death. In vitro and in vivo studies have shown that pharmacologic proteasome inhibitors can reproduce some aspects of PD pathology However, the use of inhibitors is problematic for long-term studies due to high cost, non-specific effects and toxicity. As an alternate approach to examine proteasome dysfunction, we have developed a genetic model of proteasome inhibition. This is a doxycycline-regulated expression system that allows conditional expression of a mutant catalytic β5

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

subunit of the proteasome. To create this system, the epitope-tagged wild-type or T1A mutant β5 constructs were inserted into plasmids containing the tetracycline-responsive element (pTRE) and then transfected into commercially available HEK293 cells (BD Biosciences) which stably express the Tet-On(TM) regulator plasmid. Stable, double-transfected clonal cell lines were obtained by screening with hygromycin and G418 for several weeks. This system enabled us to examine biochemical changes following induction of expression and permit examination of the long-term effects of proteasome inhibition without the non-specific effects of proteasome inhibitors. We have shown that expression of the mutant β5 subunit incorporated into proteasome complexes and impaired proteasome activity in both cell lysates and in β5-immunoprecipitated fractions. In addition, long-term exposure of cells to doxycycline resulted in chronic proteasome inhibition. With this model, we can analyze progressive changes in PD-related proteins, such as α-synuclein, that occur with proteasome inhibition. This genetic model is also being used to test the effects of oxidative stress against a background of proteasome inhibition.

85 B411

GLIAL CELL PROLIFERATION IN RAT SPINAL CORD INDUCED BY PERIPHERAL NERVE INJURY AND THE RELATIONSHIP WITH NEUROPATHIC PAIN

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Glial activation is a typical response of the central nervous system to nerve injury. In the current investigation, we characterized the temporal and spatial pattern of glial proliferation, one of the most conspicuous features of glial activation, in relation to nerve injury-induced neuropathic pain. Using bromodeoxyuridine (BrdU) as a mitotic marker, we analyzed cell proliferation in the spinal cord, identified the phenotype of dividing cells, and traced their fate, and correlated these phenomena with behavioural assays of the neuropathic pain syndrome. Our results demonstrated that peripheral nerve injury induced an early and transient cell proliferation, on the spinal cord ipsilateral to the nerve lesion which peaked at day 3 post-surgery. The majority of the proliferating cells were Iba-1+ microglia, together with some NG2+ oligodendrocyte progenitors, and GFAP+ astrocytes. These newly generated cells continued to divide and differentiate over time with the response peaking at day 14 post-injury. Microglia were always the predominant phenotype (over 50% of the newly formed cell population). There was a close temporal correlation between microglial proliferation in the spinal cord dorsal horn and the abnormal pain responses, suggesting a contribution of the new microglia to the genesis of the neuropathic pain symptoms.

86 B412

MYELINATION OF CONGENITALLY DYSMYELINATED SPINAL CORD AXONS BY ADULT NEURAL PRECURSOR CELLS RESULTS IN FORMATION OF NODES OF RANVIER AND IMPROVED AXONAL CONDUCTION

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Emerging evidence suggests that cell based remyelination strategies may be a feasible therapeutic approach for central nervous system (CNS) diseases characterized by myelin deficiency as a result of trauma, congenital anomalies or diseases. While experimental demyelination models targeted at the transient elimination of oligodendrocytes have suggested that transplantation-based remyelination can partially restore axonal molecular structure and function, it is not clear whether such therapeutic approaches can be used to achieve functional remyelination in models associated with long term, irreversible myelin deficiency. In this study, we transplanted adult neural precursor cells (aNPCs) from the brain of adult transgenic mice into the spinal cords of adult Shiverer (shi/shi) mice, which lack compact CNS myelin. Six weeks after transplantation, the transplanted aNPCs expressed oligodendrocyte markers including MBP, migrated extensively along the white matter tracts of the spinal cord, and formed compact myelin. Conventional and three-dimensional confocal and electron microscopy revealed axonal ensheathment, establishment of paranodal junctional complexes leading to de-novo formation of nodes of Ranvier and partial reconstruction of the juxtaparanodal and paranodal molecular regions of axons based on Kv1.2 and Caspr expression by the transplanted aNPCs. Electrophysiological recordings revealed improved axonal conduction along the transplanted segments of spinal cords. We conclude that myelination of congenitally dysmyelinated adult CNS axons by grafted aNPCs results in the formation of compact myelin, reconstruction of nodes of Ranvier and enhanced axonal conduction. These data suggest the therapeutic potential of aNPCs to promote functionally significant myelination in CNS disorders characterized by longstanding myelin deficiency.

87 B413

MEASURING SEVERED BRAIN'S RECONSTRUCTIVE HEALING

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Precisely severed adult rat brain tissue, marked by implanting the fine wire cutting device that had been lowered to lesion it, can undergo a reconstructive type of healing that appears to re-establish disconnected axonal trajectories either around the lesion, as detours, or across it (Foerster, 1982). Withdrawing the devices through the lower surface of the fixed brain leaves two channels that, in horizontal sections, appear as two holes that mark the ends of the cut. The initial injury disconnects intercepted tissue, with minimal displacement. HRP tracing of the rat's severed optic pathway, and microelectrode mapping of the superior colliculus, confirmed that its eventual healing was associated with the re-establishment of appropriately located, mapped and functioning, retinal connections (Foerster & Holmes, 1999). Considering the opinion that "CNS regeneration" requires heroic interventions which produce bizarre-looking axonal extensions (e.g. Steward et al., 2003), the orderliness of the reconstructive healing would have caused it to escape attention if the lesions had not been marked independently of later axonal events. Histological staining for neurons and axons (browns), myelin (normal, blue- green; degenerate, bright blue), and neuroglia and blood vessels (pinks) in the same section gives a useful view of brain architecture and its post-lesion changes. Although every structure can heal, they seem to have characteristically different ways of doing it. Lesions of pathways made 2 weeks - eight months previously are being examined now to classify these. Apparent strategies include: (i) crossing the lesion; (ii) detouring either closely together around the lesion or bulkily with a more normal separation; (iii) interweaving and abrupt bending on the proximal side of severed one-way tracts, and a smooth curving trajectory into their distal pathway. Morphological correlates of axonal neoformation are being sought to help identify healing after unmarked [recise lesions.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

88 B414

CONTROL OF CNS CELL FATE DECISIONS BY SHP-2 AND ITS DYSREGULATION IN NOONAN SYNDROME

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Within the developing mammalian CNS, growth factors direct multipotent precursors to generate neurons or glial cells. We demonstrated that the growth factor-regulated protein tyrosine phosphatase SHP-2 is essential for normal cortical cell fate determination, and that a mutation resulting in constitutive activation of SHP-2 in Noonan Syndrome, a human syndrome associated with mental retardation and learning disabilities, causes perturbations in this developmental process. Specifically, genetic knockdown of SHP-2 in cultured cortical precursors or in in utero-electroporated embryonic telencephalon inhibited basal neurogenesis and caused enhanced and precocious astrocyte formation. Conversely, expression of an activated SHP-2 mutant associated with Noonan Syndrome enhanced MEK-ERK signaling to promote neurogenesis and negatively-regulated the gp130-JAK-STAT pathway to inhibit gliogenesis. Neural cell fate decisions were similarly perturbed in the hippocampus and dorsal cortex of a mouse knock-in model that phenocopies human Noonan Syndrome. Thus, SHP-2 instructs precursors to make neurons and not astrocytes during the neurogenic period, and perturbations in the relative ratios of these two cell types following constitutive SHP-2 activation may contribute to the cognitive impairments in Noonan Syndrome patients.

89 B415

UNIQUE AND SYNERGISTIC ROLES FOR EPHA4 AND B2 RECEPTORS IN COMMISSURAL DEVELOPMENT OF MAMMALIAN FOREBRAIN

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Eph receptors represent the largest family of receptor tyrosine kinases and have been shown to regulate a variety of cell-cell sorting decisions such as neural crest cell migration, the formation of somatotopic maps, angiogenesis and axon guidance. Previous work from our laboratory has demonstrated that EphB2 is required for proper formation of the anterior commissure (AC). EphA4 has also suggested to be involved in the formation of the AC. However, the mechanism underlying these defects and potential for redundancy with respect to EphB2/A4 signaling in the AC has not been determined. In the present study, high-resolution magnetic resonance imaging (MRI) combined with in vivo retrograde labeling of CNS tracts was performed to determine the contribution of EphB2 and A4 receptors in regulating the development of interhemispheric connections of the forebrain. Using single and combinatorial null mutants for each of these receptors, we have determined the unique and synergistic contributions of each. Our findings demonstrate that EphB2 and A4 receptors exert distinct guidance roles on the anterior and posterior branches of the AC. These studies further show that these receptors also act synergistically to control aspect of AC guidance. While additional protein-protein interactions may play a role in regulating proper tract formation within mammalian forebrain, it is interesting to note that we can experimentally recapitulate all of the major interhemispheric guidance decisions in this region by proper expression of EphB2 and A4. Taken together, these results demonstrate that members of the Eph family exert both unique and synergistic functional control on the mammalian forebrain. (Supported by the Canadian Institutes for Health Research, ALS, MDAC, NARSAD).

90 B416

'THE ROLE OF SRC-LIKE KINASES IN MYELINATION OF THE PERIPHERAL NERVOUS SYSTEM'

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Myelin is an insulating membranous structure surrounding axons, which facilitates the saltatory conduction of action potentials. Deficiencies in peripheral myelin result in neuropathies such as Charcot-Marie Toothe disease, warranting further studies into its underlying molecular mechanisms. Schwann cells (SC) synthesize myelin in the Peripheral Nervous System (PNS). They provide trophic support to the axon, while axonal contact influences SC growth, maturation and myelin synthesis. The molecular mechanisms by which PNS myelination occur have yet to be fully elucidated. Fyn, a member of the Src family of cytoplasmic tyrosine kinases (SFK), plays a critical role in central nervous system myelination as suggested by the reduced myelin content in brains of fyn-deficient mutant mice (Umemori et al. 1994). Fyn transactivates MBP gene transcription (Umemori et al. 1999) and regulates OLG development in vitro (Osterhout et al. 1999). Fyn has been found in a protein complex associated with beta1 integrin/FAK and paxillin in differentiated dorsal root ganglion neurons and Schwann cell cultures (DRGN-SC) (Chen et al, 2000). However a definitive function for Fyn or other Src family kinases in peripheral myelination had not been demonstrated. To assess whether SFK are involved in peripheral myelination, DRGN/SC cultures were treated with the SFK inhibitor PP2 at the initiation of myelination, started by ascorbate addition. PP2 caused a concentration-dependent decrease in MBP levels as assessed on day 10 by both Western blot analysis and immunocytochemistry. PP2 and anti-fyn siRNA reduced the numbers of SCs expressing Krox-20, an inducible transcription factor required for peripheral myelination. Time-course experiments also revealed that PP2 had a significant effect on myelination even when cultures were treated several days after the initiation of myelination. MBP protein levels were only partially recovered in a time-dependent manner following PP2 removal. Thus, two clear phases of inhibition were evident. SFK inhibition caused a significant decrease in the length of myelinated segments following quantification on immunofluorescent digital images of MBP immunostaining. However, ultrastructural studies showed normal compaction of myelin. Similarly, the distribution of sodium channel, caspr and neurofilament expression as determined by immunofluorescence appeared normal in PP2-treated cultures. Funded by the Multiple Sclerosis Society of Canada (MSSC)

91 B417

DIFFERENTIAL REQUIREMENTS BETWEEN SKELETO-AND FUSIMOTOR NEURONS FOR BCL-2 IN MEDIATING THEIR SURVIVAL DURING CNS DEVELOPMENT

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For the nervous system to self-assemble into a complex arrangement of synaptic connections, more neurons are produced during development than are ultimately incorporated into the network. This process is believed to involve competition among neurons for neurotrophic support supplied by their appropriate innervation targets. The process of programmed cell death (PCD) has been implicated in promoting the demise of neurons that have made improper connections. Members of the Bcl-2 protein family are involved in the regulation of this process and represent a primary PCD regulatory checkpoint within both developing and postnatal neurons.

Previous analyses of bcl-2-/- mice have shown that despite the absence of any gross CNS abnormalities, some neuronal populations are more sensitive to Bcl-2 deficiency, resulting in a subtle loss of neurons during and after development. In the present study, we have analyzed in detail motor neurons in the facial nucleus (branchiomotor neurons) and lumbar spinal cord (somatic motor neurons) of bcl-2-/- mice at postnatal day 30 to contrast any differences between these two motor neuron subtypes in their dependencies for Bcl-2 for survival during development. Axonal counts and cross-sectional area measurements along with motor neuron counts in the facial nucleus revealed a significant loss of facial motor neurons in bcl-2-/- animals compared to heterozygous controls. In addition, contrary to a previous report, it was determined that subpopulations of motor neurons in the facial nucleus do not exhibit differential sensitivity to Bcl-2 deficiency. In our examination of ventral roots at the level of the lumbar spinal cord (L4), reductions in axon number and cross-sectional area were observed in Bcl-2 deficient mice. Interestingly, a shift in axon calibers seemed to have affected small caliber axons preferentially while effects on large caliber axons appeared to be minimal. By analyzing motor neuron populations in the lumbar spinal cord, we observed a corresponding loss of ChAT-positive motor neurons. The selective loss of small caliber axons suggested that they were extended by fusimotor neurons that normally innervate muscle spindles. Indeed, counts of total fusimotor fibers within the soleus muscle indicated a significant reduction in fusimotor innervation in bcl-2-/- mice. These findings indicate that fusimotor neurons are particularly sensitive to the loss of Bcl-2 and a majority of them die during development in its absence.

92 B418

OPTIMAL CA LEVELS REQUIRED FOR NEURITE OUTGROWTH IS REGULATED BY NCS-1

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Ca is an important regulator of neurite extension and growth cone pathfinding. An optimal window level of Ca may be necessary for neurite outgrowth: lower levels stabilize growth cones and higher levels stall them, in both cases preventing extension. Cytosolic Ca can come from many sources, but how these are coordinated to reach and maintain the window level of Ca is unclear. Neuronal calcium sensor-1 (NCS-1) is a member of a superfamily of proteins that respond to local Ca changes. It interacts with various proteins involved in Ca homeostasis and is a likely candidate for dynamically regulating cytosolic Ca levels. There is compelling evidence that NCS-1 is involved in neurite development. However, no study has examined how NCS-1 is involved in regulation of the window level of Ca permissive to neurite outgrowth. In this study, we examined the effects of NCS-1 on cytosolic Ca and neurite outgrowth in cultured L. stagnalis PeA neurons. Using RNA interference, we found that NCS-1 knockdown enhanced neurite extension and branching, and reduced activity-dependent Ca influx in growth cones. Using a C-terminal peptide of NCS-1, we found that the C-terminus modulates growth cone voltage-gated Ca currents, consistent with a previous finding. The peptide also affected neurite outgrowth and activity-dependent Ca influx. Interestingly, the C-terminal peptide had no effect on neurite extension as compared to the control, untreated neurons. Instead, it was more effective at enhancing neurite branching and less effective at reducing activity-dependent Ca influx as compared to NCS-1 knockdown. Our findings indicate that NCS-1 modulates neurite branching and extension by regulating Ca influx by at least two mechanisms: one specifically affecting branching, and another affecting extension. Taken together, we propose that different regions of NCS-1 regulate the cytosolic Ca concentration in growth cones in and out of the optimal window level, consequently leading to changes in neurite outgrowth behaviors.

93 B419

PHOSPHORYLATION AND CLEAVAGE OF PRESENILIN-ASSOCIATED RHOMBOID-LIKE PROTEIN (PARL) PROMOTES CHANGES IN MITOCHONDRIAL MORPHOLOGY

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Remodeling of mitochondria is a dynamic process coordinated by fusion and fission of the inner and outer membranes of the organelle, mediated by a set of conserved proteins. In metazoans, the molecular mechanism behind mitochondrial morphology has been recruited to govern novel functions, such as development, calcium signaling, and apoptosis, which suggests that novel mechanisms should exist to regulate the conserved membrane fusion/fission machinery. Here we show that phosphorylation and cleavage of the vertebrate-specific Pβ domain of the mammalian presenilin-associated rhomboid-like (PARL) protease can influence mitochondrial morphology. Phosphorylation of three residues embedded in this domain, Ser-65, Thr-69, and Ser-70, impair a cleavage at position Ser77–Ala78 that is required to initiate PARL-induced mitochondrial fragmentation. Our findings reveal that PARL phosphorylation and cleavage impact mitochondrial dynamics, providing a blueprint to study the molecular evolution of mitochondrial morphology.

94 C401

INTEGRATED DRUG DELIVERY IN CHITOSAN CHANNELS FOR PROMOTING NEURAL STEM CELL DIFFERENTIATION

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We are currently developing a strategy to induce regeneration of the spinal cord after injury by combining adult neural stem/progenitor cell (NSPC) transplants with a guidance channel implant. Earlier work has demonstrated that we can achieve good survival of transplanted NSPCs, but that almost half of these cells do not stain for markers of typical NSPC lineages. We believe that providing the proper signals to the local environment will allow greater control over NSPC cell fate and result in increased therapeutic potential. It is our goal to incorporate drug-eluting polylactide-co-glycolide (PLGA) microspheres into these guidance channels to provide localized and sustained release of factors to influence cells present within the channels. Specifically, we aim to control NSPC cell fate in vitro and in vivo through the delivery of phenotype-specific differentiation factors, resulting in enhanced populations of oligodendrocytes, astrocytes, and neurons. PLGA microspheres were prepared by a double emulsion method, with alkaline phosphatase used as a model protein. Encapsulation efficiencies were consistently above 80% and average diameters can be varied from 15 to 50 µm. To incorporate microspheres into the channel wall, microspheres were suspended in a dilute buffered chitosan solution and spun-coated onto the interior of the chitosan channel. Alkaline phosphatase release was monitored over time in physiological conditions. Similar release profiles were observed from freely suspended microspheres and microspheres incorporated into the channels, with the protein remaining bioactive for the first two weeks. Thus, the embedded microsphere strategy appears to be feasible for the delivery of large molecules from the walls of chitosan channels. Several potential NSPC differentiation factors have been identified including platelet-derived growth factor (PDGF) and bone morphogenic protein-2 (BMP2) for oligodendrocytes and astrocytes, respectively. By incorporating these factors into PLGA microspheres, we can characterize the effectiveness of our system to deliver active signals to control NSPC cell fate in vitro and eventually in an in vivo model of spinal cord injury.

95 C402

MYOTOPIC ORGANIZATION OF LIMB INNERVATION BY SPINAL MOTOR NEURONS IS DEFINED BY NETRIN AND NETRIN RECEPTOR EXPRESSION

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A remarkable feature of the developing nervous system is the establishment of topographical maps, wherein neuronal soma position is associated with the position of the innervation target. A simple and myotopic topography is evident in the organization of the lateral motor column (LMC) spinal motor neurons and their axonal projections to limb muscles. Motor neurons located in the lateral LMC innervate dorsal (extensor) limb muscles, whereas motor neurons located in the medial LMC innervate ventral (flexor) limb muscles. Limb mesenchyme axon trajectories of LMC neurons are controlled by LIM homeodomain transcription factors expressed in the LMC motor neurons (Lim1 and Isl1) and limb mesenchyme (Lmx1b). These transcription factors coordinately control the expression of Eph tyrosine kinase receptors and their Ephrin ligands, which are implicated in the specification of LMC axon trajectories. However, genetic evidence suggests the existence of additional effector molecules controlling LMC axon guidance in the limb. To uncover new LMC axon guidance determinants, we carried out a screen for molecules whose expression is restricted to the dorsal or ventral limb mesenchyme at the time of choice of dorsal or ventral axon trajectory. We found that Netrin1, a gene encoding a diffusible molecule required for attractive or repulsive guidance of many classes of axons, is expressed specifically in the dorsal limb mesenchyme and is under the control of Lmx1b. Our analysis of Netrin receptor mRNA distribution demonstrates that receptors associated with attraction towards Netrin are expressed by lateral LMC motor neurons innervating the dorsal limb, and receptors associated with repulsion from Netrin are expressed by medial LMC motor neurons innervating the ventral limb. This restricted Netrin receptor expression leads to our hypothesis that Netrin1 is a bifunctional motor axon guidance cue in the limb mesenchyme that attracts lateral LMC axons to the dorsal limb and repels medial LMC axons towards the ventral limb, thus controlling LMC myotopy. We are currently testing this hypothesis through gain and loss of function of Netrin pathway components in both the mouse and the chick.

96 C403

NEURONAL CO-CULTURE INHIBITS MYELIN PRODUCTION BY OLIGODENDROCYTES FROM DIFFERENTIATED ADULT RAT SPINAL CORD NEURAL PRECURSOR CELLS

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We recently showed that adult rat spinal cord derived neural precursor cells (NPCs) differentiate primarily into myelin producing oligodendrocytes in vitro, making them appropriate candidates for future spinal cord injury (SCI) transplantation trials since they have the potential to remyelinate demyelinated or regrowing axons (Kulbatski et al., 2007). Purpose: In the current study, we tested the in vitro ability of adult rat spinal cord NPCs to myelinate cortical neurons, as a measure of their functional capacity.
Hypothesis: We hypothesized that adult rat spinal cord NPCs will produce functional oligodendrocytes that myelinate co-cultured rat cortical neurons. NPCs were isolated and grown from adult Wistar rats as described previously (Kulbatski et al., 2007). NPCs were differentiated on Matrigel for 1 week, after which freshly isolated neonatal cortical neurons were added for an additional week. Parallel samples were prepared for transmission electron microscopy (TEM) and immunocytochemical analyses. Although double labelling immunocytochemistry showed close association of RIP+/MBP+ oligodendrocytes with MAP2+ neurons, TEM analysis showed the complete absence of myelin profiles compared with control cultures of differentiated NPCs in the absence of neurons. Moreover, immunocytochemical staining for the cell surface marker PSA-NCAM showed abundant staining on neuronal processes in co-cultured samples. Previous studies have shown that PSA-NCAM expression by immature, unmyelinated axons is inversely related to the gradient of myelination (Oumesmar et al., 1995; Charles et al., 2000), and that there is an upregulation of PSA-NCAM on neuronal fibres following demyelinating lesions (Charles et al., 2002; Oumesmar et al., 1995). The high levels of PSA-NCAM expression on neuronal fibres in the current study suggests that 1-week old cortical neurons inhibit myelin production by oligodendrocytes differentiated from adult spinal cord NPCs. Future work will examine the distribution of PSA-NCAM on neuronal processes co-cultured for three weeks with spinal cord NPC derived oligodendrocytes, with particular emphasis on the relationship between myelin production by oligodendrocytes and neuronal PSA-NCAM expression. Our current results have important implications for the design of future NPC transplantation trials, since they highlight the need to carefully time the delivery of NPCs.

97 C404

MCL-1 IS REQUIRED FOR NEURONAL SURVIVAL

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Unregulated neuronal cell death has been implicated in the pathogenesis of several neurological disorders and in acute neuronal injury such as stroke. The Bcl-2 family of proteins plays an integral role in the regulation of apoptotic cell death. Mcl-1, an anti-apoptotic member of the Bcl-2 family; is a known survival factor for hematopoietic cells. Little is known, however, regarding its function in the nervous system. The embryonic lethality of germline Mcl-1 knockout mice at E3.5 has hindered detailed examination of its role in the nervous system. To examine the role of Mcl-1 in nervous system development, we used conditional knockouts of Mcl-1 by crossing Mcl-1-floxed mice with mice expressing Cre from the Nestin, Foxg1 or CamKIIα promoter. Deletion of Mcl-1 during nervous system development with either Nestin or Foxg1 Cre resulted in embryonic lethality. Morphological analysis using Cresyl Violet staining revealed severe deterioration of the cortices of both conditional knockouts, demonstrating that Mcl-1 is required for embryonic neuronal survival. Immunohistochemical staining of brain sections with antibodies to active caspase-3 (AC3) revealed numerous apoptotic cells throughout the developing ventricular zone at E12.5 and E15.5. Double staining of AC3 with Nestin or Tuj revealed neurons are dying throughout the process of differentiation. Proliferation was assessed via phosphohistone-H3 staining and found to be comparable in the knockouts and controls, indicating that cell proliferation was not affected. Deletion of Mcl-1 in post mitotic neurons in postnatal mice with CamKIIα Cre also resulted in premature lethality at 1 and 2 months of age. Cresyl Violet staining and NeuN immunohistochemistry revealed a rapid loss of neurons in the cortices of the conditional knockouts. A lack of apoptotic markers in the knockout brains suggested a form of cell death distinct from apoptosis. Electron micrographic imaging revealed double membraned vesicles within the cortical neurons, suggestive of an autophagic form of cell death. Altogether our findings demonstrate that the loss of Mcl-1 in embryonic and post-mitotic neurons results in cell death. The two distinct forms of cell death activated indicate that Mcl-1 functions in multiple pathways to promote neuronal survival. In summary, we demonstrate that Mcl-1 is vital for the survival of neurons. Funded by Heart and Stroke Foundation.

98 C405

SEMAPHORIN 5B IS A REPELLANT CUE FOR THE CORTICOTHALAMIC PROJECTION

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Neuronal connectivity is generated by the precise guidance of neuronal growth cones in response to the spatiotemporal distribution of molecular guidance cues in the developing embryo. Connections between cortex and thalamus are among the longest and most complex projections of the mammalian forebrain, requiring many intermediate targets and guidance decisions. Although some cues are known for their roles, there remains some question as to the full complement of guidance cues necessary for the formation of this tract. We have found that the class 5 semaphorin, Sema5B, is expressed at important histogenic boundaries and regions of the subcortex flanking the internal capsule during corticothalamic axon guidance. Explants of dorsal and lateral cortex grown in culture exhibited a characteristic avoidance behavior and growth cone collapse upon contact with Sema5B expressing cells, suggesting Sema5B may function as a repulsive guidance cue. To further test this, Sema5B expressing cells were transplanted into organotypic slices along the presumptive internal capsule and ganglionic eminences. Ectopic Sema5B was sufficient to cause cortical axons to avoid their normal trajectory, resulting in either stalling at the boundary of Sema5B cells or turning into inappropriate areas of the cortex. We therefore propose that Sema5B may be an important guidance component for establishment of the corticothalamic tract.

99 C406

DEAD BOX PROTEINS ARE COMPONENTS OF DISTINCT POPULATIONS OF RNA GRANULES

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Localized protein synthesis is required for many processes from axon development to learning and memory formation. Transport of the mRNAs to sites of local synthesis is mediated through particles called RNA granules. The components of these granules are involved in mediating transport and regulating the translational competence of the mRNAs. A proteomics analysis of a heterogenous collection of RNA granules isolated from E18 rats has identified many of the proteins involved. Among the proteins identified are members of the DEAD box (DDX) RNA helicase family – including DDX 1, DDX 3, DDX 5 (p68), and DDX 6 (RCK/p54) (Elvira et al., Mol Cell Proteomics, 5(4) 635, 2006). Characterization of these proteins in cultures of early embryonic hippocampal neurons, through both overexpression of fusion proteins and endogenous staining, has revealed a punctate distribution of all four DDX proteins in both axons and dendrites. Localization studies of the overexpressed DDX proteins indicate that DDX 1 and DDX 3 are mostly localized to distinct puncta while DDX 5 and DDX 6 colocalize with DDX 3 more than with DDX 1. Nevertheless, DDX 1 colocalizes strongly with CGI99, a novel protein identified in the proteomics. This is in agreement with the CGI99:DDX 1 interaction observed by Kanai et al. (Neuron, 43(4) 514, 2004). As all the proteins were identified in the purified granules this indicates that there are multiple types of granules with distinct DEAD box protein compositions. Interestingly, DDX1 and DDX3 show a greater colocalization in axons versus dendrites, while there is no change in the colocalization with CGI99 in either case. Further, DDX1:DDX3 puncta in axons are more likely to contain P0 – a marker for large ribosomal subunits – than DDX1 puncta alone. The yeast homolog of DDX 6, Dhh1p, is a marker for processing (P) bodies - RNP particles involved in decapping and degradation of mRNAs. However, other P body components were not identified in the proteomics analysis and they do not show strong colocalization with other RNA granule components. In conclusion, distinct types of RNA granules are involved in the transport and translational regulation of mRNAs in developing neurons.

100 C407

DEVELOPMENTAL EXPRESSION OF A NOVEL PHILANTHOTOXIN-INSENSITIVE CA2+-PERMEABLE AMPA RECEPTOR IN THE MAMMALIAN RETINA

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Ca2+-permeable AMPA receptors (AMPARs) are expressed throughout the adult CNS but yet their role in development is poorly understood. In the developing retina, early Ca2+ oscillations in retinal ganglion cells are later replaced by light-induced activity mediated by NMDA receptors (NMDARs). However, NMDARs are absent from many retinal cells, suggesting that other Ca2+-permeable glutamate receptors may be important to consider. Here, we used cobalt staining to label selectively retinal cells that express Ca2+-permeable AMPARs. In addition, we made electrophysiological recordings of spontaneous synaptic activity in single retinal cells to reveal their functional properties. Specifically, we used philanthotoxin (PhTX), a synthetic polyamine previously shown to block selectively Ca2+-permeable receptors in other brain regions. We found that inhibitory horizontal and AII amacrine cells devoid of NMDARs expressed Ca2+-permeable AMPARs at all stages of development. Prior to eye-opening, Ca2+-permeable AMPARs were fully blocked by PhTX; however, cobalt staining in inhibitory cells persisted in the presence of PhTX after eye-opening. Moreover, Joro spider toxin and IEM-1460 also failed to antagonize, demonstrating that this novel pharmacology is shared by several AMPARs blockers. Electrophysiology confirmed that AII amacrine cells displayed synaptic activity insensitive to PhTX after eye-opening. Taken together, we show that expression of Ca2+-permeable AMPARs is developmentally regulated in the mammalian retina. Surprisingly, Ca2+-permeable AMPARs displayed a phenotype switch in PhTX-sensitivity that coincides with the establishment of inhibitory cell connections. We speculate that PhTX-insensitive Ca2+-permeable AMPARs may be critical for synapse maturation in the mammalian retina.

101 C408

TRK SIGNALING REGULATES NEURAL PRECURSOR CELL PROLIFERATION AND DIFFERENTIATION DURING CORTICAL DEVELOPMENT

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The neurotrophin family of growth factors is known to be essential for CNS neurons, but is not thought to regulate the function of multipotent CNS neural precursors. Here, we have asked whether neurotrophin-mediated Trk signaling is important for embryonic cortical precursor cell development by genetically manipulating cultured cortical precursors, and by performing in utero electroporation with dominant-negative TrkB and TrkC to acutely, and in a cell-autonomous fashion, disrupt Trk signaling in the embryonic cortex. These experiments demonstrated that TrkB and TrkC signaling was essential for the proliferation of embryonic cortical precursors, both in culture and in vivo. Moreover, inhibition of TrkB and TrkC signaling in embryonic cortical precursors in vivo led to a delay in the generation of new neurons, and ultimately perturbed the postnatal localization of cortical neurons. In contrast, TrkB and TrkC receptor signaling was not required for cortical astrocyte formation, at least within the first few days postnatally. Instead, inhibition of Trk signaling in embryonic cortical precursors led to a decrease in the proportion of postnatal cortical precursors that were maintained within the subventricular zone, possibly as a direct consequence of the decrease in embryonic proliferation. Together, these results indicate that TrkB and TrkC activation regulate the proliferation and differentiation of embryonic cortical precursors, suggesting that TrkB and TrkC ligands such as BDNF and NT-3 control cortical development at earlier stages than previously thought.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

102 C409

ADULT NEURAL STEM CELLS ARE COMPETENT TO RESPOND TO ENDOGENOUS FACTORS DERIVED FROM THE EMBRYONIC FOREBRAIN

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The periventricular region of the mammalian brain contains a population of neural stem cells (NSCs) that is maintained into adulthood. Individual AdNSCs and ENSCs proliferate to generate clonally derived colonies of cells termed "neurospheres" in the presence of growth factors. ENSCs and AdNSCs differ in their non-cell autonomous response to cell density in vitro. We asked if the factors responsible for the exponential increase in embryonic neurosphere formation can modify the linear increase in adult neurosphere formation to determine if AdNSCs and ENSCs were inherently different or if their differential responsiveness was due to the lack of permissive or presence of an inhibitory factor. We performed mixing experiments with tissue isolated from AdYFP expressing mice and E14/15 CD1 embryos and found that numbers of Ad neurospheres was significantly increased in primary co-cultures with E cells. Delayed mixing of cells by 2 days did not mimic this increase suggesting the effect was on AdNSC survival. Conditioned media from primary E cultures resulted in the same increase in neurosphere formation from both Ad primary and Ad secondary (passaged) neurospheres. The effect on AdNSCs can be seen in co-cultures of adult and NSCs from animals as early as E12.5 and as late as postnatal day 5 but is lost at later times. Since the effect cannot be mimicked using pure populations of neurosphere derived stem and progenitor cells, we hypothesize that the factor(s) is released from cells present within primary dissected tissue such as neurons or endothelial cells (and not glial cells since they are not present at E12.5). We are testing candidate molecules as well as using conditioned media from pure populations of candidate cells. Taken together, these data suggest that temporally distinct populations of NSCs are not inherently different and that AdNSCs maintain their competence to respond to E tissue derived cell survival factors.

103 C410

A POTENTIAL ROLE FOR THE CYTOSKELETAL LINKER PROTEIN, MOESIN, IN DROSOPHILA NEUROMUSCULAR JUNCTION MORPHOLOGY

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Although it is known that actin plays a role in synaptic development, the precise role of actin and actin-binding proteins remains unknown. Moesin is a member of the ERM family, which link F-actin to the plasma membrane. Moesin is the only ERM homolog present in Drosophila providing a unique opportunity to gain an understanding of the role of ERM proteins in actin dynamics. In the neuromuscular junction (NMJ), overexpression of Moesin was able to rescue the morphology of NSF2-induced NMJ overgrowth. This NSF mutant is known to have reduced synaptic strength and reduced F-actin in the nerve terminal. To further investigate the potential role of Moesin in NMJ development, immunocytochemical quantification of NMJ branch length was completed for gain-of-function and loss-of-function moesin in conjunction with the overgrown phenotype. Loss-of-function moesin enhanced the NMJ overgrowth indicating a likely role for Moesin in normal NMJ development. However, using electrophysiology it was determined that while overexpression of Moesin rescues the morphology, there was not a corresponding rescue of synaptic strength in the NSF2 mutant. Together this implies a role for moesin at the NMJ as well as implying the possibility that there are distinct mechanisms involved with the morphology and physiology of the Drosophila NMJ.

104 C411

NOVEL GENE TARGETS FOR MELATONIN IN A NEURAL STEM CELL LINE

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Several stem cell lines have been utilized to replace cell loss in models of neurodegeneration, including those for Parkinson's disease (PD). However, the assessment of transplanted stem cells has indicated that only 0.13-0.35% of transplanted cells acquire a dopaminergic phenotype in animal models of PD, as determined by tyrosine hydroxylase (TH). Therefore, it is of interest to characterize the factors that are necessary for survival and differentiation of precursor stem cells in order to improve the success rate of stem cell therapy. The pineal gland hormone melatonin has been implicated as having a role in neuronal survival and differentiation. Moreover, chronic treatment with a physiological dose of this lipophilic hormone protects the nigrostriatal pathway in 6-hydroxydopamine-lesioned rats, as reflected by behavioural and immunohistochemical (TH) analyses (Sharma et al., 2006). Previous studies in our laboratory, using the mouse neonate cerebellum-derived C17.2 stem cell line, indicated that these cells express the melatonin MT1 receptor subtype. In addition, melatonin treatment of C17.2 cells significantly induced the mRNA expression of glial cell-line derived neurotrophic factor (GDNF), which is known to play an essential role in dopaminergic cell survival (Niles et al., 2004). Given that melatonin enhances neuronal differentiation and GDNF expression, we hypothesized that it would also modulate the expression of genes that are integral to such differentiation and specifically to the development of a neuronal phenotype. In the current study, cultured C17.2 cells were exposed to physiological doses of melatonin (picomolar to nanomolar range) for 24 hours. RT-PCR studies revealed a significant dose-dependent increase in Nurr1 mRNA expression after melatonin treatment. The orphan nuclear receptor Nurr1 is a transcription factor that has been shown to be pivotal for the development and maintenance of dopaminergic neurons. Furthermore, increased mRNA expression of the early neuronal marker, beta-III-tubulin, was also observed following in vitro treatment. Finally, melatonin treatment modulated the mRNA expression of several histone deacetylases (HDACs). This family of transcriptional repressors is implicated in development and cellular differentiation. These findings suggest that melatonin plays a role in neuronal differentiation to a dopaminergic phenotype, possibly by modulating epigenetic targets in the C17.2 stem cell line.

105 C412

THE ROLE OF LONG-TERM IMMUNE DYSFUNCTION IN ADULT-NEUROGENESIS

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Events occurring during the early neonatal period can have significant long-term consequences on a variety of behavioural and physiological systems. For instance, rodents neonatally exposed to an early immune challenge with the bacterial product lipopolysaccharide (LPS) exhibit increased anxiety-like behaviour, cognitive deficits, greater stress-reactivity, and altered neuroimmune responses in adulthood. This is fascinating in the context that all of the above perturbations are observed in the clinically depressed population. Studies to date, however, have yet to examine depressive-like phenotypes in early immune challenged rodents. Given that deficits in neurogenesis are hypothesized to be a contributing factor in the etiology of depression, the present study examines adult neurogenesis and depressive-like behaviour in early immune challenged mice. Mice were injected with LPS (0.05 mg/kg) on postnatal days three and five. Depressive-like behaviour was assessed using the forced swim test (FST) at twelve weeks of age. Four days after the FST, mice were given pulse injections of BrdU (50mg/kg) in order to assess hippocampal neurogenesis. Preliminary

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

analysis of the FST data has yielded a surprising result: a decreased amount of immobility during the FST in LPS injected mice compared with controls. This finding may reflect an increased level of anxiety-like behaviour as has been reported previously. Immunohistochemical analysis of neurogeneic markers (BrdU & doublecortin) is currently being performed to assess hippocampal neurogenesis in early immune challenged mice. Successful completion of this work will help to reveal the contribution of the early immune environment to long-term alterations in neurogenesis and to the later development of neuropsychiatric disorders.

106 C413

THE P75 NEUROTROPHIN RECEPTOR MEDIATES AXON DEGENERATION TO REGULATE SYMPATHETIC AXON PRUNING

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One strategy used by the developing mammalian nervous system to establish neural circuitry is the overproduction of axons and the subsequent selection of only those axons which successfully compete for target innervation. The elimination of axons is termed “axon pruning”, and occurs in many CNS and PNS neuronal populations. In this study, we have focused on peripheral sympathetic neurons to study axon competition and pruning, where target-derived Nerve Growth Factor (NGF) and neural circuit activity regulate pruning during development. Using an in vitro model of axon competition, our previous study demonstrated that in the presence of NGF, local depolarization conferred a competitive growth advantage to stimulated sympathetic axons, while it disadvantaged the growth of unstimulated axons deriving from the same and neighboring neurons. In addition, we found the growth disadvantage was mediated by BDNF secreted from and acting on the unstimulated axons through p75NTR. In this study, we directly tested whether BDNF or p75NTR plays a role in sympathetic axon pruning in vivo. Using an in vivo model, we found that pruning of p75NTR -/- sympathetic eye-projecting axons was completely inhibited compared to WT controls. In addition, preliminary studies suggest that sympathetic axon pruning is also perturbed in mice lacking the activity dependent promoter element of the BDNF gene, indicating activity dependent BDNF synthesis may be required for pruning. We also utilized compartmented cultures to determine the role of p75NTR in sympathetic axon competition and pruning. Here we found that the growth disadvantage of WT unstimulated axons was completely eliminated in p75NTR -/- unstimulated axons. Furthermore, the growth disadvantage was due to p75NTR-mediated axon degeneration of unstimulated axons. Interestingly, immunocytochemistry revealed BDNF, p75NTR and Ubiquitin were enriched in degenerating beads along individual unstimulated axons, suggesting localized BDNF-p75NTR signaling induces axon degeneration. Finally, our biochemical data demonstrate that p75NTR mediates these effects in part by dampening NGF-induced MAPK signaling, and by activating a Rho-ROCK pathway. Taken together, our data demonstrate BDNF-p75NTR signaling regulates sympathetic axon pruning by inducing axon degeneration.

107 C414

NOVEL BRN3A CIS-ACTING SEQUENCES THAT MEDIATE ACTIVATION OF THE NERVE GROWTH FACTOR RECEPTOR, TRKA, IN NEURONS

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TrkA, the receptor tyrosine kinase for nerve growth factor (NGF), is critical not only for the correct spatial and temporal development of sensory neurons during embryogenesis but also for the survival of sensory neurons, the differentiation and apoptosis of neuronal tumours and suppression of latent herpes simplex virus (HSV) genomes. The transcription factor Brn3a is known to play an important role as an enhancer of TrkA transcription during development. Despite considerable information on the embryonic regulation of TrkA, the mechanisms by which the expression of TrkA is regulated in differentiated neurons, or the factors that influence its expression in tumour cells, have not been identified. By examining a segment of DNA that lies upstream from the coding sequences of human TrkA, we have identified two regions within 190 base pairs of the TrkA reading frame that bind recombinant Brn3a. This segment of the TrkA promoter is also sufficient to initiate transcription in PC12 cells, a model of rat sympathetic neurons, and in medulloblastoma cells. By introducing an active gene for Brn3a into cells we also show that the protein can enhance the expression of the endogenous TrkA. Since the expression of TrkA is required for the differentiation or apoptosis of medulloblastoma cells, our work may lead to strategies for treating this often-fatal childhood tumour.

108 C415

EFFECT OF INDUCIBLE EXPRESSION OF HOXA2 GENE ON THE PROLIFERATION AND DIFFERENTIATION OF THE RAT CG4 OLIGODENDROGLIAL CELLS

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Oligodendrocytes (OLs) are the glial cells responsible for the synthesis and maintenance of myelin in the central nervous system. Recently, Hoxa2 was found by our laboratory to be expressed by OLs and down-regulated at the terminal differentiation stage during oligodendrogenesis in mice (Nicolay et al., 2004). To further investigate the role of Hoxa2 in oligodendroglial development, a tetracycline regulated controllable expression system was utilized to establish two stable cell lines where the expression level of Hoxa2 gene could be up-regulated (CG4-SHoxa2) or down-regulated (CG4-ASHoxa2). Morphologically, no obvious differences were observed between CG4-SHoxa2 and CG4 wild type cells, whereas CG4-ASHoxa2 cells exhibited much shorter processes. Data from BrdU uptake assays indicated that an up-regulation of Hoxa2 gene promotes the proliferation of CG4-SHoxa2 cells. Transcription of PDGFR, a receptor for the mitogen PDGF and which enhances the survival and proliferation of OLs, was assessed to examine whether Hoxa2 promotes CG4-SHoxa2 cell proliferation via increasing the mRNA level of PDGFR. In addition, specific investigations of the differentiation of CG4-SHoxa2 cells were carried out by characterizing the composition of stage specific oligodendroglial subpopulations in culture. Our immunocytochemical study did not indicate the differentiation course of the genetically engineered cells was significantly altered compared to CG4 wild type cells, although results from semi-quantitative RT-PCR of oligodendrocyte-specific ceramide galactosyltransferase (CGT) and myelin basic protein (MBP) provide convincing evidence that the differentiation of CG4-SHoxa2 cells was delayed when Hoxa2 gene was up-regulated. (Supported by CIHR)

109 C416

THE ROLE OF FOXP2 IN CORTICAL DEVELOPMENT

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The FOXP2 gene, which is essential for the normal development of speech and language, encodes a zinc-finger transcription factor that is

expressed in developing neural structures, including newly-born cortical neurons (Ferland et al., JCN, 2003). Recent genetic evidence shows that the FOXP2 gene is associated with susceptibility to autism (Feuk et al., AJHG, 2006). Since disruption of FOXP2 impairs speech and motor functions, inhibits ultrasonic vocalization in mouse pups, and in an as-yet-undefined manner perturbs the early development of at least some CNS neurons (Shu et al., PNAS, 2005), we propose that FOXP2 is essential for the normal genesis and maturation of cortical neurons, and that such cortical perturbations underly the observed behavioral deficit in vocalization. Cortical development is a complex process in which extrinsic and intrinsic factors modulate the sequential generation of neurons and glial cells. The Miller/Kaplan laboratory has developed cultures of embryonic murine cortical precursors that undergo this same sequence of development in culture. Using these cultures, we performed a developmental time-line to determine the expression of FOXP2 in developing cortical precursors and neurons and tested the effects of FOXP2 overexpression and FOXP2 shRNA expression in the newly-born neurons. To determine whether these findings were relevant in vivo, we utilized the same constructs to perturb FOXP2 expression in cortical precursors and/or newly-born cortical neurons in vivo. Specifically, immnohistochemical staining reveals that FOXP2 is expressed in newly-born neurons of the cortical plate and the striatum, which co-express GAD65/67. Therefore, we performed in utero electroporation to tested the effects of ectopic FOXP2 expression in the cortical precursors and FOXP2 shRNA expression in newly-born neurons of the striatum, and then analyzed survival, migration and differentiation of the genetically-manipulated cells at various time points following electroporation.

Understanding of the nature of FOXP2 involvement in cortical development serves as an important bridge between research on molecular genetics, neuronal development and behavior. The question then is whether the differential proliferation of these precursor cells might directly alter the development and function of neuroendocrine systems that regulate behavior in mammals later in life. References: Barnabe-Heider, F. et al., Neuron (2005) 48, 253-265.; Ferland, R.J. et al., J. Comp. Neurol. (2003) 460, 266-279.; Shu, W. et al., Proc. Natl. Acad. Sci. (2005) 102, 9643-9648.; Feuk, L. et al., Am. J. Hum. Genet. (2006) 79, 965-72.

110 C417

A ROLE FOR THE P53 FAMILY MEMBER, P73, IN AGE-RELATED NEURODEGENERATION

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A central question in neurobiology is how terminally differentiated post-mitotic neurons generated during embryonic development survive throughout the duration of an organism's lifetime. Neuronal life versus death is controlled in part by a novel cell survival checkpoint comprised of p53 family members. p53 and p63 work in concert to promote neuronal death, whereas, a shortened, dominant inhibitory form of p73, ΔNp73, mediates survival. ΔNp73, the predominantly expressed isoform in the nervous system, is a key survival factor required for the survival of several developing neuron populations, and vulnerability of adult neurons to injury-induced death. The extent of p73's involvement in ensuring long-term neuronal survival has yet to be explored. Here we investigate the role of p73 in age-related neurodegeneration by examining both behaviorally and anatomically young (3 month) and old (16 month) mice which were lacking a single allele of p73. The behavioral analysis revealed that aged, but not young, p73+/- mice displayed a phenotype characteristic of mice with neurodegeneration; they exhibited a defective limb clasping reflex, decreased grip strength, and reduced anxiety. Moreover, investigation of gross brain anatomy demonstrated that whereas the brains of young p73+/- mice were virtually indistinguishable from their wild-type counterparts, the aged p73+/- mice displayed an obvious cerebral cortical atrophy. This atrophy was due to both decreased cortical thickness and neuronal loss or atrophy. These data demonstrate that p73+/- mice undergo age-dependent neurodegeneration, indicating that p73 is essential in maintaining neuronal survival throughout the lifetime of an organism, and suggest that humans with perturbations in p73 levels may show an enhanced propensity for neurodegeneration. Supported by CIHR, NeuroScience Canada, Postdoctoral Fellowship from CIHR/ Heart and Stroke Foundation of Canada

111 C418

IMPACT OF INTERLEUKIN-1β, TUMOR NECROSIS FACTOR-α, AND INTERLEUKIN-6 ON HIPPOCAMPAL CELLULAR PROLIFERATION

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Accumulating evidence indicates that alterations of hippocampal functioning may influence affective state and cognition. Specifically, disturbances of hippocampal plasticity such as impaired dendritic branching and neurogenesis have been implicated in depressive behavior. Several reports suggest that various signalling messengers, including growth factors and cytokines, might be involved, particularly proinflammatory cytokines which have been found to have potent effects upon several processes linked to depression including both central monoamine and neuropeptide activity. Recently, administration of the immunotherapeutic cytokine, interferon-γ was reported to diminish hippocampal neurogenesis and provoke behavioural signs of depression. Therefore, we assessed whether 3 additional proinflammatory cytokines (which may be altered in depressed individuals) influenced hippocampal cellular proliferation (neurogenesis and gliogenesis). Acute systemic administration of tumor necrosis factor-α (TNF-α) reduced hippocampal cell proliferation and decreased dendritic branching, yet, interleukin-1β (IL-1β) and IL-6 had no significant effects. However, chronic but not acute infusion of IL-6 and IL-1β into the hippocampus increased cellular proliferation but TNF-α had no effect in this respect. Thus, the route and chronicity of cytokine administration had a marked influence upon the nature of the alterations of cellular proliferation. These results are discussed in terms of potential mechanisms of cytokine action and functional implications.

112 C419

ELECTRICAL STIMULATION OF INTACT PERIPHERAL AXONS FROM SENSORY NEURONS IN RATE PROMOTES OUTGROWTH OF THEIR CENTRAL PROJECTIONS

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Electrical nerve stimulation (ES) promotes axonal outgrowth after peripheral nerve injury in contrast to a conditioning lesion (CL) of a nerve that promotes outgrowth and accelerates rate of axonal regeneration following a second injury. A CL also promotes regeneration of the central axon of dorsal root ganglia (DRG) sensory neurons across a spinal cord lesion site. We asked whether ES of the intact sciatic nerve promotes outgrowth of cut central DRG axons. The sciatic nerve of adult rats was stimulated at 20Hz, 7 days before harvest and primary culture of the DRG neurons on a permissive growth substrate. ES increased neurite outgrowth 4-fold as compared to non-stimulated DRG neurons. In vivo, we transected the dorsal columns at T8 in the spinal cord and, the sciatic nerve was either cut (CL), electrically stimulated (ES) for 1h at 20Hz or 200Hz. The control group had Sham sciatic nerve surgery. After 14 weeks, ES at 20Hz but not 200Hz significantly increased axon outgrowth into the lesion site but not axon regeneration as compared to the Sham control. The CL did not affect

axon outgrowth but significantly increased the number and distance of regenerating axons in the lesion site. We only found significant increase in cAMP for the 20Hz but not the 200Hz ES or the CL. We conclude that the effect of ES in promoting axonal outgrowth may be attributed to increased intracellular levels of cAMP in contrast to the effect of the CL of increasing the rate of regeneration that was not (Funded by CIHR to TG and NIH to JS).

113 A101

WILD-TYPE SUPEROXIDE DISMUTASE ACQUIRES BINDING AND TOXIC PROPERTIES OF ALS-LINKED MUTANT FORMS THROUGH OXIDATION

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Recent studies suggest that superoxide dismutase (SOD1) may represent a major target of oxidative damage in neurodegenerative diseases. To test the possibility that oxidized species of wild-type (WT) SOD1 might be involved in pathogenic processes, we analyzed the properties of the wild-type (WT) human SOD1 protein after its oxidation in vivo or in vitro by H2O2 treatment. Using transfected Neuro2a cells expressing WT or ALS-linked SOD1 species, we show that exposure to H2O2 modifies the properties of WT SOD1. Western blot analysis of immunoprecipitates from cell lysates revealed that, like mutant SOD1, oxidized WT SOD1 can be conjugated with poly-ubiquitin and can interact with Hsp70. Chromogranin B, a neurosecretory protein that interacts with mutant SOD1 but not with WT SOD1, was co-immunoprecipitated with oxidized WT SOD1 from lysates of Neuro2a cells treated with H2O2. Treatment of microglial cells (line BV2) with either oxidized WT SOD1 or mutant SOD1 recombinant proteins induced tumour necrosis factor (TNF-α) and inducible nitric oxide synthase (iNOS). Furthermore, exposure of cultured motor neurons to oxidized WT SOD1 caused dose-dependent cell death like mutant SOD1 proteins. These results suggest that WT SOD1 may acquire binding and toxic properties of mutant forms of SOD1 through oxidative damage.

Disorders of the Nervous System

115 A103

ANIMAL STUDIES OF COMORBIDITY BETWEEN MOOD DISORDERS AND COCAINE DEPENDENCE; BEHAVIOURAL CHARACTERISTICS OF AN 'AT RISK' PHENOTYPE

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The purpose of this study was to investigate the comorbidity between mood disorders and cocaine dependence using animal models of behavioral despair and cocaine seeking. One hundred and twenty five rats were tested for individual differences in active struggling in an inescapable stressor (water). The same animals were then tested in operant chambers for responding to: 1) a novel audiovisual cue; 2) the same cue after its association with infusions of cocaine (150 infusions in three sessions, 0.05 mg/kg/inf); and 3) the same cue in tests of reinstatement induced by priming injections of cocaine (15 mg/kg, IP) and exposure to foot-shock stress. It was found that rats with the least amount of struggling showed the highest level of responding for the novel cue, as well as highest levels of cocaine seeking as measured by responding for after conditioning and during both tests of reinstatement. These findings suggest that propensity to behavioral despair is positively associated with novelty seeking and cocaine seeking.
Supported by CIHR, CFI and OIT.

116 A104

GALANTAMINE: A NOVEL NEUROPROTECTANT FOR INJURED RETINAL GANGLION CELLS IN GLAUCOMA

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Glaucoma, the second leading cause of blindness worldwide, is characterized by progressive optic nerve degeneration that leads to irreversible visual field loss. A hallmark of glaucoma is the death of retinal ganglion cells (RGCs). A major risk factor for developing this disease is elevated intraocular pressure (IOP). The current standard therapy for glaucoma is to lower eye pressure by medication and/or surgery. However, a significant proportion of patients continue to experience vision loss in spite of responding well to pressure lowering drugs. Thus, it is clear that current therapeutic strategies for glaucoma are insufficient and new approaches to slow disease progression are urgently needed. Here we investigated the neuroprotective effect of galantamine, a modest acetylcholinesterase inhibitor and an allosteric potentiator of nicotinic receptors, in experimental glaucoma. Galantamine has been approved by Health Canada and the U.S. Food and Drug Administration for the treatment of Alzheimer's disease: it has few side effects, is taken orally by patients and has demonstrated efficacy in clinical trials for Alzheimer's disease. The neuroprotective effect of galantamine was tested in an experimental glaucoma model in which unilateral ocular hypertension was induced by injection of a hypertonic saline solution (1.85 M NaCl) into an episcleral vein. Rats received daily intraperitoneal injections of galantamine (3.5 mg/kg) dissolved in sterile saline solution. Treatment began on the first day of IOP increase and continued thereafter for the entire duration of the experiment. For RGC density quantification, DiI-labeled neurons were counted in 12 standard retinal areas. RGC axons were counted in optic nerve semi-thin cross sections. Counts were performed in duplicate and in a masked fashion. Daily systemic treatment with galantamine resulted in striking protection of RGCs from ocular hypertension damage. In animals with pressure increase (ΔIOP) between 5-10 mmHg, galantamine preserved 90% of RGCs at 5 weeks after ocular hypertension surgery (n=10) compared with 65% of RGCs that survived in controls treated with saline (n=7). Galantamine was also more effective than donepezil (n=8), another acetylcholinesterase inhibitor, or memantine (n=8), an NMDA receptor blocker. Our results also demonstrate that daily administration of galantamine did not reduce IOP. To further confirm that galantamine-induced neuroprotection was independent of changes in eye pressure, we examined its effect following optic nerve axotomy. In this model, intraocular injections of galantamine dramatically increased the survival of RGCs. Collectively, these results demonstrate three important properties of galantamine: i) it promotes robust protection of RGCs in different models of optic nerve damage, ii) it is effective when administered systemically or by intravitreal injection, and iii) its neuroprotective effect is not caused by reduction of IOP. Our data provide the first demonstration of the clinical potential of galantamine as neuroprotective therapy for glaucoma and other optic neuropathies.

117 A105

NEURONAL LOSS FOLLOWING CORTICAL TRAUMA: A POSSIBLE ROLE IN EPILEPTOGENESIS

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The incidence of chronic epilepsy following penetrating cortical wounds is more than 50%, however the mechanisms of post-traumatic epilepsy remain largely unknown. Here we use the model of partially isolated suprasylvian gyrus to test weather a change in the balance between the number of excitatory and inhibitory neurons might explain the increased frequency of seizures observed after head trauma. All animals with chronic partially deafferented cortex developed paroxysmal EEG activities within 1

month after cortical deafferentation. We labeled immunohistochemically all neurons with neuronal-specific nuclear protein antibody (NeuN) and only the inhibitory neurons with gamma-aminobutyric acid antibody (GABA) or its synthesizing enzyme glutamic acid decarboxylase antibody (GAD 65 and 67). The number of labeled neurons was quantified in sham animals and after chronic (2, 4 and 6 weeks) cortical deafferentation, in suprasylvian and marginal gyri, both ipsi- and contra-lateral to cortical trauma. In all animals the neuronal loss was circumscribed to the deafferented suprasylvian gyrus. The total number of neurons in the undercut was reduced by 3.81% at 2 weeks, 15.4% at 4 weeks and 17.4% at 6 weeks compared to sham animals. Nevertheless, there was no significant change in the number of neurons from the contra-lateral suprasylvian gyrus, nor from the marginal gyrus ipsi- and contra-lateral to the traumatized cortex. The number of GABA-labeled inhibitory neurons was diminished by 18.5% at 2 weeks, 23.92% at 4 weeks and 56.34% at 6 weeks. Similarly, GAD-labeled neurons decreased with 26.25% at 2 weeks, 35.87% at 4 weeks and 57.05% at 6 weeks after deafferentation. Furthermore, the ratio between excitatory and inhibitory neurons increased from 5.01 at 2 weeks following trauma to 10.02 at 4 weeks and 12.23 at 6 weeks respectively. The preferential loss of inhibitory neurons might explain the high rate of chronic epilepsy observed in patients with head trauma. Moreover, the global neuronal loss (both excitatory and inhibitory neurons) may account for the frequent cognitive impairments of children who develop epilepsy early in life. Supported by CIHR and NSERC

118 A106

ASSESSING THE SUBJECTIVE VISUAL VERTICAL AND THE PERCEPTUAL UPRIGHT IN PARKINSON'S DISEASE

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Previous reports have suggested that patients with Parkinson's disease have increased visual dominance for spatial perception tasks. We therefore assessed the relative roles of visual and non-visual cues in determining the subjective visual vertical (SVV) and the perceptual upright (PU) in patients with Parkinson's disease using a luminous line and the newly developed OCHART test (Dyde et al., 2006; Exp. Brain. Res. 173; 612). We tested the hypothesis that patients with Parkinson's disease rely more on visual cues than non-visual cues for spatial perception. Visual cues for both the SVV and PU were manipulated using a grey background, a static frame tilted +/- 18º, and a static visually enriched polarized room tilted 18º, 112.5º, 247.5º, and 342º clockwise (all orientations are relative to the observer). To measure the relative contributions of visual, vestibular and body orientation cues, patients were tested while seated upright and while lying on their right side. Parkinson's patients were tested while on and off of their prescribed dopaminergic medications and compared to an age-matched control group. No differences were found attributable to medication. Patients with Parkinson's disease were found to have significantly higher variance for both the SVV and PU tasks compared to the aged-matched control group. We confirmed that when upright, patients with Parkinson's disease are more influenced by visual cues than aged-matched controls for the SVV task. However, when tested lying on their sides, the control group was found to be more influenced by visual cues for the SVV task. When both body orientations were taken together patients with Parkinson's disease were found to be more influenced by body orientation than visual cues for the SVV task. The PU in patients with Parkinson's disease was found to be less influenced by visual cues than controls. We conclude that across different body postures, patients with Parkinson's disease are more influenced by the orientation of their body than visual cues in spatial perception tasks.

119 A107

WALLERIAN DEGENERATION INDUCED MICROGLIAL REACTIVITY GENERATES A MIGRATORY SIGNAL(S) DRIVING THE MIGRATION OF OLFACTORY ENSHEATHING CELLS (OECS)

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Olfactory ensheathing cells (OECs) are glial cells that are attractive candidates for neural repair after spinal cord injury. We determined whether OECs were induced to migrate in response to glial reactivity arising as a result of Wallerian degeneration in the right dorsal corticospinal tract (dCST; left sensorimotor cortex injury) and compared their migratory path with respect to the location of reactive microglia (i.e. inside vs outside the right dCST). Groups 1 and 2 rats (n=4 for each) each received left sensorimotor cortex lesions, whereas Groups 3 and 4 rats (n=4 for each) served as unlesioned controls. DiI-labelled OECs were injected into the right dorsal funiculus of T12 in all 4 Groups, with the timing of this injection in Groups 1 and 2 being 8 weeks after the cortical lesion. Rats received daily i.p. injections of either minocycline (Groups 1 and 3) or vehicle (Groups 2 and 4) beginning 2 weeks prior to and ending 4 weeks after the OEC grafts. All rats were killed 8 weeks after cell grafting and cell counts of DiI+ve OECs were computed separately for the right and left dCSTs and dorsal funiculus of T11. The glial reactivity was confined to the right dCST and was present only in Groups 1 and 2; in Group 1 rats minocycline diminished the Wallerian-degeneration induced microglial response. In comparison to vehicle treated unlesioned control rats (i.e. Group 4), there was a significantly higher density of DiI+ve OECs only for the right dCST of Group 2 (i.e. cortical lesion and vehicle treated) rats; thus, the migratory path was only where microglia were reactive. The density of DiI+ve OECs in the right dCST was significantly lower in Group 1 rats (i.e. minocycline treated) compared to Group 2 rats (i.e. vehicle treated); thus, dampening the microglial response also reduced the migratory signal(s). Funded by a grant from the MS Society of Canada to RD and by a scholarship from Ministry of Health and Medical Education, Iran to MB

120 A108

TEA CATECHINS PROTECT HIPPOCAMPAL NEURONS AGAINST BETA-AMYLOID-INDUCED TOXICITY

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Accumulating evidence suggests that consumption of polyphenols derived from beverages, fruits and vegetables have a beneficial impact in reducing the incidence of dementia. Considering the deleterious role of beta-amyloid (Aß) in the etiology of Alzheimer's disease (AD), we investigated green and black tea extracts and their ingredients against toxicity induced by Aß-derived peptides using primary rat hippocampal cell cultures. Both green and black tea extracts when you say extracts, how can you write (5-25 µg/ml) displayed neuroprotective action against Aß toxicity. These effects were shared by gallic acid and catechin gallate esters known as epicatechin gallate (ECG; 1-20 µM) and epigallocatechin gallate (EGCG; 1-10 µM), the former being the most potent catechin. In contrast, epicatechin and epigallocatechin were ineffective in the same range of concentrations. Moreover, EGCG, and to a lesser extent ECG and gallic acid, inhibited Aß fibrils/oligomers formation at the same concentration, they protected cells. Taken together, these results indicate that the catechin gallates (through the galloyl moiety) contribute to the neuroprotective effects of both green and black teas. Moreover, these results suggest that protective effect of EGCG is likely due, at least in part, to its inhibitory action on Aß fibrils/oligomers formation. These data also support the hypothesis that both green and black teas may prevent neurological disorders in which Aß formation likely plays a deleterious role. Supported by CIHR.

121 A109

LOSS OF HETEROZYGOSITY ON 22 CHROMOSOME IN SPORADIC SCHWANNOMA AND ITS CORRELATION TO THE PROLIFERATION OF TUMOR CELL

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Most of studies of neurofibromatosis type 2 (NF2) gene were studied in the vestibular schwannomas. It is unknown whether there are different genetic alterations between vestibular schwannoma and non-vestibular schwannoma or not. Therefore, We analyze the loss of heterozygosity (LOH) on chromosome 22 (CHR 22) in patients harboring sporadic schwannomas (including vestibular and spinal schwannomas) and correlate this genetic alteration with the tumor's proliferation. Twenty three schwannomas (42.6%) showed allele loss. The frequency of LOH in vestibualr schwannomas was significantly higher than corresponding value in spinal schwannomas ($p<0.01$). The proliferative index of schwannoma with LOH was significantly higher than those without LOH ($p<0.05$). Our findings suggest that CHR 22 LOH is the frequent event in the tumorigenesis of sporadic schwannoma. And there is the correlation between tumor harboring CHR22 LOH and its proliferative activity. The frequency of LOH in vestibular schwannoma is different from it in the spinal shcwannomas.

122 A110

AMPHETAMINE-INDUCED DOPAMINE RELEASE IN PARKINSON'S DISEASE DEPRESSION MEASURED USING HIGH-RESOLUTION POSITRON EMISSION TOMOGRAPHY WITH [11C] RACLOPRIDE

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Depression affects around 40% of patients with Parkinson's disease (PD), which is nearly double the amount of severe depression seen in comparably disabled patients with other chronic illness. Mesocorticolimbic dopamine (DA) depletion has been implicated in the pathogenesis of depression (Remy et al. 2005), and thus may contribute to the high incidence of depression in PD. Amphetamine (AMPH)-induced striatal DA release was compared between depressed (n=1) and non-depressed (n=4) patients with PD using positron emission tomography (PET). DA release was estimated by displacement of the D2/D3 receptor radiotracer [11C]raclopride (RAC). Subjects completed three scans over two days within a three month period. The first scan was a baseline scan, followed by either blinded d-AMPH (0.3 mg/kg p.o.) or placebo administration, counterbalanced across subjects. Severity of depression was assessed using the Montgomry Asperg Depression Rating Scale. The subjective response to AMPH or placebo was measured using an Amphetamine Interview Rating Scale after the PET scans. Following the bolus injection of ~350 MBq of RAC for each scan, emission data were acquired for 60 minutes on an ultra high resolution research tomograph (HRRT, Siemens) and scans were reconstructed using Ordinary Poisson-OSEM including attenuation, scatter and random correction. To correct for motion during the scans inter-frame realignment was performed using automated image registration. Circular and elliptical regions of interest of interest were placed on 9 transaxial slices for the dorsal striatum (total thickness 10.89 mm), 6 coronal slices for the ventral striatum (total thickness 7.26 mm) and 6 transaxial slices for the cerebellum. RAC binding potentials (BP) were estimated using a graphical tissue approach (Logan et al. 1996) with the cerebellum as a reference region. In non-depressed PD subjects, preliminary analysis indicates AMPH-induced DA release compared to baseline in the dorsal (caudate and putamen) and ventral striatum, with a significant effect in the latter ($p<0.03$). AMPH-induced DA release was also observed in the depressed PD subjects. Placebo-induced DA release was observed in all PD subjects, in particular the putamen of the depressed subject. As well, preliminary results suggest enhanced AMPH-induced DA release in the dorsal striatum of the depressed patient compared to non-depressed PD patients. Ongoing work will attempt to extend these findings to a larger sample.

123 A111

EFFECTS OF DIETARY OMEGA-3 POLYUNSATURATED FATTY ACID ON TOXIN-INDUCED NEURONAL DEGENERATION IN PARKINSON'S DISEASE ANIMAL MODELS

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Parkinson's disease is a neurodegenerative disorder characterized by the massive death of dopaminergic nigrostriatal neurons. The resulting diminution of striatal dopamine affects voluntary movements and causes symtoms such as resting tremor, akinesia, rigidity and postural instability. Accumulating evidences suggest that the fatty acid we eat may have a direct impact on brain health. Neuroprotective action of long chain omega-3 polyunsaturated fatty acid (n3PUFA) such as docosahexaenoic acid has been demonstrated in animal models of Alzheimer's disease and is supported by epidemiological data. We hypothesized that n3PUFA may exert neuro-protective action in Parkinson's desease as well. To test this hypothesis, we exposed mice to either low or high intake of n3PUFA before acute MPTP treatment, which included seven injections of MPTP (20 mg/kg) in five days. The duration of pre-treatment exposure to differential n3PUFA diets was of 8 months. Post-mortem analyses in the brain of mice exposed to MPTP did not show a significant effect of dietary n3PUFA on tyrosine hydroxylase (TH) striatal fibers, TH positive nigral cells and levels of Nurr1 mRNA, as assessed by in situ hybridization. However, HPLC (electrochemical detection) analyses showed that mice fed with a high n3PUFA diet were partially protected from MPTP-induced decrease in striatal dopamine and dihydroxyphenylacetic acid (DOPAC) compared with mice fed with low n3PUFA diet. These data suggest that higher n3PUFA dietary intake might exert a neuroprotective action in an animal model of parkinsonism. Since the prevalent low consumption of n3PUFA might be an important modifiable risk factor of Parkinson's disease, our data call for further investigation on the neuroprotective effect of n3PUFA in neurodegenerative diseases.

124 A112

NEUROANATOMICAL DEFICITS FOLLOWING MODERATE PRENATAL ETHANOL EXPOSURE IN THE NON-HUMAN PRIMATE

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Fetal alcohol syndrome (FAS) is a consequence of heavy maternal drinking during pregnancy and results in craniofacial dysmorphology along with an array of neurobehavioral deficits. The much more prevalent spectrum disorder (FASD), a consequence of moderate maternal ethanol consumption, also causes neurobehavioral deficits. However, despite its clinical prevalence, little is known about moderate exposure on the cortical neuronal

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

population. A small number of imaging studies of FAS/D patients suggest a reduced cranial vault volume, however these techniques lack the ability to detect cellular loss. Using a naturalistic non-human primate model of FASD, vervet monkeys (Chlorocebus aethiops sabeus) were exposed to moderate levels of ethanol during the period of rapid synaptogenesis. During the third trimester of gestation, socially-housed female vervet monkeys voluntarily drank an average of 2.22 ± 0.36 g/kg ethanol/day resulting in an average blood alcohol content of 0.01 ± 0.0016 g/dL (21.7 ± 3.5 mM), or isocaloric sucrose (control) four times a week. None of the offspring showed craniofacial dysmorphology and were neuroanatomically examined at 2 years of age. The left hemisphere along with the cerebellum were serially sectioned and stained with cresyl violet. Stereology was used to determine vulnerable brain regions. The cerebellum, cerebral cortex and hippocampus were identified as particularly vulnerable brain regions with the cerebral cortex and hippocampus showing the greatest neuronal loss. Subjects exposed to prenatal ethanol had a 30% neuronal deficit in the cerebral cortex and a 67% neuronal loss in the hippocampus. This is the first study to provide a detailed neuroanatomical analysis of the non-human primate following prenatal ethanol exposure and not only corroborates volume loss seen in FASD patients but suggest that this loss is caused by extensive neuronal loss. The model described here provides a tool for prompt and quantitative evaluation of intervention strategies and will facilitate safe and timely translation to the clinical setting.

125 A113

HIGH-DOSE METHADONE MAINTENANCE ATTENUATES COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN THE RAT

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The purpose of this study was to investigate whether high-dose methadone maintenance (HDMM) could reverse an effect of chronic cocaine exposure associated with its compulsive use, namely: behavioral sensitization. To this end, rats received one test of basal sensitivity to cocaine-induced stimulation of locomotion (15 mg/kg, IP). They then received 3 injections per day of cocaine (15 mg/kg each, IP) for 14 days. Following this period, they were implanted (SC) with osmotic mini-pumps releasing 30 mg/kg/day methadone for 14 days. Ten days following removal of the pumps, the animals received a second test of cocaine-stimulated locomotion (15 mg/kg, IP). It was found that cocaine exposure induced robust behavioral sensitization as indexed by the emergence of intense stereotypy. More importantly, this effect was significantly reduced by HDMM which, by itself, induced no cross-sensitization.

126 A114

SEX DIFFERENCES IN CAPSAICIN-INDUCED PLASMA EXTRAVASATION IN RAT FACIAL SKIN

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Capsaicin stimulates pain receptors and causes an inflammatory response characterized by vasodilation and plasma extravasation (PE). Since there are many reports of sex differences in pain, we asked whether there are sex differences in the inflammatory response evoked by capsaicin. We compared the amount of PE in the facial (V1, caudal to eye) region of male and female rats caused by subcutaneous injection of capsaicin (1%, 20uL). PE was quantified using a video camera and digital image analysis to measure changes in reflectance (pixel intensity, PI) of rat skin due to accumulation of extravasated Evans Blue (EB) dye. Capsaicin-induced PE was significantly greater in female rats in estrus (85.7 +/- 5,PI) compared to males (60.2 +/- 4.5, PI) and non-estrus females (52.1 +/- 4.8, PI) (ANOVA, Post-hoc Tukey, $P < 0.001$, $n = 6$). There was no difference in PE between non-estrus females and males. The time course of PE evoked by capsaicin also differed between males and females. The time to reach maximum PE was significantly longer in estrus females (35 minutes) compared to non-estrus females (29 minutes) and males (7 minutes). This study has revealed that PE in the facial skin is significantly greater in females in the estrus stage of their cycle compared to non-estrus females and males. We propose that differences in the inflammatory response evoked by capsaicin may be due to changes in the level of neuropeptide expression and primary afferent sensitivity caused by the surge in estrogen that occurs prior to estrus. These findings may help to explain why females are over represented in several pathological conditions associated with the trigeminal territory, such as migraine, trigeminal neuralgia and temporomandibular joint disorder.

127 A115

DETERMINATION OF THE ROLE OF SAP97 IN MOTOR ABNORMALITIES IN 6-HYDROXYDOPAMINE-LESIONED RAT MODEL OF PARKINSON'S DISEASE

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In Parkinson's disease (PD), loss of striatal dopamine causes dysfunction of multiple neurotransmitter receptor signaling pathways, in particular glutamate. This is reflected by alterations in expression levels of receptors at the synaptic membrane, such as dopamine and glutamate, which plays a role in the generation of symptoms of PD. Within the postsynaptic density (PSD), synaptic proteins play a crucial role in trafficking, anchoring and downstream signaling of receptors. Synapse associated protein 97 (SAP97), a member of MAGUK family of scaffolding proteins, is particularly well known for its role in receptor trafficking, and appropriate targeting of receptors to the synaptic membrane. Interestingly, studies have shown that, in unilaterally-lesioned 6-OHDA rats, SAP97 is dramatically decreased at striatal synapses and redistributed into the vesicular fraction (Nash et al., 2005). A similar study showed that striatal SAP97 was reduced in a PSD-enriched fraction in 6-OHDA-lesioned rats (Gardoni et al., 2006). Therefore, we hypothesized that changes in the levels of SAP97 in striatum are part of the molecular mechanism leading to the motor symptoms of PD. Thus, the effects of striatal over-expression of SAP97 on motor abnormalities displayed by 6-OHDA-lesioned parkinsonian rats were tested. The N-terminus of SAP97 appears to be necessary for trafficking and targeting of glutamate receptors to the synaptic membrane. Given the importance of N-terminal domain of SAP97 for trafficking, its involvement in parkinsonism was assessed. SAP97 mutants with N-terminal deletions of amino acids 1-65 or 66-125 were used. Adenovirus vectors encoding either, GFP (control); SAP97-GFP (wild type); SAP97Δ1-65-GFP (minus 1-65 a.a. of the N-terminal domain) or SAP97Δ66-125-GFP (minus 66-125 a.a.) were injected into the striatum of the operated side of unilaterally-lesioned 6-OHDA rats and sham-operated animals. Twenty one days post 6-OHDA lesion, forelimb asymmetry was measured. This was then repeated following intraperitoneal injection of L-DOPA methyl-ester. 6-OHDA lesions caused a 4-fold increase in limb-use assymmetry (ANOVA, $F(7,86) = 8.63$, $P<0.001$). Over-expression of wild type SAP97 and mutant constructs alone did not restore limb-use asymmetry in 6-OHDA lesioned rats. However, following L-DOPA methyl-ester administration, limb-use asymmetry was corrected in 6-OHDA-lesioned rats over-expressing wt SAP97-GFP and SAP97Δ1-65-GFP but not SAP97Δ66-125-GFP. Thus, SAP97 over-expression potentiated the anti-parkinsonian effect of L-DOPA. Furthermore, 66-125 a.a. region of N-terminal of SAP97 was found to be important for this function. These results suggest that changes in levels of SAP97 in the parkinsonian striatum may affect dopamine receptor signaling. Biochemical studies are currently underway to measure the sub-cellular distribution of SAP97 and dopamine receptors, which may be responsible for the observed behavioral changes.

128 A116

LIVE IMAGING OF ASTROGLIOSIS IN CEREBRAL ISCHEMIA REVEALS NEURONAL DAMAGE IN MALE BUT NOT IN FEMALE

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Reactive astrogliosis is one of the key components of the cellular responses to brain injuries and the passage from the quiescent to reactive astrocytes is associated with strong up-regulation of the intermediate filament, glial fibrillary acidic protein (GFAP).

The main objective of this study was to develop model-system for live analysis of brain inflammatory response in ischemic injury. Methods: Using a reporter mouse expressing luciferase gene under the transcriptional control of murine GFAP promoter (GFAP-luc mice, Xenogen) and biophotonic/ bioluminescent imaging as tools we developed a model-system for in vivo analysis of astrocyte activation/response in cerebral ischemia. Unilateral transient focal cerebral ischemia was induced by intraluminal filament occlusion of the left MCA during 1 hour followed by imaging session with a high resolution/high sensitivity CCD camera at 24 hours, 72 hours, 5 and 7 days after surgery. Results: The analysis of photon emissions from the brains of living animals revealed that the intensities of GFAP signals after stroke were significantly higher in female compared to male transgenic mice (1.652x107±0.19 p/s vs 0.747x107±0.14 p/s, $p<0.001$). This effect was abolished by ovariectomy. Similar results were obtained by quantitative immunohistochemistry (males,13.4±0.5 vs 16.96±0.64 $p<0.0001$,females). A three-dimensional analysis confirmed that GFAP signals followed the pattern of GFAP immunoreactivity and were emitted from the brain areas surrounding the ischemic lesions. Further, contrary to the positive correlation between the intensities of GFAP signals and the size of the infarction in male mice, there was no correlation between signal intensities/GFAP induction and the size of ischemic lesion in female GFAP-luc mice. Interpretation: The real-time imaging data revealed remarkable gender difference in the astrocyte response to ischemic injury. Our results suggest that GFAP up-regulation induced by ischemic injury may have different functional significance in female and male experimental animals and may not directly reflect the extent of ischemia-induced neuronal damage in female GFAP-luc mice. Using novel live imaging approach we demonstrated that early-phase brain inflammatory response to ischemia may be associated with gender-related brain injury markers.

129 A117

METHYLATION ANALYSIS OF THE 5-HT2A RECEPTOR GENE 102C ALLELE BASED ON DIFFERENTIAL EXPRESSION AND PARENT-OF-ORIGIN EFFECT IN SUICIDAL BEHAVIOR

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The serotonin 2A (5-HT2A) receptor gene has been implicated in the pathogenesis of suicidal behaviour by a genetic association between the 5-HT2A T102C silent polymorphism and suicidality in patients with major depression. However, a recent meta-analysis failed to confirm this association. We developed an improved quantitative assay for the measurement of allele-specific expression of the 5-HT2A gene, and find that the ratio of C/T allele expression in the pre-frontal cortex of heterozygous suicide victims (n=10) was significantly decreased in comparison with the non-suicide group (n=10) ($p=0.049$). Because the 5-HT2A gene is subject to imprinting, the parent-of-origin may affect the inheritance of suicidal behaviour. Thus we examined the parental origin of specific alleles for genetic association in a genetic family-based sample of major psychoses in which information on suicidal behaviour was available. This result suggests the methylation quantification of the 102C allele in suicide and non-suicide groups.

130 A118

CHANGES IN LOCAL FILED POTENTIALS IN THE CEREBELLAR THALAMUS AND SUBTHALAMIC NUCLEUS EVOKED BY STIMULATION OF THE CONTRALATERAL NUCLEI

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We recorded the evoked potentials of thalamus and sub-thalamic nucleus (STN) regions by stimulating their contralateral counterpart. Two patients participated in this study. One patient had bilateral deep brain stimulation (DBS) electrodes implanted in the ventro-intermediate nucleus (Vim) of the thalamus for treatment of essential tremor. The other patient had bilateral DBS electrodes implanted in the sub-thalamic nucleus for treatment of Parkinson's disease. Both patients were studied 2 to 5 days after the implantation of the DBS electrodes, when the leads were still externally exposed. Local field potentials were recorded from all four contacts of the DBS electrodes while stimulation was applied to the contralateral DBS electrode at different frequencies, intensities and contact combinations. Results: Right Vim stimulation elicited potentials in the left Vim electrodes with peak latencies of ~12 ms (contact 2+), ~30 ms (contact 2-) and 70 ms (contact 0-). Latencies and polarities were similar with stimulation of different contacts in the left Vim. The evoked potentials in the frequency domain can be described as the activity of two cosine oscillators, one of them starts at phase zero and oscillates 3.5 cycles and the other starts at phase Pi and oscillates at 2.5 cycles until 150 milliseconds post stimulus. Stimulation of the left STN elicited a positive potential in the right STN at contact 0 with a latency of about 65 ms. These STN potentials were most prominent with stimulation of the lowest contact (0) and diminished with stimulation of higher contacts.

131 A119

AMYGDALA RESPONSE TO BACKWARD MASKED EMOTION IN AUTISM

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Subconscious presentation of negative face stimuli to typically developed adults increases neural activity in the amygdala (Morris et al., 1998). However, Hall et al. (in press) have found that the social decisions of children with autism are less influenced by subconscious presentation of negative face stimuli, than controls. As such, it is suggested that information normally available at the amygdala through subcortical routes is reduced in autism. The purpose of this study was to examine amygdala activation induced by subconscious presentation of negative face stimuli in high functioning adult males with autism. Ten high functioning males with autism and matched male controls participated. All participants gave informed consent, were right handed and had no known neurological or psychiatric disorders, drug or alcohol abuse or history of head trauma. Stimuli were presented to the participants in the MRI scanner via an overhead visor, with responses made via a hand-held response pad. Functional BOLD imaging was done using an interleaved echo-planar imaging sequence with TR=2700ms, TE=35ms. Sixty-four neutral (32 male and 32 female) and 64 fearful face stimuli were used in an event related design. Participants were presented a neutral face (mask) and asked to identify whether the face was male or female. The presentation of neutral face was interupted at fixed intervals by two subthreshold (33 msec) presentations of a fearful face. Each trial was 2700 ms in duration, and was followed by a fixation stimulus. The intertrial presentation was jittered, with delays between 2700 ms and 8100 ms (average 5400 ms). Data analysis is underway, however, preliminary results

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

identify that in controls backward masking produced amygdala activation. In contrast, at the same threshold level, activation of visual association areas and no activation of the amygdala is seen in the data of a subject with autism.

132 A120

THE ROLE OF BRAIN INFLAMMATION IN A NEONATAL VENTRAL HIPPOCAMPUS-LESIONED ANIMAL MODEL OF SCHIZOPHRENIA

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It has been hypothesized that a viral infection during the second semester of pregnancy may alter or trigger improper brain development of the fetus. Abnormal immune functions are also among the mechanisms postulated to play a role in the pathophysiology of schizophrenia, as indicated by increased serum concentrations of interleukin-6 (IL), IL-6 receptor, IL-IR antagonist and IL-2R in schizophrenic patients. Here we investigate the contribution of the inflammatory response to this pathology using a rat model generated by ibotenic acid lesions. This model, for which the lesion is performed at post-natal day (p)7, produces an irreversible disconnection of the ventral hippocampus, which replicates a schizophrenic-like phenotype in adulthood, as assessed by amphetamine-induced behavioral analyses. The pathological and behavioral analyses are more specifically performed at two time points, p35 (corresponding to pre-puberty) and p56 (corresponding to adulthood). Preliminary results reveal a pronounced microglial activation at p17 (10 days post-lesion), as detected by the presence of several microglial cells (Iba-1 staining) and macrophages (ED-1 marker) at the site of lesion. Evidence of an inflammatory response are thus present several days before the development (p56) of behaviors corresponding to schizophrenia. Although these results are very preliminary, they suggest that an inflammatory response may be preceding and contributing to schizophrenic signs which appear during or post-adolescence. Current analyses include a time course of inflammatory events within the first 24 h after the ibotenic acid lesions. Inflammatory molecules such as Il-1, TNF-, IL-6 and Cox-2 are being analyzed in this model. Further studies include the anti-inflammatory treatment of these animals in an attempt to prevent the development of the schizophrenic phenotype.

133 A121

THE REPEATED LOW-DOSE PILOCARPINE MODEL OF STATUS EPILEPTICUS ALTERS COGNITIVE AND BEHAVIOURAL FUNCTIONING IN ADULT RATS

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Status epilepticus (SE) is defined as continuous seizure activity that lasts for at least 30 minutes. The pilocarpine model of epilepsy is widely used experimentally to investigate SE and secondary generalized seizures that evolve following a latent period (Turski et al., 1989. Synapse, 3, 154-171). Repeated low-dose administration of pilocarpine (10 mg/kg) in lithium chloride pretreated Wistar rats results in low mortality rates, while producing SE and chronic epilepsy in a high proportion of the rats (Glein et al., 2001. Epilepsy Res, 46, 111-119). We investigated the extent of cognitive and behavioural deficits, assessed through performance in the Morris water maze (MWM) and elevated plus maze, in chronically epileptic rats at 2 months following SE induction using the repeated low-dose (RLD) procedure. Approximately 60% of Wistar rats developed SE using the RLD procedure, with a low mortality rate of 18%. During MWM training, Wistar rats received 6 trials per day, for a total of 14 days to locate a submerged platform. Naïve animals and animals that received up to 6 low doses of pilocarpine without developing SE (post-pilo) learned the task (6 sec latency) within 6 days. In contrast, reference learning was grossly impaired in SE rats, with only modest improvement occurring over repeated days (32 sec latency). Reversal learning involved relocating the platform to the opposite quadrant of the pool. Naïve and post-pilo animal groups learned the task within a single day, whereas SE animals failed to show any acquisition within 5 days. The elevated plus maze is a validated test for investigating anxiety and exploratory behaviour. The naïve and post-pilo animal groups spent approximately 86% of the time shielded in the closed arm, and exhibited frequent exploratory behaviour (rearing, lookouts). In contrast, the SE animal group spent approximately 50% of the time in the closed arm, and showed virtually no exploratory behaviour. The present data demonstrates that the RLD procedure for inducing status epilepticus results in impaired cognitive function and altered anxiety and exploratory behaviour. Behavioural performance is currently being correlated with the severity of neuro-degeneration in specific brain regions. Research supported by NSERC.

134 A122

SURGICAL PATHOLOGIC FINDINGS IN THE INTRACTABLE TEMPORAL LOBE EPILEPSY

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Background and Purpose: Hippocampal sclerosis is often associated with dysplasia in the temporal neocortex from patients with intractable epilepsy. The aim of this study was identify neuropathological features in neocortex from temporal lobe epileptic patients. Materials and Methods: The study included resected specimens from 17 patients with intractable temporal lobe epilepsy. All tissues were fixed in 10% buffered formalin, embedded in paraffin, cut at 6 m thick and stained with hematoxilyn – eosin and Klüver-Barrera. In selected cases immunohistochemical reaction (GFAP and synaptophysin) was performed. Results: The studied group consisted of 10 females and 7 males, mean age of 37.7 years (range, 22 to 52 years). All patients had mesial temporal sclerosis on magnetic resonance imaging. Dual pathology was identified in 12 patients (70.5%). In 11 patients (64.7%) focal cortical dysplasia was identified according to Palmini classification. The most common patterns of dysplasia included: isolated architectural abnormalities (type IA) in 6 cases, giant neurons (type IB) in 4 cases and dysmorphic neurons (type IIA) in one case. White matter neuronal heterotopia was observed in 11 patients (64.7%). Diffuse accumulation of corpora amylacea were demonstrated in 3 patients. One case of aracnoid cyst was observed. The neuropathological features were regarded as non-specific in 29.4% of cases. Conclusions: The medial temporal lobe epilepsy is associated with neuropathological changes in the neocortex. Dual pathology was a common finding in our patients with temporal lobe resection.

135 A123

REDUCED PREFRONTAL CORTEX DARPP-32MRNA IN COMPLETED SUICIDE VICTIMS WITH SCHIZOPHRENIA

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Dopamine-and-cAMP-regulated neuronal phosphoprotein (32 kDaltons) (DARPP-32), encoded by PPP1R1B, is expressed in brain regions receiving dopaminergic projections, including the prefrontal cortex, and is implicated in the pathophysiology of schizophrenia and other neuropsychiatric disorders. As a key regulator of kinase-phosphatase signaling cascades related to dopaminergic, glutamatergic and serotonergic neurotransmission, DARPP-32 also plays a role in regulating the state of phosphorylation and

activity of phosphoproteins such as voltage-dependent Ca+ and Na+ channels, Na+/K+-ATPase, and other neurotransmitter receptors in the brain. The broad functional capacity of DARPP-32 has potential relevance to both psychotic and negative symptoms of schizophrenia. We performed RT-PCR to quantify DARPP-32 mRNA using fluorescent sequence-specific primers on brain samples donated by the Stanley Medical Research Institute (array collection). These RNA samples, from Brodmann Area 46 in the prefrontal cortex, included 35 brains each from unaffected controls (NC), patients with schizophrenia (SCZ), or bipolar disorder (BP). Relative mRNA expression was calculated from a standard curve and in relation to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). We found a significant difference in gene expression levels between SCZ patients that died by suicide (S-SCZ) vs. other causes of death (SCZ-NS) ($p<0.004$), as well as between S-SCZ and NC ($P<0.04$). DARPP-32 mRNA expression differences between S-SCZ and SCZ-NS suggests a potential molecular explanation for suicidal behaviour in this population. Replication and further work is required to understand these preliminary results.

136 A124

PROTEOMIC ANALYSIS OF NITROTYROSINE FORMATION FOLLOWING STROKE REVEALS A NEUROPROTECTIVE RESPONSE AT THE SYNAPSE F140

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Neuronal damage following stroke involves the simultaneous overproduction of nitric oxide and superoxide leading to formation of peroxynitrite. Peroxynitrite results in nitration of tyrosine residues. We hypothesized that small amounts of peroxynitrite will protect neurons from oxygen/glucose deprivation and translates to a neuroprotective role in vivo. To determine the timeline of nitrotyrosine (NT) formation following stroke, we measured its levels in tissue homogenates from ipsi- and contralateral hemispheres of animals subjected to stroke at various times following permanent focal ischemia. Here we report that NT expression precedes infarct development in stroke animals and is located exclusively within the cytoplasm of neurons. To further elucidate the proteins nitrated in stroke, immunoprecipitation was performed and then analysed by LC-ESI/MS/MS. We analysed the protein content of three distinct bands in the immunoprecipitates of protein lysates from strokes that were not present in lysates taken from control brains (~30, 70, and 100 kDa). The detected proteins were categorised as being involved in either synaptic vesicle trafficking/docking (Ap2 Complex, Synapsin, Dynamin, AP180, Tubulin), metabolism (ex. H+ transporting, ATPase; GAPDH; 3-oxoacidic CoA Transferase 1; Triosephosphate Isomerase I), or cell signalling (ex. Na/K ATPase, PKC). Our results indicate that NT is generated in neurons within the infarct region of a stroke in a time dependant manner, suggesting that a window may exist for therapeutic intervention. Further investigation revealed that nitration of tyrosine residues on proteins at the synapse may provide a neuroprotective response to ischemia. Identification of the specific pathways involved may lead to novel therapeutic innovations.

137 A125

EARLY OBJECT RECOGNITION MEMORY DEFICITS AND MITOCHONDRIAL DYSFUNCTION IN APP-TRANSGENIC :TGGCRND8" MICE

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TgCRND8 mice express a transgene encoding a double mutant (KM670/671NL plus V717F) form of human APP. These animals exhibit spatial reference memory deficits that are readily discerned in Morris water maze tasks. It has been reported that the emergence of spatial memory deficits coincides with the appearance of plaques at 15 weeks (Hyde et al., 2005, Behav Brain Res 160:344-355), although we have seen spatial memory impairment as early as 11-12 weeks (Janus et al., 2000, Nature 277:602-8). Cortical short-term memory deficits, such as object recognition impairment, occur early in development of the human disease. To determine whether cortical memory deficits precede plaque accumulation in the TgCRND8 mouse, we assessed acute Novel Object Recognition (NOR) memory. We found that TgCRND8 mice exhibit profound NOR deficits at 8 but not at 4 weeks. Eight week old TgCRND8 mice were not impaired on a Morris water maze task. Optokinetic responses and duration of object exploration did not differ between TgCRND8 and non-Tg control mice. The presence of NOR deficits in young mice suggests that rhinal cortices are affected early in the disease process. Given that protofibrillary forms of amyloid have been shown to interact with mitochondria (Devi et al., 2006, J. Neuroscience 26:9057-68), we assessed complex I + III and IV activities in the hippocampus and cortex. In aged TgCRND8 mice with extensive plaque pathology, we observed ~45% reduction in complex I + III activity. In contrast, 9 week old TgCRND8 mice exhibited increased activity of complex I + III in cortical, but not hippocampal samples. Four week old TgCRND8 and non-Tg mice had equivalent levels of complex I + III and IV activities. These findings reveal a coincidence between emergence of NOR deficits and altered oxidative phosphorylation activity. The NOR test provides an index of the behavioural impairment associated with the early stages of mitochondrial dysfunction in the TgCRND8 mouse. Supported by OMHF and OGSST (Paul and Adelle Deacon graduate scholarship).

138 A126

THE INFLUENCE OF AGE AT THE TIME OF INJURY ON MORTALITY, NEUROLOGICAL RECOVERY AND AXONAL SURVIVAL AFTER ACUTE TRAUMATIC SPINAL CORD INJURY

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The incidence of spinal cord injury (SCI) is rising in the elderly due to changing population demographics with the aging of an active "baby-boomer generation". This study examines the potential age-related differences on outcome and axonal preservation following SCI. A cohort study included all patients who were enrolled in one of the NASCIS-2 trial groups: (i) placebo, (ii) methypredinosolone (MPSS), and (iii) naloxone. Also, a molecular and cellular examination of postmortem spinal cord tissue was performed in individuals with cervical SCI and controls. Using NF200 immunostaining, the number of axons within the corticospinal tracts, dorsal column, and descending vasomotor pathways were quantitated. In alternate sections stained for myelin, the extent of degeneration was quantitated. In the cohort, there were 171 patients in the placebo group (145 M, 26 F; ages from 13 to 89 years), 162 patients in the MPSS group (140 M, 22 F; ages from 13 to 88 years), and 154 patients in the naloxone group (124 M, 30 F, ages from 14 to 86 years). In the univariate and multivariate analyses, age was not significantly associated with motor and sensory scores assessed at 6 weeks, at 6 months and at 1 year post-SCI. Elderly individuals (65 years of age or older) had significantly greater mortality rates at 30 days (25% versus 2.65%, respectively; $p<0.0001$), at 6 month (43.75% versus 4.19%, respectively; $p<0.0001$) and at 1 year post-SCI (46.88% versus 4.86%, respectively; $p<0.0001$) than younger individuals after traumatic SCI. The immunohistology included 7 SCI cases and 5 controls with comparable age and sex distribution. In controls, the number of axons within spinal tracts was

not significantly correlated with age. There were no significant age-related differences for extent of degeneration or for number of preserved axons within the spinal tracts post-SCI. Our results indicate that age at time of injury is not significantly correlated with motor and sensory recovery post-SCI. Axonal survival within selected spinal cord tracts was unaffected by age after cervical SCI. Elderly individuals were more susceptible to death within the first year post-SCI. Given this, we advocate individualizing treatment approaches for elderly patients with SCI, as the opportunity exists for neurological recovery in this patient group.

139 A127

THE DEGENERATIVE EFFECTS OF NEUROINFLAMMATION IN THE SUBSTANTIA NIGRA PARS COMPACTA: ROLE FOR NEUROPROTECTION OR NEUROTOXICITY IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by loss of dopamine (DA) neurons of the nigrostriatal pathway and corresponding disturbances of motor functioning. Accumulating evidence suggests that neuroinflammatory processes, particularly those associated with innate immune pathways, play a role in PD. In particular, pro-inflammatory cytokines, which serve as signaling messengers of the neuroimmune axis, may orchestrate the neuroinflammatory and oxidative cascades provoked in PD. For instance, tumor necrosis factor-a deficient mice were resistant to the neuro-degenerative effects of the DA toxin, MPTP, and we also found that mice lacking the cytokine, interferon-g were likewise less vulnerable to this toxin. However, ablation of other cytokines which have some anti-inflammatory functions, such as interleukin-6 (IL-6), actually enhanced DA loss in PD models. Similarly, the potent anti-inflammatory cytokine, IL-10, prevented the damaging effects of a bacterial endotoxin upon the nigrostriatal system. Accordingly, in the present investigation we provide evidence that IL-6 and IL-10 may attenuate the neurodegenerative effects of combined administered with the pesticides, paraquat + maneb that has been implicated in PD by epidemiological studies. These cytokines modified the behavioral, neurochemical and histological pathology provoked by the environmental toxins. Ultimately, these data may provide clues as to potential inflammatory factors that may be targeted to enhance the efficacy of clinical PD treatments.

140 A128

TREATMENT OF CHRONIC PAIN WITH A DELTA OPIOID RECEPTOR SELECTIVE AGONIST

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Opiates bind to and activate 3 classes of G protein-coupled receptors, namely mu (MOR), delta (DOR) and kappa (KOR). Although alkaloids activating MOR, such as morphine, are the most potent clinically used analgesics, they often induce important adverse effects such as nausea, constipation, respiratory depression or sedation. In addition to those side effects, morphine induces tolerance. For most patients, these side-effects dramatically reduce their quality of life. Agonists acting selectively at DOR (e.g. deltorphin) have lower analgesic potency, but recent data shows that under certain experimental conditions (e.g. inflammation) the analgesic potency of deltorphin can be increased. Opposing to the increase in antihyperalgesic effect of deltorphin, only few side effects are observed when the delta selective agonist is intrathecally administered to rats. Whether treatment of chronic pain with repeated administrations of deltorphin is submitted to tolerance remains to be investigated. To verify if repeated deltorphin treatments can induce tolerance, we used a model of chronic inflammatory pain induced by an injection of Complete Freund's Adjuvant (CFA) in the plantar surface of the left hindpaw. In this model, it has been demonstrated that deltorphin, 72h after CFA injection, induces a peak antihyperalgesic effect 15 min after injection. Acute tolerance was first tested following two consecutive injections of deltorphin. Pain behaviors were examined 0 to 60 min following deltorphin injection, using the plantar test (Hargreaves test). To test for chronic tolerance, we injected deltorphin twice a day (i.e. once every 12h). 72h after CFA injection, deltorphin was injected intrathecally and pain behaviors were examined using the plantar test (Hargreaves test). Development of new therapies for the treatment of chronic pain is essential. Recent findings suggest that medications acting on DOR may represent a good alternative to commonly used opioids.

141 A129

GENE EXPRESSION PROFILES OF ACTIVATED MACROPHAGES IN THE CNS

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Activated macrophages in the contused spinal cord cause extensive secondary tissue damage. However, certain conditions exist, including after the microinjection of lysophosphatidylcholine (LPC), where macrophages in the spinal cord are activated and phagocytic but not cytotoxic. Understanding the molecular and functional characteristics of these two types of macrophages is critical in controlling the inflammatory response elicited after CNS injury. We therefore assessed the gene expression patterns of macrophages isolated from the CNS at the peak of their activation after LPC microinjection and spinal cord contusion using Affymetrix GeneChips. Compared to control monocytes, there are 2354 and 2279 genes whose expression is changed in macrophages isolated from LPC microinjected and contused spinal cords, respectively ($p<0.05$, 2-fold change). The majority of these genes are GeneOntology classified as being involved in protein/receptor binding, metabolism and cytokine/cytokine receptor interactions. When comparing the two types of activated macrophages, there are a total of 344 genes whose expression are significantly altered ($p<0.05$, 1.5-fold change). Of these, 82 genes are involved in binding, 51 in metabolism, 28 in development and 18 in the immune response (ie: cytokines. The most prevalent KEGG pathways are the cell adhesion molecules and cytokine-cytokine receptor interactions. Of the 344 genes identified, over 30 were identified as potential targets to alter the cytotoxic phenotype of macrophages in the CNS.

142 A130

NEUROPROTECTIVE PROPERTIES OF CYSAMINE IN ACUTE VS CHRONIC MODELS OF PARKINSON'S DISEASE

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The molecule cystamine has recently been probed for its neuroprotective properties in Huntington's disease, but its mechanisms of action are still largely unknown. Since several neurodegenerative disorders may share similar oxidative and programmed cell death, it is reasonable to think that the therapeutic gain of cystamine can be observed in Parkinson's disease (PD). We thus recently investigated the neuroprotective effects of cystamine in acute parkinsonian mouse model generated by the toxin MPTP. Aged mice (16 months of age) were assigned to either a low dose (10 mg/kg/day) or a high dose (50 mg/kg/day) of cystamine treatment beginning: 1) 2 days prior to systemic MPTP administration or 2) on the day of MPTP lesioning. Pre-treatment with lower doses of cystamine (10 mg/kg) revealed significantly increased levels of tyrosine hydroxylase (TH) striatal fiber, significant increases in the number of TH-immunoreactive cells, significant increase of substantia nigra Nurr1 mRNA levels and in the number of cells expressing the dopamine transporter (DAT) as compared to MPTP treated mice. Concomitant treatments (i.e. when cystamine treatment was started at the

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

same time as MPTP lesioning) displayed reduced efficacy. We are now reporting the same neuroprotective properties of cystamine in cohort of 80 young parkinsonian adult mice (generated by a systemic MPTP treatment) when pre-treatment with low doses of cystamine is used. We are pursuing the investigation of the neuroprotective properties of cystamine using chronic administration of MPTP in mice. This 28 days intraperitoneal delivery produces a more progressive degeneration of the DA system and includes the induction of nuclear inclusions not seen in the acute model. Using both of these models, we intent to determine if cystamine holds neuroprotective properties in chronic neuronal degeneration paradigms. Given its neuroprotective action in either or both context, treatments using cystamine could be applied to different classes of PD patients.

143 B126

HYDROGEN PEROXIDE UPREGULATES DOPAMINE D2 RECEPTORS IN RETINOIC ACID DIFFERENTIATED SH-SY5Y NEUROBLASTOMA

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The striatum is exposed to high levels of oxidative stress, a fact that is often explained by locally high levels of dopamine and oxidation thereof. Moreover, H2O2 has recently been shown to act as a non conventional neurotransmitter at striatal synapses [1]. Little is known, however, regarding the adaptation of key components of dopaminergic neurotransmission to subacute, non lethal oxidative stress. This prompted us to investigate the effects of H2O2, a canonical oxidant, on the expression levels of dopamine receptors D2 (DRD2) and tyrosine hydroxylase (TH) in retinoic acid (RA) differentiated SH-SY5Y, an acknowledged in vitro model of dopaminergic neuron [2]. On the same model, we also tested the effect of polyinosinic-polycytidylic acid (poly (IC)), which has recently been used in preclinical models of schizophrenia and shown to disrupt dopamine homeostasis [3]. SH-SY5Y (ATCC) were differentiated for 6 days in the presence of 10 microM RA and exposed for 24 h to H2O2 (100 microM) or poly(IC). DRD2 protein levels were assessed by western blotting with ECL detection. We used quantitative real time PCR with Sybr-Green detection and beta-tubulin as a reference gene to measure DRD2 mRNA levels. Nuclear and cytoplasmic levels of NF kappa B p65 were assessed by immunoblotting after cellular fractionation. After H2O2 incubation, DRD2 protein levels were increased (+ 59% ; p=0.012) as well as mRNA levels (+ 9% ; p=0.012). Poly (IC) did not elicit any change. Under our conditions, H2O2 but not poly (IC) induced nuclear translocation of NF kappa B p 65, which mimicked their effect on DRD2 regulation. The effect was restricted to differentiated (vs undifferentiated) SH-SY5Y. TH levels were not modified under our experimental conditions. Increased striatal DRD2 binding potential has been described in restless legs syndrome, Parkinson's disease, antipsychotics side effects and schizophrenia, where it might be a vulnerability and prognosis marker. The present results suggest that oxidant status might be a relevant parameter in the control of DRD2 levels. 1. Avshalumov, M.V., et al. J Neurosci, 2005. 25(17): p. 4222-31; 2. Presgraves, S.P., et al. Neurotox Res, 2004. 5(8): p. 579-98.; 3. Ozawa, K., et al.Biol Psychiatry, 2006. 59(6): p. 546-54.

144 B127

EXAMINING SEIZURE-INDUCED EFFECTS IN THE DEVELOPING BRAIN-A NOVEL EXPERIMENTAL MODEL

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Considering that seizures are most common during childhood, understanding the possibly detrimental effects of seizures on neuronal growth during development is highly warranted. We have developed a novel model system allowing the direct examination of seizure-induced effects on neuronal growth during development. The albino Xenopus laevis tadpole is an attractive model organism as it allows for the direct imaging of neuronal growth and synapse formation, in vivo, in real-time within the intact developing brain, using multiphoton fluorescence microscopy. Further, this system is readily amenable to in vivo electrophysiology and genetic manipulation. Bath application of the convulsant pentylenetetrazol (PTZ) to freely swimming or acutely immobilized unanaesthetized tadpoles reliably elicited behavioural and electrographic seizure activity. Seizures ceased upon washout of PTZ or after bath application of the anticonvulsant valproate. The immediate and short-term effects of PTZ-induced seizures on neuronal growth in vivo were subsequently examined. Repeated time-lapse imaging of individual fluorescently labeled immature neurons in vivo revealed that prolonged PTZ-induced seizures inhibit dendritic arbor growth, eventually resulting in retraction of existing branches. Rapid time-lapse imaging during seizures, in immobilized unanaesthetized tadpoles, revealed that dendritic filopodia – dynamic ultrastructural processes involved in arbor growth –- are significantly less motile and have a lower turnover rate compared to controls. Our results strongly suggest that seizures impede neuronal growth within the developing brain. These findings are of particular clinical relevance given that the observed anatomical changes likely affect neuronal function and connectivity.

145 B128

ULTRASTRUCTURAL LOCALIZATION OF DELTA OPIOID RECEPTORS FOLLOWING PERIPHERAL NERVE INJURY: IMPLICATIONS FOR NOVEL PAIN TREATMENT?

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Neuropathic (NP) pain is defined as pain caused by a peripheral and/or central nervous system lesion with sensory symptoms and signs and is estimated to affect 2-3 % of the population. Despite its prevalence and adverse impact on functionality and quality of life, it remains a significant challenge for physicians as it is typically refractory to traditional analgesics. However, research in ours and other laboratories increasingly suggests a therapeutic role for delta opioid receptor (δOR) agonists in treating NP pain. Following induction of NP pain in rats, δOR activation produced anti-allodynia as well as enhanced antinociception in NP rats compared to controls in two acute thermal pain paradigms, suggesting injury-induced changes in δOR function. This functional enhancement does not appear to be a result of enhanced δOR biosynthesis, as δOR protein did not significantly increase. It is therefore hypothesized that alternative mechanisms, such as increased cell-surface expression, may be responsible. Accordingly, we have examined δOR sub-cellular localization by electron microscopy immunohistochemistry using immunogold labeling in the spinal cords of NP and control rats. The number and proximity to the plasma membrane of silver-enhanced gold particles were assessed within the dorsal horn. Preliminary data reveal a shift in δOR sub-cellular compartmentalization from intracellular to membrane-bound in postsynaptic profiles within the spinal cord of NP rats. Further studies will assess δOR sub-cellular localization at presynaptic sites as well as visualization of internalized fluorophore-tagged δOR ligand, as an index of functional δOR recruitment. The targeting of δORs to neuronal plasma membranes with a corresponding enhancement in antinociceptive effectiveness may represent a compensatory mechanism by which neurons may sustain an inhibitory tone during chronic pain states, and in turn, indicate a viable target for pharmacological intervention.

146 B129

MITOFUSIN 2 PROTECTS AGAINST ACUTE NEURONAL INJURY

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Formation of a mitochondrial network would be required to maintain the functional peripheral nerve axon. MFN2, a mitochondrial transmembrane GTPase, regulates the mitochondrial network architecture by fusion of mitochondria. Mfn2 plays an important role in the nervous system as point mutations of this isoform are associated with Charcot Marie Tooth neuropathy. Here, we investigate whether Mfn2 plays a role in the regulation of neuronal cell death. We first examine mitochondrial dynamics following different modes of injury in cerebellar granule neurons. We demonstrate that neurons exposed to DNA damage or oxidative stress exhibit extensive mitochondrial fission, an early event preceding neuronal loss. The extent of mitochondrial fragmentation and remodeling is variable and depends on the mode and the severity of the death stimuli. Expression of wild type Mfn2 and a hydrolysis deficient mutant of Mfn2 Mfn2RasG12V), inhibits mitochondrial fragmentation and cell death following injury. Neurons respond with a dramatic lengthening of the mitochondria, up to 30 microns within the processes. More importantly, while both Mfn2 and Mfn2RasG12V function equally to promote fusion and lengthening of mitochondria, the activated Mfn2RasG12V mutant shows a 2 fold increase in protection of neurons against cell death. These findings implicate a signaling role for Mfn2 in the regulation of apoptosis that extends beyond its role in mitochondrial fusion.

147 B130

PROTEIN NITRATION CAUSES NEURONAL DEATH FOLLOWING TRAUMATIC BRAIN INJURY

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Recently, research has focused on the role of free radicals in mediating neuronal degeneration following traumatic brain injury (TBI). The reaction between the free radicals superoxide (O2•-) and nitric oxide (NO•) to produce peroxynitrite (ONOO-) is required to be lethal in a secondary model of TBI. Additionally, inhibition of NO• production has been shown to be neuroprotective (Arundine et al., 2004). Subsequently, a whole animal model of TBI resulted in increased 3-nitrotyrosine (3-NT), a marker for ONOO- (Lau et al, 2006). What remains unclear is the temporal profile of ONOO- production, and relationship between peroxynitrite and neuronal damage. This was investigated using the lateral Fluid Percussion Injury (FPI) model of moderate TBI in rats. A temporal increase in protein nitration occurs in injured neurons immediately following injury, peaking at 12 hours, and persisting for at least 24 hours. Injured neurons exhibit nitrated proteins in the cytoplasm, have altered morphology compared to non-nitrated neurons, and undergo neurodegeneration. Meanwhile, protein nitration was not observed in astrocytes. Mass spectrometry identified several key, cytoplasmic neuronal proteins as nitrated, such as GAPDH and TPI, and using an ex-vivo system, ONOO- was shown to inhibit GAPDH activity through cysteinyl oxidation. Taken together, our results suggest that peroxynitrite is formed rapidly following FPI, inactivates key neuronal proteins, and causes neurodegeneration.

148 B131

MODELING MODEL NEURON DISORDERS IN ZEBRAFISH

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder (MND) with major symptoms and death resulting from dysfunction and loss of upper and lower motor neurons. Animal models of ALS will aid to understand the molecular mechanisms that lead to motor neuron dysfunction and death and also propose new treatments for these neurodegenerative disorders. Recently, mutations in the vesicle-trafficking protein, vesicle associated protein B (VAPB) was found to cause two types of MNDs, ALS and late-onset spinal muscular atrophy (SMA) most likely due to loss of function of the VAPB protein. We decided to develop a novel model of motor neuron disorder in zebrafish. The zebrafish VAPB gene is highly homologous to the human gene (77%). A specific antisense morpholino oligonucleotide (AMO) to this gene was designed to selectively knock-down VAPB. Injection of this AMO in zebrafish embryos (one to eight cell stage) led to a specific motor phenotype, slower swimming ability after proper touch response (30-50% affected according to the dose) as well as more generalized developmental abnormalities (30%). This response was not found in zebrafish embryos injected with a control mis-sense AMO. Even though the number of motor neurons remained unchanged in fish with swimming deficits, abnormal guidance of motor axons and decreased firing intensity and frequency was measured in motor neurons of VAPB knock-down zebrafish. The motor neuron deficit was rescued when VAPB AMO was injected with human WT RNA. However, no rescue was conferred when the mutant form of VAPB RNA found in ALS was injected alongside the VAPB AMO. The VAPB knock-down zebrafish represents an excellent animal model to study the pathophysiological mechanisms and therapeutical strategies in ALS as well as other types of MNDs.

149 B132

APOPTOSOME FORMATION CRITICALLY MEDIATES THE INDUCTION OF PROGRAMMED CELL DEATH IN MOTOR NEURONS IN VIVO

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Programmed Cell Death (PCD), is a significant contributor to a variety of central nervous system (CNS) insults. Experimental efforts to directly assess the role of the apoptosome in mediating PCD following motor neuron injury in vivo have been hindered by the embryonic lethality exhibited by cytochrome c, Apaf-1 and caspase-9 null mice. In order to determine the role of the apoptosome in regulating injury-induced PCD, we have examined motor neuron death following axotomy in mice expressing a point mutant of cyt c (K72A) which retains its ability to perform in oxidative phosphorylation but does not bind the WD-40 repeat motifs of Apaf-1. As a result, cytochrome c-dependent activation of Apaf-1 is inhibited. Facial axotomies performed on cyt c (KA/KA) mice at postnatal day 3.5 demonstrated a three-fold increase in the survival of motor neurons compared to control littermates. Elevated levels of caspase-3 activation and TUNEL immunoreactivity was observed within the facial nucleus of axotomized control mice ipsilateral to the lesion by 48 hours post-axotomy, but was largely absent in cyt c (KA/KA) mice. In addition, our results demonstrate that following axotomy, AIF translocation does not occur in the absence of apoptosome activity. Taken together, these results demonstrate that, downstream of the mitochondria, the apoptosome plays a dominant role in mediating motor neuron PCD following facial axotomy. Funded by the Canadian Institute for Health Research (CIHR), The Amyotrophic Lateral Sclerosis Society of Canada (ALS), and The Muscular Dystrophy Association of Canada (MDAC).

150 B133

CYCLOSPORIN A OFFERS PROTECTION AT THE ISCHEMIC SYNAPSE

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Ischemia and its resultant reperfusion oxidative stress, lead to immediate and delayed synaptic dysfunction characterized by decreased evoked and increased spontaneous neurotransmitter release, possibly due to presynaptic mitochondrial dysfunction. The increased intracellular Ca2+ that follows an ischemic episode can reach pathological concentrations, exhausting the cell's buffering capabilities leading to oxidative stress. This could eventually activate the mitochondrial permeability transition pore (mPTP), compromising cellular energetic metabolism and synaptic transmission. Since blocked synaptic transmission is an early event in ischemia which precedes cell death, it may be a target for neuroprotection. Since the effects of ischemia vary with age, acute hippocampal slices from both young (3-6 weeks) and aged (13-16 weeks) mice were used. We found that 8 minutes of oxygen glucose deprivation (OGD) in young control slices and 6 minutes of OGD in aged control slices were critical time points, where the decline in fEPSPs was irreversible upon oxygen and glucose reintroduction. We hypothesized that blocking the mPTP with cyclosporin A (CsA) during OGD would help recovery of OGD-induced failure in synaptic transmission, which we believe is presynaptic in origin. To test our hypothesis, 8 minutes of OGD in young slices and 6 minutes of OGD in aged slices were both administered in the presence of CsA. As well, CsA was also present during reintroduction of glucose and oxygen. Our results showed that the addition of CsA during 8 minutes of OGD and during reperfusion, allowed for substantial recovery of fEPSPs in young slices. Similar results were seen in the aged slices. This suggests that blocking the opening of the mtPTP can ameliorate OGD induced synaptic deficits. Mitochondrial dysfunction has been observed in the Alzheimer's disease (AD) brain. The dysfunction is characterized by altered mitochondrial calcium buffering capabilities and the increased open probability of the mPTP. Thus, the AD brain might be more vulnerable to ischemic insults, presumably providing a segue towards the development of Vascular Dementia (VD). Subsequent experiments will examine whether CsA can ameliorate OGD induced alterations in synaptic transmission in the hippocampal region of a transgenic mouse model of Alzheimer's disease. Supported by: CIHR

151 B134

GENERATION OF A NEW MODEL-SYSTEM TO STUDY AMYOTROPHIC LATERAL SCLEROSIS DISEASE BY NON INVASIVE IMAGING

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Amyotrophic lateral sclerosis (ALS) is a late onset neurological disease characterized by progressive spinal motor neurons degeneration associated with paralysis and eventually death. Transgenic mice expressing a mutant sodium dismutase1 (SOD1) develop phenotype with many pathological features resembling human familial and sporadic ALS. Although major clinical symptoms in ALS arise from neurodegeneration and death of motoneurons, recent studies suggest that non neuronal cells could play a role in the toxicity to motor neurons. Their precise role in onset and progression of the disease remain however unknown. To further investigate the role of non-neuronal cells in the disease onset and progression, we developed mouse model for live imaging of astrogliosis in ALS. As a glial fibrilary acidic protein (GFAP) is strongly up-regulated in ALS, we used it as a hallmark of astrogliosis to create our model. We crossed mice carrying the firefly luciferase gene under the transcriptional control of mouse GFAP promoter (GFAP-luc, Xenogen, CA) with mice carrying the SOD1G93A mutation. The double transgenic GFAP-luc/SOD1G93A mice were used in the study as well as GFAP(wt)-SOD1G93A and GFAP as controls. Live imaging was performed weekly starting from postnatal weeks 3-4 till the end stage of the disease. Loss of extension reflex, weight-loss and motor deficits were used as indicators of clinical symptoms. The results were obtained from 12 female/male mice matched-age littermates and were compared to adequate controls. Data collected by in vivo imaging showed that photon emission/GFAP signal was first detected at the lumbar spinal cord area. The signal first arose from small multiple areas of astrocytes activation which then converged into a larger signal around 80-100days of age. The correlation analysis between live imaging and behaviour data revealed that increase in GFAP signal in the spinal cord at 70-80 days correlated with the initial disease onset (loss of extension reflex). Moreover the peak signals arising from the spinal cord around 100 days correlated with the abrupt onset of sensori motor deficit and paralysis. The end-stage of the disease (approx 133d) was characterized by GFAP signal in the brainstem and in the brain and coincided with loss of body weight, suggesting distal-rostral spreading of the disease. These mice will provide unique tools for understanding disease pathology and longitudinal responses to drug testing. Acknowledgment CHIR,RRTQ, FRSQ.

152 B135

NEUROMOTOR, COGNITIVE AND SYNAPTIC ANOMALIES IN α-SYNUCLEIN-TRANSGENIC MICE: A MODEL OF PARKINSON'S DISEASE DEMENTIA?

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The accumulation of α-synuclein deposits is a hallmark of the Parkinson's disease (PD) brain and this accumulation may play an important role in disease pathogenesis. Synuclein aggregates in PD are concentrated in brainstem nuclei, including neurons of the substantia nigra. A "PD dementia" (PDD) is also observed in 40-70% of patients. The occurrence and severity of dementia may be correlated to the accumulation of α-synuclein in the entorhinal cortex, hippocampus and amygdala. Evidence of dysfunction in these areas include reports that PD patients exhibit early deficits in visual object recognition and an absence of amygdala event-related potentials in response to fearful facial expression. We probed for PD-like neuromotor and cognitive impairments in new lines of α-synuclein transgenic mice in which the hamster prion promoter controls expression of human wild type (WT) or missense mutant α-synuclein (A53T and A30P) transgenes. We found progressive neuromotor deficits and gait asymmetry in adult A30P-Tg mice. The A53T-Tg was also associated with motor impairment, when expressed in α-synuclein-knockout mice, suggesting that the endogenous murine protein abrogates effects of human A53T α-synuclein expression. We observed deficits in 3 h novel object recognition memory, suggestive of entorhinal cortical and hippocampal dysfunction, in 4 mos old A30P-Tg and A53T-Tg mice. To test amygdala function, we examined retention of an association between presentation of a tone and a mild electric shock. Tone-dependent freezing was significantly impaired in 4 mos old A30P-Tg mice. By 8 mos, A53-Tg mice also displayed deficits in fear conditioning. The constitutive activation state of stress activated protein kinase (SAPK/JNK), was examined in regionally dissected brains. Levels of phosphorylated SAPK were dramatically upregulated in rhinal cortices, amygdala, hippocampus, and cerebellum of A53T-Tg and A30P-Tg animals. Finally, levels of synaptophysin, a presynaptic marker, were lower in the hippocampus and cerebellum of animals expressing A53T, or A30P missense mutations. Upregulated levels of phosphorylated SAPK and reduced levels of synaptophysin suggest synaptic degeneration in the mutant animals. The pattern of behavioral, biochemical and synaptic anomalies in these mice implicate dysfunction in entorhinal cortex, hippocampus and amygdala and suggest that they may provide relevant models for studying mechanisms underlying cognitive dysfunction in PDD.

153 B136

THE MOVEMENT CORRECTION DEFICIT IN HUNTINGTON'S DISEASE IS SENSITIVE TO TERMINAL CONTROL REQUIREMENTS

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder affecting the striatum at early stages. HD is characterized by involuntary and voluntary movement deficits, cognitive decline and behavioral disturbances. Rapid pointing movements performed by patients are usually slower and more irregular than controls, especially in the terminal corrective phase. The movement correction deficit in HD is still poorly understood and a better characterization of this problem could help understand the role of fronto-striatal systems in movement. This study examined whether the correction deficit in target acquisition is sensitive to terminal control requirements or to visual attention load. We compared cyclical movements (back-and-forth pointing) to discrete pointing movements performed to a target surrounded or not by distractors. Sixteen patients with early HD and 16 age-matched controls participated in the study. Reducing the termination requirements (cyclical movements) significantly attenuated the correction deficit in patients as shown by a shorter and less irregular corrective phase in cyclical movements as compared to discrete movements. Distractors around the target affected the initial part of the movement (lower speed and movement irregularity) but did not affect the two groups differentially. These results suggest that the error correction problem in HD is sensitive to terminal control requirements and that this effect is independent of visual attention. Acknowledgments: This research was supported by the Huntington's Disease Society of America and by the Natural Sciences & Engineering Research Council.

154 B137

AN IMMUNIZATION STRATEGY FOR TREATING AMYOTROPHIC LATERAL SCLEROSIS

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Immunotherapy is emerging as a therapeutic approach in the neurodegenerative diseases characterized by deposition of aggregated and/or misfolded protein including amyotrophic lateral sclerosis (ALS). ALS causes progressive motor neuron degeneration in the brain and spinal cord. In familial ALS cases harboring mutations within the gene encoding superoxide dismutase-1 (SOD1), SOD1 aggregates are found in motor neurons. Although the precise molecular mechanisms of motor neuron degeneration in the presence of mutant SOD1 remain to be elucidated, it is suggested that toxicity is related to the propensity of the mutant protein to misfold and/or dissociate from a dimer into a monomer, and ultimately to form aggregates. The in vivo presence of misfolded/monomeric SOD1 is demonstrated by the recent development of an antibody, called SOD1-Exposed-Dimer-Interface (SEDI) antibody raised to an epitope that is only exposed when the homodimeric SOD1 is misfolded or dissociated. Here we report that an active immunization strategy that selectively targets disease-associated protein species, misfolded/monomeric SOD1 or SOD1 aggregates, has therapeutic benefits. Vaccination of ALS transgenic mice overexpressing the mutant SOD1 with SEDI peptide or SOD1 aggregates delayed the onset and slowed the progression of disease. Immunized transgenic mice also showed a significant increase in lifespan and improvement in motor deficits. Furthermore, accumulation of SOD1 oligomers in spinal cord extract was reduced in vaccinated mice. These results suggest that immunotherapy effectively improves motor and pathological disease outcomes as well as reduces the accumulation of SOD1 aggregates, and this therefore may provide a new means for the treatment of ALS.

155 B138

EXAMINATION OF THE THERAPEUTIC EFFECT OF AN ENGINEERED TRANSCRIPTION FACTOR PROMOTING ACTIVATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN SPINAL CORD INJURY

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Spinal cord injury (SCI) results in the initiation of multiple secondary injury cascades, one of which is the disruption of spinal cord blood flow and the onset of spinal cord ischemia. Vascular endothelial growth factor (VEGF) is a prototypical angiogenic growth factor, more recent findings revealed that VEGF also has direct effects on neural cells. An engineered zinc finger protein (ZFP) transcription factor was designed to activate expression of all the isoforms of the endogenous VEGF gene for complete biological function. The aim of this study was to evaluate the effect of a recombinant adenoviral vector or adeno-associated virus containing an engineered ZFP-VEGF-A transcriptional activator (AdV-ZFP-VEGF or AAV-ZFP-VEGF) after SCI in adult rats. In the acute phases, the adenovirus vectors encoding either Ds-Red fluorescent protein or ZFP-VEGF-A were delivered locally immediately after a 35g clip compression injury. In AdV-Ds-Red injected rats, adenovirus-infected cells were observed in gray and white matter, and the vectors transduced neurons, astrocytes and oligodendrocytes. VEGF mRNA levels encoding for VEGF 120, 164 and 188 isoforms were measured by real-time PCR and VEGF protein. Neurofilament 200 protein (NF200) levels were determined by Western blot analysis. Administration of Adv-ZFP-VEGF resulted in an increase of VEGF mRNA and protein levels at 3 days after injury. The posttraumatic degradation of NF200 was significantly attenuated in the ZFP-VEGF treated animals at 7 days after SCI. Expression of ZFP-VEGF-A was confirmed by Western blot. In the injured spinal cord area ten days after SCI and treatment, using immunohistochemistry, a significant increase in vascularization (assessed by RECA-1 immunostaining) and decreased levels of apoptosis (assessed by TUNEL) occurred in rats treated with AdV-ZFP-VEGF compared with controls. Furthermore, in the more chronic evaluation period, animals treated with AAV-ZFP-VEGF showed significant improvement in hind limb function up to six weeks after injury compared with control animals. Our finding suggests that this engineered ZFP-VEGF-activating transcription factor led to upregulation of VEGF mRNA and protein, attenuation of NF200 degradation, protection/repair of blood vessels, decreased apoptosis, and enhanced functional neurological outcome. These data suggest that VEGF gene therapy using a ZFP transcription factor plasmid holds promise as a therapy for SCI and other forms of neurotrauma.

156 B139

SHORT CHAIN FATTY ACIDS INDUCE CATECHOLAMINERGIC NEUROTRANSMISSION IN PC12 CELLS VIA A CREB DEPENDANT MECHANISM- POSSIBLE RELEVANCE TO AUTISM SPECTRUM DISORDERS

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Dietary or gastroenterological factors have been implicated in autism spectrum disorders (ASDs). Short chain fatty acids (SCFA) such as butyric (BA) or propionic acid (PPA) are present in diet or are produced by enteric bacterial fermentation of residual carbohydrates. We have shown that intraventricular infusions of PPA can elicit consistent behavioural, electrophysiological, neuropathological and biochemical effects reminiscent

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

of ASD's in rodents (MacFabe et al. 2006). Effects include activation of CREB dependent pathways, important in neurodevelopment, learning and memory. Furthermore BA can induce tyrosine hydroxylase gene expression (TH; rate limiting enzyme in biosynthesis of monoamine transmitters dopamine, epinephrine and norepinephrine) in a PC12 cell model (Santosh 2006). Since increased monoamine concentration is known to be elevated in the brain and blood of ASD patients and a number of models (Narita 2002), we hypothesized that SCFA's may directly influence brain catecholaminergic pathways. To determine whether SCFA's with known behavioral effects and putative links to ASD's (PPA; valproate, VPA) can regulate TH gene expression site-directed mutagenesis was used to introduce point and deletion mutations into the wild-type TH promoter driving the expression of luciferase reporter gene. After transfection and treatment with SCFA, PC12 cells were harvested and reporter activity measured along with endogenous TH mRNA and TH protein. We found SB, PPA & VPA induced TH promoter over a wide concentration range. The canonical CRE motif the novel BRE (butyrate response element, Patel et al., 2005 and CREB transcription factors were found necessary for the transcriptional activation of TH gene by all SCFA tested. Physiologically relevant concentrations of SCFA also caused accumulation of TH mRNA and immunoreactive protein. Intraventricular infusions of SB produced behavioral and neuropathological changes similar to those evoked by PPA (MacFabe, 2006). In conclusion, our data are consistent with a molecular mechanism through which environmental signals such as fatty acids can modulate animal behavior through effects on central catecholaminergic and CREB dependant systems and are consistent with their hypothetical role in ASDs. (Support: Mead Johnson, GoodLife Children's Charities)

157 B140

THE FATE OF THE LARGE STRIATAL INTERNEURONS EXPRESSING CALRETININ IN HUNTINGTON'S DISEASE

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The severe atrophy of the striatum that characterizes Huntington's disease (HD) is due to massive losses of striatal medium spiny projection neurons. In contrast, interneurons are relatively spared in HD, but little is known of the fate of the large interneurons that express calretinin (CR) in HD. We addressed this issue by applying a double immunofluorescent labeling technique to postmortem material obtained from HD patients and age-matched controls. Antibodies against CR and choline acetyltransferase (ChAT) were used to compare the distribution, density and degree of ChAT co-localization of the CR+ striatal interneurons in normal and HD cases. The normal human striatum was found to contain 3 types of large interneurons: a) neurons expressing CR only; b) neurons displaying ChAT only; and c) neurons co-expressing CR and ChAT. The CR+/ChAT+ neurons outnumbered neurons expressing ChAT only, which were themselves more numerous than neurons displaying CR only. A two-fold decrease in the density of CR+/ChAT+, CR-/ChAT+ occurred in the striatum of HD patients compared to that of controls, suggesting that these neurons are affected in HD. However, studies undertaken with neurokinine-1 receptor NK-1R as a marker of large CR+ and ChAT+ neurons revealed that these striatal neurons are selectively spared in HD patients. Hence, the apparent decrease in the number of CR+/ChAT+, CR-/ChAT+ neurons in HD does not appear to result from a degeneration of these cells, but rather from a marked diminution of the expression of CR and ChAT. Our data indicate that the neurodegenerative processes at play in HD affect the expression of CR and ChAT proteins without causing the death of these large striatal interneurons.

158 B141

STRESS AND GLUCOCORTICOIDS ACCELERATE DOPAMINERGIC CELL DEATH AND EXAGGERATE MOTOR SYMPTOMS IN A RAT MODEL OF PARKINSON'S DISEASE

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Stress has been one of the earliest proposed causes of Parkinson's disease. The purpose of this study was to investigate if chronic stress and elevated levels of the stress hormone corticosterone exaggerate motor deficits and neurodegenerative events in a rat model of Parkinson's disease. Rats were tested in a comprehensive test battery of skilled and non-skilled movement while being exposed to daily restraint stress or corticosterone treatment. Stress and corticosterone compromised normal motor function and exaggerated motor deficits caused by a dopamine depletion induced by unilateral 6-hydroxydopamine infusion into the nigrostriatal bundle. Evaluation of drug-induced rotation and immunohistochemistry indicated that greater motor impairments in stress-treated animals were related to accelerated death of midbrain dopaminergic neurons during the first week post-lesion. In addition, the lesioned substantia nigra of stress-treated animals revealed elevated levels of glial fibrillary acidic protein indicating increased reactive gliosis. These findings suggest that stress and elevated stress hormone levels represent key factors in the pathogenesis of Parkinson's disease that lead to earlier onset of symptoms and exaggerated neurodegenerative processes. This research was supported by: National Institutes of Health (NINDS), Parkinson's Disease Foundation, Alberta Heritage Foundation for Medical Research.

159 B142

THE IL-10 AND IL-10 RECEPTOR 1 GENES AND CHILDHOOD-ONSET MOOD DISORDERS

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IL-10 is a pleiotropic cytokine that regulates neuronal, neuroimmune and neuroendocrine substrates implicated in the etiology of depression, such as inflammatory cytokines, the HPA axis, and indoleamine 2,3-dioxygenase, an enzyme which limits serotonin availability. Accordingly, it is hypothesized that IL-10 signalling pathway genes may be involved in genetic susceptibility to depressive disorders. Here, in a family-based association study of childhood-onset mood disorders (COMD) (characterized by onset of depression before age 15), we have begun to test this hypothesis through an investigation of single nucleotide polymorphisms in the gene for IL-10 (IL10) and the gene for a component of its receptor, IL-10 receptor 1 (IL10RA). Using a sample of 386 families (386 COMD probands, 76 affected siblings, and their parents), we have analysed 3 functional promoter variants of IL10 (-1082G/A, -819C/T, and -592C/A), and 6 polymorphisms across IL10RA, including 3 coding variants (Ser138Gly, Gly330Arg, and Ser399Leu) that may alter receptor function. Genetic association analyses were carried out using the transmission/disequilibrium test (TDT), testing for biased transmission of alleles from heterozygous parents to their affected offspring. TDT analysis of the IL10 gene showed no evidence for biased transmission of the promoter polymorphisms, either individually or as haplotypes. By contrast, TDT analysis of the IL10RA gene showed significant evidence for biased transmission of one of the polymorphisms (rs9610: chi-sqared=4.173;p=0.041) and for excess transmission of one of

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

the 6-marker haplotypes (chi-squared=7.090;p=0.008). These findings suggest the possibility of IL0RA involvement in genetic risk for depression and warrant further investigation. Follow-up analyses, including analyses of an additional 200 newly recruited COMD families, are underway.

160 B143

LONG-TERM EFFECT OF SUBCHRONIC ADMINISTRATION OF (±) 3,4 - METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON SENSORIMOTOR GATING IN RATS

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Prepulse inhibition (PPI) of acoustic startle is the unlearned suppression of the acoustic startle response triggered by a weak, non-startling stimulus which precedes the startle eliciting stimulus (pulse) by 30-500 ms. PPI has been found to be reduced in schizophrenia patients and therefore, it is an operational measure of sensorimotor gating.

(±)3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is known to induce psychopathological effects in humans. MDMA acts as an indirect monoaminergic agonist and moreover, it is one of the most popular recreational drugs. In this study we investigated the long-term effect of MDMA on PPI in rats to prove the hypothesis of long-term psychotic events after MDMA consumption. Therefore, rats were treated over ten consecutive days with 20mg/kg MDMA or placebo. Two weeks after the treatment period, behavioural testing with different prepulse intensities was followed. In addition to the behavioural experiments, a quantification of brain monoamines was carried out via high pressure liquid chromatography (HPLC). We observed that (1) MDMA significantly decreased PPI but does not affect baseline startle magnitude and that (2) even weeks after MDMA treatment HPLC revealed a significant reduction of the content of serotonin and its metabolite 5-HIAA in several brain areas known to be involved in PPI modulation. Furthermore, neither the dopamine level nor the DOPAC, HVA or MT levels were affected. Preliminary results of a parallel experiment also show (3) a profound reduction of PPI in the acute state one hour after the injection of 20 mg/kg MDMA.

161 B144

PROPIONIC ACID BUT NOT CONTROL COMPOUNDS INDUCE INCREASED LOCOMOTOR ACTIVITY AND INNATE NEUROINFLAMMATORY CHANGES IN RATS FOLLOWING VENTRICULAR INFUSION- A NOVEL ANIMAL MODEL FOR AUTISM SPECTRUM DISORDERS

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Dietary, digestive system and immunological factors have been implicated in the cause and symptoms of autism spectrum disorders (ASDs). Propionic acid (PPA) is a short chain fatty acid, a product of enteric bacteria, and a common food preservative. PPA has widespread effects on cellular metabolism, neurotransmitter release, second messenger signalling, intracellular pH, and immune function. We found PPA can elicit consistent behavioural, biochemical and neuropathological changes in rodents and may be an animal model for ASDs. To examine the effects of chronic intraventricular infusions of PPA and structurally similar compounds in rodents on motor output and neuropathology, adult rats received intraventricular infusions of pH 7.5 buffered PPA (500ug/ul), isomolar 1-propanol, sodium acetate or PBS vehicle (0.1M) twice daily for 5 treatment days. Immediately following microinfusion, animals were individually placed into an automated open field (Versamax) and a variety of locomotor activity variables were assessed for 30min. Afterwards, animals were sacrificed and the dorsal hippocampal foramation and adjacent white matter examined mmunohistochemically for markers of innate neuroinflammation. Rodents which were infused with with PPA, and to a lesser extent acetate, showed significant increases in locomotor activity and turning behaviour, compared to controls. Immunohistochemical analyses of brain revealed increased reactive astrogliosis (GFAP) and microglial activation (CD68) only in PPA treated animals, reminiscent of neuropathological changes found in ASD autopsy tissue. PPA was not directly neurotoxic, as measured by direct cell pyramidal cell counts and apoptosis (cleaved Caspase 3). Only PPA infusions produced behavioural and neuroinflammatory effects in rats reminiscent of ASDs, suggesting a specific short chain fatty acid/pH dependent effect of this compound, which may involve pH dependent activation of a number of second messenger processes, including intracellular calcium release and closure of gap junctions. PPA infusions in rats may model some aspects of ASDs, and may provide a plausible dietary/gut/CNS link to this disorder. Sponsor: GoodLife Children's Charities

162 B145

SEX AND AGE DEPENDENT MODULATION OF PLASTICITY IN A MURINE MODEL OF ALZHEIMER'S DISEASE AS REVEALED BY SYNAPTIC ZINC

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Most synaptic zinc within the brain is localized within a subset of glutamatergic neurons. Once released, this zinc can modulate the activation of a multitude of receptors and therefore potently modulate synaptic plasticity. In addition, zinc has robust effects in a number of disease states, including Alzheimer's disease (AD). Zinc promotes the aggregation of beta amyloid and the pharmacological or genetic removal of zinc leads to an amelioration of AD related pathology. As well, the increased susceptibility of females to greater pathological loads is eliminated with the genetic removal of zinc. As in mouse models of Alzheimer's disease, deficits in plasticity are among the first features to be observed, we hypothesized that sex dependent deficits in plasticity would be revealed by synaptic zinc. Our laboratory has previously shown that in response to sensory deprivation, a condition in which endogenous plastic mechanisms are activated, a rapid, age-dependent increase in synaptic zinc occurs. Using this model, we utilized male and female 3xTg-AD mice (1, 3, 6, 9, 12,18 months) and age-matched C57BL/6 male and female controls. Mice were killed 48 hours following the bilateral removal of the c-row of vibrissae. The brains were then processed and stained using the Timm-Danscher method in order to visualize vesicular zinc levels in the S1 cortex. Measures consisted of comparing the staining intensities of the deprived row of barrels to the adjacent, non-deprived rows. Four major results were observed. First, in all groups, plasticity was observed in the form of an increase in vesicular zinc in the deprived barrels. Second, in both sexes and strains, an age-dependent reduction in plasticity was observed. Third, female control mice had a greater plastic response compared to male control mice between three and twelve months of age while male and female 3xTg-AD did not significantly differ from each other at any age. Fourth, male 3xTg-AD mice consistently had an increased plastic response than control male mice whereas female 3xTg-AD mice had decreased plasticity at younger ages compared to female controls. These findings demonstrate the complexity of the role of zinc in plasticity and AD. They also suggest that plasticity revealed by synaptic zinc in the non-pathological state is sex-dependent and that this effect is altered in 3xTg-AD mice. Supported by: NSERC, Scottish Rite Charitable Foundation

163 B146

CORTICAL AND THALAMIC COMPONENTS OF NEOCORTICAL KINDLING-INDUCED EPILEPTOGENESIS IN BEHAVING CATS

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The neocortical kindling in the majority of cases exhibits a resistance to produce generalized convulsive seizures compared to other brain structures, and therefore, it is rather difficult to use it to study the cortical epileptogenesis. Here, using a new method of supra-threshold cortical kindling, we report electrophysiological patterns of synchronization of field EEG (cortical areas 4, 5, 7, 21, 17, 18, 22 & thalamic VPL nucleus); and intracellular activities (area 5) in chronically implanted non-anesthetized cats, both during different states of vigilance: wake, slow-wave sleep (SWS) and rapid eye movement sleep (REM); and during acute seizures elicited by prolonged (20-60 s) electrical stimulation of high intensity (0.5-1.5 mA). The highest probability to elicit paroxysmal afterdischarges was during transition from SWS to wake. The acute elicited seizures were mainly clonic accompanied with tonic components followed by prolonged postictal depression. Delayed rhythmic outlasting activities (OA) at ~1.5 Hz followed the postictal depression and lasted up to 2 hours. These activities were clear during wake, slightly reduced during SWS and completely absent during REM sleep. They started focally in the vicinity of the stimulating electrode and generalized over the whole cortical surface following serial stimulation. Intracellular correlates of OAs consisted in neuronal discharges during the depth-negative spike of the OA, but the hyperpolarizing components were not accompanied by a profound hyperpolarization as during SWS and neuronal action potentials were built on the summation of successive synaptic FPPs. During seizures cortical activities were leading over thalamic ones, while during OA thalamic components preceded the cortical ones. Electrical stimulation of locus coeruleus or pedunculo-pontine tegmentum during OA slightly decreased their amplitude, but was not able to completely abolish the OA. The results suggest that such rhythmic long lasting oscillatory activity outlasting seizures are the key factor of epileptogenesis, leading to epilepsy. Supported by CIHR and NSERC.

164 B147

EVALUATION OF FACTORS INFLUENCING MEDICATION ADHERENCE IN PATIENTS WITH EPILEPSY IN RURAL COMMUNITIES OF KADUNA STATE, NIGERIA

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Adherence to medication is the backbone to effectiveness of a therapy. In the absence of a definitive curative therapy, antiepileptic therapy is a key intervention aimed at prolonging and improving the quality of life of epileptic patients suffering from a disease known for its stigmatization with many cultural misconception. The aim of the study is to assess the level of, and factors influencing adherence to antiepileptic therapy among patients in rural communities attending outpatient clinic in Ahmadu Bello University Teaching Hospital, Zaria and Jicon Hospital, Kaduna, northern Nigeria. A cross sectional descriptive study design was used. Interviewer-administered, structured questionnaires were administered to a sample of 272 epileptic patients attending Ahmadu Bello University Teaching Hospital/Jicon Hospital Kaduna, who had been on antiepileptic drug (AED) for at least one year. Systematic random sampling technique was used to select the patients. Information was obtained on their knowledge of epilepsy and antiepileptic therapy regimen adherence to AED and factors influencing the adherence to AED regimen. The level of knowledge of epilepsy, signs and symptoms was high of about 57.8%, 25.9% which had excellent knowledge and good knowledge respectively. The level of antiepileptic therapy was high with 78.6% having excellent knowledge. The level of adherence to AED was 32.6%. It was found that there was significant association between knowledge of AED and adherence to therapy (p=.00385) and there was no significance association between age (p=0.067), sex (p=0.182) educational status (p = 0.688), income (p=0.519) religion (p=0.69), place of residence (p=0.157) with AED adherence. The reasons for non-adherence were as a result of forgetfulness and AED fatigue and being away from home. Adherence to AED was low despite high level of knowledge due to forgetfulness, fatigue and being away from home, therefore a need for mounting adherence counseling in the clinic and health educational interventions to improve adherence in our rural communities where the disease cannot be managed. Further exploration of the relationship between clinical outcomes and other non-drug self-management strategies is needed.

165 B148

Laser microdissection and gene expression in striosomes and matrix compartments of the striatum in normal and Parkinsonian rats

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The striatum is the main input structure of the basal ganglia; it receives major projections from the cerebral cortex, thalamus and substantia nigra. The nigrostriatal projection uses dopamine as a neurotransmitter and its terminal fields in the striatum conform to the two major striatal compartments, that is, the striosomes (or patches) and the extrastriosomal matrix. These two striatal compartments differ from one another by their neurochemical makeup as well as by their afferent and efferent connections. The aim of the present study was to obtain a global picture of the different genes expressed at the level of each of these two striatal compartments and to document possible changes in gene expression at these levels consecutive to dopamine denervation of the striatum. Experiments were performed in normal rats as well as in rats in which the nigrostriatal dopaminergic pathway was lesioned with 6-OH-DA on one side of the brain. The rats were first perfused during 1 minute with a phosphate buffer solution containing 30% sucrose and their brain immediately frozen in liquid nitrogen. Brains were sectioned at 16 µm with a cryostat and the sections containing the striatum were collected on membrane-coated slides and fixed 10 min at -20 C in 95% ethanol-5% acetic acid. The sections then went though a rapid (20 minutes) immunostaining procedure to reveal striosomes, which are markedly enriched in µm-opiate receptors. The sections were sequentially incubated with: (1) µm-opiate receptor primary antibody during 10 minutes; (2) biotinylated anti-rabbit secondary antibody during 4 minutes; (3) avidin-biotin-peroxydase during 3 minutes; and finally (4) with DAB for 3 minutes. Striosomes and matrix compartments were dissected out with the help of a laser microdissection system (Leica AS LMD) and all portions of each striatal compartment on each section were collected in separated tubes. The RNA was then extracted and its quality assessed with an Aligent Bio-Analyzer. High quality RNA samples were then amplified and hybridized to rat complete genome cDNA arrays. The data obtained in control and in 6-OHDA lesioned rats are likely to shed a new light on the role of the striatal compartmentation in both normal and pathological conditions. It is hope that this approach will help identifying new avenues for the development of effective drugs to treat Parkinsonian disease.

166 B149

NORMAL AND ABNORMAL AGING ASSESSED USING OCULOMOTOR TASKS AND TESTS OF FRONTAL FUNCTION

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Alzheimer's disease (AD) is a neurodegenerative disease initially characterized by progressive memory loss and cognitive deterioration, often non-differentiable from the normal cognitive decline associated with aging and Mild Cognitive Impairment of the amnesic type (MCI). MCI is an

isolated memory deficit with normal scores in all other areas of cognition and normal daily function. Approximately 10% per year will go on to develop the symptoms of AD. The distinction between AD and MCI patient groups needs to be examined to determine if there are predictors of the conversion from MCI to AD. We compared the ability of 77 normal elderly, 14 AD, and 19 MCI patients to perform two saccadic eye movement tasks: a pro-saccade task (automatically look towards a target) and an anti-saccade task (inhibit the automatic response and instead initiate a voluntary saccade away from the target). In healthy aging, saccadic function decreased with aging (60-90 yrs), such that subjects became slower and performed with a higher proportion of errors in the anti-saccade task (i.e. initiated erroneous pro-saccades). We hypothesized that AD and MCI saccade performance would also decline with age, but in such a way that both patient groups should exhibit more severe impairments relative to controls. We also predicted that a measurable discrepancy would exist between these two populations. Saccadic reaction times (SRT) were much more variable amongst both AD and MCI patient groups relative to controls. On anti-saccade trials, the occurrence of direction errors was higher in AD and MCI suggesting an impaired ability to inhibit automatic visually-triggered saccades, and thus decreased cognitive control. Furthermore, the variability in SRT and the proportion of short-latency saccades differed between MCI and AD groups. The increased error rates and variability in SRT also correlated with altered cognitive functions, as assessed by various psychometric tests (i.e. Stroop, Wisconsin Card Sorting Test). These data provide insight into how the normal and abnormal aging processes differentially affect the neural circuitry controlling saccade initiation and saccade suppression. This also allows us to explore potential brain changes in healthy aging, and how they relate to the dysfunction in AD and MCI. Moreover, using quantitative saccade measures in combination with neuropsychological assessment may allow us the opportunity to differentiate the two disorders.

167 B150

ANALYSIS OF MITOCHONDRIAL AND LYSOSOMAL AXONAL TRANSPORTS IN CULTURED DRG NEURONS WITH VARIOUS NEURONAL CYTOSKELETON DISORGANIZATIONS

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Abnormal accumulations of intermediate filaments (IFs) are a pathological hallmark of many human neurodegenerative disorders including amyotrophic lateral sclerosis, Parkinson's disease, Charcot-Marie-Tooth and giant axonal neuropathy. Several studies provided evidence that disorganization of the neuronal IF network may be directly involved in neurodegeneration. However, the exact molecular mechanisms underlying the deleterious effects of IFs disorganization remain elusive. We postulate that changes in the organization of IFs can influence the fast axonal transport machinery either through levels of motor proteins or block of cargoe movement. To test this hypothesis, we analyzed the axonal transport of mitochondria and lysosomes in cultured dorsal root ganglion (DRG) neurons from different mouse models with various abnormalities of the neuronal cytoskeleton, including NFL knockout mice, which are characterized by the absence of axonal neurofilaments, gigaxonin-deficient mice, which develop IF accumulation in specific region of the nervous system, mice overexpressing peripherin (Per mice) that develop a motor neuron disease characterized by the presence of IF inclusions, and finally Per NFL-/- mice in which the onset of peripherin-mediated disease is precipitated. We used time-lapse microscopy to measure the movement of mitochondria and lysosomes in cultured DRG neurons after staining with respectively MitoTracker Red CMX-Ros and LysoTracker Red DND-99. We also analyzed by Western-Blot the expression of proteins involved in axonal transport (KIF1A, KIF5A, dynein, NUDEL and p150glued) in the sciatic nerve and in the optic nerve from the different mice. Preliminary results indicate that these alterations of the neuronal cytoskeleton induce no major modifications of the mitochondrial and lysosomal axonal transport. These organelles are distributed along the entire length of the axons and are transported in anterograde and retrograde directions. However some subtle changes are observed, such as a significant modification of the mean velocity of transport in some mice. Moreover, the protein analysis shows that the levels of some proteins involved in axonal transport are modified. These results suggest that the modifications of the neuronal cytoskeleton analyzed here have a moderate effect on mitochondrial and lysosomal axonal transport in DRG neurons in vitro.

168 B151

TASK-RELATED PUPILLARY ACTIVATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by cognitive decline, slowing of voluntary movements, choreiform movements and affective disturbances. Most HD symptoms are believed to be a consequence of neuronal depletion of the striatum. In two studies we use task-related pupillary responses during auditory reaction time tasks to explicitly examine attentional processes in patients in the early stages of HD and age-matched controls. Pupil size was measured with a head-mounted infrared camera. In a first study, participants had to make rapid keypress responses after hearing letters A or B presented as singletons (simple responses) or triplets (sequential responses). In a second study, a third condition was added where patients had to inverse their keypress responses (inverted simple responses), adding attentional load to the task. Our results show that patients were slower and made more errors in inverted and sequential responses than controls. There was no difference between groups in baseline pupil size. However, the amplitude of the pupillary response was smaller in HD patients than in controls and it also increased less with task difficulty. These results suggest that HD affects the recruitment of brainstem activation systems in relation to task demands. This research was supported in part by grants from the Canadian Institutes of Health Research and the Huntington's Disease Society of America.

169 B152

EFFECTS OF CHRONIC BUPRENORPHINE TREATMENT ON LEVELS OF NUCLEUS ACCUMBENS DOPAMINE AND GLUTAMATE IN COCAINE-SENSITIZED AND NON-SENSITIZED RATS

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Buprenorphine (BUP) is a partial mu-opioid receptor agonist currently being used as a maintenance treatment for opiate dependence. Previous work in our lab has shown that in rats with a history of cocaine self-administration, chronic BUP treatment prevents the reinstatement of cocaine seeking by a priming injection of cocaine and reduces cocaine self-administration, but potentiates the nucleus accumbens (NAc) dopamine (DA) response to an acute injection of cocaine. In a continuing effort to understand the effects of BUP on responses to cocaine, we investigated the effects of chronic BUP treatment on NAc DA levels and on locomotor activity in rats pre-exposed to repeated injections of cocaine. Given previous reports showing that basal levels of glutamate (GLU) in the NAc are reduced after repeated exposure to cocaine, we also examined the effects of chronic BUP treatment on NAc GLU levels. Male Long Evans rats were given daily injections of cocaine or saline for 7 days. On days 1 and 7, cocaine (15 mg/kg; i.p.) or saline was given in the testing environment; on days 2-6, cocaine (30 mg/kg; i.p.) or saline was given in the home cage. Following an 18-20 day withdrawal period, rats had BUP-containing osmotic minipumps (3 mg/kg/day) surgically implanted, or underwent sham surgery. Three days later, all rats

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

were brought to the testing environment where locomotor activity was monitored and extracellular levels of NAc DA were assessed using in vivo microdialysis and HPLC. Ten min baseline samples were collected for 1 h. Rats then received an injection of cocaine (15 mg/kg; i.p.) and samples were taken for 2 h. The cocaine-induced increase in DA levels was enhanced in cocaine pre-exposed rats, and BUP greatly potentiated this sensitized response. The cocaine pre-exposed rats also showed a sensitized locomotor response to the cocaine challenge, but paradoxically, BUP did not potentiate and even reduced this behavioral response. To explore the basis of this finding, we studied the effect of chronic BUP on NAc levels of GLU before and after an acute injection of cocaine (15 mg/kg; i.p.). Preliminary results show that basal levels of GLU were enhanced in BUP-treated rats, but cocaine had no effect. While it remains to be seen how BUP affects GLU levels in cocaine-sensitized rats, these higher basal levels of GLU induced by BUP could possibly affect responses to cocaine in cocaine-sensitized rats. Support Contributed By: Canadian Institutes of Health Research

170 C126

HYPERMETHYLATION OF THE GABAA RECEPTOR ?1 SUBUNIT IS ASSOCIATED WITH ALTERED DNA METHYLTRANSFERASE EXPRESSION IN THE BRAINS OF DEPRESSED SUICIDE VICTIMS

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Epigenetic mechanisms may be involved in long-term gene expression programming responsible for stressor reactivity. To assess the possibility, that major depressive disorder (MDD) may be similarly associated with epigenetic phenomena we examined the expression of DNA methyl transferase (DNMT) mRNA in several brain regions of MDD patients who had committed suicide. We found that DNMT gene transcripts were deregulated in several brains regions, including fronto polar cortex, amygdale and paraventricular nucleus of the hypothalamus. In addition, although transcript abundance of various forms of DNMT was highly correlated in normal controls, this coordination of DNMT isoform expression was diminished in suicide brain. Next we examined gene specific aberrations in DNA methylation in the frontopolar cortex the GABAA receptor ?1 subunit promoter region, whose transcript is under-expressed in suicide/MDD brains. Indeed, three CG sites were hypermethylated relative to controls. These data suggest that epigenetic mechanisms may alter gene expression in suicide/MDD.

171 C127

HOW MUCH CAN EMBRYONIC CORTEX-DERIVED NEUROSPHERES TRANSPLANTATION LEAD TO NEURONAL REPLACEMENT OR PARTIAL RECONSTRUCTION OF DAMAGED PERFORANT PATHWAY AND FUNCTIONAL RECOVERY?

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Neural transplantation is an attractive strategy for the replacement of neurons that have been lost because of neurodegenerative processes, vascular insults or axonal lesions. However, the effectiveness and widespread application of this approach clinically has been limited primarily because of ethical concerns and poor donor supply of human fetal neural tissue. Neurospheres have emerged as a possible donor material, particularly because they can be expanded in vitro and have a good tendency to differentiate into neurons. The purpose of this study was to assess the short and long-term survival, migration, differentiation and axonal outgrowth of neurospheres transplanted into the mouse brain. Cortex-derived neurospheres were prepared from embryonic day 15 transgenic mouse embryos expressing green fluorescent protein, and transplanted into an intact or lesioned entorhino-hippocampal formation of young adult mice. One week after transplantation, grafted cells closely attached to the host tissue and after two and three weeks a large number of grafted cells had survived and differentiated into neurons. That was confirmed by expression of neuron specific marker SMI31. After transplantation into the intact brain, short processes emerged from the transplant, which did not reach the hippocampal formation. In contrast, when neurospheres were transplanted into a host brain in which the entorhino-hippocampal projection was mechanically lesioned the neurosphere-derived cells showed a directed migration towards the dentate gyrus of the hippocampus and, in several cases, extended a strong axonal projection towards the dentate gyrus. Some of these fibers did, however, stop at the level of the hippocampal fissure and some of the outer molecular layer and of the dentate gyrus was observed. Just three weeks after transplantation of cortex-derived neurospheres immunostaining with anti-synaptophysin as a presynaptic marker revealed strong expression in the termination area of the newly developed Tau-GFP axons within the host hippocampal part indicating the formation of synapses in this area. Our results show that transplantation of immature neural cells cannot only be beneficial by supporting the local neurons in the host brain and inducing neural plasticity, but that such transplants might indeed be able to replace a degenerated or axotomized neuronal population with the restitution of the original axonal projection.

172 C128

HUMAN PROSTAGLANDIN H SYNTHASE (HPHS)-2-DEPENDENT REACTIVE OXYGEN SPECIES (ROS)-MEDIATED CYTOTOXICITY CAUSED BY DOPAMINE, ITS PRECURSOR AND METABOLITES

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PHS-2 is implicated in neurotoxicity associated with neurodegenerative diseases but the mechanism is unclear. Neurotransmitters such as dopamine (DA), its precursor L-dihydroxyphenylalanine (L-DOPA) and metabolite dihydroxyphenylacetic acid (DOPAC) may serve as substrates for PHS-dependent bioactivation in the brain to free radical intermediates that generate potentially neurotoxic ROS, as shown using purified ovine PHS or microsomal preparations. However, few studies have evaluated human PHSs in intact cells. CHO-K1 cell lines stably expressing hPHS-2 and untransfected CHO-K1 cells were used to investigate hPHS-2-dependent cytotoxicity. Cells were activated with arachidonic acid (AA), and PHS-2 activity measured by production of prostaglandin E2 (PGE2) using a PGE2 enzyme immunoassay. PHS activity in AA activated cells was up to 40-fold higher in hPHS-2 cells compared to untransfected CHO-K1 cells; activities were reduced without AA ($p<0.01$). The role of hPHS-2 in neurotransmitter-mediated cytotoxicity was measured by the release of the lactate dehydrogenase enzyme (LDH). hPHS-2 cells incubated for 6 hr with DA, L-DOPA, DOPAC and homovanillic acid (HVA) showed an increase in LDH release compared to the vehicle control ($p<0.001$). Cytotoxicity was increased further by AA-activation ($p<0.05$) while cytotoxicity was lower in untransfected CHO-K1 cells ($p<0.05$). hPHS-2 cells were preincubated with 250 U/ml of polyethylene glycol-conjugated catalase (PEG-catalase), which detoxifies ROS, and then treated with DA, L-DOPA or DOPAC at 1000 uM for 6 hr with or without AA. PEG-catalase reduced the cytotoxicity of DA, L-DOPA and DOPAC in hPHS-2 cells both with and without AA ($p<0.001$). These results suggest the cytotoxicity in hPHS-2 cells treated with DA, L-DOPA and DOPAC, and the enhanced effect of AA activation, are mediated by ROS. Further, endogenous neurotransmitters, their precursors and metabolites, can generate ROS by PHS-2-catalyzed bioactivation, so CNS hPHS-2 expression may contribute to cytotoxicity. This mechanism may be

involved in neurodegenerative changes associated with aging and drugs like amphetamines, which initiate neurotransmitter release. Since the cytosolic concentrations of precursors and metabolites are often higher than that of the neurotransmitters, bioactivation of the former may contribute substantially to PHS-catalyzed oxidative macromolecular damage and cytotoxicity in vivo. [Support: CIHR, CIHR/Rx&D HRF].

173 C129

CERULOPLASMIN IS PROTECTIVE AFTER SPINAL CORD CONTUSION INJURY

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Despite being essential for a number of metabolic processes, Iron (Fe2+) is also a potent generator of free radicals that produce tissue damage. The processes and mechanisms that control its levels in the CNS are therefore especially important in cases involving trauma, hemorrhage and inflammation. Ceruloplasmin (Cp) is a ferroxidase that converts toxic ferrous iron to its non-toxic ferric form. We have previously reported that a GPI-linked form of this enzyme is the major form of Cp in the CNS and is expressed by astrocytes. GPI-Cp is also necessary for iron efflux from astrocytes in vitro. Here we have assessed the role of Cp in preventing iron-mediated secondary damage after spinal cord contusion injury (SCI). We report that iron accumulates at the lesion site after injury and that there is greater accumulation in mice deficient in Cp. The physiological importance of Cp is underlined by the fact that locomotor functional recovery is significantly impaired in Cp-/- mice. Fewer motor neurons survive in the spinal cords of Cp-/- mice and there is evidence of greater demyelination at the lesion site. In addition, a number of other biological markers of oxidative and nitrosative damage markers are increased in the injured spinal cords of Cp -/- mice. Finally, the treatment of wildtype injured animals with an iron chelator SIH, promoted functional recovery, indicating that iron contributes to continued SCI pathology. The results we will present indicate that there is a co-ordinated iron-homeostatic response to SCI in wildtype mice, which is dysregulated in mice deficient in Cp. There is a cell specific upregulation of proteins involved in the trafficking (DMT1 and FPN1) and the storage of iron (ferritin) after SCI, suggesting that astrocytes and macrophages at the lesion site play distinct roles in this response. Taken together our data provides evidence for an iron-homeostatic response to SCI, in which ceruloplasmin plays an integral role. Ceruloplasmin is crucial in curtailing iron-associated damage after SCI. Importantly, treatment with an iron chelator is effective in promoting functional recovery in wildtype mice, suggesting that the iron-homeostatic response is amenable to pharmacological intervention.

174 C130

EFFECTS OF ONDANSETRON AND URB597 ON THE EXPRESSION OF LITHIUM-INDUCED ANTICIPATORY NAUSEA IN RATS

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Cancer patients undergoing chemotherapy treatments often report anticipatory nausea (AN) when they are re-exposed to contextual cues previously paired with treatment. AN is resistant to treatment with classical anti-emetic drugs such as ondansetron (OND). Although rats do not possess the mechanism to vomit, they do display conditioned gaping reactions elicited by an odour-laced context, previously paired with lithium-induced sickness—serving as a rodent model of AN. The principle consitituents in marijuana, psychoactive ∆9 –Tetrahydrocannabinol (∆9-THC) and non-psychoactive cannabidiol (CBD), are both effective in suppressing conditioned gaping in this model. URB597 prolongs the action of the endogenous cannabinoid anandamide (AEA) by inhibiting fatty acid amide hydrolase (FAAH), which rapidly deactivates AEA. Here, we present evidence that OND is ineffective in reducing the expression of conditioned gaping (Experiment 1), while pre-treatment with two doses of URB597 (0.1 and 0.3 mg/kg) suppressed the expression of conditioned gaping (Experiment 2) elicited by an odour-laced context, previously paired with lithium-induced sickness, as a model of AN in rats.

175 C131

ALTERED LYSOPHOSPHATIC ACID-STIMULATED CALCIUM RESPONSES IN BIPOLAR DISORDER PATIENTS SUGGESTS TRANSIENT RECEPTOR POTENTIAL CHANNEL DYSFUNCTION

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Introduction: Chronic treatment with lithium attenuates lysophosphatidic acid (LPA, 100 μM) stimulated calcium (Ca2+) responses in B-lymphoblast cell lines (BLCLs), but the mechanism(s) involved and whether there is an impairment of this response in bipolar disorder (BD) patients is uncertain. LPA shares a structurally similar fatty acid side chain with the cell permeable diacylglycerol analogue, 1-oleoyl-2-acetyl-sn-glycerol (OAG), which is a known agonist of subtypes 3, 6 and 7 of the canonical transient receptor potential (TRPC) cation channel subfamily. Accordingly, the objective of this study was to characterize the involvement of this TRPC channel subfamily in the LPA-stimulated Ca2+ response of BLCLs and the extent to which this response is altered in cell lines from BD patients as compared with controls. Methods: TRPC subtypes expressed in BLCLs were determined by immunoblotting and RT-PCR. To distinguish the TRPC-like character of Ca2+ responses in BLCLs, divalent cation (barium versus Ca2+) selectivity of thapsigargin (TG)-, LPA-, and OAG-activated responses was assessed. Gadolinium sensitivity was used to determine the store-operated nature of the responses. The phospholipase C (PLC) dependence of agonist responses was assessed by a PLC inhibitor, U73122. The magnitude and rate of LPA-induced Ca2+ mobilization was measured in BLCLs from 42 patients with a DSM-IV diagnosis of BD-I and in 25 healthy control subjects. Results: Only TRPC1, 3 and 5 are expressed in BLCLs. Significant barium influx, of a magnitude similar to OAG-mediated responses, occurred upon addition of LPA, but not TG. TG-provoked Ca2+ influx was completely inhibited by 15 μM gadolinium, whereas LPA-stimulated responses were minimally affected. Gadolinium (≤100 μM) pre-treatment potentiated the OAG-activated Ca2+ response. Cell lines from BD-I patients showed an enhanced (40%) rate of rise of intracellular Ca2+ levels ([Ca2+]i) (F1,63=5.2, p=0.03) but not magnitude of the response (F1,63=1.0, p=0.3) to 100 μM LPA compared with controls.
Conclusions: The results suggest that LPA stimulates Ca2+ entry through channels with characteristics similar to the TRPC3 subfamily in BLCLs. Thus, the findings of an enhanced LPA-stimulated rate of rise in [Ca2+]i from BD compared with healthy subjects may implicate dysfunction of TRPC3-like Ca2+ entry in the pathophysiology of BD.

176 C132

NTS1-PREFERRING AGONISTS PRODUCE SPINAL ANTINOCICEPTION IN A FORMALIN TONIC PAIN MODEL

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Neurotensin (NT) is neuropeptide that mediates, through three receptor subtypes (NTS1-3), a variety of central and peripheral functions including opioid-independent antinociception. Recent studies, using knock-down

strategies, show that NTS1 is involved in acute pain. However, little is known about the role of NTS1 in a model of persistent pain. The aim of the present study was to determine whether intrathecal administration of NT or NTS1 agonists, PD149163 and NT69L, induced antinociceptive responses in the formalin test, a model of tonic pain. All compounds were administrated intrathecally 5 minutes before the injection of 2% formaldehyde into the plantar surface of the right hindpaw. Intrathecal administration of NT (0.1-15 g/kg) causes a dose-dependent inhibition of nociceptive behaviours in phase I (0-9 min) and phase II (21-60 min) of the formalin test. Both NTS1-agonists, PD149163 (10-120 g/kg) and NT69L (0.1-100 g/kg), dose-dependently attenuated the formaline-induced behaviours. PD149163 and NT69L decrease tonic pain up to 60% and 48% respectively and their effects were more important than that of NT. Also, the co-treatment of PD149163 or NT69L and the NTS1 antagonist SR48692 markedly reversed both PD149163- and NT69L-induced antinociception demonstrating the implication of NTS1 in tonic pain. A C-Fos immunohistochemistry study on spinal cord of the tested rats confirm our behavioural results by demonstrating a reduction of neuronal activity corresponding to the antinociceptive response induced by the NTS1 agonists. Depending of the spinal laminae observed, the neuronal activity decreased up to 44% and 48% for NT69L and PD149163 respectively. These results demonstrate that NTS1 receptors are involved in the mediation of the analgesic effects of NT in persistent pain and suggest that NTS1-selective agonists may represent a new line of analgesic compounds. (Supported by CIHR, FRSQ, and NIMH)

177 C133

IN DEPTH ANALYSIS OF GRAFT IMPLANTS IN A HUNTINGTON'S DISEASE PATIENT: LEAD INTO LONG-TERM GRAFT SURVIVAL, DEVELOPMENT AND CONNECTIVITY

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Ten years ago, the first clinical trial using bilateral transplantation of fetal neural tissue in Huntington's disease (HD) patients was conducted at University of South Florida. In this series, one patient died 18 months after transplantation of causes unrelated to the surgical procedures. The post-mortem analysis was reported in 2000 and showed that six out of ten grafts survived and were not affected by the disease process. Recently, a second patient of the initial trial died, nine years post-surgery. This unique case provides the opportunity to answer important questions related to 1) the survival and neuronal organization of implants 9 years post-surgery, 2) graft health from an immunological and pathological perspective and 3) the integrative and functional connectivity of the graft within the host. Thus far, we have performed a 3D computerized reconstruction on 80% of the grafted tissue, the remaining 20% is currently being processed for electron microscopy (EM) and DiI tracing to investigate graft-host connectivity. Five graft sites have been identified in each hemisphere; which contain striatal markers such as calbindin, a marker of striatal projection neurons and calretinin, found in a sub-population of striatal interneurons. Results also indicate that the graft is almost entirely devoid of an astrocytic and macrophagial response, as assessed by the markers GFAP and HLA-DR, and is not affected by the pathology as demonstrated by the absence of EM48 (abnormal human huntingtin protein) and ubiquitin (nuclear inclusions) staining. Evaluation of this HD case will help provide knowledge regarding long-term graft survival and functionality. This new information is critical, particularly if such therapies will be utilized in young patients with an expectation of lifetime benefit.

178 C134

NEUROTOXICOLOGICAL CHARACTERIZATION OF MDMA-ANALOGS IN VITRO: IMPLICATIONS FOR THE SYMPTOMATIC TREATMENT OF PD

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The recreational drug ecstasy, 3,4-methylenedioxymethamphetamine (MDMA), has been shown to prolong the actions of L-DOPA while suppressing L-DOPA induced dyskinesias in both rodent and primate models of Parkinson's disease (PD). Despite the obvious implications of these findings, i.e. that MDMA may be of therapeutic value to PD patients, MDMA is toxic to serotonergic and dopaminergic neurons in rodents and cell culture systems. Furthermore, anecdotal evidence suggests that chronic use of MDMA may induce parkinsonism by increasing expression of alpha-synuclein and neuronal inclusions. A compound possessing the therapeutic effects of MDMA without associated neurotoxic and psychotropic properties would be an attractive candidate for development in the symptomatic treatment of PD. We therefore examined the neurotoxicity of several MDMA analogs bearing different substituents at the alpha-position. The effects of MDMA analogs ATK-0001, ATK-0090, ATK-0091, ATK-0101, ATK-0102, and ATK-0104 on cell viability were determined using AlamarBlue fluorescence assays. The catecholaminergic neuroblastoma cell line, SH-SY5Y, was exposed to full dose response curves (1μM-600μM) of each analog for 24 hours. MDMA significantly reduced cell viability by 73±6.6% at 600μM ($P<0.01$, ANOVA, Dunnett's comparisons post-hoc. (n=6)). Analogs ATK-0001 (600μM), ATK-0090 (30μM), and ATK-0102 (600μM) significantly reduced cell viability as well, though not to the extent of that of MDMA (43±3.4%, 12±2.6%, and 36±2.0%, respectively, $P<0.01$, ANOVA, Dunnett's comparisons post-hoc. (n=6)). Analogs ATK-0091 and ATK-0101 minimally reduced viability (11±2.4% and 15±0.9%, respectively) at 600μM ($P<0.01$, ANOVA, Dunnett's comparisons post-hoc. (n=6)). Analog ATK-0104 did not decrease cell viability at any concentrations examined, and indeed induced insignificant increases in cell proliferation. Given that MDMA analogs ATK-0091, ATK-0101 and ATK-0104 appear not to be neurotoxic in these studies, these compounds may warrant further investigation as symptomatic treatments for PD.

179 C135

COMPLEX PHARMACOLOGICAL EFFECTS OF BIBN 4096BS IN SUPRASPINAL CGRPRELATED BEHAVIOURS IN MICE

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Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide widely expressed throughout the central nervous system and enriched in dorsal root ganglion nociceptive neurons where it contributes to pain transmission. Although little is known about the effects of supraspinal CGRP, animal behavioural data and human clinical biochemistry point towards a contribution of CGRP to depression- and anxiety-related behaviours. This hypothesis is supported by the strong interaction between the CGRP and dopaminergic systems. To explore the contribution of CGRP to centrally mediated behaviours, we performed intracerebroventricular (i.c.v.) injections of CGRP and a non-peptidergic CGRP receptor antagonist, BIBN 4096BS, in C57BL/6 mice. Mice were tested for thermal pain thresholds using the paw-withdrawal test (PWT), or screened for anxiety- and depression-like behaviour using the elevated plus maze (EPM) and the forced swimming test (FST), respectively. In the PWT, CGRP increased latencies whereas BIBN 4096BS decreased latencies. In the EPM, BIBN 4096BS significantly

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

increased the number of open arm entries (OAE) and the time spent in open arms (OAT). CGRP showed a non-significant tendency to increase OAE, but not OAT. However, in the FST, both CGRP and BIBN 4096BS significantly decreased non-swimming time.
These results suggest that BIBN 409BS acts as a CGRP antagonist in supraspinal pathways involved in pain sensation, whereas it likely acts as a CGRP receptor agonist within brain circuits involved in anxiety- and depression-like behaviour. In this context, both molecules display an anxiolytic and antidepressant-like action in mice. This work has been supported by grants from CIHR and NIH, and a DAAD scholarship to A.S.P.

180 C136

INTERACTIVE EFFECTS OF PERINATAL AND POST-WEANING DIETS ON AMPHETAMINE-INDUCED LOCOMOTION IN THE ADULT OFFSPRING

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In rats, maternal diet during the perinatal period and lactation can change both metabolism and mesolimbic dopaminergic function in the adult offspring. However, the nature of the change in dopaminergic function is not clear. One study reports that a high-fat maternal diet during late gestation and lactation results in increased sensitization to amphetamine-induced hyper-locomotion, while a second study reports reduced amphetamine-induced locomotion. Moreover, there are indications for an interaction between the effects of maternal diet and post-weaning pup diet on dopaminergic function. Here we tested the effects of perinatal exposure to a highly palatable, high energy (HE) diet on sensitivity to amphetamine-induced hyper-locomotion, in offspring that were subsequently exposed to control (C) or HE post-weaning diet. Dams were fed C or HE diet throughout gestation and lactation. Male pups from both groups were weaned at postnatal day (PND) 23, and maintained on HE or C diet until PND 60-65. Rats were then tested for psychostimulant-induced hyper-locomotion using low (0.5 mg/kg) and high (1 mg/kg) doses of amphetamine. Post-weaning exposure to HE diet resulted in an increased body weight and percent body fat on PND 80. In concordance with previous observations, adult offspring of HE mothers weighed less than offspring of C-diet dams. There were no differences between the diet treatment groups in spontaneous or saline-challenge-induced locomotor activity. In addition, all groups showed the expected dose-dependent increase in locomotion following exposure to amphetamine. However, pups from HE mothers that were maintained on HE diet displayed a significantly greater increase in locomotion following exposure to the low dose of amphetamine, compared to the other diet groups. No differences in hyper-locomotion were observed between the diet groups following exposure to the high dose of amphetamine. In conclusion, we found that maternal diet has long-lasting effects on the bodyweight of the offspring. Furthermore, our findings imply that maternal diet interacts with post-weaning maintenance diet to induce persistent adaptations in mesolimbic dopaminergic system function in the offspring. Such adaptations may indicate a higher risk for pathologies such as obesity and drug abuse, in the adult offspring. Support contributed by: CFI, CRC (to US), CIHR (to BW)

181 C137

HSN2 OPEN READING FRAME ENCODES A NOVEL EXON FOR WITH NO LYSINE PROTEIN KINASE 1 (WNK1)

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Hereditary sensory and autonomic neuropathies (HSANs) are a group of clinically heterogeneous diseases. They are characterized by loss of pain, pressure and thermal sensation often associated with ulceration of the feet and hands with mutilation of fingers, toes, as well as Charcot's joint disease.

HSAN type II is an autosomal recessive disorder with the onset of symptoms in infancy or early childhood. This would suggest congenital onset mostly involving the distal portions of upper and lower limbs and absence or diminution of tendon reflexes. Recently, linkage analysis mapped the HSAN II locus to chromosome 12p13.33, where three different protein truncating mutations were identified in a predicted novel open reading frame embedded within intron 8 of the PRKWNK1 gene. By screening the HSN2 gene in one of our patients, we identified a heterozygous nonsense mutation in HSN2 ORF. This mutation was carried by the patient and his asymptomatic brother, and inherited from the father. No additional mutation was detected in the ORF of the predicted gene. We therefore screened the entire coding sequence of WNK1 and found a second nonsense mutation located in exon 6 of the gene. The mutation identified in the patient was inherited from the mother and was absent in the unaffected brother. RT-PCR and RACE experiments using adult mouse nervous system RNA showed fusion of HSN2 ORF with exons 8 and 9 of PRKWNK1 gene. Western blots, using an antibody against HSN2 ORF, detected a ~ 200 KD protein in the adult mouse nervous tissues. Using Northern blot analysis with a specific probe for HSN2 ORF, a ~ 9-10 kb band was also detected in RNA preparations from the adult mouse nervous tissues. We have carried out experiments using immunohistochemistry and in situ hybridization and have concluded that the WNK1/HSN2 protein is highly enriched in the peripheral nervous system and is expressed by satellite glia cells in DRGs, neurons, and Schwann cells in the adult mouse peripheral nervous system.

182 C138

HIPPOCAMPAL SITE(S) OF ORIGIN FOR HYPOGLYCEMIC SEIZURES

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Severe hypoglycemia (HG) is a detrimental consequence of diabetic therapy. A common result of HG episodes is the development of seizures, occurring most frequently in children with type I diabetes mellitus. At present, the underlying mechanism and site(s) of origin for HG-induced seizures have not been elucidated. In this study, we examine HG-seizures in both the in vivo and in vitro setting. Firstly, we have established a novel in vivo juvenile murine model that exhibits insulin-induced HG seizures with a consistency of over 80%. EEG recordings have demonstrated seizure-like activity occurring in both the hippocampus and cortex concurrently with behavioral manifestations of HG-seizures. Furthermore, we used dual extracellular field recordings to observe the hippocampal site of origin for HG-induced seizure-like activity. Our in vitro preparation consisted of 500um and 800um-thick horizontal murine hippocampal slices, where we decreased ACSF glucose from 5mM to 0.5mM. We observed that interictal and ictal discharges induced by HG first appear in either the CA3 or CA1 region, followed by the dentate gyrus. Pharmacological manipulation using AMPA/kainate, NMDA, and adenosine antagonists was also studied. Our in vivo and in vitro results help elucidate an origin for HG seizures, which will allow focused investigation upon the cellular mechanisms responsible for HG seizure generation.

183 C139

INTRAVENTRICULAR INFUSIONS OF PROPIONIC ACID REVERSIBLY IMPAIR SOCIAL BEHAVIOR AND INDUCE NEUROINFLAMMATORY CHANGES IN LONG EVANS RATS

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BClinical observations suggest that certain dietary factors may transiently worsen symptoms in autism spectrum disorders (ASDs). Propionic Acid (PPA) is a short chain fatty acid, an enteric bacterial by-product, and a common food preservative. We have recently found PPA can elicit neurobehavioral, electrophysiological and neuropathological changes in rodents consistent with human ASDs. PPA may thus be a possible target compound linking diet, gastrointestinal physiology and behavior. The present study examined the effects of repeated intraventricular infusions of PPA using a variety of behavioral tasks. Adult Long Evans Hooded rat pairs were infused with 4µl of either PPA or PBS vehicle into the lateral ventricle. Rat pairs were then immediately placed in an open-field arena for 1 hr during the light phase and for 1 hr during the dark phase in counterbalanced order. Observations of social behaviour were quantified using Ethovision software. Following social testing, animals were tested on the balance beam task and in the water maze to evaluate other behavioral deficits. Following behavioral analyses, animals were sacrificed and brains were examined immunohistochemically using a variety of neuroinflammatory markers. PPA-injected rat pairs reported impairments in social behaviour when compared to control pairs. These effects were transient and attenuated within approximately 30 min post-infusion. Furthermore, PPA rats were impaired in the water maze and balance beam tasks in a manner reminiscent of human ASDs. Neuropathological analysis of rat hippocampus and white matter showed reactive astrogliosis (GFAP) and activated microglial (CD68) response with increased blood brain barrier permeability (IgG). In conclusion, PPA infusions induce reversible behavioral impairments and neuroinflammatory changes consistent with human ASDs.

184 C140

DSCR1 FACILITATES NEURONAL APOPTOSIS

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Individuals with Down syndrome (DS) will inevitably develop Alzheimer's Disease (AD) neuropathology after their middle age, to which genes triplicated in DS may be attributable. The characteristic AD neuropathology includes neuritic plaques, neurofibrillary tangles and neuronal loss in the brains. The mechanism underlying neurodegeneration in AD and DS remains elusive. Down Syndrome Critical Region 1 (DSCR1) gene locates on Chromosome 21 and has been implicated in the pathogenesis of DS. Our data show that DSCR1 expression is elevated in the cortex of DS and AD patients. DSCR1 expression can be activated by dexamethasone and calcium stress respectively. Overexpression of DSCR1 in primary neurons activates induces apoptosis. The neurotoxicity of DSCR1 is abolished by inhibition of DSCR1. Our data suggest that DSCR1 elevation in brain contributes to neuronal death and AD pathogenesis in DS.

185 C141

ANALYSIS IN G-PROTEIN BETA3 SUBUNIT GENE (C825T) POLYMORPHISM AS A CANDIDATE GENE TO ANTIPSYCHOTIC-RELATED WEIGHT GAIN

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Despite advances in schizophrenia treatment, nearly 30% of patients do not respond to atypical antipsychotic agents, such as olanzapine, clozapine and risperidone. Atypical antipsychotics have high affinity for many G-protein coupled receptors. Little research has been done to investigate the relationship between antipsychotic treatment outcomes and genetic variability in second messengers coupled to serotonin and dopamine receptors. The purpose of this investigation was to examine associations between the second messenger G-protein beta3 subunit gene (GNB3) C825T polymorphism and antipsychotics induced weight gain treatment. . The C825T polymorphism located at exon 10 of this gene has been shown to be associated with alternative splicing of exon 9 (variant with higher activity). We conducted a pharmacogenetic association study to examine GNB3 genotypes in relation to weight gain after 14 weeks of antipsychotic treatment. Subjects included fifty-nine individuals meeting DSM-IV criteria for schizophrenia treated with different antipsychotics subsequently genotyped for the C825T in GNB3 gene. No statistically significant associations existed between our outcome variable (percentage weight change) and GNB3 genotypes (p=0.175). These preliminary results showed no statistical relationship between the C825T polymorphism and weight gain. Numerical differences in weight change measures between the TT vs. CT/CC genotype groups indicate that G-protein second messenger systems variability coupled to primary targets of atypical antipsychotics may not relate to side-effect in persons with schizophrenia and that future studies in this area are warranted.

186 C142

SKIN-DERIVED PRECURSORS DIFFERENTIATED INTO SCHWANN CELLS PROMOTE REPAIR AND FUNCTIONAL RECOVERY FOLLOWING TRANSPLANTATION INTO THE INJURED RAT SPINAL CORD

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Contusive spinal cord injury (SCI) typically results in a large cavitation with a narrow rim of spared tissue at the injury site. The loss of tissue, coupled with demyelination of spared axons around the lesion, disrupts ascending sensory and descending motor tracts, leading to a loss of function below the level the injury. Natural repair mechanisms generally fail in this setting, so the functional impairment is often permanent and severely debilitating. Transplantation of exogenous cells is one potential therapeutic strategy for the treatment of SCI, but no cell transplant has yet demonstrated the ability to fully repair the injured spinal cord. We have previously described a multipotent skin-derived precursor (SKPs), exhibiting gene expression and differentiation properties characteristic of neural crest stem cells. In vitro, SKPs generate neural and mesodermal cell types and we have recently demonstrated that SKPs predifferentiated into Schwann cells (SKP-SCs) generate compact myelin in vivo. Here we investigated whether mouse naïve SKPs or SKP-SCs were capable of remyelination and repair of the contused adult rat spinal cord. In comparison to media injection and subventricular zone neurosphere transplants, the naïve SKP and SKP-SC transplants exhibited greater survival, increased myelination and axonal sparing/regeneration, as well as enhanced invasion by endogenous SCs. Naïve SKPs showed some mesodermal differentiation and limited integration with host tissue, making them poorly suited for therapeutic transplantation. In contrast, the SKP-SCs appear ideally suited to this role, as they bridged the lesion site in a rostro-caudal orientation and integrated well with host tissue to promote axonal sparing/regeneration of a functionally appropriate orientation. They also showed the most efficient myelination of host axons and the most extensive recruitment of endogenous SCs in the spared rim. In agreement with the histological evidence, rats receiving SKP-SCs showed improved hindlimb locomotor function in open field testing relative to all other treatment groups and no indication of lowered sensory thresholds. As these cells are harvested from skin, they are of little ethical concern, and are

ideal for autologous transplantation paradigms that circumvent the need for harmful long-term immunosuppression post-transplantation. In conclusion, SKP-SCs emerge as an excellent candidate for (potentially autologous) transplantation repair strategies following SCI.

187 C143

EXPERIENTIAL DETAILS, AND NOT TEMPORAL SPECIFICITY, DETERMINES AUTOBIOGRAPHICAL MEMORY IN PATIENTS WITH UNILATERAL TEMPORAL LOBE EPILEPSY OR EXCISIONS

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The ability to retrieve autobiographical memories (AM) is dependent on the functional integrity of the hippocampus (HC). Patients with unilateral temporal lobe epilepsy, involving dysfunction or lesions to the HC, are impaired at recalling autobiographical episodes (Addis, 2005; Viskontas et al., 2002; Voltzenlogel et al., 2006). While this impairment is clear for unique AMs, it is not clear that hippocampal damage results in an equal deficit in generic (repeated) AM recall. One study from our group using fMRI showed that the hippocampus is equally engaged during retrieval of both unique and generic personal episodes (Addis et al., 2004) and the objective of the current study was to assess the impact of hippocampal damage on these two types of AM. Patients with unilateral mesial temporal epilepsy (including both pre-surgical and post-surgical patients) were compared to healthy controls on a modified version of the Autobiographical Interview (AI) (Levine et al., 2002). AM narratives produced during the AI are broken down into details that are either bound or unbound to an episode; details are further classified into categories that reflect different aspects of the memory. Our version of the AI was adapted by constructing a parallel set of questions for generic personal events. Results indicate that both patients with right and left TL dysfunction reported fewer details pertaining to the recollected event, for both specific and generic personal events. The source of this deficit was the paucity of perceptual information about the personal events. These results suggest that the hippocampus plays a key role in the reconstruction of experience-near sensory-perceptual events. The similarity of the impairment between the episodic and the generic memory conditions also suggests that the hippocampus is not sensitive to the temporal specificity of the reconstructed personal events, which confirms our previous functional neuroimaging findings with healthy controls. References: Addis (2005). Unpublished PhD thesis, University of Toronto, Toronto, Canada.; Addis et al., (2004). NeuroImage, 23, 1460-71.; Levine et al. (2002). Psychology and Aging, 17, 677-689.; Viskontas et al. (2002). The Journal of Neuroscience, 20, 5853-5857. Voltzenlogel et al. (2006). Epilepsia, 47, 1329-1336

188 C144

MEDIATION OF DELAYED HIPPOCAMPAL NEURONAL DEATH FOLLOWING GLOBAL CEREBRAL ISCHEMIA BY TRPM7 CHANNELS.

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Stroke is a leading cause of morbidity and mortality in industrialized countries. We have recently described that TRPM7, a member of the Transient Receptor Potential channel superfamily, is important in mediating anoxia/reoxygenation damage in-vitro. To determine whether TRPM7 channels mediate ischemic cell death in-vivo. TRPM7 expression was suppressed in vivo using adeno-associated virus (AAV) vector –mediated gene transfer of shRNA to effect RNA interference in mature neurons. The AAV vectors were stereotactically microinjected into adult rat brains. Efficacy of gene silencing was tested in-vitro and in-vivo by immunohistochemistry (IHC) and by RT-PCR of transfected cells isolated by laser dissection microcapture. Cerebral ischemia was induced using the four vessel occlusion (4VO) model in adult Wistar rats transfected 7 days previously with the viral vectors. Suppression of TRPM7 channel expression was confirmed at the mRNA level in-vivo with the laser dissection microcapture followed by PCR, and at the protein level both in-vitro and in-vivo using IHC. After global ischemia by 4VO, CA1 neurons in which TRPM7 was suppressed exhibited significantly greater survival (395±40 vs 25±4 EGFP positive CA1 neurons) and less TUNEL staining (601 vs 2154 TUNEL positive CA1 cells) than controls, respectively (n=6/groups). The suppression of the TRPM7 channels was confirmed both in-vitro and in-vivo using PCR and IHC techniques. TRPM7 channels play an important role mediating neuronal cell death in global ischemia. These results may have broader significance to ischemic injury of other tissues due to the widespread expression of TRPM7 channels. (This study was funded by CIHR, NIH, CSN and Krembil Seed fund - MT, and H-SS: HSFC - FOS Fellowship.)

189 C145

EXCITOTOXIC NEUROPROTECTION IN AN IN VITRO MODEL OF THE ISCHEMIC CONTINUUM

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Drug(s) required to protect rat cortical neurons from increasingly longer durations of oxygen-glucose deprivation (an OGD 'continuum') were identified, using antagonists of NMDA (MK-801 or a glycine-site sensitive L689,560) or AMPA (CNQX, NBQX or SYM2206) receptors, voltage-gated Ca2+ channels (nifedipine, Ni2+ or an extracellular pH of 6.5) or compounds reported to prevent TRPM7 receptor-mediated neurotoxicity in mouse cortical neuron cultures (Gd3+, an nNOS inhibitor, 7-nitroindazole, or the antioxidants, trolox or a metalloporphyrin, MnTBAP [Aarts et al, 2003]), at maximally effective concentrations. Protection by a cocktail of MK-801 (2 μM)/CNQX (10 μM)/nifedipine (2 μM) against 1.5 h OGD was overcome if OGD was 2 h long. Increasing MK-801 to ≥12 μM protected neurons against 2, but not 2.5 h, OGD. Replacing MK-801 with L689,560 (200 μM) protected against 2.5 h OGD, with protection largely reversed by glycine, but not against 3 h OGD. Increasing CNQX to ≥ 100 μM in the L689,560/nifedipine cocktail protected against 3 h, but not 3.5 h, OGD. Replacing CNQX with NBQX (100 μM) and SYM2206 (30 μM) was protective at 3.5 h, but not 4 h, OGD. The MK- 01/CNQX/nifedipine cocktails used at 1.5-2.5 h OGD were augmented with different drugs: (i) To determine if NMDA receptors were blocked, NMDA (0.5-2 mM) was included. NMDA was not injurious but protective, perhaps due to AMPA receptor blockade. (ii) Augmentation with MnTBAP (200 μM), but not with Gd3+ (5-100 μM), 7-nitroindazole (150 μM) or Trolox (1 mM), was protective; however, inactive ZnTBAP (50 μM) or CoTBAP (25 μM), but not a potent antioxidant analog, MnTE-PyP(2) (200 μM), were protective. Hence, TRPM7 receptors may be involved only in mouse cortical cultures (Aarts et al, 2003). (iii) Augmentation with low extracellular pH (6.5) buffer or with Ni2+ (1 mM) was protective, perhaps due to NMDA receptor blockade. (iv) In intracellular Ca2+ (Ca2+i) measurements, only protective drugs suppressed Ca2+i elevations. Results suggest inadequate glutamate receptor block by MK-801/CNQX combinations, since replacement or augmentation with compounds known to block these receptors improved protection, although non-specific blockade of other receptors by the high concentrations of receptor antagonists used cannot be ruled out. Combination therapy with potent glutamate receptor and VGCC antagonists at high concentrations are required for Ca2+i-dependent neuroprotection under stringent OGD conditions.

190 C146

DELAYED CALCIUM DEREGULATION DURING NMDA EXCITOTOXICITY MEDIATED BY PANNEXIN CHANNELS

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Over activation of glutamate receptors leads to neuronal death and is a component of several neurodegenerative disorders including stroke, Alzheimer's disease and epilepsy. A key component of this cell death is thought to be a delayed calcium deregulation, manifested as the sustained influx of calcium that persists even after washout of glutamate receptor ligands. We recently reported that ischemia (oxygen / glucose deprivation) opens very large conductance cation channels, likely pannexin 1 (panx1) hemichannels and that this may be a key component of the ionic dysregulation observed during neuronal death (Thompson et al, 2006, Science 312:924). Here we tested the hypothesis that the delayed calcium deregulation upon activation of NMDA receptors is mediated by the opening of panx1 channels. We report that a cation current is activated in acutely isolated hippocampal neurons by either intermittent (10 s applications at 1 min intervals) or continuous (5-10 min) exposure to 100 µM NMDA. The current was inhibited by carbenoxolone, a known blocker of gap junctions and pannexin or connexin channels (hemichannels). Confirmation of the role of hemichannels was achieved by measuring dye flux across the plasma membrane of acutely isolated neurons and those in brain slices. In acutely isolated neurons, NMDA evoked an intracellular calcium increase as measured by Fluo-4 that persisted after NMDA removal. This delayed calcium deregulation preceded efflux of an inert dye, calcein red-orange, from the neuron. Both the delayed calcium deregulation and calcein efflux were inhibited by carbenoxolone. Influx of sulforhodamine 101 (SR101) into neurons in the CA1 region of hippocampal slices was activated by bath application of NMDA and this was blocked by carbenoxolone. We conclude that the delayed calcium deregulation evoked by NMDA receptor activation at excitotoxic concentrations of NMDA is mediated by hemichannel opening.

191 C147

INFUSIONS OF PROPIONIC ACID INDUCES INCREASED LOCOMOTOR ACTIVITY, NEUROINFLAMMATORY EFFECTS, FATTY ACID TRANSPORT, BLOOD BRAIN BARRIER AND NEUROPLASTIC CHANGES IN RATS FOLLOWING INTRAVENTRICULAR INFUSION-RELEVANCE TO AUTISM SPECTRUM DISORDERS

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Innate neuroinflammatory changes, alterations in fatty acid metabolism and blood brain barrier (BBB) permeability may be involved in the pathophysiology of autism spectrum disorders (ASDs). Furthermore pre or perinatal infectious processes, as well as dietary or digestive system factors have been suggested in ASD cause or symptom exacerbation. Propionic acid (PPA) is a short chain fatty acid, a by-product of many enteric bacteria, and a food preservative. PPA is actively transported by monocarboxylate transporters in the gut and CNS, and has widespread effects on cellular metabolism and immune function. We found PPA that can elicit behavioural and neuropathological changes in rodents and may be a model for ASDs. To evaluate the behavioural and neuropathological effects of chronic intraventricular PPA infusions in rodents, adult Long-Evans rats received twice daily intraventricular infusions (4µl/animal) of pH 7.5 buffered PPA, or .1M PBS vehicle for 14 consecutive treatment days. Immediately following ventricular infusion, animals were individually placed into an automated open field and locomotor activity assessed for 30min (Versamax). Following behavioural analyses, animals were sacrificed and brains examined immunohistochemically. PPA treated animals showed increases in locomotor activity and repetitive stereotypies. Immunohistochemical analyses of brain revealed an innate neuroinflammatory response (GFAP, CD68, IbA1) similar to that found in human ASD brain. Furthermore, PPA treated rat brain showed increased monocarboxylate transporter I immunoreactivity, and increased BBB permeability (IgG, rat whole serum). PPA treated animals also showed increases in PhosphoCREB and vimentin immunoreactivity, both indices of a neuroplastic response. PPA effects were not grossly neurotoxic as indexed by direct pyramidal cell counts and apoptotic markers (cleaved Caspase 3). Chronic PPA in rats increases locomotor activity, and induces neuroinflammatory, fatty acid transport, BBB, and neuroplastic changes, and may offer a putative animal model of ASD's. Sponsor: GoodLife Children's Charities

192 C148

THE ROLE OF DOPAMINE D1 RECEPTORS IN FOOD DEPRIVATION-INDUCED REINSTATEMENT OF DRUG-SEEKING

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Clinical research has demonstrated that among recovering drug addicts, exposure to stressful events can pose a significant a risk for relapse. Traditionally, stress-induced relapse has been studied using an animal model of footshock-induced reinstatement. More recently, however, it has been shown that acute food deprivation (FD) stress can reinstate previously extinguished drug-reinforced behavior. It has been suggested that stress-induced reinstatement of reward seeking is mediated by an increase in the incentive salience of cues previously associated with reward. This increase in incentive salience may be dopamine (DA) dependent. The role of DA in footshock-induced reinstatement is unclear. For example, only the mixed DA antagonist flupenthixol decanoate, and not the D1 receptor antagonist SCH 23390 or the D2 receptor antagonist raclopride, has been shown to attenuate footshock-induced reinstatement. Additionally, the involvement of DA in FD-induced reinstatement has not been investigated. Recently, leptin, a hormone involved in the long-term regulation of feeding, has been found to suppress FD-induced reinstatement while having no affect on footshock-induced reinstatement. This suggests that the neuronal mechanisms mediating footshock- and FD-induced reinstatement may be different. Here we investigated this possibility by studying the role of the D1 receptor in FD-induced reinstatement. Rats were trained to lever press for heroin over a period of 10 days (.05 mg/kg/infusion, i.v.; Days 1-5: three 3-h sessions, Days 6-10: one 3-h session). Drug seeking behavior was then extinguished for a minimum of 4 days. Subsequently, rats were tested twice, in a counterbalanced order, for reinstatement: following 48 h FD, and under food-sated conditions. Prior to each test, rats were injected with one of 3 doses of the D1 receptor antagonist SCH 23390 (0 μg, 5 μg or 10 μg/kg, s.c.). It was found that SCH 23390 dose-dependently attenuated drug-seeking behavior when animals were food deprived, yet had no effect on responding when animals were not food deprived. This finding supports a role for DA in FD-induced reinstatement of drug seeking behavior.
Support contributed by: CFI, CRC (to US)

193 C149

MORPHINE AND ULTRA-LOW DOSE NALTREXONE: DIFFERENTIAL EFFECTS ON MORPHINE-INDUCED CATALEPSY, FEEDING AND ANALGESIA

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Classic pharmacological theories predict that opioid receptor antagonists block the behavioural effects of opioids. Surprisingly, co-administration of ultra-low dose (ULD) of the opioid receptor antagonist naltrexone with morphine enhanced morphine's analgesic effects. This paradoxical effect was attributed to the activation of mu opioid receptors that mediate morphine-induced catalepsy, feeding, drinking and analgesia. We hypothesized that ULD naltrexone will enhance morphine-induced catalepsy and feeding. In

experiment 1, rats (N = 56) were randomly assigned to saline, morphine (10 mg/kg), co-treatments of morphine (10 mg/kg) plus naltrexone (molar ratios of 1,000,000:1; 500,000:1; 100,000:1) or naltrexone alone groups. For 7 consecutive days, catalepsy and analgesia were assessed 30 and 60 min post injection using the bar and tail-flick tests, respectively. ULD naltrexone co-administered with morphine did not significantly affect morphine-induced catalepsy but significantly and dose-dependently attenuated tolerance to morphine's analgesic effect. In experiment 2, rats (N = 35) were randomly assigned to saline, morphine (4 mg/kg) or co-treatments of morphine (4 mg/kg) plus naltrexone groups (molar ratios of 1,000,000:1; 500,000:1; 100,000:1) and tested for food and water intake. Rats then underwent a 7-day sensitization period with a morphine dose of 10 mg/kg and naltrexone in the same ULD ratios. On days 1, 7 and 21, food and water intake were again assessed using 4 mg/kg of morphine and naltrexone in the ULD ratios. Food and water intake were augmented by morphine but were not further enhanced by co-administration of ULD naltrexone plus morphine. All together these experiments showed that ULD naltrexone co-administered with morphine specifically enhanced morphine's analgesic effects but not its cataleptic, hyperdipsic or hyperphagic effects. These data also showed that morphine's paradoxical analgesic effects are not the result of changes in morphine-induced catalepsy. Results suggest that the actions of ultra-low doses of naltrexone co-administered with morphine are specific to antinociceptive systems and do not generalize to other behavioural systems affected by morphine. (Funded by NSERC)

194 C150

BDNF AND PROBDNF IN CORTICAL SYNAPTONEUROSOMES FOLLOWING KAINIC ACID-INDUCED SEIZURES IN RATS

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Brain-derived neurotrophic factor (BDNF) plays a crucial role in activation-induced neuroplasticity. Seizures are known to increase BDNF mRNA and protein expression. Furthermore, seizures also enhance cleavage of the precursor form of BDNF, proBDNF, to BDNF by increasing the expression of various intracellular and extracellular proteases. There is increasing evidence that BDNF mRNA and protein are present at the synapse and that processing occurs close to the synapse. In this study, we asked whether seizures change the subcellular distribution and processing of BDNF. We used Western blotting to measure relative levels of BDNF and proBDNF in isolated synaptoneurosomes from cerebral cortex of rats subjected to kainic acid-induced status epilepticus seizures. The post-synaptic density protein PSD95 was used as a marker for synaptoneurosomal enrichment. All samples were normalized to β-actin as a stable reference gene. In naïve rat cortex, higher levels of BDNF were detected in synaptoneurosomes compared to cell body fractions. Seventy-two hours following kainic acid-induced seizures, there was a significant increase in BDNF protein in both synaptoneurosomes and cell bodies. Surprisingly, there was no difference in levels of proBDNF between the synaptoneurosomes and cell bodies of naïve rats, and seizures did not increase proBDNF levels in either compartment. Thus, BDNF, but not proBDNF, is preferentially localized to synaptic regions in naïve cortex. The increase in BDNF levels following status epilepticus seizures, without changes in proBDNF, may be due to localised synthesis of BDNF and regional conversion of proBDNF to BDNF, coupled with activity-induced increased expression of protease enzymes that convert proBDNF to BDNF.

195 C151

DOES ADMISSION BLOOD PRESSURE REALLY PREDICT POOR OUTCOME IN ACUTE ISCHAEMIC STROKE? - A STUDY OF 100 NIGERIAN AFRICANS

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There is a lot of controversy on the prognostic value of admission blood pressures in acute ischaemic stroke. There has been no study on the effect of admission blood pressures on stroke outcome in Nigeria. The objective of this study was to study the effect of blood pressures measured on admission on 30-day mortality and neurological handicap in Nigerians.
This study was conducted at the Lagos University Teaching Hospital, Lagos, Nigeria. Patients who met the inclusion criteria were prospectively recruited from the emergency medical unit of the hospital. Information which included age, sex, level of consciousness and stroke severity using the National Institute of Health Stroke Scale (NIHSS) was obtained in a standardized manner. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured while pulse pressure (PP) and mean arterial pressure (MAP) were derived. Patients were managed conservatively and periodically evaluated for progress and/or development of complications. Primary outcome was mortality within 30 days while secondary outcome was level of handicap on the modified Rankin Scale. 100 patients with a mean age of 58.56±14.12 years were studied; 53 of them were males. Overall mortality rate was 28%. There was no significant correlation between admission blood pressures and 30-day mortality (SBP: r = -0.05, p= 0.62; DBP: r = -0.12, p= 0.23; PP: r = 0.01, p= 0.90; MAP: r = -0.09, p= 0.36) or modified Rankin Score (SBP: r = -0.11, p= 0.29; DBP: r = -0.13, p= 0.21; PP: r= -0.06, p= 0.54; MAP: r = -0.13, p= 0.21). In conclusion, admission blood pressures do not have significant influence on 30-day mortality and level of handicap in Nigerian Africans with acute ischaemic stroke.

196 C152

IDENTIFICATION OF A CRITICAL DEVELOPMENTAL WINDOW FOR THE EFFECTS OF MORPHINE ADMINISTRATION ON THE SURVIVAL OF ADULT-GENERATED GRANULE CELLS IN THE DENTATE GYRUS

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Morphine administration reduces both proliferation and survival of adult-generated granule cells in the dentate gyrus of the hippocampus (Eisch et al, 2006; Nestler et al, 2000). To evaluate whether there is a critical period for the effects of morphine on survival, mice were injected daily with morphine (0, 10, 20 mg/kg) for 5 days either 1 week or 3 weeks after treatment with the cell proliferation marker 5-bromo-2'-deoxyuridine (BrdU). All mice were sacrificed 4 weeks after BrdU treatment. Using immunohistochemical methods, we quantified BrdU+ cells throughout the anterior-posterior extent of dentate gyrus. Numbers of BrdU+ cells were decreased in mice treated with highest dose of morphine 1 week, but not 3 weeks, after BrdU treatment. Our data provide evidence that morphine administration reduces survival of adult-generated granule cells in the dentate gyrus, and identify a critical developmental window for these effects. In the future, we will evaluate whether similar critical windows exist for the effects of other drugs of abuse on survival of adult-generated neurons in the hippocampus.

197 C153

ISOLATION AND CHARACTERIZATION OF A NOVEL MORPHINE-DEPENDENCE CANDIDATE HUMAN GENE ENCODING ZINC FINGER AND RNA-BINDING MOTIF 1-CONTAINING PROTEIN ZCRB1

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Addiction to opiates such as morphine is a major public health concern. A more thorough understanding of the molecular mechanisms of opiate

addiction can lead to better treatment options in the future. Until recently, little was known about the changes in gene expression that underlie these effects. Here we isolated a novel morphine dependence candidate human gene ZCRB1 with differential display. ZCRB1 expression is up-regulated by acute (3 hours), but not chronic morphine treatment (morphine hydrochloride, 1-10 mg/ml). The acute cellular level morphine dependence is consistent with the dynamic change of intracellular CREB concentration. ZCRB1, a novel zinc finger CCHC-type and RNA binding motif 1 containing protein, encodes a nuclear protein (217 residues). The ZCRB1 gene consists of 8 exons and 7 introns. It is mapped to 12q12, which is within a locus reported for Parkinson's disease. The 5'-flanking region contains an enhancer core motif and binding sites for AP-1, 2 and LF-A1. ZCRB1 is characterized by an RNA-binding motif and a CCHC Zinc finger motif. The latter overlaps the C..C…GH….C core nucleocapsid motif. ZCRB1 is conserved through zebrafish to human, and shares homology with cold-inducible RNA-binding protein. Transfection assay showed ZCRB1 is located in the nucleoplasm, but outside the nucleolus. ZCRB1 gene expression was inhibited by 30-36°C and upregulated by 39°C incubation in SH-SY5Y neural cells. ZCRB1 gene expression is highest in the heart and testes, lower in the cerebellum and lowest in the liver in mice. ZCRB1 mRNA expression is specifically elevated in hepatocarcinoma HepG2 cells. In this study, we have cloned and characterized a novel morphine-dependence candidate human gene encoding a multifunctional zinc-finger protein that is involved in morphine dependence, heat shock and hepatocarcinoma. (*Thanks Drs. L Li, B Wang, N Hori, K Sato, J Yeomans, and the staff of NRI for technical assistance and/or valuable discussion. This research is supported by grants to XG, HW and JY. HW is currently at the University of Toronto)

198 D401

CALCITONIN GENE-RELATED PEPTIDE IS INVOLVED IN CHRONIC MORPHINE INDUCED ACTIVATION OF P38 MITOGEN-ACTIVATED PROTEIN KINASE

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Opiates including morphine are potent analgesics to treat moderate and severe pain. The usefulness of opiates is compromised by the rapid development of tolerance to their antinociceptive effects. We have previously shown that chronic morphine administration up-regulates the pain-related peptide, calcitonin gene-related peptide (CGRP), in primary sensory neurons and CGRP receptor antagonists are able to reverse the development of morphine tolerance, suggesting the role of CGRP in the development of morphine tolerance. In this study, we explored the mechanisms how CGRP contributes to the development of morphine tolerance. Repeated intrathecal (i.t.) injection of morphine (15μg) for 7 days evoked a gradual tolerance to morphine induced anti-nociception, which was reversed by co-administration of the non-peptide CGRP receptor antagonist BIBN4096BS (0.1μg). Interestingly, i.t. repeated morphine injection also increased the phosphorylation (p) of p38 mitogen-activated protein kinase (MAPK) in glia-like cell profiles in the dorsal horn of lumbar spinal cord. These pp38 positive cell profiles were identified as microglia since they co-expressed microglia marker OX-42. BIBN4096BS was also able to attenuate chronic morphine increased pp38 in microglia. In contrast, i.t. repeated morphine injection did not alter the phosphorylation of Akt and GSK3 in the dorsal horn of lumbar spinal cord. Taken together, our data suggest that the activation of p38 MAPK in microglia is an important signalling event underlying the development of morphine tolerance. Morphine up-regulated CGRP likely plays a role in the activation of p38 MAPK signalling. (Supported by the Canadian Institutes of Health Research)

199 D402

MINOCYCLINE PROTECTS THE BLOOD-BRAIN BARRIER AND REDUCES EDEMA FOLLOWING INTRACEREBRAL HEMORRHAGE IN THE RAT

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Intracerebral hemorrhage (ICH) results from rupture of a blood vessel in the brain. After ICH, the blood-brain barrier (BBB) surrounding the hematoma is disrupted, leading to cerebral edema. In both animals and humans, edema coincides with inflammation, which is characterized by production of pro-inflammatory cytokines, activation of resident brain microglia and migration of peripheral immune cells into the brain. Accordingly, inflammation is an attractive target for reducing edema following ICH. In the present study, BBB damage was assessed by quantifying intact microvessels surrounding the hematoma and by measuring brain water content 3 days after ICH induced by collagenase injection into the rat striatum. In the injured brain, the water content increased in both ipsilateral and contralateral hemispheres compared with the normal brain. Immunostaining showed a loss of intact microvessels surrounding the hematoma, as judged by collagen type IV staining, and co-localization of MMP-12 with damaged microvessels. MMP-12 was also observed for the first time in neurons. Quantitative real-time RT-PCR revealed an up-regulation of inflammatory genes associated with BBB damage; IL-1beta, TNF-alpha, matrix metalloproteases MMP-3, MMP-9, and most notably, MMP-12. Dual-antibody labelling demonstrated that neutrophils were the predominant source of TNF-alpha protein. Intraperitoneal injection of the tetracycline derivative, minocycline, beginning 6 hours after ICH reduced microvessel loss and edema, decreased TNF-alpha and MMP-12 expression, and reduced the numbers of TNF-alpha positive cells and neutrophils in the brain. Thus, minocycline, administered at a clinically relevant time, appears to target the inflammatory processes involved in edema development after ICH.

200 D403

REGULATION OF BACE1 EXPRESSION BY INTERACTION OF HIF1 AND SP1

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The pathogenesis of sporadic Alzheimer's disease (AD) is largely unknown. Patients with stroke or cerebral infarction showed poorer cognitive performance and greater severity of clinical dementia. Vascular risk factors have been suggested to be associated with development of AD. A reduced cerebral perfusion is a common vascular component among AD risk factors. Hypoxia is a direct consequence of hypoperfusion. The hypoxia signal transduction pathway plays a major role in vascular development and ischemia, as well as neurodegeneration. Hypoxia inducible transcription factor (HIF1) is a central mediator of hypoxia signal transduction pathway through which cells in the brain respond to reduced oxygen tension. Previously we have reported that BACE gene promoter contains HIF1 and Sp1 element. Hypoxia and Sp1 overexpression increased APP CTF production by increasing BACE1 expression and transcription in cells. BACE1 expression was significantly upregulated in mice under hypoxia treatment. Knockout of Sp1 or Knockdown of HIF1 significantly inhibited BACE1 gene transcription. Interaction of Sp1 and HIF1 is critical for the BACE1 gene expression.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

201 D404

GAMMA OSCILLATIONS, TREMOR AND PARKINSON'S DISEASE

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Resting tremor, one of the main symptoms in Parkinson's disease (PD), is not a consistent feature of the disease. Unlike rigidity and akinesia there is no correlation between the severity of PD tremor and the degree of dopamine deficiency in the striatum or the progression of the disease. Moreover, PD patients with predominant resting tremor have better prognosis and slower disease progression than patients with primarily akinesia and rigidity. Recent studies in humans suggest that the akinesia in PD is related to abnormal increased beta (15-30 Hz) synchronous oscillatory activity in the basal ganglia and decreased gamma (35-80 Hz) activity. In this study, we recorded local field potential (LFP) activity from the subthalamic nucleus (STN) in 7 PD patients who exhibited intermittent periods of resting tremor during the time of functional neurosurgery. In 6 out of the 7 patients, LFP oscillatory activity in the low gamma frequency range (35-55 Hz) was found to be increased during periods of tremor and this activity was maximal in the dorsal part of STN. Furthermore, LFP gamma power was positively correlated with tremor amplitude when averaged across subjects. These results suggest that increased gamma activity in STN may be related to the mechanism of generation of resting tremor in PD patients. Since gamma LFP activity in STN is thought to reflect its role in movement, the increased gamma activity observed during PD tremor may be due to a compensatory mechanism generated in order to overcome the akinesia and which may lead to tremor as a side effect. Supported by CIHR FRN 42505.

202D405

ANALYSIS OF THE BINDING ABILITY OF MOUSE BRAIN ALPHA-SYNUCLEIN TO PRESYNAPTIC MEMBRANES.

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Alpha-Synuclein is a 140 amino acid protein, associated with presynaptic membranes in the brain and constituting one of the major components of Lewy bodies (a hallmark of Parkinson's disease). Three missense mutations (A30P, A53T and E46K) in the gene encoding alpha-synuclein are associated with rare autosomal dominant forms of familial Parkinson's disease. However, the normal conformation of alpha-synuclein in vivo, its subcellular localization in neurons, and the corresponding effects of the clinical mutations are poorly understood. In the present study, we analysed the capacity of cytosolic alpha-synuclein to interact with biological membranes. In contrast to E.coli-expressed recombinant alpha-synuclein, the cytosolic alpha-synuclein from mouse brain has significantly lower membrane binding ability. To assess whether alpha-synuclein binding properties might be regulated, we first analysed the effect of cytosolic factors (proteins and lipids), ATP and calcium on E.coli-expressed alpha-synuclein. Both ATP and cytosol increased the binding of E.coli-expressed alpha-synuclein. Although ATP also increased the binding of mouse brain cytosolic alpha-synuclein, the overall binding efficiency of the murine expressed alpha-synuclein remained poor compared to the E.coli-expressed alpha-synclein. Furthermore, a primary difference between E.coli-expressed alpha-synuclein incubated with cytosol versus cytosolic mouse brain alpha-synuclein is that the murine-expressed alpha-synuclein co-elutes with high molecular weight markers on glycerol gradients suggesting the possibility that alpha-synuclein in mammalian brain participates in high molecular weight complexes. The identification of the proteins involved in those complexes would allow a better understanding of the alpha-synuclein function.

203 D406

INCREASE IN SPONTANEOUS, ACTION POTENTIAL DEPENDENT GLUTAMATE RELEASE DURING EARLY ISCHEMIA CAN BE PREVENTED BY CALCIUM CHELATOR BAPTA-AM

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Spontaneous synaptic release of glutamate during early ischemia is neurotoxic and may cause perturbation to normal neural network functions. These releases are mediated by both action potential (AP) dependent and AP-independent mechanisms. Previous studies have shown that AP-independent (miniature) release was enhanced during ischemia, by Ca2+ release from the internal stores. However, neuronal mechanism underlying the AP-dependent spontaneous release remains unclear. In this paper, we show that the AP-dependent release is also increased in the CA1 pyramidal neurons in the hippocampus during 2 min hypoxia/hypoglycemia (H/H) episode. Enhancement in the activity of the presynaptic CA3 neurons is sufficient and necessary to cause this increase. BAPTA-AM, an intracellular calcium buffer, blocks the increase during H/H, by attenuating CA3 activity, and by compromising the ability for the action potentials in triggering spontaneous release from the CA3 terminals. Both lesion of the pre-post synaptic pathway, and buffering of the intracellular calcium, could provide significant rescue strategies to the excessive glutamate release and perturbed network functions during early ischemia.

204 D407

MEMORY DEFICITS IN ALZHEIMER'S DISEASE: A POSSIBLE ROLE OF CREB?

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Alzheimer's disease (AD) is a progressive neurodegenerative condition that is initially characterized by mild memory impairments that progress to more global cognitive deficits, behavioural impairment and eventually death. As memory deficits are the core feature of AD, novel treatments may benefit from building on basic research studying the molecular basis of normal memory formation. Amyloid plaques and neurofibrillary tangles in the hippocampus, amygdala and cerebral cortex serve as a criterion for postmortem diagnosis of the disease. As amyloid plaques consist mainly of a 40-42 amino acid peptide termed beta-amyloid peptide (A), overproduction and accumulation of A has been proposed to be a cause of AD. The mechanism underlying the mild memory deficits observed before the onset of clear brain pathology are unknown. Exogenous application of A in vitro disrupts neuronal signaling and compromises the function of CREB (cAMP responsive element binding protein), a transcription factor critical for normal memory formation. Therefore, disrupted CREB function may account for early memory deficits in AD. Here we examined if increasing CREB function improves memory in mouse that modesl AD. TgCRND8 transgenic mice overexpress a human gene associated with familial AD (APP) and recapitulate key aspects of AD, including increased levels of A and memory deficits. We, and others, have shown that the TgCRND8 mice show robust spatial memory deficits, as assessed by the Morris water maze. To examine whether increasing CREB function rescues this memory deficit, we used viral mediated gene transfer techniques to increase CREB levels and function in the hippocampus of transgenic and wild-type littermate control mice. Our preliminary results show that increasing CREB rescues some of the spatial memory deficits in this mouse model of AD. These results may serve as a step towards developing novel treatment strategies to reverse or delay the early memory deficits of AD.

205 D408

POSTERIOR HYPOTHALAMIC STIMULATION AMELIORATES HALOPERIDOL INDUCED BRADYKINESIA IN AN ACTIVE AVOIDANCE TASK

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Parkinson's disease (PD) is principally a movement disorder characterized by muscle rigidity, tremors and bradykinesia/akinesia. One of the most effective treatments of PD is deep brain stimulation (DBS). DBS in the subthalamic nucleus is particularly effective in ameliorating bradykinesia (slow movement) and drug-induced dyskineisa (involuntary movements). Thalamic DBS is particularly effective in tremor stoppage. DBS effect on akinesia (poverty of movement), however, remains less established. In rats, the stimulation of the posterior hypothalamic area (PH) elicits spontaneous, natural behaviours. The vigour and intensity of ongoing behaviour are positively related to the stimulation intensity. In this study, we investigated the effectiveness of PH stimulation on haloperidol-induced movement deficits. Haloperidol is an antipsychotic with anti-dopaminergic (predominantly at the D2 receptor) actions. It produces the rigidity, tremor and bradykinesia/akinesia seen in PD patients, hence has been used extensively to probe the movement-related deficits in PD.....Thirteen Long-Evans rats were implanted bipolar stimulating electrodes in the PH. Upon recovery, pretests determined stimulus intensities to elicit natural spontaneous behaviour. The animals were subsequently trained in an active avoidance task to reach target criterion (10 consecutive successful escapes under 10 seconds). Saline, haloperidol or no injections were administered on separate days when animals became proficient at the task. Animals received saline had similar escape latencies and success rate compared to animals that received no injections. Conversely, animals that received haloperidol were not able to escape foot shocks. Posterior hypothalamic stimulation had little effect on escape latency and no effect on escape success in animals injected with saline or received no injections. However, PH stimulation was effective to reverse haloperidol-induced bradykinesia and rescued the animals' task performance back to criterion. The data illustrates the effectiveness of PH stimulation to produce context-relevant behaviours and offer a novel locus for possible DBS in PD treatment.

206 D409

NICOTINE PROTECTS AGAINST BETA-AMYLOID NEUROTOXICITY AND ATTENUATES NUCLEAR TRANSLOCATION OF APOPTOSIS-INDUCING FACTOR (AIF) IN CULTURED RAT CORTICAL NEURONS

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Introduction and objective: Accumulation of beta-amyloid is thought to play a central role in the progressive loss of synapses and neurons occurring in Alzheimer's disease. Recent evidence indicates that beta-amyloid-induced neurotoxicity is likely associated with both caspase-dependent and -independent programmed cell death. The latter type is mediated by the nuclear translocation of the mitochondrial flavoprotein, apoptosis-inducing factor (AIF). Given the well-established neuroprotective properties of nicotine against beta-amyloid -induced neurotoxicity, here we investigated the effects of nicotine on beta-amyloid -induced caspase-independent cell death and AIF nuclear translocation in rat cortical neurons in vitro. Methods: Primary cultures of rat cortical neurons were exposed to various concentrations of beta-amyloid 25-35 and beta-amyloid 1-40 with or without 10 microM nicotine. Cell viability was measured using the MTT assay, and AIF protein expression was measured by immunoblot. Results: 48 hour-exposure to either beta-amyloid 25-35 (20, 30 or 40 microM) or beta-amyloid 1-40 (20 or 40 microM) led to significant increases in cell death. Under these conditions, AIF levels were found to decrease in the mitochondrial compartment while increased in the cytosolic and nuclear compartments. Pretreatment of neurons 10 microM nicotine for 1 hour with decreased the occurrence of beta-amyloid -induced cell death and attenuated the increase in AIF levels in the cytosol and nucleus. Conclusion: These results suggest that the neuroprotection conferred by nicotine against beta-amyloid -induced toxicity involves a reduction in caspase-independent programmed cell death through the inhibition of AIF nuclear translocation. Supported by CIHR

207 D410

PROMOTION OF SPINAL CORD REPAIR AND REGENERATION IN CERVICAL SPONDYLOTIC MYELOPATHY BY TARGETING THE FAS PATHWAY

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Cervical spondylotic myelopathy (CSM) is a chronic degenerative disorder of the spinal cord characterized by cord compression due to spondylosis, degenerative disc disease or ossification of the posterior longitudinal ligament. The autosomal recessive mutant Tip-toe Walking Yoshimura (twy/twy) mouse has a spontaneous mutation in the nucleotide pyrophosphatase (Npps) gene, and as a result spontaneously develops calcified deposits at C1-C2 vertebral levels causing cervical spinal cord compression and resulting in spinal cord pathology and neurological deficits, which mimics human CSM. Approaches to enhance neurological recovery and reduce neural cell death in CSM would be of major clinical importance. Previous work from our laboratory indicated that the Fas receptor is involved in the pathobiology of cell death in human CSM and twy/twy mice. We hypothesized that neutralization of Fas ligand (FasL) by a function-blocking-antibody will reduce cell death, the extent of inflammation and promote axonal growth resulting in functional neurological recovery in twy/twy mouse model of CSM. We tested neutralization of Fas-mediated apoptosis in the twy/twy model in vivo. We treated 4 month-old twy/twy mice with FasL specific antibody (MFL3; BD Bioscience) 50ug i.p twice weekly for up to 4 weeks (n=12 per group). Control mice were treated with IgG and artificial CSF alone. Functional neurological recovery was assessed by quantitative footprint gait analysis at weekly intervals for the 4 weeks treatment and measuring weight of body for twice (from the beginning and end of treatment). The injury sites were examined using quantitative histomorphometric and western blotting approaches to examine growth-associated protein-43 (GAP-43), NF200, NeuN, GFAP, MaC-1, FasL and Caspase-9 for role of axonal function, glial scar, inflammation and cell death. Twy/twy mice treated with FasL by function-blocking-antibody were capable of reduction of weight loss and improvement of neurological functional recovery, mirrored by an increase in regenerating fibers and upregulation of GAP43, a decrease in the infiltration of macrophages and reactive microglia and the inhibition in GFAP positive glial scar as well as a reduction in caspase 9 activation after chronic SCI.

In the present study, Our results are consistent with previous work from our and others that deficiency of Fas (Yoshino et al. 2004; Casha et al. 2005) and neutralization of Fas ligand (FasL) (Demjen et al. 2004) resulted in reducing oligodendroglial cell death and improving behavioral and histological outcomes after SCI. Neutralization of Fas and FasL in the twy/twy mouse model of CSM attenuates neuronal and oligodendroglial cell death and promotes improved functional neurological recovery. Our data will lay the foundation to develop novel therapeutic approaches to target spinal cord degeneration associated with CSM.

208 D411

AN INVESTIGATION OF THE INDUCTION AND RECOVERY OF RAT BRAIN CYP2D PROTEIN AND MRNA LEVELS BY NICOTINE

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Human CYP2D6 is involved in the metabolism of many centrally active drugs, neurotoxins and endogenous neurochemicals. CYP2D6 levels are higher in many brain regions of smokers suggesting induction by nicotine. CYP2D activity among rat brain regions correlates with protein and mRNA levels. We investigated whether nicotine can induce brain CYP2D in rat, and the recovery time course. Rats were treated either once with saline or nicotine (1 mg base /kg, s.c.), and sacrificed at 8 h after the treatment, or daily for 7 days and sacrificed 0.5-24 h after the last treatment. CYP2D protein levels were detected quantitatively by immunoblotting and in specific neural cells using immunocytochemistry. CYP2D mRNA levels were assessed by slot-blotting. There were no changes in brain CYP2D levels after a single nicotine injection. Brain CYP2D levels were maximally induced at 8 h in all three regions assessed after nicotine-treated for 7 days, which returned to control levels by 12 h. At 8 h post-nicotine treatment, CYP2D levels were significantly ($p<0.05$) higher than in saline-treated controls in cerebellum (1.4-fold), hippocampus (1.3-fold) and striatum (3.2-fold), and trended to be higher in frontal cortex, brainstem and thalamus. Induction was brain region- and cell-specific for example in some striatal neurons, and in cells in the cerebellar granular layer and white matter. There was no increase in brain CYP2D mRNA levels following 7 days of nicotine treatment. Hepatic CYP2D levels were unchanged at all times tested. This data demonstrates that CYP2D protein in rat brain, but not liver, can be induced by 7 days of nicotine treatment and that the induction of brain CYP2D levels utilizes a post-transcriptional mechanism. This model will be useful for investigating many aspects of CYP2D induction including the molecular mechanisms and behavioral consequences. The findings suggest that humans exposed to nicotine, including current and passive smokers and those on nicotine replacement treatment, may have increased in situ CYP2D-mediated metabolism of centrally acting drugs, neurotoxins, and endogenous neurochemicals owing to the higher CYP2D6 in the brain.

209 D412

ANTIGENIC-INDUCED EXPRESSION OF BRAIN DERIVED NEUROTROPHIC FACTOR IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory disease in young adults of western world which results in the demyelination, axonal lost and neurons degeneration in the central nervous system. Current treatments for MS including immunomodulation and immune deviation are only partially effective due to they are no cure in remyelination, axonal and neurons regeneration. Neuroprotection will be a promising strategy aim at prevention of the damage pf various neuronal cells and promoting regeneration. Because brain derived neurotrophic factor (BDNF) is a potent neurotrophin with the profound effects on neuronal survival and repairing, in our research, we use the myelin basic protein as the antigen to induce EAE animal model of MS in rats and determine the expression of BDNF in the dorsal root ganglia (DRG), spinal cord and brain at a series of time points with the immunohistochemistry, semi-quantitative RT-PCR and fully-quantitative Real-time PCR in data analysis. Preliminary results from our laboratory indicate that there is an up regulated expression of BDNF in EAE active group comparing to naïve group. It seems plausible that BDNF maybe exact functional consequences in the remitting phase of RRMS and will be neuroptotective implications for the therapy of MS in the future.

210 D413

DECREASED SIRT1 CORRELATES WITH TAU PATHOLOGY IN ALZHEIMER'S DISEASE

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Sirtuins have been recently shown to increase the lifespan of several animal species possibly through regulation of cellular metabolism. Since age and metabolism-related disorders are risk factors for Alzheimer's disease (AD), we have hypothesized that SIRT1, a nuclear human sirtuin, plays a role in AD. To test that hypothesis, we have compared the concentration of SIRT1 in the parietal cortex, the hippocampus and the cerebellum of AD patients (n=19) with age-matched Controls (n=20), using Western immunoblots. We have also measured SIRT1 levels in the temporal cortex of an AD mouse model (3xTg-AD) of tau and beta-amyloid pathologies (n=26) compared to control littermates (n=26). The extent of beta-amyloid pathology was determined by measuring soluble and detergent-insoluble Abeta peptide40/42 load by Elisa. Concentrations of total and phosphorylated tau in parietal cortex homogenates were determined using Western immunoblots. We found a significant decrease of the protein SIRT1 in the parietal cortex (–43% ; p=0.0014), but not in the hippocampus or the cerebellum of AD patients compared to Controls. The decrease of SIRT1 remained at –43% when expressed as a ratio over actin (p=0.0126). Levels of SIRT1 were correlated positively with brain mass (r2=+0.138 ; p=0.0199) and total soluble Tau (r2=+0.221 ; p=0.0025), and negatively with the duration of symptoms (r2=–0.549 ; p=0.0010), soluble Abeta40 (r2=–0.138 ; p=–0.0198), insoluble Abeta42 (r2=–0.157 ; p=0.0124), insoluble phosphorylated Tau (r2=–0.211 ; p=0.0033), and total insoluble Tau (r2=–0.267 ; p=0.0009), but not with insoluble Abeta40, age, post-mortem delay or brain pH. In contrast, SIRT1 levels in the cortex of 16-month-old 3xTg-AD mice with significant Abeta and tau pathologies were similar to control animals. Our results indicate that the decrease of SIRT1 in AD is associated with beta-amyloid and tau pathologies in advanced stages of the disease.

211 D414

ENVIRONMENTAL CHALLENGE DURING PREGNANCY: ANALYSIS OF DOPAMINERGIC-RELATED MRNAS IN A MOUSE MODEL TO STUDY AUTISM

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In the present study we hypothesized that prenatal exposure to an anti-epileptic drug, valproic acid (VPA) at a specific timepoint will impact the later neurodevelopment in the offspring. Indeed, environmental factors have been associated with neurodevelopmental illnesses like schizophrenia or autism. Moreover, epidemiological studies reveal that VPA exposure during the first trimester in humans could induce higher incidence of autism in the offspring. In our experiments, we examine the impact of the VPA administration in pregnant mice on pup development. Previously we demonstrated that prenatal exposure to VPA leads to alterations in postnatal growth and maturation as well as deficits in social behaviour. Here we studied the dopaminergic system at the level of gene expression using in situ hybridization. Dopamine (DA) has been intensively linked to stereotypy and this altered behaviour has been described in the autistic population. A prenatal role of DA in the incidence of autism has been suggested. Our preliminary data show a significant sex-related difference in D2 receptor (D2R) mRNA expression in the striatum. We are also analyzing D1 and D2 receptors mRNA expression in the nucleus accumbens. Additional animal

imaging experiments are underway to examine dopaminergic system function in VPA and control mice, with [123I]altropane (Boston Life Sciences) using SPECT-CT. This experiment will provide new insight in the role of dopaminergic neurotransmission in brain neurodevelopment and extend the face validity of a potential animal model to study autism.

212 D415

DISTRIBUTION OF KCNQ3 CHANNELS IN MIDBRAIN DOPAMINE CELLS OF A NEURODEVELOPMENTAL RAT MODEL OF SCHIZOPHRENIA

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Dysfunction in midbrain dopaminergic system has been implicated in schizophrenia. One known mechanism for promoting DA release is burst firing of dopamine (DA) cells. However, whether alterations in firing pattern increases DA release in schizophrenia is unclear. It has been shown that blocking M channels induces burst firing in VTA dopamine cells. Changes in M channels can be a plausible explanation for increased excitability and burst firing of DA cells in schizophrenia. In neonatal ventral hippocampal lesion rats, a neurodevelopmental model of schizophrenia, the expression and distribution of KCNQ3 subtype of M channels in midbrain, the VTA in particular, immunohistochemically were studied. The VTA of neonatal ventral hippocampal lesion group revealed a decline in immunoreactivity to KCNQ3 in comparison with the sham group. In double immunoflorecence labeling with tyrosine hydroxylase and KCNQ3 co- localization of both antagonists in DA cells was observed. In conclusion, it is possible that decreased expression of M channels in DA cells results in changes in excitability and firing pattern to increase DA transmission.

213 D416

A GIGAXONIN VARIANT PREVENTS GIANT AXONAL NEUROPATHY IN MICE.

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Gigaxonin is a ubiquitously expressed 68kDa protein known to play a role in cytoskeleton stability. Several mutations in Gan gene lead to giant axonal neuropathy, a rare autosomal recessive disorder. As in many neurodegenerative diseases, giant axonal neuropathy is associated with abnormal accumulations of intermediate filaments. To further investigate the role of cytoskeletal components on intermediate filaments toxicity, we have generated a new mouse model deficient for gigaxonin. The knock-out mice are viable, reproduce normally and do not show any overt phenotype. Interestingly, protein analyses revealed an increase in the level of various intermediate filaments, including the neurofilaments subunits, that correlates with a change in axons caliber in L5 ventral roots. However, a 47.5 kDa variant has been detected which suggest the presence of a Gan isoform only in the spinal cord. These results suggest that giant axonal neuropathy is provoked by the loss of both Gan isoforms in mice.

214 D417

CYCLIN-DEPENDENT KINASES TRIGGER NEURONAL APOPTOSIS BY REGULATING THE TRANSCRIPTIONAL INDUCTION OF PUMA

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Oxidative stress and ER stress (i.e. calcium deregulation & protein aggregation) have been implicated in acute and chronic neurodegenerative conditions. However, the signaling pathways that activate the apoptotic response in these conditions are not well understood. Here, we demonstrate that the Bcl-2 family member PUMA is transcriptionally upregulated in response to several different oxidative and ER stressors, and that PUMA is essential for neuronal apoptosis in these contexts. Furthermore, we demonstrate that the cyclin-dependent kinase inhibitor (cdki), flavopiridol, blocks oxidative and ER stress-induced PUMA transcription and neuronal cell death. Ectopic expression of either DN-Cdk4 or the Cdk4 inhibitor p16(INK4A) also significantly reduced PUMA induction and apoptosis in these paradigms. Finally, we demonstrate by chromatin immunoprecipitation that the Cdk4 targets, Rb and p130, along with their respective cognate binding partners, E2F1 and E2F4, endogenously associate with the PUMA promoter, in vitro. Taken together, these results suggest that in oxidative and ER stress-triggered neuronal apoptosis, the activation of cdks regulates the transcriptional induction of the crucial pro-apoptotic BH3-only domain gene PUMA.

215 D418

MONOAMINE NEUROTRANSMITTER METABOLISM IN POST-MORTEM BRAIN OF SUBJECTS WITH AUTISM

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Autism is a pervasive developmental disorder believed to be the result of developmental defects in the brain. Elevated monoamine neurotransmitters serotonin, dopamine, and norepinephrine in the blood of subjects with autism have been reported in the literature. Also, drugs that target these neurotransmitters have been found effective in treating some of the behavioural symptoms. These findings implicate dysregulation in these neurotransmitter systems; however the mechanism that accounts for this is unknown. To investigate whether dysregulation occurs centrally, the enzymes responsible for monoamine neurotransmitter synthesis, degradation, and reuptake were examined in the post-mortem brains of subjects with autism. The mRNA levels of tryptophan hydroxylase 2, serotonin transporter, dopamine beta-hydroxylase, and monoamine oxidase A were measured using real-time RT-PCR. Beta-actin was also measured as a reference gene. Tryptophan hydroxylase 2 is the rate-limiting enzyme for serotonin synthesis in the brain, and the serotonin transporter is responsible for serotonin reuptake into the pre-synaptic neuron. Dopamine beta-hydroxylase is responsible for converting dopamine into norepinephrine, and monoamine oxidase A is responsible for the breakdown of monoamines. These targets were measured in the fusiform gyrus and striatum, as studies have shown that these areas are structurally and functionally abnormal in subjects with autism. The mRNA levels of all targets in subjects with autism compared to controls were not significantly different. This was consistent both with and without normalization to beta-actin. There were also no correlations between any of the targets and age or post mortem interval. Our results show no differences in mRNA; however enzymatic activity has not been examined and other brain areas may be relevant.

216 D419

SELECTIVE EARLY LOSS OF THE MOST FORCEFUL MOTOR UNITS IN A MOUSE MODEL OF ALS

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Anatomical studies suggest that the largest caliber motor axons are most vulnerable to die-back in both human patients and transgenic mouse models

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

of Amyotrophic Lateral Sclerosis (ALS). In contrast, electromyographic studies of functional motor units have failed to uncover selective loss and disagree regarding the time-course of die-back. Here, electrophysiological and immunohistochemical methods were combined to enumerate and characterize functional motor units in a pre-symptomatic SOD1G93A mouse model of ALS. At 60 days of age, a full month before the reported onset of symptoms, the number of motor units in SOD1G93A mouse tibialis anterior (TA) muscle was reduced by ~60%. In agreement with anatomical data, there was a preferential loss of the most forceful motor units, and the average motor unit force declined to ~50% of controls. Despite the reduction in force, there was no parallel change in the number of muscle fibers innervated per motor unit (innervation ratio; IR). Dissociation of motor unit force and IR occurred because the average force produced by the innervated muscle fibers declined due to an increase in the proportion of smaller, less forceful type IIA and IID/X muscle fiber types. Two parallel processes, 1) preferential denervation of type IIB muscle fibers and 2) activity-dependent conversion of type IIB muscle fibers accounted for the change in fiber type proportions. In conclusion, our findings show that the less forceful motor units are preferentially spared in pre-symptomatic SOD1G93A but do not undergo compensatory functional enlargement.

217 D301

ISOLATION AND CHARACTERIZATION OF LCHN: A NOVEL FACTOR INDUCED BY TRANSIENT GLOBAL ISCHEMIA IN THE ADULT HIPPOCAMPUS

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Using mRNA differential display to identify cerebral ischemia-responsive mRNAs, we isolated and cloned a cDNA derived from a novel gene, that has been designated LCHN. Antisense mRNA in situ hybridization and immunoblotting confirmed LCHN expression to be induced in the rat hippocampus following transient forebrain ischemia. The deduced amino acid sequence of the novel LCHN cDNA contains an open reading frame of 455 amino acids, encoding a protein with a predicted molecular mass of approximately 51 kDa. Although LCHN is highly conserved between rat, mouse, and human, the deduced amino acid sequence of LCHN does not possess significant homology to other known genes. LCHN immunoreactivity is detected within the somatodendritic compartment of neurons, is also present on dendritic growth cones, but is not detected on astrocytes. The induction of LCHN in the hippocampus following ischemic injury may have functional consequences, as the ectopic over-expression of LCHN generated neurons with longer and more branched axons and dendrites. Taken together, these data suggest that LCHN could play a role in neuritogenesis, as well as in neuronal recovery and/or restructuring in the hippocampus following transient cerebral ischemia.

218 D302

INVADING MACROPHAGE DERIVED PROSTAGLANDINS PLAY A ROLE IN THE MAINTENANCE OF NERVE INJURY ELICITED NEUROPATHIC PAIN AT AN ADVANCED STAGE

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Chronic neuropathic pain is a serious clinical concern. It inflicts more than 1% population and the current treatments are not satisfactory due to uncertain mechanisms. We have previously shown that invading macrophage derived cyclooxygenase 2/prostaglandin E2 (COX2/PGE2) is involved in the pathogenesis of neuropathic pain in the acute stage. In this study, we ask whether invading macrophage derived COX2/PGE2 contribute to the maintenance of neuropathic pain at a rather advance stage. Both tactile allodynia and heat hyperalgesia, but not cold allodynia, are still exhibited in rats 18 months after partial sciatic nerve ligation (PSNL). At this stage, OX-42 or ED1 immunoreactive (IR) invading macrophages still existed in both proximal and distal stumps of ligated nerve, but much less abundant compared to 2 and 4 weeks postlesion. The up-regulation of COX2 and microsomal prostaglandin E synthase 1 (mPGES1) remained in these macrophages and endothelial cells in small blood vessels, suggesting that PGE2 is over-produced in injured nerves in these rats. Moreover, pro-inflammatory cytokine interleukin-6 (IL-6) was also increased in invading macrophages. PSNL increased phosphorylation of CREB, a transcription factor and hall marker of the cell activation, is still evident in the ipsilateral dorsal horn of lumbar spinal cord. Perineural injection of a selective COX2 inhibitor NS-398 (60µg/rat) significantly relieved both tactile allodynia and heat hyperalgesia for more than 72 hours. NS-398 also reduced IL-6 expression in injured nerves and increased pCREB in the dorsal horn. Intraperitoneal injection of NS-398 at the same dose produced a delayed relief on neuropathic pain. Taken together, our data indicate that partial nerve injury can produce a rather prolonged neuropathic pain and invading macrophage derived PGE2 is involved in the maintenance of this chronic pain condition. Local injection of selective COX2 inhibitors is a plausible avenue to treat neuropathic pain and avoid serious cardiovascular side effects. (Supported by the Canadian Institutes of Health Research)

219 D303

EFFECT OF TAU DYSFUNCTION ON NERVE GROWTH FACTOR RETROGRADE TRANSPORT

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Basal forebrain cholinergic neurons (BFCNs) are dependent on nerve growth factor (NGF) for survival and function. In aged brain and also in Alzheimer's disease, BFCNs atrophy is associated with loss of the NGF receptor, TrkA, and abnormal distribution of NGF in those neurons and in target tissues such as cortex and hippocampus. There is a defect in retrograde transport of NGF from target tissue to BFCN cell bodies in aged animals and in transgenic mice mimicking some Alzheimer's disease pathology. Hyperphosphorylation of microtubule-associated protein tau, implicated in Alzheimer's disease and in tauopathies such as Pick's disease and progressive supranuclear palsy (PSP), leads to defects in axonal transport. However, whether the defective retrograde transport of NGF in Alzheimer's disease is due to loss of TrkA or to tau hyperphosphorylation is not clear. The purpose of this study was to determine whether tau hyperphosphorylation causes NGF retrograde transport impairment, as evidenced by increased NGF levels in cortex and/or hippocampus. Western blotting for proNGF (the NGF precursor and the dominant form of NGF in the brain) was carried out on post mortem human cortical tissue from subjects with tauopathies and controls. Transgenic mice overexpressing GSK-3β, the most prominent tau kinase in brain responsible for tau hyperphosphorylation, and mice with a tau mutation corresponding to the human tauopathy FTDP-17 (P301L) were similarly tested for proNGF content in cortex and hippocampus. Increased proNGF was demonstrated in Pick's disease, confirming the effect of tau dysfunction on proNGF axonal transport. However, PSP brains and both GSK-3β overexpressing and P301L transgenic mice showed normal levels of cortical and/or hippocampal proNGF. Interestingly, it has been shown that accumulated tau in Alzheimer's and Pick's diseases is more highly modified compared to that in other tauopathies, including PSP. This may explain the normal distribution of proNGF in PSP and also in the transgenic animals, as they may have less modified tau which does not interfere with axonal transport of proNGF.

Increased proNGF levels in Pick's disease cortex shows that, similar to Alzheimer's disease, retrograde transport of this protein is disrupted in Pick's disease, which supports the hypothesis that tau dysfunction interferes with retrograde transport of proNGF.

220 D304

LONG-TERM CHANGES IN NEUROTROPHIC FACTOR EXPRESSION IN DISTAL NERVE STUMP FOLLOWING MUSCLE DENERVATION AND REINNERVATION WITH MOTOR OR SENSORY NERVES

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Following peripheral nerve injury, Schwann cells of the distal nerve stump acutely alter expression of neurotrophic factors, which may contribute to a stimulating environment for nerve regeneration. However, over time, the skeletal muscle atrophies and loses receptivity to the regenerating axon. A sensory nerve sutured to the distal nerve stump during prolonged denervation significantly improves distal nerve stump and skeletal muscle morphology and functional recovery and modulates neurotrophic factor levels in muscle. In this study we investigated whether the sensory nerve also alters neurotrophic factor expression in distal nerve stump. In our model, rat tibial nerve (mixed motor and sensory components) is transected and either left denervated ("denervated group") or the peroneal nerve (mixed nerve, "immediate repair group") or the saphenous nerve (pure sensory nerve, "sensory protection group") is sutured to the distal stump. The intact tibial nerve is used as a control. Our results demonstrate that denervated distal stump expresses mRNA for all investigated neurotrophic factors, BDNF, NGF, NT-3, GDNF and CNTF, for at least six months following injury. BDNF and GDNF mRNA levels rise dramatically in distal stump for the first month following denervation. By two to three months after injury, BDNF and GDNF levels have begun to fall, but they remain elevated above control levels for at least six months. Sensory protection significantly lowers injury-induced BDNF and GDNF mRNA levels in distal stump compared to denervated distal stump, whereas motor nerve repair is lower still and not significantly different from controls at any time. In contrast to BDNF and GDNF, CNTF levels are down-regulated with injury and remain at low levels for at least 6 months in denervated distal stump, returning towards control levels in distal stump repaired with motor nerve, and exhibiting a trend towards normal levels of CNTF mRNA in the sensory protected group. Sensory protection did not affect mRNA expression of NGF or NT-3 These data demonstrate a role for sensory protection in altering neurotrophic factor mRNA expression in distal nerve stump following motor denervation and demonstrate the ability of the distal nerve stump to contribute to a neurotrophic environment for nerve regeneration for at least six months. Furthermore, BDNF and GDNF may serve as biomarkers of successful communication between nerve and muscle.

221 D305

TDP-43 INCLUSIONS IN ALS: INSIGHT GAINED THROUGH THREE DIMENTIONAL ANALYSIS

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TAR DNA binding protein (TDP-43) is a 43 KDa protein that was first identified as a DNA binding protein of the human immunodeficiency virus 1 long terminal repeat (HIV-1 LTR). Although it has no known physiological function in neuronal cells, it is a nuclear protein known to be involved in transcriptional repression and regulation of alternative splicing. Recently, TDP-43 was found to be a major component of pathological neuronal ubiquitinated inclusions that are present in Frontal Temporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). In the present study we used immunofluorescence and deconvolution microscopy to examine the make-up and structure of ubiquitinated inclusions in sporadic ALS. Using fixed, paraffin-embedded, 6 micron thick spinal cord tissue sections from ALS cases, we labelled for ubiquitin and TDP-43. In accordance with previous immunohistochemical studies, ubiquitin and TDP-43 appeared to co-localize in both skeins and round inclusions in our 2D images. When we examined the inclusions in 3-D there appeared to be areas of ubiquitin and TDP-43 co-localization in the skeins, whereas the round inclusions appeared to have a ubiquitin core, surrounded by TDP-43. Furthermore, 3-D imaging revealed that ubiquitinated round cytoplasmic inclusions may be formed through a dynamic process, where ubiquitinated skeins eventually become round inclusions and such neuronal inclusions exist at various stages of compaction throughout the spinal cord of affected individuals.
The present study illustrates the importance of the use of 3-D imaging techniques, which enabeled us to further examine the physical interaction of the protein components of ubiquitinated inclusions in ALS. This, in turn, may offer clues to disease pathogenesis.

222 D306

ALTERED STOICHIOMETRY OF NOVEL HUMAN PERIPHERIN SPLICE VARIANT EXPRESSION CAUSES AGGREGATION

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The intermediate filament protein peripherin is a component of axonal spheroids and of Lewy-body like inclusions in amyotrophic lateral sclerosis (ALS). Three peripherin mRNA transcripts generated by alternative splicing have previously been identified in mouse and we have shown their differential expression in transgenic models of ALS. Here we describe a novel human peripherin transcript, Per 3,4, that retains introns 3 and 4. A predicted protein species of ~27.6 kDa is expressed from the Per 3,4 transcript in transfected SW13 vim(-) cells. We show that the Per 3,4 transcript is also expressed from the normal human peripherin gene and that changes in the stoichiometric ratio of peripherin splice variant expression can lead to peripherin aggregate formation. Using a unique peptide sequence created by intron 3, we made an antibody specific for Per 3,4. Here we provide evidence for the expression of Per 3,4 in human ALS spinal cord tissues and also show an upregulation of the Per 3, 4 message in ALS RNA samples. These findings suggest that alternative splicing of peripherin occurs in ALS and may contribute to disease pathogenesis.

223 D307

DELINEATION AND VALIDATION OF THE SUB-CELLULAR MECHANISMS UNDERLYING CELL DEATH IN PARKINSON'S DISEASE USING A CELL MODEL

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Idiopathic Parkinson's disease (PD) is thought to be caused by many individual cellular abnormalities such as oxidative stress, mitochondrial dysfunction and a malfunctioning ubiquitin-proteasome system (UPS). Increasing evidence has shown that a combination of interactions between each of these individual abnormalities causes cellular death. Indeed, using pharmacological tools, we have shown that inhibition of mitochondria, UPS and lysosome function interact synergistically to decrease cell viability. Given that multiple mechanisms are responsible for cell death in PD, and that these pathways synergise, development of an effective neuroprotective strategy requires elucidation of how these sub-cellular mechanisms interact. To this end, we utilized the dopaminergic neuroblastoma cell line, SH-SY5Y and determined the expression of proteins linked with neurodegeneration in PD through SDS-PAGE followed by Western blotting and immuno-cytochemistry. SH-SY5Y cells were exposed to EC50 concentrations of

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

toxins which recapitulate cell death in PD - dopamine (300 μM) (reactive oxygen species), excitatory cocktail [KCl (30 mM), glycine (30 μM) and NMDA (100 μM)] (excitotoxicity), naphthazarin (7.26 μM) (lysosome damage), proteasome inhibitor (PSI) (40 μM), and rotenone (10 μM) (mitochondrial complex 1 inhibition). Twenty four hours following exposure to toxins, α-synuclein, caspase-3 and ubiquitin levels were examined by either Western blotting of cell lysates or immunocytochemistry of fixed cells. An interaction between mechanisms is seen when two proteins, e.g. α-synuclein and ubiquitin are increased by a toxin (PSI) application which would be expected to increase only ubiquitin levels. To validate the use of SH-SY5Y cells to study the mechanisms underlying cell death in PD, the neuroprotective potential of several compounds was evaluated in SH-SY5Y cells exposed to toxins which recapitulate cell death mechanisms in PD. Salicylic acid (10 mM, 1mM), caffeine (10 mM, 1mM), nicotine (10 μM, 1 μM), creatine (25 mM, 10 mM) coenzyme Q10 (117 μM, 35 μM) and L-deprenyl (10 μM, 1 μM) were added to cells 5 minutes prior to addition of dopamine (30 μM), excitatory cocktail [KCl (30 mM), glycine (30 μM) and NMDA (100 μM)], naphthazarin (2.17 μM), proteasome inhibitor (PSI) (40 μM), or rotenone (10 μM). Cell viability was assessed after 24 hours using the redox-sensitive dye, Alamar BlueTM. The neuroprotective potential of each drug is determined by comparing with toxin alone. These studies will determine the interactions between cellular mechanisms which cause PD, as well as validating this cell model, so that it may be used to assess novel potential neuroprotective treatments in the future.

224 D308
ABSTRACT WITHDRAWN.

EDUCATION

225 A401
BEHAVIORAL NEUROSCIENCE IN ONLINE EDUCATION

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Technology is continually being applied in new and innovative ways to education in the sciences at all levels. For the first time at Drexel University, an online ePsychology program has been initiated and one of the debut classes offered is Behavioral Neuroscience. This poster will present some of the techniques used in this course to overcome some of the hardships often associated with long-distance learning, and discuss how distance learning may be advantageous in some respects. Focuses of the poster will include methods and quality of student to student and student to professor communication, how problems were handled as they arose, how experiments were conducted without a physical classroom, and the results of a class gender difference experiment. A student project using modern video and audio technology to describe neural transmission and Drexel's collaboration with the University of Pennsylvania will also be discussed. Possible future developments of the online program will be included as well as subsequent research planned to determine the efficacy of the methods of education used.

EXCITABILITY, SYNAPSES & GLIA: CELLULAR MECHANISMS

226 A201
CRITICAL ROLE FOR CREB ACTIVATION IN NEUROPROTECTION BY NMDAR-PSD95 UNCOUPLING

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Studies indicate that specific intracellular signal pathways linked to N-Methyl D-Aspartate Receptors, rather than receptor activation itself, are responsible for determining excitotoxic neuronal death in stroke. As proof of principle, success has been made in rodent models of stroke using cell-permeable peptides that uncouple NMDARs from binding to PSD95, an intracellular scaffold protein that mediates multiple protein-protein interactions and brings intracellular second messengers in close approximation to receptors. Uncoupling PSD95-NMDAR interactions provides long-term (2 months) neuroprotection and functional recovery in animal models of stroke, a property not before demonstrated for a stroke therapeutic. Pharmacologic blockade of NMDARs however provides only short-term (24h) benefits and limited functional recovery. Blocking NMDAR-PSD95 interaction reportedly impedes cell death by blocking the production of the toxic free radical, nitric oxide (NO). This is likely an overly simplistic view of neuroprotection as PSD95, and the NMDAR itself, govern multiple signals necessary for glutamate receptor communication and neuronal survival including calmodulin (CaM) and Ras-MAP kinase-linked signal cascades. If neuroprotection were conferred simply by blocking PSD-95 associated signals (such as NO production) then pharmacologic blockade should also be effective. With this dichotomy in mind we hypothesized that neuroprotection requires not only a blockade of toxic signals but that a 'normal' balance of NMDAR signalling is restored during anoxic stress. Here we show that CREB, a nuclear transcription factor phosphorylated downstream of CaM and MAP kinases, plays a key role in neuroprotection by uncoupling NMDARs from PSD95. CREB phosphorylation, activated in response to NMDA or oxygen glucose deprivation (OGD), is maintained for a longer period of time in cells treated with a peptide inhibitor of the NMDAR-PSD95 interaction (TAT-NR2B9c), a result paralleled by neuroprotection by TAT-NR2B9c in both NMDA excitotoxicity and OGD experiments. In addition, in vivo neuroprotection by TAT-NR2B9c was blocked by concomitant application of KN-93, a CaM-kinase IV & II blocker. CaM-kinase IV is a nuclear kinase that directly phosphorylates CREB and its binding protein. Thus, manipulating neuroprotective NMDAR signalling in combination with uncoupling PSD95 may yield new therapeutic strategies for neuroprotection in excitotoxic disease.

227 A202
A PHYSIOLOGICAL ROLE FOR PHOSPHATIDIC ACID AND THE PATTERN OF STIMULATION IN THE TRANSLOCATION OF THE NOVEL PROTEIN KINASE C APL II IN APLYSIA NEURONS

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Protein kinase Cs are important mediators of synaptic plasticity. In Aplysia, serotonin-mediated activation of PKC Apl II is important for some forms of synaptic facilitation. Calcium-independent or novel PKCs (nPKCs) such as PKC Apl II contain an N-terminal C2 domain, but the role of this domain is controversial. Using live-imaging of fluorescently-tagged fusion proteins of PKC Apl II and PKC Apl II ΔC2 in Sf9 cells and primary sensory neurons of Aplysia, we demonstrate that the role of this domain in nPKCs is inhibitory. Moreover, we show that phosphatidic acid, generated by phospholipase D, removes C2 domain-mediated inhibition of PKC Apl II and is required for the physiological translocation of PKC Apl II by serotonin in

sensory neurons. While imaging the physiological translocation of PKC Apl II, we made a surprising observation. Translocation of PKC Apl II is sensitive to the pattern of stimulation. Spaced applications of serotonin strongly desensitize PKC Apl II translocation, while desensitization is much weaker with prolonged applications of serotonin. PKC activation was not required for this desensitization by spaced applications of serotonin. This has important implications for understanding why these different patterns of stimulation lead to different physiological outcomes and is one possible explanation for differences between spaced and massed training in Aplysia.

228 A203

STRUCTURE-BASED DESIGN, PARALLEL SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIP AND ANTICONVULSANT ACTIVITY OF SOME N-SUBSTITUTED BENZAMIDINE

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The anticonvulsant activities of some analogues of N-substituted benzamidines were determined at three dose levels in accordance with the Antiepileptic Drug Development Program (ADD) of National Institute of Health (NIH), USA. These compounds are analogues of 4-amino-N-(2,6-dimethylphenyl)benzamide (Ameltolide ®), (LY201116) which is the most potent benzamide anticonvulsant studied to date. The benzamidines were designed as prodrugs of Ameltolide by isosteric replacement of the carbonyl oxygen with amidine (NH) group. The in-vivo metabolism of benzamidines to benzamides seems to improve anticonvulsant properties.

229 A204

TRANSIENT CHOLINE-MEDIATED DEPRESSION OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES

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Nicotinic acetylcholine receptors (nAChRs) are pentameric, ligand-gated ion channels formed from various alpha (α2-10) and beta (β2-4) subunits, and modulate network activity in the brain as a consequence of broad cholinergic innervation. While the variety of subunits allows for numerous functional combinations, one predominant type is the α7 homopentamer (α7 nAChR), which is highly permeable to Ca2+ and thought to influence both memory and neurodegeneration. Choline, a selective agonist of α7 nAChRs, is present in the synaptic cleft after degradation of acetylcholine, but little has been done to try and understand how it may affect synaptic transmission. Using extracellular recordings from acutely prepared brain slices, we attempted to determine the effects of exogenously applied choline upon synaptic activity in the dendritic region of the hippocampal CA1 subfield. Our results show that bath application of choline significantly depressed the amplitude of evoked field excitatory post-synaptic potentials (fEPSPs) in a concentration dependant manner (10, 500, and 1000 μM). Furthermore, the depression developed within minutes, and was reversed upon washout, albeit incompletely following the highest choline concentration. Given that choline (500 μm) did not affect measures of presynaptic function such as paired-pulse facilitation and stimulation-response curves, we believe that a postsynaptic mechanism is responsible for the observed depression. In order to confirm the role of α7 nAChRs, two different specific antagonists were used. Surprisingly, neither methyllycaconitine, nor α-bungarotoxin was able to completely prevent the choline-mediated effect upon evoked fEPSPs. Future experiments will attempt to clarify the underlying mechanism of choline-mediated depression by administering additional subtype-specific nAChR antagonists, and examining interactions with depression caused by low-frequency stimulation.

230 A205

ADIPONECTIN ACTIONS IN SUBFORNICAL ORGAN

I. Alim and A.V. Ferguson

Adiponectin (ADP) is a peptide produced by adipose tissue, which acts as an insulin sensitizing hormone. Recent studies using whole-cell electrophysiology in the brain have shown that neurons in both the area postrema and the paraventricular nucleus are affected by ADP. We have recently demonstrated that the subfornical organ (SFO), a circumventricular structure responsive to amylin CCK and ghrelin, also shows a high density of mRNA for both adiponectin receptors (AdipoR1, AdipoR2). These observations suggest that SFO maybe a key player in ADP signaling from circulation to the brain. Using dissociated SFO neurons maintained in cell culture we tested the hypothesis that ADP influences the excitability of SFO neurons. The effects of ADP on membrane potential of dissociated SFO neurons were recorded using whole-cell patch clamp in current clamp mode. ADP had clear effects on the excitability of SFO neurons which either depolarized (14.5 ± 3.4 mV, 9 of 23 cells) or hyperpolarized (-8.4±1.1 mV, 8 of 23 cells) following exposure to 10nM ADP. These observations indicate that ADP does play a role in modulating SFO ionic channels, suggesting that the SFO may play roles in sensing circulating concentrations of ADP and transmitting such signals to essential hypothalamic autonomic control centers.

231 A206

REAL-TIME VOLUME RESPONSES OF ASTROCYTES TO OSMOTIC AND ISCHEMIC STRESS IN CORTICAL BRAIN SLICES

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Studies of how acute osmotic challenge affects the volume of cortical neurons and glia have concentrated on isolated or cultured brain cells. Two-photon laser scanning microscopy (2PLSM) enables real-time visualization of functioning cells expressing Green Fluorescent Protein (GFP) deep (60-200 μm) within living neocortical and hippocampal slices. Using 2PLSM we recently showed that pyramidal somata, dendrites and spines steadfastly maintain their volume during osmotic stress, as do cerebellar axon terminals (Andrew et al., Cerebral Cortex, in press). Here we use similar techniques to monitor changes in astrocytic volume within hippocampal slices (400 um) from 37-40 day old mice of the FVB/N-Tg(GFAPGFP)14Mes/J strain. Single astrocytic cell bodies, their processes and capillary end-feet display GFP fluorescence (Zhuo et al. 1997, Devel. Biol. 187,36-42). Volume responses to 20 min of overhydration (-40 mOsm) or dehydration (+40 or +80 mOsm) were measured in astrocytic cell bodies and their processes (n=15 slices). Astrocytes reversibly swelled during overhydration and shrank during dehydration. These same astrocytes also rapidly swelled during O2/glucose deprivation (OGD) for 10 min (as do adjacent pyramidal neurons, Andrew et al., ibid). We then imaged four of these slices during recovery from OGD. Within 10 min, astrocytic volume recovered by 100% in 4 slices and by 80% in 4 others. Such recovery did not occur in adjacent pyramidal neurons which remained swollen with beaded dendrites post-OGD. We conclude that, in contrast to pyramidal neurons, adjacent astrocytes are clearly osmoresponsive to acute osmotic stress. We have not yet detected evidence for astrocytic volume regulation during acute challenge. Simulated ischemia induces an immediate and dramatic swelling of astrocytes upon onset of anoxic depolarization. Unlike adjacent pyramidal neurons that also swell, astrocytes display significant recovery from O2/glucose deprivation.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

232 A207

PROTEIN KINASE G INHIBITION RAPIDLY CONFERS ACUTE STRESS PROTECTION IN NEURAL CIRCUITS

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Neural circuits exposed to high but sub-lethal temperatures are impaired and cease to function long before cell death. Ectothermic animals such as the migratory locust (Locusta migratoria), which do not possess the ability to regulate their internal temperatures, are particularly susceptible to rapid changes in ambient temperatures. In response to these challenges locusts have developed a strong response to heat stress. Preconditioning treatments (3hr at 45°C followed by 1hr at room temperature; heat shock, HS) switch on physiological adaptations which subsequently provide protection. Using ventilatory central pattern generator (CPG) activity as a measure of central nervous system function we investigated the role of protein kinase G (PKG) in mitigating neural thermosensitivity to heat stress. Using a semi-intact preparation we monitored ventilatory motor pattern activity as saline temperature was heated. Prior to circuit failure we observed arrhythmias in motor pattern generation. Upon circuit failure we switched off the heat source and allowed the preparation to cool, thereby reestablishing conditions for circuit recovery. We scored the prevalence of arrhythmias, failure temperatures and recovery times. Using a pressure injection system we delivered agonists and antagonists of the PKG signaling pathway directly into the ventilator neuropil. Following nanoinjections of the PKG antagonist KT5823 we found that control animals had high incidence of ventilatory arrhythmias (60%) whereas treated animals had lower prevalence (13%). Circuit thermotolerance (failure temperature) was extended from 38°C to 47°C and the length of time taken to recover was reduced from 180 to 47 seconds. Conversely, HS animals which have protective physiological adaptations already switched on were more susceptible to thermal insults following injections of the PKG agonist 8-Bromo-cCMP. We observed a higher incidence of ventilatory arrhythmias (40% vs. 15%), a reduced failure temperature (36°C vs. 47°C), and longer recovery time (147 seconds vs. 75 seconds) then their HS counterparts. These data indicate that PKG plays an integral role in modulating stress protection in neural circuits.

233 A208

PARKIN-MEDIATED UBIQUITINATION OF THE PDZ PROTEIN PICK1 REGULATES ACID-SENSING ION CHANNEL ACTIVITY

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Mutations in the parkin gene result in an autosomal recessive juvenile-onset form of Parkinson's disease. Parkin functions as an E3 ubiquitin-ligase that promotes the attachment of the small protein ubiquitin onto specific substrate proteins. Defects in the ubiquitination of parkin substrates are therefore believed to lead to neurodegeneration in Parkinson's disease. Here, we identify the synaptic PDZ protein PICK1 as a novel substrate of parkin-mediated ubiquitination. We find that parkin binds PICK1 via a PDZ-mediated interaction and promotes PICK1 ubiquitination. Interestingly, parkin-promoting ubiquitination does not seem to target PICK1 to the proteasome for degradation. This led us to hypothesize that PICK1 ubiquitination might regulate intracellular trafficking or interactions with other PDZ ligands. Accordingly, we examined the effect of parkin-mediated PICK1 ubiquitination on the previously described PICK1-dependent ASIC2a potentiation induced by the PKC agonist OAG. We observed that wild-type parkin, but not PDZ-binding defective or E3 ubiquitin-ligase inactive parkin mutants, abolishes the OAG-induced PICK1 potentiation of ASIC2a currents in transfected COS cells. Native ASIC currents in cultured hippocampal neurons of mice lacking the parkin gene are potentiated following OAG treatment. This modulation is absent in wild-type hippocampal neurons, despite the fact that ASIC current amplitudes and kinetics are very similar in wild-type, heterozygous, and homozygous parkin KO mice. These findings implicate parkin, for the first time, in the functional regulation of an ion channel. Given that ASIC channels contribute to excitotoxic responses to brain injury, we propose that defects in parkin-mediated ubiquitination of PICK1 could enhance ASIC channel activity and thereby promote neurodegeneration in Parkinson's disease. Supported by CIHR and Parkinson Society Canada.

234 A209

MODULATION OF CHOLINE ACETYLTRANSFERASE ACTIVITY BY POLYSIALIC ACID

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Choline acetyltransferase (ChAT), the enzyme synthesizing acetylcholine, is known to be activated by brain-derived neurotrophic factor (BDNF). We investigated the possibility that removal of polysialic acid (PSA) on embryonic septal neurons, would impact the function of BDNF on these cells and influence ChAT activity. Interestingly, we found that the removal of PSA using endoneuraminidase N (endoN), significantly increased BDNF-induced ChAT activity. PSA removal did not influence cell survival and the number of cholinergic neurons present in culture. In these conditions, ChAT activity was unaltered by the removal of PSA alone (in absence of BDNF), or the application of antibodies blocking the function of to the neural cell adhesion molecule (NCAM). BDNF-induced ChAT activity required the stimulation of both BDNF's receptors, namely the p75 neurotrophin receptor and the tropomyosin related kinase B receptors. Mechanistically, PSA removal increased the maximal binding capacity of [125I]BDNF to its receptors, suggesting that this additional binding of BDNF upon PSA removal maximizes ChAT activity. We are investigating the signaling mechanisms activated by BDNF, and combined with PSA removal, which trigger ChAT activity. Furthermore, we established that PSA removal in presence of BDNF promotes a cholinergic phenotype in immature cells derived from the adult brain. Our results indicate a novel role for PSA in the regulation of cholinergic neurons, especially in presence of the neurotrophin BDNF.

235 A210

DOPAMINERGIC FUNCTION IN ADULT MICE WITH REDUCED NURR1 EXPRESSION

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The Nurr1 gene, which codes for a transcriptional factor in the nuclear receptor superfamily, plays an essential role in the development of mesencephalic dopamine (DA) neurons and an important role in the functional maintenance and survival of these neurons. Given its role in the development of the DA phenotype, it is conceivable that Nurr1 could also play a role in dopaminergic function in the adult brain. Nurr1 homozygotous knock-out (-/-) mice die at birth, probably due to developmental deficiencies of the brain stem. However, Nurr1 (+/-) mice are viable, fertile and display no apparent deficiencies. These mice have reduced Nurr1 expression throughout their life and decreased levels of DA at 2 months but normalized levels at 5 months. The objective of the present study was to investigate the physiological role of reduced Nurr1 expression on the dopaminergic function in the adult brain. First, we investigated two aspects of DA-mediated locomotor activity; 1) the basal locomotor activity in their home cage as

measured by a wheel-system, and 2) the amphetamine (AMPH)-induced locomotor activity measured in a Flex-Field apparatus. Nurr1 (+/-) mice have a reduced basal locomotor activity compared to their wild-type (WT) littermates. However, there is no difference in the locomotor activity induced by a low dose of amphetamine (2.5 mg/kg i.p.). In the second part of the study, we investigated the biochemical changes associated with Nurr1-reduced expression. As previously reported, Nurr1 expression is reduced by approximately 50% in all brain structures in Nurr1 (+/-) mice. Interestingly, an acute administration of AMPH has no effect on Nurr1 mRNA expression in the SN/VTA complex in WT animals but induces a significant increase in Nurr1 (+/-) mice bringing the levels of Nurr1 at the same levels than WT animals. Nurr1 mRNA levels in the hippocampus (CA1, CA2 and CA3) are significantly upregulated in both heterozygotous and WT mice. As expected, a single AMPH administration induces a significant upregulation of enkephalin (ENK) mRNA in the striatum and nucleus accumbens (core and shell) in Nurr1 (+/+) mice whereas this AMPH-induced upregulation is absent in Nurr1 (+/-) animals. Striatal and accumbal dynorphin mRNA levels are not modulated by the acute administration of AMPH in both mouse types. These data suggest that reduction of Nurr1 mRNA levels induces subtle changes in DA-mediated neurotransmission.

236 A211

INTEGRATION OF ASYNCHRONOUSLY RELEASED QUANTA PROLONGS THE POSTSYNAPTIC SPIKE WINDOW

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Classically, the release of glutamate in response to a presynaptic action potential causes a brief increase in postsynaptic excitability. Recent reports indicate that at some central synapses, a single action potential can elicit multiple, asynchronous release events. This raises the possibility that the temporal dynamics of neurotransmitter release may determine the duration of altered postsynaptic excitability. In response to physiological challenges, the magnocellular neurosecretory cells (MNCs) in the paraventricular nucleus of the hypothalamus (PVN) exhibit robust and prolonged increases in neuronal activity. While the postsynaptic conductances that may facilitate this form of activity have been investigated thoroughly, the role of presynaptic release has been largely overlooked. Since the specific patterns of activity generated by MNCs require the activation of excitatory synaptic inputs, we sought to characterize the release dynamics at these synapses and determine whether they contribute to prolonged excitability in these cells. We obtained whole-cell recordings from MNCs in brain slices of p21-44 rats. Stimulation of glutamatergic inputs elicited large and prolonged postsynaptic events that resulted from the summation of multiple, asynchronously released quanta. Asynchronous release was selectively inhibited by the slow calcium buffer EGTA-AM and potentiated by brief stimulus trains. Administration of these trains also caused a prolonged increase in postsynaptic spike activity that could also be eliminated by EGTA-AM. Our results demonstrate that glutamatergic terminals in PVN exhibit asynchronous release which is important in generating large postsynaptic depolarizations and prolonged spiking in response to brief high frequency bursts of presynaptic activity.

237 A212

COLOCALIZATION OF DOPAMINE AND GLUTAMATE IN AXON TERMINALS OF THE NUCLEUS ACCUMBENS

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There is increasing data indicating that the vesicular glutamate transporter 2 (VGluT2) is expressed by mesencephalic dopamine (DA) neurons in vitro and in vivo. Recently, we showed by double in situ hybridization that, 2, 6 and 11 days after the cerebroventricular administration of 6 hydroxydopamine (6 OHDA) to neonatal (P4) rats, a significant proportion of surviving neurons of the ventral tegmental area (VTA) contain both tyrosine hydroxylase (TH) and VGluT2 mRNA (Dal Bo et al., 2007, submitted). To seek evidence for the in vivo colocalization of TH and VGluT2 protein in axon terminals of DA neurons, we therefore examined the nucleus accumbens (nAcb) of P15 rats treated or not with 6-OHDA, using double labeling immuno-electron microscopy with specific antibodies against TH and VGluT2. Doubly (TH /VGluT2) as well as singly (TH or VGluT2) labeled axon terminals were readily identified in the nAcb of both control and 6 OHDA-treated rats. The total number of terminals labeled for TH was markedly diminished after the 6-OHDA lesion (38% decrease), but the proportion of dually-labeled terminals slightly but significantly higher than in controls (37% vs 28%; $p < 0.05$). As observed in single thin sections, the frequency with which labeled axon varicosities displayed a synaptic contact was higher for dually (TH/VGluT2) than singly labeled terminals in both control and lesioned rats. When extrapolated to the whole volume of varicosities, the proportion of TH/VGluT2 terminals making a synapse reached almost 100% in both groups, as opposed to respective values of 34% (control) and 55% (lesioned) for terminals labeled for TH only, and 51% (control) and 73% (lesioned) for terminals labeled for VGluT2 only. These data demonstrate that, in the juvenile rat, many axon terminals of DA neurons projecting to the nAcb have the potential to contain and release glutamate. Moreover, the synaptic or asynaptic character of these axon terminals appears to be dependent on their glutamatergic phenotype. Further studies will be needed to determine if similar characteristics prevail at maturity, and whether they are shared by DA neurons innervating other brain regions, such as the neostriatum and the prefrontal cortex (Supported by CIHR grants MOP 3544 and NARSAD).

238 A213

MYELIN BASIC PROTEIN IS A MULTIFUNCTIONAL PROTEIN: LIPID, ACTIN, AND SH3 DOMAIN BINDING

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Myelin basic protein (MBP), the second most abundant protein in central nervous system myelin, is responsible for adhesion of the cytosolic surfaces of multilayered compact myelin. A member of the “intrinsically disordered” or conformationally adaptable protein family, it also appears to have several other functions. It can interact with a number of polyanionic proteins including actin, tubulin, Ca2+-calmodulin (CaM), and negatively charged lipids, and acquires structure on binding to them. It also has a PXXP motif, predicted to bind to proteins with SH3 domains. It can be modified post-translationally by phosphorylation and deimination of Arginine to citrulline, resulting in a reduction of its positive charge. An increase in deimination occurs during development and in multiple sclerosis. Extracellular signals received by myelin or cultured oligodendrocytes cause changes in phosphorylation of MBP, suggesting that MBP is involved in signaling. We investigated the interactions of MBP with actin and SH3-domain containing proteins, ability to bind these proteins to a lipid surface, and the effect of post-translational modifications of MBP and of phosphorylation of phosphatidylinositol in the lipid bilayer on these interactions in vitro. The ability of MBP to bind actin to a lipid surface is decreased by CaM, by phosphorylation and deimination of MBP, and by phosphorylation of PI, all changes which can occur during signaling. MBP also bound to several SH3 domains on an array and bound the Fyn SH3 domain to a lipid surface. Thus it may bind proteins with SH3 domains to the cytosolic membrane surface and/or to the cytoskeleton. Further study of this very abundant protein will reveal how it is utilized by the oligodendrocyte and myelin for different purposes.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

239 A214

CLONING AND CHARACTERIZATION OF PROTEIN KINASE C (PKC) APL III, A HOMOLOGUE OF ATYPICAL PKCS IN APLYSIA

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In Aplysia californica, protein kinase Cs (PKC) are important regulators of synaptic plasticity and learning and memory. Here, we report cloning and characterization of an additional nervous system isoform of PKC in Aplysia, PKC Apl III, homologous to the non-phorbol ester activated zeta and iota isoforms in vertebrates. PKC Apl III is not activated by phorbol esters, but is inhibited by chelerythrine, a common PKC antagonist used in many physiological studies in Aplysia. In vertebrates, a nervous-system specific PKM form of PKC zeta, formed from an alternative start codon, plays an important role in synaptic plasticity; however we find no evidence for a PKM form of Apl III formed by an alternative start site. Interestingly, there is a nervous-specific alternatively spliced form of PKC Apl III that provides a calpain cleavage site for formation of a PKM. Confocal imaging of over-expressed Apl III tagged at the N-terminus with mRFP revealed a large proportion of mRFP in the nucleus; however less nuclear staining was seen with alternative antibodies to the expressed protein. Nuclear mRFP was not seen with mRFP tagged PKC Apl II or when the same tagged mRFP Apl III is expressed in heterologous cells, suggesting specific cleavage of PKC Apl III in sensory neurons, perhaps to a PKM form. Consistent with formation of a PKM, an N-terminal antibody to PKC Apl III also showed increased nuclear staining. We are further investigating the mechanism of PKM formation from PKC Apl III through cleavage in the hinge domain. In addition, we are also examining the ability of 5-HT to activate Apl III through translocation or phosphorylation, in Apl III over-expressed sensory and motor neurons.

240 A215

PRE- AND POSTSYNAPTIC LOCALIZATION OF EPHA4 AND EPHB2 BY SUBCELLULAR FRACTIONATION AND EM ANALYSIS IN ADULT MOUSE FOREBRAIN

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EphA4 and EphB2 receptors have been implicated in synaptogenesis, the maturation of dendritic spines, and long term potentiation (LTP), in the postnatal cerebral cortex and hippocampus. They are generally viewed as postsynaptic in adult CNS, although some data also suggest a presynaptic localization. To determine the precise distribution of these receptors at mature CNS synapses, we used a combination of subcellular fractionation and electron microscopic immunocytochemistry in the adult mouse forebrain/midbrain. EphA4 and EphB2 were particularly enriched in microsomes and synaptosomes. In synaptosomes, they were profuse in the synaptic plasma membrane fraction, and detectable in the synaptic vesicle fraction. In synaptic junctions, EphA4, but not EphB2, remained strongly associated with post-synaptic density (PSD) core proteins, while both were found in the presynaptic active zone fraction. At the ultrastructural level, immunocytochemistry showed EphA4 mainly associated with axon terminals and dendritic spines, in hippocampus and cerebral cortex, whereas EphB2 was mostly dendritic. EphB2 was, however, more frequent in axon terminals and dendritic spines in the ventrobasal thalamus. Consistent with the fractionation results, silver-intensified immunogold particle (SIGP) labeling for EphA4 was associated mainly with synaptic vesicles, in axon terminals, whereas the EphB2 labeling was mostly associated with the plasma membrane of dendritic shafts, in hippocampus and cerebral cortex. Both EphA4- and EphB2-associated SIGP were found on PSD, plasma membrane, and synaptic vesicles. The localization of EphA4 and EphB2 in multiple subcellular compartments of neurons suggests that these receptors interact with distinct proteins and play diverse roles at synapses. Supported by NSERC. D.B. holds a studentship from the Groupe de recherche sur le système nerveux central (Centre FRSQ).

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IMPAIRED GABAERGIC AND GLYCINERGIC NEUROTRANSMISSION INDUCES REM-SLEEP BEHAVIOUR DISORDER (RBD) IN TRANSGENIC MICE

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Chronic RBD is a neurological disorder that is characterized by excessive phasic muscle activity in REM sleep, which often leads to disturbed sleep and physical injury. It is also a harbinger of neurodegenerative disorders, with 80-90% of RBD patients eventually developing Parkinson's disease or other synucleinopathies. Although its cause is unknown, RBD is effectively treated with the benzodiazepine clonazepam (a GABAA agonist). This suggests that dysregulation of the endogenous inhibitory processes that normally suppress phasic muscle activation in REM sleep may underlie the exaggerated motor activity in RBD. We therefore hypothesize that transgenic mice with impaired GABAA and glycine receptor transmission would have excessive motor activity in REM sleep and therefore exhibit an RBD phenotype. To test this hypothesis, we used a transgenic mouse model in which both GABAergic and glycinergic neurotransmission is severely down-regulated (Becker et al., J. Neurosci, 22:2505-12, 2002). To characterize levels of somatic muscle activity, we recorded both EEG and neck EMG activity across the sleep-wake cycle in freely-behaving transgenic (Tg, n=4) and wild-type mice (Wt, n=4). While Tg mice have normal sleep-wake architecture, they have abnormal motor activity during sleep, and particularly in REM sleep. Using both videography and EEG/EMG activity, we observed that all Tg mice exhibited a clear RBD phenotype. They presented with overt periods of vigorous limb movements and jerks. Compared to Wt mice, Tg had a 217% (P=0.016) increase in muscle activity during REM sleep. Although basal levels of muscle activity were similar in Tg and Wt mice during both waking and NREM sleep, all Tg mice had regular myoclonic twitches in NREM sleep. We conclude that: 1) GABAergic and glycinergic processes regulate motor suppression in both REM and NREM sleep; and, 2) impaired inhibitory neurotransmission may underlie RBD.

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POLYSIALIC ACID REMOVAL INCREASES THE RATE OF NEURONAL MATURATION IN THE ADULT DENTATE GYRUS

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In the adult brain, the presence of the carbohydrate polysialic acid (PSA) is limited to areas of on-going neurogenesis. For example, newborn neurons of the dentate gyrus of the hippocampus express high levels of PSA and interestingly, they can develop into mature neurons and form new neuronal circuits. In this study, we use endoneuraminidase N (endoN) to specifically cleave PSA in the hippocampus and examine the impact of PSA removal in adult hippocampal neurogenesis in vivo. EndoN was injected into the anterior hippocampus followed by intrapertioneal injections of 5-bromo-2'-

deoxyuridine (BrdU) two days later. EndoN effectively removed PSA in the hippocampus for at least nine days post-injection, the longest time point tested. Within three days of a BrdU injection, BrdU-positive newborn cells, are found in clusters in the subgranular zone of the dentate gyrus and most express the cell cycle marker, Ki67. In control animals three days after BrdU injection, BrdU-positive cells expressing Ki67 and PSA are found both inside and outside of the cluster. In endoN injected animals, the number of Ki67-positive cells found outside of the cluster was significantly reduced compared to control animals. Seven days after BrdU injection, BrdU-positive cells express immature (double cortin) and mature (neuronal nuclei, NeuN) neuronal markers in control and endoN injected animals. Interestingly, endoN treated animals have a significant increase in the number of mature neuronal cells positive for both double cortin and NeuN, compared to controls. The main findings of this study indicate that PSA favors the migration of newborn cells from clusters and that PSA controls the maturation of newly generated neurons.

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RESISTANCE OF PRESYNAPTIC CALCIUM CHANNELS (CAV2.2) TO VOLTAGE-DEPENDENT INACTIVATION: DYNAMIC PALMITOYLATION AND VOLTAGE SENSITIVITY

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Presynaptic CaV2.2 (N type) calcium channels gate the influx of calcium ions to trigger transmitter release. We have previously demonstrated at the chick ciliary ganglion presynaptic calyx terminal that the bulk of these channels are highly resistant to voltage dependent inactivation. Recent studies have suggested that CaV2.2 can be rendered inactivation-resistant when expressed with the palmitoylated beta2A subunit and that this effect can be eliminated by tunicamycin, a general inhibitor of dynamic palmitoylation. We find that while tunicamycin treatment had no effect on CaV2.2 current in the inactivation-sensitive isolated chick dorsal root ganglion (DRG) neuron, it caused a 10 mV hyperpolarized shift in the profile of the inactivation-resistant presynaptic CaV2.2 population. This shift occurred without any effect on the voltage sensitivity of the inactivation process, as measured by a Boltzmann slope factor. While these findings suggests that dynamic palmitoylation contributes to the inactivation resistance of presynaptic CaV2.2 can not fully account for the differences between the somatal and presynaptic Ca channel biophysical properties.

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RESISTANCE OF NEUROPEPTIDE Y1 RECEPTOR TO MUTAGENIC INDUCTION OF CONSTITUTIVE ACTIVITY IS OVERCOME BY TMD6 CHIMERIZATION

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Neuronal receptors are generally considered to be silent in the absence of an agonist, but constitutively active G-protein coupled receptors (GPCRs) can produce a receptor signal even in the absence of agonists. Based on structural studies of crystallized rhodopsin, the activated state of a receptor can be encouraged by the loss of intramolecular conformational constraints involving transmembrane domain (TMD) 6. Specifically, site-directed mutagenesis of loci 6.30 and/or 6.34 has resulted in generation of constitutively active mutants. It has therefore been proposed that interactions with these loci may represent a common mechanism for constitutive activity among GPCRs. To test the hypothesis that mutations at these TMD 6 loci would similarly confer constitutive activity to the neuropeptide Y1 receptor, two Y1 receptor mutants with either a T258(6.30)A or N262(6.34)A point-mutation were generated. Using both biochemical and electrophysiological assays, we demonstrate that the wild-type (WT)-Y1 receptor does not possess any agonist-independent activation, and that the mutations at locus 6.30 or 6.34 do not confer constitutive activity. However, when this TMD6 region from the WT-Y1 receptor was replaced with that from the constitutively active m-opioid receptor (MOR), the resulting chimeric receptor – Y1-ICL3-MOR – was shown to be constitutively active. Constitutive activity expressed by the Y1-ICL3-MOR chimera implicates a role for the TMD6 in constitutive GPCR activation and highlights a significant structural difference between the Y1 receptor and other rhodopsin-like GPCRs. We concluded that 1) the activation mechanisms identified from the rhodopsin model are not comprehensive for all GPCRs; 2) these mechanisms do not regulate the activation of the Y1 receptor; and 3) the TMD6 of the Y1 receptor confers conformational constraints upon the receptor structure that are uniquely resistant to constitutive activation. Supported by CIHR MT10250. WFC is a Medical Scientist of the Alberta Heritage Foundation for Medical Research

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SYNAPTIC ACTIVITY AND TRIPHENYLTETRAZOLIUM CHLORIDE METABOLISM ARE CORRELATED AFTER OXYGEN-GLUCOSE DEPRIVATION IN ACUTE, BUT NOT CULTURED, HIPPOCAMPAL SLICES

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The importance of the hippocampus to learning and memory has attracted significant attention regarding how the structure responds to damage. Although many studies have used either the acute hippocampal slice preparation (AHSP) or organotypic hippocampal slice cultures (OHSC), little work has been done to examine if the choice of model is an important variable. We sought to examine whether differences exist in how each model responds to a commonly studied ischemic-like insult, oxygen-glucose deprivation (OGD). Following OGD, synaptic activity was examined by recording orthodromically evoked CA1 subfield responses, while mitochondrial activity was assessed by spectrophotometric measurement of formazan produced by metabolism of 2,3,5-triphenyltetrazolium chloride (TTC). The insult significantly decreased both synaptic and mitochondrial activity within AHSPs, but a disparity existed between these measures in OHSCs. While evoked activity was greatly reduced by a moderate duration of OGD, a much longer period was required to cause a comparable decrease in TTC metabolism. Quantitative immunoblotting revealed that one possible explanation for the discrepancy was an elevated OHSC expression of astrocytes, which are resistant to OGD. Our data indicate that acutely prepared and cultured slices respond differently to OGD, and suggest that assays examining viability in these models must consider such innate differences.

246 A221

MECHANISM OF NEUROPROTECTIVE ACTION OF THE PSD-95 INHIBITOR TAT-NR2B9C

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NMDA receptors (NMDARs) interact with the PDZ-domain-containing scaffolding protein PSD-95. Perturbing this interaction with a cell-permeable peptide that recapitulates the C-terminus of NMDAR 2B subunit (Tat-NR2B9c) reduces neuronal nitric oxide production, excitotoxic vulnerability,

and stroke size in rats (Aarts et al., Science, 2002). However, the human genome contains >250 unique PDZ domains, raising the possibility that Tat-NR2B9c may protect neurons through other PDZ-domain interactions. To determine the mechanism of action of Tat-NR2B9c, its interactions with all known human PDZ domains were determined using a ELISA-based assay for which all PDZ domains were cloned and expressed as GST:PDZ fusion proteins. The interactions of nNOS with all human PDZs were similarly tested. These screens revealed that Tat-NR2B9c interacts strongly with 5 PDZ containing proteins: PSD-95, PSD-93, SAP-97, SAP-102 and TIP1. Moreover, nNOS interacted with PSD-95, PSD-93, SAP-97 and Syn1 Alpha. The EC50s of these interactions were also studied, as well as their inhibition by Tat-NR2B9c. The IC50 of Tat-NR2B9c for inhibiting the interactions between PSD95:nNOS and PSD95:NR2 subunits was ~0.2 uM, and 0.5-8uM, respectively. To determine which of the observed interactions may play a role in the neuroprotective mechanism of Tat-NR2B9c, the expression of PSD-95, PSD-93, SAP-97, SAP-102 and nNOS in cultured cortical neurons was inhibited by transfecting them with small interfering RNA duplexes (siRNA) targeted against each of these proteins. The neurons were then subjected to NMDA toxicity in order to determine the role of each protein in their vulnerability to excitotoxicity. Only neurons lacking PSD-95 or nNOS, but not neurons lacking PSD-93, SAP-97 or SAP102 exhibited a reduced vulnerability to NMDA toxicity. Collectively, our data indicate that NMDAR-dependent excitotoxicity is mediated via the interactions of NMDAR subunits with PSD-95, and not other PDZ-domain containing proteins. Neuroprotection by Tat-NR2B9c is thus likely due to its ability to dissociate NMDARs from nNOS both by inhibiting NR2:PDS95 and PSD95:nNOS interactions.

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GLUTAMATE RECEPTOR SUBUNIT INCREASE IN THE GLOMERULAR LAYER OF THE OLFACTORY BULB MAY SUPPORT MEMORY IN ODOR PREFERENCE LEARNING IN NEONATAL RATS

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Phosphorylation of the AMPA receptor GluR1 subunit has been reported to be important for the stability of LTP (Lee et al. 2003; Whitlock et al. 2006). This phosphorylation-induced increase of synaptic receptors was critical for potentiating synaptic transmission and is proposed as a mechanism underlying the LTP stability. In odor preference learning in neonate rats, we hypothesize that phosphorylation of the AMPA receptor GluR1 subunit induces AMPA receptor increases in the glomerular layer of the olfactory bulb. This area is where the axons of the olfactory sensory neurons synapse with the dendrites of mitral cells, the major output neurons in the olfactory bulb. This increase in AMPA receptors would help support memory. Previously, we reported that under learning conditions (odor + 2mg/kg isoproterenol), PKA phosphorylation of the AMPA receptor subunit (GluR1) at serine 845 increased from the end of training to 30 min after training, reaching the maximum at 10 min after training. This increase was specific to the learning group. However, total GluR1 did not change from the end of training to one day after, as measured using Western Blot analysis of the whole olfactory bulb homogenates. To identify whether phosphorylation of GluR1 induces redistribution of GluR1 and increases GluR1 specifically to the synaptic area, instead of the whole bulb, we measured the GluR1 changes in the glumerular layer by using immunohistochemistry. One day after a 10 min training session, during which peppermint odor was paired with 2mg/kg isoproterenol, the GluR1 in trained animals increased significantly, compared to naïve animals. Further learning-specific controls are currently being examined. Our preliminary results suggest that phosphorylation through cAMP-PKA pathway enhances glutamatergic transmission in the olfactory bulb and may be a mechanism underlying the maintenance of odor preference memory. Supported by CIHR

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IMPLICATION OF THE CHEMOKINE MCP-1/CCL2 IN SPINAL NOCICEPTIVE NEUROTRANSMISSION

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An increasing number of studies describe the roles played by chemokines in the central nervous system. Here, we investigate the implication of the chemokine MCP-1/CCL2 in spinal nociceptive neurotransmission in the rat both in normal and pathological conditions. We show that the MCP-1/CCL2 is constitutively expressed by nociceptive sensory neurons in the dorsal root ganglion (DRG), especially nociceptors and in the superficial layers of the spinal cord. Using a subcellular fractionation approach, we show that MCP-1/CCL2 is enriched in large vesicles and that potassium-triggered depolarization evokes calcium-dependent release of MCP-1/CCL2 from DRG or spinal explants. In addition, we demonstrate that CCR2, the preferred CCL2 receptor, is expressed in spinal cord neurons of healthy rat and that exposure of cultured spinal neurons to MCP-1/CCL2 leads to a dose-dependent inhibition of GABA-induced currents. Finally, we report that a single intrathecal injection of MCP-1/CCL2 (1µg) induces allodynia mechanism from 2 hours to 6 days post injection in normal rat. Moreover, we observed that this treatment is also responsible for thermal hyperalgia from 90 to 240 min post injection in hot plate test (52°C). These data suggest that the chemokine MCP-1/CCL2 might be a nociceptor-expressed pronociceptive neuromediator and strongly strengthen the recent concept that the chemokines represent a new class of neuromodulators.

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CHRONIC AND ACUTE ALTERATIONS OF DROSOPHILA FREQUENINS REVEAL THEIR ROLES IN SYNAPTIC TRANSMISSION AND NERVE TERMINAL MORPHOLOGY

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Frequenin (Frq) and its mammalian homologue, neuronal calcium sensor 1, are calcium-binding proteins which enhance neurotransmitter release and facilitation. Here, we report the discovery of a second Frq-encoding gene (frq2) in Drosophila. The temporal and spatial expression patterns of the two genes are very similar, and the proteins they encode, Frq1 and Frq2, are 95% identical in amino acid sequence. We used the Gal4/UAS system to overexpress Frq1, Frq2 or both in the nervous system. Nerve-evoked excitatory junction potentials (eEJPs) of Frq1 and/or Frq2 overexpressers were significantly larger than in controls, due to a 1.4 to 2-fold increase in quantal content at individual synaptic boutons. One of the two motor neurons (MNSNb/d-Is) innervating abdominal muscles 6 and 7 had fewer synaptic boutons in Frq1 and Frq2 overexpressers, indicating selective effects on neuromuscular junction formation. We used several methods to reduce or interfere with the function of Frq: RNA interference; transgenic expression of an interfering C-terminal peptide to chronically disrupt interaction of Frq with its intracellular targets; and introduction of the same peptide into presynaptic terminals using a forward-filling method to acutely disrupt Frq's effects. Both acute and chronic disruption of Frq's actions caused a 70% reduction in quantal content per bouton. The chronic treatment also produced more synaptic boutons from MNSNb/d-Is motorneurons. All the effects are identical for both Frqs, and are consistent with gain-of-function and loss-of-function genotypes. Overall, there was a 6-fold range in quantal content that was observed in response to altered Frq levels and/or activity. We conclude that Frq has two distinct effects: one on neurotransmitter release, and a second on the formation of synaptic boutons.

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FACILIATION IS SENSITIVE TO THE NUMBER OF OPEN CA CHANNELS BUT NOT THE FLUX PER CHANNEL AT FROG NEUROMUSCULAR JUNCTION (NMJ)

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Paired pulse faciliation (PPF) is a ubiquitous phenomenon. Like transmitter release itself it is dependent upon Ca influx into the presynaptic terminal (Katz and Miledi 1967 J. Physiol. 189:535) but there are several distinct differences. For example, facilitation is more sensitive than release to presynaptic addition of Ca buffers with slow forward binding rates. We have explored the effect of changing the flux per open channel, or the number of open channels on facilitation and release by changing Ca(ext) or applying low doses of omega-conotoxin GVIA (w-CTX) while recording excitatory junction potentials (ejps) from frog pectoralis muscle. Although release is very sensitive to Ca(ext) facilitation is not. Reducing Ca(ext) to 40% of normal reduced release by more than 16-fold but had no detectable effect upon PPF and reducing Ca(ext) to 10-20% reduced release by more than 100-fold but PPF was reduced by <10% (n=9). Adding w-CTX (50-200nM) to normal (1.8 mM Ca) Ringer reduced release gradually over the course of 30-60 minutes. PPF was reduced concomitant with blockade of release: > 50% when release was reduced 6-fold and > 80% when release was reduced 10 fold (n=6). The inhibitory effect of w-CTX on PPF was not increased by applying w-CTX in reduced Ca(ext) (40% normal) Ringer (n=2; see Zengel et al., 1993 Br. Res 61:25)). Collectively our data suggest the spatial pattern of Ca influx, rather than the total influx has a strong effect on facilitation at synapses like frog nmj where release is driven by a sparse array of open Ca channels in a linearly extended active zone (Shahrezaei et al., 2006 J. Neurosci. 26:13240; Cho and Meriney, Eur. J. Neurosci. 23:3200). Biophysical modeling currently underway suggests facilitation is restricted to vesicles in a zone around an open channel that is less than the length of a single active zone and does not extend to adjacent active zones. This has implications for how neuromodulators that regulate presynaptic Ca activity could differentially affect release and facilitation depending upon the microgeometry of the active zone at different kinds of synapses. Supported by UBC CIHR Neuroscience Training Grant (LK, RM) and NSERC RGPIN 121698

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UNDER CHRONIC FLUOXETINE TREATMENT, FUNCTIONAL 5-HT1A AUTORECEPTORS ON THE PLASMA MEMBRANE OF NUCLEUS RAPHE DORSALIS NEURONS ARE REPLACED BY DESENSITIZED RECEPTORS

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The desensitization of 5-HT1A autoreceptors plays a key role in the antidepressant effects of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs), as these receptors exert a negative control on the firing and hence release of 5-HT neurons. Using quantitative immunogold electron microscopy, we have previously demonstrated an internalization of 5-HT1A autoreceptors in neurons (soma-dendrites) of the nucleus raphe dorsalis (NRD) of rats acutely treated with the SSRI, fluoxetine (Prozac®) (Riad et al., 2004). Twenty-four hours after the i.p. injection of fluoxetine, the receptors were back on the plasma membrane of the neurons. Here, we compared the subcellular localization of 5-HT1A receptors after 1 day and 3 weeks of fluoxetine treatment via Alzet minipumps (10 mg/kg/day). After the 1 day treatment, 5 HT1A receptors were partly internalized in dendrites of the NRD (autoreceptors), but not hippocampus (heteroreceptors). In contrast, after the prolonged treatment, the density of dendritic plasma membrane immunolabeling was the same between treated and control rats in both the NRD and hippocampus. This was unexpected in view of the strong evidence for a desensitization of 5 HT1A autoreceptors under such conditions. In addition, several studies have previously documented an uncoupling of 5-HT1A receptors from their G protein in the NRD (but not hippocampus) of rats chronically treated with fluoxetine. Therefore, the most likely explanation for our results is that, after repeated internalization and retargetting, functional 5 HT1A autoreceptors have been replaced by receptors uncoupled from their G protein on the plasma membrane of NRD neurons. The functional regulation of these receptors may thus depend on a dynamic balance between their production, activation, internalization and recycling to the plasma membrane in inactivated (desensitized) form. (Supported by CIHR grant MOP-3544).

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SUPPLEMENTAL OXYGEN RESCUES CHRONIC HYPOXIA INDUCED NEURONAL IMPAIRMENT

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Chronic hypoxia is one of the most common pathological processes seen in clinic and caused neural disfunction. Although oxygen supplement is a common practice in clinic for patients with severe chronic hypoxia-related disorders, its therapeutic effect remains controversial due largely to a lack of cellular and molecular evidence of improvement. In this study, we have investigated the effects of hypoxia and reoxygenation with either 40% O2 or 80% O2 on variables of neurobehaviors, hypoxia stress factors and presynaptic exocytotic proteins in a simple invertebrate model, fresh water pond snail Lymnaea stagnalis. We found that chronic hypoxia exposure caused a delayed response of the snail to light stimuli, which coincided with a suppression of locomotion activity. Supplement of 40% O2 improved, but 80% O2 aggravated the hypoxia-induced neural suppression behaviors. Semi-quantitative immunoblotting analyses showed that chronic hypoxia exposure induced up-regulation of both HIF-1 and HSP70, was significantly reduced by 40% O2 supplement. 80% O2 supplement reduced HIF-1 expression, but not HSP70. By comparison, O2 supplement prevented the hypoxia induced reduction of presynaptic exocytotic proteins. To determine the long-term effects of O2 supplement, we further studied neural behaviors and the protein levels during the post-O2 supplement periods. We found that both O2 supplements accelerated recovery of the animals from hypoxia induced neural suppression, although 80% O2 caused an initial aggravation of the symptoms. Immunoblotting analyses showed that O2 supplement groups exhibited a rapidly recovery of expression of the proteins detected, as compared to that seen in chronic hypoxia group without O2 treatment. The recovery of protein expression was closely related to neurobehavioral improvement. In conclusion, our findings provided the direct evidence that supplemental oxygen not only improves neurobehavioral dysfunctions but rescues altered stress inducible factors and presynaptic proteins induced by chronic hypoxia. Our findings thus support the notion of supplemental oxygen is necessary in the treatment of chronic hypoxia-induced neurobehavioral adaptation and impairment.

253 A228

MORPHOMETRIC ANALYSES OF DENDRITIC ARBOURIZATION OF THALAMOCORTICAL NEURONS IN SOMATOSENSORY AND MOTOR NUCLEI OF THE THALAMUS

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The thalmocortical (TC) network is organized in a loop and contributes to a variety of sensory and cognitive functions. The main gateway of TC system is the dorsal thalamus, which receives specific inputs from ascending sensory pathways. Incoming excitatory inputs terminate on separate

subcellular compartments along the TC cells dendrites. Prethalamic inputs occupy predominantly proximal dendrites in contrast, corticothalamic inputs arrive mainly distally. On the intermediate dendrites prethalamic ascending and corticothalamic descending inputs overlap. In order to reveal how dendritic arbor of TC cells provide potential membrane surface for incoming synapses, we analysed the dendritic architecture of TC cells in ventral lateral (VL) and ventral posterior lateral (VPL) nuclei of the cat. To this end, we used extra- and intracellular neuronal tracing combined with morphometric analyses. Neurons from VPL nucleus were labelled by retrograde transport of fluorescent dextrane-amine injected in the somatosensory cortex. VL neuron was visualized using intracellular injection of neurobiotin. Spatial organization of the labelled dendrites was revealed with three-dimensional reconstruction. We found that individual neurons varied considerably in overall size and had a mean soma diameter of 32.8±5.3μm. The labelled cells had usually 6-11 thick dendrites emerging from the soma. The total dendritic length was estimated (mean 16603.7±5075.9μm) and showed 2.57 fold differences among the cells. The size of dendritic trees ranged from 315 to 520μm (mean 415±66μm). The thickness of individual dendrites decreased suddenly with increasing distance from the soma, although dendritic diameter was constant from 4 to 11 dendritic orders without radical dendritic tapering of the distal segments. The mean diameter of 1st order dendrites was 3.5±0.6mm and the last order dendrite was 0.8±0.2mm. The number of branch orders of the reconstructed cells ranged between 2 and 11. Sholl-analysis revealed a peak value of dendritic bifurcations between 50-90μm from the soma. Our quantitative analyses showed that the dendritic area (total mean 43926.8±16063.7μm2) reached the maximum at 100-150 μm from soma, which corresponds to 5-6th dendritic orders. These findings indicate that the maximal membrane area for incoming inputs is present on 5 and 6 order dendritic branches, where presumably both ascending prethalamic and descending corticothalamic axons form synapses. Supported by NSERC and CIHR

254 A229

CHRONIC DEPOLARIZATION UP-REGULATES MITOCHONDRIAL PROTEIN IMPORT IN DIFFERENTIATED PC12 CELLS

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The majority of mitochondrial proteins (>99%) are nuclear-encoded and must be imported into mitochondria. While deficits in import are known to impact virtually every aspect of mitochondrial function, little is known about how mitochondrial protein import is regulated in neurons. We hypothesized that electrical activity, specifically chronic depolarization (50mm KCl), regulates mitochondrial protein import in neurons. To assess the effects of KCl on import in differentiated PC12 cells we measured the import of 3 proteins; (a) mtGFP, an inducible GFP fusion protein targeted to mitochondria, (b) mtHSP70, a mitochondrial matrix chaperone, and (c) Tom20, a key mitochondrial protein import receptor. Protein import to mitochondria, cytoplasmic levels of mitochondrial proteins and protein expression were measured by Western blot of mitochondrial fractions, cytoplasmic fractions and whole cell lysates respectively. mtGFP import in live cells and intramitochondrial turnover of mtGFP were assessed by flow cytometry. Results: Exposure to KCl (50mM, iso-osmotic) significantly increased mtGFP import; 24hrs post induction of mtGFP the fluorescence signal increased by 33%+/-7 (n=5, p< 0.01) compared to controls and 40%+/-5 (n=5, p<0.001) by 48hrs. Western blots confirmed that KCl increased mtGFP import; by 24hrs mtGFP increased by 59%+/-5 (n= 3, p< 0.05) compared to controls. mtGFP expression also increased significantly but mtGFP intramitochondrial turnover was unchanged for up to 48hrs. The KCl induced increase in mtGFP import was reversible, blocked by the L-type calcium channel antagonist, nimodipine, and further enhanced by treatment with the L-type calcium channel agonist, BayK. The effects of KCl on import were selective; KCl increased both expression and import of a physiological protein mtHSP70 but did not up-regulate Tom20 or GAPDH. Treatment with KCl wholly or partially blocked the inhibitory effects of amyloid beta, CCCP and trophic withdrawal on mitochondrial protein import. These findings demonstrate that in neurons chronic activity up-regulates the expression of some nuclear encoded mitochondrial proteins and increases their import to mitochondria. Our findings suggest that the KCl mediated increase in import is dependent, at least in part, on calcium influx through voltage gated calcium channels.

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REGULATION OF DOPAMINE SYNAPTIC TRANSMISSION IN THE MIDBRAIN

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Dopamine (DA) neurons of the Ventral Tegmental Area (VTA) contribute to reward, learning, and are involved in drug addiction and a variety of mental disorders. In addition to releasing DA at their axon terminals, DA cells are also capable of releasing DA from their somas and dendrites. This somatodentrically released DA functions to reduce neuronal excitability due to the activation of inhibitory D2 'autoreceptors' and activation of a G-protein activated inhibitory potassium (GIRK) conductance. Detecting DA by classical means using efflux or carbon-fiber amperometry has previously led to the conclusion that somatodendritically released DA acts in a paracrine manner (i.e. volume transmission, or 'spill-over'). However, we have recently found that that somatodendritically released DA can produce an inhibitory postsynaptic current (IPSC). The kinetics of this synaptic event suggest that within the midbrain, DA may be acting in a manner more analogous to classical synaptic transmission and argues against a paracrine mode of transmission (i.e. not volume transmission). To further address the manner by which DA signals in the midbrain, we have electrophysiologically recorded DA IPSCs while simultaneously measuring DA release with fast-scanning carbon-fiber voltammetry. By performing simultaneous measurements of DA signaling we have set out to determine the mechanisms by which DA is released somtodendritically. Briefly, we have found that, DA release in the midbrain is fast, vesicular, dependent upon calcium entry and not dependent upon reversal of the dopamine uptake transporter. These findings suggest that somatodendritic DA release occurs in a manner analogous to rapid synaptic transmission.

256 A231

SUBTHRESHOLD OSCILLATIONS OF MEMBRANE POTENTIAL OF RAT SUBFORNICAL ORGAN NEURONS.

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Previous work has demonstrated that SFO neurons exhibit spontaneous action potentials interspersed with periods of membrane potential oscillation (MPO). We used whole cell patch clamp recording to investigate properties of thesoscillations. The amplitude, but not the frequency of the MPO exhibited voltage dependence. The MPOs were not affected by application of blockers f ionotrophic GABA and glutamate receptors, nor were they affected by the application of Cd++ to block voltage gated Ca++ channels, or Cs+ to block HCN channels. The MPOs however were abolished by the presence of TTX in the external recording solution, indicating a critical role of voltage gated Na+ channels. Therefore, we investigated the properties of voltage gated Na+ channels using voltage clamp techniques. We found that activation of the persistent Na+ current (INaP), but not the transient Na+ current (INaT) corresponded with the voltage dependence of activation of the MPO. Moreover we found that voltage gated Na+ currents exhibited a remarkably slow time dependent recovery from inactivation, which may play a role in determining the observed frequency of the MPO.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

257 A232

LIGHT-DEPRIVATION DISRUPTS THE FUNCTIONAL AND MORPHOLOGICAL PROPERTIES OF INHIBITORY A2 AMACRINE CELLS OF THE RETINA

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Early sensory experience is crucial to the development and maturation of synaptic circuits. In the retina, plastic changes in neuronal circuits have been mainly reported for excitatory ganglion cells. In this cell-type, light-deprivation (LD) interferes with normal dendritic modeling that in turn compromises visual processing. Importantly, Ca2+ influx through NMDA-selective ionotropic glutamate receptors has been shown to be central to this process. However, not all retinal cells express NMDARs. Recently we have identified a novel philanthotoxin (PhTX)-insensitive Ca2+-permeable AMPAR that is developmentally-regulated and selectively expressed in two inhibitory retinal cells, horizontal cells (HC) and A2 amacrine cells. Interestingly, neither cell-type expresses synaptic NMDARs suggesting that Ca2+-influx through PhTX-insensitive AMPARs during development may be also important in sculpting dendritic refinement. Here, we show that light entering the eye shapes inhibitory synaptic circuit formation by triggering expression of a novel Ca2+-permeable AMPAR through a BDNF-dependent mechanism. Specifically, we have addressed three questions;1) Does LD affect the composition of AMPARs? 2) Does LD affect the morphology of retinal cells? 3) Does BDNF trigger the expression of PhTX-insensitive AMPARs? Using cobalt (Co2+) staining and electrophysiological recordings to examine the expression of Ca2+-permeable AMPARs, we observed that PhTX-insensitive receptors failed to express in dark-reared animals. In addition, the levels of expression of BDNF protein were completely abolished in light-deprived rats. Interestingly, exogenous application of BDNF (3hrs, 250ng/ml) rescued PhTX-insensitivity in dark-reared rats. Finally, morphological analysis of A2 cells revealed that LD changed dendritic arborization of A2 cells. Moreover, presynaptic OFF-cone and rod-bipolar cells also showed disrupted morphology suggesting that PhTX-insensitive Ca2+-permeable AMPARs may act as molecular guideposts for incoming axons. In summary, light entering the eye triggers expression of novel Ca2+-permeable AMPARs through a BDNF-dependent pathway that may be critical in the development and sculpting of retinal inhibitory circuits.

258 B201

NUCLEOTIDES DIRECTLY AND INDIRECTLY REGULATE NEUROENDOCRINE CELL CATION CHANNEL VOLTAGE-DEPENDENCE

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Ion channel voltage-dependence is often modulated to change neuronal output and trigger behaviour. In the bag cell neurons of Aplysia californica, opening of a voltage-gated, non-selective cation channel drives a 30 minute afterdischarge, that results in the secretion of egg-laying hormone and the initation of reproduction. It is known that protein kinase C (PKC) can closely associate with the channel, and thereby enhance both its activity and the excitability of the neuron; however, the mechanism of this modulation remains unknown. The current study used single-channel recording to examine the effects of channel PKC-dependent phosphorylation on voltage-dependence. Application of 1 mM ATP to the cytoplasmic face of cation channel-containing excised, inside-out patches yielded either an increase or no change in open probability (Po), consistent with phosphorylation by associated PKC or no kinase association, respectively. Channels whose activity was upregulated by ATP (340% increase in Po) showed a slight left-ward shift in voltage-dependence, indicated by a change in V0.5 of -3 mV. Interestingly, channels whose Po did not change (-0.07% change in Po) showed a right-ward shift in voltage-dependence, with a change in V0.5 of +14 mV. No change in voltage-sensitivity or conductance was seen with the application of ATP. Because it has been previously shown that channels whose Po does not change lack an associated kinase, the present data suggests that ATP, beyond its purpose as a phosphate donor, may directly shift the voltage required for half-maximal opening to more positive potentials. In addition, the right-shift in voltage-dependence by ATP on its own, may be masking any left-shift produced by PKC-dependent phosphorylation. This unexpected result prompted further investigation of how nucleotides may interact with the cation channel. Preliminary experiments suggested that application of 0.1 mM GTP to the cytoplasmic face of channels in excised, inside-out patches increased the Po by left-shifting the voltage-dependence and increasing the sensitivity. It remains to be determined whether GTP is having an effect through an associated G-protein or by acting directly on the channel. In general, long-lasting changes in neuronal output and behaviour may be achieved by multiple factors converging on the voltage-sensor of an ion channel and altering its sensitivity to the membrane potential.

259 B202

ACTIVITY-DEPENDENT AND RECEPTOR-OPERATED CHANGES TO INTRACELLULAR CALCIUM IN NEUROENDOCRINE CELLS.

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Intracellular calcium dynamics are influenced by both extracellular calcium influx and calcium released from stores. Depending on the amount of initial calcium influx, a range of subsequent responses can result. We examined intracellular calcium following receptor-operated or voltage-gated calcium influx in the bag cell neurons of the marine mollusc, Aplysia californica. Following a brief synaptic input, these neuroendocrine cells undergo a 30-minute period of repetitive firing, known as the afterdischarge. During this burst, a concomitant and rapid increase in intracellular calcium triggers the secretion of several neuropeptides to initiate egg-laying. Cultured bag cell neurons were injected with the high-affinity calcium indicator, fura-PE3, and subjected to simultaneous ratiometric imaging and electrophysiology. Pharmacological activation of a non-selective cation channel, previously implicated in driving the afterdischarge, produced both inward current and, as assessed by manganese-quench of fura fluorescence, calcium entry. This receptor-operated calcium influx pathway may contribute to plasticity or neuropeptide secretion during bursting. Voltage-gated calcium influx was investigated using trains of action potentials delivered at 5 Hz, 10 sec (to mimic the synaptic input that initiates the afterdischarge) or 5 Hz, 1 min (to mimic the fast phase of the afterdischarge). While both trains transiently elevated intracellular calcium, only the 5 Hz, 1 min stimulus resulted in a calcium rise that markedly outlasted the initial influx, consistent with calcium-induced calcium release. Accordingly, depletion of the smooth endoplasmic reticulum v store, with the calcium-ATPase blocker, cyclopiazonic acid, or collapse of the mitochondrial hydrogen gradient, with the protonophore, FCCP, significantly attenuated the elevation. Thus, robust levels of calcium influx are required to trigger calcium-induced calcium release in bag cell neurons; furthermore, both the endoplasmic reticulum and the mitochondria appear to sustain this release of intracellular calcium. Activity-dependent induction of a prolonged calcium elevation may be important in initiating secretion or in transcribing genes pertinent to reproduction.

260 B203

DETERMINING THE SPATIOTEMPORAL PATTERN OF SYNAPTIC INPUTS IN HIPPOCAMPAL NEURONS DURING POPULATION RHYTHMS

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In order to generate behaviourally significant activity in neurons, synapses need to be assembled into specific patterns of connectivity. For example, an inhibitory synapse can override excitatory synapses further from the soma along the same path, but has little effect on excitatory synapses nearer to the soma. In this way, dendritic integration is a function of the specific morphology and biophysical properties of the neuron. Because these properties are very complex, it is a difficult problem to determine what pattern of inputs in a neuron produces physiologically realistic output. Using an in vitro preparation, signal processing methods, and a detailed compartmental model of a CA1 hippocampal neuron, we developed methods to obtain constraints on the temporal profile of synaptic inputs necessary to generate realistic output. Time-frequency analyses were used to determine characteristics of intact hippocampal preparation field and intracellular recocordings. The problem of spatial summation in field recordings was controlled for by determining a distinct pattern between variability in the temporal and spatial domain in this preparation. The time-frequency characteristics of the real hippocampal recordings are implemented in a detailed compartmental model of a CA1 hippocampal neuron using the genetic algorithm. This generated constraints on the pattern of synaptic input to the model neuron. We found that excitatory input plays a greater role with greater distance from the soma in tuning neuronal output to physiologically accurate temporal and frequency characteristics. Inhibitory input played a greater role in tuning output near to the soma. Because diseased brain states, and specifically epilepsy, are often strongly associated with a change in the signal measures (nonstationarity and time-frequency properties) we used, our method may allow us to make inferences as to the pattern of synaptic activity present in diseased vs functional brain states and the transition between the two. (This study was funded by CIHR, NSERC, and Epilepsy Canada.)

261 B204

GLIAL CELL INDUCED NON-HOMEOSTATIC FAST SYNAPTIC SCALING

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Current concepts of activity dependent changes in synaptic function rely on our understanding of two disparate forms of plasticity: rapid, synapse specific plasticity (LTP and LTD) and more recently, slower, compensatory scaling of all synaptic inputs in response to persistent changes in neuronal activity (homeostatic plasticity). Whether a type of plasticity exists that incorporates the rapid induction of LTP as well as the global scaling of homeostatic plasticity remains to be established. In response to focal glial cell stimulation by photolysis of caged MNI glutamate, we observe a long lasting enhancement in the strength of glutamatergic synapses onto hypothalamic neurons. The induction of this plasticity occurs reliably within minutes like LTP; unlike LTP, however, the effect is not confined to specific synapses. Instead, changes are expressed globally at all glutamatergic synapses contributing to spontaneous excitatory postsynaptic currents—a result similar to homeostatic plasticity. The augmentation of synaptic strength requires the physical presence of glial processes around synaptic spaces and the activation of receptors for both glutamate and ATP. These data indicate that glial cells can induce a fast, non-homeostatic, global enhancement of synaptic strength at excitatory inputs in the hypothalamus.

262 B205

DIFFERENTIAL ROLES OF CALCIUM BINDING PROTEINS IN MEMORY CONSOLIDATION IN LYMNAEA STAGNALIS

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Consolidation of aversive operant conditioning into long-term memory requires CREB dependent protein synthesis. However, molecular pathways involved in CREB-mediated long-term memory consolidation remains to be investigated. Taking advantage of a well-established adverse operantly conditioned model of aerial respiratory behavior in the fresh water pond snail Lymnaea stagnalis, we investigated the involvement of two calcium binding proteins, neuronal calcium sensor-1 (NCS-1) and LCaBP (a novel Lymnaea calcium binding protein) in formation of long-term memory (LTM). Specifically, we found that upregulation of both total and phosphorylated CREB1 coincided with elevations of NCS-1 and LCaBP in the snails with reliable and consistent LTM formation 24 hours after operant training. To explore the role of calcium binding proteins in memory consolidation, a dsRNA approach was used to trigger gene silencing of the calcium binding proteins. Direct delivery of dsRNA specific to LCaBP, but not control dsRNA, into the ganglia of the snail prior to the training, sufficiently reduced the specific gene/protein expression and prevented snails from learning and memory consolidation; whereas a partial knockdown of NCS-1 did not affect LTM. To further address the mechanism of LCaBP in LTM formation, we screened the downstream targets of LCaBP and found that LCaBP knockdown decreased expression of CREB1, presynaptic exocytotic proteins (syntaxin) as well as endocytotic protein (dynamin), indicating that LCaBP plays a critical role in memory consolidation in Lymnaea stagnalis by regulating CREB expression. Taken together, our findings suggest that although both are upregulated, LCaBP, but not NCS-1, is essential for long-term memory formation.

263 B206

CHARACTERIZATION OF OLIGODENDROCYTE MORPHOLOGICAL DEVELOPMENT AND MOTILITY IN HIPPOCAMPAL SLICE CULTURE USING SEMLIKI FOREST VIRUS AND MEMBRANE-TARGETED FLUORESCENT PROTEINS

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Myelination is critical for the rapid propagation of action potentials in most high caliber axons. Although the development of Schwann cells, the myelinating cells of the peripheral nervous system, is well documented, comparatively little is known about the three-dimensional maturation of oligodendrocytes, the myelinating cells of the central nervous system. Here, we follow the developmental stages of oligodendrocytes in organotypic hippocampal slice cultures for 7-60 days using viral-mediated gene delivery of membrane-associated fluorescent proteins. In culture, precursors differentiate over time to form early premyelinating and later myelinating oligodendrocytes with identifiable nodes of Ranvier. Using confocal microscopy and time-lapse imaging, we find that postmigratory NG2-positive cells have relatively low process density and are structurally stable. Later stage premyelinating cells, however, have increased process density, form dynamic growth-cone-like structures at their distal ends, and undergo dramatic structural reorganization over the course of minutes. Later stage myelinating oligodendrocytes, have pruned most of their processes and are relatively stable. Our findings provide a detailed characterization of the morphology and motility of both premyelinating and myelinating oligodendrocytes and precursors as well as describe an in vitro system to study the process of myelination by oligodendrocytes positioned in a three-dimensional slice culture.

264 B207

MICROGLIA PROCESSES BLOCK THE SPREAD OF DAMAGE IN THE BRAIN

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Microglia cells, the immune cells of the brain, have recently been shown to rapidly extend processes to regions of nearby cellular damage. However the impact of this response is unresolved. Using two photon laser scanning microscopy we show that microglia process extension is an important protective response that prevents the spread of damage into surrounding undamaged tissue. Focal lesions were made in hippocampal slices and ensuing process outgrowth of activated microglia was monitored with time-lapse microscopy. We found that inhibiting microglia Cl- channels blocked the rapid outgrowth of processes in response to the lesion. Lesion volume was measured and found to decrease in control conditions where microglia processes reached and contained the lesion. In contrast, when microglia processes outgrowth was blocked by either inhibiting microglia Cl- channels or by selectively ablating microglia, lesion volume increased and damage spread into surrounding tissue disrupting dendrite morphology. These data show that microglia processes are resistant to the excitotoxic effects of factors released by damage and in fact microglia grow into regions of brain damage in order to protect surviving neurons. When microglia processes arrive at the lesion and engulf the damage region they act to contain the harmful factors released by the damage.

Glutamate is an excitotoxic factor that is released at sites of CNS damage and the GLT-1 glutamate transporter that is expressed in microglia may help to prevent the spread of damage. Therefore, the question of whether microglia are harmful or helpful in the brain appears to have a partial answer in the fact that these cells perform a highly organized and critical role in the rapid containment of damage.

265 B208

DOPAMINERGIC MODULATION OF GLUTAMATERGIC-BASED LONG-TERM POTENTIATION IN THE PREFRONTAL CORTEX, IN VIVO

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Drug addiction and behavioural sensitization are associated with a reorganization of mesolimbocortical circuitry, which we have attempted to model with glutamatergic-based long-term potentiation (LTP) and long-term depression (LTD) in the mesencephalon and medial prefrontal cortex (mPFC). The objective of the following experiments was to examine the effects of dopamine-2 (D2) receptor family specific agonists and antagonists on glutamatergic-based LTP in the mPFC in the chronic in vivo preparation using fully-awake, freely-moving rats. Male Long-Evans rats were surgically implanted with stimulating electrodes into the corpus callosum and recording electrodes into the mPFC. Subjects were systemically administered drug together with high frequency stimulation for the induction of LTP. The D2 receptor antagonist sulpiride (3mg/kg/ml, 6mg/kg/ml, 12mg/kg/ml) significantly decreased LTP, compared to the control group, in a dose-dependent fashion. The D2 receptor agonist quinpirole hydrochloride (0.025mg/kg/ml, 0.25mg/kg/ml, 0.5mg/kg/ml) significantly increased LTP in a dose-dependent fashion. The D2 receptor agonist also induced behavioural sensitization. The intensity and frequency of behavioural sensitization was positively correlated with the LTP effect, with an increased expression of sensitization in the LTP groups. These results demonstrate that glutamatergic-based LTP in the mPFC and behavioural sensitization are positively modulated by D2 receptors in the chronic in vivo preparation. As the D2 receptor-rich neurons are located largely in mesencephalic nuclei that, in turn, project to the mPFC, the D2 effects may be indirect. This plasticity modulation needs to be more deeply explored to determine its relationship to disorders such as psychostimulant addiction and schizophrenia, that are known to be due to dysregulated dopamine and glutamate function in the mesencephalon and mPFC. Support: Natural Sciences and Engineering Research Council of Canada.

266 B209

STRESS-INDUCED SPATIAL MEMORY DISRUPTION DEPENDS ON LONG-TERM DEPRESSION IN THE HIPPOCAMPUS

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Stress is often detrimental to cognitive function. For example, acute stress impairs memory retrieval in rodents. Additionally, exposure to stress alters the expression of synaptic plasticity in the hippocampal CA1 region by favoring long-term depression (LTD) over long-term potentiation. However, it is unknown if altered synaptic plasticity causes the impairment in memory after stress. The present experiments used three strategies to test this hypothesis. Current theories suggest that LTD induction in hippocampal slices depends on NR2B-containing NMDA receptors. As a result, we pretreated rats with the NR2B-specific antagonist Ro25-6981 (i.p., 6 mg/kg), which blocks LTD induction following acute stress in vivo, and successfully reversed a stress-induced memory deficit in a standard water maze task. To confirm that the effect of Ro25-6981 was due to LTD inhibition, we also blocked the expression of LTD by disrupting the regulated endocytosis of postsynaptic AMPA receptors with a GluR2 subunit derived interference peptide. Pretreatment of rats with the interference peptide also abolished the stress-induced memory impairment. Recent evidence suggests that stress may reduce glutamate transport, thereby allowing activation of NR2B-containing receptors predominantly located extra-synaptically in adult rats. To determine if induction of hippocampal LTD could mimic the effects of stress, we bilaterally infused the glutamate transporter inhibitor DL- threo-beta-benzyloxyaspartate (TBOA) into the hippocampus and found that like acute stress treatment, TBOA treatment enabled LTD and impaired memory retrieval. Most importantly, both the LTD and memory impairments were reversed with Ro25-6981. Together, these experiments support the notion that activation of NR2B-containing NMDA receptors and subsequent LTD induction causes memory impairment following acute stress.

267 B210

CHRONIC NERVE GROWTH FACTOR TREATMENT INCREASES ACETYLCHOLINE AND GLUTAMATE RELEASE FROM CHOLINERGIC NEURONS OF THE RAT MEDIAL SEPTUM / DIAGONAL BAND OF BROCA

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Nerve growth factor (NGF) is a neurotrophic factor important for the survival, growth and function of cholinergic basal forebrain neurons. However, little is known about the function of NGF on other basal forebrain neurons such as glutamatergic or GABAergic neurons. Here, we used neurons from the rat medial septum/diagonal band of Broca (MS-DBB) to test whether chronic NGF treatment affects the synaptic function of cholinergic, glutamatergic and GABAergic basal forebrain neurons. We isolated single rat MS-DBB neurons in culture, allowing them to establish synaptic contacts onto themselves. One group of neurons chronically received NGF (25ng/ml) while another group received none. After 2-4 weeks in culture, we used whole-cell electrophysiology to measure the amplitude of

evoked autaptic EPSCs or IPSCs. Cholinergic MS-DBB neurons were identified using 192IgG-Cy3, a fluorescent p75NTR receptor antibody. Neurotransmitters involved in postsynaptic currents were characterized pharmacologically. In some experiments, a trk inhibitor K252a was added with NGF to study the role of trkA-mediated signaling. To investigate the synaptic mechanisms underlying changes in evoked EPSCs, miniature EPSCs were recorded. We found that chronic NGF treatment led to a 37-fold increase in the amplitude of cholinergic EPSCs and a 22-fold increase in the amplitude of glutamatergic EPSCs in "cholinergic" MS-DBB neurons releasing both neurotransmitters. However, NGF did not affect autaptic currents in neurons releasing glutamate or GABA only. K252a prevented the NGF-induced increase in glutamatergic EPSCs but not cholinergic EPSCs. Furthermore, chronic NGF treatment led to a 2-fold increase in the frequency of miniature EPSCs in cholinergic neurons but did not affect the amplitude of the events. The present study demonstrates, for the first time, that chronic NGF treatment dramatically increases both glutamate and ACh release from cholinergic MS-DBB neurons. TrkA-mediated signaling was found to be responsible for the NGF-induced increase in glutamate release but not for the increase in ACh release. The present study illustrates that NGF is crucial not only for cholinergic function but also for glutamatergic function in the septo-hippocampal pathway, which plays an important role in memory.

268 B211

LOCALIZING CRITICAL NEURONAL CIRCUITRY BY RANDOM GENETIC SILENCING OF INTERNEURONS IN INTACT DROSOPHILA LARVAE

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We have investigated how stereotypic locomotion and sensory-motor behaviours in the Drosophila larva are encoded. Towards this goal, we employed the UAS/Gal4 and MARCM methods to express green fluorescent protein (GFP) and a temperature-sensitive mutant allele of the vesicle-recycling protein Dynamin (called shibire(ts1) or shi(ts1) in Drosophila) in various subsets of neurons. It is well known that shi(ts1) mutant larvae exhibit rapid paralysis at non-permissive temperature (29 ºC) due to disruption of endocytosis, which in turn results in synaptic vesicle depletion. It has also been demonstrated previously that overexpression of shi(ts1) in a global manner in the larval CNS results in rapid paralysis. Here, we first describe behavioral and kinematic analyses to study shi(ts1) mediated dissection of larval neuronal networks. By constructing transgenic strains that overexpress lower or higher levels of shi(ts1) in different parts of the larval nervous system, we demonstrate that an optimal level of transgenic shi(ts1) expression is important for temperature-mediated disruption of larval locomotion and a simple sensory-motor response. Next, we used the transgenic shi(ts1) strain to create a functional-MARCM (f-MARCM) system for examining the role of randomly selected candidate cholinergic neurons in the initiation and maintenance of locomotion and the touch-mediated avoidance response. In our f-MARCM system, random subsets of interneurons and/or sensory neurons are permitted to express UAS/Gal4 mediated GFP and shi(ts1) due to the homologous recombination of Gal80 repressor transgene that occurs in some precursors during embryonic development of the nervous system. We tested such mosaic larvae for temperature-dependent motor defects or normal behaviors and then mapped the projection patterns of labeled neurons. Due to the excellent GFP expression level, the brain compartments innervated by labeled interneurons could be accurately mapped and their Dynamin expression levels could also be visualized. The f-MARCM strategy constitutes an unbiased screen for identifying functionally important interneurons. Our results indicate that a limited subset of candidate cholinergic interneurons are necessary for the proper execution of larval locomotion and sensory-motor behaviours.

269 B212

MEDIAN RAPHE STIMULATION RESETS HIPPOCAMPAL THETA ACTIVITY AND RELATED CELLULAR ACTIVITY

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Hippocampal theta rhythm is thought to be under inhibitory control of the median raphe nucleus (MRn). High frequency electrical stimulation of the MRn elicits a desynchronization of hippocampal theta rhythm while MRn lesions produce the release of theta rhythm. Despite a detailed analysis of MRn stimulation on hippocampal field activity (Vertes et al., 2005), no systematic study of hippocampal theta-related cellular activity has been performed. In this experiment we stimulated the MRn at 0.5Hz and generated perievent time histograms (PETH) for ongoing EEG and single units recorded from the dorsal hippocampus in urethane anesthetized rats as the animals cycled between theta and non-theta states. Stimulation produced a reset of hippocampal theta field, without producing a change in power or frequency of the field activity. Single units were inhibited with a latency of 5 ms and duration of 68 ms before returning to baseline firing rates. In addition, a subset of phasic theta-on cells (n=14) responded with an oscillatory PETH whereas 9 cells did not exhibit an oscillatory response. Post-hoc analysis of spiking parameters (phase relation to theta, interspike interval, firing rate, strength of phase relation) during spontaneous theta identified the non-oscillatory responding cells as a unique group of rhythmic cells that differed from oscillatory responding cells according to the above parameters. Despite maintaining different phase relations to spontaneous hippocampal theta, all phasic theta-on cells were reset to a common phase following MRn pulse. All rhythmic responses were state dependent, in that they occurred when the pulse was administered during theta, but not non-theta states. Interestingly, phasic theta-off cells were also reset to the same phase as phasic theta-on cells following the MRn pulse. Tonic theta-on cells were not consistently altered by stimulation, while cells unrelated to the hippocampal field activity were not affected. These results suggest high frequency stimulation of the MRn acts to desynchronize hippocampal EEG by resetting all rhythmic hippocampal activity on a short time scale. Additionally, these data demonstrates a cell specific site of action for the MRn modulation of hippocampal theta which is dependent on brainstate. Funding support: NSERC Grant number A9935 to BHB and NSERC CGS to JJ.

270 B213

FUNCTIONAL COUPLING BETWEEN NMDA RECEPTORS AND TRPM2 CHANNELS IN HIPPOCAMPAL NEURONS

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TRPM2 is a Ca2+-permeable, non-selective cation channel that is gated by ADP-ribose (ADPR). During oxidative stress, mitochondria generate free ADPR, which is released into the cytoplasm where it activates TRPM2. This results in Ca2+ dysregulation and subsequent cell death. Since oxidative stress, generated in neurons as a consequence of the excitotoxic stimulation of NMDA receptor (NMDARs), contributes to delayed Ca2+ overload and neuronal demise, we hypothesized that TRPM2 may be activated downstream of NMDARs. We first employed RT-PCR and Western blotting to identify TRPM2 in cultured hippocampal neurons. Immunostaining demonstrated diffuse, extrasynaptic expression of TRPM2, as TRPM2 failed to colocalize with the synaptic marker PSD-95. Whole-cell patch-clamp recordings were subsequently carried out to investigate the presence of functional TRPM2 channels in these cells. Inclusion of 0.3 mM ADPR in the patch pipette induced a large (-558.5 ± 81.9 pA) inward current (IADPR) in cultured hippocampal neurons that was inhibited upon removal of extracellular Ca2+, a hallmark characteristic of TRPM2-mediated currents. Furthermore, the resulting current could be blocked by clotrimazole (10 μM), flufenamic acid (100 μM) and ACA (20 μM) but was insensitive to La3+ (0.1 mM), consistent with TRPM2 activation. Interestingly, IADPR was facilitated by the activation of NMDARs, an effect not simply attributed

to elevated intracellular Ca2+ and suggestive of a more intimate coupling between NMDARs and TRPM2. We next investigated whether a TRPM2-like conductance (ITRPM2-like) could be activated downstream of NMDAR activation. Repeated (1/60 s for 15 min) applications of NMDA (100 μM, 5 s) to hippocampal neurons caused the progressive activation of a sustained inward current (-537.3 ± 68.8 pA). As with IADPR, ITRPM2-like was abolished by the removal of extracellular Ca2+, could be blocked by clotrimazole and was insensitive to La3+. The extrasynaptic localization of TRPM2 suggested that these channels may be preferentially coupled to extrasynaptic NR2B containing NMDARs. This was confirmed in experiments demonstrating that Ro 25-6981 (0.5 μM), a NR2B selective antagonist, prevents the development of ITRPM2-like by repeated NMDA applications. Taken together, these results suggest that hippocampal neurons possess functional TRPM2 channels whose activation lies downstream of NMDARs.

271 B214

MITOCHONDRIAL REMODELLING IN DIFFERENTIATED PC12 CELLS

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Mitochondrial morphology can fairly be described as dynamic, since mitochondria are capable of markedly changing shape within minutes under basal conditions. Dramatic permanent morphological changes in entire mitochondrial populations within a cell have also been associated with disease states and acute cell injury. Little is known about how changes in morphology alter mitochondrial function or to what extent changes in shape are reversible. Hypothesis: Mitochondrial morphology is inherently plastic; specifically, mitochondria are capable of rapidly changing morphology in response to osmotic perturbations that cause cell swelling and shrinking. We used confocal microscopy to monitor mitochondrial dynamics in live differentiated PC12 cells stably transfected with an inducible, mitochondrially targeted, GFP fusion protein (mtGFP). In cells at rest, mitochondria throughout the cell displayed a characteristic elongated morphology. Individual mitochondria moved in and out of the focal plane over the course of minutes, and, over the course of 10s of minutes, changed shape. In neurons where normal media was replaced by water or diluted media, mitochondrial morphology changed markedly; within 60s mitochondria rounded up and became spherical. These changes in morphology were rapidly reversible; upon replacement of the hypotonic media with norm-osmotic media, mitochondria reverted to elongated forms within 2min. Similar results were obtained when this process was repeated for up to 4 cycles of swelling and shrinking. Multiple cycles of 'mitochondrial remodeling' did not alter cell viability 1, 5, 24 or 48 hrs later (e.g. at 24 hrs, cell viability was 89±5% versus 91±3% in control cultures). Western blot showed negligible release of cytochrome-c at 1 hr or 24 hrs. Experiments on mtGFP minus cells using each of Rhod-123 and Mitotracker Green FM to label mitochondria confirmed the mtGFP results, although Rhod-123 and especially Mitotracker showed significant bleaching. Cell swelling caused a small decrease in mitochondrial membrane potential. Our results demonstrate that neuronal mitochondria have the potential to remodel in response to osmotic perturbations and that this 'mitochondrial remodeling' is rapid, reversible, and repeatable. Further, this remodeling is not associated with decreased neuronal viability or cytochrome c release. Ongoing studies are examining the role of the membrane cytoskeleton in this mitochondrial remodeling, specifically the role of actin and spectrin.

272 B215

DYNAMICS OF SYNCHRONIZATION IN AN INHIBITORY NETWORK OF NEURONAL OSCILLATORS

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There is increasing evidence that brain rhythms play a fundamental role in the execution and regulation of cognitive and behavioral functions. Recent studies also highlight the importance of inhibitory subnetworks in rhythm generation. This has motivated the development of many experimental and computational models which reveal their ability to support a rich structure of activity patterns such as synchronous activity, clustering, and waves. However, there are very few analytical characterizations of such patterns. Here, we consider a biophysical model describing a network of hippocampal basket cells (X. J. Wang and G. Buzsáki, J. Neurosci. 16:6402, 1996) and attempt to determine conditions under which certain specific modes of activity can be observed. We use the fast/slow structure of the equations involved to derive a simpler neuronal model that captures the essential features of the Wang-Buzsáki model interneuron and allows an analytical treatment. We then consider a pair of such neurons coupled through GABA-A type synapses and describe how the synaptic time scales interact with the intrinsic dynamics to generate various stable configurations (in-phase and out of phase synchrony, suppression) depending on initial conditions and parameters. We also discuss the implications of this analysis for larger networks. This new approach gives a more precise characterization of the network dynamics and provides new insights into the synchronization of inhibitory neurons. These findings may also provide new insights into possible mechanisms of oscillatory activity in the basal ganglia which contain large numbers of GABAergic neurons and interconnections. Supported by CIHR FRN 42505.

273 B216

D1 AND D2 DOPAMINE RECEPTORS INTERACT DIRECTLY WITH N-TYPE CALCIUM CHANNELS AND DIFFERENTIALLY REGULATE CHANNEL ACTIVITY AND SURFACE EXPRESSION

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N-type calcium channels are located on dendrites and at pre-synaptic nerve terminals where they play a fundamental role in neurotransmitter release. They are potently regulated by the activation of a number of different types of G protein coupled receptors (GPCRs), which results in inhibition of channel activity. It is well established that certain PTX-sensitive Gαi/o coupled receptors, such as dopamine D2 receptors, mediate this type of inhibition of N-type channel activity. However, the coupling of D1 receptors to these channels remains poorly characterized. Using heterologous expression in HEK 293T cells, we show via whole cell patch clamp recordings that both D1 and D2 receptors mediate G protein dependent inhibition of channel activity. This data demonstrates that, similar to the D2 receptor, D1 receptor activation allows the channel to be effectively regulated by liberated Gβγ subunits. Yet, greater inhibition is observed with D2 receptors suggesting that these receptors may be more effectively coupled to the channel. Co-immunoprecipitation, pull-down assays, and confocal microscopy reveal that both D1 and D2 receptors are physically associated with N-type channels, suggesting the existence of DA receptor-N-type channel signalling complexes. These interactions were found to be mediated by distinct structural moieties on both receptor and channel. Hence, the unique physical association between channels and

receptors might serve as a key modulator of coupling efficacy. Cell surface immunoluminometry reveals that both receptor subtypes influence trafficking of the channel to and from the plasma membrane, to differential degrees. These data provide further support for stable receptor- channel signalling complexes which allow for indirect regulation of channel activity via alteration of the degree of channel surface expression. Finally, two-photon laser scanning microscopy provides evidence that activation of D1 receptors robustly inhibits N-type channel activity in pyramidal neurons of rat pre-frontal cortex. Given the fundamental nature of calcium influx through N-type channels in synaptic release, these data help to further our understanding of how neurotransmission is modulated. Furthermore, they provide insight into the mechanisms by which intracellular calcium concentrations may be fine-tuned, during dopaminergic signalling, in both cultured cells and neurons.

274 B217

ROLE OF NERVE TERMINAL CALCIUM IN TEMPERATURE-INDUCED FAILURE OF SYNAPTIC TRANSMISSION

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Failure to transmit a signal across the Drosophila neuromuscular junction occurs at elevated temperatures in concert with an elevation in resting calcium concentration above 200nM. Above this 200nM threshold the ability to stimulate a calcium response in boutons is lost suggesting either a loss of action potential propagation or a disruption of calcium channel activation. Electrotonic stimulation, which bypasses sodium channels and directly activates calcium channels, cannot fully rescue stimulus-induced calcium responses and does so only briefly; synaptic transmission also recovers with a diminished amplitude for only a brief period of time. This suggests that hyperthermia-induced failure of synaptic transmission may result from calcium channel inactivation. Nerve terminals treated to a prior heat shock or those expressing Hsp70 were better able to maintain near-normal resting calcium concentrations and synaptic transmission at higher temperatures. Calcium influx and calcium clearance were also protected by prior heat shock. Thus, Hsp70 sustains mechanisms of calcium entry and intracellular regulation. Conversely, disruption of mechanisms clearing calcium at high temperatures, the endoplasmic reticulum Ca+2-ATPase with thapsigargin and the plasma membrane Ca+2-ATPase with high pH, significantly accelerated the temperature-induced rise in resting calcium concentration and decreased the temperature at which evoked calcium responses failed.

Interestingly, disruption of the sodium/calcium exchanger by reducing Na+ in the physiological solution disrupted evoked Ca+2 responses without a significant rise in [Ca+2]r. This suggests a role for the Na+/Ca+2 exchanger in mediating hyperthemia-induced rise in [Ca+2]r. The data also suggest that disruption of evoked calcium responses does not require elevated [Ca+2]r and can result from the misregulation of other ions. In general, elevated resting calcium concentration was linked synaptic transmission failure at high temperatures. Conversely, more stable resting calcium concentration sustained evoked calcium responses and synaptic transmission. Thus, the thermal limit of synaptic transmission may be directly linked to the stability of ion regulation. (This study was funded by the Canadian Institutes for Health Research)

275 B218

SUBCELLULAR DISTRIBUTION OF LOW-VOLTAGE ACTIVATED T-TYPE CA2+ CHANNEL SUBUNITS (CAV3.1 AND CAV3.3) ON RETICULAR AND THALAMOCORTICAL NEURONS IN THE CAT

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Low-voltage activated (LVA) T-type calcium channels (IT) are implicated in the pacemaker activities of thalamocortical (TC) and GABAergic reticular (RE) cells and have a pivotal role in the thalamic sleep oscillations. Recently, three LVA Ca2+ channel isoforms (Cav3.1, Cav3.2, Cav3.3) with distinct channel kinetics have been identified in the ventrobasal complex (VB) and thalamic RE nucleus. Previous electrophysiological and modelling studies suggested that kinetically different IT channels might be expressed in a compartmentalised manner in TC and RE cells. Therefore, the knowledge of their detailed cell-surface distribution is essential for understanding the LVA responses in thalamic neurons. In order to disclose the somato-dendritc density of IT channels in VB and RE nuclei of the cat, we utilised the high-resolution immunolocalization technique combined with electron microscopy. Cav3.1 channel isoform was mainly expressed in the VB, while strongly Cav3.3-immunopositive cells were predominantly found in RE nucleus. Immunogold particles were predominantly distributed on somatic and dendritic plasma membranes, although immunosignal was also present in the cytoplasmic membrane delineated structures. In the VB thalamus, most of the neurons were strongly immunopositive for Cav3.1 subunit and less immunopositive for Cav3.3 with a labelling localised mainly in the somatic and the proximal dendritic plasma membrane. In RE cells, distribution of Cav3.1 was mainly restricted to cell bodies and proximal dendrites similar to the expression pattern of relay cells. In contrast, Cav3.3 channels were distributed over extended length of the dendritic arbour. These results suggest that Cav3.1 and Cav3.3 channel isoforms may contribute to LVA calcium-dependent responses mainly at somatic and proximal dendritic level in VB and RE nuclei, although mediation of Ca2+ influx in the distal dendritic regions of RE neurons could be controlled mainly by Cav3.3 channels. Supported by NSERC and CIHR.

276 B219

C6 GLIOMA CELLS AS TARGETS IN RECOGNITION EVENTS OF NATURAL KILLER (NK) CYTOTOXICITY

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Defence mechanisms as natural killer (NK) cytotoxic activity are key events in immune surveillance. In contrast to this general knoledge, prenatal and neonatal pig NK activity was not measurable with usual K562 erythroleukemia cell targets in comparison with high levels of adult NK cytotoxicity. Aim was to analyse pig fetal and early postnatal NK cytotoxicity with other targets .- original C6 glioma cells (ATCC CLM 107, Rockville,MD) and our modified rat C6 glioma cells transfected (t) pig lymphocyte markers (MHC Class II, CD2, etc) checked by FACS analysis. Marked NK activity was demonstrated on feta pig day 100, with increasing levels on neonatal and postnatal day 35 in blood mononuclear cells or spleen lymphocytes with (t)C6 glioma cells targets by 51 Cr-release or non-isotope method. On the other backgound levels were measured using K562- or original C6 glioma cells at mentioned age intervals, these targets were more effective in later postnatal period.. Thus use of modified (t) C6 glioma cells with combination of xenogeneic and syngeneic cell surface determinants can contribute to better recognition by NK cells not fully maturated in fetal/ neonatal period. Supported by grants MSM 0021620849, GACR 524/05/0267, MSM 6215712403 and IAA 500 2006 20.

277 B220

Aβ-INDUCED TRUNCATION OF SYNAPTIC PROTEIN DPYSL3 IN PRIMARY CORTICAL NEURONS

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Using a proteomics approach, we were able to detect a number of protein expression changes in primary cortical neurons exposed to Aβ 1-42. One such protein whose expression is strongly reduced after Aβ application is dihydropyrimidinase-like 3 protein (DPYSL3). This synaptic

protein plays a key role in cytoskeleton organization, neuronal differentiation, axonal outgrowth and neuronal regeneration. We have previously shown that glutamate excitotoxicity and oxidative stress result in calpain-dependent cleavage of DPYSL3 (Kowara et al., J. Neurochem, 2005) and that it involves nitric oxide synthase (Kowara et al. Brain Res, 2006) and phospholipase A2 signaling. We now show that this Aβ-induced decrease in DPYSL3 levels is, in fact, due to a calpain-dependent truncation and involves the activation of the NMDA receptor, because its truncation can be blocked by both calpain inhibitors and the NMDA receptor antagonist, MK-801. The presence of these inhibitors also reduces Aβ-induced neuritic damage and improves cell viability.

278 B221

AN INCREASE IN THE FREQUENCY OF QUANTAL EVENTS ACCOMPANIES THE INDUCTION OF CHEMICAL LTD AT HYPOTHALAMIC GLUTAMATE SYNAPSES

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In response to numerous physiological challenges including dehydration, stress, lactation, and parturition, noradrenaline (NA) is released in the paraventricular nucleus (PVN) of the hypothalamus to modulate neuroendocrine output. In this study, using whole-cell patch clamp recordings from both parvocellular and magnocellular neuroendocrine cells in the PVN, we show that bath application of NA (3-5 min, 100 – 200 uM) induces a robust, fast-acting, permanent chemical LTD at glutamatergic synapses. Induction of this chemical LTD is activity dependent because LTD was only observed at synapses that were repetitively stimulated during NA application. Alternatively, when EPSCs were not evoked in synapses for 5 minutes during NA application, the EPSC amplitudes were not different from control initially; however, subsequent stimulation of these synapses in the presence of NA resulted in the rapid induction of LTD. The paired pulse ratio of evoked EPSCs was increased following induction of LTD suggesting that it occurred at a presynaptic location. Interestingly, chemical LTD was accompanied by a robust increase in the frequency of quantal glutamatergic events that was reversible upon wash of NA. To determine if these opposing actions of NA on the probability of spontaneous release versus evoked release occur at the same synapses we recorded NMDA-dependent mEPSCs in 0 Mg2+ ACSF. Application of MK801 completely blocked spontaneous NMDA-EPSCs and prevented the subsequent activation of evoked NMDA currents, suggesting that spontaneous and evoked glutamate release occurs at the same synapses. By facilitating spontaneous glutamate release while depressing evoked transmission, NA places a premium on local forms of intranuclear signaling while isolating PVN neurons from changes in activity in afferent nuclei.

279 B222

CHARACTERIZATION OF TOP TRANSLATION IN APLYSIA NEURONS

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The sensory-motor reflex of the marine mollusk Aplysia is a good model for understanding the changes in behavior linked to synaptic modifications. Behavioral sensitization in Aplysia is mediated partially by an increase in synaptic strength between sensory and motor neurons called long-term facilitation (LTF). Translational regulatory systems are important for many aspects of LTF. In particular activation of the target of rapamycin (TOR) signalling pathway is activated by LTF and is important for local and somatic regulation of translation. However, the important downstream targets of TOR have not yet been determined in this system. One possibility of a TOR target is translational regulation of the components of the translational apparatus; this would lead to an increase in translational rate that may persist. Indeed, levels of S6 increase in synaptosomes in a rapamycin sensitive manner (Khan et al, 2001 J. Neurosci). S6 and other ribosomal mRNAs are regulated through a TOP (5' terminal oligopyrimidine tract) sequence that normally represses translation. This repression is relieved by TOP through a pathway independent of other TOR targets such as S6 kinase and 4EBP. In order to investigate the role of TOP mRNAs in Aplysia neurons, pleural-pedal ganglia were subjected to 10 min serotonin (5-HT) treatment, lysed and polysome profiles were generated on sucrose gradients, and were consequently analyzed by quantitative real-time PCR. In control ganglia S6 and eEF2 (TOP-containing mRNAs) were enriched in sub-polysomal fractions compared to other transcripts (Rab-3a and actin). This result suggests that TOP mRNAs are translationally repressed at rest. After 5-HT treatment, a sizeable proportion of S6 mRNA was found in the polysome fraction, indicating removal of TOP repression. Surprisingly, eEF2 mRNA was still repressed. These results are consistent with the increase in translation of a subset of TOP mRNAs after 5-HT addition. Further work will examine the dependence of this on TOR activation and the generality with which this can be extended to other TOP mRNAs.

280 B223

RELATIONSHIPS BETWEEN SUBSTANCE P, NK1 RECEPTORS AND SEROTONIN NEURONS IN THE DORSAL RAPHE NUCLEUS OF ADULT RAT

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In addition to its neurotransmitter/modulator role in pain perception, substance P (SP) is involved in a regulation of mood, and the blockade of its neurokinin-1 receptor (NK1r) has been shown to have antidepressant-like effects in human. In rodents, treatment with NK1r antagonists and induces a desensitization of 5-HT1A autoreceptors and increases the firing of serotonin (5 HT) neurons in the dorsal raphe nucleus (DRN). In a recent, double labeling, immuno-electron microscopic study, we have demonstrated the existence of NK1r in a subpopulation of DRN 5 HT (TpOH-positive) neurons mostly located in the caudal half of the nucleus (Lacoste et al., 2006). Interestingly, in these soma-dendrites, the NK1r was always found mainly in the cytoplasm, whereas it predominated on the plasma membrane of TpOH-negative dendrites. Here, we combined SP and NK1r immunolabeling in the caudal half of the DRN to examine the relationships between SP axon terminals and dendritic profiles endowed with cytoplasmic (5-HT neurons) versus plasma membrane (non 5-HT neurons) NK1r. In singly SP (DAB)-labeled material (n=3), the frequency with which SP-labeled profiles displayed a junctional complex in single thin sections (18%) indicated that many of these terminals (~ 50%) are synaptic. In doubly SP (DAB)- and NK1 (gold)-labeled material (n=3), SP terminals were frequently found in direct contact with or in the immediate vicinity of dendritic profiles endowed with cytoplasmic NK1r. This was never seen in the case of dendrites bearing membranous NK1r. These preliminary observations are consistent with the hypothesis of a tonic activation and internalization by SP of NK1r in DRN 5 HT neurons. (Supported by CIHR grant MOP 3544).

281 B224

SELECTIVE ABLATION OF PROLIFERATING MICROGLIAL CELLS EXACERBATES ISCHEMIC INJURY IN THE BRAIN

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Here we report in vivo evidence of a neuroprotective role of proliferating microglial cells in cerebral ischemia. Using transgenic mice expressing a mutant thymidine kinase form of herpes simplex virus driven by myeloid-specific CD11b promoter and ganciclovir treatment as a tool, we selectively ablated proliferating (Mac-2 positive) microglia after transient middle

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

cerebral artery occlusion. The series of experiments using green fluorescent protein-chimeric mice demonstrated that within the first 72 h after ischemic injury, the Mac-2 marker [unlike Iba1 (ionized calcium-binding adapter molecule 1)] was preferentially expressed by the resident microglia. Selective ablation of proliferating resident microglia was associated with a marked alteration in the temporal dynamics of proinflammatory cytokine expression, a significant increase in the size of infarction associated with a 2.7-fold increase in the number of apoptotic cells, predominantly neurons, and a 1.8-fold decrease in the levels of IGF-1. A double-immunofluorescence analysis revealed a approximately 100% colocalization between IGF-1 positive cells and Mac-2, a marker of activated/proliferating resident microglia. Conversely, stimulation of microglial proliferation after cerebral ischemia by M-CSF (macrophage colony stimulating factor) resulted in a 1.9-fold increase in IGF-1 levels and a significant increase of Mac2+ cells. Our findings suggest that a postischemic proliferation of the resident microglial cells may serve as an important modulator of a brain inflammatory response. More importantly, our results revealed a marked neuroprotective potential of proliferating microglia serving as an endogenous pool of neurotrophic molecules such as IGF-1, which may open new therapeutic avenues in the treatment of stroke and other neurological disorders.

282 B225

INHIBITION OF CASPASE-MEDIATED APOPTOSIS BY PEROXYNITRITE IN TRAUMATIC BRAIN INJURY +C210

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In traumatic brain injury (TBI), neurons surviving the primary insult may succumb through poorly understood secondary mechanisms. In vitro, cortical neurons exposed to stretch injury exhibited enhanced vulnerability to NMDA, apoptotic-like DNA fragmentation, peroxynitrite (PN) formation, and cytoplasmic cytochrome c accumulation. Surprisingly, caspase-3 activity was undetectable by both immunoblotting and fluorogenic activity assays. Therefore, we hypothesized that PN directly inhibits caspases in these neurons. Consistent with this, stretch injury in cultured neurons elicited tyrosine nitration of procaspase-3, but not caspase-9 or Apaf-1, suggesting a direct interaction of PN with caspase-3. In an ex vivo system, PN inhibited the activity of caspase-3, and this inhibition was reversible with the addition of the sulfhydryl reducing agent dithiothreitol, indicating that PN inhibits caspases by cysteinyl oxidation. Moreover, in cultures, the PN donor 3-morpholinosydnonimine (SIN-1) blocked staurosporine-induced caspase-3 activation and its downstream effects including PARP-1 [poly-(ADP-ribose) polymerase-1] cleavage and phosphotidylserine inversion, suggesting that peroxynitrite can inhibit caspase-3-mediated apoptosis. To examine these mechanisms in vivo, rats were exposed to a lateral fluid percussion injury (FPI). FPI caused increased neuronal protein nitration that colocalized with TUNEL staining, indicating that PN was associated with neurodegeneration. Caspase-3 activity was inhibited in brain lysates harvested after FPI and was restored by adding dithiothreitol. Our data show that caspase-mediated apoptosis is inhibited in neurons subjected to stretch in vitro and to TBI in vivo, mostly because of cysteinyl oxidation of caspase-3 by PN. However, this is insufficient to prevent cell death, indicating that the TBI therapy may, at a minimum, require a combination of both anti-apoptotic and anti-oxidant strategies.

283 B226

THE ROLE OF ZINC IN PRESYNAPTIC RELEASE MECHANISMS AT THE HIPPOCAMPAL MOSSY FIBERS

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The hippocampal mossy fiber (MF) axons arise from the dentate granule cells of the dentate gyrus and provide synaptic input to neurons in the hilus and the CA3 area of the hippocampus. One of the particularities of the MF terminals is their unusually high concentration of chelatable zinc in the synaptic vesicles. We have shown that zinc is necessary for the proper function of the presynaptic machinery. Indeed, chelation of vesicular zinc decreases the frequency of spontaneous release and compromises the vesicle refilling machinery. In the present study, we aimed to investigate the mechanisms by which zinc influences presynaptic release at the MF terminals. Our data show that the potent membrane-permeable zinc chelator DEDTC decreased the frequency of spontaneous EPSCs (sEPSCs) but had virtually no effect in the presence of TTX (mEPSCs). This result could be explained by either a cellular mechanism which is only activated at higher level of activity, or by an effect on presynaptic calcium dynamics allowing the synchronization of several release sites. In order to differentiate between these two possibilities, we have studied the effect of Brefeldin A (BFA) and caffeine on the spontaneous activity of CA3 pyramidal cells. BFA selectively blocks the AP3-dependent vesicle recycling pathway while caffeine increases the intracellular calcium concentration. BFA-treatment (10µg/ml) selectively decreased the frequency of sEPSCs with an amplitude higher then 100 pA, while sEPSCs with smaller amplitude were unaffected. It has been shown that zinc transport into vesicles (via specific transporter ZnT-3) seems to be related to the AP3 pathway. Therefore, we investigated whether the previously described effect of zinc chelation is still present in BFA-treated slices. Our data show that DEDTC does not change the frequency of sEPSCs in the presence of BFA. This suggests that the zinc-dependent presynaptic machinery and AP3-dependent endocytosis are overlapping intracellular pathways. The application of caffeine (5mM) increased the frequency and the amplitude of sEPSPs in control slices and this effect was still present in DEDTC-treated slices. These results suggest that zinc containing vesicles are closely linked to the AP3-dependent endocytosis pathway and that the proper functioning of this pathway is crucial for the generation of large amplitude events at the MF synapses.

284 B227

VESICULAR ZINC INFLUENCES PRESYNAPTIC RELEASE AT THE MOSSY FIBER TERMINALS

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The hippocampal mossy fiber axons (MF) arise from the granule cells of the dentate gyrus and provide synaptic input to neurons in the hilus and the CA3 area of the hippocampus. MF synapses have many unique anatomical and physiological properties. The number of active zones and synaptic vesicles that are stored in the presynaptic terminals are several folds higher than in “conventional” synapses and MF inputs show very high degree of short-term facilitation. Another unique feature of this synapse is the unusually high level of chelatable zinc in the synaptic vesicles. It is not currently known why MF terminals contain such a high concentration of zinc and whether there is a correlation between the unique physiological properties of this synapse and their zinc content. In the present study, we aimed to investigate how the presence of zinc in the presynaptic vesicles affects presynaptic release at the mossy fiber terminals.

We have used membrane-permeable (DEDTC) and membrane-impermeable (CaEDTA) zinc chelators to differentiate between the effect of synaptically released and vesicle-bound zinc. Recordings from CA3 pyramidal cells showed a decrease of spontaneous activity (sEPSCs) with the application of DEDTC, while CaEDTA had no effect. Recordings from CA1

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

pyramidal cells showed no change in activity which demonstrates that DEDTC effect is specific to CA3 terminals. Sucrose puffs application to DEDTC-treated slices also showed a decrease in the readily release pool (RRP), an effect not seen with CaEDTA. Next, we evoked MF and associational/collateral (AC) synaptic inputs with focal stimulation. While DEDTD did not influence basal transmission, high frequency frequency was less efficient in the absence of vesicular zinc from MF input (DCG-IV sensitive). Responses evoked with the stimulation of AC inputs (DCG-IV insensitive) were insensitive to DEDTC. Both basal transmission and short-term plasticity were unaffected by the presence of the zinc chelators.
These data suggest that the major role of zinc at the mossy fiber terminals is not the regulation of postsynaptic receptors as it was believed previously, but it is necessary for the proper function of the unique presynaptic release machinery.
* These authors contributed equally to this work.

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THE EFFECT OF ZINC ON THE ULTRASTRUCTURE OF PRESYNAPTIC MOSSY FIBER TERMINALS

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Zinc in the mossy fiber terminal is localized in synaptic vesicles. It was suggested that during synaptic transmission it is released and translocates to the postsynaptic cell. We investigated this possibility using a highly sensitive sodium tungstate Timm method to detect vesicular zinc at the electron microscopic level. Since our previous data showed that zinc in the presynaptic terminals can influence release properties, we investigated the effect of zinc chelation on the size of the vesicle pool and on the distribution of presynaptic vesicles. First, we visualized zinc content in pre- and postsynaptic elements in control and epileptic animals. This method produces round silver grains that are small enough to precisely locate zinc both inside synaptic terminals and in the synaptic cleft. Our data showed in control animals zinc-staining was restricted to presynaptic vesicles, staining was absent from the synaptic cleft. Zinc-staining was decreased after epileptic seizures inside the terminals and increased on the presynaptic membrane surface. However, staining on the postynaptic membrane or in the postsynaptic cell was not observed either in control, or in epileptic animals. Next, we chelated zinc with DEDTC before seizure induction to measure the size of the readily-releasable and total vesicle pool in the absence of zinc in the synaptic terminal. We calculated vesicle density in 4 identified compartments in mossy fibre terminals. Each compartment was 75 nm wide, and the first compartment bordered with the active zone. We found that vesicle density was significantly decreased in all 4 compartments, the most significant effect was observed in the first compartment. These data indicate that zinc can be visualized on the presynaptic membrane surface in an activity-dependent manner, and the level of zinc inside the terminal is also modified by increasing synaptic activity. In the absence of zinc the number of vesicles is decreased, indicating that refilling the large vesicle pool in mossy fibre terminals during/following high level synaptic activity requires a zinc-dependent machinery.

286 B229

STAUFEN REGULATES LONG-TERM SYNAPTIC PLASTICITY, SPONTANEOUS SYNAPTIC ACTIVITY AND SPINE MORPHOLOGY IN HIPPOCAMPAL PYRAMIDAL CELLS

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Mammalian Staufen1 (Stau1) is an RNA-binding protein involved in mRNA transport, localization and translational control. Stau1 is present in RNA granules transported along hippocampal neuron dendrites. This dendritic mRNA localization may be critically involved in long-term synaptic plasticity and memory. Thus, our aim was to investigate the implication of Stau1 in synaptic plasticity of hippocampal pyramidal cells. We used RNA interference (siRNA) to examine effects of Stau1 down-regulation in hippocampal slice cultures. We established by western blot analysis the efficacy and specificity of biolistic transfection of Stau1 siRNA on Stau1 expression in HEK293 cells and in hippocampal slice cultures. Recordings of field EPSPs were used to assess effects of Stau1 down-regulation on pyramidal cell synaptic plasticity. We observed a block of the late form of long-term potentiation (L-LTP) induced by forskolin. The early form of tetanus-induced LTP, mGluR1-induced long-term depression (LTD), and basal evoked synaptic transmission were unaffected by Stau1 down-regulation, suggesting an implication of Stau1 in transport of newly synthesized mRNA to activated synapses for local protein synthesis associated with L-LTP. Using whole cell recordings of miniature EPSCs (mEPSCs), we found a decrease in mEPSC amplitude and frequency after Stau1 down-regulation. Thus, Stau1 is also involved in regulation of spontaneous activity at excitatory synapses. Confocal microscopy was used to study the impact of Stau1 siRNA on dendritic spine morphology in EYFP-labelled CA1 pyramidal cells. We observed no change in spine density, but found a decrease in regular spines and an increase in elongated spines, as well as an increase in filopodia protrusions after Stau1 down-regulation. The shift from regular to elongated spine morphology is consistent with the L-LTP impairment, whereas the increase in filipodia protrusions is consistent with the reduction in spontaneous synaptic activity. .....These results reveal new roles for Stau1 in mRNA transport to synapses to maintain efficacy of spontaneous activity and to sustain long-term increases in synaptic strength during LTP. This Stau1 regulation of development and long-term plasticity of hippocampal pyramidal cell synapses and dendritic spines may be important for the functional connectivity changes underlying hippocampal-dependent learning and memory.

287 B230

SMALL PEPTIDOMIMETIC LIGANDS OF NEUROTROPHIN RECEPTORS REGULATE RETINAL GANGLION CELL DEATH IN VIVO: A ROLE FOR MÜLLER CELL-SPECIFIC P75NTR?

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The neurotrophins play important roles in the survival of central nervous system neurons. Two classes of cell surface receptors mediate the biological effect of mature neurotrophins: the Trk family of receptor tyrosine kinases and p75NTR, which binds all neurotrophins. Sortilin, the latest neurotrophin receptor to be identified, interacts preferentially with pro-neurotrophins. In the adult retina, TrkA is expressed by adult retinal ganglion cells (RGCs), while p75NTR is exclusively expressed by Müller glia. Here we used small and proteolytically stable ligands of neurotrophin receptors, with receptor selective activity, to unmask the role of each receptor in RGC survival. Peptidomimetic ligands (agonist or antagonist: 1 µg/µl) were independently injected or co-injected into the vitreous chamber of adult Sprague-Dawley rats at the time of optic nerve transection. For analysis of neuronal survival, RGCs were retrogradely labeled by application of FluoroGold in the superior colliculus, the main target region of RGCs in the rodent brain. Neuronal survival was quantified at 1 or 2 weeks after nerve injury. Our data demonstrate that specific activation of TrkA by a peptidomimetic agonist (D3) led to marked neuroprotection of axotomized RGCs, while intraocular administration of recombinant nerve growth factor (NGF) did not promote survival. Combination of D3 with a TrkA antagonist completely inhibited its neuroprotective effect. Remarkably, a single injection of a p75NTR receptor

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

antagonist promoted robust RGC survival after axotomy. Our results also demonstrate activation of the transcription factor nuclear factor kappa-B (NF-kB), a downstream effector of p75NTR, in the axotomized retina. In addition, we show that sortilin, which can induce cell death by interacting with p75NTR in the presence of pro-neurotrophins, is specifically expressed by Müller glia and is upregulated in these cells after injury. Collectively, our data demonstrate a neuroprotective effect of novel, small peptidomimetic ligands of neurotrophin receptors in the retina, and suggest that Müller cells are involved in the regulation of RGC death via a p75NTR-dependent mechanism.

288 B231

MULTIPLE GBETAGAMMA MEDIATED PRESYNAPTIC INHIBITORY PATHWAYS OF VESICLE RELEASE BETWEEN SYNAPTICALLY PAIRED IDENTIFIED LYMNAEA NEURONS

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Synaptic transmission involves the exocytosis of presynaptic vesicles, a process that is susceptible to modulation through the activation of presynaptic G-protein coupled receptors (GPCR). It is believed that GPCR activation leads to a pathway involving the G-protein subunit Gbetagamma, but the mechanisms involved are not fully understood. This study examined Gbetagamma-mediated calcium channel dependent and independent inhibitory pathways on presynaptic vesicle release between synaptically paired identified Lymneaea stagnalis neuons; the presynaptic neuron VD4, which possesses dopamine (DA) GPCRs, and postsynaptic neuron LPeD1. Exogenous application of DA onto the presynaptic VD4 neuron reduced EPSC amplitudes recorded from the postsynaptic LPeD1 neuron. To determine if this reduction was due to inhibition of presynaptic vesicle release, VD4 neurons were labeled with FM1-43 dye. VD4 neurons stimulated to fire APs showed reduced FM1-43 unloading in the presence of DA. This inhibition could be attenuated by acute knockdown of Gbeta using double stranded RNA (Gbeta-dsRNA). These findings suggest that DA activates a Gbetagamma dependent inhibitory pathway of presynaptic vesicle release. To test whether DA activated a Gbetagamma-mediated calcium channel dependent inhibitory pathway of vesicle release, simultaneous whole-cell voltage-clamp recordings with ratiometric FURA-2 calcium imaging of VD4 neurons were used. DA reduced the whole cell VD4 calcium currents as well as intracellular calcium concentrations. The reduction in calcium currents could be partially removed by a depolarization pre-pulse. Acute knockdown of Gbetagamma using Gbeta-dsRNA reduced the DA-mediated inhibition and abolished the prepulse relief, confirming that Gbetagamma inhibits calcium channels in VD4. To test the calcium channel independent pathway of Gbetagamma mediated inhibition, the ionophore ionomycin was used to permit calcium influx independently from calcium channels to study vesicle release. DA inhibited ionomycin induced FM1-43 release. This inhibitory effect was attenuated after knockdown of Gbeta suggesting that Gbeta inhibits vesicle release in a calcium channel independent manner. This suggests that Gbetagamma possesses multiple pathways for inhibiting vesicle release within the same cell upon dopamine receptor activation. Future study will be carried out to determine the contribution of both Gbetagamma mediated inhibitory pathways on vesicle release in these neurons.

289 B232

EFFECTS OF A CHOLESTEROL CHELATOR ON SYNAPTIC TRANSMISSION IN CRAYFISH DEPEND ON ACCLIMATIZATION TEMPERATURE

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Cholesterol is an integral part of cell membranes and a major component of vesicle membranes. The cholesterol chelator Methyl-β-Cyclodextrin (MBCD) reduces membrane cholesterol and inhibits the extent, Ca2+-sensitivity and kinetics of fusion of cortical vesicles from sea urchins (Churchward et al, J. Cell Sci. 118:4833-48, 2005). In crayfish muscle, MBCD increases spontaneous quantal release and decreases the amplitude of excitatory junctional potentials (EJPs) by blocking impulse propagation (Zamir & Charlton, J. Physiol. 571.1: 83-99, 2006). Thus, cholesterol may influence the release pathway in many ways. We examined a possible effect of temperature acclimatization on responses to MBCD in deep abdominal extensor muscles of the crayfish, Procambarus Clarkii. Crayfish were housed at 20ºC or at 14º C for 2-12 weeks, after which EJPs were recorded at room temperature to assess effects of MBCD. EJP amplitudes from cold acclimatized crayfish decreased by 15.7 ± 11.2 % in the presence of 10 mM MBCD, which was almost significantly different from cold controls without MBCD (-2.8 ± 5%; p = 0.056, n = 9). In 5 of 9 warm acclimatized animals, however, MBCD actually increased EJP amplitude. The overall change in EJP amplitude for warm acclimatized animals was 3.8 ± 2.9% in response to MBCD, compared to -6.8 ± 5.6% for warm acclimatized controls (p = 0.034, n = 9). Thus, effects of this cholesterol chelator on EJP amplitude appear to depend on acclimatization temperature in crayfish. Supported by NSERC.

290 C201

COMBINED ENVIRONMENTAL ENRICHMENT AND EXERCISE CAUSES AN INCREASE IN NEURONAL PROLIFERATION AND PARTIALLY REPAIRS THE INJURED HIPPOCAMPUS

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The dentate gyrus subfield of the hippocampus is one of two regions in the adult mammalian brain that continues to produce neurons. We examined whether these new neurons could replenish a partially damaged dentate gyrus and reverse damage-related cognitive deficits. Rats acquired two different types of memory (spatial and context memory) and then were given bilateral adrenalectomy (ADX). ADX dramatically decreases circulating corticosterone levels, which causes a slow degeneration of the granule cell layer of the dentate gyrus. Ten weeks after ADX, a period sufficient to cause significant damage to the dentate gyrus, rats were placed on a regimen of corticosteroid replacement and a 6-wk protocol of environmental enrichment and exercise in running-wheels. The latter protocol is known to increase proliferation and survival of neurons in the dentate gyrus. Rats were then tested for retention of both types of memory. On the spatial memory test conducted in the Morris water task, rats that had received ADX remembered the location of the hidden platform as well as control rats. In contrast, ADX rats were impaired on the context memory test. They displayed less freezing than control rats in the context that was previously associated with a fear-eliciting event. The combined environmental enrichment and wheel running treatment did not reverse the cognitive deficits that were caused by the ADX and related dentate gyrus damage. Nevertheless, the combined environmental enrichment and wheel running protocol was successful in increasing neuronal proliferation in the dentate gyrus and this increase tended to repopulate the granule cell layer of the dentate gyrus. Therefore, we have a model that enables the examination of replacing neurons in the injured hippocampus and its effect on cognition.

291 C202

MIGRATION OF NEURONAL PRECURSORS IN THE ADULT BRAIN IS MODULATED BY GABAERGIC SIGNALLING AND CA2+ ACTIVITY

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Adult olfactory bulb (OB) has a remarkable capacity to renew its interneuronal population throughout the entire life-span of animals, including

humans. The neuronal precursors are produced in the subventricular zone (SVZ) and have to migrate tangentially, in chains, ensheathed by astrocytic processes, in the rostral migratory stream (RMS) to reach the OB. To better understand the mechanisms of neuronal migration in adult forebrain we have established a time-lapse videomicroscopy combined with Ca2+ imaging in the acute slices of adult mouse brain. We have observed fast (80-150 μm/hour), saltatory migration of neuronal precursors with active phases of neuronal displacement interspersed amongst resting periods. Interestingly, the duration of migratory phases was correlated with the spontaneous Ca2+ fluctuations in non-migrating cells. These non-migrating cells are most likely astrocytes since i) together with neuroblasts they represent about 90% of cells in the adult RMS and ii) they do not migrate throughout the entire period of recording (more than 1 hour). Interestingly, a temperature drop of 32ºC to 22ºC decreased the frequency of Ca2+ fluctuations by 67% and blocked neuronal migration. These results suggest that Ca2+ activity in astrocyte-like cells play a crucial role in the tangential migration of adult neuronal precursors. Thereafter, we sought to determine what triggers these Ca2+ fluxes in the astrocyte-like cells. Since neuronal precursors in the adult RMS synthesize GABA and astrocytes contain GABAA receptors, we hypothesized that neuroblasts themselves control Ca2+ activity in astrocytes through gabaergic signaling. To explore this possibility and to block endogenous gabaergic signaling we applied the GABAA receptor antagonist, bicuculline. Application of bicuculline decreased the frequency of the Ca2+ fluxes in astrocyte-like cells by 41% and affected the migration of neuronal precursors in the RMS. Altogether, these results suggest that adult neuronal precursors control their own migration via release of GABA that triggers Ca2+ fluctuation in the neighboring astrocytes.

292 C203

A CHANGE IN POST-SYNAPTIC pH DURING SYNAPTIC TRANSMISSION REGULATES CaM KINASE II TRANSLOCATION

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The multifunctional Ca2+/calmodulin protein kinase II (CaMKII) is a key enzyme in the signaling processes that underlie synaptic plasticity at excitatory synapses. The dynamic translocation of CaMKII in and out of synapses, regulated by its remarkable structural and regulatory properties, might control this plasticity. Others and we have reported that a brief (< 1 min) activation of NMDA receptors in neurons leads to the activation of CaMKII and its translocation to the postsynaptic density (PSD). We also reported that a sustained activation of NMDA receptors (> 2 min) can cause an intracellular clustering of the kinase. This process is reminiscent of a CaMKII self-association mechanism, which, in heterologous cells, depends on both a rise in free Ca2+ and a decrease in intracellular pH (pHi). Previous evidence indicates that NMDA receptor activation in neurons induces a Ca2+-dependent intracellular acidification. The aim of this study is thus to determine whether i) CaMKII intracellular clustering and ii) translocation to the PSD evoked by NMDA receptor activation are regulated by pHi. Using live imaging of wild type and mutated constructs of GFP-CaMKII, fluorescent Ca2+ and pH indicators, we are monitoring CaMKII translocation and variations of free cytosolic Ca2+ and pHi in cultured rat hippocampal neurons. To evaluate post-synaptic pH, we also transfect neurons with a genetically-encoded fluorescent indicator that targets to the PSD. To control variations in pHi, we use extracellular application of NH4Cl or patch clamping with pH buffers. Our preliminary results 1) confirm that NMDA receptor stimulation causes a significant drop in pHi in somatodendritic regions; 2) indicate that post-synaptic pH also drops upon stimulation; and that 3) extracellular application of NH4Cl prevents both the evoked drop in pHi and the intracellular clustering of GFP-CaMKII. To identify pH sensitive residues on the kinase, we are testing site-directed mutations near its catalytic site. His 282 is at the hinge of the catalytic and regulatory domains. The mutation H282K, which mimics the increased protonation of His (pKa ~6.3) that occurs during a physiological decrease in pHi, facilitated GFP-CaMKII clustering. These results suggest that the Ca2+-dependent drop in pHi during synaptic activity promotes the clustering of CaMKII at the PSD, which may have a role in mechanisms of synaptic plasticity.

293 C204

PDGFR RECEPTORS TARGET NR2B-CONTAINING NMDA RECEPTORS IN THE HIPPOCAMPUS

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Glutamate is the primary transmitter at excitatory synapses in neurons, however excess glutamate levels can result in the death of neurons in the CNS by over-activating glutamate-gated ion channels such as the NMDA receptor (NMDAR). When NMDARs are activated, NMDARs increase postsynaptic calcium level and activate signaling cascades including the extracellular signal-regulated kinase (ERK) and cyclic AMP response element binding protein (CREB) pathways. Focal ischemia in rat brain is associated with a rapid (3 hr) increase in the mRNA transcripts of PDGF-B chain isoforms that peaks at 24 hours. PDGFβ receptor expression levels (but not mRNA) also rise rapidly after ischemia in rat brain. Furthermore, neuronal death in hippocampal cultures after exposure to high levels of glutamate or NMDA can be attenuated by 24 hr pretreatment with PDGF-BB. NR2B-containing NMDARs may localize to primarily extra-synaptic compartments and mediate the excitotoxic effects of ischemia and excess glutamatergic signaling. In contrast, NR2A-containing NMDARs are primarily synaptic and signaling through these receptors may be neuroprotective. To examine the mechanisms of PDGF-BB neuroprotection, we examined the localization of PDGFβ receptors in cultured hippocampal neurons. PDGFβ receptors do not colocalize with the post-synaptic protein, PSD-95. Preliminary evidence suggests a higher colocalization of PDGFβ receptors with the NR2B NMDA subunit. PDGF-BB treatment of hippocampal slices and cultured hippocampal neurons resulted in a decrease in the membrane localization of NR2B, but did not affect membrane localization of either NR1 or NR2A subunits. This work may suggest that neuroprotective effects of PDGF-BB treatment of hippocampal neurons may be due to a preferential decrease in the activity of extrasynaptic NR2B-containing NMDA receptors.

294 C205

PRENATAL INFLAMMATION AND DISRUPTED HIPPOCAMPAL FUNCTION

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Prenatal exposure to infection during a critical period for fetal brain development has been linked to increased risk for schizophrenia, suggesting that a portion of schizophrenia could be due to neurodevelopmental disruptions. The human hippocampus is compromised in schizophrenia and it is unknown whether these changes are due to neurodevelopmental alterations. Here we used bacterial endotoxin lipopolysaccharide (LPS) to simulate bacterial infection and stimulate the maternal inflammatory response during a critical period in pregnancy (i.e., second trimester) for hippocampal development in the fetus. We administered 100µg/kg LPS to pregnant rats on gestation days 15 and 16 (gestation = 21 days) and recorded extracellular responses in slices from the hippocampus of the offspring at PD20-25 to evaluate hippocampal synaptic transmission. Schaeffer collateral-evoked field excitatory postsynaptic potentials (fEPSPs) in area CA1 were significantly greater in amplitude and slope in offspring from LPS-treated dams (2.18 ± 0.25mV; 0.96 ± 0.11mV/ms) compared to those recorded from offspring of saline-injected dams (1.44 ± 0.14mV; 0.65 ±

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

0.08mV/ms). This effect was unique to the CA1 area since it was not observed in CA3 or dentate gyrus. However, input-output curves revealed that a greater amount of current applied to Schaeffer collaterals was required to evoke an equivalent amplitude in the fEPSP in the LPS group compared to saline. In CA1, changes in paired-pulse facilitation of the fEPSP, a measure of presynaptic transmitter release, were not found. Fast GABAergic inhibition of CA1 pyramidal neurons was not found to be disrupted in the LPS group when evaluated by paired-pulse inhibition of the population spike. The intrinsic excitability of CA1 pyramidal neurons was heightened because antidromic responses in the LPS group (9.41 ± 1.84mV) were significantly larger than those in control (2.86 ± 1.13mV). This increased excitability observed in field recordings was also found in whole-cell patch-clamp recordings of CA1 pyramidal cells, which revealed a more depolarized resting membrane potential in LPS group (–56.09 ± 0.84mV) compared to those in saline (-58.90 ± 0.33mV). These results show that neurodevelopmental disruption triggered by prenatal inflammation can have profound effects on hippocampal synaptic transmission and could likely contribute to the memory and cognitive deficits observed in schizophrenia.

295 C206

LONG-TERM EXPOSURE TO BRAIN-DERIVED NEUROTROPHIC FACTOR INDUCES SPECIFIC CHANGES IN INHIBITORY SYNAPTIC TRANSMISSION IN RAT DORSAL HORN NEURONS

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Neuropathic pain is a disease of abnormal and inappropriate pain signaling. It can arise from damage to the nerves that transmit pain signals to the brain either through disease, infection or trauma. Unfortunately, conventional analgesics such as the NSAIDs and opioids are not very effective in managing neuropathic pain. Therefore, the mechanisms and mediators involved in instigating neuropathic pain are attractive new therapeutic targets for the treatment of this disease. Brain-derived neurotrophic factor (BDNF), which can induce long-lasting neuronal changes, has been implicated as a mediator of neuropathic pain. We have previously shown that long-term exposure of dorsal horn neurons to BDNF increases excitatory synaptic transmission to putative excitatory neurons whilst decreasing that to inhibitory neurons. Here we investigated the effects of long-term BDNF exposure on inhibitory synaptic activity in dorsal horn neurons by recording and analyzing spontaneous inhibitory post-synaptic currents (sIPSCs). Whole-cell recordings were obtained from dorsal horn neurons in organotypic cultured slices (OTCS) incubated with 200ng/ml of BDNF for 5-6 days. Age-matched, untreated OTCS served as controls. Dorsal horn neurons were classified based on their action potential discharge firing pattern during depolarizing current steps. Generally, the frequency of sIPSCs was affected the most following long-term BDNF treatment. However, alterations in inhibitory synaptic activity were not uniform across all classes of dorsal horn neurons. In ‘tonic’ and ‘phasic’ neurons, both exhibited a significant decrease in sIPSC frequency with an increase in sIPSC amplitude (KS-test, $p<0.05$) following long-term BDNF exposure. Other classes of dorsal horn neurons, like the ‘delay’ and ‘transient’ single-spiking neurons, displayed a significant increase in sIPSC frequency following long-term BDNF treatment (KS-test, $p<0.001$) but no change in sIPSC amplitude (KS-test, $p>0.5$). These results together with the effects of long-term BDNF exposure on excitatory synaptic activity illustrate the complexity of BDNF’s effects on dorsal horn neurons. Funding support from CIHR and AHFMR.

296 C207

THE EFFECT OF PARKIN ON LONG-TERM POTENTIATION

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Long-term potentiation refers to the increase in synaptic strength following repetitive, high-frequency stimulation of excitatory synapses (Lomo, 1966; Bliss & Lomo, 1973). This synaptic enhancement lasting hours to days is the postulated corollary of learning and memory and although it was initially demonstrated in the hippocampus, this activity-dependent synaptic alteration is a fundamental property of most excitatory synapses in the mammalian brain (Sun, Zhao, & Wolf, 2005). An increase in the number and the conductance of postsynaptic (S)-a-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptors (AMPARs), a class of glutamate receptor, has been shown to underlie LTP (Isaac, Nicoll, & Malenka, 1995; Lynch & Beaudry, 1984). Despite what knowledge has been gained, the complex molecular mechanisms by which LTP is accomplished are not fully elucidated and the list of AMPAR protein partners is by no means complete.

Parkin, a 465 amino acid E3 ubiquitin ligase that mediates the attachment of ubiquitin onto substrate proteins targeting them for degradation by the 26S proteasome, is a synaptic protein containing a PDZ-domain binding motif that associates with NR2B and CAMKII, components of the glutamate receptor-signalling complex (Fallon et al., 2002). Reduced synaptic strength has been demonstrated in cortico-striatal sections from Parkin KO mice (Goldberg et al., 2003). Further, Parkin inhibits PTEN, which negatively regulates the PI3K/AKT pathway (Yang et al., 2005). PI3K associates with AMPARs at the synapse and mediates translocation of AMPARs to the synapse and hence LTP (Man et al., 2003). In the current study, we examined the effect of Parkin on post-synaptic AMPAR translocation (i.e., LTP) following stimulation with KCl. Using a synaptosome-based system that retains the molecular machinery necessary for synaptic transmission, specifically pre- and post-synaptic membranes, we compared chemically-induced LTP in WT versus Parkin KO mice. Over various durations of KCl exposure, the number of surface AMPARs increased linearly in the WT synaptosomes. Despite similar quantities of surface AMPARs at baseline, there was no increase in surface AMPAR number over longer durations of KCl exposure, hence no LTP in the Parkin-KO derived synaptosomes. We speculate that Parkin affects synaptic AMPAR trafficking by disinhibiting the PI3K-AKT pathway. Studies to directly investigate this hypothesis are planned.

297 C208

ANOMALOUS EFFECT OF LI+ ON KAINATE RECEPTORS

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Our lab has recently shown that, in addition to the neurotransmitter L-Glutamate, kainate-selective glutamate receptors (KARs) have an absolute requirement for external ions. In addition, the decay kinetics of the receptor are strongly dependent on the type of external ion present. While examining interactions between external cations and KAR agonists, we uncovered a novel ability of Li+ to regulate the gating properties of GluR6 KARs. Specifically, we found that replacement of extracellular Na+ with Li+ produced three effects that were only observed with the agonists kainate (KA) and domoate (Dom): (1) an increase in the relative peak response, (2) a slowing of decay kinetics, (3) and a substantial increase in the relative equilibrium response. Interestingly, KA and Dom possess structural similarities, specifically a pyrrolidine ring about the α-amine group which is not found in other KAR agonists, suggesting that this element of the ligand’s structure may be necessary for Li+’s effects. Cations exert their effects on KARs by binding to or near the M770 residue and the introduction of a surrogate cation into this position (ie. a K or R residue) blocks ion-dependent gating. Surprisingly, agonist-dependent modulation by Li+ persisted in the M770K mutation, suggesting that Li+ binds to a distinct site of the receptor. We identified a mutation (K531A) in the dimer interface, a region known to be involved in desensitization, which occluded the agonist specific effect of Li+. However, it is not yet known if K531 represents a structural element necessary for the binding of Li+. Moreover, whether the

Li+ site only becomes accessible following activation by KA and Dom remains an open question.

298 C209

CLIMBING FIBRE CONTROL OF PURKINJE CELL SPIKE OUTPUT

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Purkinje cells integrate multimodal afferent inputs and generate the sole output of the cerebellar cortex. Via their control over deep cerebellar nuclear neurons, they shape the final outflow of information from the cerebellum to regulate a variety of motor- and learning-related behaviours. The contributions of Purkinje cells to these functions are modified by both acute and chronic effects of climbing fibre (CF) afferents. Whereas the chronic effects of CF discharge, such as the depression of conjunctively activated parallel fibre inputs, are well established, the acute effects of CF discharge and their associated mechanisms remain poorly understood. Here we show that CF discharge presented at physiological frequencies substantially modifies the frequency and pattern of Purkinje cell spike output in in vitro rat cerebellar slices. CF discharge converts an intrinsically-generated trimodal pattern of output characteristic of Purkinje cells in vitro to a more naturalistic pattern composed of spike trains interrupted by short and long CF-evoked pauses and state transitions. The effects of CF discharge could be reproduced in the presence of synaptic blockers using current injections to simulate CF / complex spike depolarizations, revealing that CF modification of Purkinje cell output is not a product of network activity. Instead, we found that postsynaptic changes in the Purkinje cell, produced at least by an interplay between sodium channels and calcium-activated potassium channels, accounted for CF-mediated effects on spike output. Furthermore, by controlling the frequency of Purkinje cell spike output over three discrete levels, CF discharge modulates the gain of Purkinje cell responsiveness to parallel fibre inputs in vitro. The three frequencies of CF-controlled output further proved to differentially regulate the output of deep cerebellar nuclear neurons, revealing that CFs may act as a cortico-nuclear switch that either restricts Purkinje cell signaling to the cerebellar cortex or enables Purkinje cell output to influence the entire cortico-nuclear axis. These findings provide important insights into the probable cellular and network factors contributing to motor disturbances following CF denervation.

299 C210

STRUCTURAL AND MORPHOLOGICAL EFFECTS OF CHRONIC AMPA RECEPTOR BLOCKADE IN HIPPOCAMPAL SLICE CULTURES

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The maintenance of dendritic spines, the postsynaptic elements of glutamatergic synapses, within the CNS require continued activation of AMPA receptors. In organotypic hippocampal slice cultures, chronic blockade of AMPA receptors for 14 days induced a significant loss of dendritic spines on CA1 pyramidal cells. Here, using serial section electron microscopy we show a loss of spines paralleled by a significant loss of asymmetric synapses. We observed an increase in the number of asymmetric shaft synapses, suggesting that spine retraction does not necessary lead to synapse elimination. As the dendritic spines are the primary targets of excitatory glutamatergic inputs, we investigated how a decrease in spine density and an increase in the number of shaft synapses affect synaptic transmission. We observed a significant increase in mEPSC amplitude (119 ± 4% of control, $p<0.05$) but surprisingly no change in frequency of mEPSCs (97 ± 13% of control) nor in the AMPA/NMDA ratio, the latter suggesting a proportional increase in both AMPA and NMDA receptor-mediated synaptic transmission. Interestingly the decrease in synapse number was not accompaigned by a decrease in mEPSC frequency, suggesting a compensatory change in the probability of vesicular release. Moreover, we found that the observed morphological and functional changes were associated with altered bidirectional synaptic plasticity. In conclusion the continued activation of AMPA receptors is necessary for maintaining the structure and function of glutamatergic synapses.

300 C211

CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 INFLUENCES AMPA-MEDIATED TOXICITY OF HIPPOCAMPAL SLICES BY REGULATING GLUR1 SUBUNIT IN SYNAPTIC MEMBRANES

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Over the last decade, several studies have documented that alterations in calcium-independent phospholipase A2 (iPLA2) activity is associated with development of neurodegenerative disorders. The iPLA2 enzymes were found to interact with alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor function and the recent results obtained on glutamate receptor phosphorylation point to the hypothesis that iPLA2 systems could influence glutamate-induced toxicity in the brain. Experiments described here, using cultured hippocampal slices, examined this notion by investigating whether iPLA2 inhibition can interfere with AMPA receptor properties and toxicity. We observed that preincubation of organotypic hippocampal slices with the iPLA2 inhibitor bromoenol lactone (12 h; 3 µM) increased the phosphorylation on both Ser831 and Ser845 sites of GluR1 subunits of AMPA receptors, but did not affect phosphorylation on NR1 subunit of N-methyl-D-aspartate (NMDA) receptors. Also, GluR1 subunit phosphorylation levels were selectively increased by (R)-BEL, an enantioselective inhibitor of iPLA2γ, but not by (S)-BEL, an iPLA2β inhibitor. Overtime, the iPLA2γ inhibitor (R)-BEL promoted the insertion of new GluR1 subunits into synaptic membranes, an effect observed by differential centrifugation and independent biotinylation experiments. Here again, NR1 subunit level was unaffected by (R)-BEL treatment. Finally, inhibition of iPLA2γ exacerbated AMPA-mediated cell death in the CA1 region of the hippocampus, an effect that was selectively abolished by IEM 1460 and philanthotoxin-433, two antagonists specific for AMPA receptors lacking GluR2 subunits. These results provide evidence that iPLA2γ-related regulation of AMPA receptor GluR1 subunit phosphorylation could represent an important mechanism modulating hippocampal cell death induced by AMPA receptor stimulation. This investigation raises the possibility that iPLA2 mechanisms might contribute to neurodegenerative processes.

301 C212

DIFFERENTIAL EXPRESSION OF SYNAPTOTAGMIN ISOFORMS IN DOPAMINE NEURONS AND ITS IMPLICATION IN SOMATODENDRITIC DOPAMINE RELEASE

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Active mesencephalic dopamine (DA) neurons release DA in their terminal projection areas as well as locally within the mesencephalon through somatodendritic (STD) release. STD release of DA has been shown to regulate DA neuron firing through D2 autoreceptor activation and may be implicated in motor performance regulation. Under normal circumstances, this form of release appears to require some form of exocytosis. Compatible

with this, STD DA release is TTX-sensitive, calcium-dependent, sensitive to depletion of vesicular stores by reserpine and more importantly, blocked by treatment with botulinum toxins that cleave SNARE proteins. Interestingly, STD DA release displays a different calcium-dependency than terminal DA release: lowering calcium concentration to 0.5 mM prevents axonal DA release but not STD release. Considering that Synaptotagmin (Syt) is the main calcium-sensor for exocytosis and that the different isoforms of Syt have different calcium-binding affinities, we hypothesize that differences in the exocytotic mechanism between the axonal and STD compartments could be related to differential expression of Syt isoforms. Here we took advantage of a transgenic mouse expressing the EGFP gene driven by the tyrosine hydroxylase promoter to identify the Syt isoforms expressed by DA neurons. Using single cell RT-PCR, immunochemistry and cell purification by fluorescent activated cell sorting (FACS) we found that freshly-dissociated DA neurons as well as DA neurons in culture and in slices express Syt1, Syt4 and Syt7 but not Syt2 nor Syt9. Interestingly, their localization profile showed striking differences: whereas Syt 4 localizes to the STD compartment and Syt1 in fine axonal-like processes, Syt7 was found in both compartments. Moreover, the proportion of DA neurons expressing Syt7 increased with time both in vivo and in culture. Using a siRNA strategy, experiments are currently under way to evaluate the impact of synaptotagmin isoform downregulation on basal STD DA release in our culture model. Our results are compatible with the hypothesis that differences in calcium requirement between axonal and STD DA release can be explained by the differential localization of Syt isoforms in these two compartments.

302 C213

CELLULAR DISTRIBUTION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR α7 SUBUNIT IN RAT HIPPOCAMPUS

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The density of receptors present on the surface of neurons has become recognized as an important means to influence synaptic activity. While the movement of the ionotropic glutamate and GABA receptors to and from the plasma membrane has attracted significant interest, the nicotinic acetylcholine receptors (nAChRs) have received relatively little attention. The broad cholinergic innervation of many brain areas indicates that nAChRs modulate network activity, while the α7 homopentamer, which is widely distributed in the hippocampus, is believed to be important in Alzheimer's disease and schizophrenia. To study cellular distribution of the α7 subunit, we used organotypic hippocampal slice cultures (OHSCs), which express the protein in a developmentally regulated manner. Cross-linking of surface proteins, differential centrifugation, and cell surface biotinylation all revealed a very limited presence of the α7 subunit at the plasma membrane. In contrast, AMPA and GABAA receptor subunits displayed significant surface expression. To exclude the possibility that the unexpected distribution of the α7 subunit was an artifact of the culture model, experiments were completed with adult hippocampus, and revealed a similar profile. To monitor distribution of functional α7-containing nAChRs, we developed a colourimetric assay that employed α-bungarotoxin (BGT; a specific α7 nAChR antagonist) conjugated to horseradish peroxidase through biotin-streptavidin. The BGT conjugate was applied to fixated OHSCs following either non-permeabilizing (surface) or permeabilizing (total) conditions, and revealed the majority of α7 subunits that formed receptors were at the cell surface. To determine whether tyrosine phosphorylation could affect α7 subunit trafficking, we next studied the effect of altering kinase activity. OHSCs were treated with either insulin or genistein, and while stimulating phosphorylation had no effect on either the cellular distribution of α7 subunits or the level of surface BGT binding, inhibiting phosphorylation increased the presence of the subunit at the plasma membrane. Our future work will aim to clarify the molecular mechanisms underlying intracellular retention of α7 subunits, and more clearly examine regulation of their trafficking.

303 C214

DROSOPHILA NEUROPEPTIDE INDUCES MUSCLE CONTRACTION VIA L-LIKE CALCIUM CHANNELS

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DPKQDFMRFamide is the most abundant endogenous FMRFamide-related peptide in Drosophila melanogaster (Schneider & Taghert, PNAS 85:1993-97, 1988). DPKQDFMRFamide enhances neurally-evoked contractions of larval body wall muscles (Hewes et al., J. Neurosci. 18: 7138-51, 1998) and enhances excitatory junction potentials (EJPs) (Dunn & Mercier, Peptides 26:269-76, 2005). At 1 μM, DPKQDFMRFamide also induces phasic contractions and increases tonus in body wall muscles of unstimulated Drosophila larvae. These effects persist in the presence of 7 mM glutamate, which desensitizes postsynaptic glutamate receptors. Thus, the effect on tonus cannot be explained by enhanced release of glutamate from synaptic terminals but presumably represents a postsynaptic effect. The effect on tonus is abolished in calcium-free saline. Nifedipine, an L-type calcium channel blocker, reduced DPKQDFMRFamide-induced contraction in a dose-dependent manner. Nifedipine, at 30 µM, reduced the response to DPKQDFMRFamide by approximately 90%, suggesting that L-like calcium channels are required for peptide's postsynaptic effect. The effect of the peptide was not significantly reduced by 0.5 mM amiloride or 1 µM flunarizine, which would not support a role for T-type channels. Supported by NSERC.

304 C215

ADULT SPINAL CORD STEM/PROGENITOR CELLS DIFFERENTIATE INTO OLIGODENDROCYTES AND SCHWANN CELLS FOLLOWING TRANSPLANTATION INTO THE DEMYELINATED SPINAL CORD

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Stem/progenitor cells capable of generating new neurons and glia exist in specific regions of the adult mammalian CNS, including the spinal cord. Stem/progenitor cells derived from the ependymal region of the adult rat spinal cord can be cultured in vitro as neurospheres which are multipotential and can self-renew. Transplantation of these neurospheres has therapeutic potential for spinal cord injury. Following transplantation into the intact or injured rat spinal cord, these neurospheres survive, migrate and integrate along white matter tracts, and preferentially differentiate into oligodendrocytes. To study the remyelination potential of the oligodendrocytic progeny of transplanted ependymal region stem/progenitor cells, we produced focal demyelination lesions in the spinal cord with ethidium bromide injections. This treatment leaves a population of demyelinated axons in a glial-free environment that can be used to evaluate the remyelinating and differentiation potential of the transplanted cells. We hypothesized that ependymal region derived stem/progenitor cells transplanted into focal demyelination lesions in the rat spinal cord will primarily differentiate into oligodendrocytes with remyelinating potential. Using a motorized microinjector, focal demyelination lesions were produced by microinjection of 0.1% ethidium bromide into the lateral funiculus of the spinal cord at level T8. Three days after lesion induction, ependymal region derived stem/progenitors generated from transgenic GFP rats were microinjected into the demyelinated lesion. Cyclosporine A was administered daily for immunosuppression. We find that GFP expressing stem/progenitor cells survive following transplantation into ethidium bromide-induced focal demyelination lesions in the spinal cord. Following engraftment, stem/progenitor cells primarily differentiate into oligodendrocytes (~70%)

and few astrocytes (~2%). Neuronal differentiation of transplanted cells was not detected. Interestingly, some GFP positive cells expressed characteristic Schwann cell markers, demonstrating a surprising lineage fate for these cells. Therefore, adult stem/progenitor cells derived from the ependymal region of the spinal cord primarily differentiate into oligodendrocytic and Schwann cell progeny which show a myelinating phenotype following transplantation into focal demyelination lesions. These data suggest the therapeutic potential of spinal cord derived stem/progenitor cells for remyelination.

305 C216

CALCIUM CHANNEL SUB-TYPES INVOLVED IN LONG-TERM ADAPTATION OF CRAYFISH MOTOR NEURONS

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Motor neurons can be characterized as tonic (highly active) or phasic (largely inactive). Tonic motoneurons typically have low output synapses with long filiform terminals, and phasic motoneurons typically have high output synapses with large varicose terminals (Atwood & Wojtowicz, Internat. Rev. Neurobiol. 38:275-362, 1986). Increasing the electrical activity of phasic axons over 3-14 days changes synaptic properties and nerve terminal morphology to resemble tonic neurons (Lnenicka et al., J. Neurosci. 6:2252-58, 1986). These changes, referred to as long-term adaptation, require extracellular calcium and are correlated with reduced calcium influx through P-type calcium channels in the neuronal cell body (Hong & Lnenicka, J. Neurophysiol. 77:76-85, 1997). The present study sought to characterize the calcium channel types responsible for the reduced transmitter release during long-term adaptation of phasic axons. Selective calcium channel blockers were used to determine the relative contribution of calcium channel subtypes to excitatory junctional potentials (EJPs) in crayfish abdominal extensor muscles. Crayfish axons were stimulated for 1-2 hours per day for 3 days, and EJPs were recorded 1-3 days afterward. EJPs were not altered by the N-type channel blocker, ω-Conotoxin GVI A but were reduced by 60% by the P-type blocker, ω-Agatoxin IVA. Thus, 60% of transmitter release appears to be associated with P-type channels, and 40% is associated with calcium channels that are neither P-type nor N-type. Long-term adaptation did not alter the sensitivity of the EJP to either channel blocker. Thus, the reduction in transmitter release does not involve a redistribution in the percentage of calcium channel sub-types but may result from equal changes in calcium influx through P-type and non-P/non-N type calcium channels in the synaptic terminals. Supported by NSERC.

306 C217

MBP AS A "PIP2-MODULIN" PROTEIN: NEW BIOLOGICAL FUNCTION FOR AN OLD CNS PROTEIN.

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The classically accepted biological function of myelin basic protein (MBP), one of the major protein components of myelin in central nervous system (CNS), is the maintenance of myelin sheath compaction. Owing to its extreme net positive charge, MBP serves as an adhesive molecule in CNS myelin sheaths, bringing together the two apposing faces of the cytoplasmic leaflets of the cell membrane processes of myelinating cells, oligodendrocytes (OL), to effect myelin compaction around axons. During the past years, it has been shown that MBP binds and polymerizes/bundles actin and tubulin, in a manner modulated by post-translational modifications. We have also shown MBP to interact with Ca2+-calmodulin with relatively strong affinity, and that this interaction regulates the MBP's membrane association, and ability to polymerize actin in the presence and absence of lipids. Moreover, we have shown MBP to associate with lipid rafts, and that different post-translational modifications influence the partitioning of this protein into these putative signalling platforms. Here, we describe new data that depict MBP as a member of the recently described "PIP2-modulin" proteins [MARCKS, GAP-43, CAP/NAP-22]; thus, MBP's ability to sequester PIP2 may be linked to cellular functions outside its classically accepted role in myelin. These data will be discussed with respect to cellular morphogenesis of oligodendrocytes during maturation, differentiation, and myelinogenesis in health and disease.

307 C218

THE FUNCTIONAL SIGNIFICANCE OF SEIZURE-INDUCED GAP JUNCTION-BASED SYNCHRONOUS ACTIVITY AND GENE EXPRESSION IN THE ISOLATED MOUSE HIPPOCAMPUS

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There are several reviews implicating gap junctions in epileptogenesis (Dudek et al., 1986; Jeffreys, 1995; Dudek et al., 1998; Carlen et al., 2000; Perez Velazquez & Carlen, 2000; Dudek, 2002; Traub et al., 2004). Compelling evidence now exists that direct cell-to-cell communication is up regulated in neuronal hyperactivity and seizures. It is hypothesized that seizure-induced increased synchronous activity could be due to several factors including enhanced gap junctional channel function, increased gap junctional expression, or both. Studies have shown that glial gap junctions also play an important role in epileptogenesis. Increased Cx43 mRNA, presumably in glia and not in neurons, was observed in peritumoral brain tissue surgically removed from patients whom had seizures compared to those without seizures (Naus et al., 1991; Aronica et al. 2001). We used different seizure models to test the above hypothesis. The intact hippocampi isolated from 15-day-old mice was separated into dentate gyrus and CA portion and were made epileptic from chronic bicuculline (BMI, a GABAA antogonist) exposure for 6 hours or treated with 100uM Cobalt chloride for 1 hour. In the case of BMI model, extra cellular field recordings for up to one hour, demonstrated recurrent epileptiform activity, which is blocked by the gap junctional blocker, carbenoxolone. The cobalt-induced epileptiform activity was blocked by phenytoin. After washing off the BMI and cobalt with ACSF, the changes in cellular mRNA expression of connexins and pannexins were analyzed using quantitative Real time PCR method (QRT-PCR). The expression of 90-110 bp amplicons from several connexins (Cxn26, Cx30, Cxn32, Cxn36, Cxn40, Cxn43, Cxn45 and Cxn47) and pannexins (Pxn1, Pxn2 and Pxn3) were analyzed. The results indicated up regulation of mRNAs of Pxn1, Pxn2, and Cxn43 in the CA portion of the cobalt-treated hippocampi. Pannexins, Pxn1 and Pxn2, were increased by about 1 fold and Cxn43 expression went up by 2 folds. However no changes were observed in the dentate gyrus. In the BMI model, no significant change was observed in the CA portion of the hippocampi, however, the expressions of Cxn30 and Cxn43 were elevated > 2 fold in the DG region. When the level of expression between the connexins and Pannexins were compared in the CA and DG portions of the control hippocampi, the following order was observed: Cxn43 ~ Cxn30 > Px2 > Px1 ~ Cxn32 > Cxn26 > Cxn36 ~ Cxn47 ~ Cxn45 > Cxn40. Comparison of the correl

308 C219

NEURONAL AND GLIAL CONNEXINS, ELECTRCIAL SYNAPSES AND GAP JUNCTION-ASSOCIATED PROTEINS IN THE CNS

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Twelve connexins (Cx) form gap junctions between various cell types in the adult mammalian CNS: astrocytes express C26, Cx30 and Cx43; oligodendrocytes express Cx29, Cx32 and Cx47; neurons express Cx36, Cx45, Cx50 and Cx57; vascular cells express Cx37, Cx40 and Cx45. We used immunofluorescence, freeze-fracture replica immunogold labelling

(FRIL) and molecular approaches to study the distribution, cellular localization and protein interaction partners of these connexins. Among glial cells, gap junctions composed of multiple connexins exhibit regional and cellular heterogeneity, and interdependency of connexin trafficking and coupling profiles. Glial gap junctions are found to be associated with a variety of regulatory and scaffolding proteins, including ZO-1, ZONAB and MUPP1. The important functional role of gap junctions forming the pan-glial syncytium in CNS homeostasis is indicated by reports of neural pathology and physiological impairments in glial connexin knockout mice, and CNS disorders in humans with glial connexin mutations. Among neurons, gap junctions composed of Cx36 form electrical synapses that are widely distributed throughout adult brain and spinal cord, and those composed of Cx50 and Cx57 occur exclusively between neurons in retina. In addition, co-localization of Cx36 with markers of nerve terminals in some brain regions indicate the presence of gap junctions at terminals forming mixed synapses (chemical and electrical). Gap junctional and direct molecular association of Cx36 with the scaffolding proteins ZO-1, ZO-2 and MUPP1 was demonstrated by analyses of in vivo and in vitro systems. Electrical coupling between neurons is known to generate synchrony of subthreshold membrane oscillations and to promote synchronous low and high-frequency rhythmic oscillations in neuronal networks. Such synchronous rhythmic oscillations occur in nearly every part of the brain. It is widely considered that synchronous oscillations serve as a mechanism to "bind" the activity of distributed neuronal networks, and that this synchrony-mediated binding in the CNS underlies a wide range of information processing related to memory formation and retrieval, sensory perception, motor control, attention and plasticity. Thus, electrical synapses formed by neuronal gap junctions appear to play a key and indispensable role in neural network properties and cognitive function. Supported by grants from the CIHR to JIN, and by NIH NS44010 and NS44395 to JER.

309 C220

FEED-FORWARD INHIBITION DECREASES EPSP AMPLITUDE IN HIPPOCAMPAL AREA CA1

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Long-term potentiation (LTP) of glutamatergic transmission in the hippocampus is widely accepted as a cellular substrate for learning and memory in the brain. In area CA1 of the hippocampus, both high frequency presynaptic stimulation and low frequency correlated pre- and postsynaptic spiking can induce LTP, measured as an increase in the amplitude of excitatory postsynaptic potentials (EPSPs). However, stimulation of the presynaptic inputs also activates feed-forward inhibitory circuits onto the same postsynaptic CA1 pyramidal neurons. The delay separating the postsynaptic EPSP from the inhibitory postsynaptic potential (IPSP) is only 1.9 ms, suggesting that the measured peak of the EPSP may in fact be the peak of a mixed EPSP/IPSP. Using whole-cell recordings from rat hippocampal slices, we have investigated this hypothesis. By manipulating the strength of GABAergic inhibition, achieved simply by hyperpolarizing or depolarizing the membrane potential relative to the reversal potential for GABAergic currents, we show that feed-forward inhibition greatly decreases EPSP amplitude. This suggests that GABAergic plasticity is another mechanism whereby the strength of glutamatergic transmission could be altered, and therefore, might play an important role in learning and memory.

310 C221

DYNAMICAL MECHANISMS OF NEURAL CODING AND THEIR BIOPHYSICAL BASIS

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Neural coding stems from transduction of graded synaptic input into all-or-none spikes. Understanding this transduction mechanism is crucial for deciphering precisely what information is encoded by a spike train. Using computational modeling coupled with electrophysiological experiments, we show how spinal sensory neurons with distinct transduction properties encode either the integral (running sum) or derivative (rate of change) of their input. Spike generating dynamics differed fundamentally between cell types: integrators switched to repetitive spiking when sufficiently strong stimulation destabilized the system, whereas differentiators maintained stability and spiked only upon abrupt changes in stimulus intensity. Generating spikes through the former mechanism allowed stimulus intensity to be encoded with firing rate, whereas generating spikes through the latter allowed changes in stimulus intensity to be encoded with spike times. Simulations identified direction of subthreshold current (i.e. whether current active at voltages below spike threshold was depolarizing or hyperpolarizing) to be a critical determinant of the spike generating dynamics. In turn, experiments demonstrated that integrators and differentiators expressed either a subthreshold inward (calcium) or outward (potassium) current, respectively, that was necessary and sufficient to explain the spike generating dynamics. By mediating positive feedback control of the subthreshold voltage trajectory, inward current sustains depolarization and causes the neuron to spike on the basis of its integrated stimulus waveform; conversely, outward current mediates negative feedback, truncating depolarization and causing the neuron to spike on the basis of its differentiated stimulus waveform. The causal link between direction of subthreshold current and implementation of fundamental operations (integration and differentiation) is rigorously demonstrated through dynamical systems analysis and likely constitutes a universal calculus for neural computation.

311 C222

BDNF-DEPENDENT MODIFICATION OF NMDA RECEPTOR SIGNALING PATHWAYS INVOLVED IN LONG-TERM POTENTIATION

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Long-term potentiation (LTP), a long lasting enhancement of synaptic response following neural stimulation, is a proposed cellular correlate of memory. The proteins mediating this phenomenon in post-synaptic membranes include the NMDA receptor (NMDAR) and the receptor tyrosine kinase, TrkB. Activation of TrkB by BDNF increases the activity of NMDAR resulting in enhanced LTP through mechanisms that are currently being investigated. We propose that differential interactions between a brain-specific protein, RasGrf1, and NMDAR or TrkB may modify downstream signaling pathways thus altering LTP. RasGrf1 interacts directly with the NR2B subunit of NMDAR resulting in increased phosphorylation of p38-MAPkinase and increased long-term depression (LTD), a long-lasting decrease in synaptic response. RasGrf1 also interacts with and is phosphorylated by TrkB in transfected cells resulting in morphological changes such as increased neurite outgrowth in TrkB-B5 cells. Preliminary data indicates that BDNF stimulation of adult mouse (P30) cortical slices results in the loss of interaction between NMDA/KCL-stimulated NR2B and RasGrf1. This loss of interaction is also associated with a decrease in p38-MAPkinase activation and a corresponding increase in Erk activation. Could the loss of interaction between NR2B and RasGrf1, due to competitive interaction with TrkB, decrease activation of pathways generating LTD, resulting in increased LTP? Continuing research aims to further clarify the nature of the neuronal interaction between TrkB and RasGrf1 and to determine whether, in addition to modifying NMDAR signaling, TrkB and RasGrf1 could also strengthen LTP by promoting morphological changes such as the formation of neuronal processes or dendritic spines.

312 C223

SPREADING DEPRESSION IN AN INSECT GANGLION

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Spreading depression (SD) in mammalian cortical tissue is closely associated with several important pathologies including stroke, seizures and migraine. The mechanisms underlying SD in its various forms are still incompletely understood. Here we describe an SD-like event in an insect model system. Using K+-sensitive microelectrodes, we measured extracellular K+ concentration ([K+]o) in the neuropile of the locust ventilatory central pattern generator (CPG) while monitoring CPG output electromyographically from muscle 161 in the second abdominal segment. We investigated the role of K+ in failure of neural circuit function induced by various stressors. An abrupt rise in [K+]o was reliably associated with heat-induced failure of the ventilatory motor pattern, which occurred in 100% of preparations. The rise in [K+]o reached a plateau of ~52mM on average. [K+]o was restored to normal baseline levels if heat was removed and this was associated with recovery of ventilatory motor patterning. The [K+]o event was reliably triggered by several cellular stressors in addition to hyperthermia, including anoxia, ATP depletion using sodium azide, artificially increased [K+]o within the ganglion, and Na+/K+ ATPase dysfunction induced by ouabain. In many preparations failure of rhythmic bursting preceded a burst of tonic electrical activity that occurred on the rising phase of the [K+]o increase. Repetitive [K+]o surges were triggered by ouabain in a concentration-dependent manner. 10-4M ouabain bath application induced cycles of [K+]o increase and decrease that occurred every 3-5 minutes and were associated with failure and recovery of the motor pattern. We found that the [K+]o disturbance propagates from its origin at a rate of about 2 mm/min. We also measured ATP levels in the metathoracic ganglion and found no correlation between ATP levels and initiation of [K+]o surges by different stressors. [K+]o surges were not dependent on electrical activity, though reduction of electrical activity with TTX delays their occurrence. Blockage of K+ channels with TEA significantly reduces the amplitude of the surge in [K+]o. We conclude that these events represent spreading depression, and that in this model system such activity can be interpreted as an adaptive mechanism to shut down neural function and conserve energy in response to stress.

313 C224

DOPAMINE RECEPTOR AND MICROTUBULE-ASSOCIATED PROTEIN FUNCTIONS: A ROLE FOR THE CYCLIN-DEPENDENT KINASE 5

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An increasing number of evidence demonstrates that drugs affecting dopamine levels in the brain can induce cytoskeletal modifications and D1 dopamine receptors likely play a pivotal role in this process. These evolving changes may impact striatal synaptic plasticity as cytoskeletal constituents are involved in the maintenance of dendritic processes and that any changes in their stability could affect major cellular compartments of neurons like dendrites, spines and synapses. Our recent data suggest that activation of the D1 dopamine receptor could lead to enhanced phosphorylation of the microtubule-associated protein tau, normally involved in the microtubule stabilization. The purpose of the present study is to investigate the role of D1 receptor signaling constituents in this process. By using selective antibodies that recognize specific phosphorylation sites of tau, we show that in SK-N-MC cells, endogenously expressing D1 receptors, escalating doses of the D1 receptor agonist SKF 38393 (1 to 100 μM) increase the levels of tau phosphorylation at serines 199/202 (Cdk5 site) and serine 214 (PKA site). These effects are likely due to the activation of D1 receptors since they can be reversed by a 30 minutes pre-treatment with 100 µM of the D1 receptor antagonist SCH 23390. We also show that Cdk5 play a central role in tau phosphorylation induced by D1 receptor activation as roscovitine and calpeptine, a direct and an indirect Cdk5 inhibitors, respectively, reverse tau phosphorylation at serines 199/202 and serine 214. Notably, the PKA inhibitor Rp-cAMPS, reverses tau phosphorylation at serine 214 but not at serines 199-202. These data are the first in vitro demonstrations showing that activation of the D1 dopamine receptor could have profound influence on the cytoskeletal remodeling of neurons, for which Cdk5 likely play a pivotal role.

314 C225

THE EFFECT OF CONNEXIN 43 MIMETIC PEPTIDES ON NETWORK ACTIVITY IN ORGANOTYPIC RAT HIPPOCAMPAL SLICES

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Using two connexin mimetic peptides (CMPs), short synthetic peptides corresponding to selected sequences in the two different extracellular loops of connexin 43 (mainly expressed in astrocytes in contrast to favored neuronal expression of connexin 36), the possible role of glial connexin-dependent processes in the network activity was studied in cultured organotypic rat hippocampal slices. The CMPs used were: Gap 27 (amino acid residues 201-211 of the rat connexin 43, SRPTEKTIFII) and SLS-peptide (amino acid residues 180-195, SLSAVYTCKRDPCPHQ). The effects of these CMPs were compared to those induced by carbenoxolone (CBN), a widely used gap junctional blocker with no preferential activity toward cell-specific (neuron vs. glia) types of gap junctions composed of different connexin isoforms. Neither CBN, nor CMPs had significant effects on synaptic transmission in the CA1 area of the hippocampus. The peak amplitude of population spike was 4.11±0.47 (n=18), 6.19±3.28 (n=5), 4.74±0.72 (n=7) and 5.53±1.17 (n=12) mV in control conditions and after treatment with CBN, Gap 27 and SLS-peptide, respectively. At the same time, CBN strongly inhibited different manifestations of epileptiform network activity including the primary afterdischarge evoked by tetanic stimulation. In contrast, the CMPs suppressed only the spontaneous recurrent epileptiform activity without apparent effects on evoked responses. Notably, the CMPs required much longer treatment time (>10 hours) to exert marked effects. The ability of the compounds studied to suppress seizure-like activity positively correlated with the degree of gap junctional coupling measured by the extent of the fluorescence recovery after photobleaching (FRAP). The differential effects of CBN and CMPs could be due to differences in their target connexin isoforms, reflecting cell type-specific actions. Thus, stronger FRAP inhibition induced by CBN compared to CMPs could result from its non-selective inhibition of both neuronal (connexin 36) and glial (connexin 43) gap junctions, while Gap 27 and SLS-peptide inhibited FRAP only “partially” due to their possible “selective” effect on glial gap junctions. Despite some technical challenges, the CMPs are a promising pharmacological tool to study isoform-specific connexin-dependent processes in the CNS. Supported by Canadian Institutes for Health Research, Hospital for Sick Children Research Foundation and Epilepsy Canada.

315 C226

MODULATION OF P2X3 AND P2X2/3 SENSORY PURINOCEPTORS BY PHOSPHOINOSITIDES

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The phospholipids phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) are involved in many cellular

processes such as membrane trafficking and cell motility. They have also been shown to modulate several types of ion channels. We report here evidence that these phosphoinositides are able to modulate the function of ATP-gated P2X receptor-channels expressed in primary sensory neurons and in heterologous systems. In dissociated rat DRG neurons, 2-3 hours incubation with the PI3K/PI4K inhibitor wortmannin at 35 microM induced a dramatic decrease (64%) in the amplitude of alpha,beta-meATP-evoked P2X3 receptor-mediated currents. Intracellular application of PIP2 was able to fully reverse the inhibition induced by wortmannin while incubation for 2-3 hours with wortmannin at 100 nM (a selective PI3K inhibitor at this concentration) produced no significant effect on P2X3 currents. Both in Xenopus oocytes and in HEK 293 cells transiently transfected with rat P2X3, 35 microM wortmannin incubation induced a significant decrease in the rate of receptor recovery. The modulation of P2X2/3 heteromeric purinoceptors by phospholipids was also analyzed in the Xenopus oocyte expression system. Slowly-desensitizing P2X2/3 receptor-mediated currents were decreased by 56% after a 2-3 hours incubation in wortmannin 35 microM, and by 39 and 29% after incubation with wortmannin 100 nM and selective PI3K inhibitor LY294002 35 microM, respectively. The P2X2/3 current amplitude decrease induced by wortmannin could be partially reversed by application of PIP2 or PIP3, indicating that assembly of P2X2 and P2X3 subunits confers to the heteromeric complex a sensitivity to both phospholipids. In summary, our data demonstrate a novel mode of functional regulation of the homomeric P2X3 and heteromeric P2X2/3 subtypes of ATP receptors by the levels of PIP2 and PIP3 in the plasma membrane of DRG neurons. The molecular basis of these P2X-phosphoinositides modulatory interactions is currently under investigation. Supported by CIHR and NSERC-IPS (AstraZeneca R&D Montreal).

316 C227

LOW-THRESHOLD SPIKES-DEPENDENT EXTRACELLULAR CALCIUM DEPLETION CONTROLS SYNAPTIC SENSITIVITY OF THALAMOCORTICAL NEURONS TO LEMNISCAL STIMULATION

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Thalamus is the main gateway of cerebral cortex. Extracellular unit recordings demonstrated a preferential burst firing of TC neurons during slow-wave sleep and tonic firing during both REM sleep and waking states. During tonic firing mode EPSPs arising from activities in ascending pathways easily drive action potentials sending signals to cerebral cortex. During sleep, unitary prethalamic EPSPs either do not elicit neuronal firing or elicit spike burst that is followed by neuronal silence. Burst firing is mediated by low-threshold Ca2+ spikes (LTS). We hypothesize that Ca2+ influx during LTS results in a local Ca2+ depletion from extracellular space, which affected sensitivity of postsynaptic neuron to presynaptic inputs and contributes to the generation of neuronal silence that follows the burst. The objective of this study was to investigate the modulation of mediator release probability caused by postsynaptic Ca2+ spikes. We test our hypothesis by recording responses of thalamocortical neuron from VPL nucleus in rat brain slices maintained in vitro during low intensity stimulation of the medial lemniscus. LTS was elicited with hyperpolarizing current pulses. The lemniscal stimulation intensity was set to obtain minimal EPSPs amplitude and some failures. Simultaneously, Ca2+ ion-sensitive electrode was placed juxtacellularly of the recorded neurons to measure Ca2+ depletion during LTS. Minimal lemniscal stimulation induces 0.31 ± 0.06 mV EPSPs with a failure rate of 34.4 ± 22.2 % in control (n=6). When lemniscal stimuli were applied during and/or immediately after LTS the failure rate of synaptic responses increased reaching 78.6 ± 22.4 %. Lower amplitude LTS, the LTS that did not lead to the generation of action potentials increased failure rates to a lesser extent. Failure rate level was recovered to control values on average 225 ± 45 ms after the maximum of LTS. Extracellular Ca2+ depletion followed LTS with delays of several milliseconds and paralleled modulation of failure rates. Our findings thus indicate that the generation of Ca2+ spikes associated with Ca2+ entry into postsynaptic neurons, could significantly influence synaptic transmission and gate the ascending information passing through the thalamus.

317 C228

THE ROLE OF C2A AND C2B DOMAINS OF SYNAPTOTAGMIN I AT A SPECIFIED SYNAPSE DURING MEMORY CONSOLIDATION

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We are a product of what we have learned in our histories and, in such, of what we are able to recall in relation to that. Much work has been done in recent years to understand the molecular mechanisms, including necessary transcription factors and associated pathways, in memory formation. Despite these advances, much data has yet to be obtained in regards to what is occurring at the level of the synapse. Questions remain as to whether there is formation of new synapses or a strengthening of old ones, during the acquisition of a new memory. In vitro work previously conducted in our lab has demonstrated the putative calcium sensor, synaptotagmin I, is critical for the formation of presynaptic structures which leads to functional nascent synapses. This effect is mediated through a Ca2+ binding motif at the C2A domain. By introducing a synthesized HIV1-TAT conjugated C2A peptide specific to the Ca2+ binding region acting in a dominant negative manner, synaptic vesicle aggregation and synapse formation is prevented. The objective of this study thus is to investigate the contribution of new synapses in long-term memory formation in a well established operantly conditioned learning model (Lukowiak et al., 1996), using the C2A peptide as a tool. Specifically, the giant pond snails Lymnaea stagnalis were placed in a hypoxic environment, in which the snails were driven to breathe via a respiratory orifice -the pneumostome at the water surface. The number of pneumostome openings and total breathing time were measured. The snails were then conditioned by applying a tactile stimulus to the pneumostome contingent upon its opening, and following this training regime; the snails effectively learned to avoid this behaviour. After 24 hours, the memory is tested by measuring total breathing time and pneumostome openings and comparing to the results obtained prior to training. Injection of the C2A peptide 1-1.5 hrs after the final training session prevented the snail from forming a memory. Snails injected with a C2A peptide mutated (C2A-mut) to prevent Ca2+ binding behaved similar to those previously obtained for controls in which total breathing time significantly decreased after training. This group also displays no change in number of pneumostome openings from the final training session as compared to the memory test. Taken together the results demonstrate a necessary involvement of the newly formed functional synapses in long term memory consolidation.

318 C229

THE ROLE OF CALCINEURIN, CYTOSKELETON AND CALPAIN IN LOW FREQUENCY DEPRESSION OF SYNAPTIC TRANSMISSION +C339

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Transmitter release at crayfish leg extensor NMJ phasic synapses declines by over 50% in 60 min at 0.2 Hz stimulation. This low-frequency depression (LFD) is regulated by protein phosphorylation by PKA and PKC and dephosphorylation by proteinphosphatases 1, 2A and 2B (calcineurin) (Silverman-Gavrila et al., 2005, J. Neuroscience). The permeant calcineurin inhibitors FK-506 or autoinhibitory peptide abolished LFD. To determine if the site of action of calcineurin is pre or/and postsynaptic we pressure injected impermeant calcineurin autoinhibitory peptide into presynaptic axons or postsynaptic muscle cells and measured the amplitude of the intracellularly recorded excitatory postsynaptic potential (EPSP) evoked by

stimulating the phasic axons at 0.2 Hz. LFD was decreased only when calcineurin was inhibited in the presynaptic nerve terminal. The drugs have no postsynaptic effects since FK-506 or permeant peptide did not affect amplitude distribution of spontaneous miniature EPSPs; therefore they target presynaptic calcineurin, the activity of which is necessary for LFD (Silverman-Gavrila and Charlton, J. Neuroscience, submitted). Neither drug affected high frequency depression caused by 20 Hz stimulation. Since Ca2+ buffering by injected BAPTA-based Ca2+ indicators or EGTA-AM treatment did not inhibit LFD, calcineurin activation may occur very close to Ca2+ channels where a large Ca2+ signal is available. Alternatively, a limited proteolysis of calcineurin by Ca2+-dependent protease calpain might be involved in calcineurin activation during LFD. Calpain is present at crayfish neuromuscular junction as shown by immunostaining and Western blot analysis. Pharmacological inhibition of calpain with calpain inhibitor I and PD145305 blocked LFD, while the inactive negative control compound PD150606 did not affect LFD. A feed-back regulatory mechanism of LFD possibly involves TRPM7 ion channel, a calpain substrate present at the crayfish NMJ. To examine changes in phosphoproteins during LFD, we removed motor axons and nerve terminals after the induction of LFD or treatment with various phosphorylation regulators, extracted their proteins, separated them by SDS-PAGE, and stained them with phosphospecific stains ProQ-Diamond/SYPRO Ruby to identify bands for analysis by mass spectrometry. Actin and tubulin phosphorylation was decreased during LFD. Western-blot analysis and immunostaining showed calcineurin, actin and tubulin at presynaptic axons and terminals. The involvement of cytoskeletal elements in LFD was proved pharmacologically. The anti-actin drug cytochalasin and the microtubule stabilizer taxol inhibited LFD, while the tubulin depolymerizing drug nocodazole accelerated LFD with no post-synaptic effects. In conclusion, dephosphorylation of presynaptic actin and tubulin by calcineurin possibly activated by calpain may regulate LFD.

319 D201

EFFECT OF CHOLESTEROL DEPLETION ON CEREBELLAR SYNAPTIC TRANSMISSION

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Alterations in membrane cholesterol content are associated with endogenous modulation of synaptic strength and also occur in a number of neurodegenerative conditions. We have investigated the effects of reducing membrane cholesterol content on synaptic transmission between presynaptic granule cells and postsynaptic Purkinje cells in dissociated cerebellar cultures. The cholesterol extracting agent methyl-β-cyclodextrin (mβCD, 5mM) caused a significant decrease in the amplitude of evoked EPSCs measured at the Purkinje cell soma. Current clamp measurements of the somatic granule cell action potential indicated that it was unaffected by cholesterol extraction under these conditions. The mean amplitude of miniature EPSCs was unchanged by mβCD treatment and their frequency was increased approximately 10 fold. The increase in mEPSC frequency occurred in both Ca2+ containing and Ca2+ free extracellular medium. Results suggest that cholesterol extraction reduces synaptic strength by reducing quantal content, but a reduction in vesicle release probability or presynaptic excitability is unlikely to account for this observation.

320 D202

ELECTROPHYSIOLOGICAL STUDIES OF THE EFFECT OF GALANIN IN RAT SUBSTANIA GELATINOSA

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Galanin is a 29 amino acid peptide expressed in the dorsal root ganglia and spinal dorsal horn interneurons. Behavioral studies have shown that spinal galanin produces an anti-nociceptive effect. Galanin is thought to produce these effects via activation of GalR1. Galanin is also thought to produce pro-nociceptive effects via activation of pre-synaptic GalR2 receptors on primary afferents. However, the physiological role of galanin in spinal nociception is still relatively elusive. Using whole cell recordings, in both delay (excitatory) and tonic (inhibitory) and the GalR2 specific agonist ARM1896, (500nM) or the Gal1/2 specific agonist M961 (500nM) in the presence of GalR1 antagonist (M871, 2.5 microM), we investigated the actions of galanin in rat substantia gelatinosa. The most conspicuous effects included 1) a GalR1-mediated increase in outwardly and/or inwardly rectifying K+ conductances in putative excitatory, delay neurons 2) a GalR2-mediated reduction in frequency and amplitude of spontaneous EPSC's in delay cells and 3) a GalR2 agonist decrease in the excitability of tonic inhibitory cells. The first two effects would be consistent with an anti-nociceptive effect whereas the third may be pro-nociceptive. Thus, some aspects of our results fit with the notion that GalR1 activation produces analgesia whilst GalR2 activation may produce hyperalgesia (see Liu & Hokfelt, TIPs 23:468-474, 2002). Supported by CIHR.

321 D203

VASCULATURE GUIDES MIGRATING NEUROBLASTS IN THE ADULT FOREBRAIN

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The adult subventricular zone (SVZ) – olfactory bulb (OB) system is a unique region in the mammalian forebrain where massive neuronal migration is observed. First, neuroblasts migrate tangentially in the rostral migratory stream (RMS), a long and intricate migratory pathway, and then radially in the OB. Despite extensive investigations of the molecular signals involved in these two different migratory modes, it is still unknown how neuronal precursors travel through complex brain territories to reach OB, and what mechanisms confine them within the migratory pathways. We recently uncovered a completely unexpected organization of blood vessels in the migratory stream in the adult forebrain that might be related to the migratory process. While blood vessels are randomly organized in most brain regions, they parallel migrating stream in the RMS and essentially all neuroblasts migrate in their close proximity. Interestingly, in the OB core where neuroblasts have to detach from one another to start migrating individually and radially to the different bulbar layers, many blood vessels are now oriented perpendicular to the tangentially migrating neuroblasts (i.e. parallel to the radialy migrating cells). The cells that exit from RMS and start their radial migration into the bulbar layers do so along these perpendicularly oriented blood vessels. In the search for the molecular mechanism of vasculature-guided migration of neuronal precursors in the adult brain, we discovered that endothelial cells of blood vessels synthesize BDNF which is released to the extracellular space and trapped by astrocytic processes ensheathing migrating neuroblasts. Stereotaxic injection of BDNF into the striatum, a region outside the migratory pathway of these neuronal precursors, de-routes the cells from their normal migratory stream into this area. Altogether, our data demonstrates that blood vessels define the migratory stream in the adult forebrain, and guide migrating neuroblasts toward and into the OB via a local action of BDNF.

322 D204

IDENTIFICATION AND CHARACTERIZATION OF AN INVERTEBRATE T-TYPE CALCIUM CHANNEL

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T-type calcium channels are active at the resting potential, inactivate rapidly and bear small single-channel conductances. T-types generate low-threshold calcium spikes and influence action potential patterns, participate

in cardiac pacemaker activity, regulate smooth muscle tone, hormone secretion, and the acrosome reaction in sperm. T-Type channels are implicated in human pathologies such as cardiac hypertrophy, childhood absence epilepsy, cancer, and pain. Our lab has begun analyses of a neuronal, 2683 amino acid T-type channel from the pond snail Lymnaea stagnalis. Comparative analyses of LCav3 to its three human orthologs (Cav3.1-3.1) provide a unique prospective for understanding T-type channel functions and related pathologies. Les canaux de type T sont actifs au potentiel de repos, s'inactivent rapidement et soutiennent de petites conductances d'un canal unique. Les types T génèrent des pics de calcium de bas seuil et influencent les patrons de potentiel d'action, participent à l'activité de stimulation cardiaque, régularisent le tonus du muscle lisse, la sécrétion hormonale et la réaction de l'acrosome dans le sperme. Les canaux de type T sont impliqués dans des pathologies humaines comprenant l'hypertrophie cardiaque, l'épilepsie avec absences de l'enfance, le cancer, et la douleur. Notre laboratoire a débuté la caractérisation structurale et fonctionnelle d'un canal T neuronal de 2683 acides aminés provenant de l'escargot d'étang, Lymnaea stagnalis. Les analyses comparatives de LCav3 à ses trois orthologues humains (Cav3.1-3.1) fournissent une prospective unique vers la compréhension des fonctions des canaux T et les pathologies y étant reliées.

323 D205

VESICLE DYNAMICS MEASURED IN DROSOPHILA NEURONS USING FLUORESCENCE CORRELATION SPECTROSCOPY

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Although the process by which membranous vesicles move within cells is generally accepted, we are now only beginning to probe the dynamic nature of this essential cellular function. We particularly lack understanding of the dynamics of neurotransmitter-containing synaptic vesicles within the nerve terminal. Two fundamental questions remain open: Are synaptic vesicles mobile in the resting nerve terminal and if so, by what mechanism do they move? It is generally thought that the actin cytoskeleton plays a role in local vesicle trafficking but this assumption is relatively untested and the few studies to address the role of actin have yielded mixed results. Using FRAP analysis it was recently found that vesicles in the resting Drosophila nerve terminal are highly mobile at rest and that disruption of the actin cytoskeleton, either by pharmacology or genetics, reduced vesicle dynamics. To further address the question, we have performed fluorescence correlation spectroscopy experiments (FCS) in the same system, which allowed probing the local mobility of synaptic vesicles at different loci within neuronal boutons. The FCS study confirmed that synaptic vesicles were highly mobile, and showed that the motion detected is consistent with diffusion. We also confirmed that the motion of the synaptic vesicles is on average slowed down following perturbation of filamentous actin. We therefore conclude that Drosophila synaptic vesicles are moving in an actin-dependent manner and that the combination of genetics and FCS provides a powerful method for probing intracellular organelle dynamics.

324 D206

EXAMINATION OF THE ROLE OF SCRIBBLE IN CULTERED HIPPOCAMPAL NEURONS

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We have recently demonstrated that cadherin/-catenin adhesion complexes play a large role in localizing synaptic vesicles to developing synapses. A detailed analysis of -catenin functional domains revealed that the PDZ-binding motif is essential for vesicle localization. We have taken a candidate approach to identify the PDZ-domain containing protein(s) downstream of beta-catenin that mediates its effects. Scribble, a member of LAP (leucine-rich repeats and PDZ domains) family, has been shown to regulate the localization of synaptic vesicles at neuromuscular junctions in Drosophila. We have shown that Scribble is developmentally regulated in the brain, with dramatically greater expression in P1 whole mouse brains compared to adult brains, suggesting a role for Scribble in early synaptic development. Indeed, Scribble is expressed at presynaptic compartments and colocalizes with both -catenin and synaptophysin in cultured hippocampal neurons. Furthermore, Scribble can be co-immunoprecipitaed with -catenin, but this interaction has been proved to be indirect using in vitro transcription-translation assays. We are currently using an siRNA approach to determine the functional role of Scribble in hippocampal neurons. Using three siRNA sequences, we show that depletion of endogenous Scribble results in the dispersion of vesicles along the axon, and phenocopies that of -catenin ablation. We therefore conclude that Scribble may function downstream of -catenin to recruit synaptic vesicles to developing presynaptic compartments.

325 D207

TRANSLOCATION OF TRPC5 CHANNELS CONTRIBUTES TO CHOLINERGIC-INDCUED PLATEAU POTENTIALS

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Muscarinic stimulation generates prolonged depolarizations called plateau potentials (PPs) in hippocampal pyramidal neurons (Fraser and MacVicar, 1996). PP is an attractive candidate for a major intrinsic conductance observed during ictal phase of seizures. Transient receptor potential (TRP) channel subtype TRPC5 can generate non-selective cation conductances evoked by muscarinic receptor activation. Muscarinic activation also enhances TRPC5 protein translocation and insertion in the plasma membrane of cultured hippocampal neurons (Bezzrides et al., 2004). We hypothesized that plateau potentials and tail currents in CA1 hippocampal pyramidal neurons are partially mediated by the muscarinic induced membrane insertion and activation of TRPC5 channels. In order to evaluate this hypothesis, we developed an assay for biotinylation in acute hippocampal slices in order to quantify the change in membrane proteins during treatment with a muscarinic agonist, carbachol (CCH). After 15 min in CCH the surface expression of TRPC5 channels was increased by >15 fold. A control membrane protein, the transferrin receptor (TfR) showed no change. Atropine, the muscarinic antagonist, decreased the enhancement of TRPC5 surface expression by CCH. We also used whole cell patch clamping of CA1 pyramidal neurons in hippocampal slices to determine the contribution of TRPC5 currents to the tail current that generates the PP. We found that the common TRPC5 antagonists 2-APB and SKF-96365 could significantly depress the tail current as well as PP. Interestingly, the PI3K inhibitor wortmannin, which was shown to block translocation of TRP channels in recombinant systems, could also decrease PP as well as surface expression of TRPC5 channels. Our results suggested that the rapid translocation of TRP channels contributes to the generation of PPs. This study provides a further understanding into the mechanisms of Ca2+ signaling after muscarinic stimulation and the pathology of epilepsy.

326 D208

A PERSISTENT, VOLTAGE-DEPENDENT CALCIUM CURRENT IN NEUROENDOCRINE CELLS

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Prior activity can modify the intrinsic excitability of neurons. A striking example of such activity-dependent change is the afterdischarge of the bag cell neurons from Aplysia californica. Following brief synaptic input, these neurons exhibit a 30 min burst of action potentials, resulting in the secretion of egg-laying hormone and the initiation of ovulation. In cultured bag cell

neurons, stimulation with a 5 Hz, 10 second train of action potentials results in a prolonged depolarization that extends well beyond the initial stimulus and is analogous to the afterdischarge. A voltage-independent, non-selective cation current, activated by the initial calcium influx, drives the prolonged depolarization; however, this current inactivates long before the end of the depolarization. Therefore, we examined the role of a persistent calcium current, activated by modest depolarization, in the maintenance of the prolonged depolarization. Calcium currents in cultured bag cell neurons were isolated under whole-cell voltage-clamp by replacing intracellular potassium with cesium and extracellular sodium and potassium with TEA and cesium, respectively. Currents were elicited from a holding potential of -60 mV, using step depolarizations from -50 mV to -20 mV for durations of a minimum of 10 sec to a maximum of 3 min. The onset of voltage-activation for the persistent calcium current was between -50 mV and -40 mV. Once activated, the current was still present after 3 min, and displayed only a slow decay by this point. Calcium currents were completely blocked by 10 mM nickel; moreover, 20 or 50 micromolar SKF96365, an agent known to inhibit certain calcium permeable channels, partially blocked the persistent calcium current. The effect of SKF96365 appeared to be voltage-dependent, with greater block at more depolarized potentials. Because the persistent calcium current was maintained, even after 3 min of activation, it may be recruited during the initial excitation caused by the cation current. The interaction between these two, distinct currents likely maintains the prolonged depolarization, and represents a potential general mechanism for activity-dependent changes to excitability.

327 D209

REGULATION OF SYNAPTIC VESICLE DYNAMICS BY CADHERIN-ADHESION COMPLEXES

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The rapid formation and elimination of synaptic sites occurs throughout life and represents one aspect of synaptic plasticity in which synaptic communication is modified in the long-term. Little is known about what determines where synapses will form along an axon or how are they modulated by neuronal activity. Synaptic adhesion proteins are of particular interest in this context because pre- to postsynaptic membrane adhesion is one of the initial events during synapse formation and remains a fundamental component of the maintenance of synapses in maturity. We have previously shown a role for the cadherin adhesion complex in the localization of synaptic vesicles to developing presynaptic compartments. The maintenance of strong cell-cell adhesion is detrimental to the formation of new synapses in the presence of the plasticity factor brain-derived neurotrophic factor (BDNF). Using time-lapse confocal analysis we showed that BDNF mobilizes synaptic vesicles at existing synapses, resulting in small clusters of synaptic vesicles "splitting" away from synaptic sites. We demonstrate that BDNF's ability to mobilize synaptic vesicle clusters depends on the dissociation of cadherin/-catenin adhesion complexes. Artificially maintaining cadherin/-catenin complexes in the presence of BDNF, abolishes the BDNF-mediated enhancement of synaptic vesicle mobility, and also abolishes the longer-term BDNF-mediated increase in synapse number. We are now further exploring the hypothesis that enhanced synaptic vesicle mobility contributes to the formation of new synapses, and that disruption of strong cadherin-based adhesion catalyzes this event. Together, we believe that the molecular adhesive machinery required for synapse assembly in development plays an essential role in modulating synaptic architecture in the context of plasticity-related structural remodeling.

328 D210

MGLUR5-INDUCED LONG-LASTING POTENTIATION OF L-TYPE CALCIUM CHANNELS IN DENDRITES OF HIPPOCAMPAL CA1 ORIENS/ALVEUS INTERNEURONS

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Voltage-sensitive calcium channels (VSCCs) provide a major source of Ca2+ influx in neuronal dendrites and are involved in the regulation of multiple cellular functions like dendritic electrogenesis, synaptic plasticity and gene expression. However, the distribution, regulation and role of VSCCs in dendrites of inhibitory interneurons are largely unknown. Here we use two-photon Ca2+ imaging in combination with whole-cell current-clamp recordings to study the contribution of different types of VSCCs to dendritic Ca2+ transients evoked by backpropagating action potentials (bAPs-CaTs) and their regulation by the group I metabotropic glutamate receptors (mGluRs) in CA1 oriens/alveus inhibitory interneurons. We find that L-, P/Q- and R/T-types of VSCCs as well as ryanodine-sensitive Ca2+ stores contribute to dendritic bAPs-CaTs. Local activation of mGluR5 produces slow Ca2+ response followed by long-lasting potentiation of bAPs-CaTs. This bAPs-CaT potentiation does not involve P/Q- or R/T-types of VSCCs, requires ryanodine-sensitive Ca2+ stores, is associated with an increased contribution of L-type VSCCs and can be elicited by synaptic stimulation. Our results suggest that the efficacy of dendritic L-type VSCCs can be potentiated for a long term via mGluR5- and Ca2+ release-dependent mechanisms in inhibitory interneurons. Specific mGluR5-dependent potentiation of L-type VSCC function represents a powerful synaptic mechanism regulating dendritic excitability and likely synaptic plasticity in interneurons.

329 D211

CASPASE INHIBITOR (BAF) SELECTIVELY ENHANCES NEURONAL SURVIVAL IN THE ADULT DENTATE GYRUS

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Two competing processes exist in the adult hippocampus: neurogenesis and apoptosis. Thousands of progenitors are generated each day in the dentate gyrus (DG) but >50% die before reaching maturity. Apoptosis removes neuroblasts that are not recruited into some functional role by the critical second week. This process is important for both regulating the dimension and neuronal circuitry in the adult DG. The objective of this study is to clarify the role of apoptosis in DG neurogenesis. To address this, we used a broad-spectrum caspase inhibitor (BOC-Asp-CH2F or BAF) to prevent apoptosis. Caspases are a family of proteases essentially involved in apoptosis and are expressed in the adult DG. Three-month old male Sprague-Dawley rats received two injections of bromodeoxyuridine (BrdU) within one day to label proliferating cells. Nine days later, either BAF (n=7) or saline (n=5) was perfused directly into the DG over three consecutive days. Rats were sacrificed one day following the end of treatment. Immunohistochemical markers were used to identify progenitors (BrdU) and neuroblasts (doublecortin or DCX) respectively. Our results demonstrate that immediate survival of progenitors and neuroblasts was enhanced in two of three DG regions after caspase inhibition. In the medial region, number of BrdU+ and DCX+ increased by 25.7% and 36.0% in BAF-treated animals. In the ventral region, number increased by 24.4% and 39.9%. BAF did not appear to influence differentiation of progenitors; over 75% of progenitors develop into neuroblasts in both groups. Therefore, we conclude that: 1) a considerable number of one-week old progenitors face elimination through a caspase-dependent mechanism and 2) reducing caspase activity can enhance their immediate survival. As the hippocampus is involved in various forms of learning and memory, more DG neurons could improve either process. Furthermore, this study is the first to reveal a difference in sensitivity to

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

caspase inhibition across DG regions. This can be partly attributed to the variable rate of apoptosis occurring in each region. (This study is supported by CIHR).

330 D212

ULTRASTRUCTURAL LOCALIZATION OF EPHRIN-B3 IN ADULT RAT HIPPOCAMPUS

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Recent observations suggest that interactions between the transmembrane ligand, ephrin-B3, and the EphA4 or EphB2 receptors influence synaptogenesis, as well as the induction of long-term potentiation (LTP) in regions CA1 and CA3 of rodent hippocampus. A detailed analysis of the discrete cellular distribution of ephrin-B3 within the hippocampal circuitry is currently needed in order to better understand the exact role of this molecule in such processes; notably its pre- or postsynaptic interactions with any of the two receptors. In situ hybridization had previously shown ephrin-B3 expression in CA1 and CA3 pyramidal cells, and in dentate gyrus (DG) granule cells, but the subcellular localization of the protein has not been examined at the ultrastructural level. We used light and electron microscopic immunocytochemistry with a polyclonal anti-ephrin-B3 antibody, whose specificity was tested in brain sections from ephrin-B3 knockout mice (provided by Dr. Henkemeyer). Ephrin-B3 immunoperoxidase labeling was observed in the cell body and neuropil layers of CA1, CA3, and DG, where it was most frequently observed in axon terminals, including mossy fiber terminals. Labeled neuronal perikarya and astrocytic processes were also frequent. Dendritic spines, dendritic shafts, unmyelinated and myelinated axons were only occasionally labeled. Labeled axon terminals made synaptic contact with generally unlabeled dendritic spines, dendritic shafts and neuronal perikarya, in decreasing order of frequency. The synapses between ephrin-B3 axon terminals and dendritic spines were generally asymmetrical and displayed features of excitatory synapses; whereas those with dendrites or perikarya were symmetrical. The ephrin-B3 labeling of Schaffer collateral and mossy fiber axon terminals is consistent with its proposed role in LTP at both types of synapses. We recently reported that EphA4 is localized in the pre- as well as post-synaptic partners of these synapses (Tremblay et al., J Comp Neurol 501:691, 2007), whereas EphB2 was most frequently detected in dendritic shafts and less often in spines (Bouvier et al., submitted). Thus, the above ephrin-B3 localization appears more consistent with interactions with EphA4 than EphB2; which will need confirmation by ongoing dual labeling experiments. Supported by NSERC. M.-È.T. is supported by studentships from FRSQ and UdeM.

331 D213

DYNAMIC GLIAL-AXONAL INTERACTIONS AT THE NODE OF RANVIER: NORMAL FUNCTIONS AND IMPLICATIONS TO PATHOPHYSIOLOGY OF SPINAL CORD INJURY

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Glial-axonal interactions at the node of Ranvier are key factors defining the excitability of myelinated axons in health and disease. In myelinated axons, K+ channels are located predominantly under the myelin sheath, and their role in normal physiology remains unknown. These channels are typically viewed in terms of their negative effects on axonal function following injury or with disease, when they become exposed due to retraction or breakdown of the myelin sheath. We hypothesized that paranodal myelin loops, which segregate the Na+ channel-rich nodal area from the rest part of the axon, may transiently disconnect from the axonal membrane in response to increased axonal activity, providing conditions for recruitment a normally "hidden" (and thus "inactive") ion channels/transporters in modulation of axonal excitability. We tested this hypothesis by analyzing the electrophysiological properties of white matter axons in mature rat spinal cord and have found that the decay phase of their action potentials can be modulated by axonal activity. We show that the fast K+ channel blocker 4-aminopyridine, which normally does not have effects on action potentials of myelinated axons due to its inability to reach the K+ channels concealed under the myelin sheath, becomes effective after periods of increased axonal activity. These results can be interpreted in terms of transient disconnection of paranodal myelin loops from the axonal membrane, thus opening ways for 4-AP to diffuse into the adaxonal space under the myelin sheath to block K+ channels. The proposed hypothesis emphasizes a dynamically changing picture of myelin-axonal interactions at and around the node of Ranvier and may have a wide range of applications in normal physiology and pathophysiology of myelinated axons.

332 D214

VOLTAGE-DEPENDENCE OF EXCITATION-TRANSCRIPTION COUPLING: SIGNALING FROM CALCIUM CHANNELS TO CREB

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Excitation-transcription (E-T) coupling provides a vital link between electrical activity and gene expression in excitable cells. This process is of great importance for the development and remodeling of cellular structure and function but is not thoroughly understood with respect to fundamental aspects of signal transduction. Classic studies of both excitation–contraction (E-C) and excitation–secretion (E-S) coupling have demonstrated the biological output is steeply dependent upon voltage-dependent calcium channel (VDCC) activity; however, little is known about the detailed kinetics of E-T coupling. The transcription factor CREB plays important roles in long-term neuronal plasticity and is thus one of the most widely studied mediators of E-T coupling. In this study we examined the relationship between depolarization by extracellular K+ elevation and signaling to nuclear CREB, using cultured neonatal rat superior cervical ganglion (SCG) neurons as a model system. We found that depolarizing SCG neurons activated L-type channels resulting in a rapid phosphorylation of CREB that was graded by both stimulation duration and voltage. To gauge the strength of the signal, we monitored the relationship between stimulation duration and the extent of CREB phosphorylation and determined that, like E-C and E-S coupling, CREB signaling strength is steeply voltage-dependent with an e-fold increase per 5.6 mV. Importantly, this steep relationship was determined largely by voltage-dependent changes in L-type channel open probability (Po). By grading Ca2+ flux through the channels while keeping depolarization constant, we discovered that CREB phosphorylation shows a surprisingly shallow dependence on unitary Ca2+ channel flux (i). Thus, Ca2+ entry through L-type channels is essential for signaling to CREB, but the strength of the signal is only weakly affected by gradations in the magnitude of the Ca2+ flux or resulting rise in bulk [Ca2+]. We ruled out the idea that conformational changes in the L-type channel directly engage signal transduction machinery to account for the apparent voltage-dependence above and beyond that of Ca2+ flux. Rather, we propose that Ca2+ entering the cell through L-type channels saturates a Ca2+ sensor in a local Ca2+ nanodomain, such that decreases in i have little effect on sensor activation. When neurons are depolarized, increases in Po recruit more and more channel signaling modules, which mediate signaling to CREB in a cooperative manner.

333 D215

NERVE GROWTH FACTOR INCREASES THE EXPRESSION OF ΜU OPIOID RECEPTOR PROTEIN IN PC12 CELLS

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The goal of this work is to elucidate the cellular mechanisms regulating mu-opioid receptor (MOR) expression. We therefore investigated MOR protein levels in PC12 cells treated with nerve growth factor (NGF). Using Western immunoblotting with rabbit anti-MOR (abcam) and cAMP analysis, we found a significant increase in MOR expression after 72 hours of NGF (50 ng/ml) treatment. We then explored whether this NGF-mediated increase in MOR expression is regulated by nitric oxide (NO). It was found that pre-treatment of PC12 cells with NO synthase (NOS) inhibitors Nω-nitro-L-arginine methyl ester (L-NAME) (20 mM) and s-methylisothiourea (S-MIU) (2 mM) had no effect on NGF induced MOR expression, although significant increases in MOR levels were detected as a result of pre-treatment with S-MIU alone. From these data, it appears that the NGF-mediated increase in MOR protein expression is not due to NGF induced increases in NOS or NO. Supported by NSERC and CIHR

334 D216

NA+/CL- DIPOLE COUPLES AGONIST BINDING TO KAINATE RECEPTOR ACTIVATION

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Kainate-selective ionotropic glutamate receptors (iGluRs) require external Na+ and Cl- as well as the neurotransmitter, L-glutamate, for activation. Although, external anions and cations apparently co-activate kainate receptors (KARs) in an identical manner, it has yet to be established how ions of opposite charge achieve this. A further complication is that KARs are subject to other forms of cation-modulation via extracellular acidification (i.e. protons) and divalent ions. Consequently, other cation species may compete with Na+ to regulate the time KARs remain in the open state. Here we have designed experiments to unravel how external ions regulate GluR6 KARs. We show that GluR6 kinetics are unaffected by alterations in physiological pH but that divalent and alkali metal ions compete to determine the time course of KAR channel activity. Additionally, Na+ and Cl- ions co-activate GluR6 receptors by establishing a dipole accounting for their common effect on KARs. Using charged amino acids as tethered ions, we further demonstrate that the docking order is fixed with cations binding first, followed by anions. Taken together, our findings identify the dipole as a novel gating feature that couples neurotransmitter binding to KAR activation. Supported by operating grants from the CIHR to D.B. A.Y.C.W was funded by the David T.W. Lin fellowship and D.M.M by a predoctoral fellowship from the CIHR. D.B. is the recipient of a Canada Research Chair award.

335 D217

chTOG, A NEW AXONAL MICROTUBULE-ASSOCIATED PROTEIN WITH A DISTINCT PATTERN OF DISTRIBUTION

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Microtubules are critical components of the cytoskeleton that serve as structural scaffolds and tracks for axonal transport. However, the mechanism whereby these fibers are regulated during axonal growth, regeneration, and retraction is complex and poorly understood. One of the ways that MTs are regulated is through interactions with microtubule-associated proteins (MAPs). A proteomic screen of rat brain synaptosomes identified chTOG, or colonic and hepatic tumor over-expressing gene, a MAP that is well characterized in mitotic cells and essential in MT spindle formation during cell division. We have localized chTOG (CKAP5 in chicken) to the axonal cytoplasm in chick sensory ganglia using immunocytochemistry. chTOG strongly stains the axon initial segment, nodes of Ranvier, and the growth cone, which are all areas of high MT dynamics in neurons. The MT-disrupting drug nocodazole was used to investigate whether chTOG distribution is altered in the absence of MTs in the growth cone. Short interference RNAs (siRNAs) were designed against chTOG and were successfully co-transfected with GFP into primary neurons. These indicate that chTOG plays a functional role in MAP-dependent MT regulation in the axonal cytoplasm.

336 D218

THE CELLULAR MECHANISMS UNDERLYING GABAergic SYNAPTIC PLASTICITY

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Coincident pre- and postsynaptic activity decreases the strength of synaptic inhibition in the hippocampus. The magnitude and polarity of this spike-timing dependent synaptic plasticity (STDP) depends of the spike-timing interval used during plasticity induction. When the spike-timing interval is correlated (within ±20 ms, pre-post or post-pre) a persistent increase in the amplitude of the inhibitory postsynaptic current (IPSC) is recorded. This increase in IPSC amplitude is due to a positive shift in the reversal potential for GABA (EGABA), thus STDP of GABAergic synapses produces a decrease in the overall strength of inhibition. In the present study we examined the role of postsynaptic Ca2+ in the regulation of EGABA during STDP induction. We made simultaneous electrophysiological and fluorescence imaging recordings using Fluo-4AM from hippocampal neurons cultured at low-density Following the electrophysiological identification of GABAergic synapses, we determined the postsynaptic Ca2+ dynamics at the soma and synapse during the induction of GABAergic STDP. Both coincident (pre-post +10ms) and non-coincident (pre-post +100ms) protocols (5Hz, 30sec) were examined. We found that the magnitude of the postsynaptic Ca2+ increase during coincident induction protocols was dependent on both the synaptic amplitude and EGABA of the synapse. Synapses with larger currents and a more hyperpolarized EGABA produced smaller Ca2+ increases, when compared with synapses that had smaller synaptic amplitudes and more depolarized EGABA values. Non-coincident protocols produce that same magnitude of Ca2+ as compared with postsynaptic spiking alone. Thus GABAergic STDP requires a Ca2+-dependent depolarization of EGABA. We are currently examining how Ca2+ regulates K+-Cl- cotransport (KCC2) at the molecular level; KCC2 regulates EGABA in the mature CNS.

337 D219

SIMULTANEOUS TIME-LAPSE CONFOCAL IMAGING AND ELECTROPHYSIOLOGY REVEALS THAT ATP P2Y RECEPTOR-COUPLED OUTWARD POTASSIUM CURRENT UNDERLIES MICROGLIAL CHEMOTAXIS

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Microglial cells are the resident macrophages that are involved in brain injuries and infections. Recent studies using transcranial two photon microscopy have shown that ATP and P2Y receptors mediated rapid chemotactic responses of miroglia to local injury. However, the molecular mechanism for microglial chemotaxis towards ATP is still unknown. To address this question, we employed a combination of simultaneous perforated whole-cell recordings and time-lapse confocal imaging in GFP-labeled microglia in acute brain slices from adult mice. We found that ATP-induced rapid chemotaxis is correlated with P2Y receptor associated-outward potassium current in microglia. Activation of both P2Y receptor and its

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

associated potassium channels are required for ATP-induced chemotaxis and baseline motility of microglial cells. The chemotaxis required the activation of phosphoinositide 3-kinase but not mitogen-activated protein kinase pathway. Our results provide strong evidence that P2Y receptor-associated outward potassium channels and the phosphoinositide 3-kinase pathway are important for ATP-induced microglial motility in acute brain slices. This work is supported by NeuroCanada Brain Repair program. Long-Jun Wu is supported by postdoctoral fellowships from the Canadian Institutes of Health Research and Fragile X Research Foundation of Canada.

338 D220

TIMING OF ACTION POTENTIAL EVOKED CA2+ CURRENT IN TRIGGERING TRANSMITTER RELEASE AT THE CALYX OF HELD SYNAPSE

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Action potentials (APs) play a crucial role in evoking presynaptic Ca2+ currents (ICa) and controlling neurotransmitter release, but little is known about how AP waveform determines the timing of Ca2+ influx as well as its relationship to quantal output in central synapses. By paired voltage-clamp recordings of ICa and excitatory postsynaptic currents (IEPSC), we examined these issues with pseudo- and real-APs at the calyx of Held synapse. We found that at 22°C the timing of Ca2+ entry strongly depended on AP repolarization rate but not depolarization rate, being immediately after the completion of repolarization phase for short APs and advanced towards the early part of repolarization phase as APs were broadened. At 35°C, ICa evoked by real APs remained as tail currents, but Ca2+ influx evoked by pseudo-APs with slower time course than real APs could occur as early as in the depolarization phase. Comparison of voltage-dependence of ICa at two temperatures revealed that raising temperature from 22°C to 35°C led to a temperature-dependent shift in the activation threshold of ICa and an accelerated kinetics in activation and deactivation. As a result of fast gating kinetics of ICa, timing of Ca2+ entry was shifted to from the repolarization phase to the depolarization phase of pseudo-APs, provided that the latter phase is sufficiently slow (>0.5 ms). These results presented the proof of principle that timing of Ca2+ inflow is highly dependent on the AP waveform, kinetics of ICa and temperature. Interplay of these variables dictates the timing of Ca2+ influx and ultimately quantal output. We also have evidence to suggest that ICa in the form of tail currents helps preserve temporal precision in presynaptic input and postsynaptic response, and is an important adaptation for achieving high-fidelity neurotransmission at the calyx of Held synapse. Support Contributed By: BWF & CIHR

339 D221

MUTAGENIC ANALYSIS OF TRPM7 CHANNEL FUNCTION IN ANOXIA

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Neurons that die following an ischemic attack exhibit an increase in the formation of reactive oxygen species, and experience excessive influx of calcium leading to neuronal death. While it has previously been shown that specific effectors downstream of calcium-activation may mediate apoptosis and necrosis, the contribution of a novel class of ion channels, belonging to the Transient Receptor Potential (TRP) channel superfamily, has also been implicated in cellular degeneration of these cells. In previous work we have found that specific knockdown of the TRPM7 channel prevents neurons from dying following anoxic insult (Aarts et al., 2003). TRPM7 channels provide a pathway for mono- and divalent cations into the cell and are unique in that they contain a functional, C-terminal alpha-kinase domain. More relevantly, the channel is thought to be activated in response to the generation of intracellular reactive oxygen species as well as localized decreases in extracellular calcium; events known to occur in the brain during ischemia. The present study aims to isolate the regions of TRPM7 responsible for mediating its response to oxidative stress. In particular, we chose to focus on the C-terminal PDZ-binding motif. PDZ domains enable intermolecular protein assembly through PDZ-PDZ interactions and by binding to C-terminal consensus motifs. We began by generating a truncated construct of the TRPM7 channel that contained a deletion of the 9 C-terminal amino acids (corresponding to positions P0 to P-8 with respect to the PDZ-binding motif), thereby disrupting intracellular interactions mediated by the PDZ-binding motif. Briefly, constructs containing the full-length and truncated TRPM7 sequences were studied by heterologous expression in HEK-293T cells. Cells were exposed to treatment with sodium cyanide (NaCN) to mimic the anoxic conditions of cerebral ischemia. We focused on two parameters as indicators of TRPM7 activity: calcium uptake and cell death. Calcium uptake was monitored using the fluorescent intracellular calcium indicator fluo-3, and levels of cell death assessed by propidium iodide (PI) uptake. We found that expression of the full-length and truncated constructs significantly increased calcium uptake induced by NaCN. Cell death, however, was increased only in cells expressing the full-length TRPM7 channel, suggesting a role for the PDZ-binding motif in mediating cytotoxic signaling events downstream of channel activation.

340 D222

PHOSPHORYLATION OF CREB STIMULATED BY IGF-1 IN PC12 IS MEDIATED BY MITOGEN AND STRESS ACTIVATED PROTEIN KINASE (MSK1)

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Insulin-like growth factor-1 (IGF-1) is a polypeptide growth factor with a variety of functions in both neuronal and non-neuronal cells. IGF-1 promotes its biological functions by activating multiple signaling pathways including PI3/Akt and MAPK pathways. We have previously shown that IGF-1 stimulates the phosphorylation of the Ca 2+/cAMP response element-binding protein (CREB) at Ser-133 residue in PC12 cells (Zheng et al., 2006). However, the kinase involved in IGF-1-induced phosphorylation of this transcriptional factor is not clear. We report here that MSK1, a downstream target of MAPK and p38 MAPK kinase, is involved in IGF-1 stimulated phosphorylation of CREB. IGF-1 induced the phosphorylation of both CREB, MSK1, FOXO3a and Akt in PC12 cells. The phosphorylation of CREB and MSK1 was dependent on the activation of MAPK and p38 MAPK, but that of FOXO3a was relied on the PI3 kinase/Akt. Inhibition of MAPK by PD98059 or the p38 MAPK blocker PD169316 significantly inhibited IGF-1-mediated phosphorylation of MSK1 and CREB. But had no effect on FOXO3a.Moreover, H89 and Ro318220, inhibitors of MSK1, dose-dependently blocked IGF-1-induced CREB phosphorylation. These data suggest that the activation of MSK1 via MAPK and p38 MAPK is involved, at least in part, in IGF-1 stimulated phosphorylation of CREB in PC12 cells. Supported by CIHR and NFSC 30670652.

341 D223

EPHA4 SIGNALING REGULATES PHOSPHOLIPASE C GAMMA 1 ACTIVATION, COFILIN MEMBRANE ASSOCIATION, AND DENDRITIC SPINE MORPHOLOGY

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Dendritic spines are highly specialized structures protruding from dendritic shafts that form the postsynaptic sites for the majority of glutamatergic excitatory synapses in the brain. In general, a spine is composed of an enlarged head region and a constricted neck and serves as biochemical compartment that accommodates the postsynaptic density (PSD), a dense region of ion channels and receptors that are complexed with scaffolding and other signaling proteins. Spines are actin-rich structures, and undergo rapid structural remodeling. These changes are believed to be associated with modifications of synaptic physiology that underlies the cognitive processes like learning and memory. However, the mechanisms that regulate actin organization and structural plasticity of spines are only beginning to be identified. We recently reported that activation of the EphA4 receptor tyrosine kinase upon ephrin ligand binding induced spine retraction in hippocampal neurons. However, the signaling pathways downstream of EphA4 that regulates spine morphology remain unknown. Here, we demonstrate that ephrin stimulation of EphA4 leads to the recruitment and activation of phospholipase Cgamma1 (PLCg1), which is known to be involved in membrane phosphoinositide regulation. We show that the interaction between PLCg1 and EphA4 requires an SH2-domain of PLCg1 and juxtamembrane tyrosines of EphA4. We also show that PLC activity is required for the maintenance of spine morphology and ephrin-induced spine retraction in hippocampal slices. Interestingly, PLCg1 modulates the plasma membrane association of the actin depolymerizing/severing factor, cofilin, which has previously been implicated in actin filament regulation and the remodelings of dendritic spines. EphA4 and PLCg1 signaling may thus modulate the actin cytoskeleton and spine morphology through cofilin regulation at sites of ephrin-EphA4 contact.

342 D224

RAPID CHANGES IN NEURONAL VOLUME EVOKED BY SPREADING DEPRESSION

Ning Zhou and Brian A MacVicar*

Spreading depression (SD) is a propagating wave of neuronal and astrocytic depolarization important in the pathogenesis of migraines, ischemia and brain trauma. SD in the grey matter is associated with a shift in extracellular potentials and transient depression of synaptic transmission. This can be directly monitored as an increase of light transmittance of the tissue, called the intrinsic optical signals (IOSs). Interestingly, extracellular space shrinks during SD, indicating that brain swelling may involve a neuronal component. SD was induced in cortical slices by bath perfusion of high potassium (40 mM of [K+]o) for 1.5 to 2 min. Using identified yellow fluorescent protein positive neurons from the Thy1 transgenic mouse model, we measured neuronal volume and IOS with two-photon laser scanning microscopy. Additionally, simultaneous measurements of whole-cell currents or field potentials were used to synchronize electrophysiological changes to IOSs. The high K+ evoked peak amplitudes of IOS, neuronal currents and field potentials occurred simultaneously. Neuronal volume increased immediately following SD onset and peaked within about 1 minute. Interestingly, neuronal volume recovered to the control level by 4 to 5 minutes from the onset of SD. Intracellular pH, measured with DCECF fluorescence indicated a transient acidification concurrent with the onset of SD. Since it has been suggested that neurons are resistant to osmolarity-induced volume changes, we investigated the role of ion channels and transporters. Blockers of ion channels failed to affect high K+ induced neuronal volume increase. However, an inhibitor of the acid activated Na/H exchanger (NHE), amiloride partially blocked the volume increase, and prolonged the recovery from SD induced acidification. This suggested a role for additional transporters in volume regulation of neurons during SD. K/Cl cotransporter (KCC) in pyramidal neurons normally exports K+ and Cl-. However KCC2 can transport K+ inversely into cells under conditions of elevated [K+]o thereby causing cell volume increase. Blockade of KCC2 with the inhibitor DIOA, similar to amiloride partially inhibited the neuronal volume increase. When applied together, amiloride and DIOA dramatically reduced the SD induced change in neuronal volume. We conclude that neuronal volume is rapidly and transiently regulated during high K+ induced spreading depression by activation of NHE and KCC2 transporters.

343 D225

REGENERATION OF INJURED PERIPHERAL NERVES: ROLE OF THE MICROENVIRONMENT

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Despite assumptions otherwise, peripheral nerve injuries are associated with substantial, severe and irreversible disabilities that highlight the barriers to their regeneration. Over the last 3 years, our laboratory has explored early barriers that develop following transection injuries of peripheral nerves. Transection, a common clinical injury, involves the complete separation of the proximal and distal stump of a peripheral nerve trunk. It is estimated that only 10% of transected axons successfully regenerate to distal targets. Our approach involves an accessible conduit that connects proximal and distal nerve stumps and can be manipulated from a subcutaneous access port 1. We highlight several findings identified by this window on early regenerative events. Firstly, we have observed that early regeneration is highly limited and staggered indicating profound hostility to regrowth 2. Barriers to regrowth may operate in part through RhoA/Rho kinase (ROK) axon signalling pathways independent of myelin inhibitors: pharmacological inhibition of ROK with HA1077 enhanced new axon regrowth not exposed to inhibitors in the distal nerve stump 3. A number of additional features of the microenvironment impair regeneration. An intense local inflammatory response substantially impairs early regeneration, in part because of local release of nitric oxide. The presence of diabetes mellitus also adds substantial barriers to nerve outgrowth and consolidation. Secondly, early axon outgrowth rarely occurs in isolation, but instead depends on an intimate association with Schwann cell (SC) processes. This association is almost invariable, with fidelity through three dimensions of space irrespective of the irregularity of the trajectory. Regrowth depends on the ability of SCs to proliferate and guide following axons; inhibition of SC mitosis severely disrupts regeneration 4. One of the critical signals between axons and SCs cells may be axonally synthesized CGRP peptide. Its receptors CRLR and RAMP-1 are expressed by SCs and local interruption of either CGRP synthesis or RAMP-1 synthesis using siRNA, interrupts regeneration 5. Overall, understanding how axon-SC interactions overcome the local barriers of the regenerative milieu to regrow is of substantial importance in peripheral neurobiology. [Supported by CIHR, CDA, AHFMR] 1. McDonald, D. S. & Zochodne, D. W. J. Neurosci. Methods 122, 171-178 (2003). 2.McDonald, D., Cheng, C., Chen, Y.

344 D226

EFFECT OF T-CHANNEL DISTRIBUTION ON FIRING PATTERN OF THE THALAMOCORTICAL CELL

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The low-threshold calcium current (IT) underlies burst generation in the thalamocortical (TC) relay cells and plays a central role in the genesis of synchronized oscillations by thalamic circuits. In thalamic relay cells ascending and descending inputs arrive to distinct parts the dendritic tree. Active properties of the dendritic tree influence synaptic integration. Previous studies have developed some algorithms to determine active parameters of the cell, but these methods are limited by number of free parameters. Here we developed a multicompartment model of thalamocortical cell to consider effects of dendritic currents on response of the cell.We attribute uniform T-channel distribution for all compartments in model in order to find a threshold for Low Threshold Calcium Spike (LTS) leading to action potential generation. By multiplication permeability of each section to its area we found a threshold number of channel that was necessary

to reproduce an LTS. Next we tuned active parameters of dendrites by considering different Gaussian distribution of T-channel for our 1270 compartments model. We normalized our Gaussian distribution to this threshold value, then for different means and variances we examined LTS response and IV- curve of T-current. Our simulations show, independent of the Ca2+ channel distribution, for a total channel number below the threshold value, cell gives a passive response and above the threshold, model reproduces the LTS. However, in a small range below the threshold the difference between uniform and non uniform distributions becomes visible. In the range 1-2 % below the threshold value the uniform distribution of T-channel produced passive response while a non-uniform distribution reproduced LTS response. Compare to experimental data, firing patterns and IV curve of T-current, shows a non-uniform distribution with higher density in sections near to soma has more correspondence to experimental data.
Supported by NSERC and CIHR

345 D227

STRYCHNINE-MEDIATED EXCITATION OF MAGNOCELLULAR NEUROSECRETORY CELLS IS INVERSELY RELATED TO EXTRACELLULAR FLUID OSMOLALITY IN SUPERFUSED EXPLANTS OF RAT HYPOTHALAMUS

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Application of strychnine has been shown to excite magnocellular neurosecretory cells (MNCs) in the rat supraoptic nucleus (SON) in vivo, where strychnine is believed to act as a competitive antagonist to taurine-mediated activation of glycine receptors (GlyRs). In this system, taurine has been shown to be released by astrocytes in the ventral glial limitans, which arborize within and around the nucleus. Hypoosmotic conditions have been shown to increase the release of taurine, thereby inhibiting the electrical activity of MNCs in order to suppress vasopressin and oxytocin release into the bloodstream. Conversely, hypertonic conditions suppress the basal release of taurine and lessen GlyR-mediated inhibition on MNCs, leading to an increase in electrical activity and VP release. In this study we investigated if this process could be studied in vitro, in superfused explants of rat hypothalamus. We first examined the specificity of bath-applying 1 μM strychnine for blockade of GlyRs. In single-unit extracellular recordings from MNCs, bath-application of 1 μM strychnine reversibly blocked the inhibitory effect of taurine, but not that of GABA. Indeed, application of strychnine significantly reduced the per cent inhibition induced by taurine from 58.9 ± 8.4 to 18.4 ± 5.8 per cent (n=4; P<0.01), but not those evoked by GABA (44.6 ± 2.6 to 30.7 ± 5.0 per cent (n=3; P=0.07)). We next examined whether the excitatory effect of strychnine depended on the external osmolality by recording from MNCs under hypoosmotic (275 ± 5 mosmol/kg) and isoosmotic (300 ± 5 mosmol/kg) conditions. The degree of excitation induced by bath-applying 1 μM strychnine showed an inverse relationship with respect to extracellular osmolality: The excitatory effect of strychnine was significantly greater in hypoosmotic (1.0 ± 0.3 Hz; n=18) than isoosmotic medium (0.04 ± 0.3 Hz ;n=14; P<0.05). These results indicate that GlyRs mediate an inhibitory effect on firing rate in MNCs and that the strength of this inhibition varies as an inverse function of osmolality in superfused explants of rat hypothalamus. Whether this inhibitory effect is mediated by taurine release from glial cells remains to be demonstrated.

346 D228

NEUROIMMUNOMODULATORY ASPECTS OF CELL SIGNAL TRANSDUCTION

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We studied transmembrane postreceptor signalling by trimeric G proteins - alpha subunits of main types using C6 glioma cells, natural killer (NK) cell line (RNK 16) or isolated human NK cells affected by antidepressants (SSRI) fluoxetine or sertraline. Also effects of iinterleukin 2, IL-2 or adenosine (NECA) agonist was assayed. Fluoxetine effects were found to decrese significantly G alpha q/11 levels in all tested cells. Acute effect of fluoxetine is linked to translocation of membrane G alpha q/11 into cytoplasm besides lower effector response by PLC and thus decreased 1,3,5 IP3, 2nd messenger formation. We estimated similarities in G alpha profiles in comparison with IL2 or NECA. Furthermore NECA effects interfered with fluoxetine effects on G alpha levels but also on apoptosis events. On the other hand, sertraline effects influenced mainly G alpha (s) levels a no apoptosis was observed. When we summarized, acute action of fluoxetine on G protein profiles In C6 glioma cells and NK cells were comparable with immunostimulator IL-2. Thus these effects can operate via similar transduction points in both brain cells and immunocytes. Supported by grants MSM0021620849, GACR 524/05/0267 and MSM 6215712403.

347 D229

SSRI-TYPE ANTIDEPRESSANT MEDICATION MAY COMPROMISE SEROTONERGIC CELL FUNCTION IN SELECTED RAPHE NUCLEI

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Background: Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for treatment of depression, anxiety, panic, obsessive-compulsive and impulse-control disorders. It is well established that SSRI medications inhibit serotonin reuptake via blockade of serotonin transporters. Neither the onset of therapeutic properties nor the induction of persistent side effects, however, correlates with transporter blockade; this suggests a mechanism more complex than simple inhibition of serotonin reuptake. We studied the time course of changes in serotonergic neurons in four raphe nuclei after initiation of SSRI medication. Methods: Groups of Sprague-Dawley rats received daily meals of rice pudding either alone (N=9) or mixed with the SSRI citalopram 5 mg/kg/day (N=27). Rats were sacrificed at 24 hours, 7 days or 28 days after initial administration. Sections of caudal linear nucleus (CLN), median raphe nucleus (MR), dorsal raphe nucleus (DR) and raphe magnus (RM) nucleus were processed for tryptophan hydroxylase (TPH) immunohistochemistry. The number of TPH-positive cells was determined by blinded, manual counting. Results were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Tukey tests. Results: Citalopram induced a significant 41% (p=0.001) reduction in DR TPH-positive cell counts at 24 hours, 7 days (38%, p= 0.003) and 28 days (52%, p<0.001). Downregulation in the DR was paralleled by reductions in TPH-positive cell counts in the MR and RM. In the MR, citalopram caused significant reductions at 24 hours (26%, p=0.004), 7 days (16%, p=0.034) and 28 days (23%, p=0.012). Similarly, in the RM, citalopram induced a significant (45%, p=0.009) reduction of TPH-positive cell counts at 24 hours, 7 days (34%, p=0.009) and 28 days (43%, p=0.006). No significant differences between control and treatment groups were observed in the CLN at any of the time points that we studied. Conclusions: These results indicate that citalopram can induce a regionally specific downregulation of brainstem serotonergic neurons within 24 hours. This reduction in TPH-immunoreactive cell counts may play a role in mediating the therapeutic and toxic clinical effects of antidepressant use.

348 D230

5-HT REGULATES THE APLYSIA TRK-LIKE RECEPTOR (APTRKL) THROUGH TRAFFICKING OF THE RECEPTOR

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There is an important role for tyrosine kinases, especially Trk family of receptor tyrosine kinases (RTKs), in learning and memory. In Aplysia, previous study has shown that a Trk-like receptor, named ApTrkl, is required for 5-HT mediated activation of ERK and induction of LTF when 5-HT is applied to the cell soma (Ormond et al., 2004). However, the precise mechanism for the activation of ApTrkl by 5-HT is not clear. First, we examined the involvement of a neuropeptide sensorin, which was shown to be required for activation of ERK and LTF induced by 5-HT (Hu et al., 2004), on activating ApTrkl and in relation to 5-HT. Second, we overexpressed ApTrkl constructs and examined localization and activation of the receptor. We hypothesized that 5-HT would cause the trafficking of the receptor, possibly both internally and externally. Finally, we tested if the activation of ApTrkl is transactivation through 5-HT G-protein coupled receptors (GPCRs), using adenylyl cyclase (AC)-coupled 5-HT GPCRs inhibitor, methiothepin.

349 D231

ENKEPHALIN IS ALSO EXPRESSED IN GLUTAMATERGIC NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE AMYGDALOID COMPLEX

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We recently identified the enkephalin (ENK) innervation of the centromedial amygdala (Poulin et al, JCN 496 :859-876, 2006). We found that ENK fibers in the centromedial amygdala originated mainly from the bed nucleus of the stria terminalis (BST) and others amygdaloid nuclei. However, ENK could colocalize with GABA or glutamate in these structures and this information is essential to the understanding of the anxiolytic effect of ENK and its physiological relevance in adaptation to stress. This study aims at assessing the GABA and glutamate phenotype of ENK neurons of the BST and amygdaloid complex using double fluorescence in situ hybridization with combination of riboprobes specific for vesicular glutamate transporter 1 (VGLUT1) and 2 (VGLUT2), glutamic acid decarboxylase 65 (GAD65), and preproenkephalin (ppENK). We first looked at the distribution of these genes expression in the divisions of the BST and the amygdaloid complex. Although the majority of BST neurons are GABAergic, we observed an important population of neurons expressing VGLUT2 in the posterior BST, particularly in the principal, transverse and inter-fascilular nuclei. Our preliminary results indicate that the posterior BST contains two populations of ENK cells, one population expressing GAD65 and the other expressing VGLUT2 (principal, transverse and inter-fascilular nuclei). We did not observe any VGLUT2 expression in the anterior BST nuclei nor did we observe VGLUT1 expression in the whole BST. In the amygdaloid complex, the expression of VGLUT1 and VGLUT2 is mostly segregated in specific divisions. The expression of VGLUT1 was observed in the basolateral (BLA), lateral, as well as in the posterior cortical divisions of the amygdala. Expression of VGLUT2 was restricted to the medial (MEA), basomedial, and anterior cortical divisions (COAa). In the amygdala, more than 95% of ENKergic cells in the BLA expressed VGLUT1 whereas none of the ENKergic cells of the LA expressed this marker. In addition, there is a strong colocalization between ppENK and VGLUT2 in the posterior MEA, whereas most ENKergic cells in the COAa appear to express GAD65. These results have broad implications in the functional role of ENKergic neurotransmission in the BST and the amygdaloid complex.

350 D232

IDENTIFICATION OF TENEURIN C-TERMINUS ASSOCIATED PEPTIDE (TCAP)-1 RESPONSIVE REGIONS OF THE BRAIN AS DETERMINED BY IMMUNOREACTIVE C-FOS INDUCTION

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Recently, a new family of neuropeptides was discovered on the C-terminus of the teneurin type II transmembrane protein. Four peptides from each of the teneurins 1-4 were characterized and are called the teneurin C-terminus associated peptides (TCAP). TCAP shares about 20% amino acid identity to the corticotropin-releasing factor (CRF) family of peptides, and is extremely well conserved, suggesting an important role in stress and anxiety. Behavioural studies indicate that acute intracerebroventricular (ICV) TCAP-1 modulates anxiety behaviour as measured by acoustic startle. However, the specific sites of action of TCAP are still unknown. Therefore, the level of induced c-Fos protein expression was measured by immunohistochemistry to determine areas of the brain that respond to central TCAP-1 administration. Male Wistar rats were ICV injected with either saline, TCAP-1, CRF, or co-injected with both TCAP-1 and CRF. Our preliminary results indicate that TCAP may have a modulatory effect on CRF-induced c-Fos expression. This suggests that the TCAP family of peptides may be a novel system that is an important regulator of CRF action in the brain.

HOMEOSTATIC & NEUROENDOCRINE SYSTEMS

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ANTI-OBESITY EFFECT OF A NEUTRAL CB1 RECEPTOR ANTAGONIST.

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Cannabinoid 1 (CB1) receptor inverse agonists/antagonists potently inhibit food intake in animals and humans, and potentiate emesis in animals. It is not clear whether these effects result from inverse agonist properties or from the blockade of endogenous cannabinoid signaling. Here, we examine the effect of a neutral CB1 antagonist, AM4113, on food intake and emesis. Neutral antagonist properties were established in HEK293 cells transfected with human CB1 or CB2 receptors. AM4113 had no effect on forskolin stimulated cAMP production at concentrations up to 630 nM. The Ki value of AM4113 (0.89±0.44nM) was established in competitive binding assays with the CB1/2 agonist [3H]CP55,940. AM4113 was 100-fold more selective for CB1 over CB2 receptors. We confirmed that AM4113 antagonized CB1 receptors in vivo by blocking hypothermia induced by CP55,940 (0.3 mg kg-1; i.p., n=5 per group). Food intake was significantly reduced in rats (n=5 per group) treated with AM4113 (10;20 mg kg-1) within 1.5 h of administration compared with vehicle treated control rats (mean difference from vehicle; -14.3;-15.0 g), $p<0.05$. Reductions in food intake by AM4113 continued to be significant for 3 d after a single treatment, $p<0.05$. Reductions in body weight gain were significant for at least 5 d (-22.5;-34.8 g), $p<0.05$. The effect of chronic treatment with AM4113 on food intake and weight gain in rat is currently being investigated. The pro-emetic effect of AM4113 was examined in ferret. AM4113 (5-20 mg kg-1) did not significantly increase vomiting induced by the emetic (M6G; 0.05 mg kg-1 s.c.) compared with vehicle treated ferrets. We show that a centrally active neutral CB1 receptor antagonist shares the appetite suppressant and weight loss effect of inverse

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

agonist/antagonists. These data suggest that neutral CB1 receptor antagonists could be developed into a new class of anti-obesity agents if they display similar properties in humans.

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SEX DIFFERENCES IN NEURONAL EXPRESSION OF DYNORPHIN AND NEUROKININ B IN THE CONTROL OF GONADOTROPIN-RELEASING HORMONE SECRETION IN THE SHEEP

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The secretion of gonadotropin-releasing hormone (GnRH) into the pituitary portal blood supply is the final common pathway responsible for neuroendocrine control of reproduction. Prenatal exposure to androgens causes sexual differentiation of the GnRH system, including its responsiveness to the negative feedback influence of progesterone. A number of neuropeptides, including endogenous opioid peptide, dynorphin (DYN), and neurokinin B (NKB) have been implicated in mediating these feedback effects. Our recent studies in ewe have identified a subset of neurons in the arcuate nucleus (ARN) of the hypothalamus that co-express DYN and NKB. We hypothesize that sex differences in progesterone negative feedback are due to dimorphism of this neural circuitry. In the present study, we tested this hypothesis by using dual-label immunocytochemistry to compare DYN-positive cells, and their co-expression of nuclear progesterone receptors (PR), in the hypothalami of normal adult rams (n = 4) and ewes (n = 3), and adult ewes that were exposed to either testosterone (T, n = 4), dihydrotestosterone (DHT, n = 3) or control (n = 5) treatments during days 30 to 90 of gestation. In addition, we examined expression of NKB and its colocalization with PR, since NKB in the ARN has been previously shown to be sexually dimorphic. Preliminary data from normal sheep revealed significant reductions in the number of DYN- and NKB-positive cells (mean 72.1% and 86.2%, respectively) in ARN of males compared to females. A very high degree (> 95%) of colocalization of DYN and NKB with PR was observed in the ARN of both males and females. Prenatal exposure to androgens also resulted in significant reductions in the number of DYN- and NKB-positive cells in the ARN of both T- and DHT-treated animals compared to control ewes. Sex and prenatal androgen-induced differences in peptide cell number were regionally limited to the middle and caudal divisions of the ARN, sites of the majority of DYN and NKB cells. In summary, neuronal expression of both DYN and NKB is sexually dimorphic in the ARN of the adult sheep, and is likely due to the organizational influence of prenatal androgens. Differences in DYN and NKB cell number between male and female hypothalami is consistent with sex differences in responsiveness to progesterone negative feedback, and provide further support for the role of this neuronal subpopulation in controlling pulsatile GnRH secretion. The ability of both T and DHT to decrease the number of DYN/NKB cells suggests that differentiation of this circuitry is due to exposure to prenatal androgens and not estrogen (Supported by HD39916 to MNL and NIH HD41098 to VP).

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ALTERATIONS IN PVN PARVOCELLULAR NEURONS RESPONSES TO NPY AND MELANOCORTINS IN AN INBRED RAT MODEL SUSCEPTIBLE TO DIET-INDUCED OBESITY (DIO)

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Neurons of the mediobasal hypothalamus are intimately involved in energy homeostasis. While considerable progress has been made in understanding obesity caused by genetic disorders, the complex CNS pathways that regulate energy balance, and which are vulnerable to diet-based perturbations, remain poorly understood,.as do the mechanisms underlying the changes in the brain resulting in diet-induced obesity. The parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) are one of the primary targets of energy balance-related (i.e., leptin-responsive) arcuate nucleus (ARC) neurons. ARC projections release orexigenic (NPY, AgRP) and anorexic (alpha-MSH, CART) peptides, which we have shown to inhibit and potentiate, respectively, GABAergic inputs onto parvocellular neurons. To begin to test the hypothesis that diet-induced obesity (DIO) is accompanied by changes in the response of PVN synapses to energy balance-related peptides, we examined responses to different agonists of synaptic inputs in PVN slices from inbred DIO rats maintained on a normal chow diet. PVN neurons (neurosecretory and preautonomic subpopulations) in inbred DIO rats expressed electrophysiological properties (membrane potential, input resistance, firing responses) similar to those in outbred Sprague-Dawley rats. However, their sensitivity to both orexigenic (NPY) and anorexigenic (MTII) decreased significantly for neurosecretory (NPY: -29.4 vs. -55.8 %, MTII 34.5 vs. 71.9 %), but not preautonomic cells. In addition, GABA responses on 25 % of neurons displayed a qualitative change in their response to MTII, revealing small but reversible inhibition (-23.6 %). These changes were observed in animals taken prior to any significant change in body weight. Our data are consistent with a reduction in melanocortin signalling even prior to the onset of obesity in a vulnerable population of animals, and may indicate one mechanism of predisposition to obesity. Supported by CIHR operating grants OHN 63278 and MT 10520. WFC is a Medical Scientist of the Alberta Heritage Foundation for Medical Research.

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HOMER-1 IN THE NUCLEUS ACCUMBENS IS INVOLVED IN SEXUAL BEHAVIOR OF MALE RATS

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Repeated exposure to drugs of abuse induces transcriptional and structural alterations in the mesocorticolimbic system that may contribute to the development of drug addiction. However, it is largely unknown whether natural rewarding stimuli cause similar changes in the brain. In animal models, sexual behavior, and in particular ejaculation, is reinforcing and highly rewarding, but does not lead to compulsive behavior, in contrast to drugs of abuse. Therefore, our lab recently set out to study sex-induced transcriptional and structural changes in male rats. These experiments confirm that sexual behavior induces such alterations within the mesocorticolimbic system. One of the most pronounced sex-induced transcriptional changes within the nucleus accumbens (NAcc) is an up-regulation of Homer-1 (+200-420%), a scaffolding protein located in the postsynaptic density that has been implicated in the regulation of signal transduction, synaptogenesis, receptor trafficking, and regulation and maintenance of extracellular glutamate levels. There are two main splice variants of Homer-1, the immediate early gene (IEG)-like Homer-1a (~25kDa) and the constitutively expressed Homer-1b/c (45-47kDa), which compete for the same binding sites and have opposing functions. Using real time PCR, we here confirmed that both acute sexual activity and sexual experience induced an up-regulation of Homer-1b/c. Next, we studied the functional significance of that up-regulation using antisense oligonucleotides (AS) to Homer-1 to down-regulate both Homer-1a and 1b/c proteins within the NAcc. We used two different Homer-1 AS sequences, which previously have been shown to down-regulate Homer-1 protein by ~35% (Ghasemzadeh M.B. et al. 2003). Results show that continuous infusion of Homer-1 AS into the NAcc of male Sprague Dawley rats for 9-11 days severely inhibited sexual behavior. Specifically, a lower percentage of the AS animals displayed mounts, intromissions, and ejaculation as compared to control animals. Moreover, latencies to mount, intromission, and ejaculation were longer in

the AS animals as compared to control animals. In contrast, novelty-induced locomotor activity was not affected by antisense treatment, indicating no effect on general activity. These findings suggest that Homer-1 in the NAcc is essential for the initiation of sexual behavior in male rats, possibly via its role in regulating proper glutamatergic input to the NAcc. Supported by NIH DA14591 to LMC.

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INDIVIDUAL DIFFERENCES IN ANXIETY-LIKE BEHAVIOUR ARE REVERSED BY VARIATIONS IN MATERNAL CARE

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Natural variations in maternal care influence physiological responses to stress and emotional events. Previously we have shown that the adult male offspring of Low Licking and Grooming (LG) mothers show increased anxiety levels when tested for time spent in the center of a novel field. In this study we investigated the effect of maternal rearing on novelty induced anxiety in cross fostered animals to determine whether maternal care can reverse/alter both a behavioral phenotype in later life. Partial litters of High LG or Low LG dams were cross fostered on post natal day 0 to dams of the alternate phenotype or the same phenotype while un-fostered littermates were used as control subjects. The results indicate that it is the level of maternal LG demonstrated by the dam which predicts center time in the open field test in adult male offspring. Biological offspring of High LG dams reared by Low LG mothers were significantly more fearful in the novel field compared to both offspring of High LG mothers reared by a foster High LG mother, and the High LG offspring control group. The reciprocal was true for biological offspring of Low LG dams reared by High LG mothers. These results suggest that maternal care is a fundamental determinant of anxiety which can be reversed by cross fostering in early life.

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DISSECTING ANXIETY IN THE VERVET MONKEY: A SEARCH FOR ASSOCIATION BETWEEN POLYMORPHISMS IN THE CRH AND NPY GENES AND ANXIOUS BEHAVIOR

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The implication of corticotropin- releasing hormone (CRH) and neuropeptide Y (NPY) in the pathophysiology of anxiety and anxiety-related disorders is well established. Stress response and anxiety are mediated, at least in part, by CRH, a regulator of the hypothalamic-pituitary-adrenal pathway. Biological functions of NPY include the regulation of the stress response (by regulating the release of hypothalamic hormones including CRH), appetitive behaviors and emotional behavior. The objective of this study is to explore the genetic variations in CRH and NPY genes in a well-documented behavioral animal model, the vervet monkey (Chlorocebus aethiops sabaeus), in order to uncover a possible association between these polymorphisms and anxious behavior. The anxious behavior is assessed through a behavioral phenotype, based on observational and quantitative evaluation of the reactivity of 4 birth cohorts of vervet monkeys (n=167), during late infancy and adolescence, in both social and novel settings. The vervet CRH and NPY genes have been amplified and sequenced; the priority was given to the regions expanding from -1kb upstream of the transcription initiation site through the second exon. The sequences cover the promoter fragments where most of the regulatory elements are found in both genes and the regions containing polymorphisms that have been associated with anxious traits in both humans and non-human primates. Polymorphism discovery analysis (using NovoSNP and Phred Phrap) revealed the presence of a deletion and an insertion in the promoter of CRH, 8 SNPs in the CRH gene and 9 SNPs in the NPY gene; the SNPs are relatively evenly distributed in the regions covered. Three haplotype blocks were detected, one CRH block capturing 4 CRH SNPs and 2 NPY blocks, each involving two NPY SNPs. An association between one intronic NPY SNP and the behavioral response to novelty "defensive aggression" was detected.

These results are coherent with other reports implicating NPY in defensive aggressive behavior, and support the notion that fear responses are fundamental behavioral traits for the dissection of anxiety.

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ACTIVATION OF ESTROGEN RECEPTOR β IN PRIMARY RAT HYPOTHALAMIC NEURONS INCREASES NITRIC OXIDE PRODUCTION THROUGH nNOS PHOSPHORYLATION

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We have recently shown in vivo that nitric oxide (NO) in the paraventricular nucleus (PVN) of the hypothalamus mediates estrogen's effects on blood pressure responses to L-glutamate stimulation. We have also shown that estrogen modulates the expression of neuronal nitric oxide synthase (nNOS) in the PVN of hypothalamic slice cultures and increases NO production from neuroblastoma cells. Although NO is an important mediator of estrogen actions in neurons of the PVN, little is known about the mechanism(s) of estrogen's effects on NO. Furthermore, because we have shown that estrogen acts through estrogen receptor β (ERβ) to affect the NO system in the PVN, we were interested in identifying the signaling mechanisms activated by ERβ to affect NO in these cells. nNOS catalyzes NO production in neurons and it has been shown that phosphorylation of nNOSser847 or nNOSser1416 leads to decreases or increases in nNOS activity, respectively. Therefore, we tested the hypothesis that activation of ERβ in neurons increases NO production by increasing nNOS activity through the modulation of nNOSser847 and nNOSser1416 phosphorylation. Primary hypothalamic neurons from E17 rats, grown for 8-9 days in vitro, were treated for 5 minutes with the selective ERβ agonist, diarylpropionitrile (DPN, 10 -1000 nM). NO production was then measured using the 2,3- diaminonaphthalene nitrite assay and changes in nNOS phosphorylation were determined by western blot analysis. Using immunofluorescence we found that at least 90% of cultured hypothalamic neurons expressed ERβ and that more than 50% of neurons expressed nNOS. DPN (10 nM) increased NO production by 38%, decreased nNOSser847 phosphorylation by 38%, and increased NOSser1416 phosphorylation by 56%. Together, these results suggest that activation of ERβ stimulates nNOS activity. Finally, because estrogen has been shown to activate the steroid receptor co-activator (Src) pathway in endothelial cells, we determined whether stimulation of ERβ in neurons activates Src. We found that DPN (10 nM) increased SrcTyr416 phosphorylation by 64%, suggesting that the Src signaling pathway is activated by ERβ stimulation in neurons. Our findings suggest that activation of ERβ in hypothalamic neurons increases NO production through phosphorylation of nNOS and that activation of the Src kinase pathway likely plays a role in this effect.

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SUSTAINED SODIUM DEPLETION INDUCES A REVERSIBLE PLASTICITY OF THE MU-OR RESPONSE IN THE MEDIAN PREOPTIC NUCLEUS

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Salt appetite is a behavioral response to sodium (Na+) loss in the organism and there is growing evidences that the opioid system seems to play a significant role in the motivated aspect of salt intake. Since the median preoptic nucleus (MnPO) appears as an essential interface between central Na+ detection and induction of Na+ intake, the goal of the present study was

to determine whether the excitability of the MnPO neurons was modulated by the μ opioid receptor agonist DAMGO and whether the DAMGO-mediated response(s) was modified by a sustained Na+ depletion, which generates salt appetite. The experimental model of sustained Na+ depletion consisted in two subcutaneous injections of furosemide (10 mg/kg) followed by a free Na+ diet (0.03% NaCl) for 20h and ad libidum access to clear water. The electrophysiological recordings were performed with the patch-clamp technique. Our results indicate that DAMGO triggered two distinct responses in the MnPO neurons. (1) In 25 % of the neurons, DAMGO reduced the amplitude of EPSCs evoked by electrical stimulation of the SFO by 52 ± 13%. This inhibitory effect was mediated by presynaptic μOR. The DAMGO-mediated reduction in glutamatergic transmission was not affected by the sustained Na+ depletion. (2) In 41% of the neurons, DAMGO triggered a membrane hyperpolarization (11.4 ± 0.6 mV) that was mediated by postsynaptic μOR. Interestingly, sustained Na+ depletion extended the neuronal population hyperpolarized by DAMGO to 60% without affecting the amplitude of the hyperpolarization. The population sensitive to DAMGO returned to 39% 24h after Na+ repletion. It is worth noting that 70% of the neurons hyperpolarized by DAMGO under the two Na+ conditions were identified as Na+-sensing neurons. The present study indicates that the excitability of MnPO neurons including a majority of Na+-sensors is reduced by enkephalin peptides. Together with hyponatremia-induced hyperpolarization of the Na+-sensors, our results highlight the fact that humoral and neural sensory informations signaling for hyponatremia are coherently integrated within the MnPO. This study suggests that enkephalin peptides may play an important role not only in the motivated aspect of salt intake, but also in the regulation of central detection/integration of Na+ changes. Source of Research Fund: Supported by CIHR and Heart and Stroke Foundation (Québec). Didier Mouginot is a FRSQ scholar.

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NOCICEPTIN/ORPHANIN FQ INDUCES IRREVERSIBLE ACTIVATION OF GIRK CHANNELS IN OREXIN NEURONS

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Nociceptin/orphanin FQ (N/OFQ) is an opioid-like peptide known to act as a functional “anti-opioid” because it opposes the effects of opioids on the activity of the mesolimbic pathway as well as nociception. Orexin neurons located exclusively in the lateral hypothalamus/perifornical area (LH/PFA) have known projections to the ventral tegmental area (VTA) where they exhibit an excitatory effect on dopamine neuron activity and subsequent dopamine release in the nucleus accumbens and prefrontal cortex. As it has been shown that the N/OFQ peptide (NOP) receptor is expressed in the LH/PFA, we hypothesized that the inhibitory action of N/OFQ on the mesolimbic pathway is at least partially due to inhibition of orexin neurons. To test this, whole-cell patch clamp recordings were preformed on orexin neurons using acute hypothalamic slices obtained from male SD rats. We found that 2-5 min application of N/OFQ (0.1 – 1microM) induced an irreversible, sustained outward current that lasted up to 80 min. N/OFQ effect was blocked NOP receptor antagonist UFP101, but not the broad spectrum opioid receptor antagonist naloxone. However, once the effect was induced, UFP101 was unable to reverse the effect. Therefore, the induction of the effect required N/OFQ binding to NOP receptors but the maintenance was independent of ligand binding. The N/OFQ-induced current reversed at the potassium reversal potential, and was sensitive to Ba2+. Furthermore, the effect was abolished when GDPbetaS was included in the internal solution. These results suggest that N/OFQ irreversibly activates GIRK channels in orexin neurons. Ba2+ had no effect on its own, indicating that there is no constitutive activity of GIRK channels in basal condition. In current clamp mode, N/OFQ induces a persistent hyperpolarization and cessation of firing activity. In conclusion, our study demonstrates that N/OFQ causes a tonic suppression of orexin neurons. This may in turn reduce the excitatory influence of orexins on the VTA and tone down the activity of the reward circuit. Supported by CIHR and NSERC.

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NESFATIN-1 MODULATES THE EXCITABILITY OF NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

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Nesfatin-1 is a newly discovered satiety molecule produced in the appetite control centers of the hypothalamus including the lateral hypothalamic area, the arcuate, the supraoptic and the paraventricular nucleus (PVN). Through an unidentified mechanism acute intracerebroventricular injections of nesfatin-1 dose dependently inhibit feeding while chronic injections reduce body weight. Using acutely prepared hypothalamic slices we have investigated the effects of nesfatin-1 administration on the membrane properties of PVN neurons through current-clamp recordings. The majority of neurons examined hyperpolarized in response to 10 nM nesfatin-1 (-7.6 ± 1.4 mV, 13/21) while others depolarized (6.3 ± 1.0 mV, 3/21) or did not respond (5/21). Additionally neurons which hyperpolarized showed one of two response profiles: a continuous hyperpolarization which reaches a maximum and then recovers (8/13) or the induction of a slow wave oscillation in the membrane potential (5/13). These data are the first to demonstrate the effects of nesfatin-1 on the excitability of PVN neurons and thus further highlight the PVN as a potential target where nesfatin-1 exerts its effects on feeding behavior.

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ACUTE STARVATION ALTERS THE FEVER RESPONSE TO LIPOPOLYSACCHARIDE IN RATS

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Insufficient energy supply, such as that experienced under starvation, can cause a number of physiological changes including suppression of immunity and decrease in thermogeneis exemplified by a reduction in fever response to exogenous pathogens. The underlying mechanisms regulating this alteration of fever are not clear and whether the mediators of fever (cytokines) are involved has not been investigated in depth. The aims of the present study therefore were 1) to confirm that starvation attenuates the fever response to LPS, an exogenous pyrogen and a potent activator of cytokines, 2) to characterize the plasma cytokine levels following LPS injection, 3) to investigate the expression levels of brain IL-1β and COX-2, important regulators of the central response in fever generation. Adult female rats were fasted for 48 h following which they received a single intraperitoneal injection of LPS (100 μg/kg) or sterile saline. Over the period of fasting (48h) the baseline body temperatures was lower in fasted rats as compared to the ad lib fed group (36.5 ± 0.3 vs. 37.4 ± 0.1 C°). Saline injection caused a transient stress-induced hyperthermia in both groups reaching a similar peak temperature (37.6 ± 0.4 vs. 37.8 ± 0.3 C°) indicating that the thermogenic ability is preserved in the fasted animals. Both fasted and fed rats responded to LPS with an initial hypothermia followed by an increase in body temperature. However, the fasted animals showed bigger hypothermia (36.1 ± 0.2 vs. 36.7 ± 0.2 C°) and smaller fever (38.2 ± 0.2 vs. 38.9 ± 0.2 C°) as compared with the fed group. In a separate study, plasma cytokine levels and brain gene expressions were measured 2 h after injection of LPS or saline. Both fasted and fed animals responded to LPS with a dramatic increase in the plasma levels of TNFα and IL-6 as compared with the saline-treated group. The fasted animals however had significantly lower TNFα levels than the fed group (1.6 ± 0.6 vs. 3.8 ± 1.2 ng/ml), whereas interestingly, IL-6 levels were similar in both fasted and fed rats (4.1 ± 0.9 vs. 3.1 ± 0.5 ng/ml). In the brain, LPS increased the expression of IL-1β and COX-2 mRNAs to a similar magnitude in both fasted and fed animals. These results demonstrate that acute starvation attenuates the fever response in rats and that this alteration is accompanied with attenuated

production of TNFα but not IL-6. Surprisingly, given the attenuated fever, neither COX-2 nor IL-1β expressions was affected by fasting.

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THE EFFECT OF PACED COPULATION AND STRAIN CONDITIONING ON THE DEVELOPMENT OF PARTNER PREFERENCE IN MALE RATS

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Although male rats are believed to show greater sexual arousal and mating preference for a novel female compared to a familiar one, we have shown that after repeated copulation to ejaculation with a female bearing a neutral odor in pacing chambers bisected by a 1-hole divider, male rats display a conditioned ejaculatory preference for a female bearing the odor relative to a female not bearing the odor. The aim of the present study was to examine if males might develop an ejaculatory preference for a familiar female of the same or different strain after repeated copulation with the same female in a pacing chamber bisected by either a 1-hole or 4-hole divider. In this experiment, male Long-Evans rats were given 10 copulatory trials with the same Long Evans or Wistar female in either the 1-hole or 4-hole condition. Copulatory preferences were then examined in an open field where the males had the choice to copulate with either the familiar female or a novel female of the same or other strain. Results indicated that males trained in the 1-hole condition with the same Long Evans female displayed a conditioned ejaculatory preference for the familiar vs. novel female. However, males trained in the 1-hole condition with the same Wistar female at every trial copulated indiscriminately with the familiar and novel females. No preference was detected in males trained in the 4-hole condition. These findings suggest that males display an ejaculatory preference for strain cues if the familiar female is of their own strain following training in a 1-hole paced copulation condition. Supported by CIHR to JGP.

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SEXUAL BEHAVIOR IN AGED ACYCLIC FEMALE RATS

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Normally cycling female rats display a cell cycle pattern of estrus, proestrus, metestrus and diestrus, occuring over 4-5 days, as determined by vaginal smears, and are receptive during the proestrus and estrus phases. As the female ages, the cycles become irregular, eventually leading to persistent estrus (PE) and finally anestrus. Many of the neuroendocrine fluctuations that occur during this transition have been studied, however these changes have not been fully investigated with respect to sexual behavior. The present study observed the vaginal smears of nineteen acyclic female Wistar rats (age >15 months) over a 15-day period to confirm irregular cyclicity, and females were subsequently subjected to sexual behavioral testing in bi-level chambers with sexually experienced Long-Evans males. 84% of the females had estrus or proestrus vaginal cytology on the test day. Of those females, 94% expressed defensive behaviors, 68% received a mount, 68% performed olfactory investigations on the male, 63% expressed level changes, 53% displayed hops and darts, 44% showed aggressive behaviors and 38% showed a lordosis of at least magnitude 1. None of the females received an ejaculation from the male, presumably because of their defensive and rejection responses. This pattern of data is strikingly similar to what is found in female rats during estrus termination, in which females drop out of heat following repeated vaginocervical stimulation. These findings suggest a similar inhibitory mechanism may be involved in the two states and leads to reduction or absence of female appetitive and consummatory female sexual behaviors. Furthermore, these results suggest that decreased sexual responsiveness occurs prior to changes in vaginal cell morphology in response to irregular ovarian hormone output. Supported by CIHR and NSERC to JGP.

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DEVELOPMENT OF THE ADRENOCORTICAL STRESS RESPONSE IN ARTIFICIALLY REARED RAT PUPS

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Prolonged maternal separation has been consistently shown to have long-term consequences on the neural and behavioural development of infant rats. Studies suggest that these effects are mediated through the sensitization of the developing stress response system. Complete deprivation of maternal care, by way of artificial rearing, leads to comparable neural and behavioural changes in later-life, but little is known about the effects of this procedure on the development of the stress response system. To investigate the adrenocortical response to stress during artificial rearing of rat pups, basal and stress induced levels of the hormone corticosterone (CORT) were measured on post-natal day (PND) 6 and 12. Two types of stressors were used: an intraperitoneal injection of saline on PND 6 and 3 hrs of food deprivation on PND 12. To assess the physiological impact of artificial rearing and exposure to stressors, blood glucose was also measured. In PND 6 pups, there were no differences between basal and injection stress induced levels of either CORT or glucose. In PND 12 pups, food deprivation stress resulted in an increase in CORT levels in both artificially and maternally reared groups, and a corresponding decrease in glucose levels. Surprisingly, there were no differences between artificially and maternally reared rats in basal or stress induced levels of CORT or glucose on either PND. These findings suggest that, unlike maternal separation, artificial rearing does not lead to a sensitization of the adrenocortical stress response in rat pups. Thus, the effects of artificial rearing on neural development and later-life behavioural functioning may be mediated through other mechanisms.

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SIMULATION OF VASOPRESSIN RELEASE IN A QUANTITATIVE MODEL OF HYDROMINERAL HOMEOSTASIS

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Vasopressin is the antidiuretic hormone that is released in the general blood circulation when plasma tonicity is elevated. The release of vasopressin from axon terminals of magnocellular neurons (MCNs) is highly regulated and is achieved during depolarization of the MCNs. The change in MCN excitability is mediated by the intrinsic property of the MCNs (osmoreceptors) and by their integration of synaptic signals as they belong to a hydromineral neuronal network. In the context of creating a computational model of that hypothalamic neuronal network controlling vasopressin release, the goal of the present study was to design a physiologically realistic test-bed that mimicked the MCNs and kidney dynamic in response to a plasma osmotic challenge. This model is implemented in Scicos, a module of the Scilab software. Currently, the model is at a high level and contains three distinct interconnected objects : the “plasma”, the “kidneys” and the “hypothalamic hydromineral network”. Those objects are modeled with particular mathematical functions that have been implemented with physiological parameters collected from a thorough analysis of the appropriate literature. The test-bed functions as following: the object “plasma” contains three distinct informations: the plasma volume (ml), the plasma vasopressin (pg) and the plasma Na+ (mmol). All these parameters represent the chemosensory signals. The object “hypothalamic hydromineral network” represents the integrator and it dynamically adjusts the secretion of vasopressin after having analyzed the chemosensory signals. It also activates a drinking response when needed. Finally, the object “kidneys” represents the effector by adjusting urine volume and Na+ concentration in order to restore the plasma to the osmotic set-point. Despite its apparent simplicity, the presented model works within the biological limits to mimic the dynamic changes in the plasma, vasopressin release and urine content occurring during a hyperosmotic challenge. This model represents therefore a realistic test-bed for a computational model of the hydromineral network.

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IMPACT OF GUT MICROFLORA ON LEARNING, BEHAVIOUR AND CENTRAL NERVOUS SYSTEM GENE EXPRESSION

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Germ-free mice have no intestinal microflora and thus exhibit an undeveloped immune system. Previous work has demonstrated that in conjunction with these gastrointestinal and immune deficits, germ-free mice exhibit an altered central nervous system (CNS). Specifically, when compared to conventional animals with normal gut flora, germ-free mice demonstrate an altered hypothalamic-pituitary-adrenal (HPA) stress axis, with increased corticosterone and adrenocorticotropin hormone (ACTH) released in response to restraint stress. Additionally, they show decreased hippocampal protein levels of brain derived neurotrophic factor (BDNF), and decreased NMDA receptor subunit NR-2A mRNA levels. To date, no studies examining the behavioural phenotype of the germ-free mouse have been conducted. As both BDNF and NR-2A are molecules key to hippocampal-dependent learning and plasticity, we hypothesized that the germ-free animals would show learning deficits specific to the hippocampus. Our results demonstrate that unstressed germ-free mice show basal deficits in contextual learning when compared to conventional animals. In addition, they exhibit basal learning deficits in the elevated plus maze (EPM). Through in situ hybridization experiments we are currently examining the mRNA levels of key molecules in hippocampal-dependent learning and HPA axis functioning. Our future work aims to examine the impact of specific immune deficits on the development and function of the CNS.

367 C307

NEUROPEPTIDE W HAS COMPLEX EFFECTS ON THE MEMBRANE POTENTIAL OF ARCUATE NUCLEUS SOMATOSTATIN NEURONS

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Somatostatin neurons in the hypothalamic arcuate nucleus help to regulate the release of growth hormone. Intracerebroventricular administration of the peptide neuropeptide W (NPW) results in lowered plasma growth hormone but has no direct effect on growth hormone release from somatotropes. Therefore using a combination of whole cell patch clamp recording from hypothalamic brain slices containing the arcuate nucleus and single-cell reverse transcriptase polymerase chain reaction we have characterized the response of arcuate somatostatin neurons to NPW. In current clamp recordings neurons mainly depolarized when exposed to nanomolar concentrations of NPW. However, some neurons hyperpolarized or showed a mixed depolarization-hyperpolarization. When arcuate neurons were pre-treated with the voltage-gated sodium channel blocker TTX, the depolarizing effect of NPW was largely inhibited while the slowly developing hyperpolarization was preserved. Likewise in the presence of the ionotropic glutamate receptor antagonists APV and DNQX depolarizing responses were inhibited. Therefore somatostatin neurons in the arcuate nucleus are mainly depolarized by NPW, through a mechanism that involves the elevation of glutamatergic synaptic drive onto the neurons. In addition somatostain neurons are directly hyperpolarized by NPW.

368 C308

IL-6 AND LEPTIN DIFFERENTIALLY ACTIVATE STAT3 AND THE COX2-MPGES-PATHWAY IN THE BRAIN DURING LPS-INDUCED SYSTEMIC INFLAMMATION

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Systemic inflammatory insults result in CNS mediated sickness like symptoms including fever and anorexia. Signaling pathways regulating these responses involve a multitude of immune mediators belonging largely to the 'cytokine' family. Among those, IL-6 plays a major role. Recently, leptin, an adipose-derived hormone known to regulate appetite and energy balance, has also emerged as a mediator of inflammation. To address the relative contribution of IL-6 and leptin as mediators in immune-to-brain communication, the activation of brain cells during lipopolysaccharide (LPS)-induced systemic inflammation was assessed. In order to differentiate between activities resulting from the action of IL-6 from that of leptin, both of which signal through STAT3, IL-6 knockout (IL-6KO) mice were used. Wild type and IL-6KO mice were injected with a moderate dose of LPS (50 μg/kg, i.p.) and STAT3 activation in the brain assessed by immunohistochemistry. The majority of STAT3 activation located primarily in endothelial cells throughout the brain appeared to be IL-6 mediated. Using RT-PCR we show that LPS-induced upregulation of mRNA expression of the suppressor of cytokine signaling 3 (SOCS3), a marker of STAT3 activation was significantly reduced but not abolished in the hypothalami of KO animals. This was accompanied by a reduction in cyclooxygenase (COX)2 and microsomal prostaglandin E synthase (mPGES), the rate limiting enzymes for synthesis of PGE2, an important mediator in brain derived sickness responses. In these mice we observed LPS-induced STAT3-activation in the organum vasculosum of the lamina terminalis, the supraoptic nucleus, the raphe pallidus nucleus and in meningial cells and distinct large blood vessels throughout the brain. The LPS induced response in these specific brain structures was drastically reduced in the KO animals in the presence of a mouse leptin-antiserum, which also significantly inhibited the upregulation of mRNA expression of SOCS3 and COX2 but interestingly not mPGES in the hypothalamus. In conclusion, these observations confirm that the role of IL-6 in immune-to-brain communication in LPS-induced systemic inflammation involves the activation of the STAT3-COX2-mPGES-pathway in the brain. The contribution of leptin to this response appears to be limited to distinct areas of the brain/cell types and does not appear to involve mPGES induction.

369 C309

CELL SURFACE REMODELING AND THE MEMBRANE SKELETON IN OSMOTICALLY PERTURBED MAMMALIAN NEURONS

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Neuronal surface area regulation (SAR) is the homeostatic process by which neurons reorganize their plasma membrane during swelling and shrinking. Membrane recruited to the surface during swelling is retrieved during shrinking, generating transient vacuole–like dilations (VLDs) at the adherent surface. Previous studies in molluscan neurons show that VLD formation and VLD recovery is characterized by the easily detectable reorganization of the actin-spectrin membrane skeleton around VLDs. Here we extend these studies on cell surface remodeling to mammalian neurons. In PC12 neurons VLD formation and recovery was associated with dynamic changes in F-actin. On collagen the half-time for VLD recovery was 3 min

versus 20 min on a more adhesive substrate, poly-L-ornithine. In cells treated with reagents that interfere with actin and myosin (cytochalasin B, latrunculin A, and N-ethylmaleimide), F-actin did not associate with VLD membranes and VLD recovery was delayed or blocked. In PC12 neurons spectrin is also associated with VLD membranes. Reagents that interfere with spectrin remodeling (calpeptin, MDL-28170) did not prevent the F-actin/VLD association, but did impair VLD recovery. Oxygen-glucose deprivation did not prevent VLD recovery in PC12 neurons or in hippocampal neurons. In PC12 neurons subjected to severe ATP depletion, F-actin associated with VLD membranes but was non-contractile and VLD recovery was blocked. Recovery resumed upon restoration of energy metabolism.

These results indicate that mammalian neurons demonstrate robust SAR and that VLD formation and VLD recovery are differentially affected by agents that disrupt the membrane skeleton. VLD formation is resistant to severe ATP depletion, myosin inhibition and depolymerization of F-actin. In contrast to VLD recovery is sensitive to ATP depletion, disruption of actin polymerization, disruption of existing F-actin pools and inhibition of myosin ATPase.

370 C310

DAYTIME SLEEPINESS IS IT RELATED TO THE PREMENSTRUAL SYNDROME IN STUDENTS?

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Diurnal somnolence is a frequent phenomenon in the young people. It could have an impact on the cognitive performances in the university students. Girls were reported to present more daytime sleepiness than boys. The premenstrual syndrome (PMS) is often accompanied by some psychic syndromes, which could be related to diurnal somnolence in the girls. Objectives of the study: to determine the relationship between the diurnal somnolence and the premenstrual syndrome in the girls. A sample of 407 students, including 172 of men and 235 of women, filled out the Epworth sleepiness scale (ESS). The girls filled out a questionnaire on the premenstrual syndrome (PMS). The sleepiness score of girls was more elevated than that of boys (p=0.0002). Girls also slept (p=0.0001) and woke up (p=0, 0001) earlier than boys and presented a longer night time sleep duration (p=0.038). The comparative analysis showed that the scores of the PMS presented a significant (P <0.008) relationship with the scores of sleepiness. Indeed, the ESS is higher in the girls who presented the extreme syndromes of the PMS (ESS= 10.26) in comparison with those who had less severe symptoms (ESS = 9.31, p=0.0406), or moderate (ESS = 9.05, p=0.0079). The sleep deprivation did not seem to explain the higher sleepiness scores in girls, since the preferred sleep duration is not higher in the girls. Conclusion: Diurnal somnolence in girls was related to the PMS. One of the perspectives of this study is to determine the attenuating factors of diurnal sleepiness.

OTHER

371 A401

LGI1 IS A NOVEL ANTAGONIST OF MYELIN-BASED GROWTH INHIBITORS

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The failure of damaged adult central nervous system neurons to regenerate can have devastating consequences, often resulting in permanent loss of sensory and motor function. Growth inhibitors present in myelin have been identified as one of the major factors preventing CNS axonal regeneration. Current evidence points to a multicomponent receptor complex comprised of p75NTR, NgR and Lingo-1 that responds to these myelin-associated inhibitors and transduces the inhibitory signal to the neuron. We have identified a protein, termed LGI1 that interacts with components of the myelin-associated inhibitor receptor complex and functions to antagonize the growth inhibitory effects of myelin. We will present data concerning our identification of LGI1 as a myelin-based growth inhibitor antagonist, and discuss avenues we are exploring to determine how LGI1 mediates this effect.

372 A402

THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS AS A REGULATOR OF TONIC DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS

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The shell of the nucleus accumbens (NacSh) is strongly innervated by dopamine (DA) neurons in the ventral tegmental area (VTA) and glutamate neurons in the paraventricular nucleus of the thalamus (PVT). The present in vivo voltammetry study examined if electrical stimulation of the PVT evoked DA release in the NacSh of urethane anesthetized rats. Stimulation of the PVT (40 Hz, 1.0 ms, 400 µA, 5s) resulted in a brief increase in the electrochemical current detected in the NacSh. Inhibition of dopamine uptake (GBR 12909, 10 mg/kg i.p.) increased the magnitude of the evoked response while inhibition of serotonin (clomipramine, 10 mg/kg i.p.) or norepinephrine uptake (desipramine, 10 mg/kg, i.p.) had no effect on the PVT-evoked responses. Blocking of ionotropic glutamate receptors in the NacSh with local administration of kynurenic acid (50 nM, 500 nl over 3 min) attenuated the PVT-evoked responses. In contrast, removal of the influence from DA neurons in the VTA with 4% lidocaine (500 nl injected in the medial forebrain bundle) or apomorphine (0.15 mg/kg; i.v.) had no effect. Stimulation of the PVT for a longer period (mins) at lower current intensity (10 Hz) produced an increase in dopamine oxidation current which was time-locked with the stimulation. This study suggests that glutamate release from PVT terminals can act on glutamate receptors in the NacSh to induce DA efflux. Since the PVT is activated by arousal and stress, we propose that the PVT modulates arousal state and tonic levels of DA in the NacSh.

373 A403

APOLIPOPROTEIN E GENOTYPE AND CONCUSSION IN VARSITY ATHLETES: A PROSPECTIVE COHORT STUDY

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Concussion is a common sport-related injury and it is hypothesized that possession of an epsilon-4 allele in the apolipoprotein E gene (APOEe4) may increase concussion risk. Understanding the association between APOEe4 and concussion would be useful for developing prevention strategies targeted to APOEe4 carriers. To determine the association between possession of an APOEe4 allele and concussion. A prospective cohort study was conducted from September 2002 to April 2006. Subjects included University of Toronto varsity athletes participating in sports deemed high risk for concussion. Full informed consent was obtained from 318 athletes with no concussion in the two years prior. Collected blood samples were genotyped and the presence of APOEe4 was described dichotomously. There was no loss to follow-up. Time to concussion was measured in two ways: athletic-exposures (number

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

of games and practices); and number of games played. Only the first concussion sustained by an individual was included in the analysis. Sport-medicine professionals who were present on the sidelines identified the concussions. To assess misclassification, athletes self-reported if they incurred a concussion that was missed by the sport-medicine professional. The unadjusted hazard for concussion was 18% greater in individuals with the APOEe4 allele than in those without (Hazard ratio (HR): 1.18 (95% confidence interval (CI): 0.52, 2.69)). Adjustment for sex, weight, height, and team type resulted in a HR of 1.06 (95% CI: 0.41, 2.72). The estimates including self-reported concussions were 0.96 (95% CI: 0.50, 1.85) for the unadjusted HR and 0.81 (95% CI:0.38, 1.73) for the adjusted HR. Similar results were obtained for the number of games played measure.
Our data indicate no statistically significant evidence for an association between the possession of an APOEe4 allele and concussion. Additional study is required before we can be certain there is no relationship between possession of APOEe4 and risk of concussion.

374 A404

INTRACEREBRAL HEMORRHAGE MODELS IN RAT: COMPARING COLLAGENASE TO BLOOD INFUSION

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The autologous whole blood and bacterial collagenase models of intracerebral hemorrhage (ICH) are routinely used to study the pathology of ICH and putative treatments. By directly comparing models, we aim to highlight differences between the models and limitations of each in experimental ICH studies. Thus, we assessed the time course of bleeding and tracked the progression of injury and behavioral deficits in the whole blood and collagenase ICH models. In Experiment 1, we assessed hematoma volume using a spectrophotometric hemoglobin assay at 1, 2, and 4 h after ICH (N = 60). Blood volume significantly increased from 1 to 4 h in the collagenase model, but not after blood injection. In Experiment 2 (N = 40), we attempted to match the collagenase insult to the standard 100 ?L whole blood model. Thus, we used 0.2 U of bacterial collagenase to produce similar hematoma volumes. In Experiment 3, magnetic resonance imaging (MRI) was used to track the ICH over 6 weeks (N = 40). T2 weighted images were obtained at 6 h, 12 h, 2 d, 4 d, 1 wk, 2 wk, 4 wk, and 6 wk following ICH. We also assessed neurological deficits 1 - 28 days after ICH. Damage to the striatum and to distal regions including the cortex, substantia nigra, and white matter tracts was measured at 6 weeks. Hemoglobin breakdown occurred faster after blood injection than after collagenase-induced ICH. Analysis of MR images revealed that early hematoma volume (e.g., 6 h after ICH) significantly correlated with eventual tissue loss (at 6 wk) in the blood infusion model, but not in the collagenase model. Neurological deficits completely recovered by 3 weeks in the whole blood model, whereas recovery in the collagenase rats was more gradual. Long-term histological injury included damage to the striatum and distal regions, and was much greater in the collagenase model, despite a slightly smaller initial hematoma volume. Subtle functional deficits and limited injury after blood infusion make this model less suitable for long-term studies testing therapeutics.

375 A405

TEMPORAL PATTERNS OF PERIPHERAL AND CENTRAL CYTOKINE EXPRESSION AND GLIAL REACTIVITY PROVOKED BY INFUSION OF LIPOPOLYSACCHARIDE INTO THE SUBSTANTIA NIGRA PARS COMPACTA

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Neuroinflammatory processes have been implicated in several neurological conditions, including those in which environmental insults are believed to play a prominent role, such as Parkinson's disease (PD). Infusion of the bacterial endotoxin, lipopolysaccharide (LPS), into the substantia nigra pars compacta (SNc) has been reported to provoke many histological signs of PD, including a loss of dopamine neurons and activation of microglia. Several pro-inflammatory cytokines, including interleukin-1beta (IL-1beta), IL-6 and tumor necrosis factor-alpha (TNF-alpha) modulate the central effects of LPS. Accordingly, the present study evaluated the temporal sequence of PD-like behaviors and glial activation, as well as changes in a panel of 10 pro- and anti-inflammatory cytokines within the SNc, striatum and circulation following LPS infusion into the SNc. Indeed, intra-SNc endotoxin administration reduced home-cage activity 90 min and 2 days after treatment, suggesting that transient motor impairment was provoked by nigral inflammation. Although microglial activation was evident at 2 and 7 days after LPS, astrocyte immunoreactivity was unaffected. Time- and dose-dependent cytokine changes occurred following LPS, with levels of IL-6 and TNF-alpha, together with the anti- inflammatory cytokine, IL-10 being increased within circulation and the SNc at the early 90 min interval. In contrast, circulating levels of IL-1beta, IL-2 and interferon-gamma (IFN-gamma) were elevated at the 7 day interval but only IL-1beta was increased within the SNc following LPS. Within the striatum, LPS induced elevations of IL-1beta and IL-6, which were sometimes relatively protracted. These data reinforce the notion that complex cytokine interactions and glial changes may be important for the behavioral pathology observed in PD.

376 A406

A POSSIBLE NEUROPROTECTIVE ROLE OF NICOTINE THROUGH INDUCTION OF BRAIN CYP2D6

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CYP2D6 is an enzyme which is expressed in liver and at lower levels in number of extra hepatic tissues including the brain. It is involved in the metabolism of numerous CNS drugs (e.g. antidepressants, neuroleptics), endogenous neural compounds (e.g. catecholamines) and the inactivation of neurotoxins such as pesticides and 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). Association studies indicate that poor metabolizers of CYP2D6 are over represented in Parkinson's Disease (PD) and show an even greater risk when exposed to pesticides. Conversely smokers display a 50% reduction in risk for developing PD; smokers also have higher levels of brain CYP2D6. Therefore, one possible mechanism of neuroprotection against PD by smoking may be through induction of the neurotoxin-inactivating enzyme CYP2D6 in the brain. To test if nicotine, found in cigarette smoke, induces CYP2D6 in the brain, non-human primates were chronically treated with nicotine (0.3 mg/kg b.i.d., s.c., 18 days) and immunoblotted for brain CYP2D. In these monkeys we identified three different CYP2D6 like proteins in the brain with molecular weights of 55, 52, and 49 kDa. The major CYP2D forms (55 & 52 kDa) showed an induction upon treatment with nicotine in various regions including those affected in PD such as substantia nigra (2.4-fold, p=0.016), putamen (3-fold, p=0.01), caudate (2-fold, p=0.03) and brainstem (2-fold, p=0.001). Preabsorption with the human CYP2D6 peptide confirmed that the antibody is specifically binding to CYP2D6-like proteins. In addition, antibody purification using monkey liver CYP2D indicates that all three bands show homology to the single form of CYP2D detected in monkey liver. Comigration and spiking studies suggest that the 49 kDa protein has the most similarity to the monkey liver CYP2D. The two major brain CYP2D forms (55 & 52 kDa) may be brain specific isoforms or post-translationally modified CYP2D proteins. In conclusion, these results suggest that monkey brain expresses three forms of CYP2D and two are induced in a brain-region specific manner upon treatment with chronic nicotine. Funded by: Tobacco Use in Special Populations, CAMH, CIHR MOP14173, and CRC.

377 A407

GLOBAL ISCHAEMIA INDUCES ENHANCED BRAIN DAMAGE DURING PREGNANCY: ASSESSMENT OF HIPPOCAMPAL INJURY AND MEMORY

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The female rat undergoes myriad physiological, endocrine and neurological alterations during pregnancy. Some of these changes are known to have differential effects on the susceptibility to, or outcome following, a cerebrovascular event. Clinical evidence indicates that females are at greater risk for thromboembolic stroke and ischemic injury during pregnancy. Although the increased risk has been linked to hormonal surges that encourage a pro-coagulant cardiovascular state, the precise mechanisms have not been resolved. In order to further explore the nature of the association between stroke and pregnancy, we used the two vessel occlusion (2VO) method to induce a brief (15 min) period of global ischemia in either pregnant (day 17) or virgin Sprague-Dawley rats. Three days following 2VO, rats were assessed in the open field for anxiety and locomotor behaviours, or for learning and memory in a contextual fear conditioning paradigm. Following the behavioural examinations, rats were sacrificed for histological inspection of the hippocampus. Preliminary results revealed that pregnant rats exhibited increased behavioural deficits in the conditioned fear test and enhanced neuronal injury subsequent to 2VO compared to virgin females. Thus our initial experimental findings support the clinical association between stroke-induced damage and pregnancy. This stroke model is currently used to uncover the mechanisms of stroke-induced enhanced brain damage during pregnancy.

378 A408

DISTRIBUTION AND ULTRASTRUCTURAL FEATURES OF THE ACETYLCHOLINE INNERVATION IN ADULT RAT THALAMUS

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The rodent thalamic nuclei share an heterogeneous acetylcholine (ACh) innervation arising essentially from the brainstem cell groups Ch5 and Ch6 (pedunculopontine and laterodorsal tegmental nucleus). We used a highly sensitive monoclonal antibody against rat choline acetyltransferase (ChAT) (Cozzari et al., 1990) to quantify this innervation and determine its ultrastructural characteristics in three functionally distinct thalamic nuclei: the parafascicular nucleus (PF), reticular thalamic nucleus (Rt) and dorsal lateral geniculate body (DLG) of adult rat. At the light microscopic level, the density of ACh innervation was quantified by unbiased, stereological counts of ChAT+ axon varicosities (terminals) with the optical fractionator (Stereo Investigator, MicroBrightField). It was the highest in PF (2.1 millions varicosities/cu. mm), followed by Rt (1.7 millions) and DLG (1.3 millions). Based on cell counts in adjacent Nissl-stained sections, the corresponding numbers of ACh axon varicosities per thalamic neuron were 43 in PF, 57 in Rt and 33 in DLG. At the ultrastructural level, ACh axon varicosities in the three nuclei were comparable in shape, vesicular content and frequency of mitochondria, but were significantly larger in PF than in Rt and DLG. In single thin sections, the proportion of ChAT+ varicosity profiles displaying a symmetrical or asymmetrical synaptic contact was 25% in PF, compared to only 10% in Rt and 8% in DLG. When extrapolated to the whole volume of varicosities, these values indicated that the ACh innervation of PF is entirely synaptic (109%), at variance with Rt and DLG, in which only 39% and 33% of the ACh varicosities are synaptic. Thus, in Rt and DLG, modulatory effects of the ACh input from brainstem are presumably conveyed by diffuse transmission and spread onto a large number of units within the nucleus. In contrast, in PF, the entirely synaptic ACh input would allow for a more faithful relaying of this excitatory drive from brainstem to extensive cortical area and to the striatum. (Supported by an H.H. Jasper fellowship and CIHR grant MOP-3544).

379 A409

CLUSTERED OBSTRUCTIVE APNEAS INDUCE LONG-TERM FACILATATION OF UPPER AIRWAY MOTOR OUTFLOW IN SPONTANEOUSLY BREATHING RATS IN-VIVO

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Objectives: Respiratory long-term facilitation (LTF) is a long-lasting increase in respiratory motor outflow that is induced by episodic but not continuous hypoxia. While three 5-minute periods of hypoxia have been shown to induce LTF, it is unknown if brief periods of asphyxia produced by apneas that are physiologically relevant (as in obstructive sleep apnea) also induce LTF. Therefore, the aim was to determine whether clustered (repeated) airway occlusions evoke respiratory LTF. Methods: Experiments were performed on anesthetized (2% Isoflurane in 50% O2 / 50% N2), tracheostomized, spontaneously breathing adult male Sprague-Dawley rats (n=17; 300-380g). Respiratory motor activity was determined by recording the EMG activity of both diaphragm and genioglossus muscles. Airway occlusions (apneas) were induced by obstructing tracheal airflow using a specially-constructed device. Following a 30-45 minute stabilization period, one of 2 experimental protocols was executed. Protocol 1, rats (n=8) were not exposed to apneas, and respiratory motor activity was recorded for 120 minutes. This group served as a control. Protocol 2, respiratory motor outflow was recorded for 60 minutes before and after exposure to a cluster of ten 15-second airway obstructions, each separated by 1 minute (n=9). This protocol was hypothesized to evoke respiratory LTF. Results: Respiratory frequency and amplitude of both the diaphragm and genioglossus muscle activities remained unchanged throughout the 120-minute recording period in the control experiments (1-way ANOVA, $P>0.05$). During each of the occlusions, the inspiratory amplitude of both diaphragm and genioglossus muscles increased. After the ten airway occlusions, the amplitude of the genioglossus inspiratory motor outflow transiently returned toward baseline levels and then over the subsequent 60 minutes, increased to levels significantly greater than baseline (168 ± 12%; 1-way ANOVA, $P<0.05$). Respiratory frequency, and the amplitude of diaphragm inspiratory activity remained stable and were unchanged during the post-apneic period (1-way ANOVA, $P>0.05$; fig. 1). Conclusions: These observations demonstrate that repeated airway obstructions evoke LTF in genioglossus motor activity. Accordingly, we suggest that respiratory LTF of upper airway muscles may be a protective mechanism for maintaining airway patency, which may play a role in obstructive sleep apnea.

380 A410

CEREBELLAR NUCLEI OF THE GOAT(CAPRA HIRCUS)

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This study, aimed to investigate the microscopic anatomy and topography of the goat (capra hircus) cerebellar nuclei, was carried out on eight cerebellums of local breed of goats aged less than two years. These cerebellums were subjected to a histological study through various plans of anatomical cuts (sagittal, transversal and frontal), using haematoxylin- eosin and toluidine blue stains. The comparative anatomy of the goat cerebellums enabled us to detect the analogies and differences with the other species of mammals including the weight, dimensions and the morphology. The histological study of the cartography of the cerebellar nuclei highlighted the similarities as well as the specific characteristics concerning the situation, the shape, the delimitation, the cytology of these cerebellar nuclei. The cerebellar nuclei of the goat form a nuclear mass of 0.9 cm length, 1.3 cm width and 0.7 cm height. As a whole, the goat cerebellar nuclei is closer to that of the sheep and the bovine on the frontal cuts, with some similarities with camelids, equids and rodents on the other cuts.

381 A411

EFFERENTS OF AMYGDALA TO HIPPOCAMPUS IN PROTEIN DEFICIENCY

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Amygdala and Hippocampus are important parts of the brain and proteins are essential cellular elements in all live beings.Protin deficiency is one of the commonest kinds of malnutrition in the world.Therefore we investigated the effect of low protein diet on efferents of amygdala to CA1 of hippocampus. In this study,26 Rats randomly divided into two groups(case & control).During 7 months control group was fed with normal diet(18% protein)and case group was fed with low protein diet(8% protein).After 7 months HRP injected into CA1 of hippocampus in two groups.After 48 to 72 hours of survival time each animal was deeply anesthetized and perfuzed transcardialy with fixative solution.The brains were removed from skull postfixed. sections were cut at 40 micro meter by using cryostat microtom along the frontal plane. For the histochemical demonstration of HRB, serial sections were treated with TMB reaction. The sections were mounted on gelatin - coated slides and constrained with neutral red. Topographical study of labeled neuronal cell was performed by light microscope. Selected sections were drowing by microprojector. We used image TOOL 2 and SPSS 11.0 (T test & mannwithney) software for analysis of results. Findings: following injection HRP to CA1 region of hippocampus in the control group rats. labeled neurons were more density in the centrolateral regions of BLP, BMP,LaVM, Aco, PMCo, MePD and MePV nuclei of amygdala that these neurons decrease toward rostral and caudal ends of these nuclei.Also these neurons were more density in the posterolateral region of BLA,BLV,BMA,LaVL,PLco and MeAD nuclei.Labeled neurons were more density in the anterolateral of the LaDL nuclei of amygdala that decreased toward caudal ends of its nucleus. Conclusions:Our finding show that different nucleus of amygdala send projections to CA1 region of hippocampus. Among of these nuclei,basolateral nuclei group sends the most projections. In comparison of the case and control groups we found that effect of low protein diet on efferent from basolateral, cortical and medial nucleus of amygdala to the CA1 region of hippocampus are decreased.

Sensory & Motor Systems

382 A131

ADAPTATION AND HABITUATION OF VISUAL RESPONSES IN THE SUPERFICIAL AND INTERMEDIATE LAYERS OF THE SUPERIOR COLLICULUS (sc)

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One neural correlate of visual attention is a decrease in the neural response to a target after prior presentation of an orienting 'cue' (e.g. inhibition of return). This decreased responding could be the result of repeated stimulation of a neuron's receptive field resulting in either 'adaptation' – a lower level mechanism related to neural fatigue, or 'habituation' – where an organism stops responding to an irrelevant stimulus but recovers the response after a change in stimulus properties. We dissociated adaptation from habituation in superficial (SCs) and intermediate (SCi) layer neurons of the SC, a hub of oculomotor and attentional processing. SCs receives visual input from the retina directly or via V1, while the SCi receives convergent input from visual and motor areas. Monkeys were rewarded for fixating a central point while a series of 7 successive stimuli were briefly flashed (100 ms duration; 100-400 ms interval) in the receptive field of the neuron. On 70% of trials all flashed stimuli were identical, while on others, the 4th was either brighter, dimmer or absent (10% each). If reduced neural response is due to habituation, some recovery of the response (dishabituation) should occur to any oddball stimulus. However, if the reduced response is due to adaptation, the response should be further reduced after the brighter, but recover after the dimmer or absent stimulus. The largest decrease in response (often > than 50%) was to the second stimulus, and subsequent stimuli resulted in only small further reductions. The shorter the inter-flash interval, the greater these reductions. The pattern was globally similar in SCs and SCi, but there was a greater reduction to the 3-7th stimuli in SCi. Responses to oddball stimuli in SCs neurons were suggestive of adaptation, while responses in SCi neurons showed features of both adaptation and habituation.

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ACTIVATION OF PPTg NEURONS INHIBITS AUDITORY EVOKED EPSCs IN STARTLE MEDIATING NEURONS BY A MUSCARINIC MECHANISM

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Giant neurons in the caudal pontine reticular formation (PnC) are the central structure of the mammalian startle response. They receive input from sensory nuclei and directly project onto motoneurons. Furthermore they integrate different modulatory inputs either enhancing or inhibiting startle response. One important modulation is prepulse inhibition (PPI) of the startle by a preceeding nonstartling stimulus. PPI reflects a sensory filtering mechanism and is impaired in many neurological disorders. Different behavioural studies indicated that PPI of the startle response is mediated by an cholinergic projection from the pedunculopontine tegmental nucleus (PPTg) onto PnC neurons activating muscarinic receptors. Electrophysiological studies confirmed a strong inhibition of trigeminal and auditory evoked currents in PnC giant neurons by the application of muscarinic agonists. We here combined presynaptic extracellular stimulation of auditory fibres and descending fibres from PPTg to PnC in rat slices in order to show that the activation of PPTg neurons indeed inhibits sensory inputs to the PnC at different interstimulus intervals (ISI). Furthermore, we applied the cholinergic blockers mecamylamine and scopolamine in order to determine the involved receptors. Burst stimulation of PPTg fibers prior to auditory fiber stimulation significantly inhibited auditory evoked EPSCs at ISIs of 300 and 1000ms (83.6 ± 6.3%, n=11 and 81.9 ± 4.5%, n=11 of control amplitude, respectively). In addition, paired-pulse ratio was significantly increased at an ISI of 1000ms. The application of mecamylamine, a nicotinic antagonist, had no significant effect on burst induced inhibition at ISIs of 300 and 1000ms. In contrast, the muscarinic antagonist scopolamine significantly blocked PPTg induced inhibition in the PnC as well as the changes in paired-pulse ratio at 1000ms ISI (p=0.036 and p=0.029, n=4). We therefore conclude that PPTg activation inhibits sensory signals in PnC giant neurons at ISI of 1000 ms in vitro by the activation of presumably presynaptic muscarinic receptors.

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RESPONSES IN THE PRIMARY AUDITORY CORTEX IN TINNITUS SUFFERERS AFTER INDUCTION OF RESIDUAL INHIBITION BY MASKING SOUNDS

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Tinnitus, a phantom auditory sensation in the absence of external stimuli, may be briefly suppressed after the cessation of a masking sound. This is called residual inhibition (RI) in the tinnitus literature. Typical tinnitus

sufferers experience 20-30 seconds of RI although some subjects experience no RI. In this study tinnitus sufferers who have previously demonstrated RI were compared to normal hearing controls without tinnitus. In our model, tinnitus sensations are generated by abnormal synchronous neural activity that develops in the primary auditory cortex (PAC) and other auditory regions consequent on weakened intracortical inhibition associated with hearing loss or the aging process. Neural responses in the PAC can be measured using 40 Hz amplitude-modulated (AM) probe tones which produce a 40-Hz auditory steady-state response (ASSR) in the EEG.

Tinnitus subjects and normal hearing controls underwent two conditions in which ASSR amplitude was expected to differ. One condition consisted of only probe tones (TINN condition) while the second condition included probe tones which followed maskers designed to elicit RI in the tinnitus group (RI condition). It was hypothesized that there will be an increase in ASSR amplitude in tinnitus subjects during the RI condition compared to the TINN condition, as neurons temporarily desynchronized by the masker are available for entrainment by the AM envelope. Analysis showed that ASSR amplitude increased in the tinnitus group after the masker (RI condition) compared to the TINN condition but decreased in the control group. This interaction was significant at p=.026. In addition, there was a main effect of group (tinnitus versus control) showing aSSR amplitude to be larger overall in tinnitus (p = 0.01). These results are consistent with the view that hypersynchrony in auditory regions of the brain generates tinnitus, and that desyncronization by masking sounds underlies RI. Supported by CIHR, NSERC, and the American Tinnitus Association.

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ONTOGENETIC DEVELOPMENT OF ACOUSTIC FEATURES OF ISOLATION CALLS IN WISTAR RATS

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Rat pups are known to emit an acoustically heterogeneous group of ultrasonic isolation calls, while adult rats develop two distinct ultrasonic vocalization types with the respective peak frequencies of 22 kHz and 50 kHz. The goal of the present study was to analyze acoustic features of isolation calls emitted by individually studied Wistar pups from 3 to 20 days of age and observe a postulated transition from the infantile to adult type of ultrasonic calls. Pup vocalizations were induced by a mild and unpredictable air puff delivered from above the animal. Most animals emitted calls, particularly at younger ages. Overall, the duration of individual calls increased with age, while the individual call frequency showed a decreasing tendency. The isolation calls were divided into those with short call duration (less than 100 ms) and those with long call duration (longer than 100 ms), as well as into those with high peak frequency (higher than 50 kHz) and low peak frequency (lower than 50 kHz). Calls longer than 100 ms showed a significant negative correlation with age for sound frequency ($r = -0.88$, $p< 0.0001$) and positive correlation with call duration ($r = 0.65$, $p< 0.003$). Thus in this category, the call duration lengthened and frequnecy decreased with increasing age of the pup. These calls had also increasingly flat sonographic appearance (decrease in their bandwidth, $p < 003$) and their number increased with age ($p < 001$). Calls with duration under 100 ms decreased with age ($p < 0006$), but did not show any significant change in peak frequency or call duration. These changes were consistent with those observed for calls under 50 kHz. Their frequency decreased ($r = -084$, $p < 0.0001$) and call duration increased with age ($r = 0.7$, $p < 0.001$). Calls with a peak frequency higher than 50 kHz did not show significant changes in duration, however their peak frequency significantly increased with age ($r = 0.8$, $p< 0.0003$). Overall, the results indicate that the call pattern, which is compatible with the adult 22 kHz alarm calls gradually develops and emerges early in infancy.

Study supported by NSERC of Canada

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PERCEIVED PAIN INTENSITY MODULATES BRAIN RESPONSES TO FACIAL EXPRESSIONS TO PAIN

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Results of brain imaging studies on pain empathy suggest that the neural substrate of the perception of pain in others includes cortical areas involved in the affective dimension of the perception of pain in the self. In this case, it is likely that at least some brain areas responsive to the perception of pain expression should code for the intensity of the pain expressed. This study investigated the differential engagement of emotional, sensory and motor brain areas in observers evaluating dynamic facial expressions of pain, and how activation in these areas is modulated by intensity of the pain as perceived by the subject. BOLD-signal changes were measured in a 3T fMRI scanner using an event-related design in 18 normal volunteers. Subjects viewed a series of 1-sec video clips displaying facial expressions of pain of varying intensity. In each trial, subjects were cued to perform either a pain evaluation task or a movement discrimination task. After each stimulus, subjects rated the intensity of the pain expressed (pain task) or the relative magnitude of movements in the lower versus upper face (control movement discrimination task). Brain response to pain was first examined by contrasting activation in response to pain versus neutral expressions in both task conditions. Pain-related activity was observed in both emotional- and motor-related areas including medial prefrontal cortex (mPFC), cingulate cortex (CC), insula, pre- and primary motor areas; and secondary sensory areas ($t > 4$; $p < .001$). A contrast of the pain-related activation in the two task conditions was then done in order to identify areas of activation associated with attention to, and evaluation of, the pain expressed. Activation during the pain evaluation task was significantly stronger in medial PFC areas including perigenual and dorsal ACC. We then examined the relationship between the magnitude of brain response and the intensity of the pain as perceived by the observer. A modulatory effect was found in medial PFC as well as in bilateral insula, suggesting that assessment of the amount of pain expressed by another involves emotional and social cognition systems in the observer.

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GLUTAMATERGIC CONTROL OF SOMATIC MOTONEURONS IN FREELY-BEHAVING RATS

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Skeletal muscle activity is potently suppressed in sleep and particularly during REM sleep. Abnormalities in muscle tone underlie most of the major sleep disorders including narcolepsy, REM-sleep behaviour disorder and obstructive sleep apnea. The biochemical mechanisms that mediate suppression of muscle tone in sleep are unclear. In the current study, we hypothesize that withdrawal of excitatory glutamatergic inputs onto somatic motoneurons may be responsible for sleep-dependent reductions of muscle tone. To test this hypothesis, we exogenously applied (using microdialysis probes) either glutamatergic agonists (glutamate, NMDA and AMPA) or antagonists (CNQX and D-AP5) onto trigeminal motoneurons in freely behaving rats (n=24) while recording masseter EMG activity across the natural sleep-wake cycle. Glutamate receptor antagonism led to significant decreases in masseter activity in waking (p=0.001), but had no effect during either NREM (p=0.879) or tonic REM sleep (p=0.939). However, we found that blockade of non-NMDA receptors abolished phasic muscle activity during REM periods. While application of glutamatergic agonists significantly increased masseter activity during both waking (p=0.001) and NREM (p=0.002), they had no effect on EMG activity in tonic REM sleep (p=0.916); however, glutamatergic agonists significantly enhanced muscle activity during phasic REM periods. We conclude that: 1) glutamatergic

inputs play a predominate role in motoneuron excitation during wakefulness but play a minor role during NREM and tonic REM sleep; 2) since exogenous application of glutamate onto motoneurons is unable to overcome the atonia of REM sleep, other powerful inhibitory mechanisms must be involved; and, 3) phasic REM events are mediated by glutamate acting primarily on non-NMDA receptors.

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SEPARATE EXTRASTRIATE VISUAL REGIONS PROCESS FORM AND TEXTURE IN THE ABSENCE OF EXPLICIT DEPLOYMENTS OF ATTENTION

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We recently demonstrated that attending to either the form or surface properties of objects activates anatomically distinct regions of occipito-temporal cortex (Cant & Goodale, 2007). Specifically, attending to form activated the lateral occipital area (LO), whereas attending to texture activated the collateral sulcus (CoS). Although these regions showed preferential activation to one particular stimulus dimension (e.g. texture in CoS), they also showed activation to other, non-preferred stimulus dimensions (e.g. form in Cos). Thus, one might question whether the activation to form in CoS, for example, represented form processing, or represented activation to changes in texture while people attended form. To investigate this, we conducted an fMR-adaptation experiment which allowed us to examine the response properties of regions specialized for processing form, texture, and colour when participants were not explicitly attending to a particular stimulus dimension. Participants passively viewed blocks where only one dimension varied and blocks where no dimensions varied, while fixating a cross in the centre of the display. Area LO was most sensitive to variations in form, whereas CoS was most sensitive to variations in texture. As in our previous study, no regions were found that were most sensitive to variations in colour. Taken together, these results replicate the findings from our previous study but also suggest that area LO and CoS can respond in a very stimulus-driven manner in the absence of explicit deployments of attention. Furthermore, these results provide additional evidence for the existence of separate processing pathways for form and texture in occipito-temporal cortex.

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OBSTACLE AVOIDANCE WHILE REACHING: EFFECTS OF OBSTACLES AND NON-OBSTACLE OBJECTS ON REACH TRAJECTORY

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When reaching to objects, our hand and arm rarely collide with non-target objects, even if our workspace is cluttered. The simplicity of these actions hides what must be a relatively sophisticated obstacle avoidance system. Recent studies on patients with optic ataxia and visual form agnosia have demonstrated that obstacle avoidance is an automatic process, likely governed by the dorsal stream (Schindler et al., 2004, Nature Neuroscience, 7(7):779-784, Rice et al., 2006, Experimental Brain Research, 174(1):176-188). The current study sought to quantify how normal participants react to changes in the size and position of non-target objects in and around their workspace. In the first experiment, 13 right-handed subjects performed reaches to a target strip in the presence of two non-target objects, which varied in depth and horizontal configuration. We found that objects with horizontal alignments that were asymmetric about midline created systematic deviations in reach trajectory away from midline, with participants seeming to maximize the distance away from the two objects. These deviations were significantly greater for objects nearer in depth and nearly disappeared when the objects were placed beyond the target strip, suggesting the obstacle avoidance system is sensitive to the depth at which an object becomes an obstacle. In a second experiment, we varied the height of the two objects, as well as the depth. Object pairs could now be both tall, both short, or one short and one tall (with the tall on the right or on the left). We replicated the first experiment, extending the finding to include sensitivity to the size of the objects. Here the deviations induced by short objects, while still significant, were significantly less than the tall-object deviations. Taken together, these experiments indicate a sophisticated obstacle avoidance system that is extremely sensitive and conservative in evaluating potential obstacles and deviating reach accordingly.

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HEAD-FREE ELECTRICAL STIMULATION OF THE LATERAL INTRAPARIETAL AREA (LIP) AND THE SUPERIOR COLLICULUS (SC) OF THE MACAQUE MONKEY

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Previous neurophysiological studies on head-unrestrained monkeys established that coordinated eye and head movements (gaze shifts) are evoked by stimulating the superior colliculus (SC - Klier et al. 2001). The evoked gaze shifts are encoded in an eye-fixed frame. However stimulation of LIP in head-restrained and head-partially-restrained monkeys evokes saccadic eye movements (Thier & Anderson, 1996, 1998). It is not known whether LIP stimulation evokes coordinated eye and head movements (gaze shifts) in head-unrestrained monkeys and what is the frame of reference used by LIP to encode gaze. The goal of the present study was to examine if LIP and SC play a comparable role in gaze motor control. To test this we implanted recording chambers over the stereotaxic coordinates of the LIP and SC (two monkeys each) and eye search coils for 3-D recordings. The animal was trained to fixate targets while we electrically stimulated the SC (with stimulation trains of 20-50 μA and 200 ms) and the LIP (with stimulation trains of 150-200 μA and 200 ms). The head was unrestrained during training and stimulation. Stimulating SC we evoked contralateral gaze shifts with amplitude varying from 4.52° to 90.43°, with a large range of eye and head as oppose to stimulating LIP which evoked smaller amplitude gaze shifts, varying from 2.45° to 18.2°, with a less than 1° head movement component (in the majority of sites). Using gaze shifts evoked from a variety of initial eye and head positions, and a method described by Klier et al (2001) we examined the reference frame used by the two brain areas. The results suggest that both structures use an eye-fixed frame to encode gaze. The study suggests that although LIP stimulation produces small-medium saccades and SC produces a large range of eye-head gaze shifts, they form a continuum along an eye-fixed curve, in terms of their reference frame coding.

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EJACULATION-INDUCED ACTIVATION OF NMDA RECEPTORS AND MAP KINASE IN SPINOTHALAMIC CELLS IN THE LUMBAR SPINAL CORD OF MALE RATS

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Previously we identified a population of lumbar spinothalamic (LSt) cells that play a pivotal role in the control of ejaculation and comprise an essential part of the spinal ejaculation generator in the male rats. Lesions of the LSt cell population completely eliminate expression of ejaculation, while other elements of male sexual behavior remain intact. Furthermore, LSt cells

express Fos, a marker for neural activation, only with ejaculation, but not during other elements of sexual behavior. Currently, it is unknown which signal transduction pathways or neurotransmitters are involved in the activation of LSt cells. Therefore, the goal of the present study was to investigate expression and activation of glutamate receptors as well as phosphorylation of the MAP kinase pathway in LSt cells. Male Sprague Dawley rats were perfused with 4% paraformaldehyde immediately (0 minutes) or 30 minutes after display of one ejaculation or after expression of 8 intromissions without display of ejaculation. Control males were taken from their holding cages and perfused. Spinal cords were removed, sectioned, and immunoprocessed for galanin as a marker for LSt cells and NMDA receptor subunit 1 (NR1), galanin and phosphorylated NR1 (pNR1; 30 minute groups), or galanin and phosphorylated ERK (pERK; 0 minute groups), using dual immunofluorescence. Results showed that the majority of the LSt cells contained NR1. Moreover, NR1 receptors were activated in males that displayed ejaculation but not in males that expressed intromissions and mounts, but did not ejaculate, evidenced by a significant increase in the percentage of LSt cells expressing pNR1 (68.3% following ejaculation vs 36.3% in the control group and 41% after intromissions). The percentages of LSt cells that contained pERK were increased in males that displayed ejaculation (90.4% vs 6.5% in control males). However, ERK phosphorylation was also induced in males that displayed intromissions and mounts, but no ejaculation (49.8%). Together these results indicate that the glutamate receptor NR1 is activated in LSt cells specifically with ejaculation and may in turn mediate the expression of ejaculatory reflexes. Phosphorylation of ERK is correlated with intromissions as well as ejaculations and it is not clear if this signal transduction pathway is therefore specifically involved with control of ejaculation. These experiments form a first step towards a better understanding of the spinal control of ejaculatory reflexes.

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POTENT ANTINOCICEPTIVE EFFECTS OF NTS1 AGONISTS IN A MODEL OF NEUROPATHIC PAIN

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The tridecapeptide neurotensin (NT) induces central and peripheral effects through three receptor subtypes : NTS1, NTS2, and NTS3. Both NTS1 and NTS2 receptors are involved in mediating the naloxone-insensitive antinociceptive effects of NT in a variety of analgesic tests including hotplate, tail-flick, and writhing tests. However, the role of these receptors has never been documented in chronic pain models. The goal of this study was therefore to evaluate the analgesic effects of NTS1 specific agonists in a rat neuropathic pain model. Neuropathy was induced by sciatic nerve constriction (Bennett's CCI model), and the development of mechanical allodynia and thermal hyperalgesia on the ipsi- and contra-lateral hindpaws was examined 3, 7, 14, 21 and 28 days post-surgery. Paw withdrawal latencies to heat stimulation or von Frey filament application were assessed, before and 20 min after NT, NT69L, PD149163 administration, using the Plantar and the von Frey tests, respectively. Rats exhibited heat hyperalgesia and tactile allodynia over a 28-day testing period. Mechanical and thermal sensitivity in sham-operated and saline-treated rats remained unchanged throughout the testing period. Intrathecal injection of NT (1 and 6 µg/kg) dose-dependently attenuated the mechanical allodynia and thermal hyperalgesia on days 7, 14, 21 and 28 post-surgery. Administration of the NTS1 agonists PD149163 (30 and 90 µg/kg) and NT69L (5 and 25 µg/kg) also produced both anti-allodynic and anti-hyperalgesic effects in the CCI model of neuropathic pain. Importantly, suppressive effects of NT, NT69L, and PD149163 on heat hyperalgesia and mechanical allodynia were also observed on the contralateral paws of CCI rats. In conclusion, intrathecal administration of NTS1 agonists decreases pain behavior in neuropathic animals for four consecutive weeks. Thus, these results demonstrate that NT analogs may be an interesting way for the therapy of painful neuropathies without having the unfavourable side effects of opioids. (Supported by CIHR, FRSQ, and NIMH).

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SENSORY PROTECTION OF RAT MUSCLE SPINDLES FOLLOWING PERIPHERAL NERVE INJURY AND REINNERVATION

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Skeletal muscle function and structural integrity are dependent upon intact innervation. Following peripheral injury resulting in muscle denervation, the outcome of nerve surgery is likely to be poor if reinnervation is delayed. Prolonged muscle denervation results in irreversible muscle fiber atrophy, connective tissue hyperplasia, and deterioration of the muscle spindles, specialized sensory receptors necessary for proper skeletal muscle function. The protective effect of temporary sensory innervation on denervated muscle, prior to motor nerve repair, has been shown in the rat. Sensory protected muscles exhibit less fiber atrophy and connective tissue hyperplasia while maintaining greater functional capacity than denervated muscles. The purpose of this study was to determine whether temporary sensory innervation also protects muscle spindles from degeneration. The results document deterioration of muscle spindles in muscle denervated for six months and demonstrate a significant preservation of spindle number and morphology in sensory protected muscle, adding to the known means by which sensory nerves exert protective effects on denervated muscle. This finding further promotes the use of sensory protection for improving the outcome after peripheral nerve injury.

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THE EFFECT OF INC (INTERSTITIAL NUCLEUS OF CAJAL) STIMULATION / INACTIVATION ON NECK MUSCLE SYNERGIES

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Research has shown that the interstitial nucleus of Cajal (INC) acts as a neural integrator for torsional/vertical components of eye position/head posture. Unilateral stimulation of the INC produces torsional eye and head movements to positions that are maintained until stimulation is removed. Also, unilateral INC inactivation produces relatively coordinated position-holding deficits in both the eye and head. However, the recruitment of neck muscles during INC stimulation/inactivation is unclear. We investigate the recruitment of 6 pairs of bilateral neck muscles in two head-unrestrained monkeys (Macaca mulatta) before, during stimulation, and 40 minutes after inactivation of the INC. We identified INC sites by unit recording, and subsequently stimulated (50 microamp., 200 ms, 300 Hz) and inactivated (by injecting 0.3 microliter of 0.05% muscimol) each site. We recorded three-dimensional eye/head movements using search coils within magnetic fields. By surgically implanting bipolar hook electrodes we recorded EMG (electromyographic) activity in these neck muscles (Bilaterally): occipital capitis inferior (OCI), rectus capitis posterior major (RCPmaj.), biventor cervicis (BC), complexus (COM), splenius capitis (SP) and

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

sternocleidomastoid (SCM). Inactivation of the INC produced eye and head position-holding deficits (drift): clockwise (CW) / counterclockwise (CCW) after left/right INC inactivation. In addition, the range of eye and head position tilted torsionally, similarly CW/CCW after left/right INC inactivation. Our preliminary analysis of the EMG data from one animal (5 left INC sites, 6 right INC sites) revealed an overall reduction in EMG activity of both ipsi- and contralateral neck muscles. Although the severity of this reduction was different for ipsi- versus contralateral muscles. INC stimulation, on the other hand, caused an increase in overall EMG activity of both ipsi- and contralateral muscles. The initial comparison between our stimulation and injection data did not suggest a simple 'opposite' pattern. Further analysis of both stimulation/injection data of more INC sites should provide a better perceptive of neck muscle synergies.

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NEURAL CORRELATES FOR PRO-SACCADES AND ANTI-SACCADES IN SUBSTANTIA NIGRA PARS RETICULATA

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The substantia nigra pars reticulata (SNr) is hypothesized to mediate the initiation of saccades through GABAergic projections to the superior colliculus (SC). The goal of the present study was to determine if these inhibitory inputs are involved in providing the SC with increased inhibition when suppression of an automatic saccade is required. To test this hypothesis we recorded from neurons in the SNr of two monkeys trained to perform a randomly interleaved pro/anti-saccade task. The color of the central Fixation Point instructed the monkeys to either generate a pro-saccade towards the stimulus or to suppress this automatic response and instead generate a volitional anti-saccade away from the stimulus. These tasks allowed us to determine if and how SNr activity changed with task instruction. We found that neurons in SNr that increase their activity for saccades (burst neurons; n=20) had more pre-stimulus and more peri-saccade activity when the monkey executed an anti-saccade and they had less activity when the monkey mistakenly made a pro-saccade on an anti-saccade trial. SNr neurons that have high levels of activity during fixation and decrease their activity for saccades (pause neurons; n=18) did not display differences in pre-stimulus activity between pro and anti-saccade trials, but had less peri-saccade activity when the monkey executed a correct anti-saccade. These findings suggest that the SNr burst neurons may provide the SC with the extra inhibition required to suppress the automatic pro-saccade on anti-saccade trials, while the SNr pause neurons may be involved in the generation of the volitional anti-saccade away from the stimulus.

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CONTROL OF THE SPATIAL ORIENTATION OF THE HAND DURING REACHING MOVEMENTS IN HUMAN SUBJECTS

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Control of the spatial orientation of the hand is an important component of many reaching movements. To investigate the contribution of vision and proprioception in perception and control of spatial orientation of the hand, right handed subjects were tested in perceptual and motor tasks. In the perceptual task, subjects were asked to passively align a rotatable rectangular handle held in the right hand (match) to that of a second handle held in the left hand (reference) fixed in different orientations. In the motor task, subjects actively reached and oriented the right hand to insert the match handle into a slot fixed in the same range of orientations. Orientations of the match handle were captured by a motion analysis system (Optotrak). ANOVA analysis shows a strong effect of task ($p<.05$) with smaller errors in the motor than in the perceptual task. We found that without vision of the hand, constant spatial errors of final hand orientation were smaller when the subjects simultaneously reached out and rotated their hand to align it with the target, than when they held their arm extended and passively matched the orientation of their hand with the target, even though the latter task is biomechanically simpler. This difference may reflect the engagement of on-line control mechanisms during reaching movements. To investigate this hypothesis, subjects were instructed to first align their hand to the angle of the target, and then to reach out to the target without changing the hand orientation. Subjects actively oriented their right hand then reached with the right arm to insert a rectangular handle into a slot fixed in different orientations, with and without vision of their hand. Errors in hand orientation of subject were compared for the initial and the final orientations of the hand, before and after each reach. ANOVA analysis showed smaller errors at the end of the reach than at the beginning ($p<.05$). Although subjects were instructed not to change their hand orientation while reaching, hand orientation did change during reaching in a way that reduced the final constant spatial orientation error. The finding that the initial orientation error is reduced despite the instruction not to correct is further evidence that automatic error-correction mechanisms are activated during voluntary reaching movements.

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EFFECT OF ARM ORIENTATION ON MOTOR TASK LEARNING AND GENERALIZATION

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Reaching studies suggest that humans learn novel task dynamics by adapting internal models that transform planned changes in limb state into motor commands. Learning one task interferes with the ability to learn another in a closely adjacent workspace location, consistent with broad spatial generalization (e.g., up to 80 cm away). Nonetheless, two different tasks can be simultaneously learned for relatively small spatial separations of 14-24 cm. To explain these observations, the internal model was proposed to be comprised of basis elements that reflect a multiplicative interaction between signals coding movement direction/velocity and limb position (Hwang et al., 2003). In the current study we investigated whether the coordinate system of this representation reflects the extrinsic coordinates of the hand or the intrinsic coordinates of the joints/muscles. We compared learning in four groups of subjects that made reaching movements from three pseudorandomly chosen start positions, each associated with distinct task dynamics (left: CW curl-field A; center: null; right: CCW curl-field B). Groups C1 and C2 encountered fields A and B during movements along hand paths that were close in cartesian space (1 cm separation). C1 subjects made all movements with the arm in the horizontal plane, whereas C2 subjects encountered field A with the arm in the horizontal plane but field B with the arm in the parasagittal plane. Thus, movements in these two groups were close in cartesian but distant in proprioceptive space. Groups D1 and D2 performed movements that were distant in both cartesian (24 cm separation) and proprioceptive space (D1: all horizontal plane; D2: two arm orientations). When initial limb state was similar in both cartesian and proprioceptive space (C1) subjects failed to learn the two tasks. In contrast, when limb state differed in proprioceptive space (C2, D1, D2) significant learning took place. Learning failed to generalize across significantly different initial joint angles as C2 subjects showed generalization between field A and center but not between field B and center. The results demonstrate that differences in limb joint angles/proprioceptive state are sufficient to support simultaneous learning of multiple task dynamics. This observation provides further confirmation that the internal model is composed of neural elements that encode limb state in intrinsic coordinates.

Supported by NIH and CIHR.

398 A147

THE FEEDBACK PATHWAY OF THE ONLINE CORRECTION MECHANISM INCLUDES COMPENSATION FOR LIMB DYNAMICS

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Reaching movements to visual targets have been shown to be under fast feedback control. This is evident from the ability to rapidly adjust ongoing movement to a change in the location of its goal, i.e. online correction. This study addresses the organization of a feedback controller underlying online correction by comparing performance of a virtual model arm to human movements. Human experiments were conducted with eight subjects, who performed reaching movements toward visual targets. In half of the trials, the target abruptly changed location by different amounts (3.5 – 14 deg along 15 cm radius) during the ocular saccade. The virtual arm was a dynamic 2-link model of a human arm with viscous joints built in MatLab software. A closed-loop controller drove the model arm by combining the feed-forward torque command to the original target location with either a position error between desired and delayed actual positions of the arm endpoint (Kinematic Feedback or KFb) or with a corrective torque based on this position error (Dynamic Feedback or DFb). Furthermore, delay-compensated feedback controllers, which included a forward dynamic model to predict current hand position from the efference copy of the motor command, namely Kinematic and Dynamic Feed-forward controllers (KFf and DFf), were also tested. The human results showed that the hand trajectories deviated from the control trajectory proportionally to the size of the target jump. Target jumps in different parts of the workspace resulted in different amplitudes of online correction. This scaling of online correction for movements in different directions was simulated well only by the model driven by DFb and DFf controllers, while the model driven by KFb and KFf controllers produced asymmetric trajectory adjustments due to the inability of these controllers to compensate for limb dynamics. The trajectories produced by the DFf controller showed the closest fit to the human position and velocity trajectories across a range of model parameters and this controller was found to have the lowest parametric sensitivity. Furthermore, the DFf controller was the only one to show best fits to human data within the range of physiological joint viscosities. These results support an online controller design that uses an error signal derived from the difference between the original motor program and feedback about the actual movement, and which emits a corrective motor command that takes into account limb dynamics.

399 A148

PRIMARY MOTOR CORTEX ACTIVITY EXHIBITS SIMILAR REPONSES TO TRANSIENT AND CONSTANT LOADS DURING POSTURE

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It is well known that primary motor cortex (M1) is an important neural structure for performing volitional motor behaviours. A hallmark of M1 is that the activity of individual neurons is commonly modulated by mechanical loads applied during various motor tasks. It is less appreciated that M1 neurons exhibit relatively short-latency (<100 ms) phasic responses to mechanical perturbations. We have studied the relationship between phasic and tonic responses to torques imposed at the shoulder, elbow, or both joints in monkeys during whole-limb postural maintenance. Phasic responses were measured 25–100 ms after the onset of brief torque perturbations (300 ms) that were applied while the hand was stabilized at a spatial target. Tonic responses to constant torques were measured 1000–3000 ms after posture was maintained at the same spatial target. The relationship between phasic and tonic responses was investigated by examining their directional tuning properties in joint-torque space (planar regression fits, $P < 0.01$). This analysis revealed 43 neurons with significant phasic responses, of which many of these neurons exhibited burst onsets within 50 ms of perturbation onset. Of these 43 neurons, 39 exhibited significant tonic responses (χ2 test, $P < 0.001$). Of particular interest, the preferred directions of phasic and tonic responses were tightly correlated (Rayleigh test, mean vector = 0.59, $P < 0.001$). The distribution of preferred directions in M1 was also highly similar to those of upper arm muscles which showed similar conservation of preferred directions between phasic and tonic responses. These findings suggest an important link between the neural processing in M1 for on-line control in which short-latency bursts can be viewed as rapid motor responses.

400 A149

ADAPTIVE RESCALING EXTENDS THE DYNAMIC RANGE OF CENTRAL VESTIBULAR SIGNALS IN THE ALERT CAT

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Adaptive rescaling adjusts the sensitivities of sensory responses for efficient signal transmission under varying stimulus conditions. The possibility that rescaling could improve the performance of the vestibulo-ocular reflex (VOR) after sensory loss has not been investigated. We recorded from isolated vestibular neurons in alert cats that had recovered from peripheral vestibular damage. Stimuli consisted of rotation at 1 Hz with peak velocities of 10-120 deg/s. The sensitivities and dynamic ranges of vestibular neurons were measured. Significant rescaling was seen both ipsilateral and contralateral to the damaged side. When the peak velocity increased by a factor of 8, the average sensitivity to rotation of the sample of neurons decreased by roughly a factor of 2. The dynamic ranges of central neurons and of the VOR appeared to increase at higher peak velocities. Our results suggest that after vestibular damage, adaptive rescaling improves signal transmission by central vestibular neurons and may act to restore the dynamic range in the response of the VOR to rotation at high speeds.

401 A150

BLOCKING THE GLUCOCORTICOID RECEPTOR NEUTRALIZES MOTOR FUNCTION IMPAIRMENT ASSOCIATED WITH STRESS

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Stress is one of the most critical influences on behaviour and performance. While most research has focused on the effects of stress on limbic system functions, including learning and memory, recent findings have shown that stress is also a modulator of motor control. For instance, stress can impair skilled and non-skilled movements in intact rats. The mechanisms by which stress and stress hormones exert these effects, however, have not been determined yet. Previous data suggest that the stress hormone corticosterone mediates at least some of the effects of stress on the motor system. The purpose of this study was to investigate if the impact of stress and corticosterone on motor function can be neutralized by a glucocorticoid receptor (GR) antagonist. Groups of male and female rats were tested in a skilled reaching task while receiving daily treatments of either immobilization stress or oral corticosterone. On Day 1 and Day 13 of treatment, rats were administered the glucocorticoid receptor antagonist RU-486. While both acute and chronic stress and corticosterone treatments reduced skilled reaching success, administration of RU-486 neutralized these effects by protecting skilled reaching success. There was no difference between male and female rats in the reaction to any of the treatments. These observations suggest that corticosterone plays a central role in modulating motor system function, and that these actions are mediated through GR activation. Supported by: Alberta Heritage Foundation for Medical Research and Canadian Institutes of Health Research.

402 A151

PHASE AND TASK SPECIFIC MODULATION OF THE SOLEUS MOTONEURON EXCITABILITY INDUCED BY RHYTHMIC ARM SWING MOTION IN UPRIGHT STANDING POSTURE

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While some evidence of contribution of interlimb neural connection for the generation of locomotive motor output has been suggested, the extent to which upper limb motion can modulate lower limb motoneuronal excitability is not fully understood. In the present study, we aimed to examine how the spinal motoneuronal excitability of the lower limb soleus muscle is modulated with rhythmic upper limb motions during passive standing posture. Soleus H-reflex responses were obtained at eight different motion phase instances during rhythmic arm swing motion in twelve able-bodied subjects. The amplitude of the H-reflex showed remarkable modulation in accordance with the phase of arm motion. It is notable that the H-reflex amplitude during arm swing tended to be larger than that obtained in static (arm rest) condition. Moreover, the degree of the H-reflex modulation tended to be larger when the arms swung bi-directionally (anti-phase) as compared to the case in which the arms swung in the same direction (in-phase). Two possible neural mechanisms can be hypothesized to induce the modulation of the H-reflex: (1) a voluntary neural command which is concurrent with the voluntary arm movement (corticospinal pathway); and (2) an interlimb neural pathway which is activated by afferent inputs from the upper limb (interlimb pathway). In order to examine whether the corticospinal excitability modulation involves the above H-reflex modulation, the modulation of the motor evoked potential elicited by transcranial magnetic stimulation (TMS) was evaluated. The results showed a similar modulation manner for the H-reflex, suggesting that corticospinal modulation may include the H-reflex modulation. These results indicate that there is a neural system to modulate soleus spinal motoneuronal excitability in accordance with the phase of upper arm motions. These results might provide useful information for how the upper limbs contribute to generating locomotive motor output in human bipedal walking.

403 A152

LONG-LATENCY REFLEXES OF SHOULDER MONOARTICULARS REFLECT BOTH SHOULDER AND ELBOW MOTION

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While the planar arm model has provided a fertile ground for motor control theories of volitional movement, relatively little is known about reflex coordination in this paradigm. Here we examined whether upper limb reflexes possess a sophisticated representation of limb mechanics as seen during voluntary movements? To test this hypothesis we imposed torque perturbations that induced nearly identical displacements at the shoulder joint paired with a range of displacements at the elbow joint (KINARM robot; load range ±2Nm; 10 subjects) and recorded reflex activity with surface EMG. If reflex activity of shoulder monoarticulars reflected motion at just the spanned/shoulder joint then identical responses would occur across conditions whereas a more sophisticated representation would result in different responses for different amounts of elbow motion. This paradigm revealed a qualitative shift between the early and late reflex activity: the short latency/spinal reflex (20-45 ms) was influenced by shoulder motion only whereas the long-latency/supra-spinal reflexes (50-75ms and 75-100ms) were increasingly modified by elbow motion. For example, the short-latency reflex of shoulder flexors varied only with shoulder extensor motion whereas the long-latency reflex was sensitive to shoulder motion but also decreased with increasing elbow flexor motion. A second set of perturbations further confirmed the influence of elbow motion on long-latency reflex responses. Perturbations that induced large elbow motion and minimal shoulder motion were sufficient to elicit or depress long-latency reflexes from shoulder monoarticulars. Although we've presented our results in term of motion-dependency, the pattern of long-latency reflexes were also appropriate to counter the underlying perturbing torque. This appropriate mapping of motion to torque is evidence that long-latency reflexes possess a sophisticated representation of limb mechanics, i.e. an internal model. Future studies will determine whether this sophisticated representation can adapt to novel dynamics as seen during voluntary movements.

404 D125

MICROSTIMULATION-INDUCED INHIBITION OF FIRING OF CELLS IN THE HUMAN SUBSTANTIA NIGRA

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Parkinson's disease (PD) is a movement disorder caused by degeneration of dopaminergic cells in the substantia nigra pars compacta (SNpc). Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a viable treatment for alleviating tremor in PD patients while avoiding the debilitating dyskinesia caused by long-term use of dopaminergic medication. The mechanisms by which DBS works, however, are still unclear. In order to better understand the effects of high-frequency stimulation on local cellular activity, we compared the effects of stimulation with short trains of high-frequency, low-current pulses delivered through the recording electrode on the firing of cells in the substantia nigra pars reticulata (SNr) and the STN. The SNr is located immediately ventral to the STN from which it receives excitatory projections. We have examined 142 neurons in the SNr of 33 awake PD patients undergoing stereotactic surgery. A majority (82%) of cells identified to be in the SNr on the basis of location and spontaneous activity were inhibited by high frequency (200-300 Hz), low current (< 10μA) stimulation. For example, with 0.5s trains of 2 μA pulses at 200 Hz, the duration of inhibition ranged from 264 ms to close to 2 sec (avg 600 ±88 ms). Firing of cells located in the STN, just dorsal to the SNr, was typically not inhibited by stimulation with low-current stimulation. This suggests that inhibition of SNr cells by low current, high-frequency stimulation through the recording electrode can be used as an additional criterion to identify the demarcation between the STN and SNr, thereby facilitating the determination of the surgical target. The inhibition observed in SNr neurons may be due GABA release evoked by stimulation-induced excitation of GABAergic striatal and pallidal afferents. This has been proposed as a mechanism for similar microstimulation-induced inhibition observed in the internal globus pallidus (Dostrovsky et al. 2000). Supported by CIHR FRN 42505

405 D126

RACLOPRIDE-INDUCED MOTOR LEARNING IMPAIRMENT IN PRIMATES: ROLE OF THE DOPAMINE TYPE-2 RECEPTOR IN MOVEMENT CHUNKING INTO INTEGRATED SEQUENCES

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Results obtained in patients with schizophrenia have shown that antipsychotic drugs may induce motor learning deficits correlated with the striatal type-2 dopamine receptors (D2R) occupancy. Other findings suggest that the role of the striatum in motor learning could be related to a

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

"chunking" process of discrete movements into motor sequences. We therefore hypothesized that a D2R blocking substance, such as raclopride, would affect motor learning by specifically disrupting the grouping of movements into sequences. Two monkeys were first trained to perform a baseline overlearned sequence (Seq A). Then, a new sequence was learned (Seq B) and the overlearned sequence was recalled off-drug (Seq A recall OFF-drug). The effect of raclopride was then assessed on the learning of a new sequence (Seq C), and on the recall of the overlearned sequence (Seq A recall ON-drug). Results showed that performance related to the overlearned sequence remained the same in the three experimental conditions (Seq A, Seq A recall OFF-drug, Seq A recall ON-drug), whether the primates received raclopride or not. On the other hand, new sequence learning was significantly affected during raclopride treatment (Seq C), when compared with new sequence learning without the effect of any drug (Seq B). Raclopride-induced disturbances consisted in performance fluctuations, which persisted even after many days of trials, and prevented the monkeys from reaching a stable level of performance. Further analyses also showed that these fluctuations appeared to be related to monkey's inability to group movements into single flowing motor sequences. The results of our study suggest that dopamine is involved in the stabilization or consolidation of motor performances, and that this function would involve a chunking of movements into well-integrated sequences.

406 D127

INVESTIGATION OF EXPECTATION AND THE PLACEBO EFFECT IN PARKINSON'S DISEASE USING HIGH-RESOLUTION POSITRON EMISSION TOMOGRAPHY (PET) WITH [11C] RACLOPRIDE

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Expectation of therapeutic benefit plays a crucial role in the mechanism of the placebo effect in Parkinson's disease (PD), and has also been shown to increase striatal dopamine (DA) release. We used the ability of [11C] raclopride to compete with DA for D2/3 receptors as measured by PET to investigate DA release associated with expectation strength of levodopa delivery in PD patients. Eleven subjects (mean age 61 years) with mild-moderate PD (mean Hoehn and Yahr 2.2) underwent 3 PET scans on a high resolution research tomograph (HRRT, Siemens) over 2 consecutive days under the following conditions: baseline, following oral administration of 250 mg levodopa, and following placebo administration. Subjects were divided into 4 groups based on their verbal instructions, and were told that they had a 25%, 50%, 75% or 100% chance of receiving levodopa when in fact they all received placebo. Emission data were acquired for 60 minutes following bolus injection of 370 MBq [11C] raclopride (RAC) for each scan, and reconstructed using Ordinary Poisson-OSEM including attenuation, scatter and random correction. Emission data were corrected for motion by inter-frame realignment with automated image registration. Elliptical and circular regions of interest were placed on 9 consecutive transaxial slices (total thickness 10.89 mm), and 6 consecutive coronal slices (total thickness 7.26 mm) in which the dorsal and ventral striatum were best visualized, respectively, as well as on the cerebellum. RAC binding potentials (BP) were estimated using a graphical tissue approach (Logan et al. 1996) with the cerebellum as a reference region. Levodopa administration caused a significant reduction in RAC BP in the putamen bilaterally (p=0.007and p=0.04) indicating increased DA release. Preliminary analysis indicated placebo-induced DA release in the dorsal and ventral striatum in groups with an expectation strength of 50% and greater. In addition, patients who perceived benefit following placebo administration tended to have increased DA release in the dorsal striatum as compared to those who felt no benefit. This dissociation was not seen in the ventral striatum, supporting our earlier results (de la Fuente-Fernandez et al. 2002). These results support striatal placebo-induced DA release in PD. Ongoing work will attempt to extend these findings to a larger sample.

407 D148

CORTICAL AREAS AND CIRCUITS MEDIATING SOUND LOCALIZATION IN THE CAT

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For most mammals the ability to localize a sound in space plays a vital role in the detection and tracking of prey and the avoidance of predators. In the cat, there are 13 recognized regions of acoustically-responsive cortex. In this study, we behaviorally examined the individual contributions of each auditory cortical region to sound localization during both unilateral and bilateral reversible deactivation. Cats were trained to attend to a central visual stimulus and then approach a 100-ms broadband, white-noise stimulus emitted from one of 13 loudspeakers arranged at 15-deg intervals of azimuth. Following training, each cat was bilaterally implanted with cryoloops over different regions of auditory cortex. Unilateral deactivation of primary auditory cortex (A1), the dorsal zone (DZ), the posterior auditory field (PAF), or the auditory field of the anterior ectosylvian sulcus (AES) resulted in severe sound localization deficits confined to the contralateral hemifield, whereas sound localization to positions in the ipsilateral hemifield remained unaffected. Bilateral deactivation resulted in a profound sound localization deficit throughout both hemifields. Neither unilateral nor bilateral deactivation of any other regions yielded sound localization deficits. In addition to the cortical control of sound localization, the superior colliculus (SC) also plays a key role in accurately orienting the head and eyes to acoustic stimuli. Therefore, it is expected that A1, DZ, PAF, and AES must be incorporated into a processing system that transmits signals to the SC. To examine this circuitry, biotinylated dextran amine (BDA) or wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was utilized to reveal the underlying corticocortical and corticotectal acoustic pathways, respectively. SMI-32 antibody was used to identify areal boundaries throughout the auditory cortex and confirm the accuracy of cryoloop placements and tracer deposits. Deposits of WGA-HRP into acoustically responsive SC layers and deposits of BDA into cortical areas PAF and DZ show overlapping cellular and terminal field labeling in AES. The absence of labeling within A1, DZ and PAF from SC deposits eliminates the possibility that the pathway between these areas and tectum is direct. Therefore, these studies of underlying cortical circuitry suggest that the auditory cortex utilizes a multisynaptic circuit to transmit spatial signals to the tectum for accurate orienting to an acoustic target.

408 D128

ANKLE EXTENSOR ACTIVITY DURING STANDING WAS DRAMATICALLY ATTENUATED WHEN THE BIOMECHANICAL NECESSITY OF ANKLE TORQUE WAS ELIMINATED

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The ankle extensors play a dominant role in controlling the equilibrium during bipedal quiet standing. Their primary role is to resist the gravity toppling torque that pulls the body forward. The activity level of the ankle extensors is modulated by the biomechanical necessity of ankle torque, i.e., when the body is located in the forward position, the required ankle torque is large and thus, the muscle activity becomes large. In previous studies that investigated the neural circuits controlling the muscle activity, a back rest supporting the slightly rotated body against gravity has been frequently used

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

to diminish the muscle activity in standing posture. However, considering the abovementioned role of the ankle extensors in standing posture, it is believed that eliminating the need of the ankle torque by providing a backward torque instead of modifying the standing posture by means of the back rest should be more relevant with respect to the attenuation of the activity. The purpose of this study was to demonstrate that the ankle extensor activity was dramatically attenuated when the biomechanical necessity of ankle torque was eliminated. Ten healthy adults stood on a force plate. Electromyograms in the ankle extensors, the ankle angle and torque were measured. In the first trial, the subjects stood naturally. In the second trial, the subjects were supported below both patellas by a ground-fixed device. The device was located at a height level that sufficiently compensated the gravity toppling torque. The whole body was supported via this small device instead of several braces so that this manipulation did not distract each subject's natural posture. In particular, the subjects felt that they were standing naturally, being unaware of any changes in the muscles. As a result of this manipulation of body support, the muscle activities of the ankle extensors were dramatically attenuated and approximately the same as during the resting condition, with the ankle angle set constant between the trials. Thus, we demonstrated that, when the biomechanical necessity of ankle torque was sufficiently eliminated, the ankle extensor activity became negligible for keeping the natural standing posture. Although the neural mechanism of this phenomenon is unclear at the moment, considering the minimum manipulation in the experimental setup compared to the bracing of the joints, this experimental model could be advantageous in investigating the neural mechanism of standing.

409 D129

EXPERIENCE- AND SEX-RELATED DIFFERENCES IN THE INITIAL PERFORMANCE AND LEARNING OF NOVEL VISUOMOTOR TASKS

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Experience and sex-related differences in bimanual coordination have been found in skill performance and in underlying brain activity patterns (Bryden et al., 2005; Haslinger et al., 2004). Established sex-based differences in neural connectivity (Marion et al., 2003) and an elite-athlete's extensive experience performing both practiced and novel complex visuomotor skills would seem to account for these behavioural and functional differences. We related visuomotor skill performance to sex and athletic experience in order to indirectly gain insight into the neural processes that underlie this advanced level of eye-hand coordination. In our assessment of initial performance, elite-level male and female hockey players, recruited from the National Hockey League (NHL) and the National Women's Hockey League (NWHL), were compared to control subjects on novel bimanual and unimanual visuomotor tasks. A modified washer-peg bimanual task required coordinating alternating hands. An ANOVA on bimanual task times revealed a significant main effect of sex. Post-hoc analyses revealed that females performed more quickly relative to males, with elite females significantly outperforming all other groups. In the unimanual task, subjects navigated a cursor around virtual pylons by manipulating a robotic arm in a three-dimensional acceleration-dependent force field. Analyses of the inital performance of this task revealed a significant main effect for experience, with the elite males significantly outperforming all other groups. Interestingly, the initial performances of both elite and non-elite females on this task were virtually identical. We examined this result by extending the unimanual testing protocol to examine the effects of experience on the ability of elite and control females to learn the unimanual task. While elite females learned the task quickly and achieved a higher level of performance overall, the non-elite females achieved significant learning throughout the entire task. These behavioral differences suggest both sex-related and experience-related differences in the neural processes that underlie bimanual and unimanual coordination. These data may reflect recent findings of increased bilateral activity in females during visuomotor skill performance (Gorbet et al., 2007(in press)). Similarly, distinct cortical networks for complex visuomotor control may underlie the effects of experience observed in both initial performance and learning of the unimanual task.

410 D130

THE EFFECT OF DIFFERENT EXPOSURES ON MOTOR LEARNING

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Learning new motor skills and operating off a learned motor program is an integral part of our everyday lives. In order to properly consolidate a new motor program the brain requires a certain amount of practice to the particular stimuli. It has been shown that the brain can learn to correct for altered visual feedback when performing reaching movements. However, in order to properly learn, the brain must receive repeated exposure to a small number of target locations. As the number of training targets increases, the rate of learning subsequently decreases, as does the saturation of the adaptation. After adaptation has occurred the brain can transfer this skill to reach properly to target locations that have not been practiced. But we are limited in our ability to generalize this learned skill to novel target locations (Krakauer et al., 2000). Is the brain able to adapt to altered visual feedback through different training conditions? For instance, can the brain learn when practicing only once to each target in an array? In this study we investigated the question of how much exposure is required to accurately reach to visual targets under altered visual feedback, i.e. a 30º rotation. We also examined the extent to which this learning could transfer to target locations not specifically trained to. Twenty-seven subjects adapted to two separate training conditions, both under the visual distortion: one with repeated reaches to a small number of targets (Multiple exposure) and one with a single reach to many different targets (Single exposure). In the Multiple exposure condition, subjects trained to the same 4 target locations repeatedly. In the Single exposure condition, subjects trained to 81 different target locations only once. After learning to reach accurately subjects were then tested, under the same 30º rotation, to targets that they were not previously exposed to assess the extent of generalization. We found there is very little difference in learning rate between the two different exposure conditions. This suggests the brain is able to learn a visual rotation similarly while being exposed to varying targets a single time and that repeated reaches to the same target locations is not necessary. We also found the generalization of learning to new target locations between the two conditions to be very similar. This again suggests the brain can consolidate and apply this learned motor skill to new situations through both of our exposure conditions.

411 D131

BI-DIRECTIONAL SYNAPTIC PLASTICITY IN PALLIDUM OF CERVICAL AND GENERALIZED DYSTONIA PATIENTS

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Aberrant forms of synaptic plasticity may underlie the pathophysiology of dystonia, a movement disorder characterized by sustained muscle contractions and abnormal postures. The internal segment of the globus pallidus (GPi) is the primary somatomotor output of the basal ganglia and tonically inhibits corticothalamic and brainstem premotor circuits. The remarkable efficacy of GPi high frequency stimulation (HFS) for surgical therapy of dystonia suggests that aberrant plasticity at the output of basal ganglia may be involved. Synaptic plasticity in GPi was induced by HFS in awake patients with generalized (whole body) and cervical (neck only)

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

dystonia. Dual microelectrode wide band (10 - 5kHz) recordings were made in one cervical and one generalized dystonia patient undergoing implantation of deep brain stimulation electrodes in the GPi. Evoked field potentials (eFPs) were recorded while stimulating with single pulses (50uA, 0.3 ms biphasic pulses, 1 Hz) from the second electrode located about 1 mm away at the same dorsoventral level. After establishing a stable baseline, standard tetanizing HFS (100 Hz, 2 s trains repeated 4 times at 10 sec) was performed and the effects on eFP amplitudes were measured for up to 5 minutes. Then low frequency stimulation (LFS, 20uA, 2 – 5 Hz, 10 – 20 s) was given through the recording electrode to test for post-synaptic depression of the eFP amplitudes. A final HFS confirmed the range of bi-directional plasticity. HFS potentiated eFPs by similar degrees in both groups (gen. by 65%, cer. by 71%), while LFS depressed eFPs in generalized dystonia to a much greater extent than in cervical dystonia (eFP amplitude ranges of 106% and 19% respectively). The final HFS potentiated both groups to pre-LFS levels. Patients with cervical and generalized dystonia demonstrate similar induction but different reversal of plasticity, indicating an asymmetric bi-directional plasticity in GPi neurons. These preliminary results suggest that more severe forms of dystonia are associated with greater post-synaptic depression of GPi neuronal responsiveness.

412 D132

RAPID EMG AND CORTICAL RESPONSES TO PERTURBATIONS ARE MODIFIED BY VISUO-SPATIAL GOALS

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Previous studies have shown that upper-limb reflexes are task-dependent, however, the extent of reflex flexibility remains unknown and largely unlinked to theories of motor function. To further explore the task-dependency of reflexes, we have created a multi-joint paradigm where human or non-human primates respond to sudden arm perturbations by quickly placing their hand into a visually-defined target. Subjects were shown a target located medial or lateral to their hand while they countered a constant background load. After a random hold time, a step-torque displaced the hand either into the target area (requiring no correction) or out of the target area (requiring rapid correction). Hence, we were most interested in the differences across muscle/cortical responses for the same imposed perturbation but different spatial target locations. Human EMG responses recorded from arm flexors and extensors exhibited a three-peaked series of reflex activity with increasing sensitivity to target position. The earliest reflex responses (20-50ms) were determined entirely by perturbation direction regardless of target position. In contrast, responses between 50-75ms were evoked by perturbation direction but scaled by target position and responses from 75-100ms were often evoked or suppressed entirely by target position. Motor cortical (M1) neurons recorded from non-human primates reveal that even the earliest cortical responses (20-50ms) are modulated by target position, indicating that M1 processing is influenced by task goals and suggesting that M1 activity underlies the task-dependent EMG differences observed in humans. Future experiments will explore whether rapid EMG and cortical responses are tuned to more complex visuo-spatial features.

413 D133

THE PERCEPTION OF TIME IS INFLUENCED BY LOW-LEVEL CHARACTERISTICS OF VISUAL MOTION

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Our perception of the duration of a visual stimulus does not always corroborate with the actual time the visual stimulus is presented. It has been shown that an oddball stimulus observed after a train of repeated stimuli appears to be longer in duration. This effect is referred to as the subjective expansion of time. However, most previous studies used complex visual shapes, which made it difficult to identify specific brain mechanisms that may underlie this perceptual effect. Therefore, we were interested to know if this subjective expansion of time can occur for a low-level visual stimulus such as linear motion. Because visual cortical neurons are stimulated by a preferred direction of motion, we asked how does this contrasting population activity contribute to our perception of time. Using a random dot kinematogram (75% coherent motion pulses of 200 ms separated by random motion of 400 ms), subjects reported the duration of an oddball motion direction that followed a series of standard motion pulses. The oddball motion pulse moved in one of five directions relative to the standard pulses and varied in duration relative to the duration of the standard pulse trains. The resultant psychometric curves show different magnitudes of time expansion for different oddball directions. In addition, the results suggest that changing the expectancy of an oddball direction based on the pattern of standard pulses affects the subjects' perception of time. Together, these results propose a link between neuronal activity in areas of visual cortex that encode a low-level motion stimuli and our subjective perception of time.
Supported by operating grants from CIHR and NSERC.

414 D134

VISION DOES NOT RECALIBRATE OUR KINESTHETIC SENSE OF HAND POSITION

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During motor learning, multiple senses interact to produce desired movements. When faced with inconsistent sensory feedback, people subconsciously use context and modality specific weightings to determine the optimal use of this sensory information. We know that vision plays a dominant role in motor learning and can even override other senses: if the hand is represented by a cursor and this visual feedback is rotated 30˚ counterclockwise (CCW), people will adapt their movements to move up and to the right in order to produce seen movement of the cursor straight forward, despite conflicting visual and kinesthetic information. Even in complete darkness, people continue to make these adapted movements due to miscalibration of the arm motor system. Currently it is not known whether the visual recalibration of the arm motor system also recalibrates the kinesthetic sense of hand position. Here, we investigated whether learning to reach with the left hand with altered visual feedback of hand position (visuomotor adaptation) affected subjects' ability to locate their unseen left hand with their right hand. To measure subjects' kinesthetic sense of left hand position, we used a localization task where subjects grasped a robot manipulandum underneath a tinted surface so their left hand was not visible. The robot was programmed to restrict movements to a single angle along a 'kinesthetic' wall. Subjects would move their unseen left hand to the end of this wall, and then point to its felt position with their right hand. We found that subjects generally overreached targets (unseen left hand) along the direction of extent. We then fitted confidence ellipses to reaches for each target and found that all ellipses were oriented towards the subject. For the visuomotor adaptation, subjects adapted their left hand to rotated visual feedback of 30˚ CCW, which was introduced gradually so that they wouldn't be aware of this adaptation. Following adaptation, we retested subjects' kinesthetic sense of left hand position, and found that there was no difference in localization errors, suggesting that while vision may recalibrate the motor system of the arm to ensure consistency across senses, it does not recalibrate arm kinesthesia. However, we found that the orientations of the ellipses were significantly different, suggesting that following adaptation, the trajectories taken to targets may have changed.

415 D135

UPDATING SPATIAL MEMORY ACROSS DIFFERENT EYE MOVEMENTS FOR ARM MOTOR CONTROL

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We use visual information about our surrounding environment in order to generate movements to objects within that environment in our daily lives. It has been well demonstrated previously that when an object is selected as the target of an action, its location is stored in an eye-fixed frame (Henriques et al, 1998). Given that the eyes move frequently (i.e., ~ 4 -5 times per second), these eye-fixed spatial representations must be updated for the arm-motor system. Other studies have shown that eye-fixed spatial updating applies also to targets at different distances (Medendorp et al., 2002), to tactile and auditory targets (Pouget et al., 2000), and to implicit targets defined by expanding motion patterns (Proljac & van den Berg, 2003). But spatial updating for arm control has not been demonstrated for other types of eye movements (i.e., smooth-pursuit). The goal of our study is first to determine if the remembered locations of visual targets are updated following a smooth-pursuit eye movement as they have been found to following a saccade. And second, to determine if visual information plays a role in estimating eye movements for updating spatial memory. Participants (N=8) pointed to remembered targets under three separate conditions. In the first, participants viewed a briefly flashed target and then looked to one of seven locations after the target disappeared, by either making a saccade or a smooth-pursuit. They then pointed to the remembered location of the original target. The second condition was identical to the first, but, had a series of dots above and below the stimulus which moved in either the same or opposite direction as the eyes. The third condition was similar, but, required no eye movements, allowing us to glean the impact of the moving background on estimating eye motion. Participants always pointed in complete darkness without visual feedback. We found no differences in pointing responses following saccades and smooth-pursuits, but found that when the background moved in the opposite direction of a smooth-pursuit, pointing responses did differ from those made when the background moved in the same direction as the eye or was absent. This suggests that the location of a visual target is updated following a smooth-pursuit movement of the eyes, and that visual information plays a role in estimating eye movements for updating spatial memory when smooth-pursuits are made but not when saccades are made.

416 D136

RAPID CONSOLIDATION OF MOTOR MEMORY IN THE VESTIBULO-OCULAR REFLEX

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Newly acquired motor memory is relatively labile, and susceptible to disruption, but can become more resilient through consolidation. We investigated the time course of consolidation of motor memory in the vestibulo-ocular reflex (VOR). Learned decreases were induced in the gain of the VOR by 60 minutes of passive sum-of-sines rotation of cats wearing telescopic miniaturizing lenses. We then attempted to disrupt the new memory using rotation in darkness. If rotation in darkness immediately followed learning, disruption was successful; the VOR gain increased towards its pre-learning value. For 2-Hz rotation, if immediately after learning the cat spent an intervening 30- or 60-minute period stationary without form vision ("neutral period"), the subsequent disruption was considerably less effective, suggesting that memory had consolidated. However, at 0.5 Hz, we did not consistently observe consolidation on this short time scale. We conclude that the newly formed memory in the VOR is initially labile, as they are in other systems. However, VOR motor memory can consolidate rapidly if the learning process is stopped. The process of consolidation may depend on the test frequency.

417 D137

MOTOR SEQUENCE LEARNING AND D2 RECEPTORS

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Motor learning disturbances have been shown in clinical conditions characterized by a striatal dopamine dysfunction, such as in Parkinson's disease or patient with schizophrenia treated with antipsychotic agents. In monkey (Cebus Apella), our group (Lévesque et al., 2007) has shown some specific motor learning deficits following the systemic administration of raclopride, a selective D2-receptor antagonist. These deficits were mainly characterized by a lack of stabilization that is persistent fluctuations of performance from trial to trial, suggesting a difficulty in consolidating the motor sequence. Moreover, these fluctuations were found to be related to a difficulty in chunking new movement components to old and well established ones. These specific deficits were not observed following the administration of a D1-receptor blocking agent. In order to further assess the role of dopamine in the chunking process of motor sequence learning, we verified the reversibility of the raclopride (0,05 mg/kg) induced deficit, by administering sumanirole (1 mg/kg), a highly selective D2 agonist agent. Two monkeys were trained to execute an overlearned sequence, that was recalled under no drug effect (baseline), under raclopride effect, and under sumanirole effect (administered 15 minutes after raclopride). The same was also done with a new sequence that has to be learned without drug (seq 2), under the raclopride effect (seq 3), and under sumanirole (seq 4). Results showed a raclopride-induced disturbance for the new sequence learning (seq 3) but not for the overlearned one (seq 1). As previously demonstrated (Lévesque et al., 2007), these deficits where characterized by persistent fluctuations of performance from trial to trial (even after hundreds of trials), and by a deficit in chunking a new movement to an old and well established one. The reversibility of these effects with sumanirole will be presented.

418 D138

GRASPING THE FUNCTION OF TOOLS: FMRI SUGGESTS THAT THE VENTRAL BUT NOT THE DORSAL STREAM CODES THE FUNCTIONAL SIGNIFICANCE OF FAMILIAR OBJECTS

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When grasping-to-use a familiar tool we often make use of stored information about the functional identity of the tool in order to guide our actions. At present, exactly which brain areas play an important role in representing this information is poorly understood. For example, is information regarding the functional significance of familiar objects stored within those areas mediating object-directed actions or does this information stem from other areas of the brain? During functional magnetic resonance imaging (fMRI), participants viewed short movies depicting different types of grasping actions made towards familiar tools. In one condition, the tool was grasped appropriately such that its function could be performed without further postural adjustments (e.g. a fork was grasped by the handle with the tines facing away from the actor). In the other condition, the tool was grasped inappropriately such that its function could not be performed without further postural adjustments (e.g. a fork was grasped by the handle with the tines facing toward the actor). We hypothesized that the viewing of functionally appropriate grasping actions would resonate more strongly with those areas involved in the processing of object function. Our results showed significantly stronger activity for the viewing of functionally appropriate as

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

compared with inappropriate grasping actions within several brain areas previously implicated in higher-level object perception. In contrast, areas implicated in the control of object-directed actions did not distinguish between the two conditions. These findings suggest that information about the functional significance of objects is stored within areas specialized for object perception and not within areas specialized for object-directed action. Thus, object utilization based on stored knowledge of object function must involve a complex interplay between systems specialized for object perception and those specialized for the control of actions.

419 D139

ROLE OF HUMAN PPC IN THE INTEGRATION OF INITIAL HAND POSITION INFORMATION INTO THE REACH PLAN

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Lesion and transcranial magnetic stimulation (TMS) studies in humans implicate the posterior parietal cortex (PPC) for the planning and control of actions. We previously reported that TMS reveals a hemispheric asymmetry in the early stages of the putative spatial processing in the human PPC. Moreover, we postulated that this brief TMS pulse modifies the output of the right PPC in motor coordinates, rather than modifying the upstream visual coordinates of the memory representation (Vesia et al., J. Neurophysiol, 2006). Alternately, TMS could have altered the state estimate of the initial hand position. To test this hypothesis, we investigated the memory-guided pointing accuracy in six subjects during TMS of the left and right PPC while varying visual information of the effector. We tested four conditions: 1) initial and final vision of hand positions (IFV); 2) mid and final vision of hand positions (MFV); 3) final vision of hand position (FV); and 4) full vision of hand position (IMFV). In accordance with previous findings, subject's pointing errors and biases varied as a function of stimulation site during the FV condition - left parietal stimulation significantly increased endpoint variability, while right parietal stimulation produced a significantly systematic leftward directional shift in both visual fields during the FV condition. In addition, these systematic pointing errors and biases significantly decreased during the IMV, IFV, and IMFV conditions. These data causally demonstrate the important role of the human PPC in the formation of internal 'forward' state estimates that may be used to recursively plan and control visually-guided reaching movements.

420 D140

SEROTONINERGIC INNERVATION OF HUMAN BASAL GANGLIA

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Serotonin (5-hydroxytryptamine, 5-HT) occurs in most major brain structures and is involved in various state-dependent activities, and in a wide range of cognitive and behavioral functions, including the control of movement. The basal ganglia are motor-related structures that receive a particularly dense 5-HT innervation, but the role that this neurotransmitter plays in this complex set of nuclei in both normal and pathological states in poorly known. Although the 5-HT innervation of basal ganglia has been the subject of detailed studies in rodents and monkeys, the pattern of 5-HT innervation of human basal ganglia has received very little attention. We have addressed this issue by applying an immunohistochemical approach to postmortem human brain material gathered from normal individuals. We used specific antibodies directed against the 5-HT transporter (SERT) and the 5-HT synthesizing enzyme (tryptophane hydroxylase) to visualized, respectively, axons and cell bodies that contained 5-HT. Some section were also immunostained for tyrosine hydroxylase to compare the location of 5-HT and dopaminergic (DA) axons and terminals. Human basal ganglia are principally innervated by 5-HT axons that emerge from the dorsal and median raphe nuclei. These axons formed distinct fascicles that fragmented themselves as they penetrate the decussation of the superior cerebellar peduncles. They regroup within the ventral tegmental area and ascend along the medial forebrain bundle immediately below the DA ascending fibers. At regular intervals along their ascending course, axons detach themselves from the main bundle and sweep laterally to arborize within the various basal ganglia components. The 5-HT innervation of these nuclei is highly heterogeneous; the caudate nucleus is poorly innervated by comparison to the putamen and globus pallidus, whereas the 5-HT innervation of the subthalamic nucleus displays a mediolateral decreasing gradient. At variance with the situation in monkeys, 5-HT terminals in the human striatum are not distributed according to the patch/matrix organization and 5-HT axons do not form bands at pallidal level. This study has delineated the pattern of the 5-HT innervation of human basal ganglia, the knowledge of which is essential to correctly interpret the complex neurochemical changes that occur within these nuclei in neurodegenerative diseases. [Supported by grant MT-5781 of the Canadian Institute for Health Research].

421 D141

THE PERCEIVED TIMING OF THE ACTIVE AND PASSIVE COMPONENTS OF TOUCH

Rebecca Winter, Marta Gozdzik*

Tactile stimulation usually occurs as a result of an active movement (e.g., reaching out to touch a surface) and a passive sensation (feeling the surface against the skin). The brain knows about the active component (a motor command) before it has even occurred through efferent copy, while the passive component must be processed. Since the timing of the two components are very different, determining the time of the touch requires either backwards calculation from the passive sensation, and/or worked forward from the active motor command. In order to determine which process is responsible for the perceived temporal properties of touches, we varied the relative delay between the two touch signals and determined the delay regarded as simultaneous. Since the perception of simultaneity between two stimuli can be affected by repeated exposure to asynchronous presentation, we exposed subjects to an active key press with a passive touch delayed by 250 ms. This caused subjects to accept a wider range of inter-stimulus delays as simultaneous; was this due to altering the active or passive component? We tested the two components separately against a common stimulus. The results are discussed in the context of simultaneity constancy during the perception of active movement.

422 D142

ACTIVE AND PASSIVE ESTIMATES OF FELT HAND PATH ARE NOT RECALIBRATED BY VISION

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Motor control relies on multiple sources of information. For instance, to estimate the location of our hand, the brain uses both vision and body-position sense. And performance is better when more than one sensory modality is present, i.e., we can reach more accurately when we can both see and feel our hand. But in cases of sensory conflict, that is when sensory modalities disagree, one sense may reshape a motor system to ensure online consistency across the senses. Although the online consistency is important for controlling and adapting movements, it may be that the recalibration that produces this consistency does not persist when the conflict no longer exists, as in the case when the dominant sense (e.g., vision) is removed. In this experiment, we tested whether vision merely overrules kinaesthesia or whether it recalibrates our felt sense of hand path. We adopted a task

developed by Henriques & Soechting (2003) for measuring kinesthetic sensitivity of hand path geometry. We began by mapping out subjects' sensitivity to circles by having them actively trace elliptic contours while gripping the handle of a programmable robot and estimate whether the ellipses are tall or wide. This was followed by a visual adaptation task where subjects learned to move their unseen hand along an elongated ellipse to transport a cursor along a circle. We then quantify whether this sensitivity becomes biased after motor commands are calibrated by false visual feedback. Our data show that subjects' sense of circularity does not become biased in the direction of the distortion. That is, after learning to make tall elliptic hand paths to follow a seen circular path, they did not mistake tall ellipses for circles. In a second experiment, we tested whether subjects' sense of circularity is even more vulnerable to visual calibration when subjects judge whether ellipses are tall or wide after having their hand passively moved by the programmed robot, given that estimates of passive hand motion are less accurate than those of active motion. Our results show that subjects' sense on passive hand motion remained did not change after adapting to false visual feedback. The above results are consistent with our pervious experiment measuring the effect of visual adaptation on kinaesthetic sensitivity for different hand path geometries (i.e. tilt and curvature), and strongly demonstrated that subjects' kinaesthetic sensitivity does not recalibrate after false visual feedback.

423 D143

MOTOR CORTEX CORRELATES OF POSTURE AND MOVEMENT IN INSTRUCTED-DELAY REACHING TASK IN CATS

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Reaching movements in cats are preceded by anticipatory postural adjustments (pAPA) to compensate for movement-evoked postural disturbances. During reaching the supporting limbs also produce postural adjustments accompanying the movement (aAPA) to ensure further postural stabilization of body. Thus, the neural system responsible for the control of reaching integrates both movement and posture-related signals to coordinate the execution of the movement. In the present study, we tested the hypothesis that the motor cortex contributes not only to the execution of the movement, but also to the APAs that precede them. We trained three cats to perform a task in which they were required to stand quietly and then reach to and press on a lever with either the left or right forelimb for a food reward. Following an instruction tone of random duration (0.5 - 1.5 s), the cats reached forward and depressed the lever with the instructed limb. During the task single neurons were recorded from the motor cortex with conventional microelectrodes mounted in a mechanical microdrive attached to the cranium. Electromyographic activity (EMG) was recorded from the major contralateral forelimb muscles and selected muscles of the hindlimbs and the ipsilateral forelimb. Kinetic and kinematic parameters were recorded using force sensitive platforms and a motion capture system. We recorded 219 motor cortical cells of which 82% were pyramidal tract neurons (PTNs). The database of cells included 38% (84/219) that showed an initial change in activity that was better related to the Go signal (stimulus) than to the movement produced by that stimulus. All of these neurons had a subsequent period of activity that was better correlated to the movement. In a previous study (Schepens and Drew 2004) we have argued that cells in the brainstem reticular formation with activity time-locked to the Go stimulus contribute to the production of the APAs preceding movement. In agreement with this view we found that the discharge activity of the Go-related cells in this study was also strongly related to the temporal characteristics of the force and EMG changes occurring during the pAPA. These results provide support for the view that the motor cortex contributes to the anticipatory postural adjustments that precede reaching in addition to the movement itself.

424 D144

EFFECTS OF GESTATIONAL SEROTONIN DEPLETION ON FUNCTIONAL DEVELOPMENT OF PAIN DESCENDING PATHWAYS

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Serotonin (5-HT) regulates cell differentiation during development of the central nervous system. The aim of this study was therefore to determine the effects of 5-HT depletion, on postnatal pain sensitivity, during neurogenesis of the brainstem nuclei involved in descending pain modulation. Gestational rat Sprague-Dawley dams were injected with para-chlorophenylalanine (pCPA, 400 mg/kg, ip) at embryonic day (E) 10 and E14 to deplete serotonin during neuronal proliferation of descending pathways and axonal migration, respectively. Neuronal proliferation was assessed injecting bromo-deoxyuridine (BrdU) at E12. Offspring acute pain was tested with thermal plantar test, measuring fore- or hindpaw withdrawal latency at postnatal day (P) 7, P14 and P21. Formalin test was used at P25 to evaluate the effects of 5-HT depletion on persistent pain. Nociceptive responses to formalin administration were divided in 3 phases: acute (phase I), interphase and late (phase II). Immunohistochemistry staining was performed for tryptophan hydroxylase, dopamine beta-hydroxylase and BrdU, coupled with unbiased stereology to count immunoreactive neurons in the raphe dorsalis, raphe magnus and locus coeruleus. Control pups tested for thermal pain were less sensitive for the forepaw than the hindpaw only at P7 and the response latency increased at P14 and P21 compared to P7. Following pCPA injections at E10, the forepaw becomes less sensitive than the hindpaw at P14. When pCPA was injected at E14, latency responses of both paws increased in comparison to controls at P14. pCPA treatments did not alter acute thermal pain responses at P21. In formalin test, pCPA E10 pups pain score was lesser than controls in phase II and males showed less pain in interphase. No significant change was observed for any E14 pCPA groups. An increase in immunoreactive neurons, by stereology analysis, will support the nociception decrease observed in behavioural assessments. In conclusion, targeting proliferation period of descending pain pathways with gestational 5-HT depletion diminishes both acute and tonic pain. However, 5-HT depletion during axonal migration only reduces acute pain. Since changes in thermal pain were seen only at P14, we suggest that the decrease in acute nociception caused by 5-HT depletion results from the post-natal maturation of the descending pathways. Supported by CIHR, CRSNG and FRSQ.

425 D145

MULTISENSORY CONVERGENCE IN THE PERIPHERAL FIELD REPRESENTATION OF PRIMARY VISUAL CORTEX OF THE CAT

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Sensory systems do not operate in isolation and must have the ability to influence each other. On a behavioral level, we have been examining the influence of auditory cortex on basic visual functions. Therefore, we are interested in identifying possible pathways that may serve to mediate these interactions. In adult cats (>6 months), sources of auditory cortical projections to primary visual cortex (areas 17 & 18) were studied using injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into area 17 or areas 17 &18. Two groups of animals were studied. In the first group, multiple injections into the representations of both the central and peripheral visual fields were made on the vertical meridian representation along the border between areas 17 and 18. In agreement with previous studies, characteristic patterns of cell body labeling were identified in extrastriate visual cortex (areas PMLS, PLLS, AMLS, 19, 20, & 21) and the visual thalamus (LGN, MIN, & LPl), thus confirming the efficacy of the tracer injections. Labeled neurons were also identified in the visual area of

the anterior ectosylvian sulcus (area AEV). In auditory cortex, of the four tonotopically-organized regions, labeling was identified in the supragranular layers of the posterior auditory field (PAF). Little or no labeling was evident in the primary auditory cortex (AI), the anterior auditory field (AAF), or the ventral posterior auditory field (VPAF). Furthermore, little or no labeling was identified in the remaining nine generally-recognized regions of auditory cortex. In the second group of cats, single injections were made into the central or peripheral field representations of area 17 or areas 17 &18. Between the two groups, while similar labeling patterns were identified in visual cortex, labeled cells were only identified in PAF following injections into regions of primary visual cortex representing peripheral, but not central, visual field representations. Therefore, in auditory cortex, while no projections originating in A1 could be found to terminate in primary visual cortex, projections were identified from non-primary PAF. Furthermore, these acoustic projections specifically terminate in the peripheral field representations of primary visual cortex.

426 D146

VISUAL AND TACTILE INTERACTIONS DURING VISUALLY GUIDED POINTING

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Electrophysiological studies have reported the existence of visual-tactile bimodal cells in parietal, premotor, and subcortical areas in the macaque monkey. Many of these cells have tactile receptive fields on the hand and face with overlapping visual receptive fields that extend into nearby space. The additional recruitment of bimodal cells by visual stimuli presented near the hand may account for human behavioural findings of enhanced visual processing of stimuli near the hand. This study investigates whether areas on the hand with higher tactile receptor densities (glabrous skin of the palm) benefit from greater bimodal cell representation relative to areas with lower tactile receptor densities (hairy skin on the back of the hand). We predicted that such a difference in bimodal cell representation would result in better visual processing of stimuli near areas with higher densities of tactile receptors compared to areas with lower densities of tactile receptors. We tested this idea by asking healthy undergraduates to perform rapid pointing movements to targets projected onto the palm or onto the back of a left hand. On some trials, the left hand was their own, but on other trials, the left hand was a realistic replica. Targets were presented visually only and had no tactile dimension. We found that subjects pointed more accurately to targets presented on the palm of the real hand than to targets presented on the back on the real hand, but that there was no such difference for the fake hand. We also found that pointing to the real hand was more accurate than pointing to the fake hand. The greater accuracy of pointing movements to targets presented on the real hand compared to the fake hand is consistent with reports of enhanced visual processing of stimuli presented near the body. This advantage may be due to bimodal cell recruitment by visual stimuli near the hand. Also, the higher accuracy of pointing movements to targets on the palm of the subject's real hand relative to on the back of their hand may reflect greater bimodal cell representation of areas of the body with higher tactile receptor density.

427 D147

FAILURE TO COMPENSATE FOR LIMB MECHANICS IN PROPRIOCEPTIVELY-GUIDED SACCADES

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Does the saccade generator have a complete internal model of limb mechanics? In our previous paper (Ren et al. 2006), anisotropic errors were observed during saccades to handheld targets, suggesting that the saccade generator does not have a complete internal model of limb mechanics. Here, we tested this hypothesis by measuring saccades to targets held in both the left and right hand, varying the initial posture of the body and tilting head position leftward or rightward. All experiments were done in a dark room. Saccades and hand movements were measured using search coils and an Optotrak device respectively. 8 radial target locations on a frontally placed table were used in five different paradigms: 1) right hand (body and head centered); 2) left hand (body and head centered); 3) right hand (body left); 4) right hand (head roll contra-clockwise); 5) right hand (head roll clockwise). The anisotropy of saccade errors reversed symmetrically with right vs. left hand, and tilted with body left rotation, but did not change with head roll rotations. This supports our hypotheses that the anisotropy of the hand-guided saccade errors is mainly due to the incomplete compensation in the saccade generator for the anisotropy of limb mechanics.

TECHNIQUES IN NEUROSCIENCE

428 B302

APPLICATIONS OF FUNCTIONAL NEAR INFRARED SPECTROSCOPY (fNIRS) TO PSYCHIATRIC AND NEUROLOGICAL DISORDERS

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Functional near-infrared spectroscopy (fNIRS) is an emerging functional neuroimaging technology that offers a relatively non-invasive, safe, portable, and low-cost method of both indirect and direct monitoring of brain activity. Most exciting is its potential to allow more ecologically valid investigations that can translate laboratory work into more realistic, everyday settings and clinical environments. The purpose of this poster is to acquaint clinicians and researchers with the unique and beneficial characteristics of fNIRS by reviewing the relative merits and limitations of this technique vis-à-vis other brain-imaging technologies such as functional magnetic resonance imaging (fMRI). Cross-validation efforts between fMRI and fNIRS and the possible hesitations for its deployment in clinical research and practice will be discussed. Finally, because there is no comprehensive review of the applications of fNIRS to brain disorders, the findings from the few studies that have utilized fNIRS to investigate neurocognitive processes associated with neurological (Alzheimer's Disease, Parkinsons' Disease, epilepsy, traumatic brain injury) and psychiatric disorders (schizophrenia, mood disorders, anxiety disorders) will also be reviewed. The potential for fNIRS to provide more ecologically-valid indices of brain function during these investigations will be highlighted throughout.

429 B301

SPATIOTEMPORAL INVESTIGATION OF HIPPOCAMPAL ELECTRICAL ACTIVITY USING ADAPTIVE FILTERS

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A large area of research is currently focused on the development of signal processing tools able to quantify the level of association, synchrony and/or commonality within and between different regions of the brain, as the initiation, maintenance and spread of electrical activity in the brain is still not well understood. Traditional tools have provided effective analysis options, but are constrained by limitations, such as stationarity conditions, time-frequency resolution and the inability to identify non-concurrent

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

commonalities arising from shifts or delays in signal conduction. We are proposing the application of adaptive filters for tracking of spatiotemporal commonalities of electrical activity in the brain. Adaptive filters are advantageous in system identification, as they are able to self-adjust their frequency response with time, as a function of the input signal. We use an adaptive filter model with the least mean square (LMS) optimization algorithm, to track the changes of the frequency response of the system. Here we have implemented our adaptive filter model to characterize the short term changes from four simultaneous extracellular field recordings in the CA1 region of the intact mouse hippocampus under control conditions and in a low-Mg2+ epilepsy model. The adaptive filter system provided the input-output response of our model temporally, across individual channels, and spatiotemporally, between neighbouring recording sites. The development of effective signal processing tools-which aid in characterizing the spatiotemporal spread of electrical activity in the brain-would lead to a deeper understanding of cellular and network function during normal and pathological neuronal processes.

430 B303

AN INJECTABLE DRUG DELIVERY SYSTEM FOR THERAPEUTIC AGENTS TO THE INJURED SPINAL CORD

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After the initial impact of a traumatic spinal cord injury (SCI), a secondary injury occurs that leads to further tissue degeneration and greater neurological deficit. Current drug administration for this condition is limited by the inability of drugs to cross the blood-spinal cord barrier (BSCB), as well as side affects from high dosage required to reach the site of injury. We have developed a biopolymer blend of hyaluronan and methylcellulose (HAMC) as an injectable drug delivery system that can be administered in the intrathecal space at the level of SCI. Previous work with this material shows that it is safe for in vivo use, and further work is currently being performed to characterize material degradation, drug release, and in vivo utilization of this as a drug delivery system (DDS). The degradation properties of the HAMC material were investigated with Spraque Dawley rats. Prior to injection, HA was labelled with Flourescein and MC with Texas Red. After a laminectomy at T1-2, the HAMC blend was injected into the intrathecal space. Tissue was harvested over 5 days, frozen and cut into 2mm longitudinal sections. Analysis suggests that HA degrades faster than MC in the intrathecal space, and MC is visible for up to 3 days after injection. To investigate the efficacy of HAMC as a DDS a model protein, erythropoietin (EPO), was chosen due to its potential as a neuroprotective drug. An in vitro ELISA assay was used to determine the temporal release profile of EPO from HAMC. Results showed sustained release of EPO from HAMC occured over a 16hr period. A TF-1 cell line responsive to EPO was used to ensure that bioactivity was maintained after release from HAMC. Following this characterization, an in vivo study has been performed to investigate the efficacy of EPO delivered with HAMC into the intrathecal space. Following a 35g clip injury at T1-2, one of four injections was performed: 1) intrathecal injection of HAMC, 2) intrathecal injection of HAMC containing EPO, 3) intrathecal injection of EPO, or 4) intraperitoneal injection of EPO. Behavioural analysis with BBB and grid walking was performed over 6 weeks, and subsequently tissue was harvested. Immunohistochemistry was performed on frozen, sectioned tissue to investigate inflammation, cavity volume, and neural sparing. Results suggest that EPO is capable of crossing the BSCB, and there was limited benefit for local delivery of EPO to the injured spinal cord.

431 B304

TRANSPLANTATION OF BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS AS A SCAFFOLD FOR ADULT NEURAL STEM/PROGENITOR CELLS IN THE INJURED ADULT RAT SPINAL CORD

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Transplantation of adult rat neural stem/progenitor cells (NSPCs) into the injured spinal cord results in neural differentiation but poor survival. In contrast, bone marrow derived mesenchymal stromal cells (BMSCs) survive well, but do not differentiate into cells of neural lineage. The purpose of these experiments was to examine the survival and fate of NSPCs after transplantation into the injured adult rat spinal cord 9 days after transplantation of a BMSC scaffold. Functional neurological recovery was also examined at 12 weeks. BMSCs were cultured from adult male rats and transplanted immediately after clip compression injury (27g force). NSPCs were cultured from either the brain or the spinal cord of rats expressing enhanced green fluorescent protein (eGFP) and transplanted 9 days later. Controls included transplantation of BMSCs only, NSPCs only, and culture medium. Rats were sacrificed at 12 weeks after NSPC transplantation. Immunohistochemistry was used to identify cell survival and fate. Functional neurological recovery was also studied with weekly locomotor and ladderwalk testing over a 12 week period. A significant functional recovery was seen in rats receiving spinal cord derived NSPCs only. Rats receiving either BMSCs alone, or both BMSCs and NSPCs did not show functional recovery. Cell survival of BMSCs was better than NSPCs in all groups, and transplantation of the BMSC scaffold did not affect either the survival or fate of the NSPCs. BMSCs did not differentiate into cells of neural lineage and produced a large amount of collagen. NSPCs differentiated mainly into astrocytes and oligodendrocytes, and were seen in association with both axons and myelin. Transplantation of adult rat spinal cord derived NSPCs can provide a significant functional improvement in a 27g clip model of spinal cord injury. The strategy of BMSC transplantation to provide a biological scaffold for the BMSCs did not result in a functional improvement, and the BMSC scaffold attenuated the functional recovery seen with NSPCs alone. This may be the result of the large amount of collagen produced which may contribute to the scar at the site of injury.

432 B305

FNIRS MEASUREMENT OF CEREBRAL BLOOD FLOW IN A SUBJECT WITH UNILATERAL LESION OF THE LEFT FRONTAL LOBE

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Functional near infrared spectroscopy (fNIRS) is an emerging brain imaging technology that permits 24/7 ambulatory non-invasive monitoring of functional cerebral blood flow during the performance of motor and cognitive tasks. fNIRS measures relative changes in oxygenated (oxy-Hb) and deoxygenated (deoxy-Hb) hemoglobin using infrared light that is differentially absorbed by biological tissues in the range of 700-1000 nm. While some studies have inspected the validity of fNIRS by comparing its signals with the blood-oxygen level dependent signal of fMRI, no investigation has examined the validity of fNIRS as it relates to measurement of hemodynamic activity in lesioned cortical regions. The aim of the present study is to examine functional cerebral blood flow using fNIRS in a 59-year old right-handed subject with a discrete evacuated subdural hematoma in the dorsolateral region of the left frontal lobe extending to insula and left temporal pole. The 18 cm ? 6 cm ? 0.8 cm probe consisting of four light sources surrounded by 10 photo-detectors was placed in two separate

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

positions during testing. The first position was over standard EEG placements F7, Fp1, Fp2, and F8, and the second position was just anterior to placements C3, Cz, and C4. Testing was repeated after 21 days. During scanning, the subject completed three tasks: Valsalva maneuver, verbal (phonemic) fluency, and right- and left-hand finger tapping. The verbal fluency task resulted in significant increases in oxy-Hb relative to rest in the spared left medial area of the prefrontal cortex (Brodmann's area 9). Finger tapping with the right hand activated more voxels in the ipsilateral premotor area than in homologous but lesioned contralateral cortical regions. The results suggest that fNIRS provides valid measurements of hemodynamic activity in cortical areas associated with generation of words and sequential finger tapping. In line with expectations of attenuated signal in lesioned cortical regions, fewer voxels were activated in lesioned areas of the left frontal lobe during simple motor movements. Overall, the findings provide preliminary support for the validity of fNIRS as a measure of hemodynamic activity for structurally intact regions of the cortex.

433 B306

PREDICTORS OF HEAD COMPUTED TOMOGRAPHY USE FOR PATIENTS WITH MILD TRAUMATIC BRAIN INJURY IN THE PRIMARY CARE SETTING

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OBJECTIVE: To determine which patient characteristics predict the use of head CT for mild traumatic brain injury (TBI) in the primary care setting. DESIGN: Retrospective study. SETTING: Emergency Departments (ED) around Ontario, Canada. METHOD: 855 cases of potential mild TBI were collected from 3 months of health records provided by 9 participating EDs. Pearson Chi-squared test for nominal data and Student's unpaired two-tailed t test for continuous data were used to determine the univariate association of patient characteristics with use of head CT. Logistic regression with conditional forward stepwise selection analysis was used to identify significant patient characteristics (independent variables) associated with the use of brain radiography (dependent variable). RESULTS: It was found that geographical location of the hospitals (urban vs. rural), arrival mode, presence of Loss-of-Consciousness (LOC) or Post-Traumatic Amnesia (PTA), and nausea were significant predictors of brain radiography use in the primary care setting for mild TBI. CONCLUSION: The results support our hypothesis that geographical location does indeed affect whether a patient with mild TBI receives head CT or not. Furthermore, the findings highlight the variability in current diagnostic procedures. Additional analyses will be performed to determine if variability exists between trauma vs. non-trauma centers and teaching vs. non-teaching hospitals. The results will aid in determining feasible recommendations to improve the knowledge of clinicians in all regions of Ontario regarding early detection and assessment of mild TBI. Key words: Traumatic Brain Injury, Head Imaging, Primary care.

434 B307

BIOENGINEERING BRAIN-DERIVED STEM CELL-COATED TUBES FOR SPINAL CORD INJURY REPAIR

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We are currently developing bioengineered, brain-derived stem cell (BSC)-coated tubes for implantation into the injured spinal cord. This involves an entubulation strategy for regeneration after injury to the spinal cord based on a combination therapy that includes BSCs, biodegradable chitosan tubes and drug delivery systems (DDS). BSCs used in this study were isolated from the subependymal region of the forebrains of adult EGFP transgenic rats. These BSCs were seeded into the inner lumen of chitosan tubes to generate uniform multicellular layers lining the inner tube wall. The neurosphere tubes were then implanted into the rat spinal cord transection site at T8 to determine cell survival and differentiation in vivo. Results of the 5 week in vivo study showed excellent cell survival of BSCs, as well as differentiation into primarily astrocytes and oligodendrocytes. In addition, in vitro differentiation studies were carried out in which BSCs were treated with several reagents including BMP-2, CNTF, dbcAMP, and PDGF-AA for 7 days, in the absence of mitogens. The effects of different treatments were determined on the basis of changes in cellular morphology and appropriate marker expression (Beta 111-tubulin for neurons, GFAP for astrocytes, RIP for oligodendrocytes). Results obtained from the in vitro screening of exogenous factors for their differentiation potential on BSCs, will inform our choice of the appropriate factor for incorporation into the DDS being developed in our laboratory. These tissue-engineered DDS tubes will be implanted in vivo to determine their ability to promote axonal regeneration after traumatic injury to the spinal cord.

https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

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https://doi.org/10.1017/S0317167100017066 Published online by Cambridge University Press

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