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Perspective

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Exploring the potential link between ΔFosB and *N*-acetylcysteine in craving/relapse dynamics: can *N*-acetylcysteine stand out as a possible treatment candidate?

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Abstract

From a neuroscientific point of view, one of the unique archetypes of substance use disorders is its road to relapse, in which the reward system plays a crucial role. Studies on the neurobiology of substance use disorders have highlighted the central role of a protein belonging to the Fos family of transcription factors, ΔFosB. Relying on the roles ΔFosB plays in the pathophysiology of substance use disorders, we endeavour to present some evidence demonstrating that *N*-acetylcysteine, a low-cost and well-tolerated over-the-counter medicine, may influence the downstream pathway of ΔFosB, thereby serving as a treatment strategy to mitigate the risk of relapse in cases of substance use.

Summation

- ΔFosB is a critical component in relapse and reinstatement to substance use disorders that is highly expressed after repeated chronic administration of drugs of abuse, targetting glutamate release, spine density, transcriptional factors, and epigenetic mechanisms.
- Modulation of ΔFosB's targets and upstream pathways might be a strategy to prevent relapse.
- Evidence suggests that N-acetylcysteine can potentially help reduce drug use relapse and craving. Here, we explored potential mechanisms through which N-acetylcysteine impacts dendritic arborisation, synaptic plasticity, transcriptional downstream targets, and epigenetics. These mechanisms may provide a possible link to ΔFosB-related signalling in drug use relapse and craving.

Perspective

- N-acetylcysteine influences glutamatergic and dopaminergic neurotransmission and modulates downstream signalling transcription factors and pathways altered by ΔFosB, making it a promising treatment option for preventing relapse in substance use.
- N-acetylcysteine's safety profile, tolerability, and accessibility give this compound a significant advantage for use in this context, particularly since patients with substance use disorders are prone to the risk of overdose.
- Further extensive and robust preclinical and clinical research is needed to confirm the efficacy of N-acetylcysteine and to uncover the underpinning mechanisms behind its effects.

A glance at the reward system in substance use disorders

Although different drugs of abuse act on distinct neurotransmitter systems of the brain and engender various psychoactive effects, all of them converge on the brain's reward system (Nestler, 2005). Preclinical studies on substances abused by humans have provided us with better insights into addiction's cellular and molecular pathways (Lynch *et al.*, 2010). Studies on experimental animals have shown that both acute and chronic drug self-administration

dramatically enhance the firing of the dopaminergic neurons of the ventral tegmental area (VTA) of the midbrain, an area with reciprocal projections to and from the mesocortical and mesolimbic pathways, including the nucleus accumbens (NAc) of the limbic forebrain, amygdala, cingulate gyrus, basal ganglia, and prefrontal cortex (PFC) (Nestler, 2005; Willuhn *et al.*, 2010; Volkow and Morales, 2015).

All drugs of abuse are potentially rewarding. The NAc, amygdala (particularly the basolateral amygdala), ventromedial prefrontal cortex (including the orbitofrontal cortex), and posterior cingulate cortex are among the cortical and subcortical areas involved in the valuation and reward process. These areas evaluate the value, history, and cost of a reward and whether a rewarding activity needs to be repeated (Nestler, 2005; Wassum and Izquierdo, 2015; Loganathan and Ho, 2021). Increased VTA's dopaminergic signalling will cause a sudden rise in the cyclic AMP (cAMP) and Ca^{2+} concentration in the NAc, following the activation of D_1 receptors and, therefore, activate adenylyl cyclase (Muschamp and Carlezon, 2013). All these events subsequently lead to the activation of the cAMP response element-binding protein (CREB) through the phosphorylation of Ser^{133} (Muschamp and Carlezon, 2013).

CREB is a transcription factor that can either enhance or repress the expression of several genes. Phosphorylation of CREB at Ser^{133} results in the increased expression of dynorphin, which occupies kappa opioid receptors located on the VTA neurons and hampers the dopamine signalling of the mesocorticolimbic pathway of the reward circuitry. Thus, tolerance and dependence instigate, causing patients with substance use disorders to use more of a substance (Nestler, 2005; Muschamp and Carlezon, 2013).

On the other side, with chronic use of addictive substances, several structural modifications and many cellular adaptations will occur (Robinson and Kolb, 1997; Spiga *et al.*, 2014; Zhang *et al.*, 2017). Dopamine signalling of the VTA–NAc pathway also produces another protein named ΔFosB, which leads to the activation of several other genes switched on by phosphorylated CREB and suppression of dynorphin synthesis (McClung and Nestler, 2003; Nestler, 2005, 2012). Activation of these genes will result in the production of proteins in charge of responses sensitised to drugs of abuse, including nuclear factor kappa B (NF-κB) and cyclin-dependent kinase 5 (Cdk5), both of which can also result in structural alterations of the NAc, making it hypersensitive to addictive substances and drug-related cues (McClung and Nestler, 2003; Nestler, 2005, 2012). In this stage, the hippocampus also plays a part in the formation of context-specific memories of such experiences to remember the who, the where, the when, and the how of drug self-administration (contextual conditioning) and altogether build a road to relapse (Goodman and Packard, 2016; Silva *et al.*, 2016). ΔFosB also contributes to the growth of NAc's dendritic spines where it has been generated, and these sprouted branches of NAc's dendritic spines make it more sensitized to the VTA signalling and will last even months after degradation of ΔFosB (Maze *et al.*, 2010; Nestler, 2005, 2012). Upon withdrawal or abstinence, this established hypersensitivity and strong memory will result in craving, compulsive drug-seeking behaviours, and relapse (Nestler, 2005; Milton and Everitt, 2012).

After days of abstinence, the level of CREB remarkably wanes, while ΔFosB's level, due to its considerable long half-life, will remain steady and reach a plateau for weeks and months even when substance use has ceased. Hence, the diminished concentration of CREB will open a door for the domination of ΔFosB's deteriorating effects (Figure 1), leading to craving and drug-seeking behaviours (Nestler, 2005, 2012).

We are still in search of efficient, improved treatment strategies to markedly hamper craving and reduce relapse and reinstatement associated with chronic use of drugs of abuse. Having better insights into the driving forces behind reinstatement can potentially shed light on the better management of substance use disorders in the future. Based on the accumulating evidence on ΔFosB's role in substance use relapse, the following section digs deep into how ΔFosB plays a crucial role in substance use relapse, laying the foundation for understanding how targeting this molecule and its associated signalling pathways could be a way to lessen craving.

ΔFosB as a molecular target in substance use disorders treatment

ΔFosB is a 33–37 kDa protein that belongs to the Fos family of transcription factors, which can form either a heterodimer with the Jun family of proteins, particularly JunD or form stable molecular assemblies on its own (Wang *et al.*, 2012; Yin *et al.*, 2020). Such heterodimerization shapes functional activator protein 1 (AP-1) complexes that can be attached to the AP-1 sites, regulating the transcription of various genes (Nestler, 2001, 2008).

Distinct from other FosB proteins, ΔFosB is bereft of 101 C-terminal amino acids, making it lack two degron domains and, consequently, less prone to degradation (Figure 2) (Carle *et al.*, 2007; Wang *et al.*, 2012; Zhang *et al.*, 2014). Moreover, phosphorylation of a well-preserved serine residue (Ser27) of ΔFosB *via* either previously surmised casein kinase 2 (Ulery *et al.*, 2006) or more recently explored Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) (Robison *et al.*, 2013) prevents degradation of this protein, leading to its long half-life (around 8 days in vivo) (Figure 2). Such longevity confers disparate responses in gene expression after acute and chronic administration of drugs of abuse and makes ΔFosB stable enough to remain active for several weeks even after cessation of drug exposure or drug withdrawal (Figure 1) (Nestler, 2008). Data have shown that such durability is not correlated with ΔFosB's mRNA long life but rather the protein per se (Kelz and Nestler, 2000).

Unlike most Fos family members whose expression is triggered by acute exposure to drugs of abuse and only lasts for hours, ΔFosB is highly expressed in the striatal regions in a medium spiny neuron (MSN)-subtype-selective pattern after repeated chronic administration (Olsen, 2011; Lobo *et al.*, 2013a; Zhang *et al.*, 2014). Accordingly, numerous drugs of abuse induce ΔFosB only in MSNs-expressing dopamine D1 receptors, whereas others, such as opioids, induce ΔFosB equally in D1- and D2-MSNs (Lobo *et al.*, 2013b). Similarly, a recent study has shown that chronic cocaine use induced broad changes in ΔFosB binding in both D1- and D2-MSNs in NAc (Yeh *et al.*, 2023). On the other hand, prolonged administration of some antipsychotic medications induces ΔFosB exclusively in D2-MSNs (Lobo *et al.*, 2013b).

Additionally, recent data indicate that, besides the MSN subtype, ΔFosB mediates distinct transcriptional effects in males and females (Lardner *et al.*, 2021). Induction of ΔFosB in each subtype of MSNs (D1- vs. D2-MSNs) led to sex- and MSN-specific control of transcripts. In female NAc, induction of ΔFosB in D1-MSNs resulted in opposite regulation compared to D2-MSNs (Lardner *et al.*, 2021). Moreover, little overlap was seen among regulatory downstream genes when males and females were compared after induction of ΔFosB in each specific MSN subtype (Lardner *et al.*, 2021). Although the sex-specific transcriptional effects of ΔFosB are less studied, these findings are of utmost

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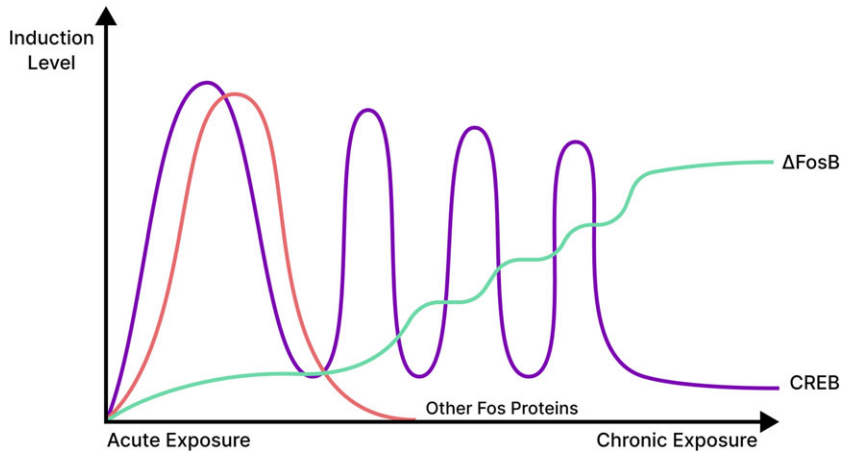

Figure 1. Temporal induction of ΔFosB, CREB, and other Fos proteins. Other Fos proteins (shown in orange) are rapidly induced by acute drug exposure and face a rapid decline afterward, while ΔFosB (shown in green) gradually increases and may persist for days. After a rapid increase following acute drug exposure, cAMP response element-binding protein (CREB; shown in purple) goes through a fluctuating pattern in chronic drug exposure until it wanes after days of abstinence.

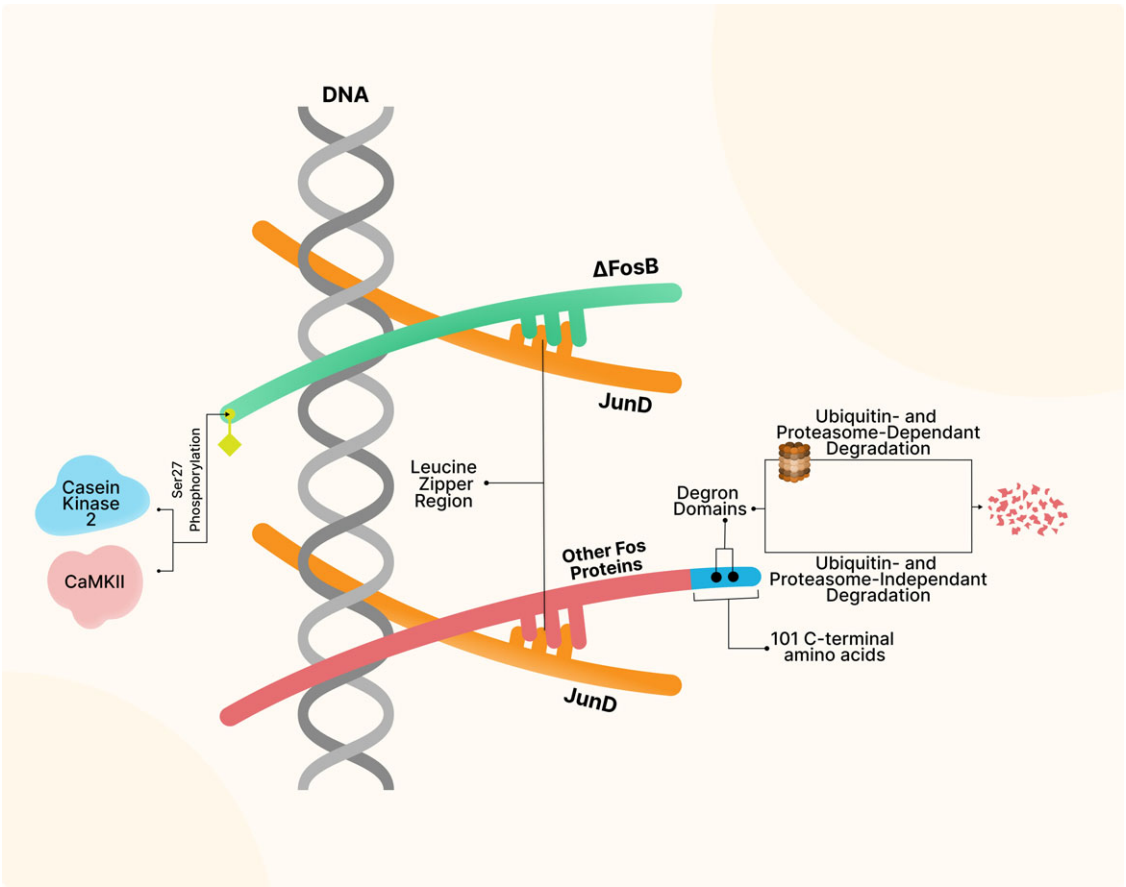

Figure 2. Structure of ΔFosB and other Fos proteins. Compared to other Fos proteins, ΔFosB lacks the 101 C-terminal amino acids and the degron domains responsible for ubiquitin- and proteasome-dependent and independent degradation, thus enhancing its stability. Additionally, Ser27 phosphorylation by Casein Kinase 2 and Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) prevents decomposition of this protein, leading to its long-term half-life.

importance since there are notable sex differences throughout the progression of various substance use disorders and their comorbid conditions (Daiwile *et al.*, 2022b; Chapp *et al.*, 2022a, 2024). Although women tend to be engaged with drugs less frequently than men, they often progress more rapidly to meet the criteria for substance use disorders and may experience more severe psychiatric disturbances compared to men (Cadet, 2021). Additionally, women are generally more prone to relapse (for a comprehensive review, see (Becker and Koob, 2016)). Therefore, the preliminary findings of Lardner et al., on sex-specific transcriptional effects of ΔFosB have significant implications for understanding the pathophysiology underlying relapse and the use of potential drugs in preventing relapse (Lardner *et al.*, 2021).

Although it has been shown that ΔFosB's distribution in the different parts of the striatum is diverse when various drugs are abused, nearly all of them induce the accumulation of ΔFosB in the

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NAc (Perrotti *et al.*, 2008). Besides, findings have suggested that the accumulation of ΔFosB induced by addictive substances targets the dynorphin-containing class of MSNs (Kelz and Nestler, 2000).

Gajewski et al., have also demonstrated that the expression profile of ΔFosB differs in various brain regions associated with substance use disorders. They reported a reduced level of ΔFosB in the hippocampus but not in the prefrontal cortex, which is supported by the observation that even some investigated upstream target genes, such as GluA2 and CaMKII, are also downregulated solely in the hippocampus (Gajewski *et al.*, 2016).

These observations suggest that ΔFosB could be one of the main contributing molecules of reinstatement. In this regard, studies on animal models of relapse have demonstrated that overexpression of ΔFosB not only sensitises the rewarding effect of drugs of abuse but also produces increased drug-seeking behaviour that leads to relapse (Kelz *et al.*, 1999; Colby *et al.*, 2003; Zachariou *et al.*, 2006). Of note, McClung et al., have implied that short or long-term expression of ΔFosB acts oppositely and thus leaves distinct outcomes (McClung and Nestler, 2003). They showed that ΔFosB's short-term expression in the NAc upregulates many of the same genes as CREB, presumably *via* direct effect. However, the scenario turned back in case of either overexpression or prolonged elevation of ΔFosB, which facilitates the induction of transcriptional downregulation of genes that CREB had upregulated and vice versa (McClung and Nestler, 2003).

Besides preclinical studies, post-mortem analyses of the brain tissues of chronic opioid abusers have revealed enhanced expression of ΔFosB in the NAc that was followed by a rise in the level of its downstream targets such as Cdk5, NF-κB, CREB, brain-derived neurotrophic factor, and JunD in both the hippocampus and NAc (Seltenhammer *et al.*, 2016). They confirmed that ΔFosB and its downstream transcriptional targets are critical to inducing sustainable brain changes and establishing a strong memory associated with drugs of abuse on the road to dependency and relapse (Seltenhammer *et al.*, 2016).

The dimerisation of ΔFosB with JunD possibly circumvents CREB on being attached to the cAMP response element (CRE) site and, as a result, acts contrariwise to that of CREB. It can also be postulated that higher levels of ΔFosB may act as an AP-1 activator (McClung and Nestler, 2003). Finally, CaMKII, a protein upregulated as ΔFosB is continuously overexpressed, has been introduced as another potential candidate for these observations (McClung and Nestler, 2003; Robison *et al.*, 2013). CaMKII hinders the dimerisation of CREB with CREB binding protein via phosphorylating CREB (McClung and Nestler, 2003).

In a study conducted by Vialou et al., following chronic cocaine administration, expression of both CREB and serum response factor (SRF) were shown to be essential for the accumulation and regulation of NAc's ΔFosB (Vialou *et al.*, 2012; Eagle *et al.*, 2019). They concluded that upstream modulation of ΔFosB requires the adequate expression of both transcription factors and deletion of either could not prevent the accumulation of ΔFosB in the NAc (Vialou *et al.*, 2012; Eagle *et al.*, 2019).

There are also studies pointing to RNA-binding proteins involved in regulating ΔFosB. Recent evidence suggests synergistic effects of D1 dopaminergic and activin receptor-like kinase 4 (ALK4) signalling, mediated by activation of poly-binding protein 1 (PCBP1) and Smad3 in MSNs of NAc, induce ΔFosB production and its reward-related behaviour (Krapacher *et al.*, 2022). Enhanced expression of an RNA-binding protein called poly-pyrimidine tract-binding protein 1 (PTBP1) was suggested to cause reduced transcription of ΔFosB (Alibhai *et al.*, 2007; Bryant and Yazdani, 2016). Another study pointed out that elevation in the level of ΔFosB can be modulated by intraperitoneal administration of molecular hydrogen in methamphetamine-dependent mice (Wen *et al.*, 2020). Wen et al. also showed that molecular hydrogen (delivered by the administration of hydrogen-rich saline) reduces behavioural sensitisation by acting as an antioxidant, inhibiting the production of superoxide anion, and diminishing the amount of phosphorylated ERK and ΔFosB in the NAc (Wen *et al.*, 2020).

Interestingly, this observed overexpression is not only limited to the drugs of abuse but also other compounds, such as sweeteners that activate the brain's reward system (Salaya-Velazquez *et al.*, 2020) and even stressful conditions (Perrotti *et al.*, 2004; Scalize Hirata *et al.*, 2019). Keeping all these in mind, it is worth mentioning that stress and stressful adverse events in life can not only lead to addiction but also predispose abstained patients with substance use disorders to reinstate and relapse (Goeders, 2003; Sinha, 2008, 2012). Though different mechanisms contribute to such phenomena, assessing the role stress plays in manipulating ΔFosB toward an increased susceptibility to relapse is not out of reason. A study has shown that repeated social defeat stress significantly enhances the expression of ΔFosB in the frontal cortex, shell, and core of the NAc, and the medial, central, and basolateral amygdala (mesocorticolimbic areas) that started after the last imposed stress and lasted for 21 days which was correlated with upregulation of μ-opioid receptor in the VTA-NAc pathway (Nikulina *et al.*, 2008). A report also indicates that chronic restraint stress induces higher locomotor activity after amphetamine administration, resulting in cross-sensitization that is associated with enhanced ΔFosB expression levels in the NAc of only adult and not adolescent rats (Carneiro De Oliveira *et al.*, 2016).

A path to relapse: a concise look into possible mechanisms in which ΔFosB begets drug-seeking behaviours and craving and how N-acetylcysteine may rectify these alterations

Human studies are indicative that behavioural changes associated with addiction are exceptionally persistent. Therefore, researchers started figuring out the cellular and molecular mechanisms involved in enduring behavioural abnormalities. In this section, we briefly enumerate potential targets that ΔFosB aims at on various levels and determine whether N-acetylcysteine could alter these pathways (Figure 3).

N-acetylcysteine is an accessible, well-tolerated, over-the-counter medicine of low cost with a safe profile regarding adverse effects. It serves as the N-acetylated derivative of the amino acid cysteine and, as such, acts as a prodrug by binding to the cysteine-glutamate exchanger. N-acetylcysteine also promotes the efflux of glial glutamate while importing cysteine, resulting in a net increase in the extracellular levels of glutamate in the brain.

Dendritic arborisation and synaptic plasticity as common targets of different pathways

ΔFosB is presumed to influence AMPAR-containing GluR2 receptors and modify glutamatergic signalling by altering the neural excitability of NAc neurons due to overexpression of these receptors. Reduced neural conductance followed by diminished Ca^{2+} permeability can affect the excitability of neurons, and prolonged exposure to addictive drugs can change the susceptibility of neurons to rewarding stimuli (Kelz *et al.*, 1999; Nestler, 2001; Nestler *et al.*, 2001). This phenomenon has been shown to

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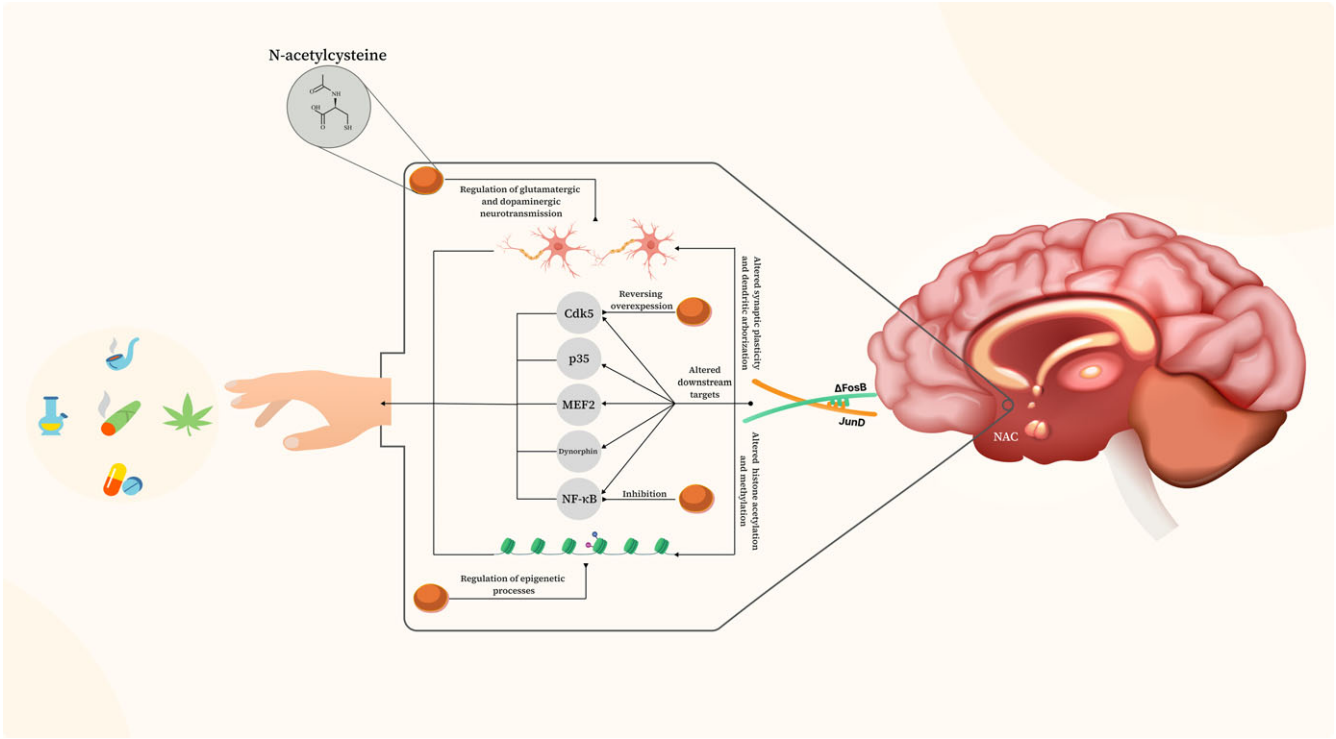

Figure 3. Mechanisms of ΔFosB involvement in relapse and rectifying actions of N-acetylcysteine. ΔFosB contributes to drug-seeking behaviours and relapse through various mechanisms, including enhanced expression of glutamatergic receptors, increasing spine density of medium spiny neurons, modulation of downstream transcription factors [including cyclin-dependent kinase 5 (Cdk5), p35, dynorphin, nuclear factor kappa B (NF-kB), and presumably myocyte enhancer factor 2 (MEF2)] and through epigenetic mechanisms namely by altering histone acetylation and methylation. N-acetylcysteine influences these signaling pathways, holding the potential to reduce drug craving and mitigate the risk of relapse.

exacerbate responses to cues and subsequent increases in craving and relapse (Kelz *et al.*, 1999; Todtenkopf *et al.*, 2006). An additional study exploring the mechanisms underlying resilience and antidepressant response has further affirmed that ΔFosB can regulate the expression of GluR2 and exert control over complex behaviours (Vialou *et al.*, 2010). However, in a preclinical study conducted by Conrad et al., a significant connection was established between the presence of increased GluR2-lacking AMPARs in the NAc of rats following extended periods of withdrawal from cocaine, leading to the hypothesis that such an increase may also contribute to enhanced cocaine craving (Conrad *et al.*, 2008).

Short-term expression of ΔFosB also leaves different outcomes on D1-type versus D2-type MSNs of the NAc (Grueter *et al.*, 2013). Observations indicate diminished AMPA-related synaptic transmission (reduced excitatory synaptic strength) in D1 receptor-expressing neurons in both the NAc's shell and core via the direct pathway that shapes silent synapses (increased silent synapses onto D1-type). In contrast, an increase in D2-type excitatory transmission through an indirect pathway only on the shell of NAc has been reported (Grueter *et al.*, 2013). Grueter et al. further depicted that morphological changes of the immature dendritic spines are under the influence of direct D1-type MSNs of the NAc and attributed the cocaine-evoked abnormal behaviours to the D1 direct but not D2 indirect pathway, therefore highlighting the cell-type and sub-regional-specific manner of ΔFosB's mode of action to cocaine-associated behavioural responses (Grueter *et al.*, 2013). In contrast, Lee et al., have demonstrated that ΔFosB participates in the short-term induction of increased spine density in both D1- and D2-containing MSNs of NAc (Lee *et al.*, 2006). However, such an enhanced density of dendritic spines and ΔFosB overexpression are maintained and remain stable, specifically in the D1 type MSNs of NAc, even after a month of cocaine withdrawal (Lee *et al.*, 2006). Moreover, elevated levels of ΔFosB can enhance the expression of NMDA receptors of the mesocorticolimbic pathway, especially on the MSNs of the NAc, which further leads to long-term potentiation and hijacks the associated memory pathways (Daneff and Jadavji, 2019).

Transcriptional downstream targets

Evidence has pointed out that ΔFosB is one of the primary regulators of transcriptional phenotype changes that occur with repeated consumption of addictive substances that ultimately result in not only addiction-related behavioural responses but also natural rewards (Nestler, 2008; Wallace *et al.*, 2008; Bali and Kenny, 2019).

Studies on transgenic animals overexpressing ΔFosB suggested the prominent role of this transcription factor in liability to relapse after the termination of drug consumption and even when the level of ΔFosB backs to its normal concentration (Colby *et al.*, 2003; McClung and Nestler, 2003; Nestler, 2008). Findings on the expression profile of ΔFosB have delineated that the ongoing predisposition to relapse and drug-taking/seeking behaviours might not directly be affected by ΔFosB *per se* (Larson *et al.*, 2010) but instead can trigger several transcriptional cascades that have impacts on the morphology and neural circuits of NAc neurons, thus mediating neural adaptation (Lee *et al.*, 2006; Ang *et al.*, 2008; Maze *et al.*, 2010; Gajewski *et al.*, 2016).

Enhanced expression and accumulation of ΔFosB raise levels of both mRNA and protein of Cdk5 and p35, which is regulated by

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the AP-1 site on the gene's promoter (Chen *et al.*, 2000; Bibb *et al.*, 2001; Benavides and Bibb, 2004). Cdk5/p35 involves diverse central nervous system processes, including synaptic plasticity, learning, and memory (Takahashi *et al.*, 2022). Cdk5 further dampens D1 dopaminergic signalling, possibly via augmented phosphorylation of dopamine- and cAMP-regulated neuronal phosphoprotein DARPP-32 (PPP1RB) (Bibb *et al.*, 2001; Benavides and Bibb, 2004).

In addition, myocyte enhancer factor 2 (MEF2) has been introduced as another critical regulator of structural and behavioural plasticity and is involved in developing various neuropsychiatric disorders (Pulipparacharuvil *et al.*, 2008; Zhang and Zhao, 2022). Reduction in the striatal level of MEF2 has been implicated in causing increased dendritic spine density after chronic administration of cocaine. This event is induced by the release of cAMP through the activation of D1 receptors that prevents the further activity of calcineurin at the inhibitory Cdk5 site (Pulipparacharuvil *et al.*, 2008). However, the possible correlation between diminished MEF2 and increased expression of ΔFosB should be sought.

Additionally, the opioid peptide dynorphin is a target through which ΔFosB exerts its behavioural phenotype (Zachariou *et al.*, 2006). It is also shown that prodynorphin, the dynorphin precursor, is a known target for CREB and that chronic consumption of cocaine is associated with CREB-mediated dynorphin expression in NAc (Teague and Nestler, 2021).

Another putative target of ΔFosB is another transcription factor named NF-κB. Enhanced expression of NF-κB in the NAc only after chronic but not acute treatment with cocaine has raised the idea that NF-κB may be under the control of ΔFosB. NF-κB engages in long-term plasticity and enhances dendritic density as Cdk5 does (Bibb *et al.*, 2001; Ang *et al.*, 2008; Boersma *et al.*, 2011; Dresselhaus *et al.*, 2018), thus contributing to craving and drug-seeking behaviours.

Epigenetics

There are emerging lines of evidence that ΔFosB might leave its long-lasting effect by epigenetic alterations since studies have signified that even after protracted withdrawal and return of ΔFosB's level to normal, the corresponding effects of it still abide (Mews *et al.*, 2018).

ΔFosB alters neuronal morphology by targeting histone methyltransferase G9a. Maze et al. have reported that enhanced expression of ΔFosB can result in the repression of lysine dimethyltransferase G9a, which further reduces the dimethylation of histone 3 lysine 9 (H3K9) in the NAc during repeated cocaine exposure. Such a downregulated level of G9a and the subsequent decrease in the process of histone methylation leads to dendritic arborisation and an enhanced tendency to reinstate cocaine abuse (Maze *et al.*, 2010).

Acetylation of H3 and H4 histone subtypes at the promoters of plasticity-associated genes, including Fos, has also been shown to be increased following cocaine exposure (Stewart *et al.*, 2021). Furthermore, a recent study demonstrated that inhibition of histone deacetylase results in an increase of ΔFosB gene expression in the NAc and medial prefrontal cortex while also reducing reinstatement of morphine-induced conditioned place preference in rats, confirming the role of epigenetic mechanisms, namely histone acetylation, in the adjustment of drug-induced plasticity (Saberian *et al.*, 2021). Promoters of ΔFosB and CREB genes involved in glutamate transmission are thought to be hyperacetylated in the NAc after cocaine administration (Kennedy *et al.*, 2013), and sustained inhibition of histone deacetylase can stimulate G9a (Kennedy *et al.*, 2013; Eagle *et al.*, 2019). ΔFosB and CREB can facilitate signalling transmission through enhanced permeability of Ca^{2+} that is modulated by histone acetylation (Mews *et al.*, 2018). By using H3.3 histone barcoding of NAc, Wimmer et al. reported an increase in upregulation of the FosB gene in cocaine self-administration mice and the implication of other novel molecular cascades in cocaine-induced neuronal plasticity (Wimmer *et al.*, 2019). Moreover, following administration of amphetamine, histone deacetylase budges to the Fos gene promoter and controls its expression (Torres *et al.*, 2015). Therefore, it should be explored in-depth whether ΔFosB can broadly affect histone modification and coding (Eagle *et al.*, 2019).

N-acetylcysteine and preclinical and clinical studies on relapse to substance abuse

There are comprehensive reviews on preclinical and clinical studies of N-acetylcysteine in substance use disorders and other psychiatric disorders (Dean *et al.*, 2011; Minarini *et al.*, 2017; Smaga *et al.*, 2021a). Here, we only focus on evidence with a direct nexus to relapse/cravings and drug-seeking behaviours.

Preclinical studies

The majority of studies investigating the effect of N-acetylcysteine on relapse or cravings have primarily focused on alcohol and cocaine use. In a preclinical study, N-acetylcysteine was able to attenuate the accumulation of ΔFosB in the mPFC, leading to reduced craving and prevention of ethanol-induced neuroadaptions associated with ΔFosB in a mouse model of behavioural sensitisation (Morais-Silva *et al.*, 2016). Although, unlike most studies, the authors could not observe an increased ΔFosB expression in the NAc (Morais-Silva *et al.*, 2016). Another preclinical report of such an effect was published later, indicating that N-acetylcysteine diminishes ethanol-seeking behaviour to a great extent of 77 percent along with reacquisition after protracted abstinence in rats self-administering ethanol chronically by 78 percent, pointing to the application of N-acetylcysteine as a treatment to prevent relapse (Lebourgeois *et al.*, 2018).

Recently, Fredriksson et al., have also presented evidence that N-acetylcysteine improves impulse control and attenuates the likelihood of relapse in male rats with long-term alcohol consumption (Fredriksson *et al.*, 2023). However, no significant difference was observed in the quantity of alcohol consumed or the motivation to drink (Fredriksson *et al.*, 2023). Using another animal model to evaluate ethanol-relapse drinking behaviour, the alcohol deprivation effect model, N-acetylcysteine was shown to be effective in preventing relapse by reducing the heightened intake of alcohol following a period of abstinence (Cano-Cebrián *et al.*, 2021). The effective dose range of N-acetylcysteine on ethanol in these preclinical studies was between 60 and 120 mg/kg.

Besides alcohol, some preclinical studies have also addressed N-acetylcysteine's impact on cocaine-seeking behaviour. Findings from a study by Amen et al., demonstrated that repeated N-acetylcysteine administration (60 mg/kg for 7 days) resulted in a significant decrease in cocaine-seeking behaviour in rats, and these findings were further validated in the same study through reductions in craving in cocaine-dependent humans as well (Amen *et al.*, 2011). Additionally, findings from another study

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have demonstrated that N-acetylcysteine (100 mg/kg) prevented cocaine and alcohol seeking following acute restraint stress (Garcia-Keller *et al*., 2020). In another study, N-acetylcysteine amide has also been shown to effectively block cocaine-seeking behaviour in rats (Jastrzębska *et al*., 2016). Moreover, N-acetylcysteine has been demonstrated to prevent cocaine-primed reinstatement, suggesting that this treatment can alter the plasticity-dependent effects of cocaine (Madayag *et al*., 2007).

Clinical trials

Several clinical studies have also investigated the impact of N-acetylcysteine on craving and substance use relapse in populations with various substance use disorders (Table 1). In a human study involving veterans with both post-traumatic stress disorder (PTSD) and substance use disorders, the administration of N-acetylcysteine (1200 mg twice a day) led to improved craving compared to the placebo (Back *et al*., 2016). A recent double-blind placebo-controlled trial has also demonstrated that supplementation with N-acetylcysteine (2700 mg daily) resulted in significant benefits on measures of drug craving among treatment-resistant PTSD individuals. However, it did not affect other PTSD symptoms (Kanaan *et al*., 2023).

There have also been several studies exploring N-acetylcysteine in cocaine use disorder. In a double-blind placebo-controlled trial aiming at evaluating the efficacy of N-acetylcysteine (2400 mg daily) for treating cocaine use disorder, no correlations were found between measures related to relapse (LaRowe *et al*., 2013). Nevertheless, a subgroup of enrolled patients who were already abstinent before the trial's commencement exhibited a longer duration of time to relapse, indicating that N-acetylcysteine might have a protective role in abstained patients with cocaine use disorder (LaRowe *et al*., 2013). Findings from the same authors also showed that there were trends for a greater reduction in cocaine withdrawal symptoms and craving within N-acetylcysteine treatment compared to the placebo (LaRowe *et al*., 2006). In a more recent trial by Woodcock et al., N-acetylcysteine treatment (3600 mg/day for 1 week) suppressed cocaine-seeking behaviour (Woodcock *et al*., 2021). However, another trial failed to show N-acetylcysteine's effect on craving, number of abstinent days, and days until relapse in cocaine-dependent individuals. Nevertheless, the study showed that N-acetylcysteine was associated with lower positive urine tests, lower cocaine use-related problems and inhibition (Schulte *et al*., 2018).

Studies on the effects of N-acetylcysteine on methamphetamine use disorder have been inconclusive so far, with one study showing no reduction in craving (McKetin *et al*., 2021), but another study reporting a decrease in methamphetamine craving (Mousavi *et al*., 2015). Similarly, trials on cannabis use disorders have been inconsistent, with studies showing improved cannabis abstinence following N-acetylcysteine treatment in adolescents (gray *et al*., 2012), but not in adults (gray *et al*., 2017).

In terms of nicotine use disorder, in a pilot randomised placebo-controlled trial, no significant decrease in craving after short-term abstinence (3.5 days) was observed. However, participants receiving a daily dose of 3600 mg N-acetylcysteine reported their initial smoking experience being less rewarding after abstinence. This finding suggests a potential avenue for further research into the viability of N-acetylcysteine as a good candidate for relapse prevention in nicotine use disorder as well (Schmaal *et al*., 2011). In later studies, while some trials reported no significant outcomes following N-acetylcysteine in nicotine-dependent individuals (Schulte *et al*., 2017; McClure *et al*., 2021), several other trials reported that N-acetylcysteine treatment resulted in less craving (Froeliger *et al*., 2015; Harlivasari *et al*., 2024), nicotine dependence (Grant *et al*., 2014), and reduction in cigarettes smoked (Knackstedt *et al*., 2009; Prado *et al*., 2015).

When it comes to alcohol use disorder, a recent trial by Morley et al., has shown that N-acetylcysteine reduced the number of drinks but did not affect craving (Morley *et al*., 2023). Another trial demonstrated that N-acetylcysteine increased the odds of alcohol abstinence, fewer drinks per week, and fewer drinking days per week (Squeglia *et al*., 2018). However, two other studies showed that N-acetylcysteine did not affect drinking outcomes (Stoops *et al*., 2020) (Squeglia *et al*., 2016).

While human clinical results remain inconsistent, two systematic reviews and meta-analyses have concluded the superiority of N-acetylcysteine to placebo in the reduction of cravings among individuals with substance use disorders (Duailibi *et al*., 2017; Chang *et al*., 2021). Although these findings are obtained from the pooled analysis of different abused substances, the compelling outcome stimulates further studies to elucidate the specific and nuanced impact of N-acetylcysteine on craving symptoms in individuals with substance use disorders.

Mechanistic insights into how N-acetylcysteine can affect drug use relapse and craving

There is evidence on how N-acetylcysteine can potentially contribute to decreasing drug use relapse and craving. Here, we provide potential mechanisms for N-acetylcysteine's impact on dendritic arborisation, synaptic plasticity, transcriptional downstream targets, and epigenetics, which offer a possible link with ΔFosB-related signalling in drug use relapse and craving (Figure 3).

N-acetylcysteine rectification of altered dendritic arborizations and synaptic plasticity

The ability of N-acetylcysteine to directly and indirectly modulate glutamatergic neurotransmission, which becomes dysregulated post-withdrawal and contributes to drug-seeking behaviours, *via* interactions with glutamate-transporter type 1 (GLT-1) (Knackstedt *et al*., 2010), as well as NMDARs and AMPARs (Smaga *et al*., 2021b), positions it as a promising candidate for substance use relapse prevention. Reissner et al., reported that in a rat model of cocaine relapse, N-acetylcysteine effectively inhibited cue-induced cocaine reinstatement through the engagement of GLT-1 (Reissner *et al*., 2015). Furthermore, another study also indicated that N-acetylcysteine restores the decrease in GLT-1 levels in both the NAc and dorsal striatum during prolonged but not short cocaine self-administration in rats. This phenomenon was accompanied by an upregulation in the expression of the transcription factor Zif268 (Ducret *et al*., 2016). Moreover, a recent study by Siemsen et al. demonstrated that heroin self-administration and extinction increased prelimbic cortical astrocyte-synapse proximity and circuit-level adaptations in cortical dendritic spine morphology. At the same time, N-acetylcysteine reversed these adaptations in corticostriatal neurons and astrocytes (Siemsen *et al*., 2023).

N-acetylcysteine has also been shown to exhibit a dose-dependent bidirectional effect on the release of dopamine (Gere-Pászti and Jakus, 2009). At the same time, higher doses of N-acetylcysteine hamper striatal dopamine release. Lower doses

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Table 1. Clinical trials exploring the effects of N-acetylcysteine on substance use abstinence, relapse, and craving

Studied population diagnosis	Trial design	N-acetylcysteine dose and duration	Findings	Reference
PTSD and substance use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 8 weeks	N-acetylcysteine improved craving compared to the placebo	(Back *et al.*, 2016)
PTSD	Double-blind randomised placebo-controlled trial	2700 mg/day for 12 weeks	N-acetylcysteine improved substance craving duration and resistance, but not other PTSD symptoms compared to the placebo	(Kanaan *et al.*, 2023)
Cocaine use disorder	Double-blind randomised placebo-controlled trial	1200 or 2400 mg/day for 8 weeks	Individuals who were already abstinent before the trial's commencement exhibited a longer duration of time to relapse and lower craving rates in the 2400 mg/day N-acetylcysteine group compared to the placebo	(LaRowe *et al.*, 2013)
Cocaine use disorder	Double-blind placebo-controlled crossover trial	3600 mg/day for 1 week	N-acetylcysteine suppressed cocaine-seeking behaviour	(Woodcock *et al.*, 2021)
Cocaine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 25 days	N-acetylcysteine did not affect craving, number of abstinent days and days until relapse. However, N-acetylcysteine was associated with lower positive urine tests, lower cocaine use-related problems and inhibition	(Schulte *et al.*, 2018)
Cocaine use disorder	Double-blind placebo-controlled crossover trial	2400 mg/day for 3 days	There were trends for a greater reduction in withdrawal symptoms and craving within N-acetylcysteine treatment	(LaRowe *et al.*, 2006)
Methamphetamine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 12 weeks	No reduction in craving, severity of dependence and withdrawal was seen in the N-acetylcysteine group compared to the placebo	(McKetin *et al.*, 2021)
Methamphetamine use disorder	Double-blind placebo-controlled crossover trial	600 mg/day for 2 weeks followed by 1200 mg/day for 2 weeks	N-acetylcysteine resulted in a decrease in methamphetamine craving	(Mousavi *et al.*, 2015)
Cannabis use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 8 weeks	Adolescents receiving N-acetylcysteine had improved cannabis abstinence compared to placebo	(Gray *et al.*, 2012)
Cannabis use disorder	Double-blind randomised placebo-controlled trial	1200 mg/day for 12 weeks	N-acetylcysteine and placebo groups did not differ in cannabis abstinence among adults	(Gray *et al.*, 2017)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	3600 mg/day for 3.5 days	Patients receiving N-acetylcysteine reported their initial smoking experience being less rewarding after abstinence, but no significant effects were seen in craving and withdrawal symptoms	(Schmaal *et al.*, 2011)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 3.5 days	Smokers in N-acetylcysteine group reported less craving compared to placebo	(Froeliger *et al.*, 2015)
Nicotine use disorder and pathological gambling	Double-blind randomised placebo-controlled trial	1200–3000 mg/day for 12 weeks	Significant benefit of N-acetylcysteine was seen in nicotine dependence scores compared to placebo	(Grant *et al.*, 2014)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 4 weeks	Participants treated with N-acetylcysteine reported a reduction in cigarettes smoked	(Knackstedt *et al.*, 2009)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	3000 mg/day for 12 weeks	N-acetylcysteine significantly reduced the daily number of cigarettes used and resulted in greater quit rate	(Prado *et al.*, 2015)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 2 weeks	N-acetylcysteine did not affect craving or withdrawal symptoms	(Schulte *et al.*, 2017)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 4 weeks	N-acetylcysteine group had a higher abstinence rate and lower craving than the placebo group	(Harlivasari *et al.*, 2024)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	1800 mg/day for 16 weeks	There was no significant difference in smoking outcomes between N-acetylcysteine and placebo groups	(Arancini *et al.*, 2021)
Nicotine use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 8 weeks	No differences between N-acetylcysteine and placebo groups in terms of early abstinence, time to relapse and end-of-treatment abstinence	(McClure *et al.*, 2021)

(*Continued*)

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Table 1. (*Continued*)

Studied population diagnosis	Trial design	N-acetylcysteine dose and duration	Findings	Reference
Alcohol use disorder	Double-blind randomised placebo-controlled trial	2400 mg/day for 4 weeks	N-acetylcysteine had effects on number of standard drinks per drinking day, but not on craving compared to placebo	(Morley *et al.*, 2023)
Alcohol use disorder	Double-blind randomised placebo-controlled trial	1200 mg/day or 2400 mg/day for 5 days	N-acetylcysteine did not influence alcohol self-administration or other subjective effects	(Stoops *et al.*, 2020)
Alcohol use in cannabis-dependent individuals	Double-blind randomised placebo-controlled trial	2400 mg/day for 12 weeks	N-acetylcysteine increased the odds of alcohol abstinence, fewer drinks per week and fewer drinking days per week	(Squeglia *et al.*, 2018)
Alcohol use in cannabis-dependent individuals	Randomised placebo-controlled trial	2400 mg/day for 8 weeks	N-acetylcysteine did not affect abstinence and binge drinking days compared to the placebo. However, in the N-acetylcysteine group, but not the placebo group, less marijuana use was associated with less alcohol use	(Squeglia *et al.*, 2016)

are associated with an increased release of dopamine in the striatum (Gere-Pászti and Jakus, 2009). Besides, in a primate study, N-acetylcysteine protected against the reduction of striatal dopamine transporter availability, followed by chronic administration of methamphetamine (Hashimoto *et al.*, 2004).

The impacts N-acetylcysteine exerts on dopamine availability and the regulation of NMDAR activity can, in part, be germane to facilitating glutathione production (Dean *et al.*, 2011). Glutathione can then affect the glutamatergic signalling without altering the level of glutamate (Bradlow *et al.*, 2022). Elevated levels of accumbal glutathione in the brains of rats have been reported following treatment with N-acetylcysteine. The increase is believed to result from cell-type specific shifts in glutamatergic inputs to the NAc's MSNs (Zalachoras *et al.*, 2022).

On the other hand, there are studies on the alterations in long-term potentiation (LTP) and long-term depression (LTD) during drug withdrawal or reinstatement. For instance, Qian et al., demonstrated that morphine withdrawal brings about the down-regulation of mGluR2/3, impairing NMDA receptor-dependent LTD in the NAc (Qian *et al.*, 2019). Moreover, in a study using a reinstatement animal model of drug-seeking behaviour, it was demonstrated that cocaine-induced metaplasticity impaired the induction of LTP and LTD at the PFC-NAc, a phenomenon linked to relapse vulnerability (Moussawi *et al.*, 2009). Interestingly, N-acetylcysteine not only prevented relapse and cravings but also restored the induction of LTP. This restoration was presumably achieved through indirect stimulation of mGluRs (mGluR2/3 and mGluR5 primarily), possibly associated with the activation of the cysteine-glutamate exchange system (Moussawi *et al.*, 2009). The cysteine-glutamate antiporter regulates glutamate release, which in turn activates presynaptic neuronal mGluR2/3, thereby modulating the vesicular transmission of glutamate (Berk *et al.*, 2013).

Taken together, all these lines of evidence suggest that N-acetylcysteine is a potential candidate to rectify the altered glutamatergic and dopaminergic neurotransmission in individuals vulnerable to relapse and reinstatement. The shared dynamics of N-acetylcysteine's interaction with ΔFosB signalling warrant further research to comprehensively understand its potential therapeutic role.

N-acetylcysteine impacts on altered transcriptional downstream targets

N-acetylcysteine can be a modulator of redox hemostasis by mitigating the activity of NF-κB through inhibition of IkappaB kinases (Oka *et al.*, 2000; Farid *et al.*, 2005; Fischer *et al.*, 2006; Mackenzie *et al.*, 2006). AP-1 has also been demonstrated as a target for N-acetylcysteine, where its activity is diminished via the production of glutathione and the suppression of reactive oxygen species generation (Sen and Packer, 1996; Mackenzie *et al.*, 2006; Shi *et al.*, 2017). A recent study has also provided evidence that N-acetylcysteine is also capable of diminishing and normalising the hyperactivity of Cdk5, showcasing therapeutic potential in disorders where the excessive enzymatic activity of Cdk5 is implicated (Saha *et al.*, 2023), including relapse to substance use and reinstatement behaviour.

Considering the significance of these three downstream entities of ΔFosB in the pathophysiology of relapse, N-acetylcysteine has the potential to reverse the elevated levels of CREB and Cdk5 induced by the overexpression of AP-1 while also addressing heightened levels of NF-κB and Cdk5, which have established roles in craving and drug-seeking behaviour.

N-acetylcysteine's impact on epigenetics

Given the role of epigenetics in the pathophysiology of relapse, it is of note to mention that in mammalian cells, histone H3 undergoes S-glutathionylation, introducing glutathione as a novel post-translational participant of the modification of the histone code (García-Giménez *et al.*, 2017). However, glutathione's impact on epigenetic processes goes far beyond H3, and a substantial body of evidence supports the notion that glutathione plays a significant role in epigenetic regulation at various levels (García-Giménez *et al.*, 2017). This includes its influence on DNA methylation, histone post-translational modification, non-coding RNAs, and even modulation of chromatin structure (Cyr and Domann, 2011; García-Giménez *et al.*, 2017)

This regulatory effect of glutathione on epigenetic processes positions N-acetylcysteine, its precursor, as a prospective candidate for potential epigenetic dysregulation influenced by

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substance use disorders and implicated in the pathophysiology of relapse and cravings. Nevertheless, it is crucial to emphasise that further in-depth explorative and confirmatory studies are needed to assess N-acetylcysteine's efficacy and involvement in this context fully. The establishment of a robust scientific foundation through comprehensive research may help determine whether N-acetylcysteine could act as a potential therapeutic tool for modifying altered epigenetic processes induced by substance use disorders.

Concluding remarks

Relapse and reinstatement to drugs of abuse, even after successfully quitting them, is a deadlock to reaching the ultimately desired treatment goals in substance use disorders. To overcome the relapse, the biological components behind such an unbridled craving should intensively be sought. ΔFosB is one of the presumed candidates responsible for morphological and psychobiological alterations in an addicted brain. In specific brain parts, ΔFosB can modulate several downstream transcriptional factors, such as NF-κB and Cdk5. It also affects AMPA and NMDA receptors through glutamatergic neurotransmission and controls synaptic plasticity. One other explanation of how ΔFosB leaves such permanent effects is epigenetic modifications. Though we have good evidence to rely on, there is much ground to be made up, and further studies are needed to illuminate the role of ΔFosB in relapse and craving comprehensively (Question Box).

Knowing the intricate roles and mechanisms behind ΔFosB's mode of action in substance use disorders, we might be able to target several upstream and downstream related components to obliterate the associated memory with drugs of abuse and introduce a new treatment strategy to avoid relapse and reinforcement. N-acetylcysteine has demonstrated effectiveness in preventing relapse, reducing craving, and curtailing drug-seeking behaviours in both preclinical and clinical studies, although some discrepancies exist. Due to its potential to modulate glutamatergic and dopaminergic neurotransmission, along with its ability to affect downstream signalling transcription factors and pathways altered by ΔFosB, N-acetylcysteine holds promise as a potential treatment strategy for preventing relapse to substance use. Nevertheless, a more extensive body of both preclinical and clinical research is warranted to establish its efficacy and unravel the elusive mechanisms underlying its effects. Its well-tolerated nature, safety profile, and accessibility make it an ideal candidate for patients with substance use disorder who are already at risk of toxicity and overdose.

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Question box

- Can keeping the NAc's CREB level high, and preventing the CREB's level from declining in the NAc be a potential target to reduce the rate of relapse after reinstatement (by affecting dynorphin activity)?
- Is ΔFosB's effects on relapse to substance use sex-specific? What can be the controlling mechanisms?
- How can the induction of ΔFosB in different subtypes of MSNs influence its mode of action?
- Can having the phosphorylation of ΔFosB targeted therapeutically reduce the relapse by reducing the half-life of ΔFosB?
- Can alteration of ΔFosB's dimerization pattern be of potential therapeutic importance?
- How can CaMKII be selectively modulated in substance use disorders?
- Can modulation of ΔFosB's production through upstream signalling (CREB, SRF, ALK4, PCBP1, etc) be of therapeutic value in relapse to drugs of abuse?
- How significant is the role of NMDARs in relapse and can alteration of long-term potentiation be targeted to reduce the craving and reinstatememnt?
- Is there a possible correlation between diminished MEF2 and increased expression of ΔFosB in substance use disorders?
- Can epigenetic alteration of chromatin accessibility be a promising therapeutic target in substance use disorders?
- Do different levels of steroid hormones affect the liability to relapse in substance use disorders by changing chromatin accessibility?
- Is ΔFosB's distinct effect in sexes controlled with sex hormones effect on chromatin remodeling?
- What are the roles of silent synapses in the pathophysiology of relapse and how does ΔFosB modulate them?

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