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# Letter

**Cite this article:** Schmidt M (2021). Response to Hofmann, 2021. *Visual Neuroscience* 38:E004. https://doi.org/10.1017/S0952523821000018

Received: 10 January 2021 Received in revised form: 00 Accepted: 11 February 2021

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University Press.



# Response to Hofmann, 2021

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In my recent study, I described two different areas in the nucleus glomerulosus of a pufferfish that receive information from different layers in the tectum opticum by neurons in different portions of the nucleus corticalis (Schmidt, 2020). These findings were challenged in a reply by M. Hofmann, in which he states that an injection site would be located in another brain structure than the one I have reported (Hofmann, 2021). This is actually not clear from the supplied material in my article. For that reason, I will present the essential part of the brain section series (Fig. 1) and provide this part as an aligned series of photomicrographs (Supplementary material). On this occasion, I will react to the other labeled structures and the topographical organization that have been addressed by M. Hofmann as well.

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#### Identifying the injection site

M. Hofmann presents a photomicrograph apart from the one in Fig. 1A of my article (Schmidt, 2020), and alleges that the true injection site is located in the tegmentum, but he does not present a series of brain sections with heavily labeled structures in the nearby brain region including the brain section in my article (Hofmann, 2021: see Section "Alleged 'nucleus corticalis injection"). I did that now with a series of 10 semi-sections (Fig. 1; see also Supplementary material). They are arranged from rostral (Fig. 1A) to caudal (Fig. 1J). M. Hofmann used the track of the glass micropipette as evidence to justify his allegation. It is indicated in Sections 4-8 (Fig. 1D-1H; arrowheads). Section 4 (Fig. 1D) includes Fig. 1A of Schmidt (2020) and Section 8 (Fig. 1H) includes Fig. 1A of Hofmann (2021). The injection site in Fig. 1A of Hofmann (2021) may look like a single entity, but in fact, it consists of two parts, a dorsal and a ventral part of labeled fiber bundles, and therefore, it cannot be the injection site (Fig. 1K and 1L). These two fiber bundles can be followed rostrally and clearly seen in Section 7 (Fig. 1G). The true injection site is located more rostral. It can be found in the Sections 4 and 5 (Fig. 1D and 1E; arrows). Perhaps it would have been better to show the injection site in Section 5 (Fig. 1E) instead of Section 4 (Fig. 1D) in my article, but I think that this is not a mistake. I wrote in the second paragraph of the Results section that the injection site lies in the "entire caudal portion" of the nucleus corticalis (Schmidt, 2020). This would mean Sections 4 and 5 (Fig. 1D and 1E).

Consequently, the labeled processes in the tectum opticum in Fig. 1B of Schmidt (2020), observed after tracer injection into the nucleus corticalis, are the dendrites of the neurons of the nucleus corticalis. Their distribution in the tectum opticum serves as reference for the labeled structures shown in Fig. 1F and 1J (Schmidt, 2020).

### **Other labeled structures**

M. Hofmann presents a set of photomicrographs of other brain structures with labeled cells in the whole brain for the selected injection cases in my article. I did not check them. All these afferents to the nucleus corticalis (Fig. 1C–1P; Hofmann, 2021) or tectum opticum (Figs. 2B–2J and 3B–3L; Hofmann, 2021) would not contribute to the pattern in the nucleus glomerulosus in Fig. 2 of my article (Schmidt, 2020). It depends on the neurons of the nucleus corticalis and summarizes the findings of Fig. 1. The connection is "tectum opticum (dendrites of nucleus corticalis neurons)—nucleus corticalis (cell bodies of nucleus corticalis neurons)—nucleus glomerulosus (terminating axons (glomeruli) of nucleus corticalis neurons)". As it can be inferred from the title of my article, the major aim was to describe these "two different areas of the nucleus glomerulosus" (Schmidt, 2020).

However, important labeled structures regarding my article are neglected. This applies, for example, to the labeled cells in the dorsal telencephalon whose projections to the tectal layer stratum griseum centrale I cited as "unpublished observations" in the first paragraph of the Discussion section (Schmidt, 2020). I would have expected them among the photomicrographs in Fig. 3 of Hofmann (2021). They are not shown.

## **Topographical organization**

M. Hofmann concluded that the projection pattern in the nucleus glomerulosus may be caused by a topography, because one injection into the tectum opticum is very dorsal (the stratum fibrosum et griseum superficiale injection: Fig. 1E; Schmidt, 2020) and the second one extreme



**Fig. 1.** Identifying the injection site. A series of 10 transverse semi-sections of the brain of *Colomesus asellus*. The sections are arranged from rostral (**A**) to caudal (**J**). Section 4 (**D**) includes Fig. 1A of Schmidt (2020) and Section 8 (**H**) includes Fig. 1A of Hofmann (2021). The track of the glass micropipette is visible in Sections 4–8 (**D**–**H**; arrowheads), and the injection site is indicated in Sections 4 and 5 (**D** and **E**; arrows). Higher magnification of Section 8 (**H**) is shown in (**K**). The large boxed area in (**K**) corresponds to Fig. 1A of Hofmann (2021) and his identified injection site. Higher magnification of the small boxed area in (**K**) is shown in (**L**). It reveals two labeled fiber bundles (dashed lines). Lateral is to the left. NC, nucleus corticalis. Scale bars = 500 μm (**A**–**K**); and 100 μm (**L**).

lateral (the stratum griseum centrale injection: Fig. 1I; Schmidt, 2020) (Hofmann, 2021: see Section "Tectal injections"). I disagree with his conclusion. The connection "tectum opticum—nucleus corticalis—nucleus glomerulosus" can be considered as one system that consists of two subsystems due to the different layers in the tectum opticum (Fig. 2; Schmidt, 2020). These two subsystems are the connections "stratum fibrosum et griseum superficiale—nucleus corticalis—nucleus glomerulosus" and "stratum griseum centrale—nucleus corticalis—nucleus glomerulosus". In my opinion, a topographical organization may occur within each of the two subsystems, but not across them. Investigations regarding a topographical organization, however, would be subject of further

studies, as I mentioned in the third paragraph of the Discussion section (Schmidt, 2020).

**Supplementary Materials.** To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0952523821000018.

Brain section series presented in Fig. 1.

# References

Hofmann, M.H. (2021). Letter to the editor. Visual Neuroscience 38, (in press). Schmidt, M. (2020). Two different areas of the nucleus glomerulosus in the South American pufferfish, Colomesus asellus. Visual Neuroscience 37, E003.