

Rebuttal sent on July 28, 2020

Dear Claude, Dear reviewers,

We are sad to hear that we failed to convince you of the significance of our study. There are a few points that we would like to address in the following.

We agree that functional experiments probing the role of the discovered neurotransmitter receptors would be very interesting. However, the goal of this study was to lay the foundation for functional experiments by describing the distribution of some of the neurotransmitter receptors in T4/T5 neurons. Without the design and creation of the adequate genetic tools and the knowledge about receptors involved, functional investigations like RNAi or pharmacological experiments would be tapping in the dark.

Although the receptor distributions described in our work broadly fit the synapses mapped in EM studies, our findings are not trivial. We found it very intriguing that they exactly match the EM data and that we are able to resolve this with light microscopy. For instance, various indirect evidence made us expect that GluCl α would form the synapse between Mi9 and T4, however, it was never demonstrated that this is really the case. It still would have been possible that other Glu receptors sit on the tips of T4 dendrites. While we cannot exclude that there are other Glu receptors present on T4 dendrites, as pointed out by reviewer 4, we at least set the starting point for this type of investigations.

Along these lines, we wanted to highlight again the novelty of the tools we created and respond to some misleading comments. Reviewer 4 stated that “a recent publication in eLife demonstrated that GluCl α can be tagged conditionally as well (Molina-Obando et al., 2019).” This statement is incorrect since the Molina-Obando et al. (2019) study did not create a conditional tagging tool for GluCl α . It merely used the already existing MiMIC GFSTF line to conclude that GluCl α is broadly expressed in the visual system in the fly (Fig. 4). This MiMIC line is a pan-neuronal, endogenous GFP-tagged allele of GluCl α , but it is not conditional and it cannot be used to assign expression of this receptor to a specific cell-type, as we pointed out in our study. Furthermore, they created a GluCl α -FlpStop line and a GluCl α -allele insensitive to picrotoxin for functional investigations, but again no conditional labeling strategy for this receptor (Fig. 5 and Fig. 7). Taken together, there is no other tool for conditional tagging of GluCl α existing to our knowledge.

Furthermore, reviewer 4 called the T2A-Gal4 knock-in study (Kondo et al., 2020) as an already existing library for tagged neurotransmitter receptor lines. While this study is extremely useful to the community, it mainly generated T2A-Gal4 lines for studying the cells expressing a specific NT receptor of interest. They also generated a Venus-tagged version of ChAT, which is endogenous, but not cell-type specific. It only constitutes an alternative approach to the MiMIC GFSTF insertions and cannot be applied to study subcellular receptor distributions. Here, we would also like to point out that creating tagged lines is a very delicate process heavily depending on the 3D-structure of the protein and the attachment site of the tag. Hence, not every C-terminal tagging strategy is generalizable and works in a straightforward way for every gene. The optimal location of the tag has to be found and re-considered for every protein of interest.

The second mayor request raised by two of the reviewers was to expand the FlpTag method to other genes. We have already done this, but did not include it in this manuscript. Attached, you can find images of three more FlpTag lines we generated for the voltage-gated ion channels para and Ih and the metabotropic GABA receptor Gaba-b-r1. We find in general that the FlpTag approach seems to work, whenever the corresponding MiMIC GFSTF line of the same MiMIC insertion site is showing GFP signal. On the contrary, if the MiMIC GFSTF line shows no signal, the FlpTag approach is also not functional. This is what we observed for

attempts in making D α 7 and Rdl FlpTag lines. The location of the MiMIC insertions we tested seem to be suboptimal for GFP-tagging of these proteins. We are currently testing the CRISPR HDR FlpTag construct for Rdl and D α 7. Maybe it would have been beneficial for our manuscript to emphasize more on these technical aspects of the tool development, especially for the 'Tools and Resources' format which we aimed for in the final submission. In fact, reviewer 2 states: 'As such, I find the manuscript in its current form better suited for a Tools and Resources article.', which, in fact, it was.

Our question is whether you would reconsider our ms if we rewrite it and focus on the 'Tools and Resources' aspects of it. In this case, we would also include the other FlpTag lines we generated to demonstrate that our FlpTag approach can be used as a general strategy for cell-specific labeling of membrane proteins.

Looking forward to hearing from you

Sandra, Renee & Axel