Optomotor response screening

Laboratory course report

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Abstract

In the visual system of *Drosophila*, T4/T5 neurons have been characterised as first stage of computation of the direction of visual motion, as blocking these neurons results in a lack of optomotor response. However, until now it has still not been completely revealed, how these neurons acquire their properties essential for sensing motion. Drgx and CG14340, which had been identified as possible candidates, do not seem to affect motion detection, as silencing or knocking down did not impair the optomotor responses. Furthermore, there seems to be a strong selectivity regarding different effector lines, as blocking T4/T5 neurons in order to abolish the optomotor responses was only successful with one *UAS-TeTxLC* line. Blocking the T4/T5 neurons via Kir 2.1 did not impair the optomotor response with either of the different UAS-Kir2.1 effector used.

1 Introduction

To safely navigate through a constantly changing space, it is indispensable for flies to integrate self-motion and environmental information in order to be able to perform very precise and robust flight maneuvers (Sherman and Dickinson, 2004; Budick et al., 2007). When tethered flies are exposed to optical stimuli in a rotating arena, they actively try to steer in the direction of motion in order to stabilize the visual image on the retina, a behaviour known as optomotor response (Götz, 1968).

In *Drosophila melanogaster*, T4/T5 neurons are known as the first stage of computation of the direction of visual motion. They both exist in four subtypes in which each responds to one of the cardinal directions and project axons into one of the four lobula plate layers (Maisak et al., 2013). T4 neurons receive input from neurons brightness increments, whereas T5 neurons receive input from neurons encoding brightness decrements (Joesch et al., 2010; Maisak et al., 2013; Shinomiya et al., 2014). Previous studies have already shown the impact of these neurons on motion detection as blocking T4/T5 neurons resulted in a lack of optomotor response (Schnell et al., 2012; Bahl et al., 2013; Maisak et al., 2013). However, which molecular components are involved for T4/T5 neurons to acquire their properties essential for sensing motion, is still not fully understood. Drgx, which encodes a paired-like homeobox transcription factor, was found to be a potential candidate as it is expressed in T4/T5 neurons and silencing drgx resulted in anatomical changes in the T4/T5 neurons. CG14340 is expressed in Tm1 and T4/T5 neurons and predicted to activate drgx expression (Rass, 2021).

This work focuses on this topic by screening the optomotor behaviour of drgx knock down flies and a CG14340 mutant line. Furthermore, it was tried to reproduce the previous achieved results of flies with blocked T4/T5 neurons by using different *UAS-Kir2.1* and *UAS-TeTxLC* effector lines.

2 Materials and methods

2.1 Fly stocks and maintenance

Flies were maintained in plastic vials with standard cornmeal agar medium at 25°C and 60% humidity on a 12h/12h light and dark cycle. The following strains were used as driver lines: *R9B10-Gal4*, *R39H12-Gal4*, *R42F06-Gal4, nsyb-Gal4*. For knock-down experiments, *UAS-Drgx-Rnai*, *UAS-Cherry-Rnai* (control) were used. The *CG14340-lexA* line was produced via CRISPR/Cas9 gene knock-in (Rass, 2021). Two different *UAS-Kir2.1* effector lines (341, BL#6596; 342, BL#6596) and three different *UAS-TeTxLC* effector lines (351, BL#28837; 353, BL#28841; 211, BL#28838) were used for blocking experiments (first number indicates the number that is used for the fly line in the fly stock of the lab (Brembs' lab), #BL = Bloomington ID). To label T4/T5 neurons, the *UAS-CD8::GFP* fly strain was used. Crossings with *Canton S* wild type flies served as controls. In all experiments, 1–3-day old adult flies were tested. Additionally, 2 weeks old *CG14340-lexA* flies were tested. Only female flies were tested in the experiments except for the R9B10-Drgx and R39H12-Drgx flies. For blocking the T4/T5 neurons, the crossings were set up reciprocally (**Figure 1**). The crossing with one of the *UAS-TeTxLC* effector line (352) turned out to be lethal in both directions: Larvae were unable to pupate. Crossing with another *UAS-TeTxLC* effector line (211) was only lethal when females of the *R42F06-Gal4* driver line were used.

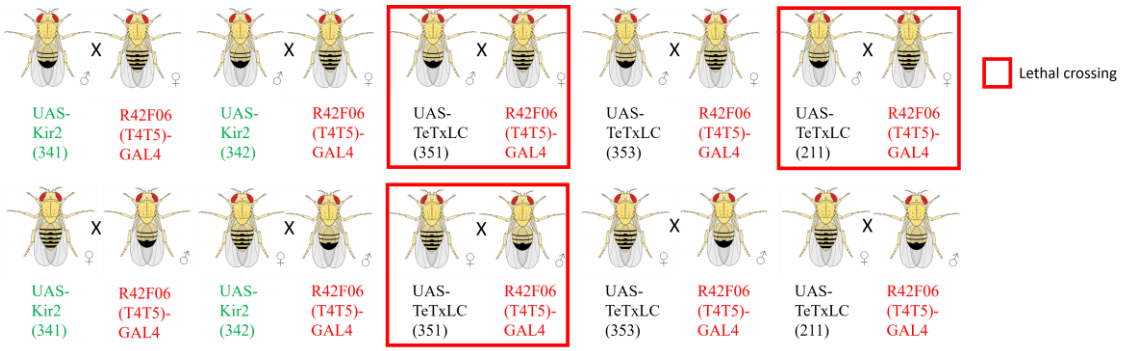

Figure 1: T4/T5 blocking crossing scheme: Number in brackets indicates the number of the fly line in the brembs' lab fly stock. Red borders show that this crossing was lethal.

2.2 Behavioural essay and analysis

2.2.1 Optomotor setup

Flies were cold-anesthetized and a small stick of fishing line (length of ~ 2 cm) was glued on the centre of the thorax via a UV sensitive glue. The flies were still able to freely move their head and wings and stored for at least 1 hour prior to the experiments in a thin ring of foam rubber attached to a petri dish. Via a clamp the animals were positioned with their sticks on a platform in the middle of the arena. To test the optomotor response of walking flies, the setup of von der Linde (Linde, 2018) was used (**Figure 2**): The arena of this optomotor setup is illuminated by a 100 W light bulb with a filter for heat generating wavelengths. Transparent plastic tubes are responsible for a homogeneous distribution of the light. On the inside of the cylindrical arena, an even pattern of black and white stripes can be found. The rotation of the arena is achieved via a motor controlled by a motor unit that receives signals from a Digital-Analog-Converter (DAC) connected to a computer. The platform consists of a bent tin strip where the fly is placed on the horizontal plane and able to push the platform to the left and right. This movement of the platform is then detected by a photoelectric sensor. Using a python-based script, the platform signal will be read at 100 Hz and a live graph showing the platform position as well as the arena position can be seen. The duration of each experiment was 5 minutes, with alternating rotations for 30 seconds and the metadata for each experiment were saved as an xml-file.

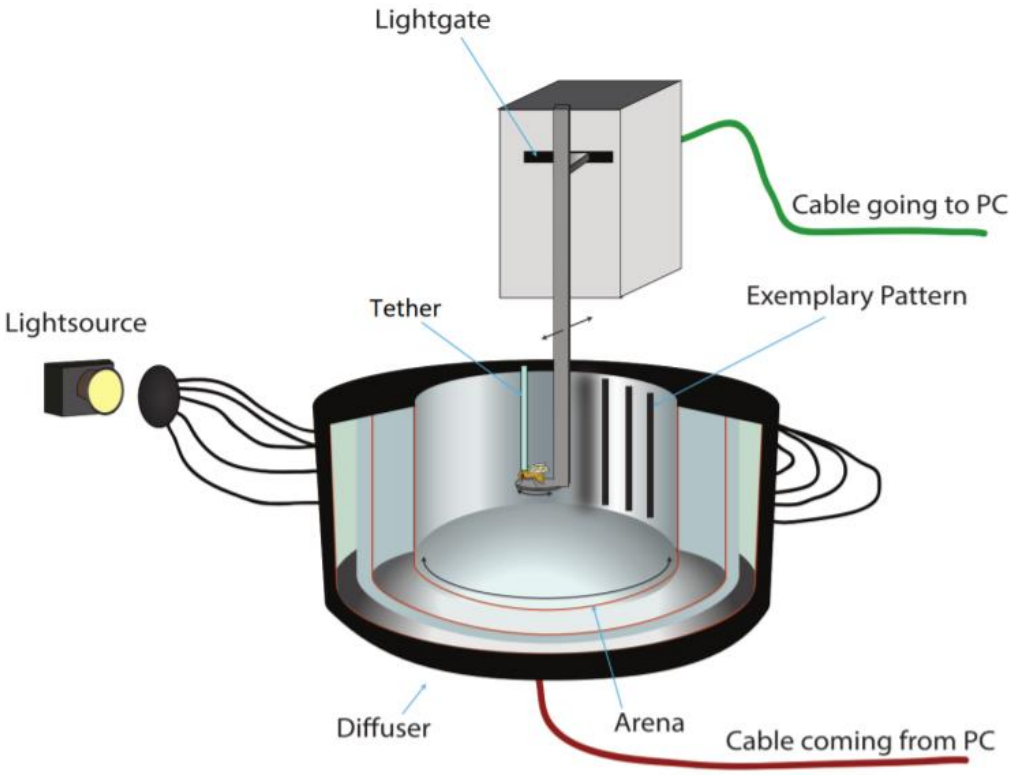

Figure 2: Optomotor setup (Linde, 2018): Basic schematic of the experimental setup. The arena is able to rotate clockwise and counter-clockwise (indicated by black arrow).

2.2.2 Evaluation of the optomotor data

Using a R based script, the individual mean optomotor traces for each rotation (left and right turning of the arena) can be plotted. Flies performing an optomotor response will push the platform to one side as an attempt to follow the rotation of the patterning. When switching the direction of the rotation, the fly will push the platform towards the opposite direction. As a result, the traces of flies showing an optomotor response tend to look like a double-sigmoidal curve. On the other hand, it is of course possible that some flies lack an optomotor response. In this case, a double-sigmoidal model would not fit and therefore a linear model is needed. To determine, whether the observed traces would fit a double-sigmoidal model, four criteria need to be fulfilled (**Figure 3**): The mean difference between the mean optomotor responses at the beginning (0-10/30-40 s) and the end (20-30/50-60 s) of the right and left rotations needs to be above a certain value. The same applies for the slope of a linear fit of the optomotor traces at the first half (first 15 s) of clockwise and counter-clockwise rotation. Furthermore, this slope must be positive for the right turning optomotor traces and negative for the left turning optomotor traces. To

look for differences in optomotor behaviour, the script is able to calculate four different parameters: optomotor magnitude (OM), optomotor slope (OS) and the asymmetry indices for the optomotor magnitudes and slopes. The optomotor magnitude indicates how strong the optomotor response was, whereas the optomotor slope is an indicator of how fast the optomotor response war. For the sigmoidal model, the OM is calculated as the difference between the maximum and the minimum asymptote. The slope is detected as the mean of the first and second slope. For the linear model the OM is determined as the difference between the mean optomotor responses of the right rotation traces and the mean optomotor response of the left rotation traces. The slope is calculated as the mean of the slopes of the linear fit of the right and left turning optomotor traces. Furthermore, the asymmetry indices of the optomotor magnitude and the slope can be calculated in both models. A Mann-Whiney U test is performed to look for statistical differences. The script is available at "https://github.com/brembslab/DTSevaluations".

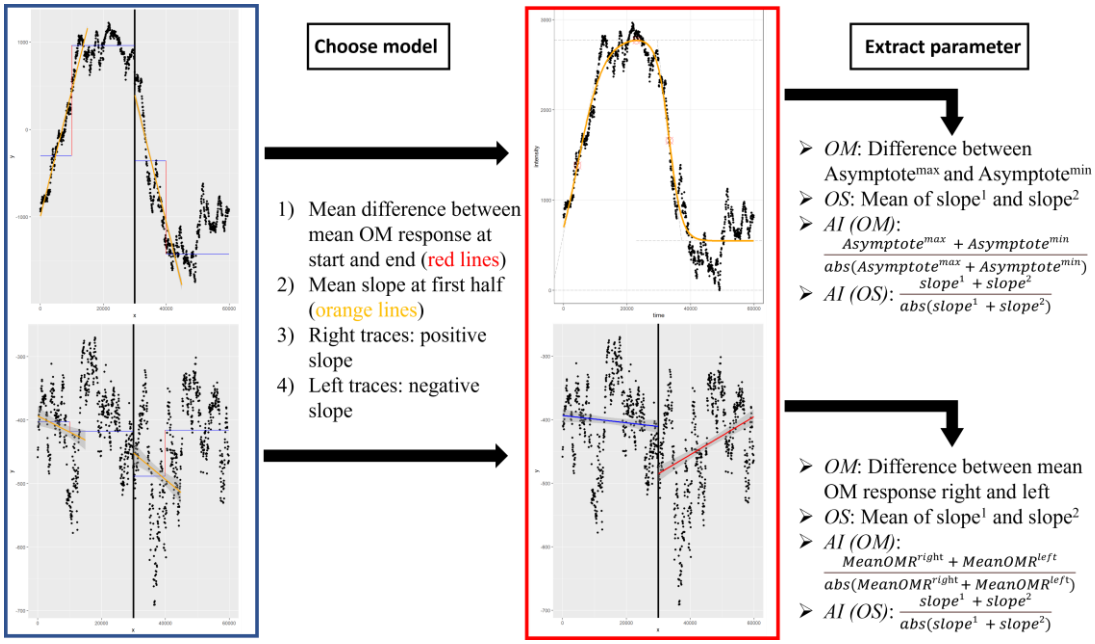

Figure 3: Process of analysing and evaluating of the optomotor data: Plots in the blue box represent examples of optomotor traces of individual flies. Above is an example of a fly showing a solid optomotor response whereas the example below does not seem to show any optomotor response. Graphics in the red box show the plotted double-sigmoidal and linear model for these examples. Based on the choice of the model, further evaluation parameters (OM, OS and AIs) are calculated.

2.3 Brain dissection and imaging

Brains of female adult flies were fixated for 2 hours in 4 % PFA-TX100 and subsequently dissected in PBS. After that, they were washed three times with PBST (0.1 % TX-100) for 10 minutes and one time with PBS for 5 minutes and then mounted in VectaShield H-1000. A total of 5 brains were dissected. Imaging was performed with a Leica TCS SP8 confocal microscope.

3 Results

3.1 Silencing drgx in T4/T5 neurons did not impair the optomotor response

A specific knockdown of drgx was performed by combining *UAS-Drgx-Rnai* effector lines with two different Gal4 driver lines. The *R39H12-Gal4* driver line drives expression in T4/T5 neurons from late L3 larval stage onwards (Schilling et al., 2019) and the *R9B10-Gal4* driver line already at an earlier stage in neuroblasts (Rass, 2021). Using either the *R39H12-Gal4* or the *R9B10-Gal4* driver line to knock down the drgx expression in T4/T5 neurons at different developmental stages did not impair the optomotor response, as in both cases no significant differences in the optomotor magnitudes as well as optomotor slopes were found compared to control flies that were crossed to a *UAS-Cherry-Rnai* effector line. (**Figure 4**).

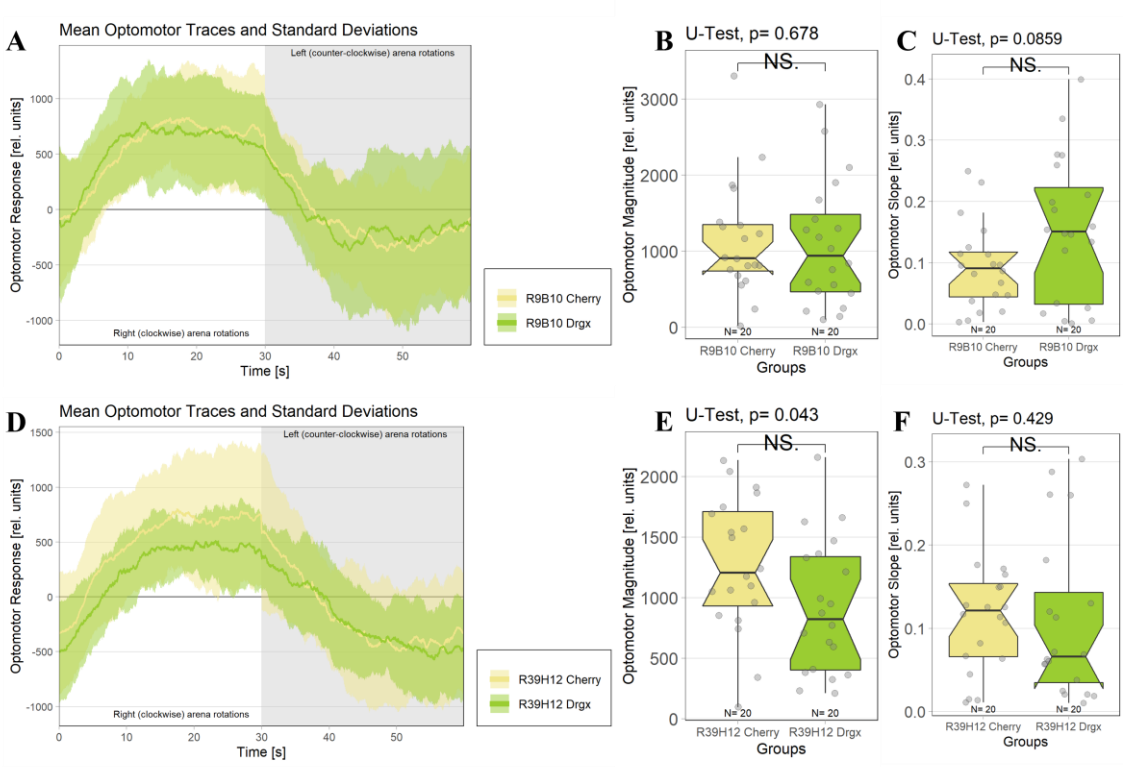

Figure 4: Optomotor response of drgx knockdown flies: (*A,D)* Mean optomotor traces with standard deviations of flies expressing *Drgx-Rnai* or *Cherry-Rnai* in T4/T5 neurons. *(B,E)* Mean optomotor magnitudes. *(C,F)* Mean optomotor slopes. Bars represent standard deviations. Single data points are shown as grey circles. U-Test was performed to test for statistical significance. Stars above brackets indicate p value (*p < 0,005, **p < 0,001, ***p < 0,0001, NS. p > 0,05).

3.2 CG14340 mutant flies showed solid optomotor responses

The *CG14340-lexA* mutant line was produced via CRISPR/Cas9 gene knock-in (Rass, 2021). A heterozygous line served as control (*CG14340-lexA/+)*. Similar to the previous results, 1-3 day old *CG14340-lexA* mutant flies were able to perform solid optomotor responses, resulting in no significant differences regarding the optomotor magnitude (U-test, p=0,247) and slope (U-test, p=0,971) compared to the heterozygous control flies (**Figure 5**). Similar results were obtained when using two week old *CG14340-lexA* flies.

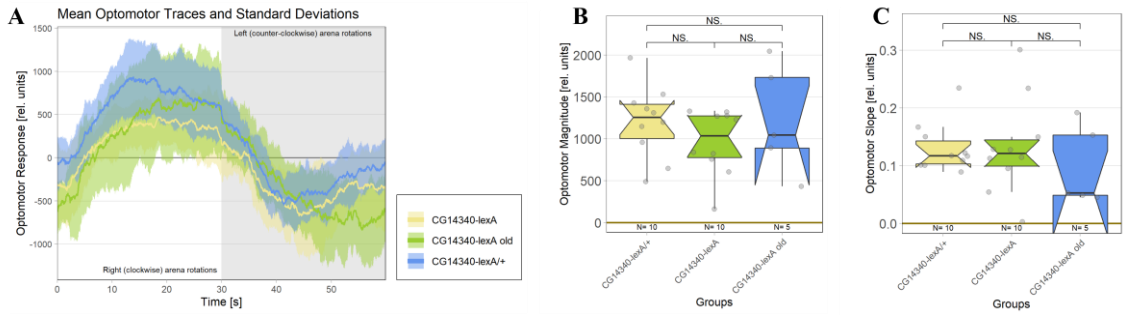

Figure 5: Optomotor response of *CG14340-lexA* mutant flies: *(A)* Mean optomotor traces with standard deviations of *CG14340-lexA* mutant flies and heterozygous control flies. *(B)* Mean optomotor magnitudes. *(C)* Mean optomotor slopes. Bars represent standard deviations. Single data points are shown as grey circles. U-Test was performed to test for statistical significance. Stars above brackets indicate p value (*p < 0,005, **p < 0,001, ***p < 0,0001, NS. p > 0,05).

3.3 Silencing T4/T5 neurons did not impair the optomotor response for all effector lines

In order to silence T4/T5 neurons, the Gal4 driver line R42F06 was used, leading to selective expression in T4 and T5 cells (Schnell et al., 2012). Interestingly, only one *UAS-TeTxLC* effector line (TeTx 211) seemed to be able to completely block T4/T5 neurons resulting in a significant lack of optomotor response (**Figure 6 G-I**). These flies showed significantly lower optomotor magnitudes and significantly slower reactions to the change in direction of rotation (optomotor slopes) compared to the effector control flies (OM: U-test, p < 0,0001; OS: U-test, p < 0,001) as well as to the driver control flies (OM: U-test, p < 0,0001; OS: U-test, p < 0,005). Blocking via Kir 2.1 did not seem to impair the optomotor response (**Figure 6 A-C**) as there was no significant reduction in optomotor responses or slopes compared to the effector control flies. Furthermore, the optomotor magnitudes and slopes of *T4T5-Gal4/UAS-Tetox353* flies were not significantly lower compared to the driver control flies (OM: U-test, p= 0,185; OS: U-test, p about < 0,054). However, looking at the individual data points revealed, that the

optomotor magnitudes and slopes of *T4T5-Gal4/UAS-Tetox353* flies appeared to be strongly reduced for about half of these flies, suggesting that T4/T5 neurons might have been blocked by only some of these flies (**Figure 6 E and F**).

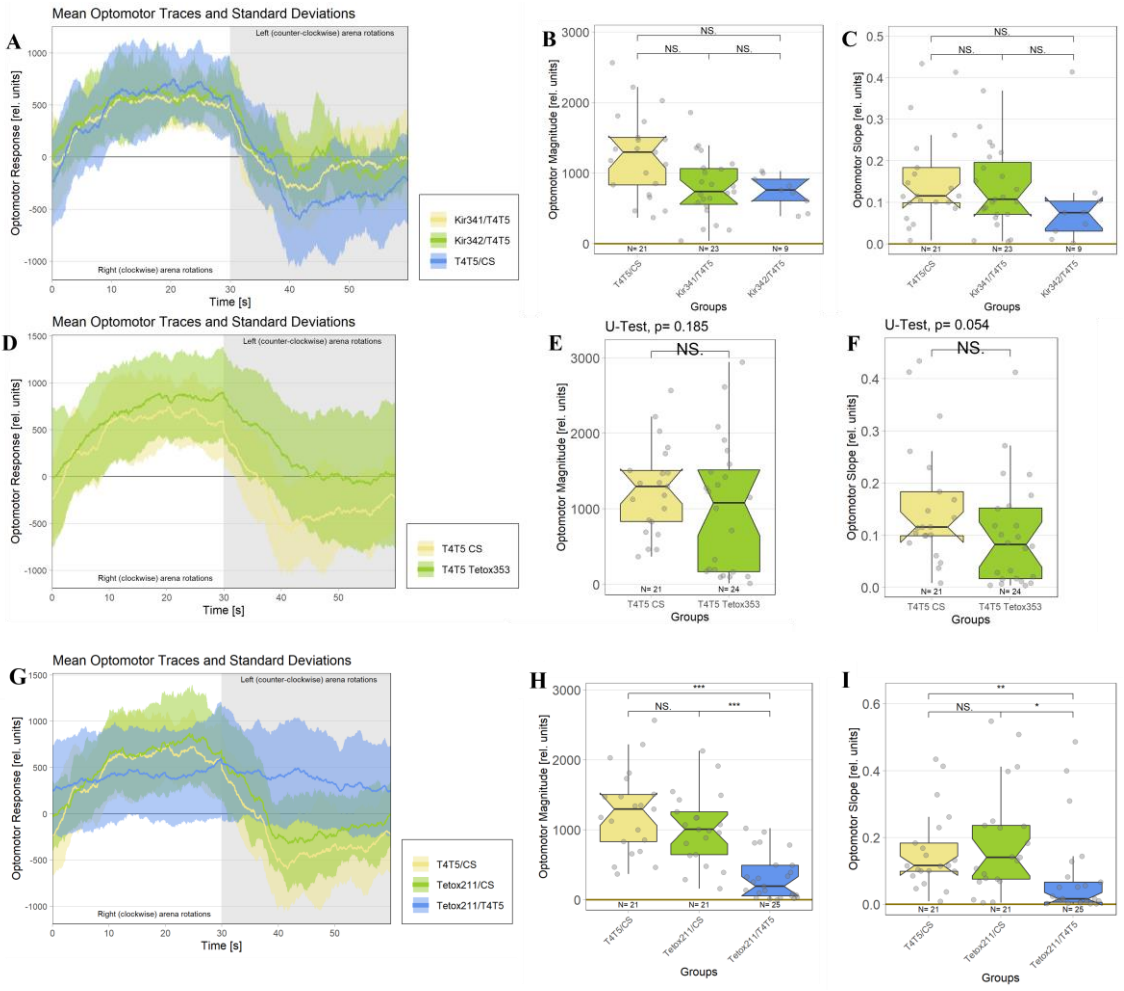

Figure 6: Optomotor response of flies expressing Kir 2.1 or tetanus toxin in T4/T5 neurons *(A,D,G)* Mean optomotor traces with standard deviations of flies expressing Kir 2.1/ tetanus toxin in T4/T5 neurons. *(B,E,H)* Mean optomotor magnitudes. *(C,F,I)* Mean optomotor slopes. Bars represent standard deviations. Single data points are shown as grey circles. U-Test was performed to test for statistical significance. Stars above brackets indicate p value (*p < 0,005, **p < 0,001, ***p < 0,0001, NS. p > 0,05).

To test whether the effectors used would in principle be able to block neurons, all effector fly lines were crossed with *nsyb-Gal4* flies. These crossings turned out to be lethal for all effector lines. Interestingly, Kir342/nsyb flies died only in the approximately second larval stage.

3.4 Expression pattern of the *R42F06-Gal4* line

To ensure that the *R42F06-Gal4* driver line selectively expresses in T4/T5 neurons, the brains of adult *R42F06-Gal4*/*UAS-CD8::GFP* flies were examined. All dissected brains revealed GFP expression in the T4 and T5 neurons of the optic lobes (**Figure 6**).

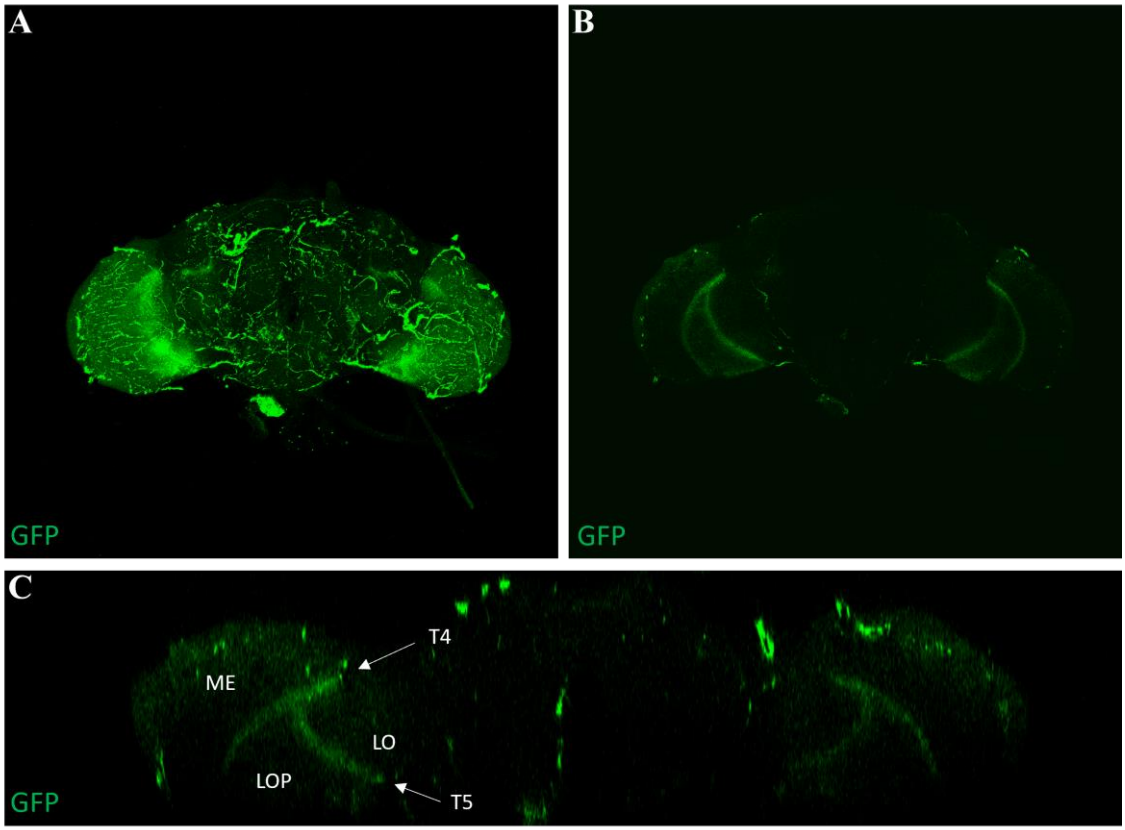

Figure 7: GFP expression pattern of *R42F06-Gal4*/*UAS-CD8::GFP* fly brain: *(A)* Z-Projection of frontal view *(B)* Frontal view of single slice *(D)* Horizontal view of single slice. White arrows indicate T4/T5 neurons. ME=Medulla, LO=Lobula, LOP=Lobula Plate.

4 Discussion

In all of the experiments that were carried out there was a small positive shift of the optomotor traces, which was also detected by the asymmetry indices of the optomotor magnitudes. This shift could be explained by various factors. First, given the fact that the horizontal plane of the tin strip where the flies were positioned might not be totally smooth or flat, the flies could have been able to push the platform to one side more easily. An alternative explanation could be a systematic error when gluing the flies or positioning them on the platform.

Looking at the results, silencing drgx or knocking down CG14340 does not seem to impair the optomotor response (**Figure 4 and 5**). However, as there was a small reduction in optomotor magnitudes of flies expressing *drgx-Rnai* from late L3 larval stage onwards, further studies would be needed to reveal whether this effect was actually caused by silencing the drgx expression. One possibility to detect smaller differences in optomotor responses would be to increase the width of the black stripes making it harder for the flies to follow the rotation of the patterning.

Furthermore, using two different fly lines to hyperpolarise T4/T5 neurons with the inward rectifier K^+ channel Kir 2.1 (Johns et al., 1999) did not impair the optomotor response (**Figure 6 A-C**). On the other hand, using one of the *UAS-TeTxLC* effector lines (211) to block T4/T5 neurons via tetanus toxin was sufficient to completely abolish the optomotor responses (**Figure 6 G-I**). In contrast to Kir 2.1, tetanus toxin cleaves the synaptic vesicle protein synaptobrevin, resulting in a loss of neurotransmitter exocytosis (Sweeney et al., 1995). Interestingly, blocking with the other *UAS-TeTxLC* effector line (353) resulted in strongly reduced optomotor magnitudes and slopes of about half of these flies, indicating that T4/T5 neurons were only blocked in some of these flies (**Figure 6 D-F**). A third *UAS-TeTxLC* line (351) could not be tested, as the crossings with *R42F06-Gal4* turned out to be lethal.

Schnell, et al., which had previously shown that blocking T4/T5 neurons resulted in a lack of motion responses, used a effector line, carrying *UAS-Kir2.1-EGFP* on the second and *tub-Gal80^{ts}* on the third chromosome. These flies were raised at 25 °C and shifted to 31°C, 2–5 days prior to hatching to inactivate Gal80ts. As a second effector strain they used a fly line, carrying *UAS-shi^{ts}* on the third chromosome. Shipire^{ts} is a temperature-

sensitive mutation of a dynamin orthologue gene which blocks synaptic transmission by inhibiting vesicle endocytosis (Schnell et al., 2012). Given the fact, that although both the effector lines, whose crossings with *nsyb-Gal4* were lethal, as well as the *R42F06-Gal4* driver line, which indeed selectively expressed in T4/T5 neurons, appeared to work in principle, it seems as if some of these neurons were somehow able to compensate the blocking. Consequently, further investigations would be needed to explain the observed selectivity regarding these effector lines in order to reveal whether, for example, *tub-Gal80^{ts}* would be needed to reproduce the previous accomplished block of T4/T5 neurons via Kir 2.1(Schnell et al., 2012). Furthermore, it would be interesting to see, whether a similar selectivity could be observed when using different *UAS-shi^{ts}* strains as effector lines.

5 References

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