Defined subpopulation of dopaminergic neurons mediates avoidance behavior under optogenetic activation

Bachelor Thesis

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Abstract

Dopamine plays a role in reinforcement in *Drosophila melanogaster* as well as in other organisms. Dopaminergic neurons involved in this process are considered to mediate either approach or avoidance behavior to help the organism making the right decisions and forming memories. These results rely mostly on classical condition experiments. Here, two subpopulations of dopaminergic neurons were tested in an operant behavioral paradigm. The ``Joystick´´ is a single fly optogenetic experimental setup which allows the fly to choose activation or inactivation of specific neurons without external stimuli. The findings suggest the tested neurons do not mediate one consistent value, instead the behavior changed over time between aversive and neutral behavior towards the optogenetic activation.

Zusammenfassung

Dopamin spielt sowohl in *Drosophila Melanogaster* als auch in anderen Organismen eine Rolle beim „Reinforcement‘‘. Es wird davon ausgegangen, dass die an diesem Prozess beteiligten dopaminergen Neuronen entweder Annäherungs- oder Vermeidungsverhalten vermitteln, um dem Organismus zu helfen, die richtigen Entscheidungen zu treffen und Gedächtnisse zu bilden. Diese Ergebnisse beruhen zumeist auf Versuchen klassischer Konditionierung. Hier wurden zwei Subpopulationen von dopaminergen Neuronen in einem operanten Verhaltensparadigma getestet. Der ``Joystick" ist ein optogenetischer Versuchsaufbau, der es einer einzelnen Fliege ermöglicht, die Aktivierung oder Inaktivierung bestimmter Neuronen ohne äußere Reize zu wählen. Die Ergebnisse deuten darauf hin, dass die getesteten Neuronen nicht einen einheitlichen Wert vermitteln, sondern dass sich das Verhalten im Laufe der Zeit zwischen aversivem und neutralem Verhalten gegenüber der optogenetischen Aktivierung ändert.

1. Introduction

Animals depend on learning to adapt their behavior according to the environment for avoiding harm and choosing benefits. In *Drosophila* the neurotransmitter dopamine (DA) is involved in reinforcement learning. Different subsets of dopaminergic neurons (DANs) are considered to play a role in punishment prediction (Riemensperger et al. 2005). First indication of DA playing role also in appetitive learning was as dDA1 receptor mutant showed impaired reward learning (Kim et al. 2007). DANs signaling reward during odor memory formation are localized in the protocerebral anteriomedial (PAM) cluster (Liu et al. 2012). These findings are mainly observed in classical olfactory conditioning experiments.

Since it is assumed that DANs encode for a certain value, either reward or punishment, this might also be applicable for operant conditioning experiments. Rohrsen et al. (2021) examined different subpopulations of DANs in four operant conditioning experiments for approach or avoidance behavior. The conclusion was that flies behaved differently depending on context and no constant behavior was visible across different experiments.

Experiments like this are enabled by the availability of many different GAL4-driver lines which specifically label different subsets of DANs (Xie et al. 2018). In this thesis the driver line TH-C-AD;TH-D-DBD was used which specifically labels for DANs, two projecting into the wedge-neuropil (WED) (Liu et al. 2017) and two into the ventrolateral protocerebrum (VLP) (Ito et al. 2013). They are part of the posterior inferiormedial protocerebrum (PPM2) cluster (Mao und Davis 2009).

This cluster is also targeted by the TH'-GAL4 driver line used in experiments of Rohrsen et al. (2021). This specific line avoided activation of the neurons over all four optogenetic experiments. In a rescreen test in the Joystick the interesting trend of a shift from significantly negative values indicating avoidance to not significantly positive values was observed. This change in behavior over time could speak against the hypothesis of one certain value encoded by specific DANs (Rohrsen et al. 2021).

In this thesis a similar Joystick experiment was performed with the TH-C-AD;TH-D-DBD line. The single-fly operant behavioral paradigm was used to investigate the role of the specific target neurons in value computation. If these DANs were responsible for the shift from avoidance to approach observed in the TH'-GAL4 line in Rohrsen et al. (2021), this behavior should be reproducible in the Joystick. If not, it would still be interesting to investigate whether the DANs investigated in this thesis really encode for one consistent value

(aversive) regardless of the context or if this does not apply to operant testing. To verify whether observed effects were DA-dependent flies were fed the DA synthesis inhibitor 3-iodo-tyrosine (3IY) and tested again.

2. Materials and Methods

2.1 Fly stocks and maintenance

All flies used in the following experiments were maintained in plastic vials with cornmeal/molasses medium and fresh yeast. Vials were stored in a climate chamber with controlled temperature at 25°C, 60 % humidity and 12/12h light/dark cycle. Flies were transferred into fresh vials every day. Hatched flies were transferred to fresh vials and stored for one day.

For the experimental group, males containing the dopaminergic driver line TH-C-AD;TH-D-DBD, which specifically labels two PPM2 WED and two VLP DANs (Xie et al. 2018), were crossed with norpA^{P24};20xUAS-CsChrimson female virgin flies. These flies are expected to be blind due to the no receptor potential A mutation in the effector line to exclude interference with the innate positive phototaxis of *Drosophila*.

For the control groups w;Gr28bd-Gal4;TrpA1-Gal4 males were crossed with the same effector line as the experimental group. These flies express CsChrimson in heat sensitive neurons expressing Gr28bd (Mishra et al. 2018) and TrpA1 channels (Tang et al. 2013). The CsChrimson channel needs supplementation with retinal precursor All-Trans-retinal (ATR) to be activated (Yu et al. 2015). Therefore, the flies could be used as both, positive control with ATR treatment and negative control without ATR.

The brain dissection was performed with the offspring of TH-C-AD;TH-D-DBD flies crossed with UAS-mCD8::GFP flies.

2.2 Joystick

The joystick is a single-fly operant behavioral paradigm that allows flies to choose between optogenetic activation or inactivation of target neurons in the absence of other stimuli. Decisions are tracked over time, and preference indices are calculated to provide insight into whether flies showed appetitive or aversive behavior in response to the activation.

Preparation protocol

One-day old flies were anaesthetized on a cooling station. 15 to 30 males were selected and gently transferred into small glass vials containing medium without yeast and 15 µl of 200mM ATR dissolved in ethanol. Negative controls were fed with food containing the same amount of Ethanol but without ATR. Aluminum foil was then wrapped around the vials to

prevent any light exposure prior to the start of the experiment. All vials were stored at 25°C for 48 hours before being tested. After the main experiment the dopamine (DA) synthesis inhibitor 3-iodo-tyrosine (3IY) was used to verify a DA-dependent behavior. Flies were fed with different amounts of 10 mg/ml 3IY dissolved in water with different concentrations of sucrose and ATR on the cornmeal/molasses medium or instant medium for different timespans (**Table 1**).

Table 1: Feeding solutions

Food	Sucrose concentration in %	3IY	ATR
Instant	5	1.5 – 2 ml	5 µl
Instant	10	1.5 - 2 ml	5 µl
Instant	10	1.5 - 2 ml	10 µl
Normal	5	30 µl	15 µl
Normal (4 days)	5	45 µl	15 µl
Normal (half full)	5	30 µl	15 µl
Normal (half full)	5	45 µl	15 µl

Gluing

Flies were individually glued to fishing lines only using a blue light source. The fishing lines with a diameter of 0.6 mm were cut into approximately 2 cm long sticks. The end was glued to the dorsal side of the flies' thorax, using UV-sensitive glue and a corresponding lamp. This created a right angle between fly body and fishing line, allowing the fly to move its legs and wings. Prepared flies were kept in darkness at 25°C for one hour and fed a sucrose solution for five to ten minutes before testing.

Test setup

The test setup, called "Joystick" (**Figure 1**) consists of a clamp holding the fishing line attached to a fly which is then placed over a small platform. The fly stands on the platform with all six feet and can move the platform to both sides. A light guide is placed directly above the fly's head and connected to an LED, with sufficient power to illuminate the entire brain. This leads to an activation of CsChrimson channel containing neurons. The red light has a wavelength of 660 nm, the yellow light 590 nm and a 20 Hz pulse with 50 ms pulse width and no cycle delay. The platform acts as a light switch, so lateral movement of the fly results in light activation or deactivation. The position of the platform gets saved at a 20 Hz

rate. Three Joystick-machines were used simultaneously, each covered with a box blocking out any external light.

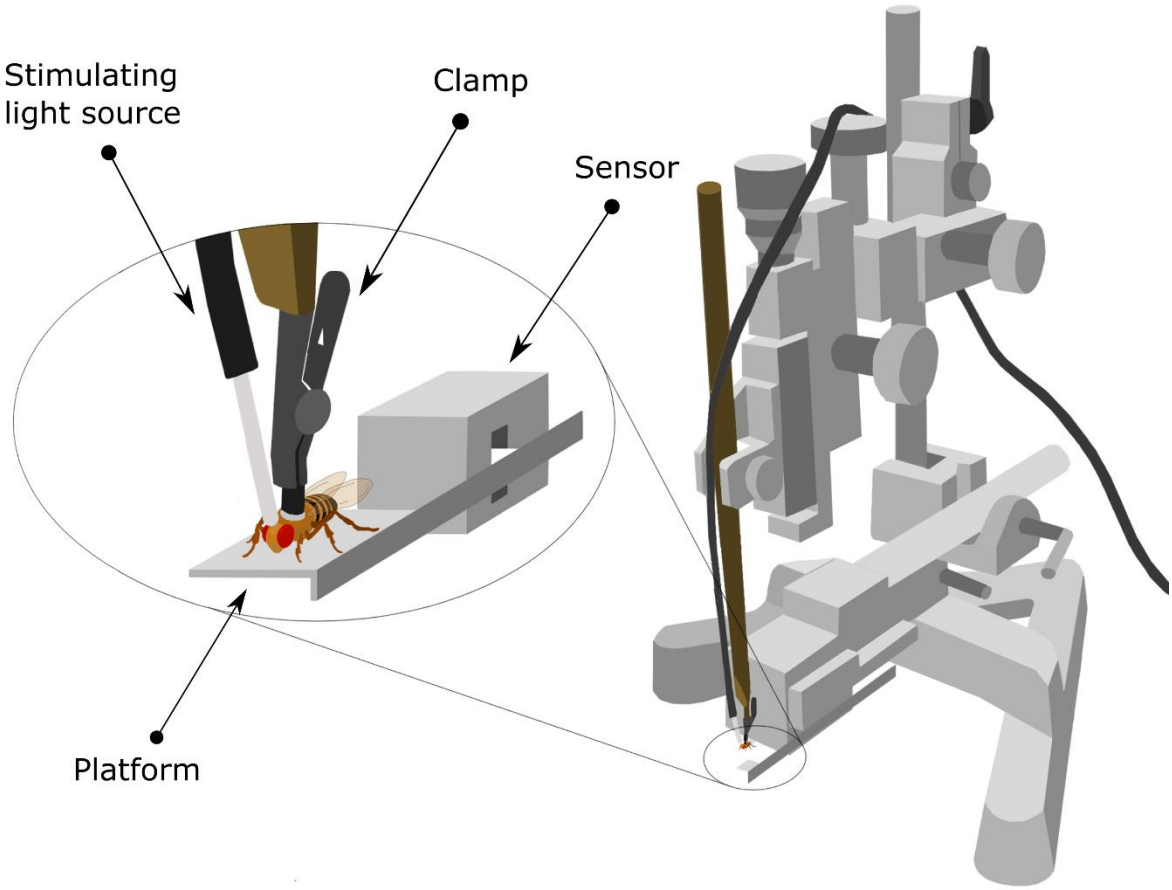


Figure 1: Schematic of the Joystick: The fishing line attached to the fly is held by a clamp that can be positioned so that the fly can use its legs to control the lateral position of the platform. The sensor records the platform's position, and the PC activates or deactivates the light based on the fly's choice, one side is preset to be the activated side during training periods. The LED light source is directed over the fly's head by a light guide.

Test protocol

The training protocol consisted of 10 60s long periods (**table 2**), including period 1 and period 10, where no optogenetic activation was performed. The light activating side was the same for all training periods of one test. The side was altered randomly between tests to achieve a balanced number of flies showing an initial preference for the activated side compared to flies showing an initial preference for the non-activated side.

Table 2: Test protocol

Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8	Period 9	Period 10
Pre-test	Training	Training	Training	Training	Training	Training	Training	Training	Post-test
60 sec.	60 sec.	60 sec.	60 sec.	60 sec.	60 sec.	60 sec.	60 sec.	60 sec.	60 sec.

Evaluation

From the obtained data sets a preference index (PI) was calculated for each period.

$$PI = \frac{(x - y)}{(x + y)}$$

x represents the number of data points referring to time spent in the domain in which the target neurons were optogenetically activated. y represents the number of data points referring to time spent in the non-illuminated domain. The PI is a value between +1 and -1 with positive values pointing to a preference for the optogenetic activation of the targeted neurons. For evaluating the raw data, a R script was used which can be accessed online at: „https://github.com/brembslab/Platform-Drosophila/tree/master/Platform-Optogenetics''. For data analysis R version 4.2.1 was used.

2.3 Pre-test: T-maze

The T-maze is an often used tool mainly for olfactory learning experiments (Tully und Quinn 1985). Here it was used as a low effort opportunity to test a high number of flies in a short time regarding phototaxis.

Test setup

The setup consists of three removable plastic tubes attached to a core structure which includes an elevator. Approximately 45-50 flies were transferred into the first tube for 10 minutes. Next, the flies were transferred into the elevator by gently tapping the T-maze on a soft surface. Once all flies were in the elevator, it was moved downwards to the level of both the transparent and the opaque testing tubes. The flies were able to move freely within both tubes and choose between the illuminated and the darkened tube for one minute. They were then counted under CO_2 -anesthesia.

Evaluation

For the T-maze a preference index (PI) was calculated:

$$PI = \frac{(x - y)}{(x + y)}$$

x represents the number of flies counted in the illuminated tube, y the number of flies in the dark tube. The resulting PI is again a value between +1 and -1, with positive values indicating positive phototaxis.

2.4 Dissection of adult *Drosophila* brains

2.4.1 Dissection without antibody staining

The TH-D-DBD/TH-C-AD > GFP flies were fixated in 4% paraformaldehyde (PFA) for two hours at room temperature, then transferred to phosphate-buffered saline, 0.1% Triton X-100 (PBST). The brains were dissected in 1x phosphate-buffered saline (PBS) and washed with PBST afterwards. They were then mounted with VECTASHIELD® *Antifade Mounting Medium* on a microscope slide.

2.4.2 Dissection with antibody staining

For the dissection with antibody staining, the flies were fixated, and the brains dissected in the same way as described in **2.4.1**, next the brains were washed 6x for 10 minutes with PBST. The unspecific binding domains were blocked by incubating the brains in 500µl 10% NGS in PBST for one hour. The brains were then incubated in primary antibody solution **(Table 3)** at 4°C over-night.

Table 3: Primary antibody solution

459 µl	PBST 0.1% TX-100%
15 µl	NGS
25 µl	Mouse-anti-Bruchpilot
1 µl	Rabbit-anti-GFP

Then the brains were warmed up at room temperature for 30 minutes and washed 6x in PBST. The brains were incubated at 4°C overnight in secondary antibody solution **(Table 4)** while protecting them from light exposure.

Table 4: Secondary antibody solution

480 µl	PBST 0.1% TX-100%
15 µl	NGS
2.5 µl	Goat-anti-Rabbit AF488
2.5 µl	Goat-anti-Mouse AF555

After the incubation the brains were again washed 6 times and mounted with VECTASHIELD® *Antifade Mounting Medium* on a microscope slide.

The brains were visualized using a confocal microscope (Leica SP8, RRID: SCR_018169) and edited in Image-J 1.53t and Inkscape 1.1.

3 Results

3.1. Pre-test: T-maze

The T-maze experiment (**2.3**) was performed to verify whether the norpA^{P24};20xUAS-CsChrimson flies were blind. Although previous joystick experiments performed in this laboratory seemed not to be affected by visual stimuli, flies used in this thesis were also to be tested in various optogenetic experiments which rely on vision. The T-maze was used to monitor phototaxis which only occurs in seeing flies (**Figure 2**). The calculated PI for the effector line, with a median of around 0.6, was similar to that of Wild Type Berlin (WTB) flies tested as positive control, indicating a regain in vision. Subsequently, two different copies of norpA flies without the UAS construct were also tested. In addition, copy 1 was kept in two populations at either 25°C or 18°C to rule out the possibility of a temperature effect. Both populations showed even higher PIs of around 0.8. Only the second copy of norpA flies had a PI close to zero with a median around -0.1, indicating blindness.

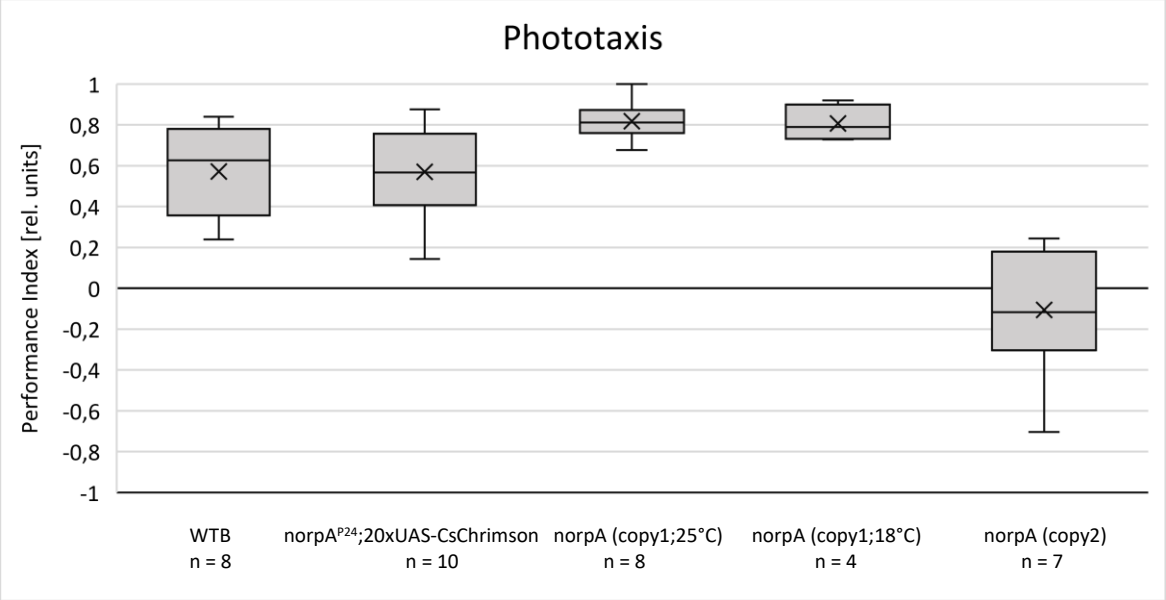


Figure 2: T-maze Phototaxis: Boxplots depicting the PI of approximately 45-50 flies with positive values indicating positive phototaxis, values around zero indicating no phototaxis. Solid lines within boxes depict medians, boxes represent the middle 50% of data points, whiskers represent non-outlier ranges. Wild Type Berlin flies were tested as control and showed positive phototactic behavior as well as all other tested groups except norpA (copy2) depicted on the right which did not show a preference for light or darkness.

3.2. Optogenetic testing with the Joystick

TH-C-AD;TH-D-DBD driver line crossed with norpA^{P24};20xUAS-CsChrimson flies were tested in the Joystick. Because of time issues no blind effector line was used in the following

experiments. Positive and negative controls, as well as experimental group were tested in parallel.

3.2.1 Red light

Red light optogenetic activation during trainings resulted in strong avoidance of the positive control group, with median PI values between -0.3 and below -0.6. In the post-test, the flies continued to show avoidance of the previously light-associated side with a median PI between -0.2 and -0.3 (**Figure 3A**). The negative control group's PIs were all negative during the training periods. Overall, the medians for this group did not reach values below -0.2 (**Figure 3C**). Training PIs for the experimental group started at approximately -0.3 following a pre-test median of zero. The median PIs increased throughout the training periods, with the highest value near zero in training 8. In the post-test phase the median PI increased to around 0.15 (**Figure 3B**).

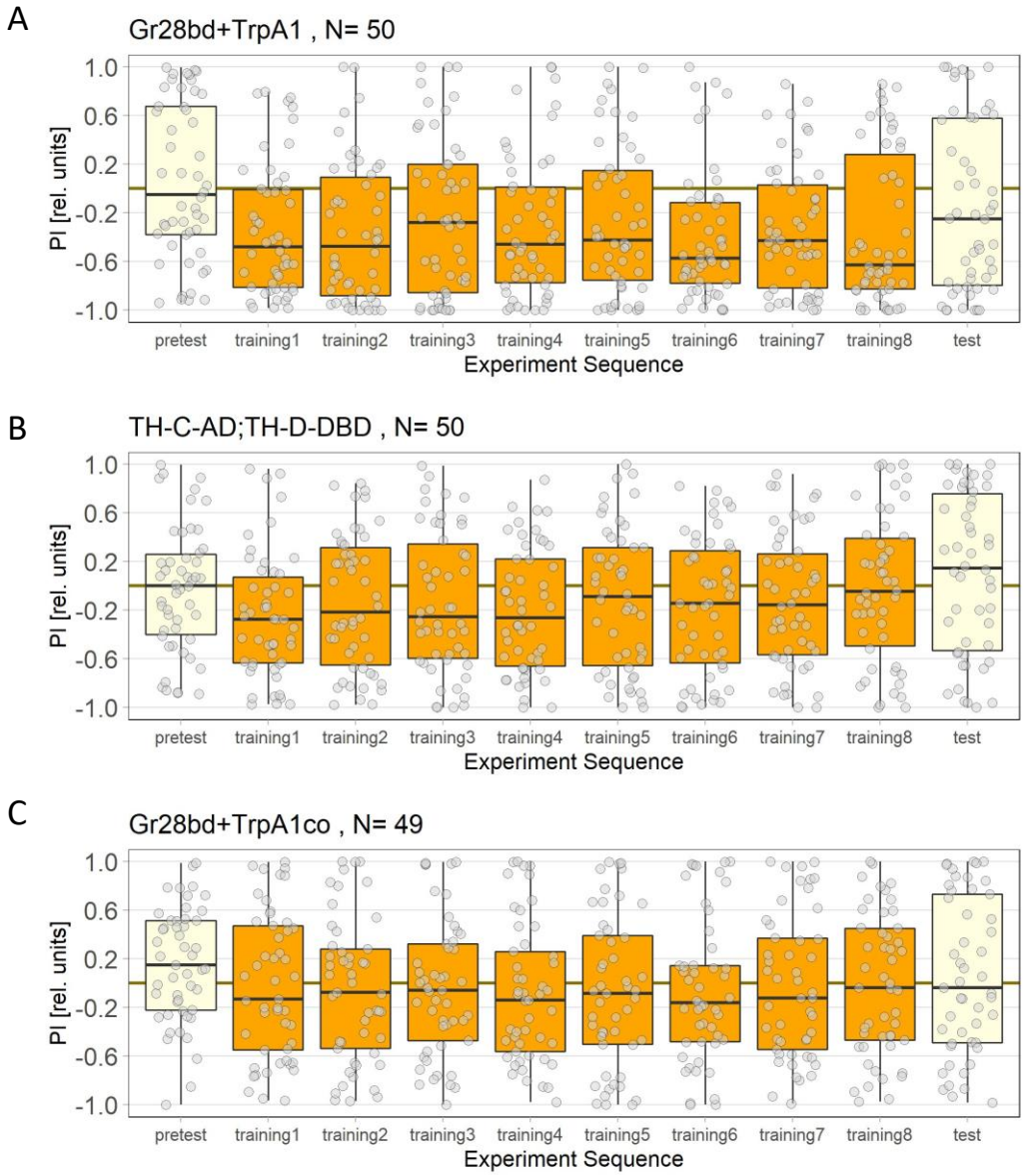
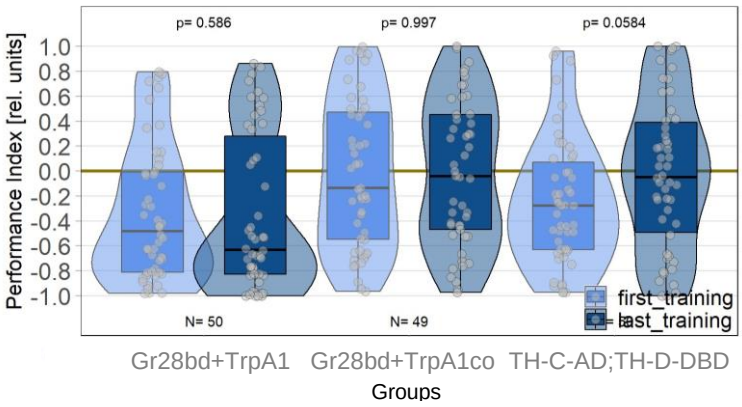


Figure 3: Time course of Joystick experiments with red light: (**A**) Control flies expressing CsChrimson in heat-sensing neurons preferentially keep the optogenetic light switched off throughout the training periods (orange), with an effect on platform position with the light permanently switched off (white/yellow). (**B**) Flies expressing the optogenetic channel in TH-C-AD;TH-D-DBD positive neurons starting their training phase by avoiding the light and finishing the last training period close to zero. (**C**) Negative control flies expressing CsChrimson in heat-sensing neurons with no optogenetic activation do not show a strong avoidance or preference for the light switched on or off.

The increase in PIs of the experimental flies was also evident when only the first and last training PIs were compared (**Figure 4**). There was tendency to difference between these two values ($p = 0.0584$) with a Bayes Factor larger than one indicating the rejection of the null hypothesis. The middle 50 % of data points were mainly negative in the first training and distributed around zero in the last training period. The first median PI was significantly negative ($p = 0.00131$) indicating avoidance, the last median PI was not significantly different

compared to zero (**Figure S1**). The controls did not show a significant difference between these two values, indicating a consistent behavior over time (**Figure 4**).



	bf	error
Gr28bd+TrpA1	0.211	0.000255
Gr28bd+TrpA1co	0.213	0.000247
TH-C-AD;TH-D-DBD	1.19	0.000124

Figure 4: Comparison of approach/avoidance behavior during first and last training period: Left: Light blue shows the PIs during the first training period and the distribution of values. Dark blue shows the PIs during the last training period. In positive and negative control, the difference between the two periods was not significant. TH-C-AD;TH-D-DBD flies showed the strongest change in behavior (p = 0.0584). **Right**: Bayes Factors for all three groups: Bayes Factor of TH-C-AD;TH-D-DBD of larger than one giving light evidence against the null hypothesis of no difference between the values.

3.2.2 Yellow light

PIs of the test group showed similar trend as red light testing when performed with yellow light. However, highest PIs during training periods were observed during training 6 with a median close to zero (**Figure 5B**). A tendency to approach was measured in the post-test. The results for the positive control group indicated stronger effect compared to red light testing (**Figure 5A**). Here, the median PIs were lower (around -0.6) and the middle 50% of the PIs were negative during all the training periods. The negative control had negative median PIs between -0.4 and 0, the whiskers reached almost from -1 to 1, indicating high dispersion of data points (**Figure 5C**).

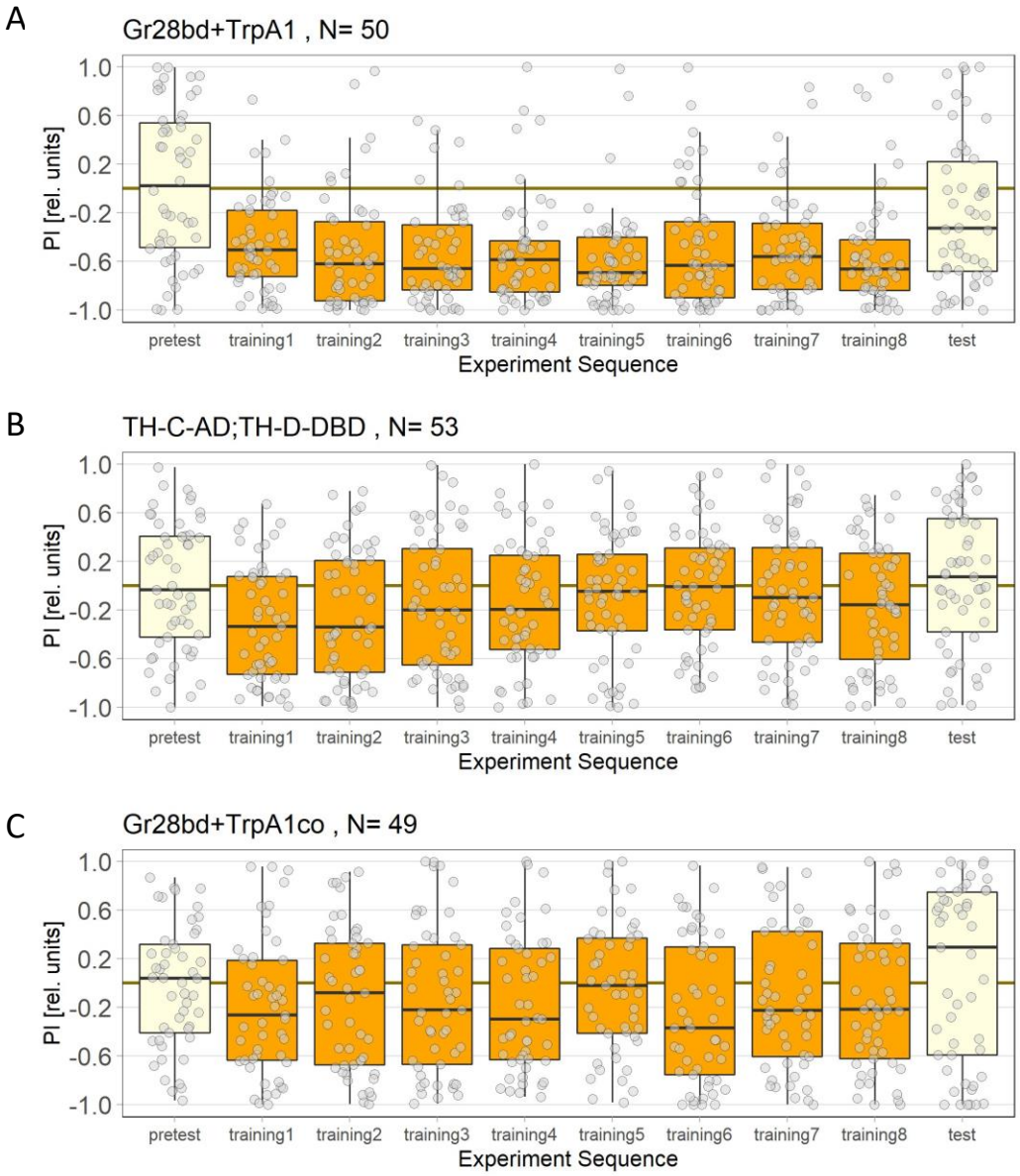
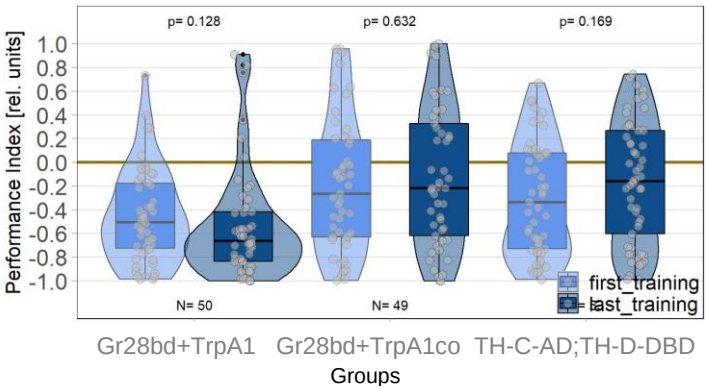


Figure 5: Time course of Joystick experiments with yellow light: (A) Control flies expressing CsChrimson in heat-sensing neurons showing strong avoidance of the light (orange), with an effect on platform position with the light permanently switched off (white/yellow). (**B**) TH-D-DBD/TH-C-AD flies show gradually increasing PIs and decreasing ones after the peak in period 6. (**C**) Negative control flies with no optogenetic activation show light tendency for the light switched off during the training but positive PIs in the post test with the light permanently switched off.

The difference between PIs during first and last training period were not significant for all three groups (**Figure 6**). Median PIs were significantly negative for all groups during training 1 ($p < 0.05$) indicating avoidance and stayed significantly negative in the last training for experimental group and positive control (**Figure S2**).



	bf	error
Gr28bd+TrpA1	0.296	0.000224
Gr28bd+TrpA1co	0.245	0.000234
TH-C-AD;TH-D-DBD	0.541	0.000185

Figure 6: Comparison of approach/avoidance behavior during first and last training period: Left: Light blue shows the PIs during the first training period and the distribution of values. Dark blue shows the PIs during the last training period. The difference between first and last training was not significant in all three groups. **Right:** Bayes Factors for all three groups are below one indicating light evidence for the null hypothesis.

3.3 3IY

To verify whether the observed behavior was DA-dependent flies were fed the DA synthesis inhibitor 3IY to reduce DA production. Flies were fed 3IY in addition to the ATR or ethanol and tested in the Joystick. Different feeding protocols (**Table 1**) were tested. Joystick experiments were performed with yellow light and most of the groups showed an effect similar to that described in **3.2.2**. Testing group of flies which were fed 45µl of 10mg/ml 3IY dissolved in 5% sucrose solution and 15µl of 200mM ATR dissolved in EtOH on approximately half the amount of medium as before for two days, is depicted in **Figure 7.** The time course showed similar trend with negative median PIs in the beginning and the highest median PI in training 7. Median PIs varied between -0.1 and 0.15.

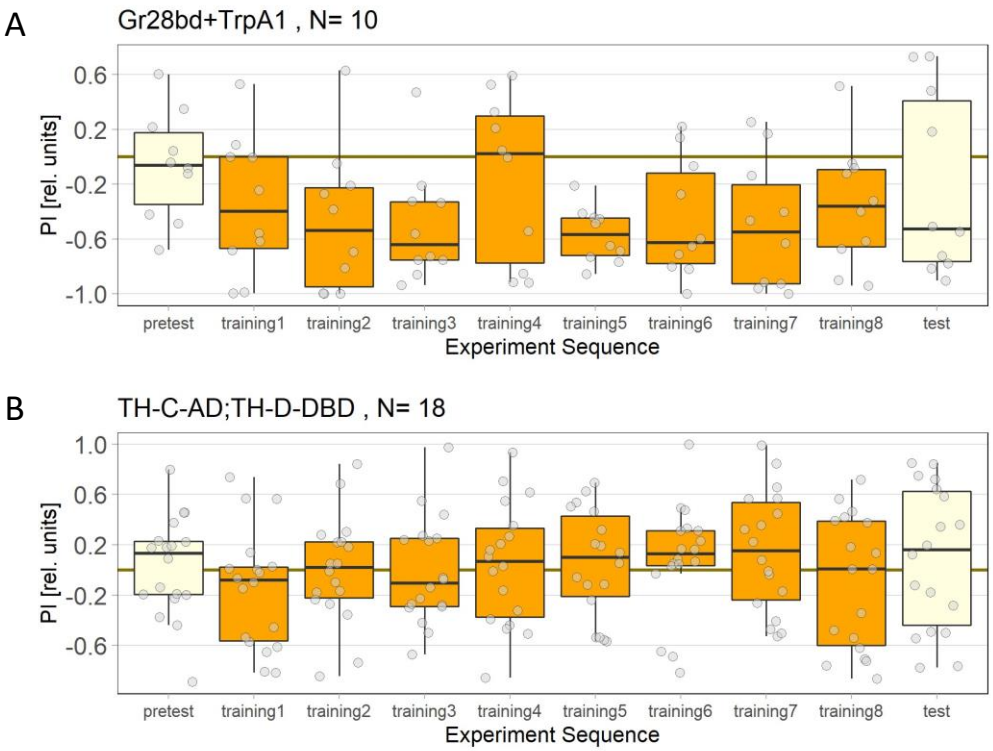


Figure 7: Time course of Joystick experiments with yellow light with 3IY: (A) Control flies expressing CsChrimson in heat-sensing neurons showing strong avoidance in all training periods except training 4 (orange), with an effect on platform position with the light permanently switched off (white/yellow). (**B**) TH-C-AD;TH-D-DBD flies fed with 3IY show increasing PIs and decreasing ones after the peak in period 7. Median PIs vary between -0.1 and 0.15.

3.4 Brain dissection

3.4.1 Brain visualization without antibodies

To prove whether the correct neurons were targeted TH-C-AD;TH-D-DBD>UAS-mCD8::GFP fly brains got dissected (**2.4.1**) and analyzed (**Figure 8**). The driver line was described by (Xie et al. 2018) and targets two PPM2 WED and two VLP DA neurons which are localized in the posterior inferiormedial protocerebrum (Mao und Davis 2009). Only in one hemisphere of one image four cell bodies were detected in the correct localization, only part of the stack was visualized here to show the fluorescence in this brain area (**Figure 8A**), the other images only showed three or less cell bodies of the DANs. Additionally, there were also other stained cells. Kenyon cells (KC) from three different clusters (**Figure 8B**) projecting in supposedly the α/α' and β/β' lobes of the MBs (**Figure 8D**). There was also a fluorescent signal in the optic lobes with the cell bodies located in the lobula or lobula plate and projecting into the optic lobes (**Figure 8D**).

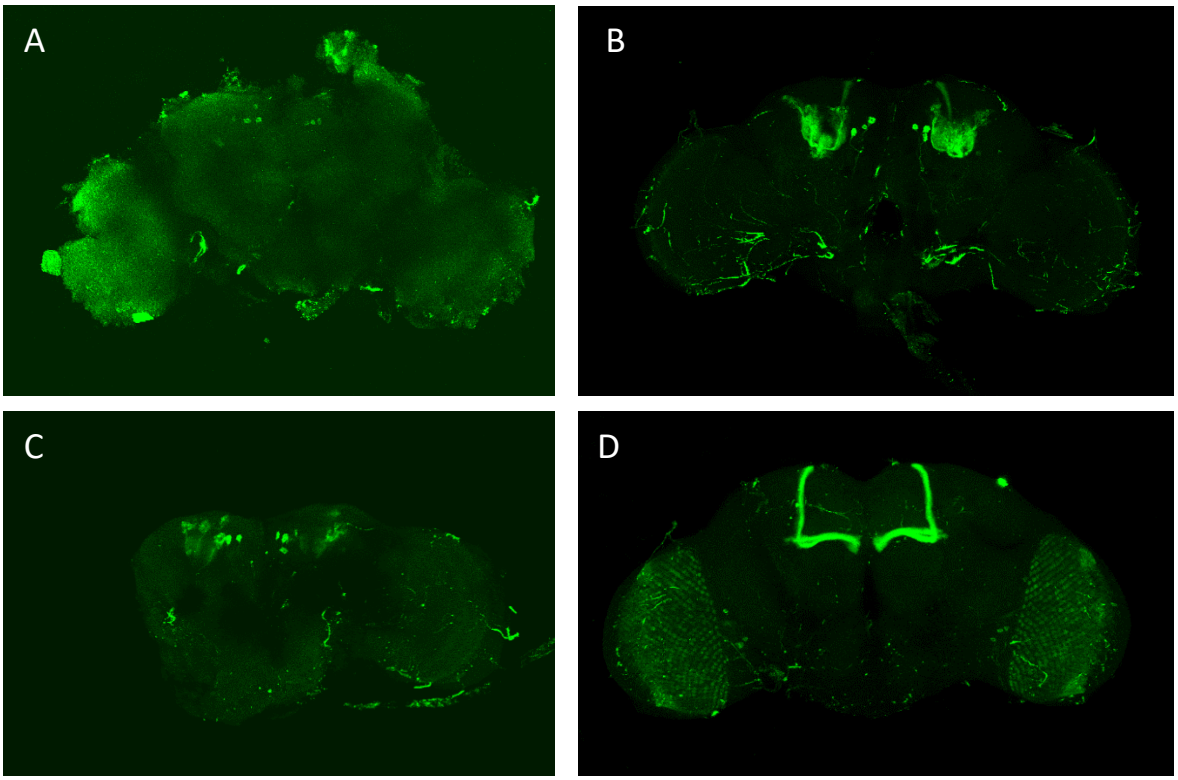


Figure 8: Confocal images without antibody staining of TH-C-AD;TH-D-DBD>UAS-mCD8::GFP fly brains: (A) Only posterior stacks of image with DAN cell bodies detectable, four in the left and three in the right hemisphere. (**B**) GFP expression in three detectable DANs per hemisphere and cells in the region of MB calyces (**C**) Brain with one optic lobe missing expressing GFP in at least three DANs in the right and two DANs in the left hemisphere. (**D**) GFP expression in the MB-lobes and the optic lobes.

3.4.2 Brain visualization using antibodies

For more detailed images TH-C-AD;TH-D-DBD flies were crossed again to UAS-mCD8::GFP flies. Brains were then dissected and stained (**2.4.2**). The images (**Figure 9**) revealed similar results as described in **3.4.1**. The four DANs were stained consistently in at least one hemisphere in each of the analyzed brains, even though they were partially not detectable in the whole images because of overlapping Kenyon cells. In some brains the number of DANs in one of the hemispheres varied between three and four. It could be verified that the stained Kenyon cells are organized in three different clusters (**Figure 9A**). The staining of the MBs was localized in the same lobes as described in3.4.1 (**Figure 9 B,C)**. Neurons projecting into the optic lobes were more prominent with antibody staining. There were four cell bodies in each hemisphere localized in the lobula or the lobula plate verified. These neurons were projecting through the lobula plate and the medulla. Unlike images without antibody staining it was also demonstrated that these neurons also project into more central regions of the brain, possibly the ventral lateral protocerebrum (**Figure 9 B,C**) (Otsuna und Ito 2006).

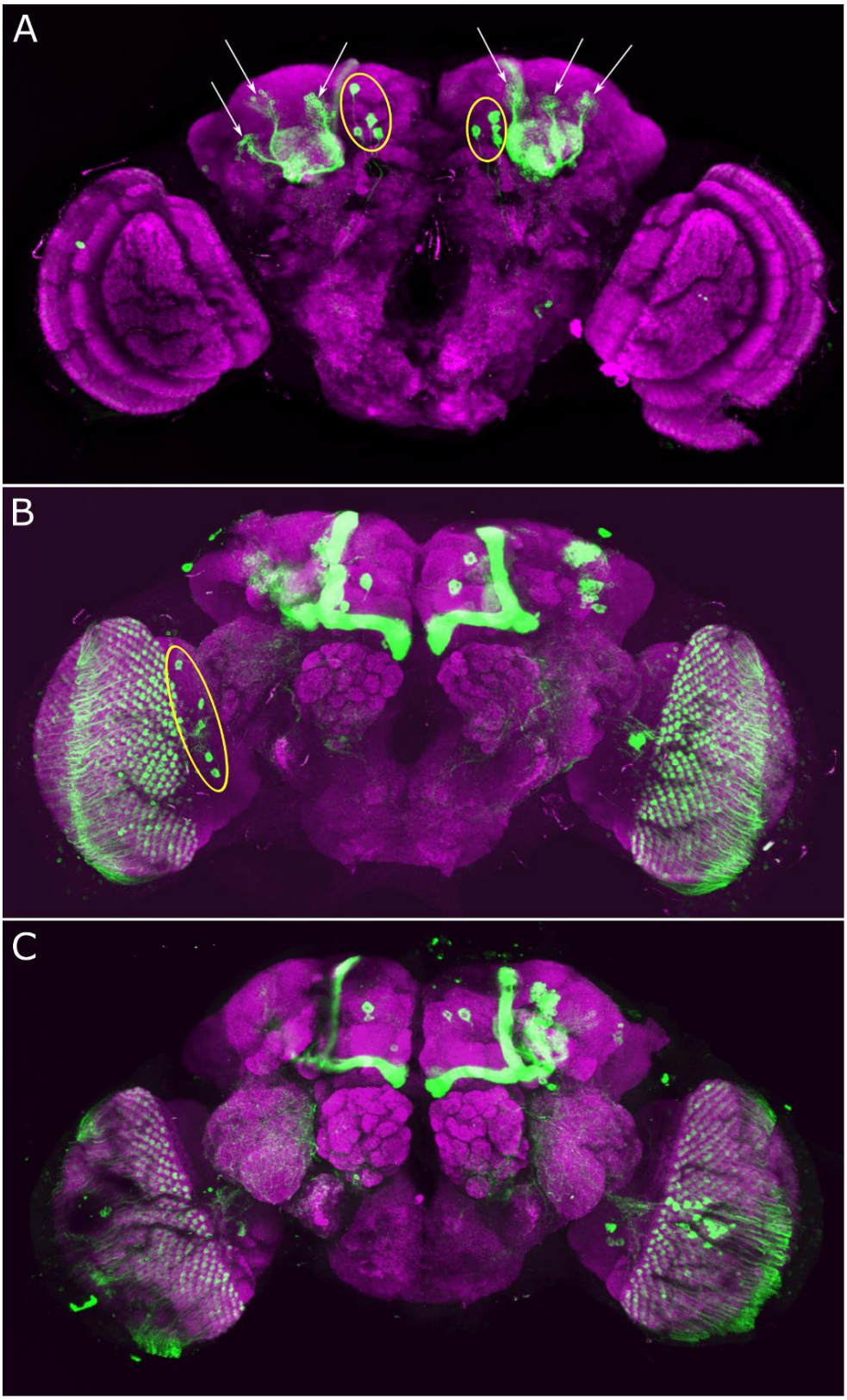


Figure 9: Confocal images with anti-GFP and anti-Brp staining of TH-C-AD;TH-D-DBD > mCD::GFP fly brains: (A) 4 PPM 2 DANs per hemisphere marked with yellow circles, three clusters of Kenyon cell bodies per hemisphere marked with white arrows. (**B)** Five cell bodies of unidentified neurons marked with a yellow circle in the left hemisphere. The neurons project into the lobula plate and the medulla. Strong fluorescence of cell bodies of the Kenyon cells projecting into the Mushroom bodies could be observed. (**C**) Unidentified neurons projecting into structures outside of the optic lobes.

4. Discussion

In this thesis, the role of specific DANs labeled by the TH-C-AD;TH-D-DBD driver line in reinforcement during operant learning in the Joystick (**Figure 1**) was examined. Before the experiment, visual competence of the norpA^{24};20xUAS-CsChrimson effector line flies was tested in the T-maze. Visual restoration was revealed (**Figure 2**) but experiments were performed nevertheless because of earlier experiences with the Joystick gained by Prof. Björn Brembs indicating blindness was not crucial for testing with the Joystick.

Optogenetic activation of DANs in the PPM2 cluster revealed a change in behavior over time. While flies avoided activation of the targeted DANs in the beginning, avoidance decreased over eight minutes in one experiment and increased again after six minutes in a second experiment (**Figure 3** and **5**). Only difference between these two tests was the wavelength used for optogenetic activation. The used CsChrimson channel has its optimum at 590 nm (Klapoetke et al. 2014) corresponding to the yellow light used in the first experiment. Red light (660 nm) used in the second experiment is still able to activate CsChrimson. However, a weaker effect in red light could result in a delayed behavioral response. The stronger avoidance observed in the positive control with yellow light stimulation compared to red light supports this hypothesis. To verify if avoidance increases again in red light testing as well, it could be a possibility to extend testing periods.

Compared to results by Rohrsen et al. (2021) avoidance in the positive control was stronger for the same wavelength and had greater impact on behavior after the experiment resulting in ongoing avoidance of the before light-associated behavior. This could be due to the use of a thinner fishing line allowing smoother handling and easier adjustment of the fly's head close to the light guide. Except for the person carrying out the experiment, no other parameters were changed on purpose. The only other parameter that differed in the experiments shown here from the ones in Rohrsen et al. (2021) was the flies' visual restoration. The negative control with supposedly no optogenetic activation showed significant avoidance during at least one training period for yellow light testing (**Figure S2**). Yellow light is in the visible spectrum of *Drosophila* as opposed to red light and the negative control showed avoidance of the light without any optogenetic activation. Since this was only the case for yellow not for red light testing it could imply an effect of vision on the observed behavior. This is unlikely because flies usually show positive phototaxis not negative and previous Joystick experiments performed in this laboratory did not show this effect. If this was the case, vision could also impact the experimental groups' behavior. Because of this possibility, it should be considered

to repeat yellow light testing with blind flies. It seems unlikely the avoidance occurred because of optogenetic activation since a cofactor like all-trans-retinal is considered to be essential for the activation of opsins like CsChrimson and has to be fed because Drosophila lack endogenous ATR (Yu et al. 2015).

DANs are considered to mediate either avoidance or approach behavior in classical conditioning. Rohrsen et al. (2021) found that DANs, which are reported to convey information about either aversive or appetitive unconditioned stimuli (US) in classical conditioning, did not show consistent behavior in different operant paradigms and with different optogenetic activation in the same operant paradigm. In this thesis, only one operant paradigm was tested, so there is no knowlegde about possible differences to other screens. The flies showed different behavior over time for the two wavelengths of stimulating light. Rohrsen et al. (2021) assumed the more effective activation of CsChrimson with yellow light changed the function of the neurons instead of only increasing it. This could also be the case for the DANs observed here, although the hypothesis of a somehow delayed effect due to reduced neuron function in the red light presented earlier is not ruled out.

Overall, the observed behavior showed avoidance to neutral behavior, which is similar to classical conditioning experiments that report mainly PAM DANs not PPM DANs convey appetitive stimulus information. However, the shift from avoidance to neutral behavior shows a less consistent behavior than avoiding activation of these DANs at a constant level. The driver line should be considered to be tested in other operant paradigms as well to get more detailed picture of the function during operant behavior.

The line tested here has been described to specifically label two WED and two VLP DANs of the PPM2 cluster (Xie et al. 2018). Confocal imaging revealed additional stained cells in the mushroom bodies, presumably KCs, and cells in the lobula or lobula plate projecting into the optic lobes (**Figure 8** and **9**). It is not clear whether activation of these cells could affect behavior during operant experiments. The neurons projecting to the optic lobes presumably do not influence decision making but may have changed the behavior in the yellow light screen because of visual input.

Kenyon cells have been reported to be involved in forming aversive and appetitive memory in classical conditioning (Krashes et al. 2007). To investigate whether the observed behavior was DA-dependent and not due to KS activation. Flies were fed the DA synthesis inhibitor 3IY to reduce DA production. 3IY inhibits tyrosine hydroxylase enzyme (TH), which

catalyzes the conversion of L-DOPA, a precursor of DA (Thoener et al. 2021). A similar behavior as before was observed in yellow light Joystick testing (**Figure 7**), even after trying different concentrations and different media (**Table 1**). As next step, it would be useful to verify the 3IY efficiency. Flies treated with 3IY have been reported to show reduced activity and increased resting periods (Andretic et al. 2005), which could be tested using 3IY-fed wild-type flies in the Buridan's paradigm. Another easy possibility could be a T-maze phototaxis test, since genetically induced DA deficient flies show a lack of phototaxis (Riemensperger et al. 2011).

4.1 Summary

Optogenetic testing of DANs labeled by TH-C-AD;TH-D-DBD led to dynamic behavioral changes over time. Initially, flies avoided activation but showed varying responses to different wavelengths of light. Confocal imaging revealed that more neurons than expected were labeled by the line, which may have affected the test results.

Investigating extended testing periods and repeated yellow light testing with blind flies in the Joystick, as well as testing in other operant behavior paradigms would be of interest. Further testing with 3IY to investigate if the observed behavior was DA dependent is also recommended.

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6. Attachment

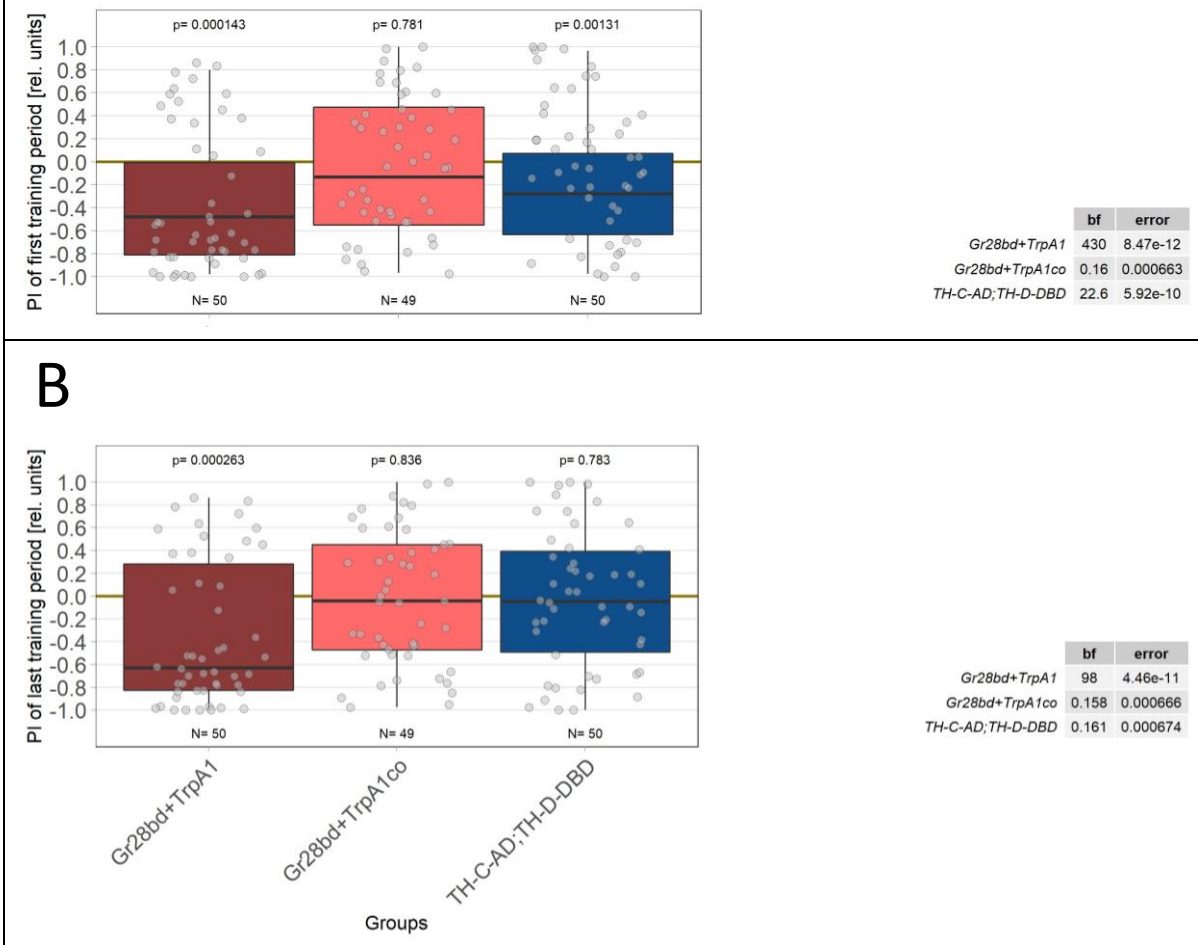

Figure S1: Joystick performance during first and last training period for red light: (**A**) First training period. **Left**: Positive control and test group show significantly negative test PIs indicating avoidance, negative control is not significantly different from zero. **Right**: Bayes factors of the significant groups are greater than one, confirming rejection of the null hypothesis of no difference from zero. (**B**) Last training period. **Left**: Only positive control group shows significant avoidance. **Right**: Bayes Factor larger than one indicates rejection of the null hypothesis.

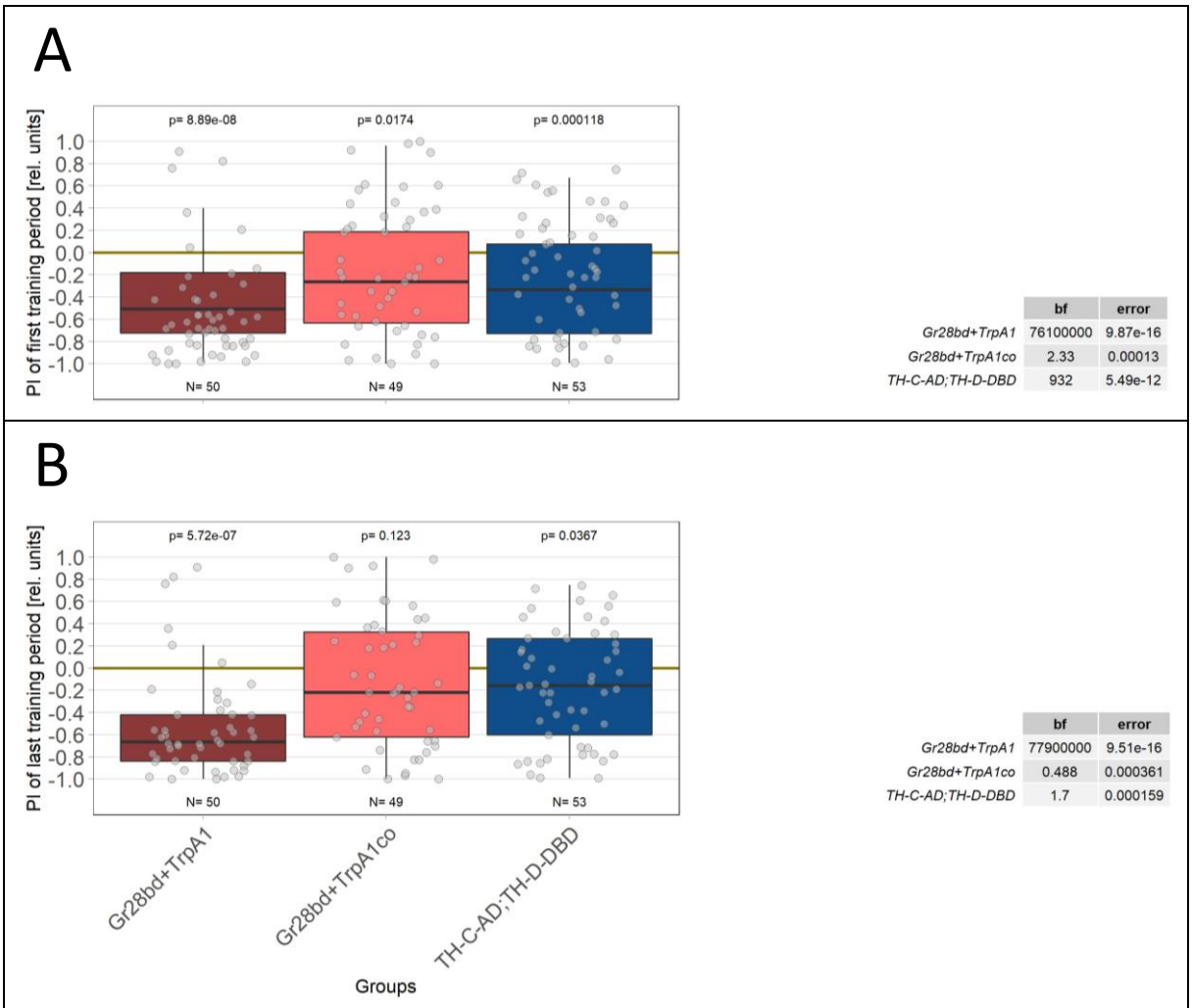

Figure S2: Joystick performance during first and last training period for yellow light: (A) First training period. **Left**: PIs are significantly negative for the testing group as well as for both control groups, indicating avoidance. **Right**: Bayes factor larger than one, indicating rejection of the null hypothesis for all three groups. (**B**) Last training period: **Left**: Positive control and test group show significantly negative PIs, negative control does not show significantly lowered PIs. **Right**: Bayes factors of test group and positive control greater than one support rejection of the null hypothesis.

7. Declaration of authorship

The submitted printed copies and the submitted electronic version of the thesis are identical. I have written the thesis independently, have not used any sources or tools other than those indicated and have not already submitted the thesis to another university for the purpose of obtaining an academic degree. Furthermore, I confirm that I am aware of the legal consequences provided in § 27 (5) of the current examination regulations.

Die vorgelegten Druckexemplare und die vorgelegte elektronische Version der Arbeit sind identisch. Ich habe die Arbeit selbstständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und die Arbeit nicht bereits an einer anderen Hochschule zur Erlangung eines akademischen Grades eingereicht. Weiterhin bestätige ich, dass ich von den in § 27 Abs. 5 der geltenden Prüfungsordnung vorgesehenen Rechtsfolgen Kenntnis habe.

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