Is anxiety a factor influencing photo-preference in *Drosophila melanogaster*?

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Declaration of self-reliance

I, Lena Matzeder, hereby declare that the work submitted is my own and that all passages and ideas that are not mine have been fully and properly acknowledged.

Ich habe die Arbeit selbständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und bisher keiner anderen Prüfungsbehörde vorgelegt.

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Signature, Date

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Abstract

This thesis investigates if anxiety is a factor influencing photo-preference in *Drosophila melanogaster*. Photo-preference is defined as a hard-wired stimulus response link to light, which can be positive or negative (Gorostiza et al. 2015). While flies with intact wings are highly attracted by light, wing-clipped flies show light avoidance. Because of the limited ability to escape, flies without wings are more likely to hide and avoid high exposure in the purpose of self-protection. To investigate if this is a behavior generated by anxiety several assays were performed such as Benzer's counter-current paradigm, the T-Maze paradigm, and the Individual T-Maze. Therefore, flies were treated with Diazepam to test if there is an anxiety component in photo-preference that can be changed. To corroborate the hypothesis it was expected that the anxiolytic effect of Diazepam would decrease the light avoidance in wing-clipped flies. The results in this thesis reveal a tendency of anxiolytic effects. Only the individual T-Maze showed a difference, but for a definite statement more experiments will be necessary.

Zusammenfassung

Im Fokus dieser Arbeit steht die Hypothese, dass die Fruchtfliege *Drosophila melanogaster* ein Angstverhalten besitzt und Photo-Präferenz von Angst beeinflusst wird. Eine Präferenz zeichnet sich durch die Bevorzugung eines bestimmten Reizes aus. Photo-Präferenz bezieht sich daher auf das Verhalten im Bezug zu Licht. Im Gegenteil zu normalen Fliegen, die von Licht stark angezogen werden, bevorzugen es Fliegen mit gekürzten Flügeln Licht zu meiden. Aufgrund der Flugunfähigkeit und eingeschränkten Möglichkeiten zur Flucht vor Prädatoren ist es möglich, dass dieses Verhalten dem Selbstschutz dient und dabei Angst eine Rolle spielt. Dazu wurden verschiedene Versuche durchgeführt: Benzer's counter-current Apparatus, das T-Maze Paradigma und eine weitere Form des T-Maze, um Fliegen individuell testen zu können. Um feststellen zu können, ob Photo-Präferenz in Fliegen von Angst beeinflusst wird, wurden sie mit Diazepam behandelt. Um die Hypothese zu bestätigen, sollte die angstlösende Eigenschaft von Diazepam ein gegenteiliges Verhalten in den Fliegen mit gekürzten Flügeln hervorrufen. Dies konnte in den Experimenten nicht eindeutig bestätigt werden, denn lediglich eine Tendenz einer zunehmenden Photo-Präferenz war zu erkennen. Der Individual T-Maze zeigte den Effekt am deutlichsten, somit könnten weitere Experimente zu einem aussagekräftigeren Ergebnis verhelfen.

1. Introduction

What is anxiety?

According to Curran and Chalasani, Anxiety is a behavioral response to a perceived, not real threat (Curran and Chalasani 2012). It is a natural and necessary component of emotional behavior for the purpose of self-protection and self-defense. On a molecular level the serotonergic and GABAergic neuronal pathways are involved in the development of this behavior. Alterations of these signaling pathways can cause anxiety disorders. They can be treated amongst others with selective serotonin reuptake inhibitors (SSRI) or Benzodiazepines like Diazepam, which blocks the excitation of GABAergic neurons and will be important for this thesis.

Rodent anxiety paradigm

A typical anxiety paradigm for rodents is the light/dark-box (Bourin, Michel, and Martine 2003), which is a box that contains a partition wall to separate a darkened room from a lit one. Rodents show anxiety when they spend more time on the dark side and show a low rate of transitions. Another similar assay is the Elevated Plus Maze (EPM) (Komada et al. 2008), which consists of an elevated platform with two open arms and two dark arms. In this case, the time they spend in the open arm and the transitions between the two environments are also measured. The difference between these two assays is that in the light-dark box the animal is caged on both sides, whereas in the EPM the animal is more or less free on the open arm, which can also be interpreted as high exposure and hence induce anxiety. As a last example, the Open field test was invented to test for centrophobism and explorative behavior. Here rodents show anxiety when they avoid the center and instead spend more time near walls and corners.

New Anxiety Models

Another animal model for neurobehavioral research is the zebrafish (*Danio rerio*) (Stewart et al. 2012). Numerous paradigms have been adapted from rodents, where anxiety is identified by decreased exploration and advanced vigilance in a novel tank similar to the rodent open field test. The light-dark box can be realized for zebrafish as well using a rectangular tank, which is

divided into a light and a dark area by coloring the tank half black and half white. This experiment measures scototactic behavior, which is the locomotory movement towards darker environments. Another popular research model is the fruitfly (*Drosophila melanogaster*), whose genome is already largely understood. Moreover, the fruitfly provides several experimental advantages like easier housing, experiments can be performed with higher sample sizes and in case of pharmacological testing less drug is needed. Flies placed in an arena avoid the center, which is called centrophobism, and instead prefer to stay near walls and corners. It is suggested that this behavior might be caused by self-protection because of the high exposure to possible predators in the center, which can be interpreted as anxiety.

Photo preference

Photo-preference is a hard-wired stimulus response link to light, which can be positive or negative. It is known that flies are highly attracted by light and wingless flies show light avoidance. The locomotory movement to the light is established as positive Phototaxis, while moving away from light is called negative Phototaxis. If photo-preference in flies is influenced by anxiety, wing-clipped flies that avoid the light should show an opposite behavior when they are manipulated by anxiolytics. Here normal and wing-clipped flies are treated with Diazepam and tested for photo-preference in the Benzer counter-current apparatus, the T-Maze and the individual T-Maze. Flies with intact wings showed no difference in photo-preference after they were treated with Diazepam, while wing-clipped flies showed an increased positive light preference after the treatment. This change in behavior was most considerable when they were tested individually.

2. Methodology

Fly strain and care

For all the experiments *Drosophila melanogaster* wild-type Berlin (WTB) flies from the stock in our lab in Regensburg were used. They were kept at 25 °C with 60 % humidity on a 12/12 h light and dark cycle. Furthermore the flies were bred on standard cornmeal-agar medium, where I added a blot of fresh and living yeast paste and a filter paper. To generate a controlled density and a daily hatching, 20–30 flies were transferred to new vials every 24 h. The breeding flies were not used for the behavioral assays and were replaced at least every 2 weeks. For the experiments only 2 - 5 days old flies were used.

Wing clipping

24 h before the experiments the flies were briefly anaesthetized using carbon dioxide. Then the distal 2/3 of the original wing length was cut off. Afterwards, the flies were divided into two groups and put in separate vials, because only half of them will be tested with Diazepam. In order to recover from the anesthesia and the clipping procedure, the flies were placed at 25 °C.

Diazepam treatment

Diazepam (Fig. 1) belongs to the group of Benzodiazepines and is also known under the trade name Valium. In therapy it functions as an anxiolytic, as an anticonvulsive and as a hypnotic. Moreover, it has a relatively long half-value period of 24-48 h and it's soluble in Dimethylformamide (DMF) and Ethanol, which leads to an easy transition of the blood-brain barrier (BBB). Diazepam acts in GABAergic neurons on the G-protein-coupled $GABA_A$-Receptor as an allosteric modulator and intensifies the inhibitory effect of the neurotransmitter γ-Aminobutyric acid (GABA). The $GABA_A$-Receptor is a chloride channel that changes its spatial conformation when Diazepam binds, which makes it easier for GABA to bind at the main binding site. This leads to an increased chloride influx, which induces a hyperpolarization of the postsynaptic cell. As a result the cell is less excitable.

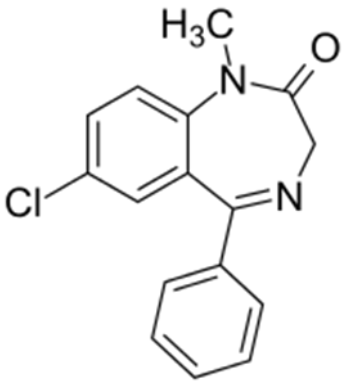


Figure 1: Molecular structure of Diazepam ($C_{16}H_{13}ClN_2O$). Diazepam is a Benzodiazepine and acts as an agonist on the G-Protein-coupled $GABA_A$-Receptor. It has an anxiolytic, anticonvulsant, muscle relaxant and sedative effect (Figure from sigmaaldrich.com).

First, flies were starved overnight using H_2O soaked filter paper and then fed for 6 h using filter paper soaked with a 5 mM Diazepam (Sigma Aldrich, Munich, Germany, Cat. No. D0899) solution in 10 % Ethanol, 5 % Sucrose, 5 % yeast on water (Mohammad et al. 2016) and blue food dye, to ensure the food delivery was effective. Only flies showing a blue belly were used for the experiments. They were divided into two groups. One group was treated with Diazepam and the other group was treated without Diazepam but the same amount of Ethanol. In Mohammad's research paper different concentrations from 1 mM up to 10 mM were applied. According to their results, a concentration of 5 mM provided the biggest effect. Because of that I started the open field assay with 5 mM and remained with it for the rest of the experiments, since the effect appeared to be very small. The animals were tested at 25 °C during the Open field Assay and at 22 °C in both T-Maze experiments and the counter-current apparatus.

Open field assay

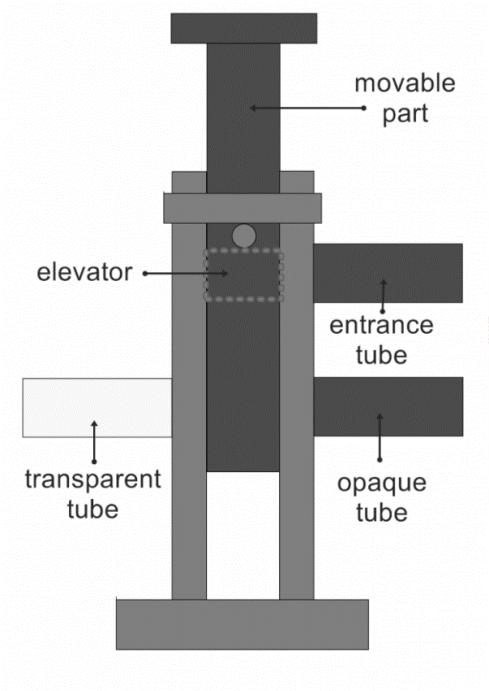
I tried to replicate an experiment published recently in Mohammad et al. 2016 to ensure the delivering of the treatment was effective. The trajectory of a fly is filmed in an arena, which is built by a small container turned upside down. The setup was modified from the original one, trying to be conservative. I used a single well of a 24 well plate, which had a circular shape with a diameter of 16 mm, a minimized height of 7 mm after cutting it horizontal and was lit from below using a luminous plate. It was adapted from Mohammad's methods, where they used a

square chamber (10 x 10 x 1.6 mm) which was lit from the sides. The flies were divided into two groups and starved overnight, where only one group was treated with Diazepam. Each fly was tested individually by sucking one fly from the vial using a flexible tube and then blowing it into the arena. A piece of cotton wool in the flexible tube avoids to accidentally swallow the fly. A tracking camera was placed above the arena to film the locomotion of the fly for 10 min. While they are in the arena, they can take three positions: the bottom, the wall and the ceiling. Spending more time distant from the center of the arena represents anxious behavior. For the measurements I used BuriTrack, which is an open source software designed to track a single animal walking in a homogenous environment (Colomb et al. 2012). It records the position of the fly referring to the coordinates X, Y in pixels to measure the distance from the center. In addition, the arena can be separated to an inner and outer zone in order to measure the time the fly spends in each sector. Not moving flies were excluded from the analysis.

T-maze paradigm

Similar to the light/dark-box assay of anxiety research in mammalians (Bourin, Michel, and Martine 2003) flies in the T-Maze apparatus also have to choose between light and dark (Fig. 2). It is made of an opaque acrylic PVC and has a wall thickness of 5 mm. One test group contained 40 flies with intact wings and 40 wing-clipped flies. First, they have to be put in a cylindrical entrance tube (15 x 100 mm) for 10 minutes for the purpose of dark adaptation. Second, they are pushed in a movable part inside the T-Maze, where they have to stay for 30 s to rest. The elevator is used to transfer the flies down to the light/dark choice point. After pushing it down, the flies have 30 s to choose between a darkened opaque tube and a transparent one, which is lit from above. Pulling the elevator up closes both tubes and carries the individuals that didn't pick a side back in the entrance tube. For the analysis I calculated a Choice Index (CI) out of the number of choices. Therefore, the flies in each tube were counted. A Choice Index of 1 means all the flies chose the Light, while a CI of -1 means all the flies chose the dark.

$$CI = \frac{[(\#Light) * 1 + (\#Dark) * (-1) + (\#Elevator) * 0]}{(\#Total\ of\ flies)}$$



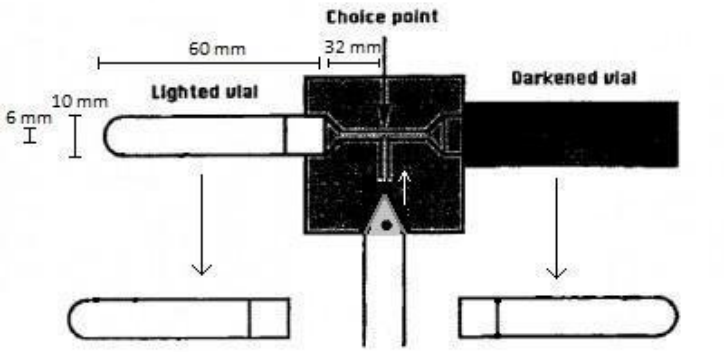


Figure 2: T-Maze apparatus.
Experimental setup to detect light-dark preference in *Drosophila melanogaster* (Figure from lab.brembs.net).

Figure 3: Individual T-Maze apparatus.
Experimental setup to detect light-dark preference in individuals of *Drosophila melanogaster* (Figure from lab.brembs.net).

Individual T-Maze

The individual T-Maze is inspired by the regular T-Maze paradigm with the advantages of looking at the behavior of a single fly and the opportunity to test the same fly several times. For this thesis, the apparatus was handmade using a T-shaped plastic cable connector, which had to be fixed in an upright position (Fig. 3). The arms of the cable connector had a length of 32 mm and a diameter of 6 mm. They end up in two plastic vials, which have a length of 60 mm and a diameter of 10 mm. The plastic vials were covered with a sponge that had a hole in it, which closes when the vial gets removed from the T-maze. Thus the fly cannot escape. The flies were kept in two groups, where one group was treated with Diazepam. At the beginning of the experiment, a single fly has to be sucked from the vial using a flexible tube. Then it gets blown into the apparatus from below, which forces them to decide immediately between one arm that is lit from above, and one arm that is darkened with aluminum foil. After the fly ended

up in one of the plastic vials, the vial was removed and the fly was transferred back in the T-Maze to test it again. For the analysis a Choice Index is calculated by counting how many times the fly chose the dark or the light. A CI of 1 means the fly always chose the light, while -1 means the fly always chose the dark.

$$CI = \frac{[(\#Light\ choices) * 1 + (\#Dark\ choices) * (-1)]}{(\#Total\ of\ choices)}$$

Benzer's counter-current apparatus

The counter-current concept, invented by Seymour Benzer, separates flies on the basis of differences in phototaxis behavior (Benzer 1967; Bonini 2008). The apparatus that was used for this thesis has 6 tubes in the lower frame and 5 in the upper frame (Fig. 4). Before the start, a group of flies is placed in the first tube for 10 min in darkness for the purpose of dark adaptation. One test group contained 40 normal flies and 40 wing-clipped flies. To avoid geotactic effects the apparatus has to stand horizontal. First, a light source is turned on and the apparatus is laid down pointing the upper frame to the light. Second the upper frame is moved to the left making the first tubes face each other. The flies have 15 seconds to either move to the light entering the upper tube or to stay in the lower tube. Then the upper frame is moved back and the apparatus is tapped down, thus the flies, which moved to the light, end up in a new tube next to where they started. The principle is demonstrated in Figure 5. After 5 repetitions, the flies that never went to the light are still in the first tube, and the ones that always went to the light are located in the last tube. For the analysis of the counter-current apparatus I calculated a Preference Index by counting the flies in each tube. The higher the numerical value the more positive is the response to the light.

$$PI = \frac{(\#F5 \times 5) + (\#F4 \times 4) + (\#F3 \times 3) + (\#F2 \times 2) + (\#F1 \times 1) + (\#F0 \times 0)}{\#FT}$$

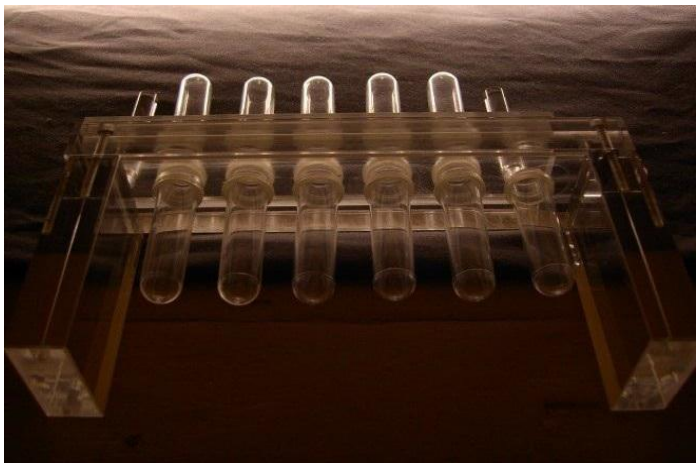


Figure 4: Benzer's counter-current apparatus. Apparatus invented by Seymour Benzer to quantify light preference in Drosophila melanogaster (Figure from lab.brembs.net).

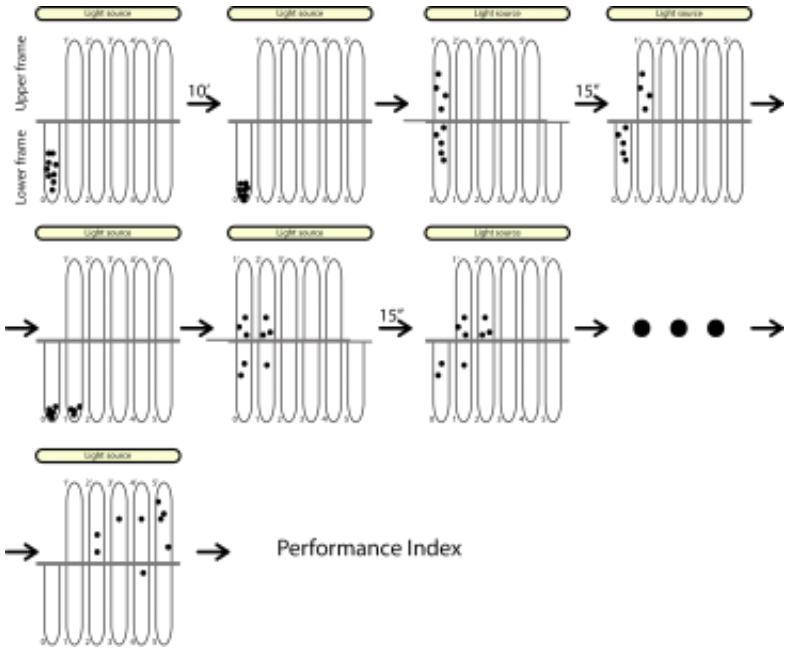


Figure 5: Principle of the Benzer counter-current apparatus. By moving the upper frame to the left making the first tubes face each other, the flies can either move to the light entering the upper tube or stay in the lower tube. After 15 seconds the upper frame is moved to the right and the flies get tapped down. The flies which moved to the light end up in a new tube next to where they started (Figure from lab.brembs.net).

Analysis

For the analysis of the open field assay, I used the Centroid Trajectory Analysis software (CeTrAn) (Colomb et al. 2012). It calculates several metrics, but for this thesis only centrophobism moving, centrophobism sitting and the transition plots are important. For statistical analysis for all experiments I used InfoStat. In addition, the effect size Hedges [g] was calculated for all the experiments using an online calculator available in http://www.socscistatistics.com/effectsize/Default3.aspx. Hedges is standardized with a pooled SD, which allows comparison across different experimental systems. The following formula shows how the effect size is calculated.

$$g = \frac{\overline{x_1} - \overline{x_2}}{s^*}$$

$$s^* = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

3. Results

Open field assay

The purpose of this assay was to decrease the avoidance of the center by treating flies with Diazepam. It showed a very small decrease in centrophobism in Diazepam treated flies (Fig. 6) but still a consistent tendency. The transition plots (Fig. 7) represent the time flies spend at a specific position with colors from red to blue. In red areas flies spend more time, while in blue areas they spend less time. White colored zones have not been entered. Diazepam treated flies showed more transitions across the center than the control flies. Looking at the individual trajectories (Fig.8), there might be only a small effect of Diazepam, thus I decided to go on with the following experiments testing changes in photo-preference.

A **B**

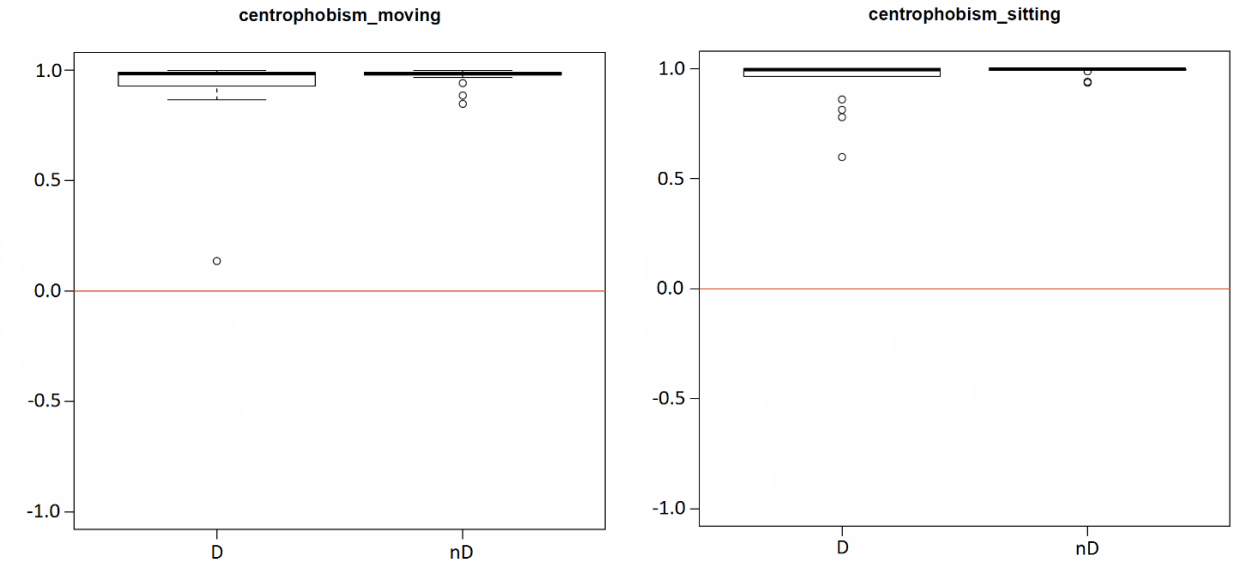


Figure 6: (A) Centrophobism moving (B) Centrophobism sitting. 1 equals flies spend all the time on the wall and -1 equals flies spend all the time in the center (n=17).

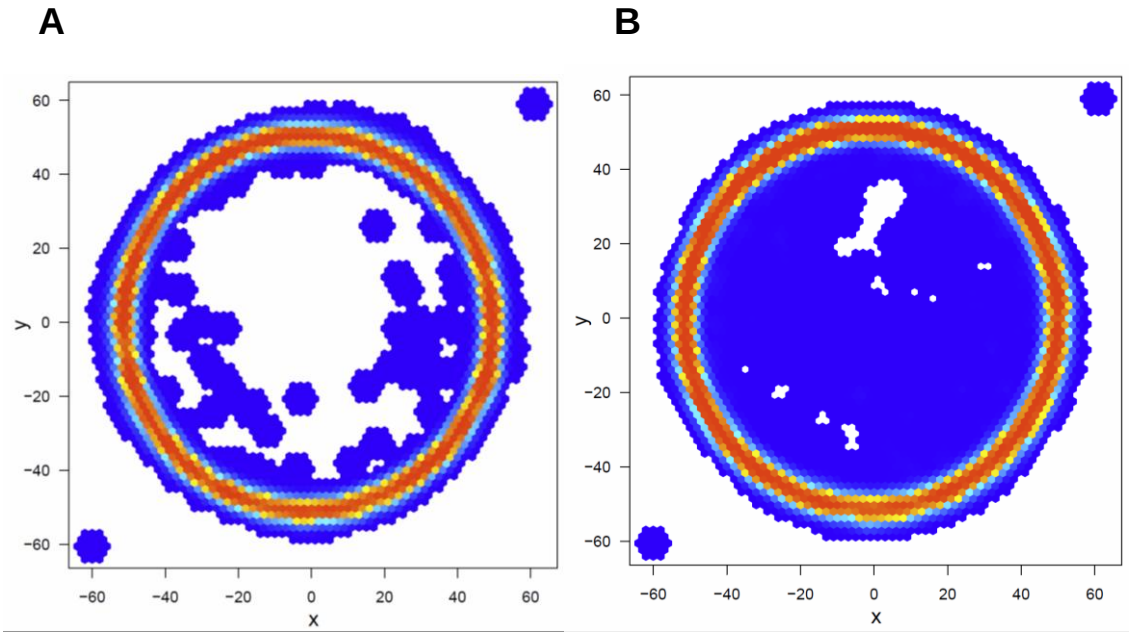


Figure 7: Transition plots for (A) the control group and (B) Diazepam treated flies. Blue color represents, that a fly spends less time in that area, while red equals spending more time there. The white zones have not been entered. Flies fed with Diazepam cross the center more often than the control flies (n=17).

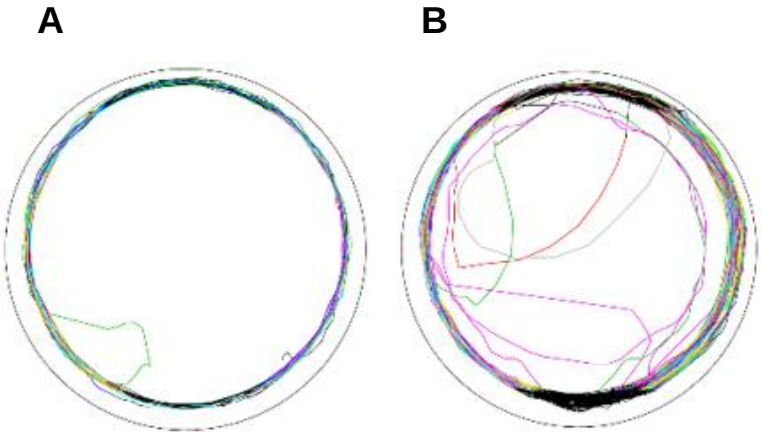


Figure 8: Individual Trajectories. (A) Fly without Diazepam. (B) Fly treated with Diazepam, which crosses the center more often.

T-Maze paradigm

Since wing-clipped flies avoid light and prefer to hide in the darkness, the expectation for the T-Maze experiment was to get an increased tendency for them to decide to go to the light after treating them with Diazepam. First normal flies and wing-clipped flies were put in the same vial and then tested together, but the response to the light was only slightly higher in Diazepam treated flies than in the control group (Fig. 9A). In many samples, half of the individuals did not even choose a side and stayed in the elevator instead. Therefore, to avoid a potential distraction by the normal flies in the matter of spatial reasons the experiment was repeated only with wing-clipped flies (Fig. 9B), but the result was almost the same.

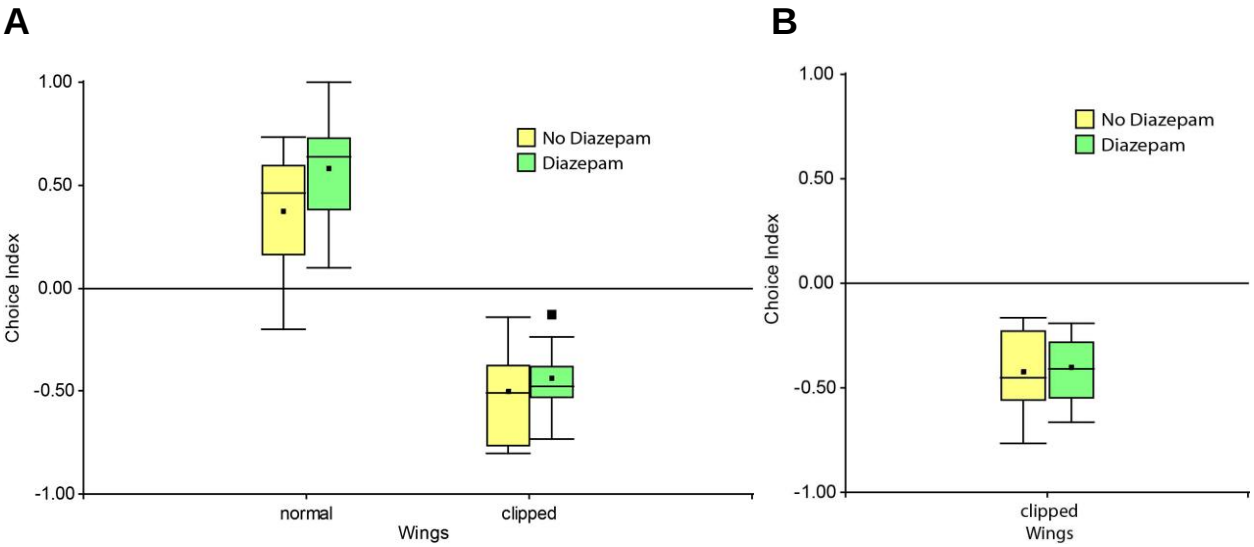


Figure 9: Results of the T-Maze experiment. (A) Flies with and without wings (n=16). (B) Flies only without wings (n=16). The light preference slightly increased in wing-clipped flies after the Diazepam treatment.

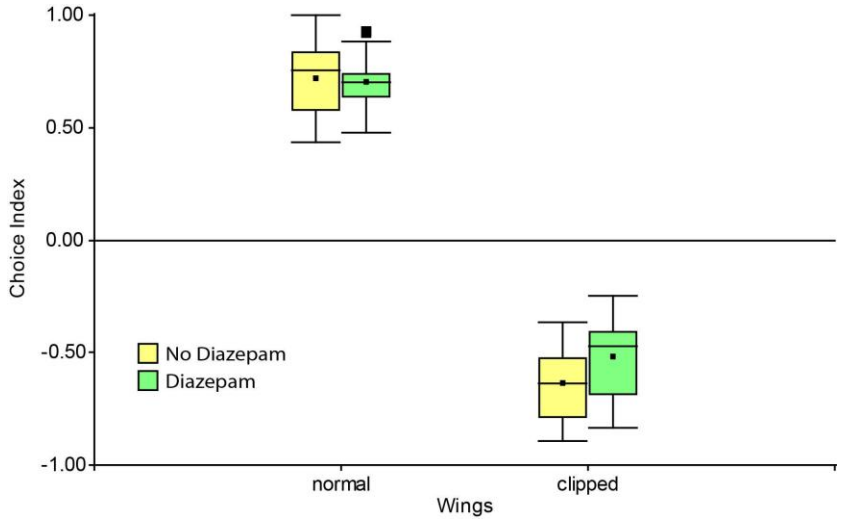


Figure 10: Results of the T-Maze experiment. Flies with and without wings. Repeated with new Diazepam at the very end of the experiments (n=16). Compared to Figure 9 the effect is the same.

Benzer's counter-current apparatus

At this particular time a supply of new Diazepam had arrived, which was then used for the counter-current apparatus as well as for the remaining experiments. To ensure that there is no difference between the old and the new Diazepam, the T-maze experiment was repeated (Fig. 10). After the findings, that a high ratio of flies in the T-maze choose neither light nor dark but rather stay in the elevator instead, I decided to do some experiments in Benzer's counter-current apparatus, because of the possibility that the pure darkness also has an anxiety-inducing effect. This assay was performed similar to the T-Maze, once with normal and wing-clipped flies together (Fig. 11A) and afterwards exclusively with wing-clipped flies (Fig. 11B). There was no perceivable change after Diazepam administration in both performances. Because of that, I started testing flies individually in a smaller T-Maze apparatus to see if the social interaction in the group experiments in general had an anxiety inducing effect.

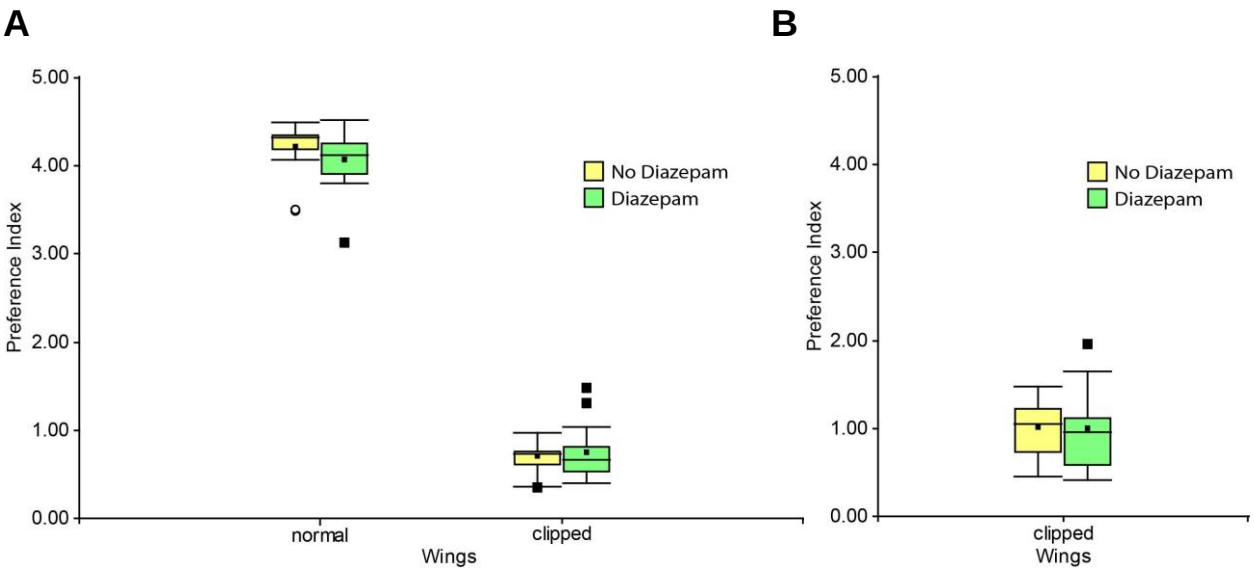


Figure 11: Results of Benzer's counter-current apparatus. (A)Flies with and without wings (n=16). (B) Only flies without wings (n=16). The Diazepam treatment showed almost no effect.

Individual T-Maze

Since the group experiments did not show a significant difference, a slightly modified version of the T-Maze paradigm was used to test wing-clipped flies individually. In the first attempt each fly was able to choose 6 times between light and dark and the results were surprisingly auspicious, because the averaged Choice Index of Diazepam treated flies reached a slightly positive score. To prove that the principle of the hand-made small T-Maze really works, the assay was repeated with 10 choices. As a control I also tested flies with intact wings to see if they show a characteristically positive light response. Figure 12 shows that the normal flies showed a positive light response no matter if they got the drug treatment or not. Furthermore, wing-clipped flies without the drug treatment preferred the darkness and when they were fed with Diazepam mostly chose the light and reached a positive Choice Index.

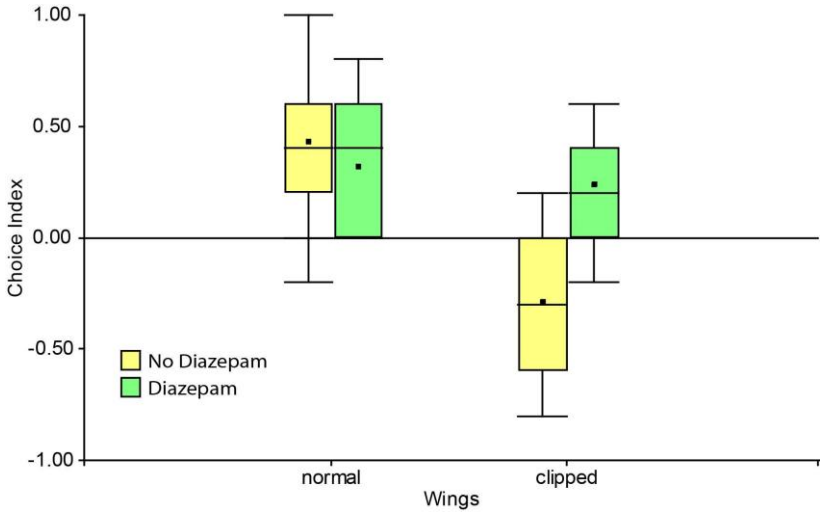


Figure 12: Results of the Individual T-Maze. Each fly had to choose 10 times between light and dark (each group n=20). Wing-clipped flies, which were treated with Diazepam showed a positive light response.

Same fly tested twice

As a final step, flies were tested twice in the individual T-Maze to get insight of the exact behavioral change in one individual. First they were starved overnight and then tested without the Diazepam treatment. To being able to recognize them, they were individualized by placing them in numbered 1.5 ml Eppendorf tubes and then starved overnight again. The next day half of the flies were treated with Diazepam and the other half was treated without Diazepam. Then all flies were tested again. This experiment was performed with wing-clipped flies (Fig. 13B) and as well as with normal flies (Fig. 13A) and retained the setup of 10 Choices. As expected, the normal flies show the same positive light response on both days no matter if they got the drug treatment or not. In contrast, there was an increase of light preference in wing-clipped drug-treated flies, but not as big as when flies were tested only once. The averaged Choice Index was still below 0.

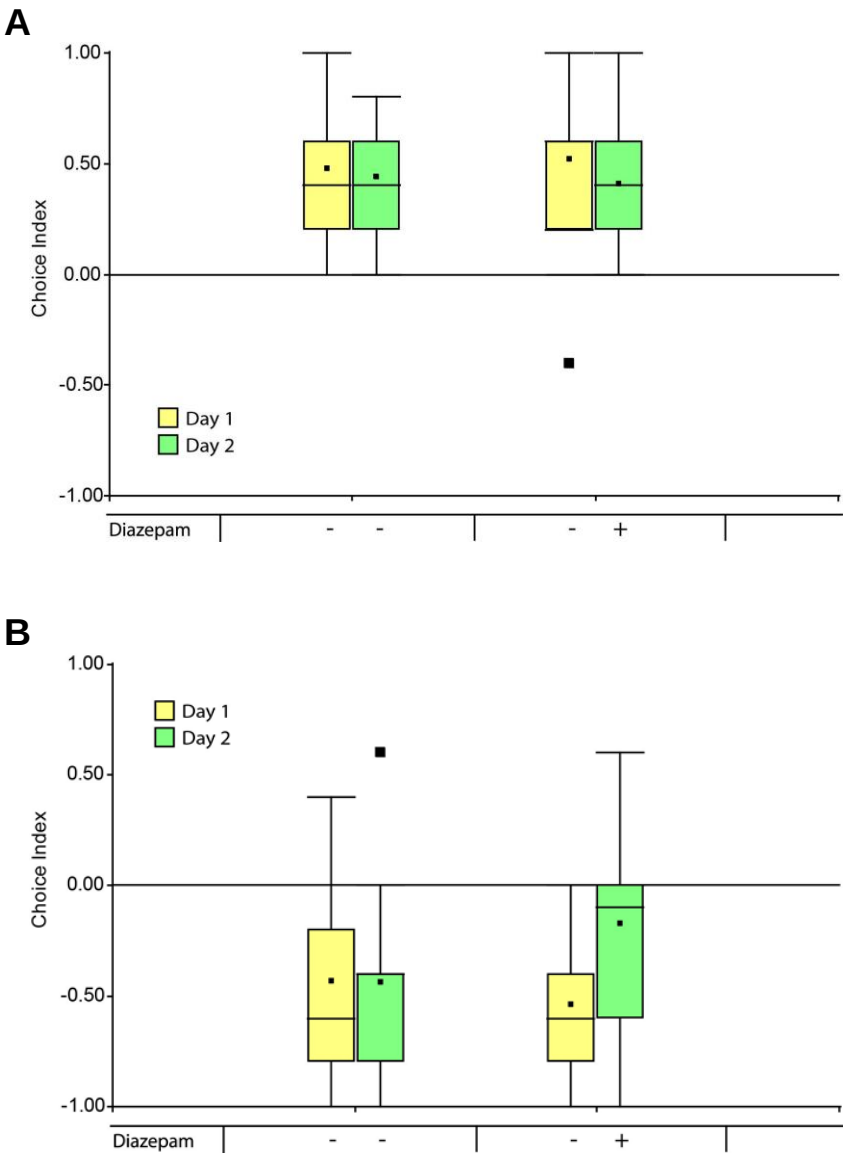


Figure 13: Results of testing the same flies twice in the individual T-maze. (A) Only flies with wings (n=20). (B) Only wing-clipped flies (n=20). Wing-clipped flies, which were treated with Diazepam on the second day of testing showed a more positive light response.

4. Discussion

The open field assay was reproduced from Mohammad et al. 2016 to test if the delivery of Diazepam is effective. That the treatment was successful can be seen in the results that have been similar to their findings. Analyzing the open field data (Fig. 6), the transition plots (Fig. 7), but especially the individual trajectories (Fig. 8) clearly show the diverse behavior with Diazepam, even though the walking distance varied extremely in some individuals. A possible reason for that could be the time of day a fly was tested, which varied between 2-5 pm. Male individuals tend to have a siesta in the afternoon, which perhaps led to less activity during the tests. Another influencing factor that has to be considered is the size and shape of the arena. On the one hand choosing a circular shape instead of a square had the advantage that the options are limited to center and wall. A square offers corners that a fly will prefer, because of less exposure than on a straight wall (Soibam et al. 2012). On the other hand the arena used in this thesis is higher, which could have influenced the trajectory. Seen from the camera perspective, the higher the arena, the bigger is the difference between a position on the wall near the bottom and near the ceiling. In the end the latter appears to be farther from the center. Concluding, the negative geotactic behavior of flies can cause a balance shift towards the ceiling, but occasional sittings on the bottom wall will be recorded as closer to the center and could cause changes in the data. Anyway, a small decrease of centrophobism was noticeable, so I decided to go on with testing changes in photo-preference in flies, which are treated with Diazepam.

First, experiments in a T-Maze were performed with normal and wing-clipped flies together, but the outcomes were not promising (Fig. 9) and many individuals ended up in the elevator again. It was repeated only with wing-clipped flies to lower the group size in the purpose of less distraction between each other. But the results were the same. At this point, it is difficult to say if the flies in the elevator still have been in the process to decide or did not choose at all. The latter could be caused by the darkness as another anxiety inducing factor.

Alternatively, I continued with the counter-current apparatus. Since darkness is not involved in this setup and the evaluation is more distributed, I expected a clearer difference between Diazepam treated and control flies. But the results did not show any change in Diazepam treated flies (Fig. 11). Due to the fact, that the change was already very small in the T-Maze experiment, these outcomes can be explained by the less significant contrast in light conditions. While the animals in the T-Maze have to choose between lightness and darkness, the

possibilities in the counter-current apparatus consist of going to the light and staying in less bright. Because of that, the preference of light appears to be less strong and an influence of Diazepam maybe cannot be recognized.

After the effect in group experiments was very small, I tested flies in the individual T-Maze. In this experiment factors of distraction by other flies or shortage of space caused by the huge sample size were eliminated. Figure 12 shows that the effect of the treatment induced a positive light preference. To explore this results, an assay was performed where the same flies were first tested with normal food and the following day tested with Diazepam. Unlike expected, the effect was smaller, but still there was an increase of light choices (Fig. 13). This can be explained in different ways. First, the procedure can be very stressful and violating by repetitive sucking them in the flexible tube and blowing them into the apparatus. These methods could be improved by finding a more harmless way of transferring them to the start position. Second, after the first measurement the flies were individualized in numbered 1.5 ml Eppendorf tubes to be able to recognize them the next day. But shortage of space and isolation can increase anxiety, which could have lowered the impact of Diazepam. Moreover, it has been shown that social behavior is correlated with social interactions, but relatively independent of the group size (Simon et al. 2011). This could explain why excluding the normal flies in the T-Maze and the counter-current apparatus showed no difference, but excluding all social interactions by individualizing them showed an effect.

For further comparison to the results of the replicated experiment the raw data of this thesis was converted in the effect size Hedges [g] (Fig. 14). While the effect size of Benzer's counter-current apparatus (WOW -0.03; WOW+WW 0.16) and the regular T-Maze (WOW 0.19; WOW+WW 0.36) is less significant, the Individual T-Maze experiments (Single T-Maze 1.8; Same fly 1.07) are highly comparable to the effect size of fly WAFO ("Wall following"; -0.83) and effects in rodents (-0.85). It is important only to attend to the actual size of the effect, because whether the effect size is positive or negative depends on what variable is measured. For example in the paper a *decrease* of WAFO was measured, while this thesis focuses on the *increase* of positive light response.

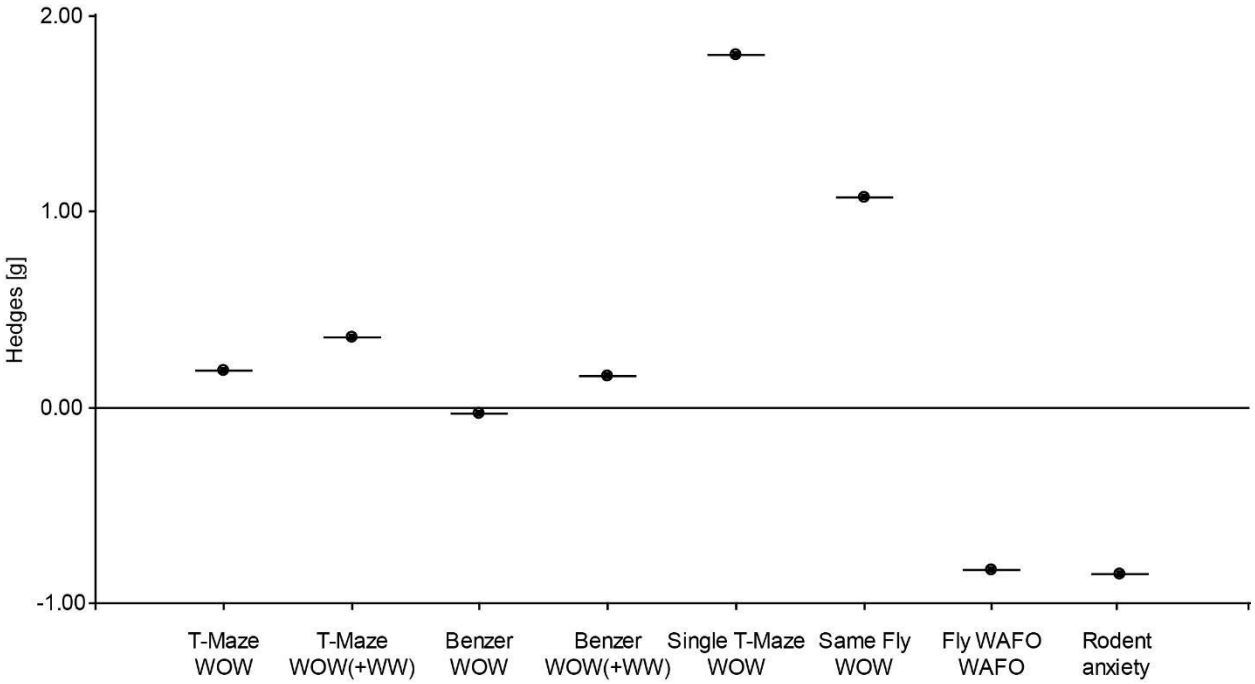


Figure 14: Effect size comparison between the photo-optical assays of this thesis and a standardized mean effect size of Diazepam on fly WAFO and rodent anxiety. The data from fly WAFO and rodent anxiety is presented in Mohammad et al. 2016 (WOW = without wings; WW = with wings).

Concluding this thesis, it could be helpful to do more experiments. For example, experiments in slow Phototaxis by extending the time to choose in the T-Maze and the counter-current apparatus. It would be interesting to see how many flies stay in the elevator, when they have more time to decide. To improve the setup of the Individual T-Maze could also be helpful to confirm the hypothesis. Nevertheless, the consistent tendency in all experiments shows that after treating flies with Diazepam there is an anxiolytic effect on photo-preference, which turned out to be very small.

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