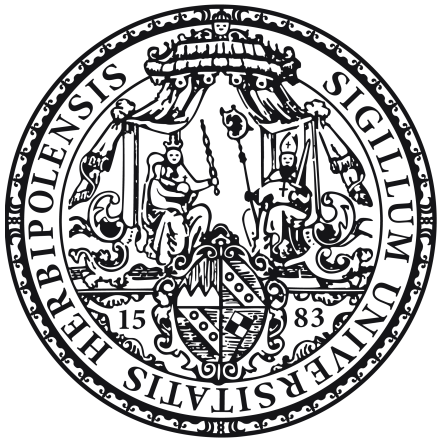


A systematic study of learned helplessness in *Drosophila melanogaster*

Eine systematische Untersuchung der erlernten Hilflosigkeit in *Drosophila melanogaster*

Doctoral thesis for a doctoral degree
at the Graduate School of Life Sciences,
Julius-Maximilians-Universität Würzburg,
Section Neuroscience

Zhenghong Yang

Würzburg**,** 2015

Submitted on: ……..

Members of the *Promotionskomitee*:

Chairperson:	**Prof. Michael Sendtner**
Primary Supervisor:	**Prof. Martin Heisenberg**
Supervisor (Second):	**Prof. Bertram Gerber**
Supervisor (Third):	**Prof. Li Liu**

Date of Public Defence: ……

Date of Receipt of Certificates: ……

1. Introduction **5**

1.1 Learned helplessness **5**

1.1.1 Learned helplessness in varied animal models 6

1.1.2 Sex dimorphisms in learned helplessness 7

1.2 The model organism Drosophila melanogaster **8**

1.3 Biogenic amines in learned helplessness **9**

1.3.1 Serotonin system 9

1.3.2 Dopaminergic system 11

1.4 Aims of this work **12**

2. Material and Methods **14**

2.1 Fly rearing **14**

2.2 Heatbox and controlling software **14**

2.3 Experimental setup **15**

2.3.1 No-idleness experiment 15

2.3.2 Setup of parameters 16

2.4 Monoamine experiments **17**

2.4.1 Pharmacological treatment 17

2.4.2 Genetic manipulation 17

2.5 Statistical analysis **18**

3. Results **19**

3.1 No Idleness experiment with Canton S flies **19**

3.1.1 Learned helplessness in Canton S flies 19

3.1.2 Experiments with test phases under high temperature 25

3.1.3 Experiments with repeated training 28

3.1.4 Experiments with different training durations 33

3.1.5 Control experiments with different temperatures 37

3.2 Serotonin in learned helplessness **40**

3.2.1 Pharmacological treatments 40

3.2.2 Genetic manipulations 51

3.3 Dopamine in learned helplessness **53**

3.3.1 Pharmacological treatments 53

3.3.2 Genetic manipulations 58

4. Discussion 61

4.1 Learned helplessness in *Drosophila melanogaster* 61

4.1.1 Sexual differences 64

4.2 Monoamines in learned helplessness 65

5. References 67

6. Summary 77

7. Zusammenfassung 79

8. Affidavit/ Eidesstattliche Erklärung 81

9. Curriculum Vitae 82

10. Acknowledgements 84

1. Introduction

1.1 Learned helplessness

In rodents and humans the learned helplessness effect describes a specific deficit in behavior to control aversive stimuli that is induced by prior exposure to uncontrollable aversive stimuli. In past decades it has been considered one of the important animal models of depression in humans. In this model, different groups of animals are exposed to either controllable or uncontrollable stressful events for a certain time, then tested on a new task in which all animals are given the opportunity to escape from the punishment, usually by jumping over a partition in the cage. In most cases, animals that are exposed to uncontrollable stressful events do not learn to escape during testing on the new task as fast as the other animals do.

In the initial experiments of Seligman and Maier (1967) three groups of dogs were placed in harnesses. Dogs in the first group were given electric shocks, which could be terminated by pressing a lever. After several trails the animals had learned this and pressed the lever to stop electric shocks. Dogs in the second group received shocks whenever the first group did, with identical intensity and duration, but couldn't stop the electric shocks. Thus, the shocks seemed to happen randomly and were uncontrollable for the dogs in the second group. Afterwards, both groups of dogs were put in a shuttle box and all animals had the opportunity to escape from the punishment by jumping over a low partition. There, the dogs that previously had experienced uncontrollable shocks stayed in the box for a longer time and suffered the punishment, even though they could easily avoid the shocks in this new task. Seligman and his colleagues assumed these dogs had learned in the first part of the experiment, that they had no control of the shock and that termination was independent of their behavior.

1.1.1 Learned helplessness in varied animal models

Learned helplessness as a model of a major depression disorder has been most intensively investigated in rats and mice. The main features of all the conditioning procedures for rats are similar to those for dogs: an aversive stimulus is presented that is unpredictable and uncontrollable, for rats it is usually foot shock or tail shock. For example, in an experiment of Vollmayr and Henn (2001) the rats are given 0.8mA foot shocks in varying time lengths over 40min. The animals are then tested 24h later in the same cage, which contains a bar that terminates the shock when pressed. If the animal presses the bar within 60s of the initiation of shock the trial is termed a success, if not a failure. The rats are given 15 trials and 10 or more failures are considered 'helplessness', animals with five or fewer failures are considered non-helpless. Usually the frequency of helpless rats is 15–20% in such a procedure (Vollmayr and Henn, 2001). Changes in norepinephrine, serotonin and immune system in rats were observed in studies of Anisman and colleagues (1992). Other studies have described significant variations in behaviors and neurobiological responses in different mouse strains they utilized (Anisman, 1984; Francis, 1995; Prince, 1984).

Learned helplessness can also be observed in fish. In Giacalone's group, goldfishes were divided into two groups. One group was given uncontrollable electric shocks, and 24h later together with the other control group, their escape behavior was tested. In this part, a red light was presented to the fishes; if they didn't swim to the other side of the tank, they were shocked for 5s. As a result, goldfishes that experienced uncontrollable shocks showed significantly less avoidance behavior than fishes in the control group (Padilla, 1970).

Learned helplessness has also been investigated in invertebrates. Different groups of cockroaches (*Periplaneta americana*) were exposed to either escapable orinescapable shocks for three days and then all were tested in a new escape task. It has been observed that the animals of the 'inescapable' group showed longer escape latencies and a larger number of escape-failures than the animals of the other group (Brown, 1988).

In *Drosophila melanogaster*, the learned helplessness phenomenon was first investigated by Brown (1996). The study showed that *Drosophila* flies exposed to inescapable mechanical shaking in a black-white Y-maze escape task had longer escape latencies 12h later in a shuttle box escape task than groups with

escapable or no shaking. Furthermore, another learned helplessness experiment was performed by Bertolucci (2008). He found in his doctoral thesis, that flies that experienced uncontrollable heat pulses had decreased learning performance in a new place-learning task. They spent significantly more time on the heated side than flies, which had received escapable or no shocks.

1.1.2 Sex dimorphisms in learned helplessness

It has been reported, that the major depression disorder is twice as common in women as in men (Marcus et al. 2005). With respect to the serotonergic system, whole brain serotonin synthesis and 5-HT_2 receptor binding capacity were found to be decreased in several brain regions of women compared to men (Rubinow, 1998). Moreover, recent evidence points towards a sex-specific antidepressant response. It suggests that women may respond better to selective serotonin reuptake inhibitors (Kornstein, 2000; Hildebrandt, 2003).

As an animal model of depression in humans, the sex dimorphism in learned helplessness has been investigated in the last years. A few studies have shown that female rats do not express learned helplessness behavior as males do. It has been reported, that male rats which have been exposed to uncontrollable footshock stress in a shuttle-box cannot learn to escape in a new task. But on the other hand, the female rats have learned to escape when tested under the same conditions (Shors, 2007). Additionally, recent findings from Papadopoulou-Daifoti lab (Dalla, 2005; Dalla, 2008) indicate a decrease in hippocampal serotonergic activity and a decrease in cortical dopaminergic activity in females, but no neurochemical alterations in male rats. In his doctoral thesis Bertolucci (2008) has reported a sex dimorphism in learned helplessness in *Drosophila*. Only female flies which were exposed to inescapable heat pulses showed decreased learning ability in the new place learning task. Male flies which went through the same procedure behaved like control animals.

1.2 The model organism *Drosophila melanogaster*

The fruit fly *Drosophila melanogaster* is a classical model organism in genetics and developmental biology. It is also considered a crucial model organism in research of human diseases, since approximately 75% of known human disease genes have recognizable matches in the genome of *Drosophila melanogaster* (Adams 2000, Reiter et. al. 2001).

With about 135,000 neurons in the brain *Drosophila* has a relatively simple nervous system in comparison to the complex brains of vertebrates. Thus, the tasks of mapping neuronal networks and understanding interactions of neurons are less complicated with them. Work on *Drosophila* has successfully identified different networks of neurons that govern circadian timekeeping (Nitabach and Taghert 2008), courtship (Villella et al. 2008), memory (McGuire et al. 2005), sleep (Crocker and Sehgal 2010), feeding (Melcher et al. 2007), and decision-making (Dickson 2008; Peabody et al. 2009). The most important advantage of using *Drosophila* as genetic research model is the multitude of genetic tools available for it. One of the most powerful and widely used techniques is the controlled expression of genes by using the UAS-GAL4 system (Brand, Perrimon, 1993). *Gal4* is a gene of yeast encoding the transcription factor GAL4. It contains three domains, a DNA-binding domain specifically recognized by the "Upstream Activating Sequence (UAS)", a transcriptional activator domain, which can activate any gene under the control of UAS, and a regulatory domain binding the galactose-sensitive inhibitory protein GAL80. The GAL4 gene is inserted randomly into the *Drosophila* genome to drive GAL4 expression from one of a multitude of tissue-specific genomic enhancers. A GAL4-dependent target gene can then be constructed by cloning the desired cDNA sequence behind the UAS binding element for GAL4. The target gene is silent in the absence of GAL4. To activate the target gene in a cell- or tissue-specific pattern, flies carrying the target (UAS-Gene X) are crossed to flies expressing GAL4 in the relevant cells(Fig.1).

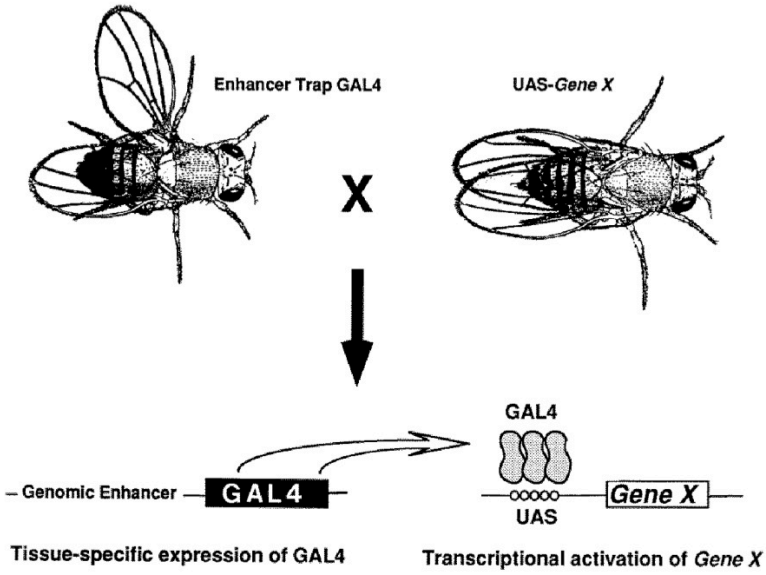


Fig. 1: UAS-GAL4 system in *Drosophila*. The GAL4 gene is inserted at a genomic enhancer site with tissue-specific expression. A target gene of interest is inserted downstream of the UAS binding site for GAL4. The target (Gene X) can be activated in a cell- or tissue-specific pattern, by crossing flies carrying the target to flies expressing GAL4 (Enhancer GAL4). Figure from Brand, Perrimon.

1.3 Biogenic amines in learned helplessness

Biogenic amines are metabolic derivatives of amino acids, and are found in several tissues of vertebrate and invertebrate species. In the nervous system they are detected in distinct neurons from where they are excreted as chemical messengers controlling neural activity. They have functions in different physiological states and behaviors of the organisms. Disruption of the biogenic amine systems has been related to various neurological diseases in humans.

1.3.1 Serotonin system

Serotonin (5-hydroxytryptamine, 5-HT) acts as a messenger substance in most animal species. It controls and modulates a great variety of important physiological and behavioral processes such as aggression in lobsters, feeding and learning in snails, locomotion in lampreys, and pain perception, sleep,

appetite, and mood in mammals (Weiger, 1997). Disruption of the serotonergic system was linked to some human diseases, such as schizophrenia, migraine, depression, suicidal behavior, infantile autism, eating disorders, and obsessive-compulsive disorder (Jones and Blackburn, 2002).

In *Drosophila*, serotonin is synthesized from tryptophan by two tryptophan hydroxylase homologues: DTRHn (*Drosophila* tryptophan hydroxylase, hydroxylates tryptophan) and DTPHu (Drosophila tryptophan-phenylalanine hydroxylase, hydroxylates both tryptophan and phenylalanine) in the presynaptic serotonergic neuron (Neckameyer and White 1992; Neckameyer et al. 2007). Serotonin is packaged into vesicles with DVMAT (Drosophila vesicular monoamine transporter) (Greer et al. 2005). These vesicles fuse with the cell membrane and serotonin is released into the synaptic cleft and bound to four classes of serotonin receptors on the surface of postsynaptic cells. Serotonin left in the synaptic cleft is removed by serotonin transporter protein DSERT (Demchyshyn et al. 1994). A catabolic enzyme, monoamine oxidase (MAO), metabolizes serotonin to non-active aldehyde derivatives (Horvitz et al. 1982; Kandel et al. 2000; Chase and Koelle 2007).

The fly brain is composed of multiple cell clusters containing serotonin. In early studies, ~84 larval and >100 adult serotonin-immunoreactive neurons have been identified in *Drosophila melanogaster* (Vallés and White, 1988; Monastirioti, 1999). In a recent study, using a monoclonal antibody against serotonin, Sitaraman et al. (2008) have identified between 38 and 41 serotonergic neurons per brain hemisphere in adult flies. Similar results have been obtained by Alekseyenko et al. (2010) using TRH-Gal4-driven GFP expression.

In adult flies, serotonergic neurons participate in many processes, such as the regulation of insulin signaling and organismal growth (Kaplan et al., 2008), locomotion (Neckameyer et al., 2007), aggression (Dierick and Greenspan, 2007; Johnson et al., 2009; Alekseyenko et al., 2010), circadian rhythms (Yuan et al., 2005; Nichols, 2007), sleep (Yuan et al., 2006), and reproductive function (Lee et al., 2001). In *D. melanogaster* larvae, serotonin modulates heart rate (Zornik et al., 1999; Dasari and Cooper, 2006) and is involved in olfactory processing (Python and Stocker, 2002), responses to light (Rodriguez Moncalvo and Campos, 2009), and feeding behavior (Neckameyer et al., 2007).

Serotonin plays a crucial role in human depression. Several classes of antidepressants target the serotonergic system. The selective serotonin reuptake inhibitors (SSRIs) are a class of compounds typically used in the treatment of depression. They inhibit the reuptake of serotonin from the synapse cleft. This leads to a higher concentration of serotonin molecules in the synaptic cleft and an increased probability of them binding on the receptors of the postsynaptic cells. Another class of antidepressant is the monoamine oxidase inhibitor (MAOI). It prevents the degradation of monoamine neurotransmitters, including serotonin.

In rats, several research groups provided evidence for the involvement of serotonergic pathways in learned helplessness. Edwards suggests the serotonergic mechanisms with a limbic-hypothalamic circuit serving as a center for adaptation to uncontrollable stress (Edwards et al. 1991, 1992). And in helpless rats, 5-HT_{2a} receptor density was found to be decreased, as compared to control rats (Wu et al. 1999). In addition, changes of presynaptic serotonergic activity caused by uncontrollable shocks had been described in detail in rats. A recent study suggests an important role for serotonergic neurons in the dorsal raphe nucleus (DRN) in mediating learned helplessness (Maier and Watkins, 2005).

In invertebrates the role of serotonin in learned helplessness stays unclear. So far there are only few studies about learned helplessness in *Drosophila* (Brown, 1996; Bertolucci, 2008). In Bertolucci's doctoral thesis it was described that, after being shocked by uncontrollable heat pulses, female flies showed decreased learning ability in a subsequent place learning task. This defect could be fixed by feeding the flies with antidepressants.

1.3.2 Dopaminergic system

Dopamine is another important neurotransmitter that is highly conserved throughout evolution. In mammals, dopamine plays key roles in motor coordination as well as motivation, reward, addiction, learning, and memory. Disruption of dopamine signaling has been implicated in a variety of human disorders (Fahr, Jankovic, Hallett. 2011).

Most genes involved in synthesis, transport, secretion, signal reception, and signal transduction are conserved between Drosophila and mammals. In the *Drosophila* central nervous system, dopamine is synthesized by tyrosine hydroxylase and Dopa-decarboxylase in presynaptic dopaminergic neurons. Then it is loaded in vesicles by VMTA (vesicular monoamine transporter). After releasing through exocytosis, dopamine binds to receptors present on the postsynaptic neurons and triggers a signaling cascade. Excessive dopamine is metabolized by enzymes such as Ebony, Black, Tan, and aaNAT.

Out of the ~100,000 neurons in the adult Drosophila brain, only ~130 cells are dopaminergic (Mao, Davis, 2009). In the larval central nervous system, this number is even smaller (70–90 cells) (Selcho et al., 2009). Despite their relatively small number, dopaminergic neurons are involved in many biological processes. Dopamine has been shown to play key roles in regulating locomotion, learning and memory, courtship, and addiction in flies. More recently, the involvement of dopamine in more complex behaviors such as attention, decision making, and appetite have also been reported (Arnsten, 2007; Roesch, 2007).

Increasing evidence from human and animal studies suggests a relationship between dopamine transmission in the central nervous system and depression. In depressed patients, an up-regulation of D2 receptor density was observed in the basal ganglia/cerebellum in comparison to healthy subjects (D'haenen H.A., Bossuyt A., 1994). The animal models of depression also suggest an implication of dopamine in the depression-like behaviors (Cervo L. et al., 1990; Papp M.et al., 1994; Renard C.E. et al., 2001; Duman R.S., 2004). Furthermore, the relationship between dopamine and depression was confirmed by the fact that antidepressants act on the dopamine system (Plaznik A., 1987; Durlach-Misteli C., 1992; Pozzi L., 1999; Page M.E., 1999).

1.4 Aims of this work

The main goal of this study is to investigate learned helplessness in *Drosophila melanogaster* and the role of the biogenic amine systems in learned helplessness and its sexual dimorphisms. The study consists of three parts. In the first part the learned helplessness behavior in *Drosophila* was investigated using the

heatbox (see M&M). Flies were tested in a variety of no-idleness experiments to gain a better understanding of their helpless behavior. As mentioned before, different biogenic amines are considered to play a crucial role in human depression and in learned helplessness in animal models. Thus, in the other two parts of this work, the serotonergic and dopaminergic neuron systems in *Drosophila* were investigated. The neuron networks of these two systems were manipulated using the UAS-GAL4 technique, and, in the third part, the levels of serotonin and dopamine were altered by drug treatment. Flies were tested in the no-idleness experiment to study whether these manipulations changed some of the behaviors.

2. Materials and Methods

2.1 Fly rearing

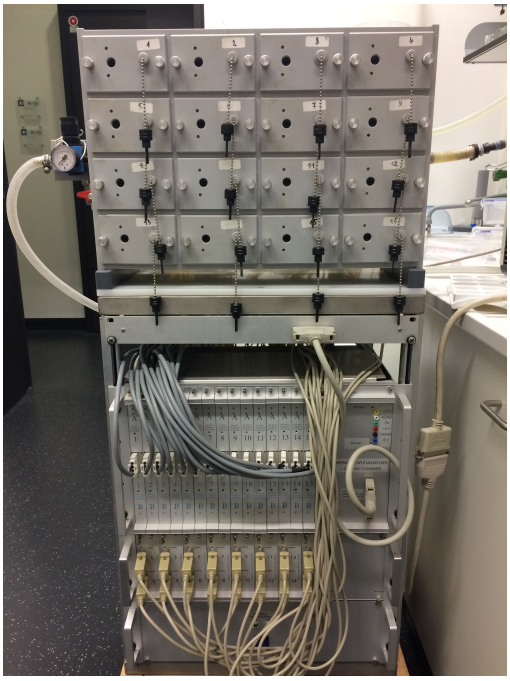
For the culture medium for the flies, 212g corn meal was cooked in 750ml water then left for maceration over night. 40ml syrup and 40ml malt were then added to the soaked mash and cooked together. Additionally, 18.5g dry yeast, 7g agar and 10g soya meal were dissolved in 150ml water and stirred with the mash. After cooling down to 80°C, one tee spoon of methyl-4-hydroxybenzoate was added to the mash as fungicide. Still liquid mash was poured into the food vials 2cm high and stored in 4°C until used.

Flies were kept at 25°C and 60% relative humidity under 14h/10h light/dark cycle. Canton S flies were used for all the wild type behavior experiments. All the Gal4 and UAS lines used in this work were ordered from Bloomington stock centre. Drugs for the monoamine experiments were from SIGMA company (5-HTP: H9972; α-MT: 120693; α-MTP: M8377). All flies tested were 3 to 4 days old, unless otherwise specified.

2.2 The heatbox and controlling software

The Heatbox set-up consists two parts: upper the experimental and lower the electronic control part (Fig.2A). The former includes 16 units as shown in Fig.2B. Inside each unit is a chamber with size of 29x4x2 mm (LxWxH). The two long sides of the chamber are transparent. An infrared LED and a sensor from a bar code reader are on the two long sides. The sensor detects the shadow of the fly and monitors its position. The length of the chamber is defined as 128 position units. Top and bottom of the chamber are equipped with Peltier elements allowing for quick heating and cooling of the chamber. An aim temperature in the range of 24˚C and 41˚C can be reached within 2 seconds.

A



B

Fig. 2 (B): Schematic diagram of one chamber from heatbox. Single flies can run in this small chamber, while its positions are recorded by computer continually. With peltier elements chamber can be heated or cooled very quickly.

Fig. 2 (A): The heatbox. Heatbox consists of 16 boxes in total, each of which is wired with electric part in the bottom. The whole heatbox is connected with a computer, which allows us to control the experiment and see status of each chamber.

The program to operate the heatbox and gather data is HeatGui. It was written by Andreas Eckert (Biocenter, University of Wuerzburg). All the parameters for the experiment can be set with it, such as lengths of test/training phases, normal/punish temperature, master/yoked pairs, etc. The positions of each fly and actual temperature in every chamber are recorded by HeatGui at a particular frequency which was set to 10 cycles per second for all experiments in this work.

2.3 Experimental setup

2.3.1 No-idleness experiment

Flies were gently transferred from vials to the chamber by an aspirator. Their positions in the chambers were continuously recorded. The experiment consisted always of 3 phases: pretest, training and test. In the pretest, there was no punishment, and temperature stayed at 24˚C. Flies ran back and forth in the dark chambers. In the training phase, if they stopped running, (so-called master)

flies (see below) would be punished by being heated at a high temperature after one second. As soon as they ran again, chambers were quickly cooled down. In the test phase, like in pretest flies were not heated any more but could freely walk in chambers.

In each experiment, 16 flies were divided in 2 groups: 8 master and 8 yoked flies. Each master chamber was bound with one yoked chamber. While the master group could control their chamber temperature by running or staying, as described above, the yoked flies didn't have this ability. Their chambers were only heated or cooled whenever their master chambers were. Therefore, for yoked flies the heat pulses were random. They experienced the heat events at the same time, with the same duration as their master flies did. A third group of flies was also tested in the heat box. To them no heat pulses were presented. The temperature during the whole experiment stayed at a constant value.

2.3.2 Setup of parameters

For master/yoked experiments, the normal (unpunishing) temperature was 24˚C, and the punishing temperature was 37˚C. For control experiments, the temperature stayed at 24˚C or 27˚C, depending on experiments. Master flies were punished if they were "idle" (i.e. not walking) for longer than 0.9 seconds. Flies were recognized as "idle", if their position value did not change by more than 3 points within 0.9s . The heat pulse started at 1.0s and lasted until a position change of at least 4 position points was recorded. "Activity" was defined as the total time minus the sum of all "idle" periods.

"Escape latency" for master flies was calculated as the duration from the beginning of a heat pulse to the time when it stopped, which was the time point a fly was active again. This also equaled the idle time of the flies minus 1s. For yoked flies, since the heat pulses were random for them, they could be shocked while active or inactive, so another evaluating process was used. Only those events for yoked flies were calculated, in which they had already being sitting at least for 1s when a heat pulse began. "Escape latency" for these yoked flies was the duration from the beginning of that heat pulse to the time they ran again, irrespective of when the heat pulse actually stopped.

“Turn around” behavior referred only to yoked flies. Flies often changed their walking direction if heat arrived while walking. The frequency was the number of turning around during heat pulses divided by total number of heat events while walking.

2.4 Monoamine experiments

2.4.1 Pharmacological treatment

10ml of fly food mentioned above was melted in the microwave and then different drugs were added to it: 110mg 5-hydroxyl tryptophan (5-HTP), 3.9mg α-methyl-p-tyrosine (α-MT), 38mg α-methyl tryptophan (α-MTP), to make the concentrations 50mM, 20mM, 2mM, respectively. After cooling down of the food, about 50 newly eclosed flies were put into the vials and kept in incubator. Flies were transferred to new food vials every day.

After 4 days treatment, these flies were divided into 2 groups. Flies in one group were put into the heatbox and trained in the no-idleness experiment, both female and male flies. Together with these also control flies tested, which underwent the same feeding procedure without drugs added to their food.

Flies from the other group were used for detecting the monoamine concentrations in their brains. They were stored in a freezer at about -18°C and later were transferred to plastic tubes and put into liquid nitrogen for several minutes. After being vibrated on a vortex mixer, their heads and bodies were separated. About 20 heads were stored in an Eppendorf tube and then put in liquid nitrogen. Then serotonin and dopamine levels in fly brains were then detected in HPLC in Department of Botany I, University of Wuerzburg.

2.4.2 Genetic manipulation

The flies ordered from Bloomington were at first reared in our laboratory for 2 generations. The homozygotes UAS- and GAL4-lines were crossed to our Canton

S flies and the offsprings were used for comparison. To obtain mutant flies, two genotypes of flies were crossed: UAS-TNT/TH-GAL4 and UAS-TNT/TRH-GAL4.

2.5 Statistical analysis

T-test was used for normally distributed data and Mann-Whitney U-test for not normally distributed data. If more than two samples needed to be compared, Kruskal-Wallis test was used. P-value < 0.05 is considered as significant (* for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$).

3.Results

3.1 Learned helplessness in Canton S flies

3.1.1 No-idleness experiment with Canton S flies

In the no-idleness experiment sixteen flies were put in the heatbox, each chamber one. Eight of them were marked as “masters”, the other eight were “yoked” in the controlling program. A pretest phase with length of 30s and temperature at 24℃ was presented to the animals at first, followed by a 10min training phase. There the master flies were punished by being heated at 37℃ , when they stop walking in the chamber for 0.9s. A heat pulse was over as soon as this fly ran again. A yoked fly chamber was only heated when its master chamber was. After training there was a 30s test phase, during which the chamber temperature was at 24°C. Both groups of flies could move or stop without being heated. Another group of flies, the controls were also tested in the same protocol, however, without being punished at 37℃. Instead a constant temperature of 27 ℃ was given throughout the experiment.

All three groups of flies showed the same activity in 30s pretest phase (Fig.3), since they were facing the same situation. In the 10min training phase, yoked flies had a lower activity curve than masters. And the difference was getting bigger in the first 5 minutes. In the last minute of training, master flies were 38% more active than the yoked ones. Their difference persisted in the following 30s test: although there were no uncontrollable heat pulses any more, yoked flies were still 46% less active than master flies. On the other hand, the control group showed a higher activity throughout the experiment. This is probably because they didn’t get any heat pulses as punishment, but a constant temperature. Although a slightly higher temperature (27℃) was chosen, it did not compensate for the stressful condition master and yoked flies underwent. So the control flies were most active. The decreasing activities in all 3 groups were another evidence, that being in dark, narrow and heated chambers was stressful for the animals.

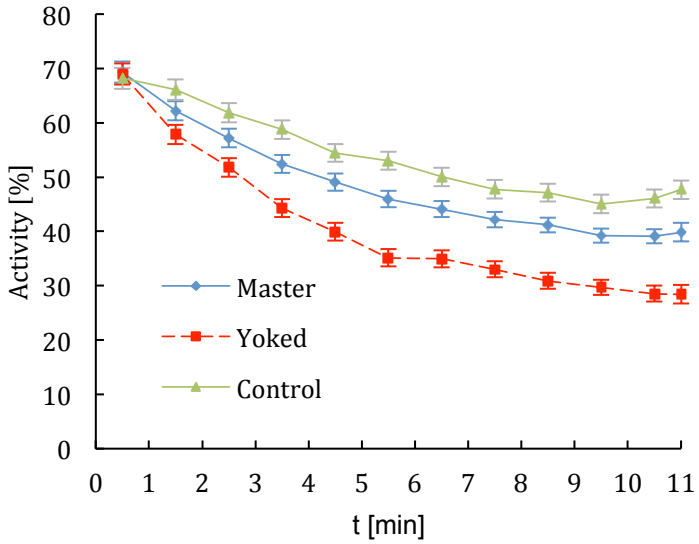


Fig. 3: Walking activities of master, yoked and control flies in no-idleness experiment (n=180 for each group). All three groups are about 70% active in pretest. Then their activities drop in training over time. But yoked flies become inactive faster than the other two groups do. In test, yoked flies still have the lowest activity compared to master and control flies.

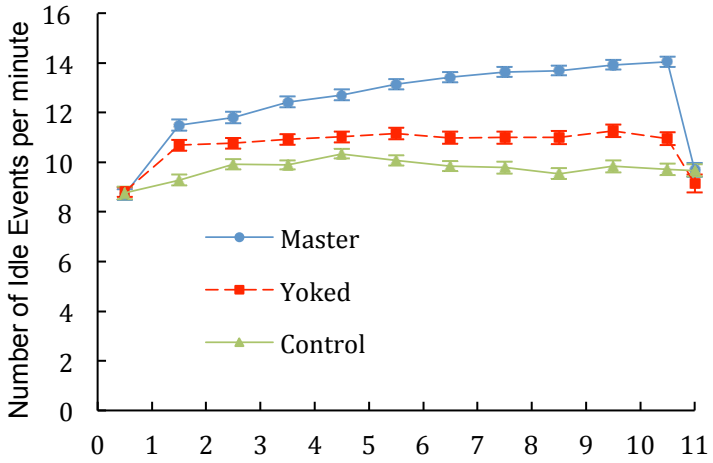


Fig. 4: Number of idle events of flies (n=180 for each group). Only master flies keep increasing number of idle events during whole training phase.

The frequency of idle events was also different between the 3 groups (Fig.4). The yoked and control flies didn't change much in 10 training minute. They stopped

about 11 and 10 times per minute, respectively. In comparison to this, the master flies, which could control their environment by their own, kept increasing their number of idle events in this phase. It increased up to 14 times per minute in the last training minute. This is highly significantly more than yoked or control flies did. It seemed like master flies have developed an efficient strategy to balance their energy consumption and avoiding being heated, namely making many but short pauses. Interestingly the number of idle events dropped to the same level in the three groups, once the conditioning period was over. They were not significantly different from each other in the test phase any more.

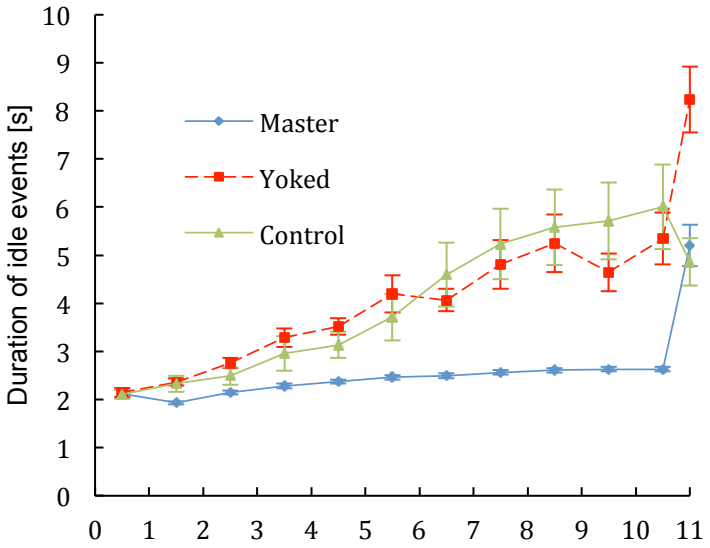


Fig. 5: Duration of idle events of flies (n=180 for each group). Master flies show shortest idle durations in training. In test phase, yoked flies have significantly longer idle durations than master and control flies.

The duration of idle events represented the resting time of flies in single idle events (Fig.5). It shows the time from one fly stop to it resumed walking. For masters this period could be divided into two parts: the 0.9s idle allowance time and their escape latencies.

The duration of idle events also differed much between master and yoked flies. In the pretest, durations of idle event were about 2s for both master and yoked flies. The masters only increased their idle duration from 2,1s to 2.6s within 10min training. However, yoked flies' duration of idle events developed much

more dramatically: in the last training minute, yoked flies rested for 5.3s per time on average. This was highly significantly longer than in master flies.

Since no heat pulses were presented after the conditioning phase, both groups increased their idle durations radically. But still, yoked flies sat with 8.1s duration significantly longer than masters (5.1s). The control group's curve looked like that of the yoked flies' in the training phase and then slightly fell to masters' level in the last 30s test. This also led to a significant difference to yoked flies.

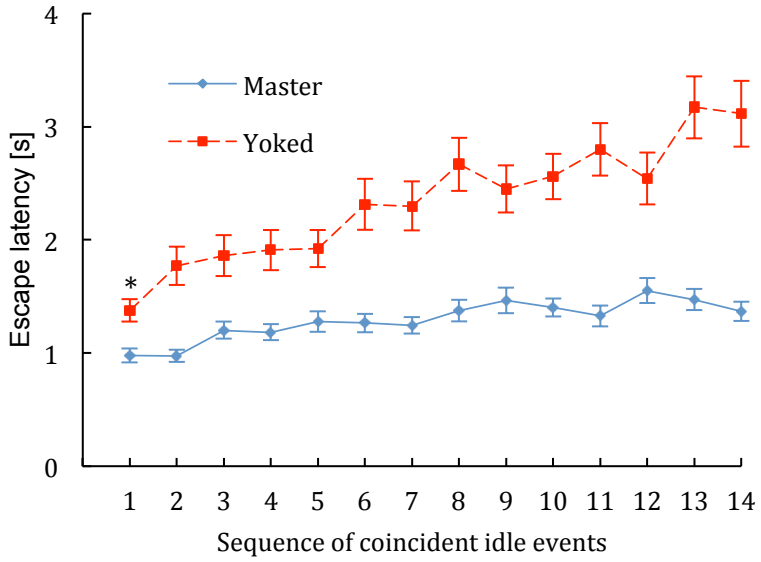


Fig. 6: Escape latencies of master and yoked flies (n=180 for each group). In the first 14 coincident events, yoked flies react slower than masters to heat pulses. Even in the first event, yoked flies have a significantly longer escape latency. It is probably because the first value in this evaluation is about the fifth heat pulse in the whole experiment on average.

Fig.6 shows the response latencies (escape latencies) for master and yoked flies. The escape latency for masters was the time from the beginning of a heat pulse (0.9s after flies have been sitting), until it stopped (flies ran again). To investigate the escape behavior of master and yoked groups, they were compared under similar conditions. For this reason, only those idle events from yoked flies were included in the average, in which the yoked flies already had been sitting at least for 0.9s (the idle allowance time) before a heat pulse started. As shown in Fig.6, while the master flies' escape latencies lasted only slightly longer than 1s in the first 14 events, yoked flies spent significantly more time to response to heat

coming. It is also to note, that yoked flies increased their response latencies over time, whereas masters only weakly changed them. It seemed that the yoked flies learned from one event to another, that there was no chance for them to affect the heat on and off.

Already in the first event, which on average is preceded by five events that did not meet the criteria for the yoked flies, the mean escape latency in the yoked flies was significantly longer than in the master flies. It suggested that, only after about 5 heating events, the yoked flies could have learned the uncontrollability of their environment, and not spent as much energy as the master flies on escaping.

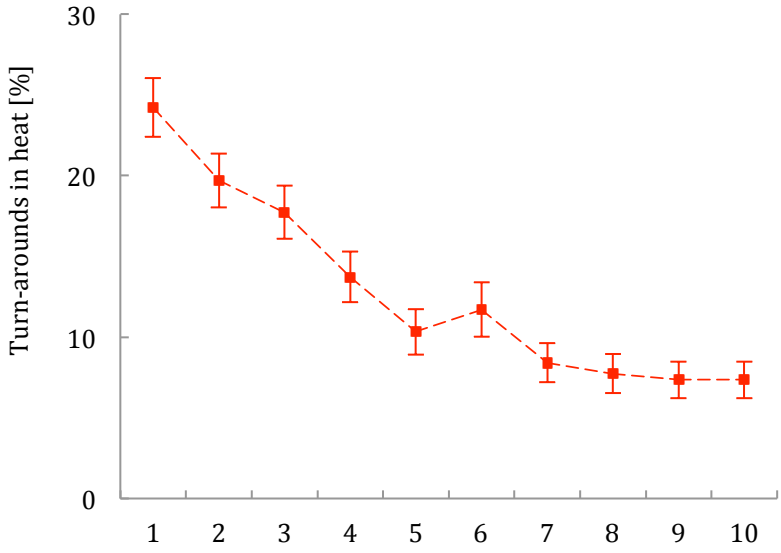


Fig. 7: Turn-around behavior of yoked flies under heat (n=190 female flies). A heat encounter is scored if the fly has been walking for 1s when heat is switched on. A turn-around has to occur within 2s after heat onset to be scored.

If heat arrived while a yoked fly was walking, it might change its walking direction and turn around immediately (Fig.7). Since the temperature in a chamber could keep rising for up to 2 seconds, it was tempting to assume that the fly interpreted the increasing temperature during forward walking as a spatial gradient. Thus, turning around and trying to escape from high temperature was an innate and also reasonable reaction for yoked flies. As in fact, the heat might continue to rise after a turn-around, the fly occasionally even

quickly made another turn and resumed the previous direction. In the first training minute, in 24.2% of all heat events while walking, yoked flies chose to change their walking directions. This value fell to about 7% in the last 4 minutes of the training phase.

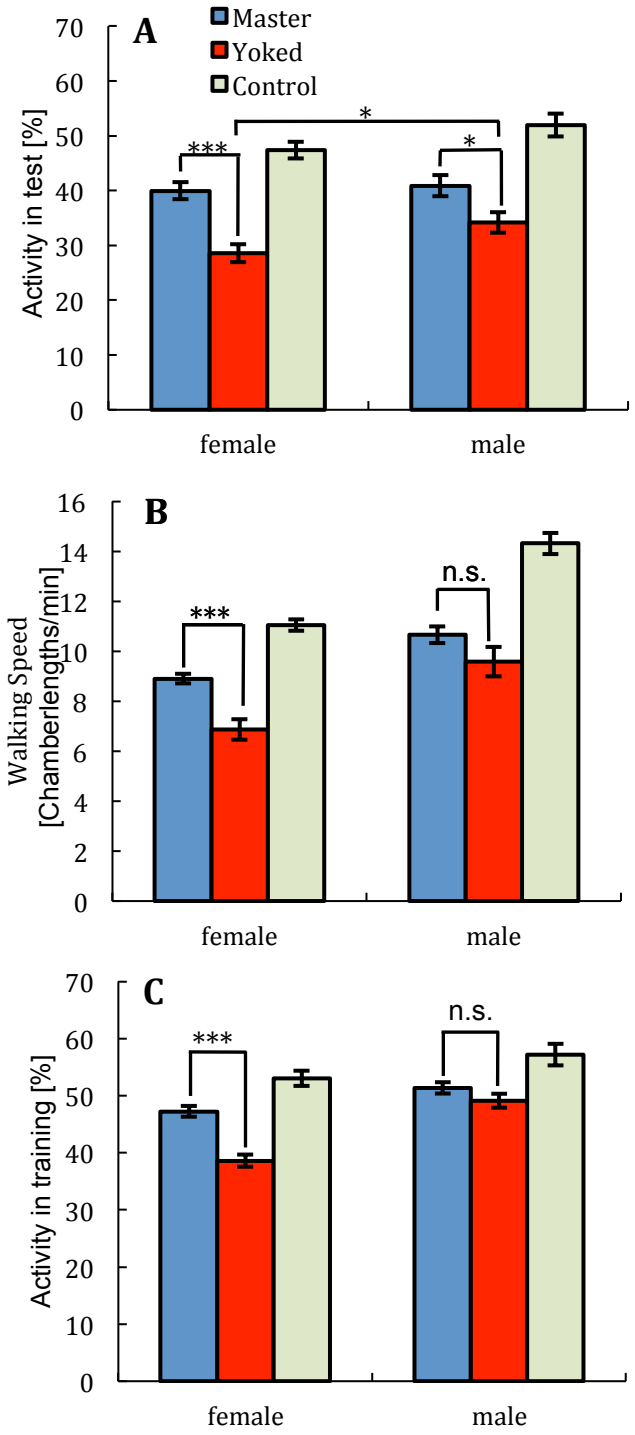


Fig. 8: (A) Walking activity in test phase. Both in female and male flies, master flies walk significantly more than yoked flies in test. But the difference in male flies is smaller than it in female flies. Male yoked flies walk more than female yoked flies.

(B) Walking speed of flies in test. A difference between master and yoked flies can be found in female flies not in males, although all three groups of flies walk faster than female.

(C) In the training phase, there is no significant difference between master and yoked flies in males. (female master and yoked pairs: n=180; male master and yoked pairs: n=143; control females: n=180; control males: n=126)

With respect to learned helplessness, the data reveal interesting differences between female and male flies (Fig.8A). For the master group in the test phase, female and male flies were almost equally active (40%, 41%). However, female yoked flies were significantly less active than male ones. In another word, the master/yoked difference in female animals was more pronounced than in males. A similar gender difference was observed for walking speed in the test phase (Fig.8B). In spite of a higher walking speed for all 3 groups of male flies, there was no significant difference observed between the master and yoked group in male flies. And the situation for activity in 10min training phase was the same: while female master flies were more active than their yoked flies, these two group of male flies were not statistically different from each other (Fig.8C).

3.1.2 Experiments with test phases under high temperature

The original no-idleness experiment, as described above, consisted of a test phase at the end, in which a constant "normal temperature" at 24℃ was present for all experimental flies. In this phase the masters showed higher activity and shorter rest periods than yoked animals. In the following experiment, it was investigated whether this was still true, when flies were tested under a constant high temperature after training.

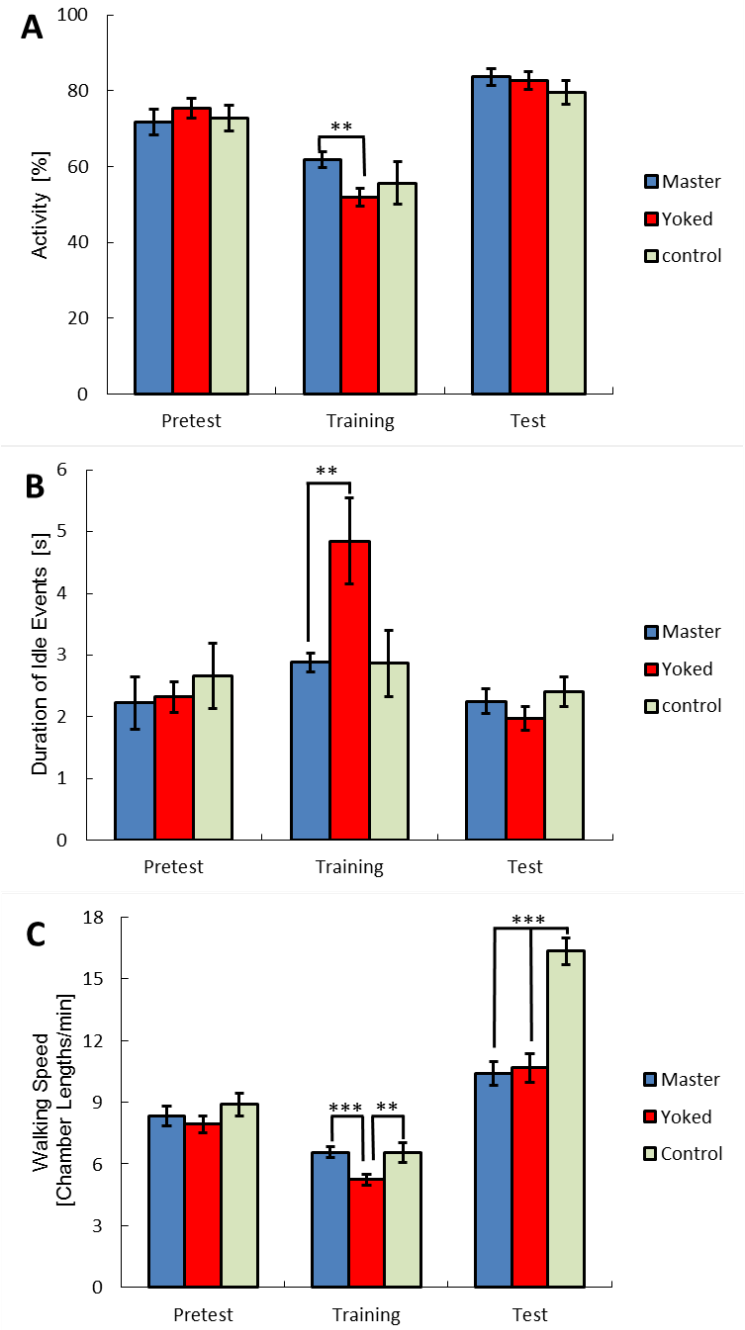


Fig.9: Walking activity and rest periods of flies in No-Idleness experiment with a 37°C test phase. In 30s pretest the temperature in chamber is 24°C; in 10min training phase the normal temperature is still 24°C, punishing temperature is 37°C. For the control group "training" is 10min in chambers under 24°C. In the following test phase, all three groups have to experience constant 37°C chamber temperature for 30s.

(A) A significant difference is only between master (n=48) and yoked (n=48) groups in training phase. There the activity of master flies is 61.7% and yoked is 51.9%. Activity of control group (n=24) is between them (55.6%). In test phase, all three groups show the highest activity levels in the whole experiments (master: 83.6%, yoked: 82.7%, control: 79.6%). But a difference between them is not to observe.

(B) The yoked flies take significantly longer pauses than masters in training phase. The average resting time per event of master flies is 2.8s, while it is 4.8s for yoked and 2.8s for control flies. In the following test phase, all three groups of flies shorten their resting time (master: 2.2s, yoked: 1.9s, control: 2.4s). None of them is significantly different from the other.

(C) Walking speed is evaluated in Chamber Lengths per minute (CL/min). All three groups of flies have lower walking speed in training phase than in pretest. In the training phase, yoked flies (5.2CL/min) walk significantly slower than masters (6.5CL/min) or controls (6.5CL/min). In test phase, all three groups increase their walking speed. Master and yoked flies walk significantly faster than they did in pretest or training phase ($p=0.0063$ and <0.0001 compared to pretest and training for master flies; $p=0.0009$ and <0.0001 compared to pretest and training for yoked flies). In test phase, control flies walk 16.5CL/min. This is highly significant faster than the walking speeds of master and yoked flies.

Three groups of flies were tested again, master, yoked and control flies. The condition in the pretest was the same for them: 24℃ for 30s. As shown in Fig.9, there were no significant differences between them in activity, duration of idle events or walking speed in the pretest phase. In the following 10 minute training phase, unpunished temperature was 24℃ and punished was 37℃ for master and yoked pairs, while the control group experienced constant 24℃. It could be observed in the figure, that master flies were significantly more active than yoked and had shorter average durations of stops; they also walked faster than yoked flies, as already shown in the previous results. Control flies also stopped shorter and walked more than yoked flies. But no differences between master and control groups were observed.

As shown in the previous chapter, if the temperature fell to 24℃ after the conditioning phase, differences between master and yoked flies remained at least for 30s. Surprisingly, under stable 37℃ condition, yoked flies were as active as masters; and they were all about at the control flies' level (Fig.9A), which had the highest activity in test phase under 24℃ (Fig.3). Not only the difference between master and yoked flies was altered, but also the absolute values of activity changed at higher temperature. The activities of all 3 groups in the test phase increased to about 80% of total time; this was even higher than values in the pretest.

Similar results were to be observed in the duration of idle event of flies (Fig.9B). Durations of all 3 groups dropped to around 2s in test, about the level in the pretest. Not like in the original experiment under 24℃, yoked flies didn't spent more time on sitting than masters or controls under this condition. They acted like the master and control flies, made short pauses and resumed running quickly.

Furthermore, in the 37℃ test phase, all 3 groups walked faster than before (Fig.9C). Master and yoked flies showed similar walking speed in the last 30s, ~11 chamber-length per minute (CL/min). Control flies, which hadn't experienced any heat pulses in the chamber previously, walked 16 CL/min in the 30s test phase. This was almost a 3-fold increase to their walking speed during training with the chamber temperature kept at 24℃. Although the activity of control flies was on the same level as master and yoked flies in test, their walking speed was much higher than the other two groups, which had undergone heat

pulses. This is another evidence that stressful heat pulses were one important reason for decreasing activity of master flies in training.

3.1.3 Experiments with repeated training

In learned helplessness experiments for rats it is common that animals are tested repeatedly, over days or even weeks under stressful, uncontrollable stimuli. The no-idleness experiment we used so far lasts only 11min including pretest and test phases. Next we designed a repeated no-idleness experiment for the flies, to investigate if a repetition of presenting uncontrollable heat pulses could affect flies more severely.

Canton S flies at 3 days of age were put into chambers of the heatbox and tested in no-idleness experiment with master/yoked groups. The protocol was like the original one: 30s pretest, 10min training and 30s test. After that all master and yoked flies were put back into two food glasses separately and were stored in the incubator. Two hours later, the same flies were transferred back into the heatbox and tested in the no-idleness experiment again, with identical protocol. After that, a third experiment followed after a further two-hours interval. In all three sessions, there was no switch between master and yoked flies. Walking activity and resting behaviors of flies were evaluated, for the three phases of the three experiments.

Fig.10 shows results in the pretest phase. As expected, no differences were found in the pretest of the first experiment (Fig.10A). Both master and yoked flies explored their chambers for the first time; their walking and resting behavior didn't differ from each other. Surprisingly, when flies were in the chambers for the second time, after the first no-idleness experiment, yoked flies were more active than masters in the pretest, although there was no environmental difference for them. This effect remained in the third experiment. There yoked flies also walked more than masters. Furthermore, an increase in activity itself was found in repetitions of experiments (Fig.10A). Both master and yoked flies increased their activities when they were put back into chambers. Especially in second experiment, yoked flies were over 80% of pretest time active. This result was opposite to our expectation. It could be explained by the fact, that masters were aware of their environment better than yoked flies did. As yoked flies only experienced uncontrollable shocks in previous training, they became more

aroused than masters when they were put into these dark, narrow chambers again.

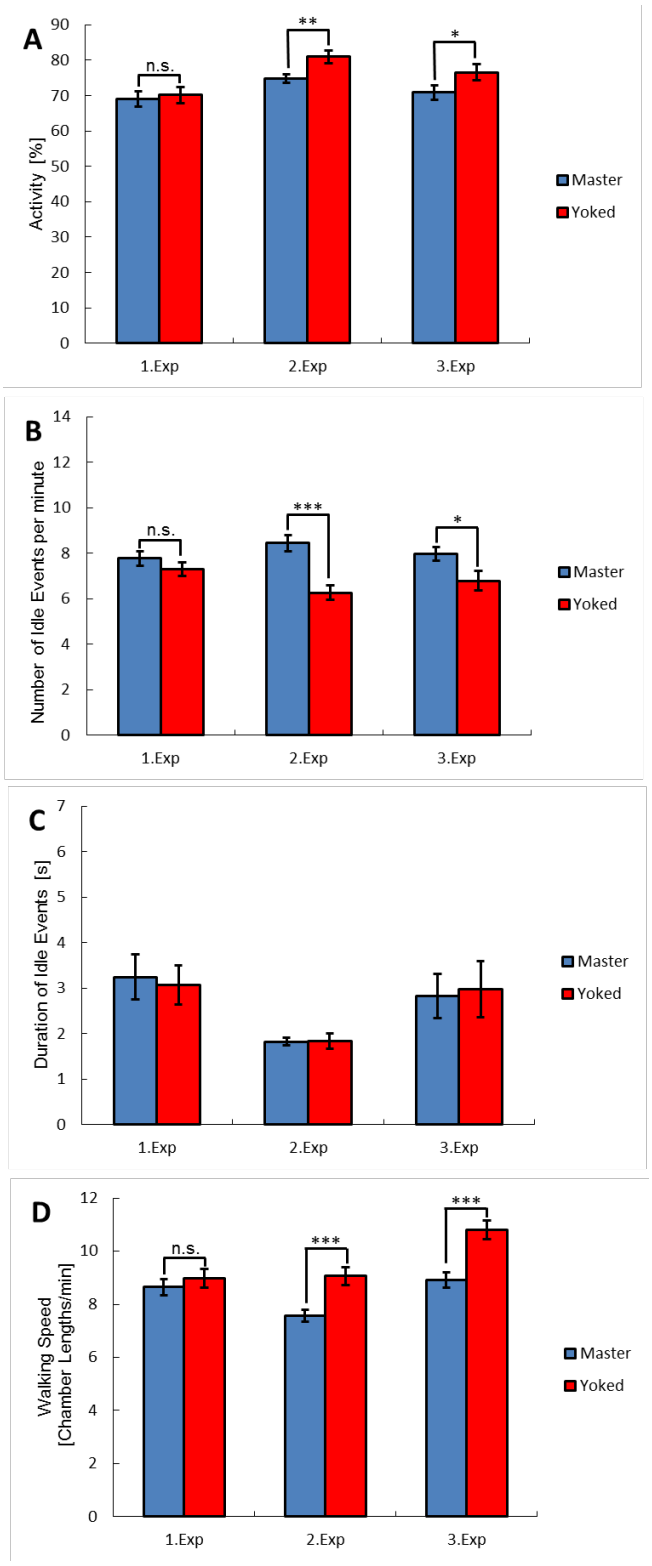


Fig. 10: Flies are tested in no-idleness experiment for three times in 2-hours intervals. Their behaviors in pretest are shown here (n=120 for master and for yoked in first, 108 in second, 101 in third experiment).

(A) The activities of master and yoked flies in pretest phase. They do not differ from each other in the first experiment (p=0.75). After 2 hours, both master and yoked flies increase their activities in the first 30s in second experiment. And yoked flies are more active than masters (p=0.0035), even if they are facing a same situation without any heat pulses. In the third experiment, yoked flies are still more active than masters (p=0.0115).

(B) Master and yoked flies make similar numbers of idle events in pretest of first experiment. In second and third experiments, frequency of yoked flies taking rest is significantly less than that of the masters (p<0.0001 for 2.Exp and p=0.026 for 3.Exp).

(C) None of the master/yoked pairs differ from each other in idle duration in pretest. But in the second experiment, both master and yoked group make shorter breaks compared to them in first experiment (p=0.0051 between masters and p=0.0079 between yoked).

(D) Results for walking speed are like activity of flies. Differences exist in second and third experiments. Yoked flies walk faster than masters in the pretest (p=0.0002 in 2.Exp and p<0.0001 in 3.Exp).

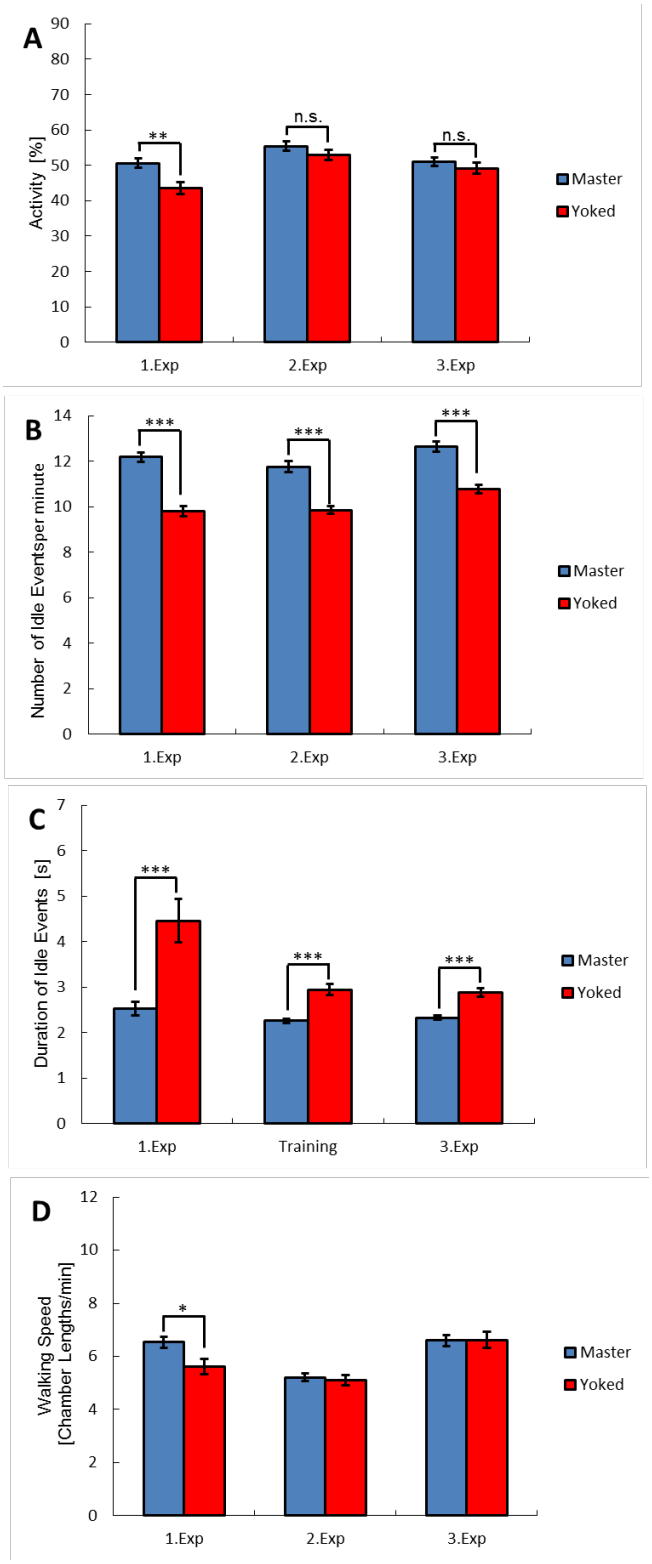


Fig.11 Behaviors of flies in training phases of three experiments.

(A) In the first No-Idleness experiment, master flies walk more than yoked in the 10min conditioning phase ($p=0.001$). For the second and third time in chambers, master/yoked flies do not differ from each other in activity. But increases of activities in repeated experiments can be observed: yoked flies in 2. and 3. exp are more active than in 1. exp ($p<0.001$, $p=0.01$); master in 2. exp are more active than in 1. exp ($p=0.02$).

(B) (C) In all three experiments, master flies make more but shorter pauses than yoked in training.

(D) Masters are walking faster than yoked flies, when they are trained for the first time ($p=0.011$). In second and third repetitions no master/yoked difference is found. However, walking speed of master flies decreases in 2.Exp ($p<0.0001$) and increases again in 3.Exp ($p<0.0001$). Also yoked flies increase their walking speed in 3.Exp compared to 2.Exp ($p<0.0001$).

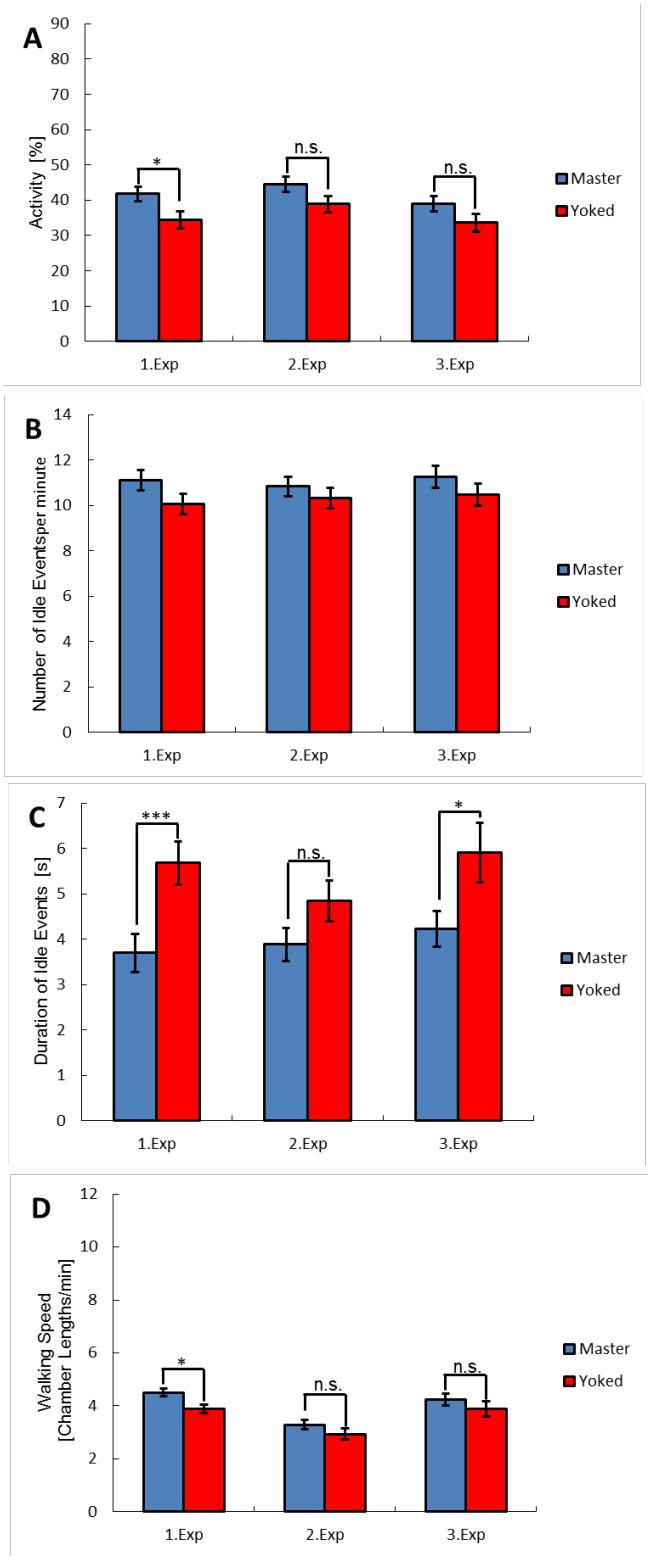


Fig.12 Behaviors of flies in test phase

(A) In all three experiments, masters are more active than yoked flies after the conditioning phase. But they are only significant different in 1.Exp (p=0.017).

(B) No significant differences between master and yoked flies are found in number of idle events in test phase.

(C) Master flies' idle durations are shorter than yoked flies'. The differences are significant in first and third tests. (p=0.0021 for 1.Exp, p=0.09 for 2.Exp, p=0.028 for 3.Exp)

(D) Walking speeds of all groups drop in test phase to a level lower than 5CL/min. A significant difference between master/yoked can only be observed in 1.Exp (p=0.0049).

Similar results were found in number of idle events (Fig.10B) and walking speed (Fig.10D). In pretests of second and third experiments, yoked flies decreased their frequencies of resting and increased their walking speed compared to master flies. In last repetition yoked flies walked even 20% faster than they did walking in the chamber for the first time. Interestingly, differences between master and yoked flies in duration of idle events could not be observed. However, both groups shortened their idle duration significantly in second experiment.

In the 10min training phase, no differences in activity between master and yoked flies were found in the second or third experiment (Fig.11A). Compared to first experiment, yoked flies increased their activities to masters' levels. Activity of master flies in second experiment was also significantly higher than in first experiment, which indicated they had learned how they could escape from aversive heat pulses in chambers. Furthermore, another disappearance of the differentiation between master and yoked flies was found in walking speed of flies in training phase (Fig.11D). Interestingly, both master and yoked flies' walking speed decreased in second and then increased again in third experiments. The number and duration of idle events did not change much in second or third experiments (Fig.11 B, C), except that yoked flies made shorter breaks in second and third trainings. It was to note, that their error bars were also smaller compared to yoked flies in first training. This is probably because some yoked flies had very long pauses in first training, but did not in following training anymore.

In test phases the results looked similar as in training (Fig.12). Master flies were significantly more active than yoked only after the first conditioning phase (Fig.12A). After two or three times of training, differences in activity between master and yoked flies became smaller. The same effect was found also in walking speed, whereas master flies were not walking significantly faster than yoked in second or third experiment (Fig.12D). The differences between two master and yoked flies in idle duration became smaller with repetitions (Fig.12C). No significant differences were found in number of idle events in test phases (Fig.12B).

3.1.4 Experiments with different training durations

The no-idleness experiment in chapter 3.1.1 consisted of 3 phases: pretest, training and test. After 10 minutes conditioning, master group differed in many aspects from yoked group. Would master and yoked flies also behave differently, when training phase were shorter or longer? Would the difference become more pronounced as the training time increased? Or in other words, would yoked flies become more helpless, if they experienced longer and more uncontrollable heat pulses? The following experiments were done, in order to try to answer these questions.

Six groups of master/yoked flies were tested in no-idleness experiments with six different lengths of training phases. The durations of training varied between 5 and 30 minutes. Flies were punished by being heated at 37℃, when masters stopped walking for over 0.9s. The unpunished temperature and temperature in pretest and test phase was 24℃. As mentioned before, the aim was to investigate the after-effects of master and yoked flies, so the evaluations were focused only on the 30s test phase.

Fig.13 shows the activities of flies in test phases for the different training durations. The lengths of training phases are indicated on the x-axis. Results of master and yoked flies are shown separately in Fig.13A and Fig.13B, and the differences between them in panel Fig.13C; positive value means masters are more active than yoked flies. In master group (Fig.13A), flies tested for 5, 7 and 10 minutes all had similar activity levels at around 50%. If training lasted for 15 minutes, activity decreased to 30%. With increasing length of training, the activity values was getting lower; with 30min training the master flies spent only less than 20% of total time moving. This was a highly significantly shorter total active period than after 5, 7 and 10 min of training.

A similar result could be observed in yoked flies: the longer the training phase was, the lower the activity became. However, data for yoked flies were different to masters' at two points. First, the highest activity values in 5 and 7min groups were about 40%, not 50%. Second, a drop of activity happened in the 10min training group, whereas masters in this group showed same activity as in shorter training groups. What made master and yoked flies differ from each other in the test phase of the 10min-training group, was this second point. In Fig.13C, this

was the only value significantly different from zero. Although the activity differences in 5, 7 and 15min groups were positive, they were too small to reach significance. The value for 20 and 30min lay on the negative side, which meant yoked flies were even more active than masters in the test phase.

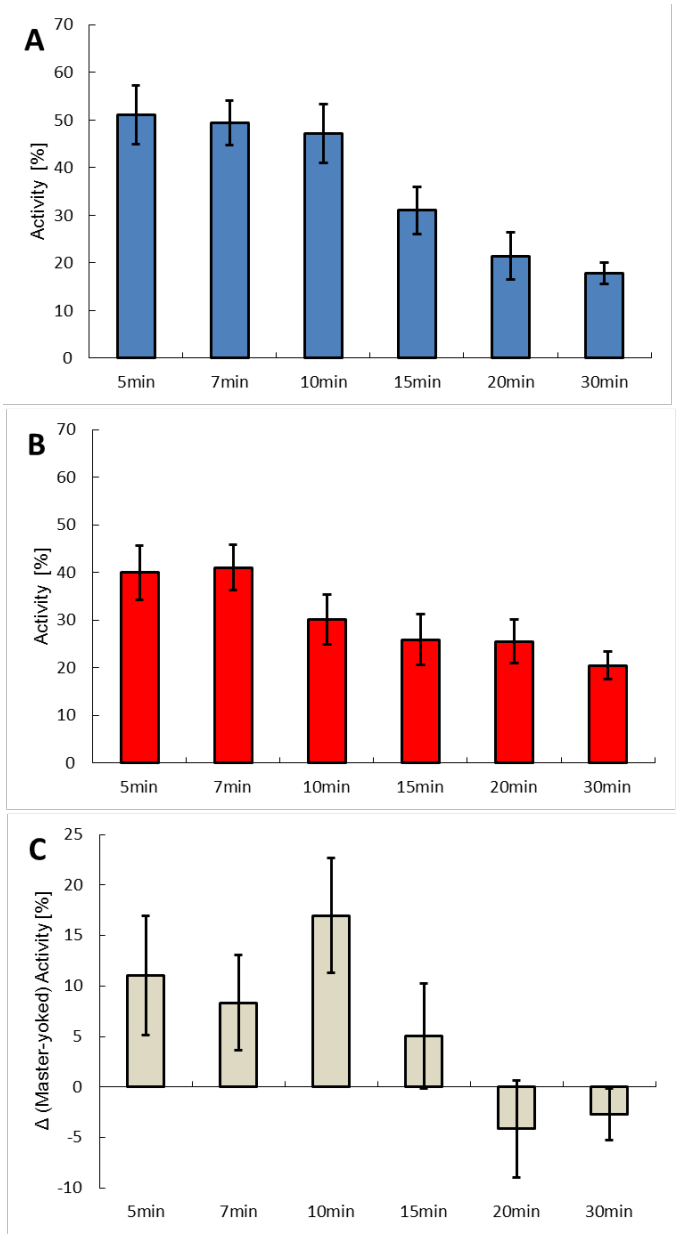


Fig.13 Activities of different groups of flies tested in No-Idleness experiments with different lengths of training phases. The duration varies from 5min to 30min. n=16 for groups 5min to 20min, 32 for 30min.

(A) Activity of master flies in test phase after different lengths of training phases. With 5min, 7min and 10min training, master flies show higher activities (51%, 49.3%, 47.1%) in test phases. For 15min training, the activities of master flies drop to 30.9% ($p=0.05$ compared to activity for 10min group). With longer durations, activities drop to 21.4% and 17.8% for 20min and 30min. They are both significantly lower than the activity for 10min training ($p=0.0029$ and $p<0.0001$).

(B) Activity of yoked flies in test phase after different lengths of training phases. The decrease of activity for yoked flies begins already with 10min training (30.1%). Longer training durations than 10min do not make the yoked flies significantly less active ($p=0.57$, 0.51, 0.085 for 15min, 20min, 30min compared to 10min).

(C) Differences between master and yoked flies are calculated by subtracting activity of yoked flies from that of masters. With varied lengths of training, masters are more active than yoked flies if conditioning phase is not longer than 15min. The only significant difference between master and yoked flies in test is found for 10min training ($p=0.0094$ against zero).

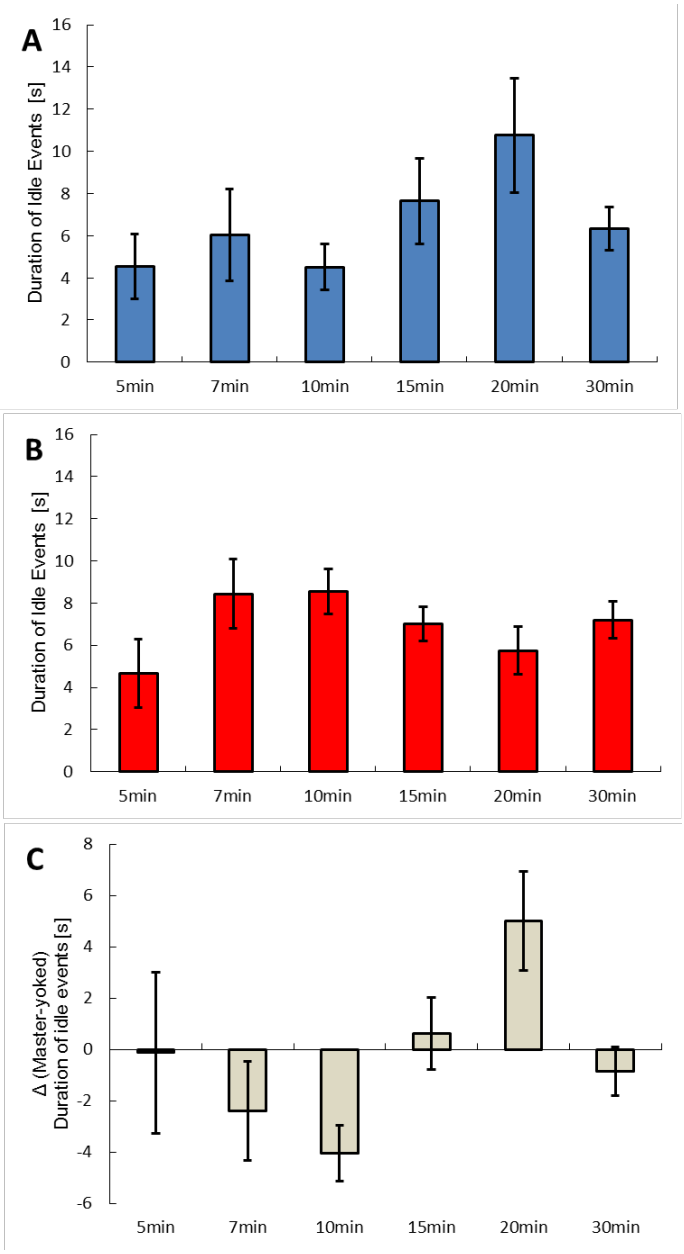


Fig.14 Durations of idle events for different groups in test phase.

(A) Durations of idle events for master flies increase with longer durations of training phases until 20min and drop again in 30min group. After 20min training, master flies rest on average 2.4 times longer than flies after 10min training per event.

(B) Yoked flies show shortest idle duration after 5min training. The longest durations are found in 7min and 10min groups. With 20min training phase, yoked flies have a relative shorter idle duration at 5.7s.

(C) Differences in duration of idle events between master and yoked flies is calculated by subtracting yoke flies' values from masters'. The difference in 10min group is significant (p=0.0019 against zero). After 20min training, master flies rest significant longer than yoked in test phase per time (p=0.019 against zero).

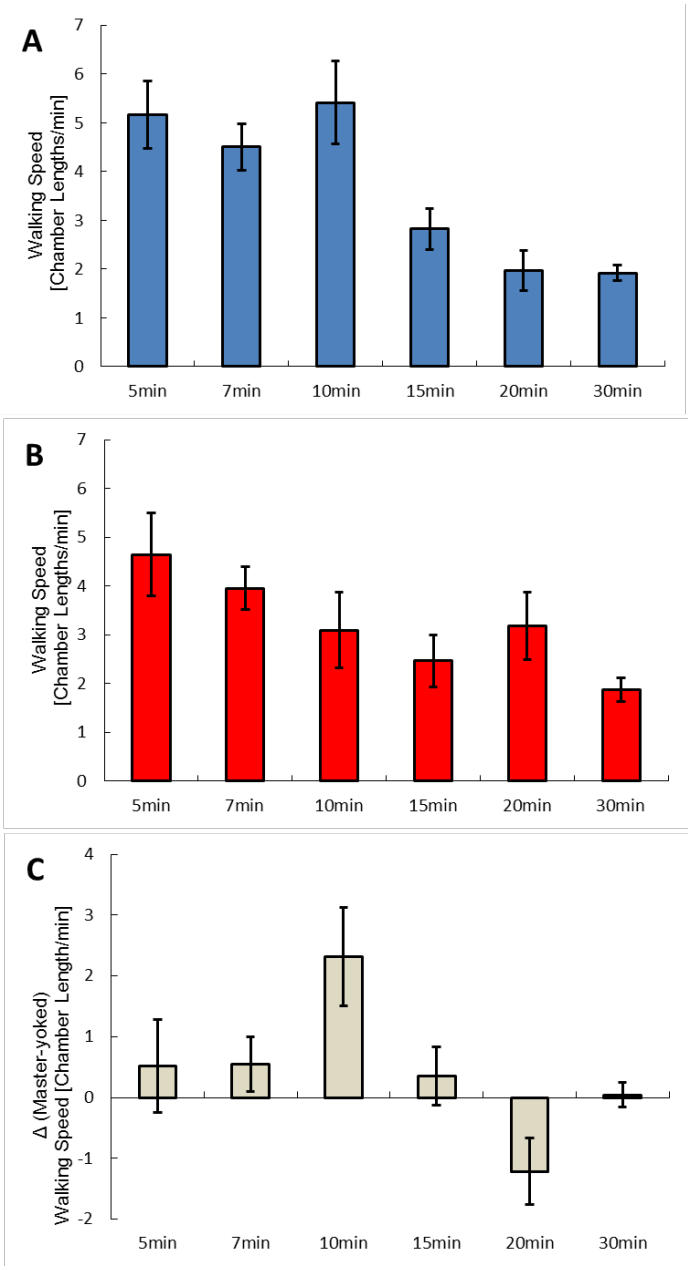


Fig.15 Walking speeds for different groups in test phase.

(A) Master flies walk faster after shorter training phases. With 5min, 7min and 10min training, the walking speeds in following test phase are 5.15, 4.49 and 5.4 CL/min. As the training phase prolongs, the walking speeds of master flies decrease (2.82 for 15min, 1.97 for 20min, 1.91 for 30min).

(B) Yoked flies show the highest walking speed after 5min training and lowest after 30min training.

(C) Differences in walking speed between master and yoked flies. After 10min of training master flies walk faster (p=0.012) after 20min of training master flies walk slower than yoked flies (p=0.043).

In 'durations of idle events' in Fig.14 the flies with 5min training showed the shortest durations in both master and yoked groups. A significant difference existed in the 10min-training group. Master flies had shorter mean idle events than yoked flies. After a 20min training phase, surprisingly, duration of idle events in masters was significant longer than in yoked flies.

Fig.15 shows the walking speed of master (A) and yoked flies (B), as well as their differences (C) in the test phase. In general, flies walked faster, if they were trained for shorter times. For example, both master and yoked flies walked about

5 chamberlengths per minute after a 5min training, they accomplished less than 1 chamberlength (equal to about 2CL/min) after 30 min of training. Again, a highly significant difference could only be observed between master and yoked flies, which had been trained for 10min. After 20 minutes of training, the masters showed slower walking speed on average than yoked flies.

In conclusion from the experiment with different durations of training phases the choice of 10 minutes has turned out the best condition for studying the current symptoms of learned helplessness in the heatbox.

3.1.5 Control experiments with different temperatures

In the original no-idleness experiment, a constant temperature of 27℃ was chosen for control flies throughout the experiment, trying to compensate for the heat of the heat pulses for master and yoked flies in the training phase. However, it was not known what kinds of effects different temperatures would have on control flies. Would they behave alike under lower and higher temperatures? To answer these questions walking in the heatbox was studied at different temperatures.

All 16 chambers were filled with control flies, which were tested at 3 different, but constant temperatures: 24℃, 27℃ and 30℃, separately. The experiments lasted 11min in total.

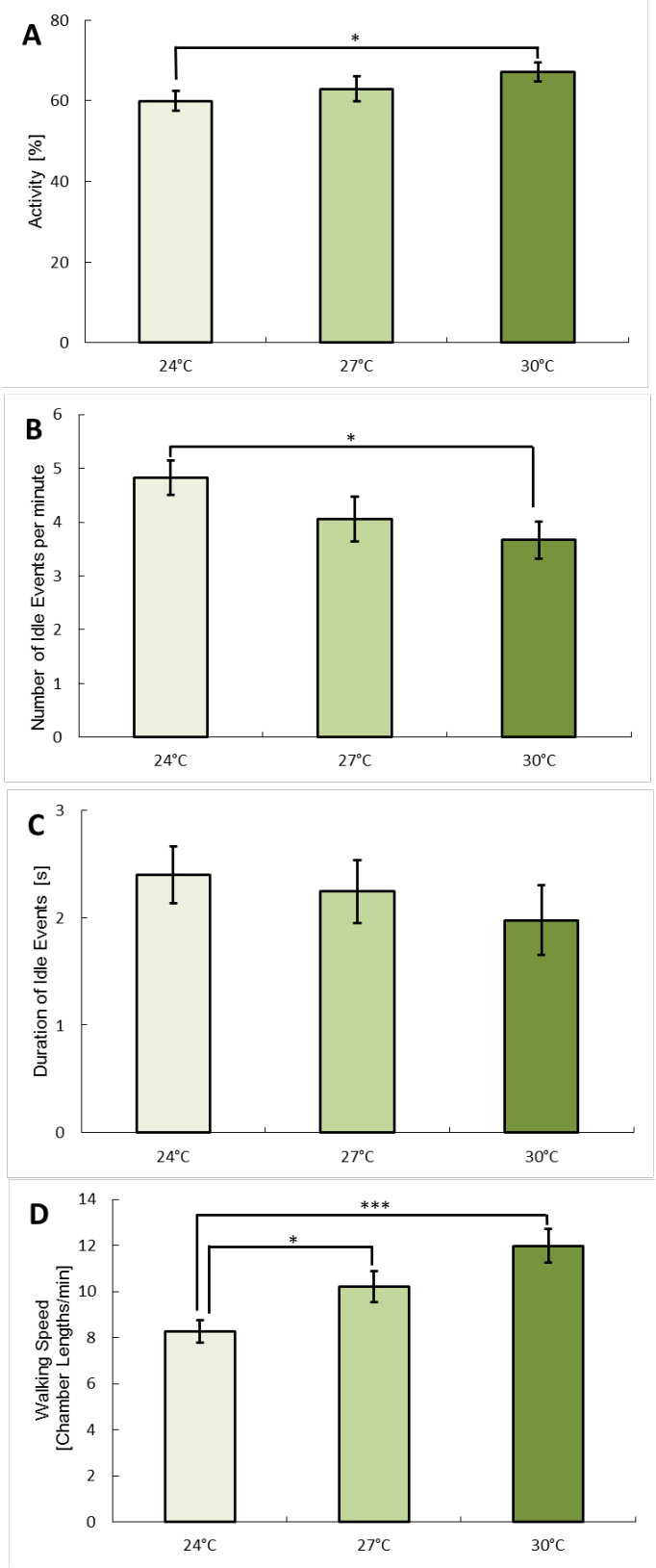


Fig.16 Behaviors of flies under different temperatures in the heatbox. Flies are put into chambers in Heatbox for 11 minutes. Three groups of flies are tested at different temperatures: 24℃, 27℃ and 30℃ (n=28 for each group).

(A) The activity of flies increases as experimental temperature rises. Flies at 30℃ show significantly higher activity than flies at 24℃ (p=0.039). Activity at 27℃ is not significantly different from that of the other two groups.

(B) Flies at 30℃stop significantly less often than flies at 24 ℃ (p=0.021).

(C) Durations of idle events decrease as experimental temperature rises, but none of them differ significantly among the three groups.

(D) Walking speed differs between groups. Flies at27℃ and 30℃ walk faster than flies at 24℃ (p=0.026 and 0.0003). The difference between 27℃ and 30℃ is not significant (p=0.08).

The result was shown in Fig.16. Under 24℃ flies had an overall activity of 60% of total time (Fig.16A). This value increased as the chamber temperature increased. As the temperature was raised to 30℃, flies became significantly more active. They also made less stops than at 24℃ (Fig.16B). Although the durations of idle event of the three groups were not statistically differed from each other, a decreasing trend could also be observed with increasing temperature (Fig.16C).

Another more pronounced effect of experimental temperature on flies was in walking speed (Fig.16D). Flies walked with a speed of 8 CL/min on average under 24℃ in experiment. When the temperature was raised 3℃, to 27℃, they walked two more chamber-lengths in one minute. If the temperature was raised to 30℃, flies walked 12 CL/min on average.

It was shown in this control experiment that flies walked more in time and in distance under a higher temperature. This is also consistent with the result in chapter 3.1.2, where flies showed hyperactivity under 37℃ test condition.

3.2 Serotonin in learned helplessness in *Drosophila*

In many studies, it was reported that serotonin plays an important role in depression in humans [Zitate]. One group of antidepressants aims at increasing the concentration of serotonin in serotonergic neurons [Zitate]. Serotonin selective reuptake inhibitor (SSRI) inhibits the reuptake of serotonin from the synapse cleft, so that the chance of serotonin to bind on receptors of the postsynaptic membrane is getting bigger [Zitate].

As already described in previous chapters, yoked flies in the heatbox showed symptoms of learned helplessness after experiencing inescapable heat shocks. They suppressed their innate responses by reducing walking activity and walking speed. Their attempts to escape from aversive conditions were also suppressed (longer escape latencies and lower turning around frequency). Considering the importance of serotonin in depression and more important, in learned helplessness experiments in other animal models [Zitate??], it is worth investigating the role of this biological amine in learned helplessness in *Drosophila*.

3.2.1 Pharmacological treatments

The serotonin level in the brain of flies was manipulated in two ways: pharmacologically and genetically. Using the former methods, experimental flies were fed by different drugs, which act either as a precursor or inhibitor in serotonin metabolism. The concentration of serotonin was measured by HPLC (by Markus Krischke in Institute of Pharmaceutical Biology). Furthermore, such flies were tested in no-idleness experiments to find out their behavioral changes.

Both female and male flies were fed on food containing serotonin precursor 5-hydroxyl tryptophan (5-HTP) or the serotonin synthesis inhibitor alpha-methyl tryptophan (α-MTP) with the concentration 50mM and 20mM, respectively. The feeding procedure lasted 4 days. On the 5th day, flies were decapitated and the serotonin concentrations in their heads were measured. Female and male flies were evaluated separately.

The untreated flies, which were fed on normal food without drugs for 4 days, showed about the same serotonin level in males and females, 14.26pg/head and 15.89 pg/head respectively (Fig.17). After 4d treatment with 50mM of 5-HTP, both gender flies' serotonin levels in the brain increased dramatically, however, with different intensity. The male flies showed a roughly 50-fold increase over base line, while the average value for females reached 2400 pg/head, a 150-fold increase compared to untreated flies. No explanation and no related reports have been found for this sex-specific difference. Serotonin levels of flies treated for 4d with 20mM αMTP could not be detected in our experiment, probably because concentrations were too a low .

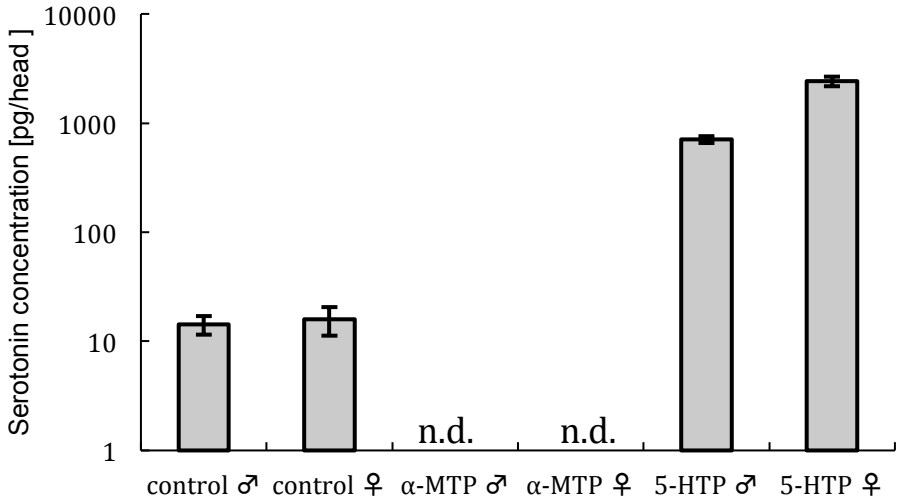


Fig.17 Serotonin concentration after pharmacological treatment (control male: n=7; control female: n=6; 5-HTP male: n=10; 5-HTP female: n=10). Serotonin levels in female and male flies' brains increased strongly after feeding with 5-HTP. The increase rates are different between the two genders. While serotonin concentration in male flies increased from 14.2 pg/head to 709.2 pg/head, that in female flies increased from 15.8 pg/head to 2416.9 pg/head. Concentrations of serotonin in brains after treatment with α-MTP cannot be detected in our experiments, probably because of their extreme low values.

Flies from the same population used for concentration evaluation were tested in the standard no-idleness experiment. The results were focused on two aspects. First, it was asked whether the walking activity and duration of stops had changed in master and yoked flies; second, whether the differentiation between master and yoked flies had changed. Female and male flies were evaluated separately.

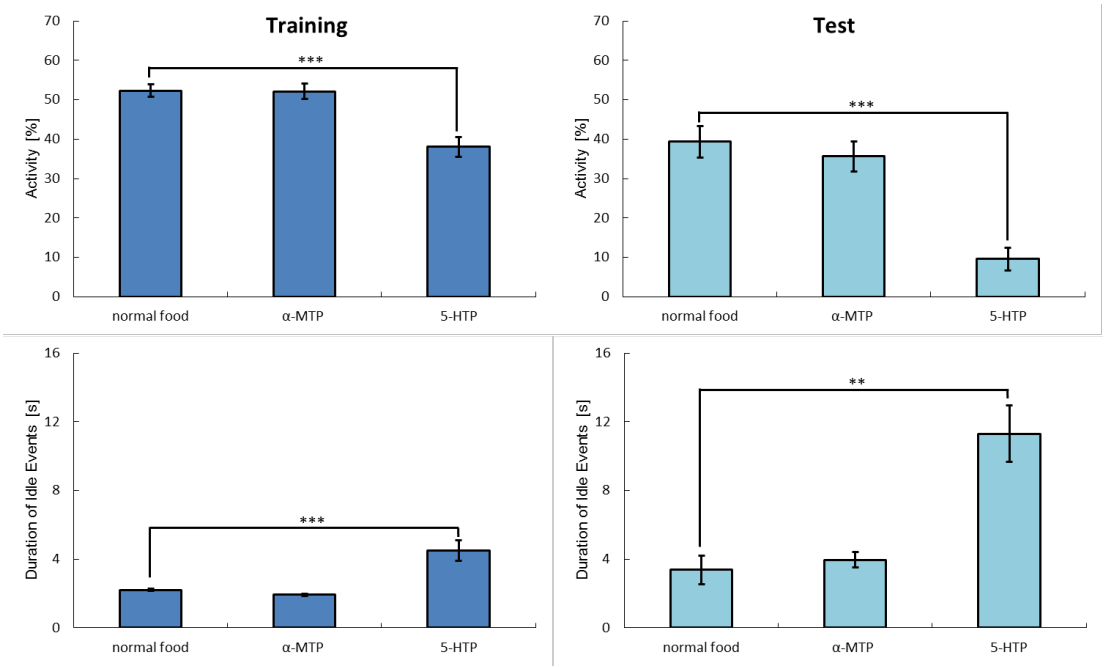


Fig.18 Walking activities of female master flies in training and test phases after treatment with serotonin inhibitor and enhancer (n=40 for control; n=40 for α-MTP; n=42 for 5-HTP). Activities of flies treated with 5-HTP decrease in training and in test phase significantly compared to control flies. In test phases, their activity is lower than 10%. These flies also increase their idle durations in both phases. Female master flies treated with α-MTP do not show any significant differences compared to controls.

Fig.18 shows the female master flies' activities in training and test phases. The active time of the group fed with normal food(control) averaged in 10 training minutes at 52.3% of total time. The flies treated with α-MTP were not different from controls; their activity was 52.1% in training. However, flies bred on food with 5-HTP had a significantly lower activity than the other two groups. It was only 38%. Furthermore, this effect remained after training. In the 30s test the 5-HTP group with 9.5% activity walked much less than the groups grown on normal food and α-MTP (39.3% and 35.6% respectively). 5-HTP -treated master flies showed not only lower activity. They also had longer durations of idle events in training and test. If during training a 5-HTP- master stopped walking, it rested for 4.5 s on average. This was highly significantly longer than the pauses of flies grown on normal food orα-MTP. Their average 'idle' time of 11.3s in the test phase was also much longer than that of the other two groups.

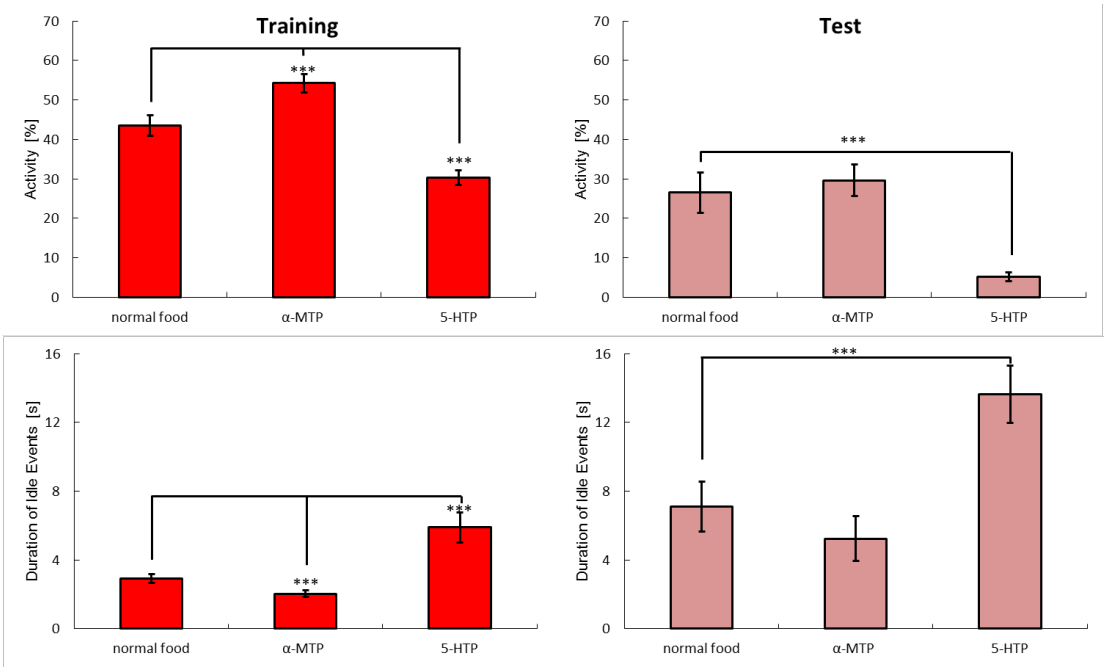


Fig.19 Walking activities of female yoked flies in training and test phases after treatment with 5-HTP and α-MTP enhancer (n=40 for control; n=40 for α-MTP; n=42 for 5-HTP). Like masters, female yoked flies also reduce their activities and prolong their durations of idle event in training and in test. Interesting is that female yoked flies fed with α-MTP become more active in training compared to control flies. They show higher activity and shorter idle durations.

5-HTP not only affected the activity of female master but also that of female yoked flies (Fig.19). Grown on serotonin precursor 5-HTP the yoked flies showed significantly lower activity and longer duration of idle events in training and test phases. The absolute value of activity in the test phase fell even to only 5%, which meant they were only 1.5s active out of 30s on average.

Interestingly the yoked flies grown on α-MTP showed a significantly increased activity and a decreased duration of idle event in training phase compared to the normal-food group. In the test phase, small differences between control and α-MTP groups could be observed in activity and duration of idle event, but they were not statistically different. Since α-MTP is a serotonin synthesis inhibitor, this indicated that a lower serotonin level enhanced the yoked flies' walking activity in the conditioning phase in the heatbox.

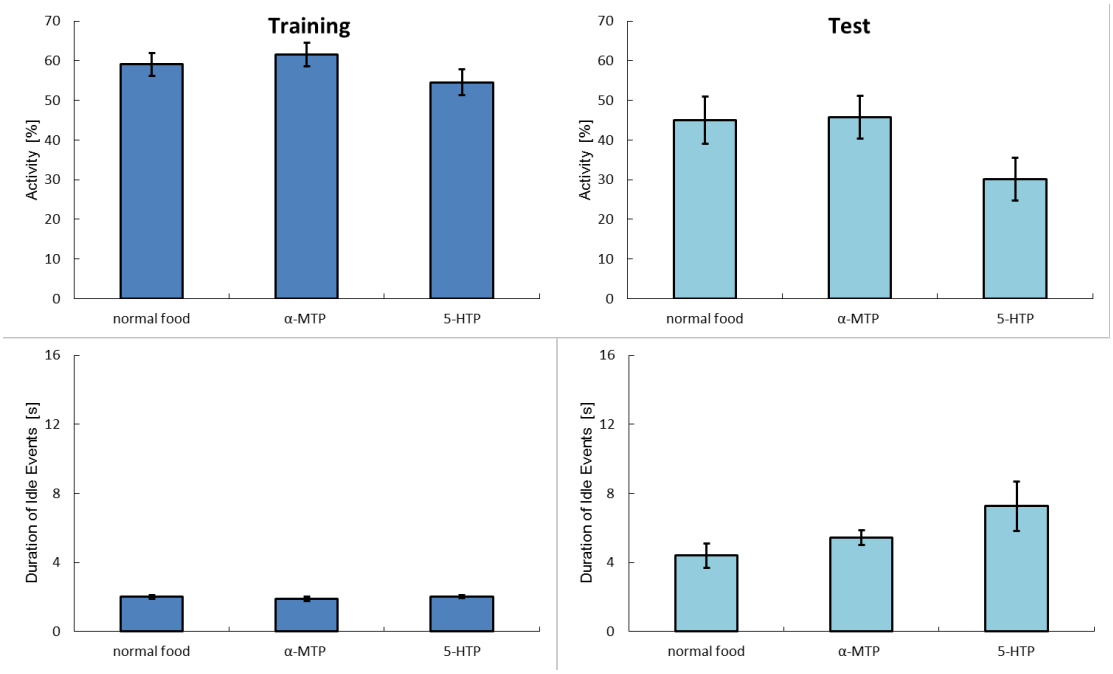


Fig.20 Walking and rest of male master flies after pharmacological treatments (n=30 for control; n=30 for α-MTP; n=40 for 5-HTP). Male master flies treated with serotonin enhancer or inhibitor do not change their activity level significantly compared to control group. There is a small decrease of activity and increase of idle duration in the 5-HTP group in the test phase, but these differences are not significant.

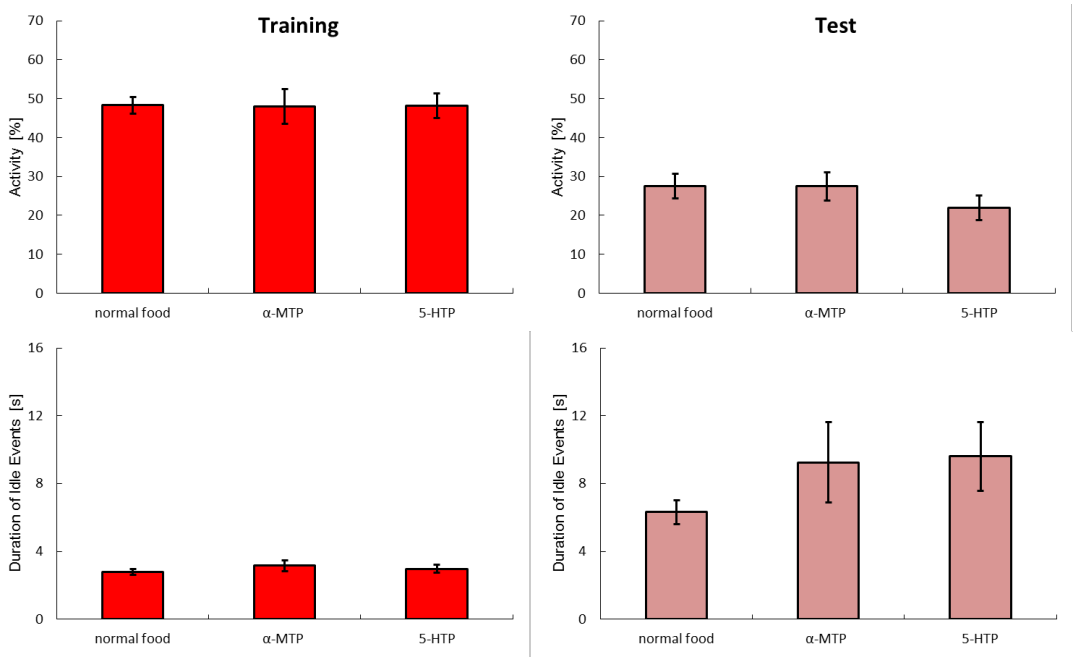


Fig. 21: Activity levels of male yoked flies after pharmacological treatments (n=30 for control; n=30 for α-MTP; n=40 for 5-HTP). Like the master flies, no significant differences can be found in flies treated with α-MTP or with 5-HTP.

The results for male flies are not the same as in females (Fig.20 and Fig.21). A change in walking activity or duration of idle events was to be observed neither in the α-MTP nor the 5-HTP group. They were all not statistically different from control flies. Although there was a small decrease in activity of 5-HTP male masters, it didn't reach significance.

To summarize this part, serotonin does plays a role in flies' walking activity and rest in the heatbox, but these effects differ regarding the female/male and master/yoked groups. The following tables are an overview (Tab.1 and Tab.2).

female	Master	Yoked	M/Y difference
normal food			√ , √
α-MTP	—,—	↑ , —	X, X
5-HTP	↓ , ↓	↓ , ↓	√ , (√)

Tab. 1: Activities of female master and yoked flies after treatment with serotonin inhibitor and enhancer. First sign for training and second for test phase in each group. — means no significant change, ↑ means increase, ↓ means decrease. X indicates no difference between master and yoked flies, √ indicates difference. Brackets means not significant difference.

male	Master	Yoked	M/Y difference
normal food			√ , √
α-MTP	—,—	—,—	√ , √
5-HTP	—,—	—,—	√ , (X)

Tab. 2: Activities of male master and yoked flies after treatment with serotonin inhibitor and enhancer. Meanings of signs same as in Tab.1

How about the activity differences between master and yoked flies? Were they also affected by changing the serotonin level? The following figures show these differences in training and test (Fig. 22 to Fig. 25).

Training

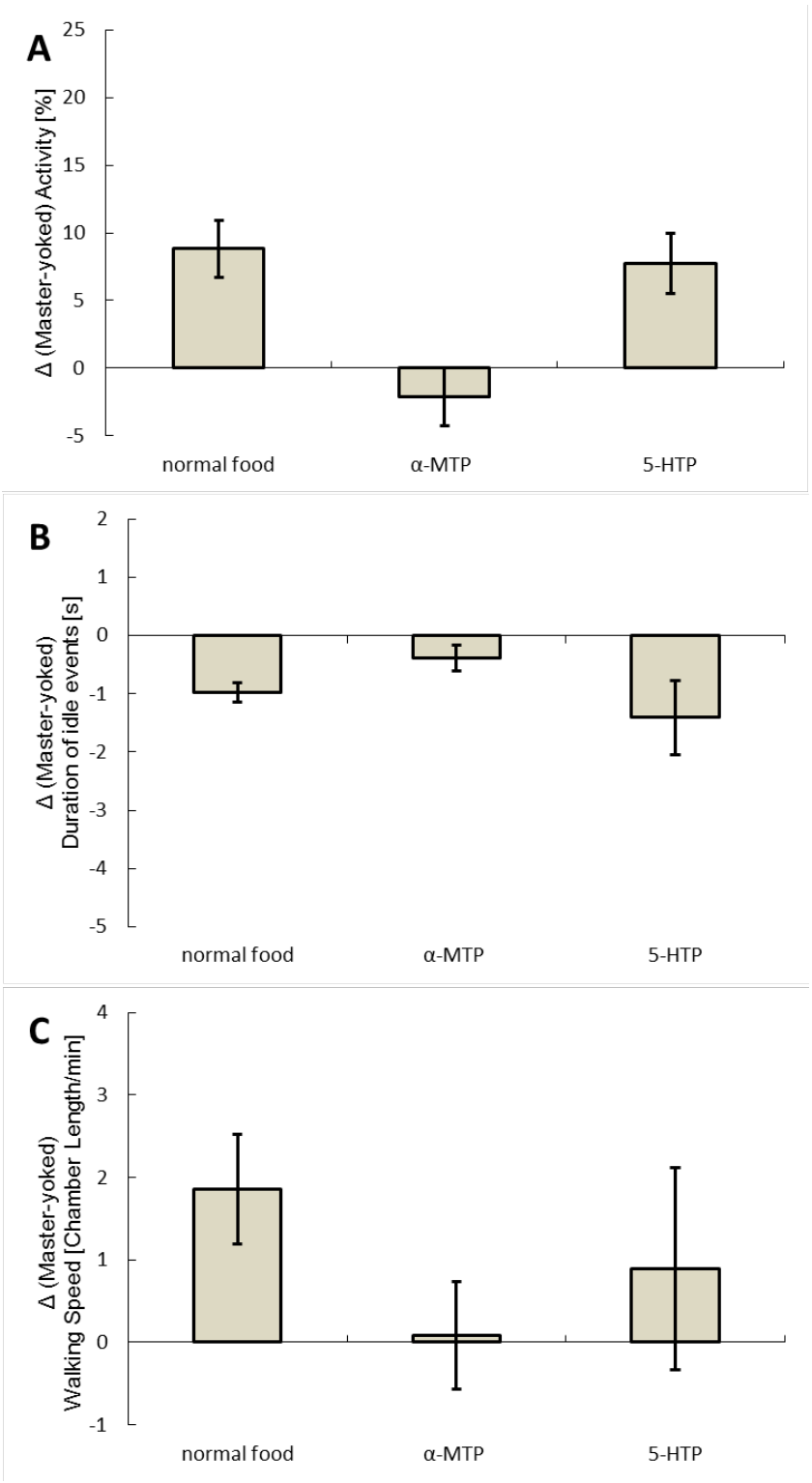


Fig. 22: Differences of activity level between female master and yoked flies in training (n=40 for control; n=40 for α-MTP; n=42 for 5-HTP). In 10min training phase, flies treated with α-MTP do not show a master/yoked difference. No differences in activity, idle duration or walking speed can be found in the α-MTP group. Female flies treated with 5-HTP show differences between master and yoked flies that are similar to those of the 'normal food' group. (Difference in walking speed is not significant.)

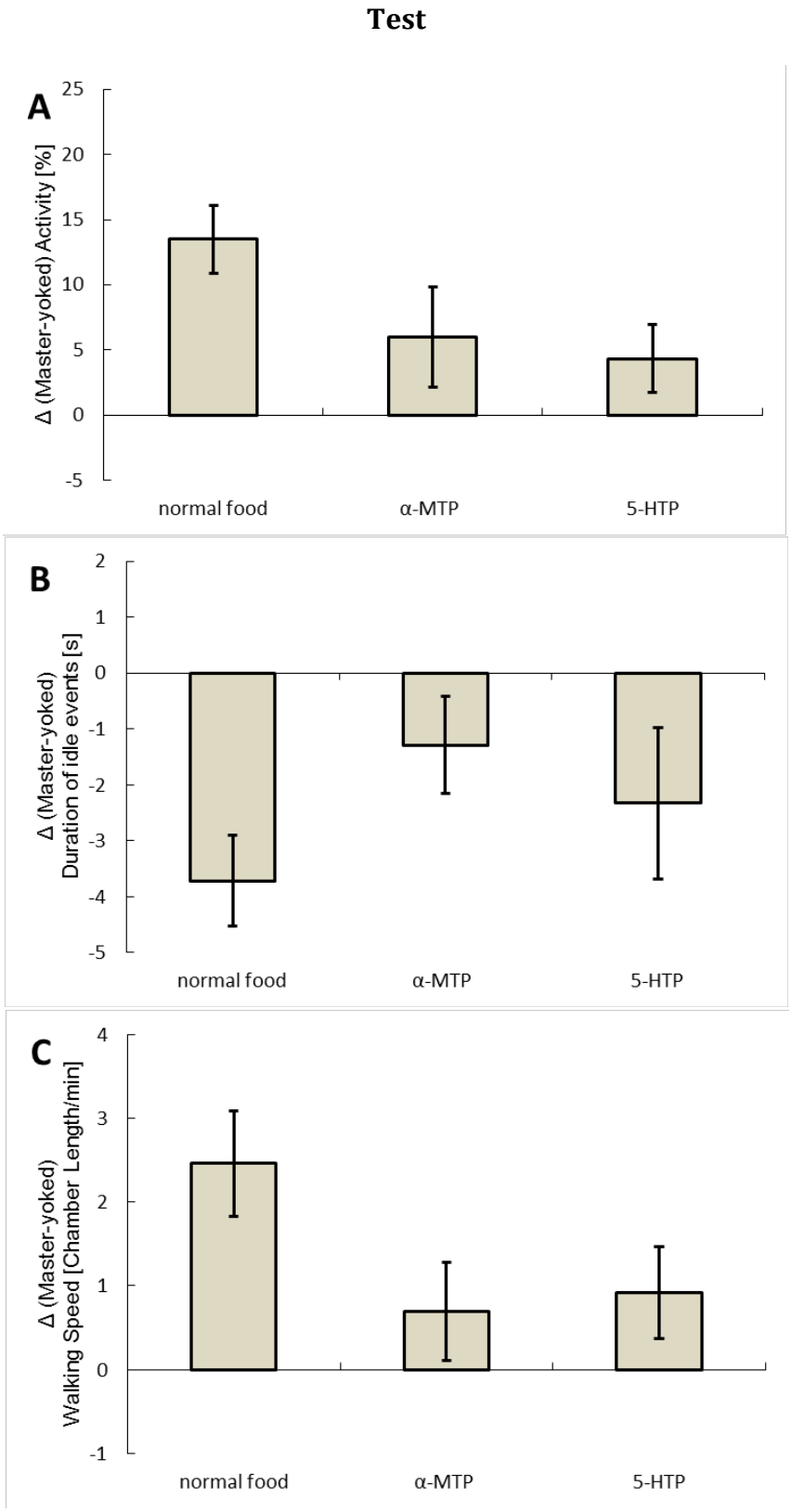


Fig. 23: Differences of activity level between female master and yoked flies in test (n=40 for control; n=40 for α-MTP; n=42 for 5-HTP). While control animals show a difference in activity at 13.4%, α-MTP and 5-HTP groups show decreased differences at 6% and 4.3%. Similar situations can be found in differences in idle durations and walking speed. The flies treated with drugs show a reduced master/yoked difference (i.e. differences for α-MTP and 5-HTP groups are not significant).

As mentioned repeatedly, the control flies showed different activity, duration of idle events and walking speed between master and yoked flies in training phase (Fig.22). The yoked flies, which had no control of their environment, walked less and slower. This is also (partly) true for female flies treated with serotonin precursor 5-HTP. Master female flies had higher activity than yoked; and they made shorter breaks but the difference in walking speed was not significant. However, those female flies, which were fed on food with serotonin inhibitor α-MTP, did not show any differentiation between master and yoked flies at all. They walked about the same time with the same speed and their idle events had about the same duration . The yoked, but not the master flies on α-MTP increased their activity during training (Fig.19).
After the training phase, masters were still more active than yoked in the control group. In the α-MTP group, as in the training phase, no significant difference between master and yoked flies could be observed, although the masters did walked a little more (Fig.23). Surprisingly, the master/yoked difference in the 5-HTP group had also disappeared in the test phase. There were differences in activity, duration of idle event and walking distance, but none of these differed significantly from zero. Therefore, a decreased serotonin level abolished the difference between master and yoked flies in both training and test phases, whereas with an increased concentration of serotonin the difference was still to be observed in the training phase but not in the test phase (Tab.1).

In the male control group, master flies were more active than yoked during training (Fig.24). Unlike the female flies, the α-MTP male flies showed a difference between master and yoked flies during training. This difference was potentially even stronger than that in the control group (difference normal food / α-MTP not significant).

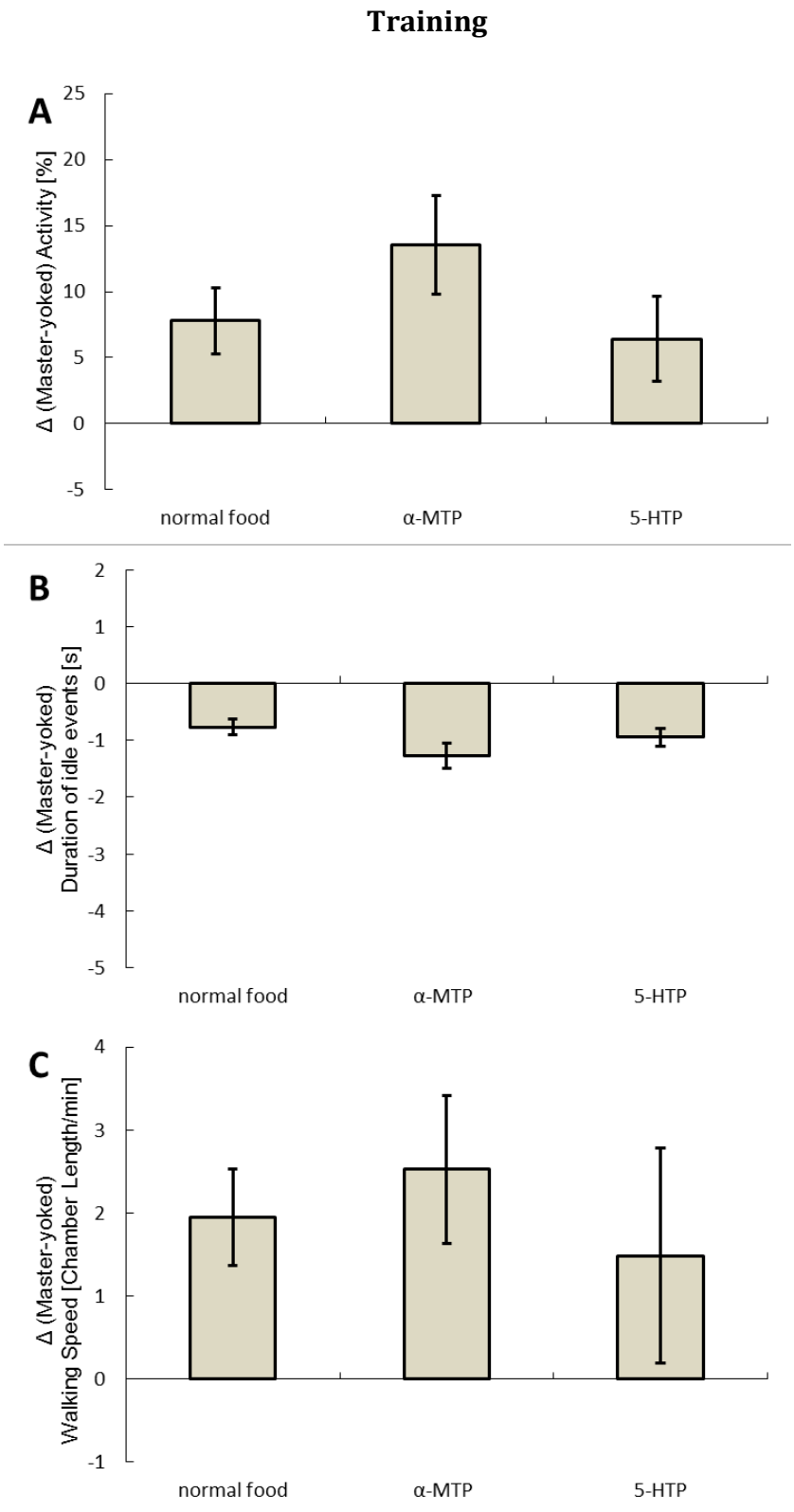


Fig.24 Differences of activity level between male master and yoked flies in training (n=30 for control; n=30 for α-MTP; n=40 for 5-HTP). Male flies still show differences between master and yoked after 4d treatment with α-MTP and 5-HTP. Changes in serotonin levels do not affect male flies very much. Only in the walking speed no significant difference can be found in 5-HTP group.

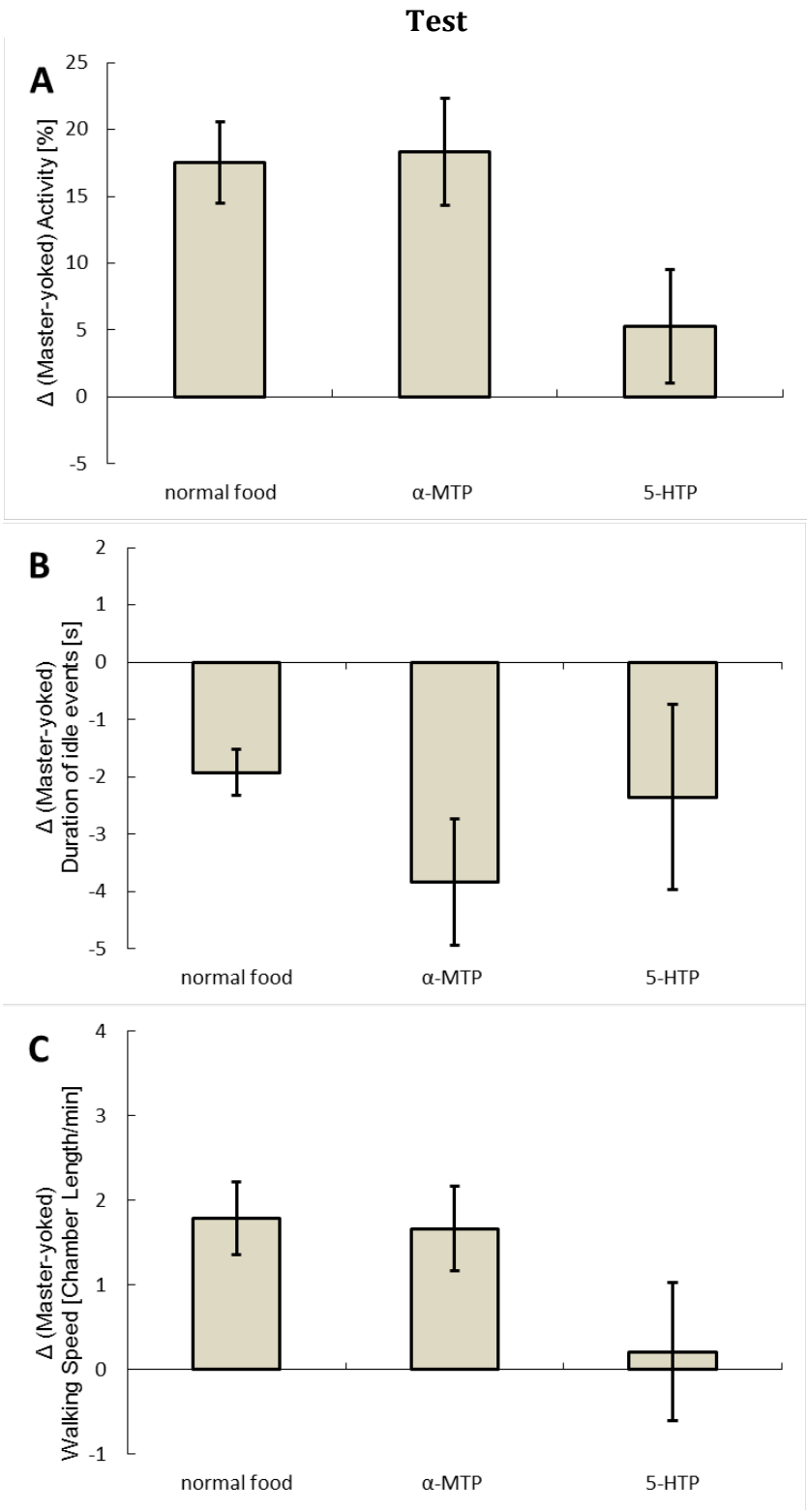


Fig. 25: Differences of activity level between male master and yoked flies in test (n=30 for control; n=30 for α-MTP; n=40 for 5-HTP). In test phase, master flies fed with α-MTP are more active than yoked flies. In duration of idle events, this difference is even larger than in control group, but not significant larger. After treatment with 5-HTP male flies show reduced differences between master and yoked flies compared to control or α-MTP group.

This effect remained after the training: α-MTP master flies walked for a longer time and longer distance than yoked in the test phase (Fig.25). They also took shorter pauses than control flies. On the other hand, flies treated with 5-HTP did show differences between master and yoked male flies, but only the difference in duration of idle events in the training phase was statistically significant. In summary for the male flies: a decreased serotonin level caused by inhibitor α-MTP did not affect the master/yoked differences; but these differences could be reduced, not abolished by an increased concentration of serotonin through 5-HTP (Tab.2).

Manipulation of serotonin had different effects on female and male, on master and yoked animals. Decreased serotonin level through α-MTP reduced the difference between master and yoked flies only in female animals, not in male. Increased serotonin level through 5-HTP led to reduction of activity only in female, not in male flies. Furthermore, change of serotonin level could affect flies differently according to experiment phase. With α-MTP, the female yoked flies increased their activity in training phase, but had the same level as control flies in test phase. Again in female flies, 5-HTP reduced the master/yoked difference only in test phase, not in training phase.

3.2.2 Genetic manipulations

Next, we manipulated the serotonin level of flies using genetic tools. Tryptophan hydroxylase is the initial and rate-limiting enzyme in the biosynthesis of serotonin. It catalyzes the hydroxylation of tryptophan to 5-hydroxyl tryptophan, which is further decarboxylated to serotonin. Flies carrying the UAS-TNT effector transgene together with the TRH-GAL4 driver are tested in the no-idleness experiment. Fig. 26 shows the differences between master and yoked flies of different genotypes. Both female and male TRH GAL4/UAS TNT flies showed significant master/yoked differences during training. Their values were at about wild type flies' levels (Fig. 26A). These results have to be taken with some reservation because in the two parental control lines these differences were small and in one case (TRH GAL4) not significant. In the test phase, the

results were inconclusive because differences in both driver- and effector-controls were not significantly different from zero.

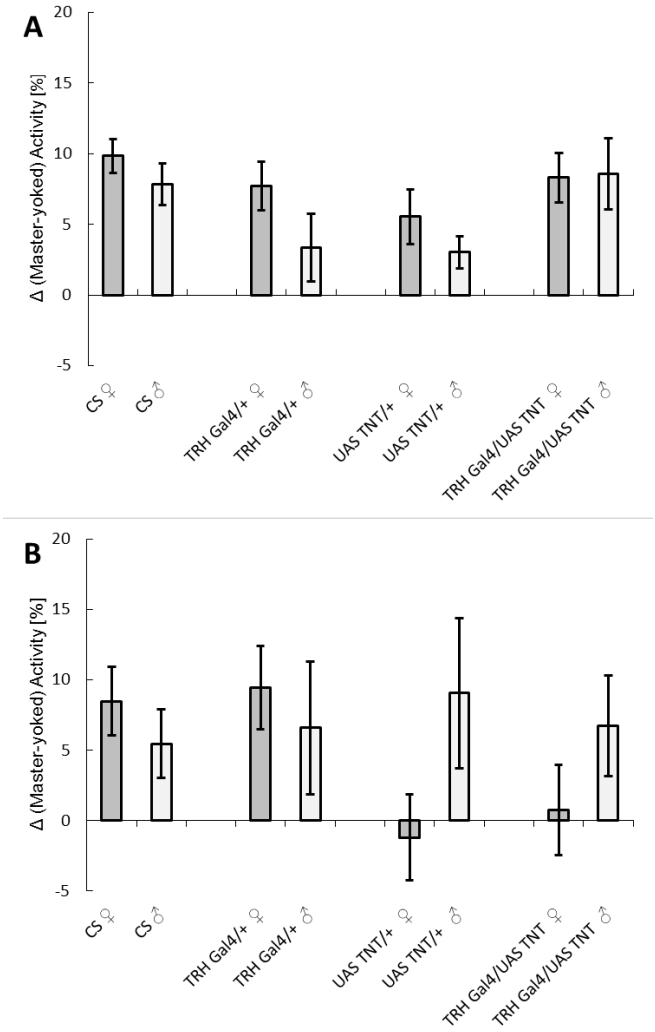


Fig.26 Female and male flies with UAS-TNT transgene expressed with TRH-GAL4 driver are together with all control groups tested in No-Idleness experiment. The differences between master and yoked flies in training and test phase are presented. Positive values represent higher activities in master than in yoked flies.

(A) Both female (n=42) and male (n=35) mutant flies show significant differences between master and yoked in training phase ($p<0.0001$ for female and $p=0.0019$ for male group). To note is here two control line for male flies: TRH-Gal4/+ (n=35) and UAS-TNT/+ (n=21) have smaller values than mutant, but not significant ($p=0.14$ and 0.1).

(B) Activities in test phase indicate sex dimorphism. The TRH-GAL4/UAS-TNT female flies do not show difference between master/yoked (Δ activity=0.75%, n=42), while male flies have a difference at control flies level (Δ activity= 6.73%, n=35), but because of bigger error bar, it doesn't reach significance ($p=0.06$).

3.3 Dopamine in learned helplessness

3.3.1 Pharmacological treatments

Another important biologic amine for many organisms is dopamine. It is involved in many biological processes. It was reported that dopamine has a role in human depression as well as in learned helplessness in rats. Here, we investigated the influence of dopamine on activity of flies in no-idleness experiment and master/yoked differences.

As described before, flies were treated with 2mM α-methyl tyrosine (α-MT) for 4 days, which reduced the concentration of dopamine. Together with untreated flies, the levels of dopamine are shown in Fig.27. Control males and females show mean dopamine levels of 21.6 pg/head and 37.1 pg/heat, respectively. After a 4d treatment, both of them decreased to about only 5 pg/head (about 23% and 13% of normal).

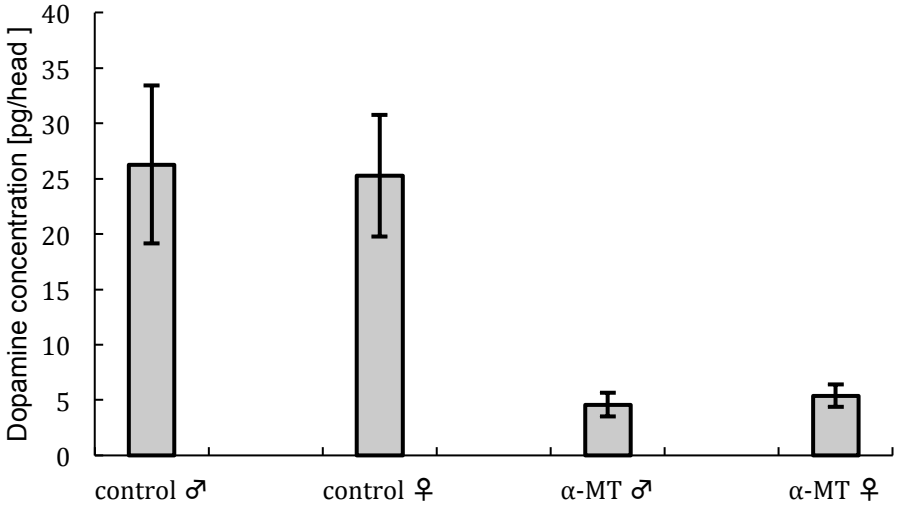


Fig.27 Dopamine concentrations after pharmacological treatment (control male: n=7; control female: n=6; α-MT male: n=10; α-MT female: n=10). Female and male flies have similar dopamine levels in their brain. These decrease significantly after 4d treatment with α-MT, a dopamine inhibitor. Not like treatment with 5-HTP for serotonin level, no difference between master and yoked flies can be observed.

Next, flies with reduced dopamine were tested in no-idleness experiment and the walking activity of female and male flies was calculated. Furthermore, it was

investigated, whether the master/yoked difference changed in comparison to control group.

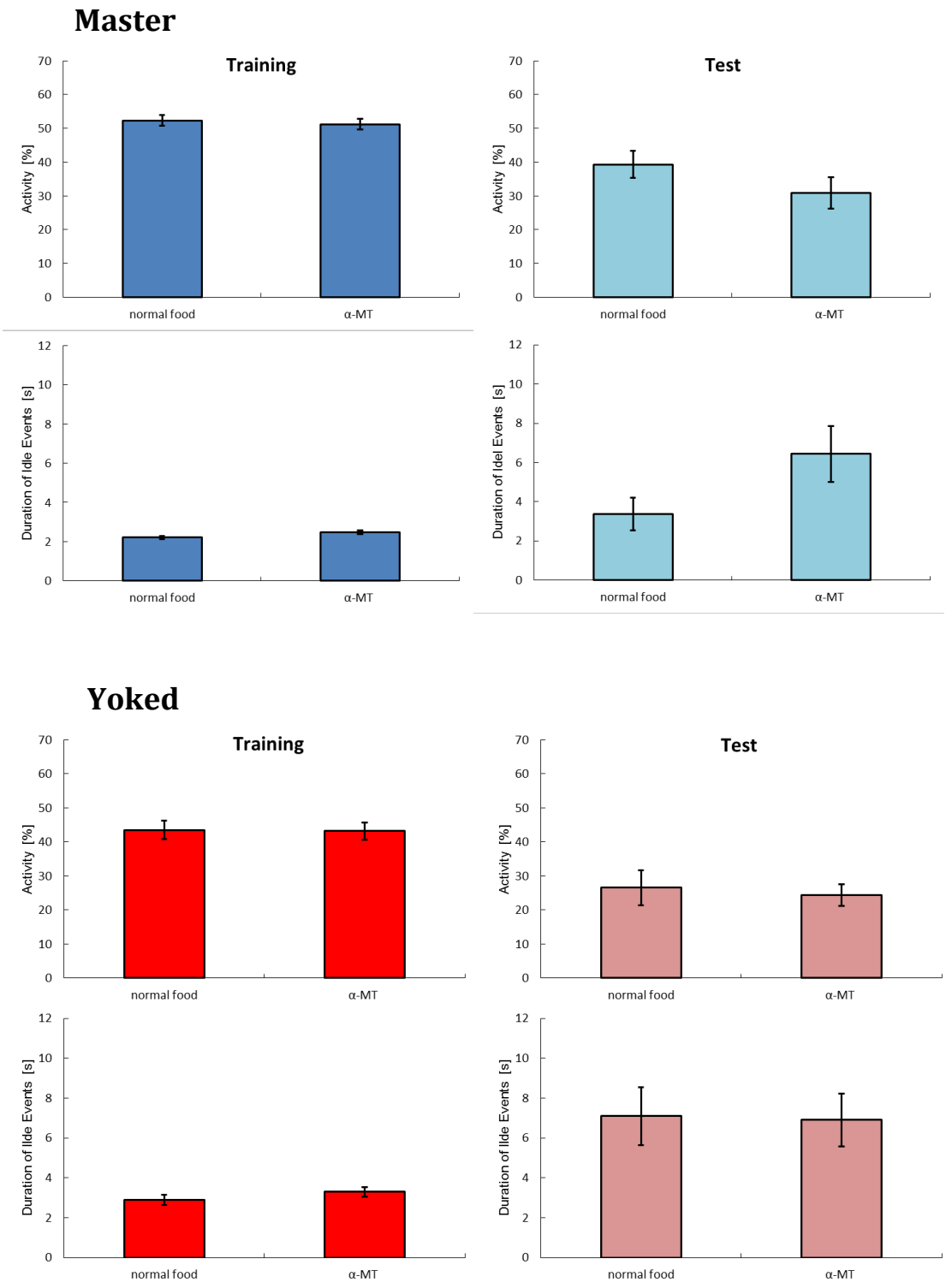


Fig.28 Activity levels of female flies after treatment with dopamine inhibitor (n=48 for control and α-MT). Both master (blue) and yoked (red) flies are not affected by α-MT. Reduced dopamine levels in their heads have not changed their activities.

Female flies with lower dopamine level did not change walking activity (Fig.28). In both master and yoked flies, there were no significant differences between α-

MT and control groups in activity and duration of idle events. The activity level of master α-MT flies was lower than the one of control master flies in test phase but this difference did not reach significance.

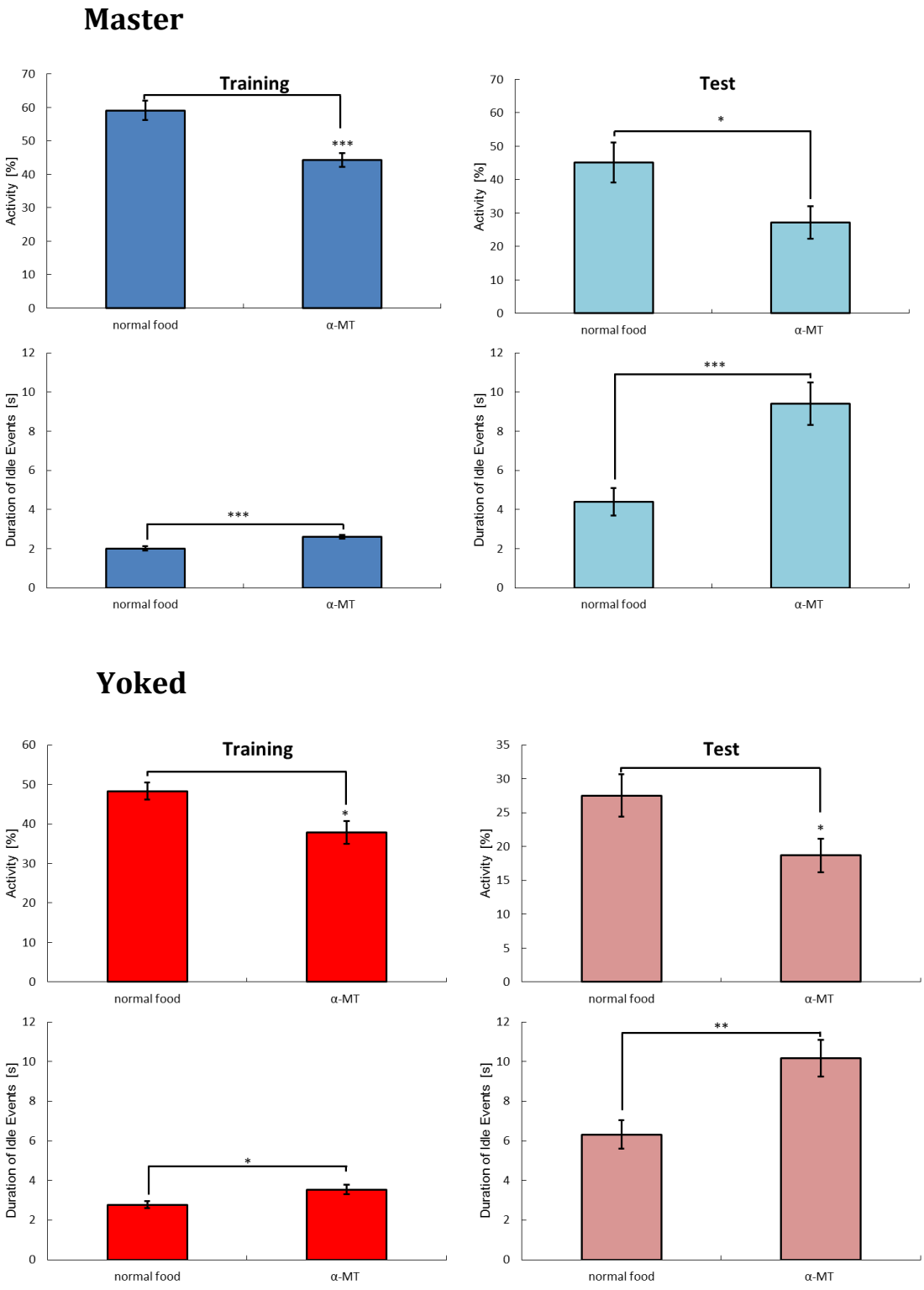


Fig.29 Activity levels of male flies after treatment with dopamine inhibitor (n=40 for control and n=36 for α-MT). Interestingly, reduced dopamine levels lead to lower activities of male flies in no-idleness experiment. Male flies treated with α-MT show shorter active time and longer idle durations in both training and test phases.

Different to female flies, the α-MT treated male flies showed decreased activity levels (Fig.29). α-MT treatment reduced activities of master flies by about 30% during training and 38% in the test phase compared to flies grown on normal food. The duration of idle events of these master flies were also significantly longer. Particularly in the test phase, master α-MT flies stopped twice as long as control flies on average. The male yoked flies in the α-MT group reduced their activities not as much as masters (20% in training, 36% in test), but they were also significantly less active compared to the yoked flies without drug.

In addition, the differences between master and yoked flies were compared. In the training phase, female master/yoked differences were similar in α-MT and control groups (Fig.30). Female masters walked more and longer than yoked, made also significantly shorter pauses. In the test phase, although master flies were ≈5% more active than yoked, this difference was not significant (Fig.30). The differences in idle durations and walking speed were also decreased. Similar results could be observed in male flies. After treatment with α-MT, differences between male master and yoked flies existed in the training but not in the test phase (Fig.31).

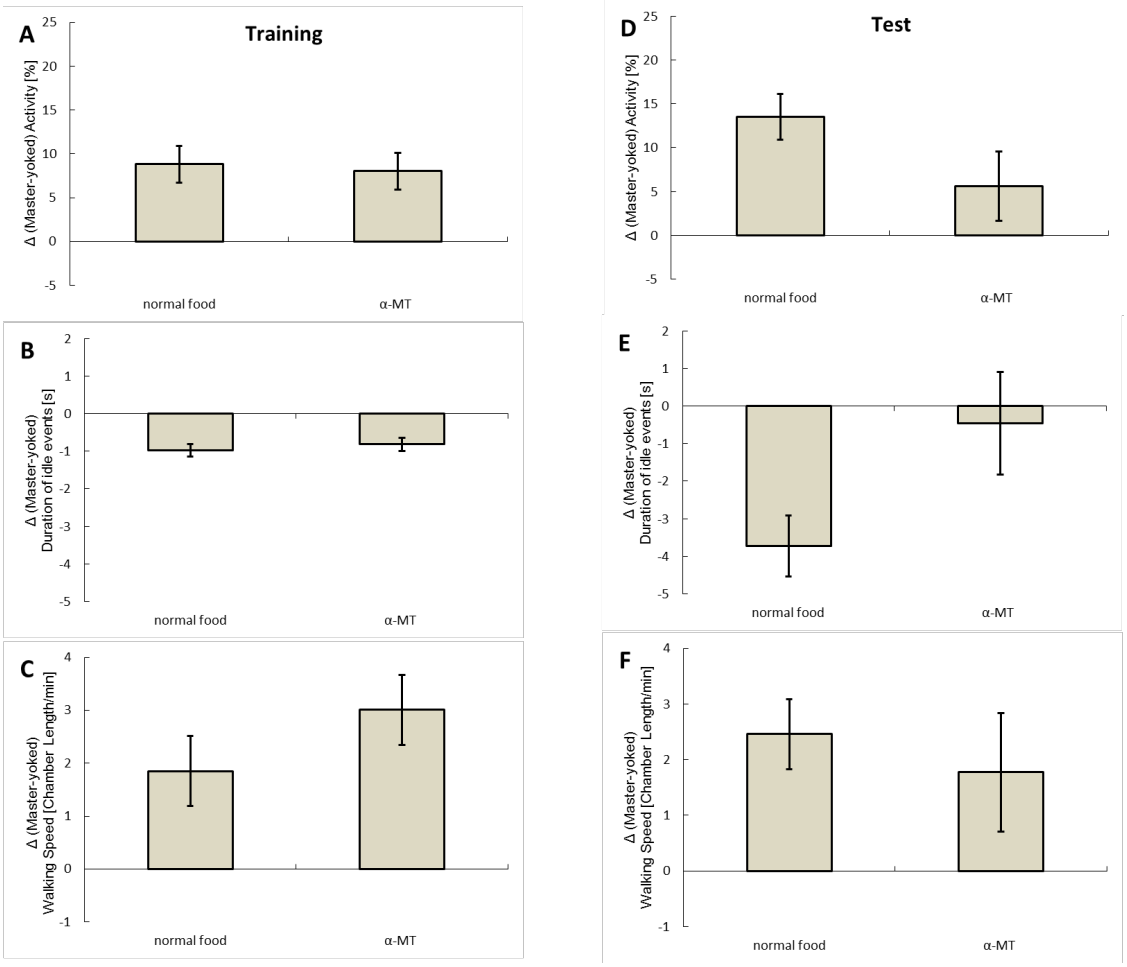


Fig. 30: Effect of α-MT on master/yoked differences in female flies (n=48 for control and α-MT). Compared to control flies, flies treated with dopamine inhibitor α-MT show similar master/yoked differences in training. but differences in the test phase are not significant in activity, duration of idle events or walking speed.

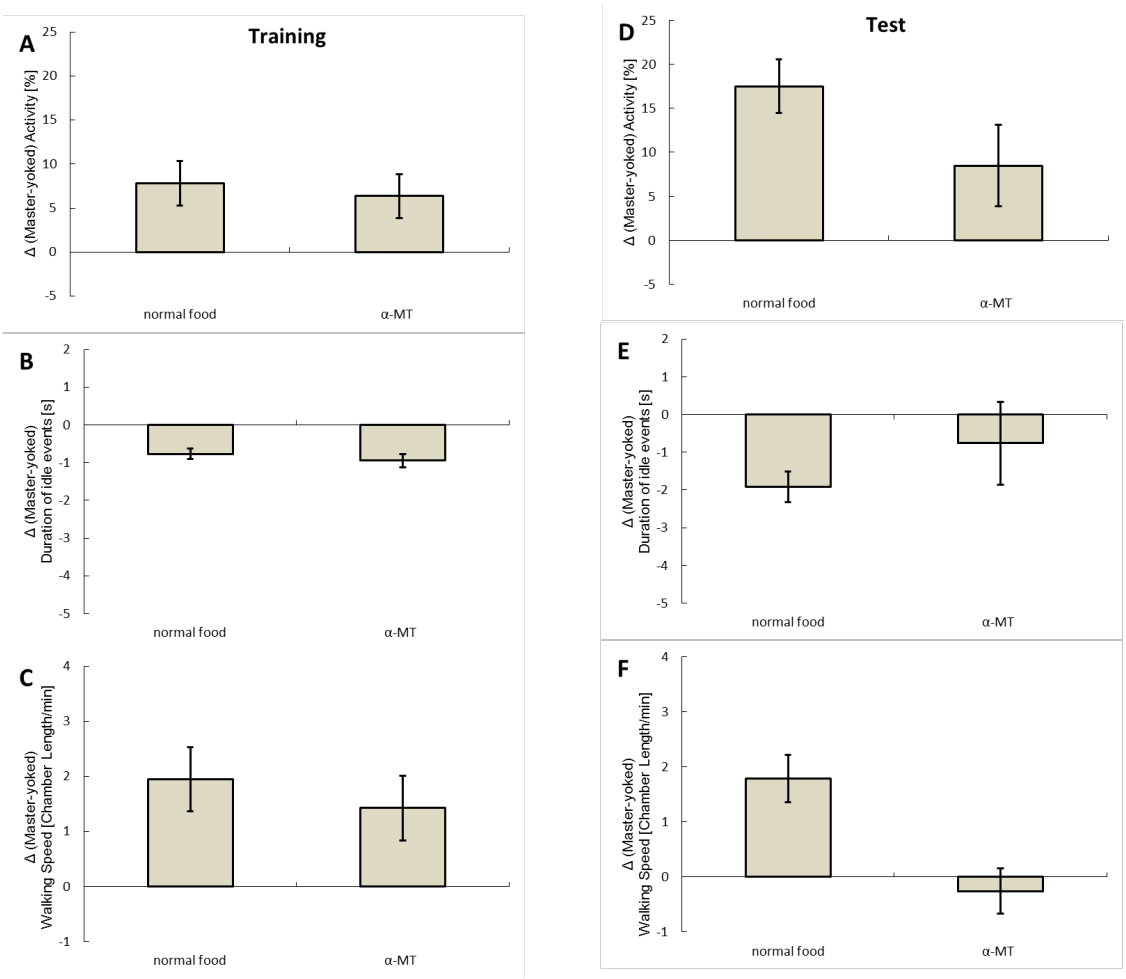


Fig.31 Differences between master and yoked in male flies. A similar situation as in female flies can be observed in males (n=40 for control and n=36 for α-MT). While the difference between master and yoked flies is not altered by the lower dopamine level during training, it is abolished in the test phase. Master flies walk only 8% more than yoked flies after treatment with α-MT.

3.3.2 Genetic manipulations

Additional support for the role of dopamine in learned helplessness can be gained from manipulating the function of the dopaminergic neural systems. Furthermore, one might learn more about the role of dopamine in the no-idleness experiment with the help of the UAS-GAL4 system. The tyrosine hydroxylase (TH)- GAL4 driver is expressed in dopaminergic neurons. The TH-GAL4 driven expression of the TeTxLC (UAS-TNT) transgene had no effect on the difference between master and yoked flies in the training phase (Fig. 32A). Both female and male masters were ≈10% more active than male yoked flies. For the

test phase the experiment was inconclusive, because not only the flies expressing TeTxLC in the TH-positive neurons had lost the master/yoked difference in activity but also the two parental lines UAS-TNT and TH-GAL4 (Fig. 32B).

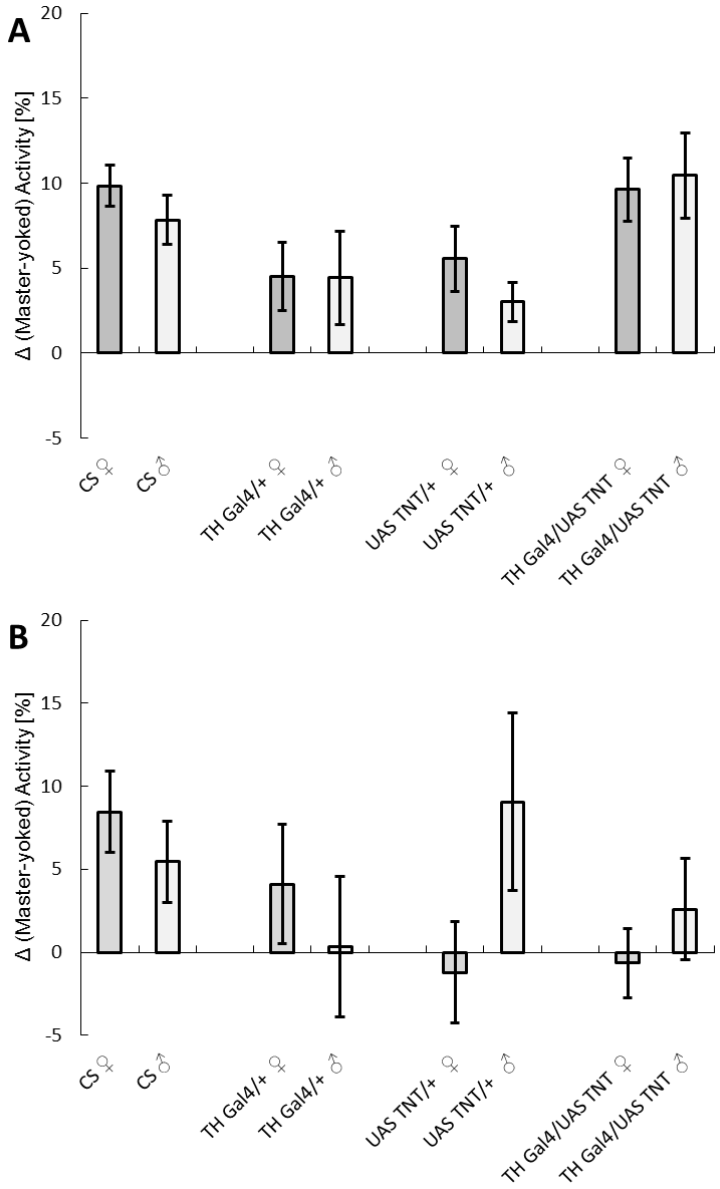


Fig.32 UAS-TNT transgene is expressed with TH-GAL4 driver in female and male flies. They are tested in No-Idleness experiment and compared with control lines. The differences between master and yoked flies in training and test phase are presented. Positive values represent higher activities in master than in yoked flies.

(A) Both female (n=49) and male (n=27) master mutant flies are more active than yoked in training phase ($p<0.0001$ for both). These differences are even larger than in control lines.

(B) In test phase, both female (n=49) and male (n=27) TH-GAL4/UAS-TNT flies do not show significant differences between master and yoked flies ($p=0.75$ for female and 0.4 for male). The values for two control lines, TH-GAL4/+ and UAS-TNT/+ are also near zero.

In summary, the results from experiments using transgenes and drug treatment experiments were consistent in that a decreased dopamine level in flies did not abolish differences between master and yoked flies in conditioning phase. Moreover, α-MT reduced the activity level in male but not female flies suggesting that dopamine has different functions in male and female flies (Tab.3).

α-MT	**Master**	**Yoked**	**M/Y difference**
female	—, —	—, —	√ , X
male	↓ , ↓	↓ , ↓	√ , X

Tab. 3: Activities of female and male master and yoked flies after treatment with dopamine inhibitor α-MT. First sign for training and second for test phase in each group. — means no significant change, ↑means increase, ↓means decrease. X indicates no difference between master and yoked flies, √indicates difference.

4. Discussion

4.1 Learned helplessness in *Drosophila melanogaster*

Learned helplessness is one of the important animal models of major depression disorder (MDD) in humans. Most studies to understand the learned helplessness phenomenon are from rats. Two studies on *Drosophila* (Brown, 1996; Bertolucci, 2008) have provided the first clues that the fruit fly with its multitude of genetic tools could help to understand learned helpless behaviors and the underlying neuronal networks.

In the present work, learned helplessness was systematically investigated in *Drosophila* for the first time. Flies were tested under various conditions in the heatbox using heat pulses and a tracking device. We analyzed the flies' walking behaviors with 10Hz recording frequency. Not only the time they spent on walking or sitting, but also their escape latencies from a heat pulse or turning-around behaviors under higher temperature could be quantified. In this way, we were able to compare master, yoked and control flies and to study several aspects of the learned helplessness phenomenon. Other advantages have also contributed to an easy and objective evaluation of flies' behaviors, for example, except for putting flies into chambers, the process of experimentation was independent of the experimenter. Moreover, up to 8 pairs of flies could be tested at the same time, which made collecting data more efficient.

In the first part of our work, we investigated the learned helplessness phenomenon in wild type flies. The classical design of learned helplessness experiments in rats involves exposing subjects to aversive stimuli in one environment and testing for aversive stimulus escape behaviors in a different environment, e.g. in the tail suspension test, forced swimming test or shuttle box. The escape behaviors and the learned uncontrollability of animals are evaluated in the second paradigm.

In our experiment, instead of giving flies a new learning task we concentrated on the conditioning phase and a short test phase after it. In this way, it was possible to observe the changes of the flies' behaviors, not just their decreased learning abilities. Yoked flies, which experienced uncontrollable heat pulses in the heatbox showed several different behaviors in comparison to master and control

flies: they walked less and slower (Fig.3 and Fig.4B), made longer stops (Fig.5); they took longer time to respond to heat (Fig.6). Moreover, frequency of turn-around behaviors of yoked flies decreased over time (Fig.7).

The typical symptoms of depression can be viewed in three disrupted states: emotional, motivational and cognitive. As one of the most used animal models, learned helplessness is said to exhibit similar changes in these three domains (Maier & Seligman, 1976). In our study with *Drosophila* changes in only two of these domains are demonstrated.

In the established view of learned helplessness, the cognitive part is the animal's reduced performance in an operant learning task. In our experiment, there was no second paradigm serving as cognitive test; both master and yoked flies stayed in the chambers facing one main task, namely how to avoid being heated. Thus, the cognitive component of learned helplessness changed its meaning here. Yoked flies learned in the aversive conditioning phase, that the environment was inescapable for them, so that we could observe already in the training phase, that yoked flies showed longer escape latencies not only compared to master flies, but also to their own responses in an earlier phase (Fig.6). The reduced frequency of turn-around behavior under shocks was another indication showing that yoked flies had learned that heat pulses were inescapable (Fig.7). Therefore, the cognitive part of learned helplessness means here, that yoked flies adapted their outcome expectations of their innate responses to heat pulses such as running and turning, to an uncontrollable environment. This led to suppression of these behaviors.

On the other hand, the motivational process was represented by the reduced walking activity of yoked flies. Although they experienced the same aversive shocks as master flies, yoked flies spent less time on walking and made longer pauses (Fig.3 and Fig.5). Their motivation to explore the environment and to escape from it decreased in comparison to master or control flies. Furthermore, this motivational state remained for a while after training. In the following test, despite the fact that all flies were facing the same external conditions, yoked flies were still less active than the others. This indicated that not heating itself led to reduced activity of yoked animals, but the uncontrollability of heat pulses.

The emotional component can not be demonstrated directly in animal studies. In rats, it is usually deduced from the observation of physical states, e.g.

decreased appetite, weight loss, sleep disturbance, increased ulceration and heart rate. No attempts have been made so far to assess such parameters in *Drosophila* learned helplessness. I think it is still too early to talk about emotion of flies. However, decreased activity and slower walking speed of yoked flies after experiencing aversive shocks give us a hint to think about it.
Learned helplessness in our study is not described as disorders, and certainly should not be considered as disease or trauma. What the yoked flies did in the chambers is, that they learned that they could do nothing about the heat pulses and as a consequence they did not try as much as master or control flies to escape. They suppressed their innate responses by reducing walking time and speed. They also suppressed their runaway/escape behaviors to heat shocks. Such adjustments have evolutionary significance: flies try to optimize the balance between enduring stressful environment and saving energy to escape from it later.

For a better understanding of the learned helplessness effect in flies it is important to know, how critically it depends upon the intensities and durations of the stimuli used. Learned helplessness studies with rats have shown that the extent to which an initial treatment leads to a generalized helplessness is very likely to depend on the severity of the aversive stimulation. We have also treated flies with different durations of the training phase in order to answer such questions. The results suggest that neither too short nor too long a conditioning phase would make flies more helpless in our paradigm (Fig.13-15). In a short training, like 5 or 7 minutes, there was not enough time or numbers of uncontrollable shocks presented to yoked flies, so that they didn't have enough opportunities to learn to be helpless. On the other hand the more severe the aversive stimuli were for the yoked group, then the more severe they were for the master group also. This might lead to stronger helplessness of yoked flies, but might also affect the master group. In our experiments, master flies even showed a lower activity level than yoked flies when they were trained for 20 minutes (Fig.13-15). In this case, we assume that it was the aversive stimulus per se but not its uncontrollability that had more effect on the experimental animals.
Not only longer durations of training could influence the learned helplessness effect, but also a repetition of the experiments. In one of our experiments, flies were trained three times in a day. They also didn't show an enhanced learned

helplessness effect (Fig.11-12). To our surprise, yoked flies became more active than masters after the first no-idleness experiment. They walked more and faster in the pretest phase of the 2nd and 3rd experiments (Fig.10). The disappearance of the differences between master and yoked flies might be explained by the increased activity of yoked flies, as they walked significantly more in the 2nd and 3rd training than they did in the first (Fig.11).

It is difficult to explain, why yoked animal became more active when they were put into chambers again after experiencing the training and test phases. In the first experiment, yoked flies showed activity at 34.4% in the last 30s test. After two hours rest in the food vial, they showed activity at 80.9% in the pretest of the second experiment, which was a 135% increase, while increase in master flies was only 78%. A similar effect happened in the last pretest: yoked and master flies had 97% and 59% increases of activity after the second pause. One possible reason is that the arousal of flies in the dark, narrow chambers is more pronounced after experiencing uncontrollable heat pulses. However, if this is true, it means that the learned helplessness effect last only a very short period in flies that are transferred back to their normal environment. Thus, more experiments must be done in order to understand this effect better.

4.1.1 Sexual differences

As mentioned in previous chapters, major depression is twice as common in women as in men. Moreover, gender differences are common in antidepressant responses. Whole brain serotonin synthesis and 5-HT_2 receptor binding capacity were found to be decreased in several brain regions of women compared to men. Furthermore, women may respond better to selective serotonin reuptake inhibitors (SSRIs). Not surprisingly, such sex dimorphisms have also been reported in animal models. Female rats spend more time immobile than male rats during the second session of the forced swim test (FST) after exposure to chronic mild stress (CMS). On the other hand, other studies have showed male rats do not learn to escape when tested under the same conditions in a learned helplessness experiment (Shors, 2007; Dalla, 2008).

What makes our study particular interesting is that we have observed sex dimorphisms also for learned helplessness in *Drosophila* (Fig.8). Female flies

show larger differences between master and yoked groups than males. Male yoked flies are more active than female yoked flies, and the difference to male masters is smaller. For walking speed, no significant difference is found between male master and yoked flies. However, there are many essential questions in order to have clearer understanding of sex dimorphisms in flies, e.g. to what extent are the sex dimorphisms. What are the reasons for such dimorphisms? Do the differences in serotonergic and dopaminergic nervous systems in female and male flies play a role? To answer these questions, more work need be done. In my opinion, feminization and masculinization of flies using genetic tools could be a good start for investigating sex dimorphisms in learned helplessness in flies.

4.2 Monoamines in learned helplessness

Serotonin and dopamine play crucial rolls in human depression. Also in animal models of depression, they were found to be important. Evidence suggests an important role for serotonergic neurons in the dorsal raphe nucleus in mediating learned helplessness (Maier and Watkins, 2005). Decreased serotonergic activity has also been reported in the hippocampus and hypothalamus of rats in forced swim test. One study has shown that manipulations increasing central 5-HT or activity of 5-HT neurons, in the absence of stress, are sufficient to produce behaviors resembling those produced by uncontrollable stress (Brown *et al.* 1982).

Our study shows that both serotonin and dopamine can affect learned helplessness in *Drosophila*. Female flies treated with serotonin inhibitor α-MTP do not show significant master/yoked differences, neither in training nor in the test phase (Fig.22-23). However, reducing serotonin by genetic manipulations does not lead to a suppression of the learned helplessness effect during training (Fig.26). This suggests serotonin is crucial for female flies learning to be helpless after experiencing uncontrollable stimuli, but might not necessary for them to behave helplessly under such stimuli. Surprisingly, the learned helplessness effect in male flies seems not to be affected by a reduction of serotonin, neither via drug treatment nor by genetic manipulation (Fig.24-26). Both leave the master / yoked differences unaffected. This reminds of sex dimorphisms in learned helplessness of other animals. It is reported, for instance, that the

decrease of activity in serotonergic neurons in rats is different in females and males (Drossopoulou, 2004). Possibly with a reduced serotonin level only males but not females might still be able to deal with uncontrollable aversive stimuli.

Increasing evidence from human and animal studies suggest a relationship between dopamine transmission in the central nervous system and depression (D'haenen, 1994; Laasonen-Balk, 1999; Lambert, 2000; McLean, 2004). Moreover, the relationship between dopamine and depression was confirmed by the fact that antidepressants act on the dopamine system. In the frontal cortex of rats, antidepressants such as desipramine, a potent inhibitor of the noradrenaline reuptake carrier, increases extracellular concentrations of dopamine by preventing the dopamine reuptake into noradrenergic neurons (Carboni, 1990; Pozzi, 1994). Fluoxetine, a selective serotonin re-uptake inhibitor also increases the extracellular dopamine concentration in the prefrontal cortex by a mechanism not dependent on serotonin (Pozzi, 1999).
As shown in our drug treatment experiment, flies with lower dopamine level do not display the learned helplessness effect in the test phase (Fig.30-32). This result suggests that with low dopamine the motivational change in learned helplessness in *Drosophila* may decline faster than with a normal dopamine level. Another interesting finding in this study is the effect of serotonin on flies' locomotion. As already reported in early studies, serotonin plays an important role in the regulation of locomotion (Segalat *et al.*, 1995; Lundell and Hirsh, 1994). In our study, elevated serotonin markedly reduces the activity of female flies (Fig.18-19). Such a decrease can only be observed in females, not in males, suggesting a sexual dimorphism of serotonin function in locomotion. We cannot rule out, however, that it is because of the different concentrations of serotonin in the brain (Fig.17), since after pharmacological treatment the level of serotonin was much higher in females than in males.

References

Adams MD. *et al.* (2000). The genome sequence of *Drosophila melanogaster*. Science. 24: 287(5461):2185-95.

Alekseyenko OV., Lee C., Kravitz EA. (2010). Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male *Drosophila melanogaster*. PLoS One 5, e10806.

Arnold AP. (2004). Sex chromosomes and brain gender. Nat Rev Neurosci. 5: 701–708.

Arnsten AF. (2007) Catecholamine and second messenger influences on prefrontal cortical networks of "representational knowledge": a rational bridge between genetics and the symptoms of mental illness. Cereb Cortex. 17(Suppl 1): i6–i15.

Barr CS., Newman TK., Schwandt M., Shannon C., Dvoskin RL., Lindell SG. *et al.* (2004a) Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. Proc Natl Acad Sci USA. 101: 12358–12363.

Barr CS., Newman TK., Shannon C., Parker C., Dvoskin RL., Becker ML. *et al.* (2004b) Rearing condition and rh5-HTTLPR interact to influence limbic–hypothalamic–pituitary–adrenal axis response to stress in infant macaques. Biol Psychiatry. 55: 733–738.

Bertolucci F. (2008) Operant and classical learning in *Drosophila melanogaster*: the *ignorant* gene (*ign*). Doctoral thesis.

Brand AH., Perrimon N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 118(2): 401-15.

Brown GE., Mitchell AL., Peercy AM., Robertson CL. (1996) Learned helplessness in *Drosophila melanogaster*? *Psychol. Rep.* 78: 962

Brown GE., Stroup K. (1988) Learned helplessness in the cockroach (*Periplaneta anmericana*). *Behav. Neural. Biol.* 50: 246-250

Caldarone BJ., George TP., Zachariou V., Picciotto MR. (2000) Gender differences in learned helplessness behavior are influenced by genetic background. Pharmacol Biochem Behav. 66: 811–817. doi: 10.1016/S0091-3057(00)00271-9.

Carboni E., Tanda GL., Frau R., Di Chiara G. (1990) Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. J. Neurochem. 55: 1067–1070.

Cervo L., Grignaschi G., Samanin R. (1990) The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. Eur. J. Pharmacol. 178: 129–133.

Chase DL., Koelle MR. (2007) Biogenic amine neurotransmitters in *C. elegans*. WormBook, Pasadena, 1–15

Chen TY., Duh SL., Huang CC., Lin TB. & Kuo DY. (2001) Evidence for the involvement of dopamine D1 and D2 receptors in mediating the decrease of food intake during repeated treatment with amphetamine. J Biomed Sci. 8: 462–466.

Crocker A., Sehgal A.. (2010) Genetic analysis of sleep. Genes Dev. 24: 1220–1235

Dalla C., Antoniou K., Drossopoulou G., Xagoraris M., Kokras., Sfikakis A. *et al.* (2005) Chronic mild stress impact: are females more vulnerable? Neuroscience. 135: 703–14.

Dalla C., Antoniou K., Kokras N., Drossopoulou G., Papathanasiou G., Bekris S. *et al.* (2008) Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. Physiol Behav. 93: 595–605.

Dalla C., Edgecomb C., Abigail S., Whetstone AS., Shors TJ. (2008) *Neuropsychopharmacology.* 33: 1559–1569

Davies W, Wilkinson LS. (2006) It is not all hormones: alternative explanations for sexual differentiation of the brain. Brain Res. 1126: 36–45.

Dasari S., Cooper R.L. (2006) Direct influence of serotonin on the larval heart of Drosophila melanogaster. Journal of Comparative Physiology [B]. 176: 349–357

Francis DD., Zaharia MD., Shanks N., Anisman H. (1995) Stress-induced disturbances in Morris water-maze performance: interstrain variability. Physiol Behav. 58(1): 57-65

Demchyshyn LL., Pristupa ZB. *et al.* (1994) Cloning, expression, and localization of a chloride-facilitated, cocaine-sensitive serotonin transporter from Drosophila melanogaster. Proc Natl Acad Sci USA. 91(11):5158–516

D'haenen H.A., Bossuyt A. (1994) Dopamine D2 receptors in depression measured with single photon emission computed tomography. Biol. Psychiatry. 35: 128–132

Dickson BJ. (2008) Wired for sex: the neurobiology of Drosophila mating decisions. Science. 322: 904–909

Dierick H.A., Greenspan R.J. (2007) Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. Nature Genetics. 39: 678–682

Divya Sitaraman D., Melissa Zars M., Holly LaFerriere H., Yin-Chieh Chen Y.C., Alex Sable-Smith A., Toshihiro Kitamoto T., George E. Rottinghaus GF., Zars, T. (2008) Serotonin is necessary for place memory in *Drosophila*. *PNAS.* 105: 5579-5584

Drossopoulou G., Antoniou K., Kitraki E, Papathanasiou G, Papalexi E, Dalla C *et al.* (2004) Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. Neuroscience.126: 849–57.

Duman RS. (2004) Role of neurotrophic factors in the etiology and treatment of mood disorders. Neuromol. Med. 5: 11–26.

Durlach-Misteli C., Van Ree JM. (1992) Dopamine and melatonin in the nucleus accumbens may be implicated in the mode of action of antidepressant drugs. Eur. J. Pharmacol. 217: 15–21.

Edwards E., Harkins K., Wright G., Henn FA. (1991) 5-HT1b receptors in an animal model of depression. Neuropharmacology. 30:101–105

Edwards E., Kornich W., Houtten PV., Henn FA. (1992) Presynaptic serotonin mechanisms in rats subjected to inescapable shock. Neuropharmacology. 31: 323–330

Fahr, S., Jankovic, J., and Hallett, M. (2011) Principles and Practice of movement Disorders. Elsevier, Philadelphia.

Padilla AM, Padilla C., Ketterer T., Giacalone D. (1970) Inescapable shocks subsequent escape an avoidance conditioning in goldfish, *Carassius aurotus*. Psychonomic Science. 20: 295-296.

Greer CL., Grygoruk A. *et al.* (2005) A splice variant of the Drosophila vesicular monoamine transporter contains a conserved trafficking domain and functions in the storage of dopamine, serotonin, and octopamine. J Neurobiol. 64(3): 239–258

Hildebrandt MG., Steyerberg EW., Stage KB., Passchier J., Kragh-Soerensen P. (2003) Are gender differences important for the clinical effects of antidepressants? Am J Psychiatry. 160: 1643–50.

Horvitz HR., Chalfie M. *et al.* (1982) Serotonin and octopamine in thenematode *Caenorhabditis elegans*. Science. 216(4549): 1012– 1014

Johnson O., Becnel J., Nichols C.D. (2009) Serotonin 5-HT2 and 5-HT1A-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. Neuroscience. 158: 1292–1300

Jones BJ., Blackburn TP., (2002) The medical benefit of 5-HT research. Pharmacology Biochemistry and Behavior. 71: 555-568.

Kandel ER, Schwartz JH *et al.* (2000) Principles of neuroscience. McGraw-Hill. New York

Kaplan DD., Zimmermann G., Suyama K., Meyer T., Scott MP. (2008) A nucleostemin family GTPase, NS3, acts in serotonergic neurons to regulate insulin signaling and control body size. Genes and Development. 22: 1877-1893.

Kornstein SG., Schatzberg AF., Thase ME., Yonkers KA., McCul- lough JP., Keitner GI. *et al.* (2000) Gender differences in treatment response to sertraline versus imipramine in chronic depression. Am J Psychiatry.157: 1445–52.

Laasonen-Balk T., Kuikka J., Viinamaki H., Husso-Saastamoinen M., Lehtonen J., Tiihonen J. (1999) Striatal dopamine transporter density in major depression. Psychopharmacology (Berl). 144: 282–285.

Lambert G., Johansson M., Agren H., Friberg P. (2000) Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. Arch. Gen. Psychiatry. 57: 787–793.

Reiter L., Potocki L., Chien S., Gribskov M., Bier E. (2001) A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. Genome Res. 11(6): 1114-25.

Lee G., Villella A., Taylor BJ., Hall JC. (2001) New reproductive anomalies in fruitless-mutant *Drosophila* males: extreme lengthening of mating durations and infertility correlated with defective serotonergic innervation of reproductive organs. Journal of Neurobiology. 47: 121–149

Lundell MJ., Hirsh J. (1994) Temporal and spatial development of serotonin and dopamine neurons in the Drosophila CNS. Dev Biol. 165(2): 385–396

Maier, SF. (1984) Learned helplessness and animal models of depression. *Neuro-Psychopharmacol. & Biol. Psychiat.* 8: 435-446

Maier SF., & Watkins LR. (2005) Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev. 29: 829–841.

Mao Z. and Davis R.L. (2009) Eight different types of dopaminergic neurons innervate the Drosophila mushroom body neuropil: anatomical and physiological heterogeneity. Front. Neural. Circuits 3: 5.

Marcus SM., Young EA., Kerber KB., Kornstein S., Farabaugh AH., Mitchell J. *et al.* (2005) Gender differences in depression: findings from the STAR*D study. J Affect Disord. 87: 141–50.

McGuire SE., Deshazer M., Davis RL. (2005) Thirty years of olfactory learning and memory research in Drosophila melanogaster. Prog Neurobiol. 76: 328–347

McLean A., Rubinsztein J.S., Robbins T.W., Sahakian B.J. (2004) The effects of tyrosine depletion in normal healthy volunteers: implications for unipolar depression. Psychopharmacology (Berl). 171: 286–297.

Melcher C., Bader R., Pankratz MJ. (2007) Amino acids, taste circuits, and feeding behavior in *Drosophila*: towards understanding the psychology of feeding in flies and man. J Endocrinol. 192: 467–472

Monastirioti M. (1999) Biogenic amine systems in the fruit fly *Drosophila melanogaster*. Microscopy Research and Technique. 45: 106-121.

Neckameyer W.S., Coleman C.M., Eadie S., Goodwin SF. (2007) Compartmentalization of neuronal and peripheral serotonin synthesis in *Drosophila melanogaster*. Genes. Brain and Behavior. 6: 756-769.

Neckameyer WS., White K. (1992) A single locus encodes both phenylalanine hydroxylase and tryptophan hydroxylase activities in *Drosophila*. Journal of Biological Chemistry. 267: 4199-4206.

Nitabach MN., Taghert PH. (2008) Organization of the Drosophila circadian control circuit. Curr Biol. 18: R84—37215–R93

Overmier JB., Seligman MEP. (1967) Effects of inescapable shock upon subsequent escape and avoidance responding. *J. Comp. Physiol. Psychol.* 63: 28-33

Page ME., Detke MJ., Dalvi A., Kirby LG., Lucki I. (1999) Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology (Berl). 147: 162–167.

Papp M., Klimek V., Willner P. Parallel. (1994) Changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine. Psychopharmacology (Berl). 115: 441–446.

Peabody NC., Pohl JB., Diao F., Vreede AP., Sandstrom DJ., Wang H., Zelensky PK., White BH. (2009) Characterization of the decision network for wing expansion in *Drosophila* using targeted expression of the TRPM8 channel. J Neurosci. 29: 3343–3353

Plaznik A., Kostowski W. (1987) The effects of antidepressants and electroconvulsive shocks on the functioning of the mesolimbic dopaminergic system: a behavioral study. Eur. J. Pharmacol. 135: 389–396.

Pozzi L., Invernizzi R., Garavaglia C., Samanin R. (1999) Fluoxetine increases extracellular dopamine in the prefrontal cortex by a mechanism not dependent on serotonin: a comparison with citalopram. J. Neurochem. 73: 1051–1057.

Pozzi L., Invernizzi R., Cervo L., Vallebuona F., Samanin R. (1994) Evidence that extracellular concentrations of dopamine are regulated by noradrenergic neurons in the frontal cortex of rats. J. Neurochem. 63: 195–200.

Prince CR., Anisman H. (1984) Acute and chronic stress effects on performance in a forced-swim task. Behav Neural Biol. 42(2): 99–119. doi: 10.1016/s0163-1047(84)90942-7

Python F., Stocker R.F. (2002) Immunoreactivity against choline acetyltransferase, gamma-aminobutyric acid, histamine, octopamine, and serotonin in the larval chemosensory system of *Dosophila melanogaster*. Journal of Comparative Neurology. 453: 157–167

Renard CE., Fiocco AJ., Clenet F., Hascoet M., Bourin M. (2001) Is dopamine implicated in the antidepressant-like effects of selective serotonin reuptake inhibitors in the mouse forced swimming test? Psychopharmacology (Berl). 159: 42–50

Rodriguez Moncalvo VG., Campos AR. (2009) Role of serotonergic neurons in the Drosophila larval response to light. BMC Neuroscience. 10: 66

Roesch MR., Calu DJ., Schoenbaum G. (2007). Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. Nat Neurosci. 10: 1615–1624

Rubinow DR., Schmidt PJ., Roca CA. (1998) Estrogen-serotonin interactions: implications for affective regulation. Biol Psychiatry. 44: 839–50.

Shors TJ., Mathew J., Sisti HM., Edgecomb C., Beckoff S., Dalla C. (2007) Neurogenesis and helplessness are mediated by controllability in males but not in females. Biol Psychiatry. 62: 487–95.

Segalat L., Elkes DA. *et al.* (1995) Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. Science. 267(5204):1648–1651

Selcho, M., Pauls, D., Han, K.a., Stocker, R.F., and Thum, A.S. (2009) The role of dopamine in *Drosophila* larval classical olfactory conditioning. PLoS ONE. 4: e5897.

Seligman MEP., and Maier SF. (1967) Failure to escape traumatic shock. J. Exp. Psychol. 74: 1-9

Sitaraman D., Zars M., Laferriere H., Chen Y.C., Sable-Smith A., Kitamoto T., Rottinghaus G.E., Zars T. (2008) Serotonin is necessary for place memory in Drosophila. Proceedings of the National Academy of Sciences USA. 105: 5579-5584.

Sjoberg RL., Nilsson KW., Nordquist N., Ohrvik J., Leppert J., Lindstrom L. *et al.* (2006) Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. Int J Neuropsychopharmacol. 9: 443–449.

Vallés AM., White, K., (1988) Serotonin-containing neurons in *Drosophila melanogaster*: development and distribution. Journal of Comparative Neurology. 268: 414-428.

Villella A., Hall JC. (2008) Neurogenetics of courtship and mating in *Drosophila*. Adv Genet. 62: 67-184.

Vollmayr B., Henn FA. (2001) Learned helplessness in the rat: improvements in validity and reliability. Brain Res Brain Res Protoc. Aug; 8(1): 1-7.

Weiger WA., (1997) Serotonergic modulation of behaviour: a phylogenetic overview. Biological Reviews. 72: 61-95.

Weiss LA., Abney M., Cook Jr EH., Ober C. (2005) Sex-specific genetic architecture of whole blood serotonin levels. Am J Hum Genet. 76: 33–41.

Wu J., Kramer G L., Kram M., Steciuk M., Crawford IL., Petty F. (1999) Serotonin and learned helplessness: a regional study of 5-HT-1A, 5-HT-2A receptors and the serotonin transport site in rat brain. J Psychiatr Res. 33:17–22

Zacharko RM., Bowers WJ., Kokkinidis L., Anisman H. (1983) Region-specific reductions of intracranial self-stimulation after uncontrollable stress: possible effects on reward processes. Behav Brain Res. 9(2): 129–141. doi: 10.1016/0166-4328(83)90123-7

Zornik E., Paisley K., Nichols R. (1999) Neural transmitters and a peptide modulate *Drosophila* heart rate. Peptides. 20: 45–51

Summary

The learned helplessness phenomenon is a specific animal behavior induced by prior exposure to uncontrollable aversive stimuli. It was first found by Seligman and Maier (1967) in dogs and then has been reported in many other species, e.g. in rats (Vollmayr and Henn, 2001), in goldfishes (Padilla, 1970), in cockroaches (Brown, 1988) and also in fruit flies (Brown, 1996; Bertolucci, 2008). However, the learned helplessness effect in fruit flies (*Drosophila melanogaster*) has not been studied in detail. Thus, in this doctoral study, we investigated systematically learned helplessness behavior of *Drosophila* for the first time.

Three groups of flies were tested in heatbox. Control group was in the chambers experiencing constant, mild temperature. Second group, master flies were punished in their chambers by being heated if they stopped walking for 0.9s. The heat pulses ended as soon as they resumed walking again. A third group, the yoked fly, was in their chambers at the same time. However, their behavior didn't affect anything: yoked flies were heated whenever master flies did, with same timing and durations. After certain amount of heating events, yoked flies associated their own behavior with the uncontrollability of the environment. They suppressed their innate responses such as reducing their walking time and walking speed; making longer escape latencies and less turning around behavior under heat pulses. Even after the conditioning phase, yoked flies showed lower activity level than master and control flies. Interestingly, we have also observed sex dimorphisms in flies. Male flies expressed learned helplessness not like female flies. Differences between master and yoked flies were smaller in male than in female flies. Another interesting finding was that prolonged or even repetition of training phases didn't enhance learned helplessness effect in flies.

Furthermore, we investigated serotonergic and dopaminergic nervous systems in learned helplessness. Using genetic and pharmacological manipulations, we altered the levels of serotonin and dopamine in flies' central nervous system. Female flies with reduced serotonin concentration didn't show helpless behavior, while the learned helplessness effect in male flies seems not to be affected by a reduction of serotonin. Flies with lower dopamine level do not display the learned helplessness effect in the test phase, suggesting that with low dopamine

the motivational change in learned helplessness in *Drosophila* may decline faster than with a normal dopamine level.

Zusammenfassung

Das „learned helplessness“ Phänomen ist ein spezifisches Verhalten nach vorheriger Exposition von unkontrollierbaren aversiven Stimuli induziert. Es wurde zuerst von Seligman und Maier (1967) bei Hunden und dann in vielen anderen Tierarten berichtet, z.B. in Ratten (Vollmayr und Henn, 2001), in Goldfische (Padilla , 1970), in Kakerlaken (Brown, 1988) sowie in Fruchtfliegen (Brown, 1996; Bertolucci, 2008). Allerdings wurde das learned helplessness Phänomen in Fruchtfliegen (*Drosophila melanogaster*) noch nicht genau erforscht. Somit wird in dieser Doktorarbeit haben wir erlernten learned helplessness von Drosophila zum ersten Mal systematisch untersucht.

Drei Gruppen von Fliegen wurden in Heatbox getestet. Die Kontrollgruppe war in den Kammern erlebter konstant milder Temperatur. Die zweite Master Gruppe wurde in ihren Kammern erhitzt, wenn sie blieb stehen für 0,9 s. Die Hitze endete, sobald sie sich wieder bewegten. Eine dritte Gruppe, die Yoked Fliegen, war in ihren Kammern gleichzeitig. Doch ihr Verhalten keine Auswirkungen auf die Hitze hatte: Yoked Fliegen wurden erhitzt, wenn Master Fliegen wurden, mit gleichen Zeitpunkt und Dauer. Nach gewissen Hitze Veranstaltungen, Yoked Fliegen assoziierten ihre eigenen Verhalten mit der Unkontrollierbarkeit der Umwelt. Sie unterdrückte ihre angeborene Reaktionen, wie die Verringerung ihrer Laufaktivität; verlängerte mehr Fluchtlatenzzeiten und weniger Umdrehen Verhalten unter Hitzen. Auch nach der Konditionierungsphase zeigte Yoked Fliegen niedrigeren Aktivität als Master und Kontrolle Fliegen. Interessanterweise haben wir auch Sex Dimorphismus in Fliegen beobachtet. Male Fliegen haben learned helplessness nicht wie weibliche Fliegen ausgedrückt. Die Unterschiede zwischen den Master und Yoked Fliegen waren bei männlichen kleiner als bei weiblichen Fliegen. Ein weiteres interessantes Ergebnis war, dass längere oder sogar wiederholte Trainingsphasen die lerned helplessness Wirkung bei Fliegen nicht verstärken könnten.

Darüber hinaus haben wir serotonergen und dopaminerge Nervensysteme in learned helplessness erforscht. Mit genetischen und pharmakologischen Manipulationen, haben wir das Niveau von Serotonin und Dopamin im zentralen

Nervensystem der Fliegen geändert. Weibliche Fliegen mit reduzierten Serotoninkonzentration zeigten kein hilflos Verhalten, während die learned helplessness Wirkung in männlichen Fliegen schien nicht durch eine Reduktion von Serotonin beeinflusst zu werden. Fliegen mit niedrigerer Dopamin Konzentration zeigten keine learned helplessness Wirkung in der Testphase an, was darauf hindeutet, dass mit niedrigen Dopamin die Motivationsänderung in learned helplessness in *Drosophila* kann schneller als mit einem normalen Dopaminspiegel sinken.

Affidavit

I hereby confirm my thesis entitled “” is the result of my own work. I did not receive any help or support from commercial consultants. All sources and /o or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar from.

Wuerzburg, 10.01.2015

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „“ eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg, 10.01.2015

Curriculum Vitae

Zhenghong Yang

PERSONAL DATA

Rudolf Virchow Center
University of Wuerzburg
Josef-Schneider-Str. 2, Building D15
97080 Wuerzburg, Germany

Tel:
Email:
Date of birth: 13.02.1985
Place of birth: Zhengzhou, China

EDUCATION

02.2011 – present Ph.D., Behavioral Neurobiology
Rudolf Virchow Center, University of Wuerzburg
Wuerzburg, Germany
Advisor: Prof. Martin Heisenberg

10.2005 – 01. 2011 Diplom, Biology
University of Wuerzburg
Wuerzburg, Germany
Advisor: Prof. Martin Heisenberg

RESEARCH EXPERIENCE

02.2011 – present Rudolf Virchow Center, University of Wuerzburg
Ph.D. thesis title: "A systematic study of learned helplessness in Drosophila melanogaster"
Advisor: Prof. Martin Heisenberg

03.2010 – 01. 2011 Department of Neurobiology and Genetics
University of Wuerzburg
Diplom thesis title: "Learned Helplessness in Drosophila melanogaster – a Consolidation Attempt"
Advisor: Prof. Martin Heisenberg

FELLOWSHIP

02.2011 – 07.2014 PhD Fellowship from the Excellence Initiative of the German Federal and State Governments (DFG) grant to the Graduate School of Life Sciences, University of Wuerzburg, Germany

PUBLICATIONS

Zhenghong Yang, Franco Bertolucci, Reinhard Wolf and Martin Heisenberg (2013). Flies cope with uncontrollable stress by learned helplessness. Current Biology. 23: 799-803

CONFERENCE CONTRIBUTIONS

Zhenghong Yang, Reinhard Wolf and Martin Heisenberg (2014). Learned helplessness of Drosophila in the heatbox. Poster presented at the 79th CSHL Symposium: Cognition, Cold Spring Harbor Laboratory, USA

Zhenghong Yang, Reinhard Wolf and Martin Heisenberg (2013). Operant Learning in Heatbox. Poster presented at 10th Goettingen Meeting of the German Neuroscience Society, Goettingen, Germany

Zhenghong Yang, Reinhard Wolf and Martin Heisenberg (2012). Learned Helplessness in Drosophila melanogaster. Talk presented at Honeybee-Drosophila meeting, Konstanz, Germany

Zhenghong Yang, Reinhard Wolf and Martin Heisenberg (2012). Operant Behavior of Drosophila in the Heatbox. Poster presented at European Fly Neurobiology biennial conference (Neurofly), Padova, Italy

Wuerzburg, 10.01.2015

Acknowledgements

First of all I would like to give my deepest gratitude to my Doktorvater Prof. Heisenberg. He not only brought me to the wonderful world of *Drosophila*, taught me scientific knowledge to explore it, but also provided all kindness of help for my life in Germany. Without him it would not be possible for me to finish my doctoral thesis. I would also like to thank Reinhard Wolf for teaching me uncountable skills in science and for his ideals and discussion.

I would like to thank Prof. Gerber for giving helpful advices, Markus Krischke for doing HPLC tests and Konrad Oeschner for giving me technical assistance through my whole doctoral study. I would also like to thank Graduate School of Life Sciences, University of Wuerzburg for providing me most part of financial support of my PhD research.

Most Importantly, I would like to give my sincere thanks to my parent and my family, who always supported me in last 9 years. There is long distance between us and we can see each other only in few days per year, but I can always feel their warm support around me. Without their understanding and support it would not be possible I finish my study in Germany.