LUDWIG-MAXIMILIANS-UNIVERSITÄT
MÜNCHEN

BACHELOR THESIS

Neuropeptide Signaling in the Perception of Polyamines

Anastasia Zindrou

6. August 2014

Erklärung zur Bachelorarbeit

Hiermit versichere ich, dass die vorliegende Arbeit von mir selbstständig verfasst wurde und dass keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden.

Diese Erklärung erstreckt sich auch auf in der Arbeit enthaltene Graphiken, Zeichnungen, Kartenskizzen und bildliche Darstellungen.

Bachelor's thesis statement of originality

I hereby confirm that I have written the accompanying thesis by myself, without contributions from any sources other than those cited in the text and acknowledgements.

This applies also to all graphics, drawings, maps and images included in the thesis.

…… ……..

Ort und Datum Unterschrift

Place and date Signature

Table of contents

Table of figures

List of tables

Abbreviations

AKH	Adipokinetic hormone
AL	Antennal Lobe
ANOVA	Analysis of Variance
AstA	Allatostatin A
Burs	Bursicon
Capa	Capability
CNS	Central Nervous System
Crz	Corazonin
DH	Diuretik Hormone
Dsk	Drosulfakinin
DTK	*Drosophila* Tachykinin
DTRPA1	*Drosophila* transient receptor potential 1
ETH	ecdysis triggering hormone
GFP	Green fluorescence protein
hs-hid	heat shock-inducible Head involution defective
GABA	Gamma-aminobutyric acid
GFP	Greeb Fluorencing Protein
GPCR	G protein-coupled receptor
LN	local interneurons
Lk	Leukokinin
MIP	Myoinhitory Peptide
Ms	Myosuppressin
NP	Neuropeptide
ORN	Olfactory Receptor Neurons
OR	Odorant Receptor
PN	Projection Neuron
Proc	Proctolin
PI	Preference Index
SP	Sex-peptide
SPR	Sex-peptide receptor
UAS	Upstream Activating Sequence

Summary

Current research in our laboratory examines the behavioral response of the fly *Drosophila melanogaster* to the diamines putrescine and cadaverine. It was found that flies are attracted to these compounds which also guide egg-laying behavior (oviposition). Flies sample food substrates in which this comounds are disolved and make judgements where to lay their eggs relative to the diamine distribution. Furthermore, it was observed that virgin flies are less attracted to these two diamines than mated flies are. This fact demonstrates a change in the flies' behavior according to olfaction after they were mated.

Post-mating changes have been observed in many species. After copulation *Drosophila* females start laying more eggs, mate in a lower frequency and actively reject male courtship. Responsible for this post-mating behaviors has been shown to be the sex-peptide, which is transferred from the male to female fly during copulation and triggers the female fly's behavior through binding with the sex-peptide receptor (Nilay et al, 2007). The change in the fly's preference to diamides demonstrates a post-mating switch that hasn't been described before.

We would like to investigate which neural circuits mediate the response of the fly to putrescine and how they work. We would also like to find out how the function of these neural circuits changes after the fly is mated. For this project a number of neuropeptides (NPs) was tested as potential signaling molecules in this circuit by observing fly behavior after hyperactivation of the NP producing neurons.

We used NP-GAL4 lines, in which the NP upstream sequence leads to expression of the GAL4 driver. These transgenic male flies were mated to female UAS- dTrpA1 flies. The resulting progeny with the genotype NP-GAL4-UAS- dTrpA1 could thus be hyperactivated at high temperature (32ºC). The preference of mated flies for H_2O or putrescine was tested in the T-maze set-up.

Here we were able to identify six lines and five different NPs, which showed significantly different response to putrescine. Hyperactivation of Capa, Crz, Lk, Dtk and MIP caused inhibition of the attraction to putrescine.

This experiment is the first step in identifying which neural circuits are associated with the response to putrescine and with the observed post-mating change in fly's preference to the odor. Certainly, fly's behavior is a complex system

depending on different cues and thus many different aspects should be considered in the further research.

Zusammenfassung

Aktuelle Forschung in unserem Labor untersucht die Reaktion der Fliege *Drosophila melanogaster* auf die Diamine Putrescine und Cadaverine. Es wurde herausgefunden, dass die Gerüche dieser Diamine für die Fliegen anziehend wirken und dass sie bei der Eiablage eine Rolle spielen. Die Fliegen testen die Substrate, die diese Gerüche ausströmen und legen ihre Eier in der Nähe ab. Weiterhin wurde beobachtet, dass jungfräuliche Fliegen weniger von diesen beiden Diaminen angezogen werden als Fliegen, die sich bereits gepaart hatten. Diese Tatsache stellt eine Veränderung des Verhaltens der Fliegen bezüglich ihres Geruchssinns dar nachdem sie sich gepaart haben.

Veränderungen nach der Paarung wurden schon in vielen Spezies beobachtet. *Drosophila* Fliegen insbesondere fangen an mehr Eier zu legen, sich mit niedrigerer Frequenz zu paaren und Männchen aktiv zurückzuweisen, nachdem sie sich gepaart haben. Es wurde gezeigt, dass für diese Veränderungen nach der Paarung ein Sex-Peptid verantwortlich ist, das von der männlichen auf die weibliche Fliege während der Koppulation übertragen wird und das Verhalten der weiblichen Fliege durch das Binden an den Sex-Peptid-Rezeptor auslöst (Nilay et al). Die Veränderung in der Preferenz der Fliege zu Diaminen stellt eine Verhaltensveränderung nach der Paarung dar, die noch nie zuvor beschrieben wurde.

Wir möchten untersuchen welche neuronalen Schaltkreise für diese Reaktion der Fliege auf Putrescine verantwortlich sind und wie sie funktionieren. Außerdem, möchten wir herausfinden wie die Funktion dieser neuronalen Schaltkreise sich verändert nachdem sich die Fliege gepaart hat. In diesem Projekt wurden einige Neuropeptide als potenzielle Bestandteile dieser Schaltkreise getestet indem das Verhalten der Fliegen nach der Hyperaktivierung der NPs beobachtet wurde.

Wir haben NP-GAL4 Linien benutzt, in denen die NP-upstream-Sequenz zu einer Expression des GAL4-Drivers führt(Hergarden, 2012). Diese transgenen männlichen Fliegen wurden mit weiblichen UAS-TRPA1 Fliegen gepaart. Die NP-Expression in den Nachkommen, die den Genotyp NP-GAL4-UAS-TRPA1 tragen, konnte daher bei hohen Temperaturen hyperaktiviert werden. Die Preferenz gepaarter Fliegen zu Wasser oder Putrescine wurde in T-maze set-up getestet.

Hier konnten wir sechs Linien und fünf verschiedene NPs identifizieren, die signifikant verschiedene Reaktionen auf Putrescine zeigten. Eine Hyperaktivierung von Capa, Corazonin, Leukokinin, Drosophila Tachykinin und Myoinhibitory Peptide verursachte eine Hemmung der Anziehung zu Putrescine.

Dieses Experiment ist der erste Schritt der Identifizierung neuronaler Schaltkreise, die mit der Reaktion auf Putrescine und mit den beobachteten Veränderungen der Preferenz der Fliege nach der Paarung zu dem Geruch assoziiert sind. Das Verhalten von Fliegen ist sicherlich ein komplexes System, das von verschiedenen Reizen abhängt, daher sollten in die weitere Forschung viele verschiedene Aspekte miteinbezogen werden.

1. Introduction

1.1. Olfaction and decision making

Chemotaxis, the ability of the organisms to perceive chemical substances from their environment, is required for discovering food and mating partners, as well as avoiding enemies and poisons. Developed multicellular organisms can perceive chemical substances through olfaction as well as through gustation. Gustation requires contact to the food or the sexual partner, and works through diffusion, whereas olfaction works over long distances for substances that can be transmitted through the air. Olfaction is in general more sensitive and more specific than gustation.

The detection of chemicals through olfaction can lead to different behaviors, for instance attraction or avoidance of the source. These behaviors, however, also depend on the context of additional external and internal signals. For instance, according to different publications, the metabolic state of an animal influences its response to odors and the way they are perceived (Braecker et al., 2013, E T Rolls 2011; Huetteroth 2011 Root et al. 2011; Moss & Dethier 1983; Schloegl et al. 2011; Gruber et al. 2013; Y. Wang et al. 2013; Siju et al. 2010). Thus, the olfactory system and the underlying neuronal circuit are a great model to investigate how context-dependent decisions are taken.

1.2. Olfaction in Drosophila

1.2.1. Drosophila as a model organism

The development of the available genetic and behavior-testing toolset in combination with its well-studied genome makes *Drosophila* the most appropriate model organism (Simpson, 2009). Every sequence of the genome can thus be studied, for example by knocking particular genes down. The resulting phenotype will show the probable function of the gene. Here is one common and very useful example: w^- flies have knocked down the gene “white”. The phenotype of w^- flies is white eyes. That led to the conclusion that the w gene is eventually responsible for the red color eyes, and it was indeed proved that this is the case (Hazelrigg, 1984). Another powerful tool that helped analyzing Drosophila's genes by making able their targeted expression for the first time is the GAL4/UAS system (Brand and Perrimon, 1993). Alhough the UAS/GAL4 system started as a tool for misexpression, this is rapidly changing and the

bipartite system is also commonly used in different ways, as for example to identify genes involved in the process of interest via enhancer- or gene-trapping (Duffy, 2002).

Behavior has already been studied before as a consequence of neural activity, using different ways of genetic manipulations so that specific genes or neurons that are presumable associated with olfaction can be tested. Based on the GAL4/UAS system, specific parts of the olfactory system could be studied. For instance, development of local interneurons (LNs) in the antennal lobe (Das et al, 2008) and of olfactory receptor neurons and projection neurons (Rodriguez, Hummel, 2008) were able to be studied due to this system. A large number of transgenic fly lines that carry this system and make possible the manipulation of the olfaction system are currently available (Bloomington Drosophila Stock Center). In addition, different methods for testing fly's behavior have been developed and can be used (T-maze, free walking behavior essay).

1.2.2. Anatomy of the *Drosophila* olfactory system

Our model organism *Drosophila* is able to detect chemical substances primarily via two pairs of chemosensory organs, the antennae and the maxillary palps (MP), which are located on the fly's head. These two organs are covered by different types of sensilla, which differ in size and shape. Drosophila melanogaster antennae house three different types of sensilla- basiconic, trichoid and coeloconic, while the MP is covered only by basiconic sensilla. (Figure 1.1) (Vossshall, L. B., & Stocker, R. F 2007, 12)

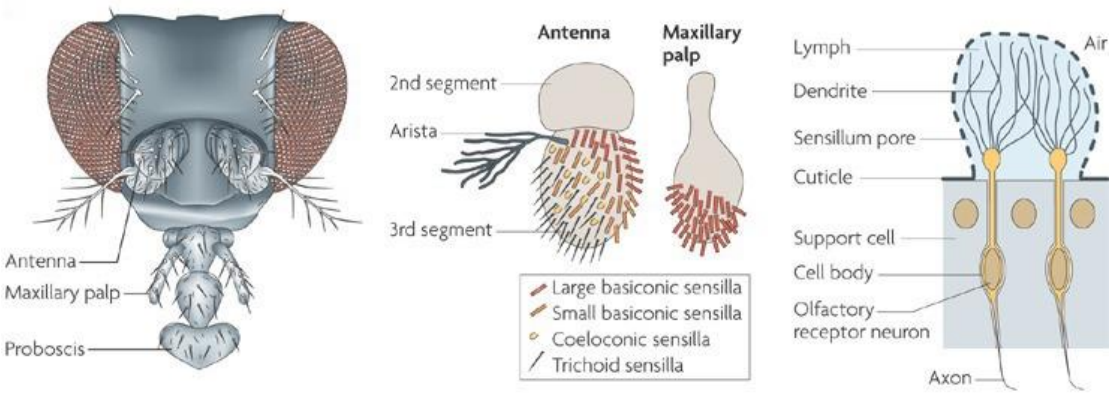

Figure 1-1 Overview of the olfactory system of the fly.

The olfactory receptors can be found on the antennae and the maxillary palps (left image). Both organs are covered by thin hair called sensilla. On the antenna three types of sensilla can be found, while on the maxillary palp only one (middle image). On the

right the olfactory receptor neurons and their projections at the surface of the sensilli are to be seen (Kaupp, 2010).

Inside the protecting sensilla are housed the olfactory receptor neurons (ORN), who are able to detect chemical molecules and transport the information to the brain. From the apical side, each ORN extends a sensory dendrite ending in ciliated projections into the shaft of the sensillum (Figure 1.1., right). There, odors are recognized by odorant receptor molecules expressed in ORNs. A given sensillum houses between one and four ORNs that are surrounded by support cells, which secrete sensillum lymph and keep each sensillum electrically insulated from its neighbor. Each ORN expresses only one type of odorant receptor gene. ORNs which express the same OR gene converge to and activate the same glomerulus. The glomeruli are spherical structures that build the antennal lobe (AL), the primary olfactory center of the brain. Olfactory neurons within sensory hairs send projections to 1 of 43 glomeruli. Each OR can detect different odor molecules, and each odor molecule can be detected by more than one OR, resulting that every odor activates a particular pattern of glomeruli in the antennal lobe (AL) (Figure 1.2.) (Couto, 2005). Drosophila has approximately 50 Glomeruli in its AL.

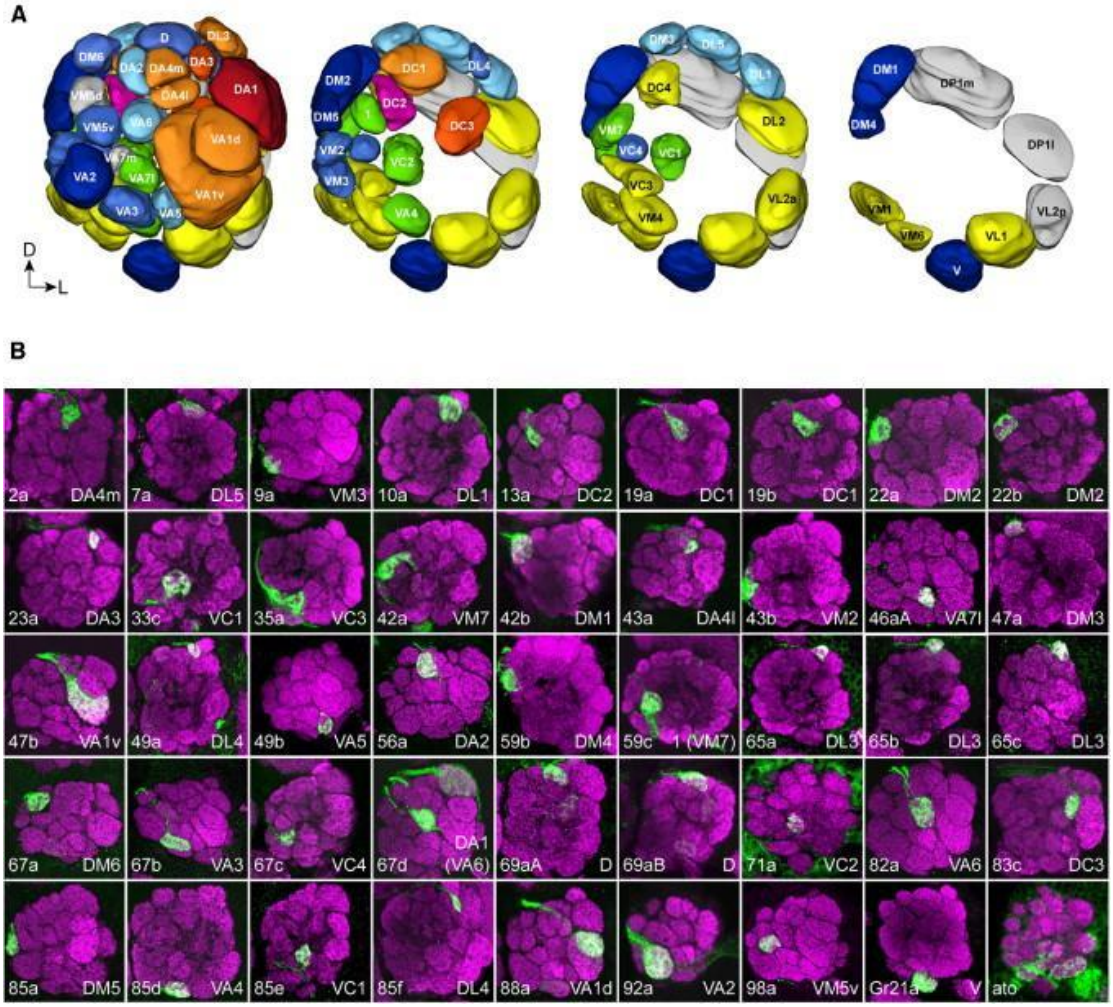

Figure 1-2 Map of ORN Projections in the Antennal Lobe:

A: 3D reconstruction of a male AL, showing the position of 49 Glomeruli. B: Antennal lobes of various ORtransgenic reporter lines, as published in Couto, 2005, Molecular, Anatomical, and Functional Organization of the Drosophila Olfactory System. Or-mCD8-GFP reporter lines were stained with anti-GFP to visualize the ORN axons (green), and the synaptic marker mAb nc82 was used to visualize the glomerular structure of the antennal lobe (magenta) (Couto, 2005).

In the glomeruli a highly specific connection takes place, while ORNs synapse in the glomeruli onto projections neurons (PNs) which grow to higher centers of the brain like the Mushroom body or the Lateral horn (Jefferis, G., and Hummel, T., 2006).

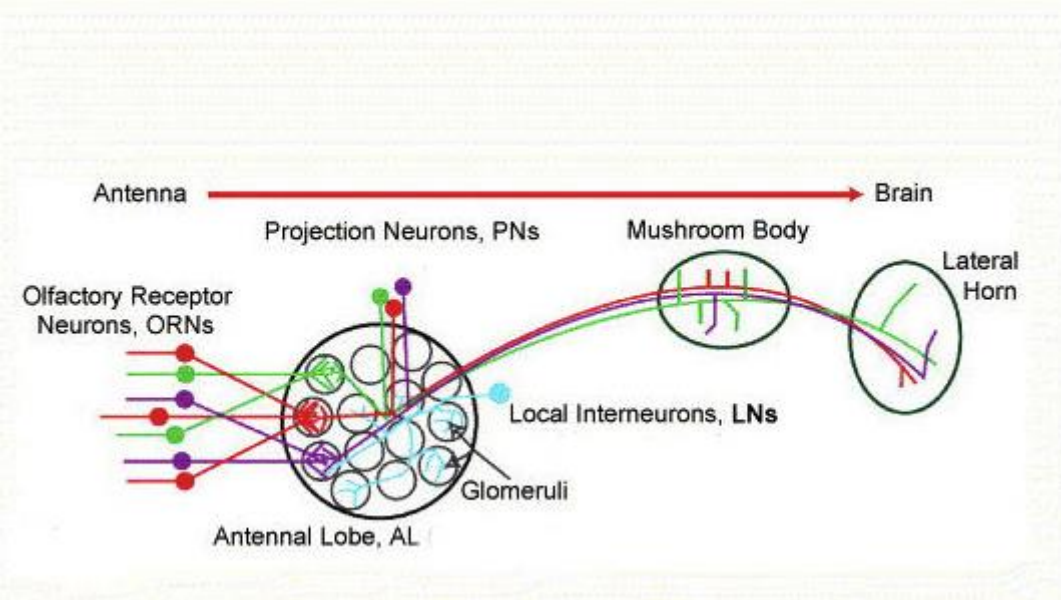

Figure 1-3 Schematic of the olfactory pathway

Schematic of the olfactory pathway from the peripheral sense organs to higher olfactory centers (Jefferis, Hummel, 2005).

Inside the AL, local interneurons (LN) connect glomeruli with each other. The functional role of the LN is not well understood yet, while we know that they provide transfer of information between glomeruli (Chou et al, 2010; Stocker, 1994), modulate glomerular signal activity and that most of them have an inhibitory function. LN can be divided in different classes (figure 1.3.), which present morphological and physiological diversity (Seki and Kanzaki 2008, Husch et al. 2009), with probably different roles each (Seki, Y. et al, 2010).

Presynaptic GABAergic inhibition of the ORNs has been shown in both Drosophila (Olsen SR, 2008) and in mammals (Murphy GJ, 2005, McGann, 2005). Conversely, cholinergic LNs in the Drosophila antennal lobe have been suggested to increase and redistribute odor-evoked activity at low odor concentrations (Shang, 2007, Olsen, 2007). Some subpulations of LNs have been interestingly shown to be peptidergic (Ignell, 2009).

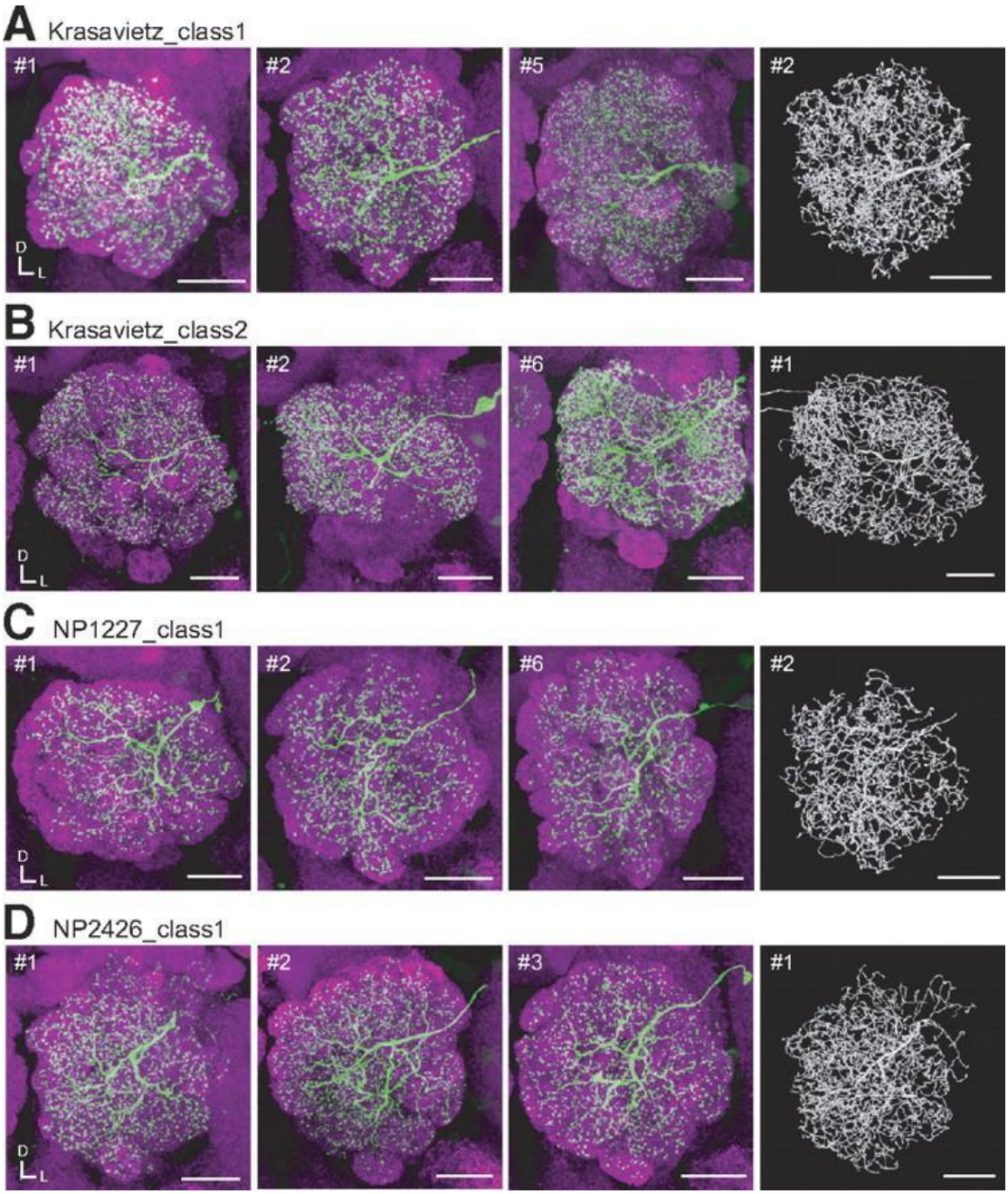

Figure 1-4 Local Interneurons

Examples of the morphology of the four LN classes identified by Seki, Y. LN appear green on the magenta Antennal Lobe, projection from confocal stacks. In each example one neuron is to be seen. Right: A complete reconstruction of one neuron (Seki, Y. et al, 2010).

1.3. Neuropeptides

Neuropeptides are small protein-like molecules used by neurons to communicate with each other. Many neuropeptides (NPs) identified in *Drosophila* are associated with behavior and physiology of the fly, like response to odors, feeding behaviors- either decreasing (Meng et al, 2002) or promoting feeding (Lee et al, 2009)- courtship and reproduction, metabolism control, circadian clock system, as well as with locomotion. Neuropeptides are used as neuromodulators in the AL circuitry of insects (Naessel, 2006), as for example was shown for the NP DTK (Ignell, 2009), which seems to act inhibitory on the ORNs and influencing olfactory responses.

In Drosophila's brain 75 different NPs are predicted to be encoded, however some of them are actually most probably not produced at all, while only 42 are identified (Yew et al., 2009).

With only some exceptions, the neuropeptides identified and studied so far act on G protein-coupled receptor (GPCRs). In most of the cases, neuropeptides activate only one single GPCR, but in a few cases peptides can activate two different GPCRs (Naessel, 2006).

According to studies (Nusbaum, 2001, Kupfermann, 1991), peptides act as co-transmitters with GABA to shape the olfactory responses of projection neurons. They are thus co-localized with a classical neurotransmitter that acts on an ion-channel-type of a receptor (Burnstock, 2004). When the neuropeptide is co-released with the neurotransmitter, the activation of the peptide GPCR leads to a modulation of the ion-channel-mediated signaling.

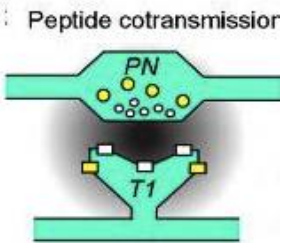

Figure 1-5: Peptide cotransmission

A neuron with a colocalized neurotransmitter and a neuropeptide, located in large densecore vesicles in the perisynaptic region. Peptide release is likely to occur perisynaptically and peptide acts on GPCRs also located perisynaptically. Peptide release may require stronger depolarization of the presynaptic neuron than neurotransmitter release (Naessel & Winther, 2010)

1.3.1. The NP-GAL4 lines

Peptidergic signaling is very complex and hard to study in more evolved animals. The development of genetic tools like GAL4/UAS system in the fly made able the cell specific expression of neuropeptides and their receptors and thus their analysis and better understanding. Through this system it was able to identify peptidergic neuron populations so that many neuropeptides have been demonstrated in interneurons of various types.

The first NP that was tested through the GAL4/UAS system was Allatostatin A (AstA). AstA was suggested to promote food abversion and the brain parts of its expression could be identified (Hergarden et al). Subsequently, more NP-GAL4 lines could be created. Anne Hergarden and Timothy Tayler cloned the putative regulatory regions of several NP genes upstream of GAL4 as AstA-GAL4 was constructed for that very first time. The research that followed has focused a lot on feeding behaviors and other NPs that play a role in feeding could be identified.

1.3.2. Postmating switch behavior

At various stages in their lifespan, animals can undergo marked switches in their innate behavioral patterns. A cue that induces a dramatic switch in female insects' behavior is mating. In *Drosophila melanogaster* responsible for postmating behaviors is the Sex Peptide(SP), which is produced in the male accessory gland, is transferred to females through mating and modulates their behavior through binding at the sex peptide receptor(SPR)(Yapici, 2007). The sex-peptide represses female sexual receptivity, stimulates oviposition (Chen, P. S. et al., 1988) and feeding (Gioti, 2012).

1.3.3. Myoinhibitory Peptides (MIPs)

SPR is not only found in females but also in male flies, as well as in insect species where no sex peptide is produced. This fact urged scientists to search for additional ligands and subsequently they identified a second family of SPR ligands, the Myoinhibitory peptides (MIPs). MIPs, in contrast to SP, are not produced in male reproductive organs and are likely to mediate functions other than the regulation of females reproductive behaviors (Young-Joon Kim, 2010).

1.4. Polyamines

Polyamines are small organic molecules that play an important role in the animal physiology, affecting growth and development. Mammalian, plants, bacteria as also other organisms contain polyamines in their tissues, among others also the diamine putrecine.

Although there are a lot of publications made regarding polyamines, current studies are mostly focused in those molecules' role in human diseases, connoting interest of their effects inside the same organism where they've been observed. Little research is done on how polyamines of the environment are detected by the olfactory system as well as on how they contribute in inducing specific animal behaviors. We know that the death-associated polyamines, putrescine and cadaverine, molecules emanating from decaying flesh, are strongly repugnant to humans, but can cause diverse behaviors depending on the species.

Zebrafish response to those two polyamines has been shown to be avoidant through the activation of some of its olfactory sensory neurons. (Hussain, 2013) In contrast, the same diamines work as feeding attractants for goldfish (Rolen, 2013) and rats (Heale, 1996). According to other studies the odors of putrescine and cadaverine function as social cues: In feline species, for example, the two molecules function as territorial markers (Burger BV, 2008), and in rats they signal the burying of conspecifics (Pinel, JP, 1981)

The perception of polyamines has not been systematically studied in the fruit-fly *Drosophila* before. In particular, the function that these compounds might play in the life of the fly were unknown. Current research of our laboratory (Hussain et al., in preparation) shows that putrescine and cadaverine induce attractive behavior in *Drosophila melanogaster*. However, while *Drosophila* likes putrescine odor, it does not lay eggs on putrescine containing substrates but in their closer vicinity. As also in previous studies has been shown, different sensory cues seem to affect the decision to lay eggs (Jeffrey A. Riffell, 2013, Joseph RM, 2009, Chung-hui Yang, 2008). The opposite effect of putrescine in those two behavioral drives provides the molecular basis

for studying neural circuits connecting sensation, perception and decision-making innate behaviors.

2. Aim of the thesis

Putrescine was seen to play an important role in the fly orientation. Experiments showed that flies are attracted to putrescine, they don't choose to lay their eggs on it, though. Furthermore, virgin flies are less attracted to putrescine than mated flies. Thus, many question were raised due to the different response of mated and non-mated flies. Why is the response of the virgin flies different? What kind of processing in the fly brain causes the different response to putrescine? The decoding of the corresponding neural circuit would help us answer this questions. We chose to start the analysis by identifying NPs that could play a role in this response.

The aim of the thesis was to unravel whether and which neuropeptides modulate the perception, neural processing and behavior to polyamines in a mating state-dependent manner in Drosophila females. Because putrescine was studied as an oviposition cue, it's important to mention that the flies were kept on food till one hour before the experiment took place. Therefore, we could limit the case that flies would be attracted to putrescine as a cue for food intake.

This could be accomplished through screening of 30 genetic modified lines, with a modification of a different NP each, to identify the ones that show a different phenotype than the wild type as response to putrescine. The wild type of *Drosophila* is attracted to putrescine in simple T-maze essays. The identification was accomplished through testing of each line in a T-maze set-up. The flies that were tested were mated. The NPs were hyperactivated at 32ºC through a binary system including the transactivator GAL4 which could initiate transcription of the receptor TRPA1 through an Upstream Activating Sequence (UAS). Thus, TRPA1 was expressed at the positions where normally NPs are expressed and activated the respective neurons when stimulated at 32ºC.

The thesis was part of a bigger project regarding putrescine and cadaverine and how the odors regulate oviposition and position of the fly.

3. Materials & Methods

3.1. Materials

3.1.1. Stock

Following lines were used for the crosses:

Table 3-1 Males

w[1118]; P{w[+mC]=Capa-GAL4.TH}4F
w[1118]; P{w[+mC]=Capa-GAL4.TH}5F
w[1118]; P{w[+mC]=Proc-GAL4.TH}2M/TM6B, Tb[1]
w[1118]; P{w[+mC]=Proc-GAL4.TH}6M
w[1118]; P{w[+mC]=Tk-GAL4.TH}2Ma
w[1118]; P{w[+mC]=Tk-GAL4.TH}3Ma/TM6B, Tb[1]
w[1118]; P{w[+mC]=Tk-GAL4.TH}5Fa
w[1118]; P{w[+mC]=Crz-GAL4.391}3M
w[1118]; P{w[+mC]=Crz-GAL4.391}4M
w[1118]; P{w[+mC]=AstA-GAL4.2.1}3M/TM6B, Tb[1]
w[1118]; P{w[+mC]=AstA-GAL4.2.1}5
w[1118]; P{w[+mC]=Burs-GAL4.TH}4M
w[1118]; P{w[+mC]=Dsk-GAL4.TH}3M
w[1118]; P{w[+mC]=ETH-GAL4.TH}1M
w[1118]; P{w[+mC]=Mip-GAL4.TH}1M/TM6B, Tb[1]
w[1118]; P{w[+mC]=Mip-GAL4.TH}2M
w[1118]; P{w[+mC]=Ms-GAL4.TH}1M/TM6B, Tb[1]
w[1118]; P{w[+mC]=Ms-GAL4.TH}6Ma
w[1118]; P{w[+mC]=Dh44-GAL4.TH}2M
w[1118]; P{w[+mC]=Dh31-GAL4.TH}2M
w[1118]; P{w[+mC]=Dh31-GAL4.TH}5F
w[1118]; P{w[+mC]=FMRFa-GAL4.TH}1M
P{w[+mC]=sNPF-GAL4.TH}2, w[1118]
w[1118]; P{w[+mC]=Lk-GAL4.TH}1
w[1118]; P{w[+mC]=Lk-GAL4.TH}2M
w[1118]; P{w[+mC]=AstC-GAL4.TH}1M/TM6B, Tb[1]
w[1118]; ;TH-Gal4
w[1118]; 5HT1B-GAL4
w[1118]; DDC-GAL4
w[1118]; TDC-GAL4/cyO
[1118]; ILP7-GAL4
[1118]

Table 3-2 Females

w[*]; P{y[+t7.7] w[+mC]=UAS-TrpA1(B).K}attP16

3.1.2. Solutions

0,001% Putrescine solution

3.2. Methods

3.2.1. Setting crosses

Virgin females for the cross were collected using the "virginator" UAS-TRPA1 strain, which contains a heat shock-inducible Head involution defective (Hid). Hs-hid transgene induces ectopic cell death in wild-type embryos following heat shock. The hs-Hid transgene inserted on the Y chromosome had selectively kills males after 2 h heat shock at 37°C during the pupal stage.

Males from the NP-GAL4 lines and UAS-TRPA1 females were kept in bottles for three days. After that the parental generation was trashed. Progeny was collected after 13 days and kept on fresh food for two days before the experiment.

Flies were raised in bottles containing standard fly food consisting of yeast and other ingredients.

The incubator was set to 25° C at around 60–70 % humidity.

3.2.2. NP-GAL4 Lines

The NP-GAL4 lines were used to target the neurons where the NPs of our interest are expressed. The NP-GAL4 lines were constructed by Anne Hergarden and Timothy Tayleras as described in the construction of P{Crz-GAL4.391} and P{AstA-GAL4.2.1} in Hergarden et al. and Tayler et al.

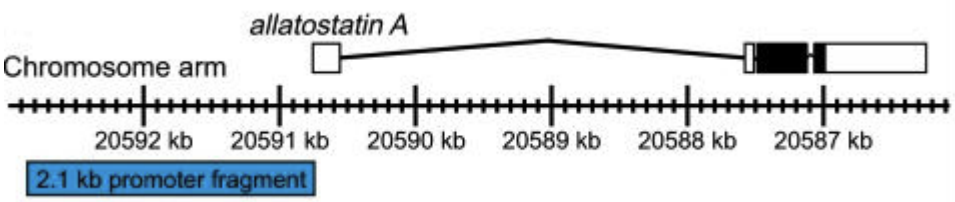

Figure 3-1: Structure of Drosophila

Structure of Drosophila AstA gene and origin of upstream sequence in AstA–GAL4 transgenic flies (blue box). Black boxes are coding exons.

The constructed *AstA* promoter–*GAL4* transgenic flies contain 2.1 kb upstream of the predicted transcription start site of the *AstA* gene

3.2.3. GAL4/UAS system

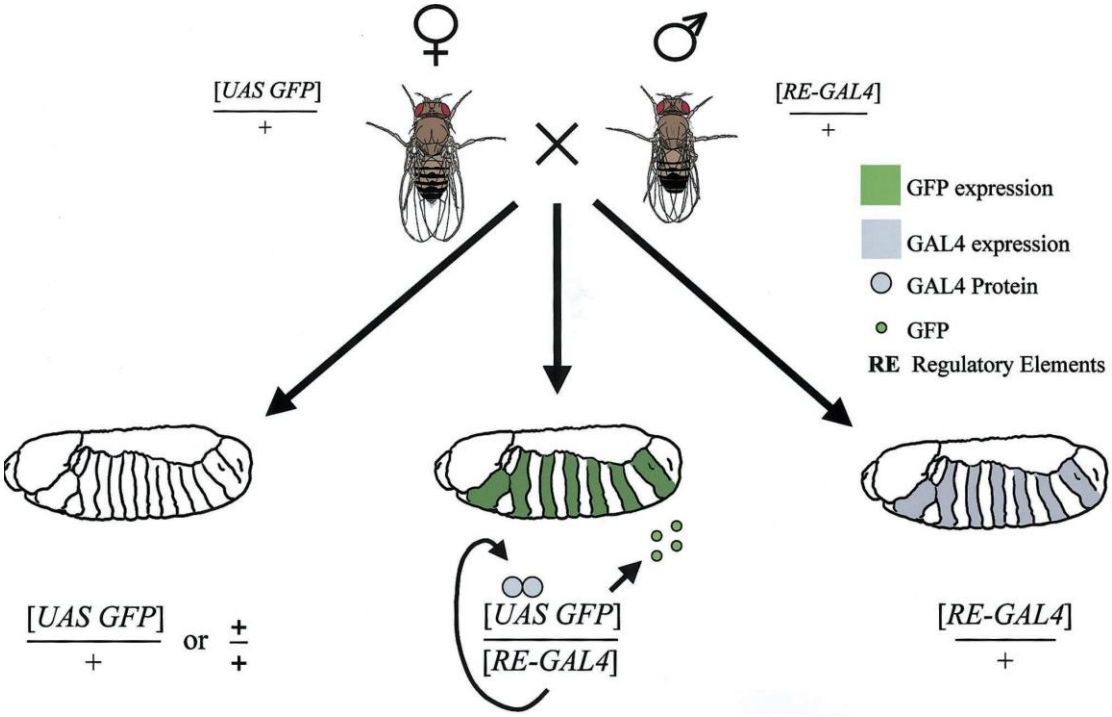

Figure 3-2 The bipartite UAS/GAL4 system in Drosophila.

When females carrying the UAS responder are mated with males carrying the GAL4 driver progeny containing both element of the system are produced. The presence of GAL4 in an altering segmental pattern in the depicted embryos then drives expression of the UAS responder gene in a corresponding pattern (Duffy, 2002).

This system was used to drive the expression of distinct genes. It consists of the yeast transcription activator protein (GAL4) and the upstream activation sequence (UAS). If the GAL4 protein is activated by a specific promoter such as the promoter of a NP, it binds to the enhancer UAS (Brand, A. and Perrimon, N., 1993). Then the transcription of the genes downstream of the UAS sequence will start.

3.2.4. DTRPA1

Drosophila transient receptor potential 1(DTRPA1) is an ion channel that functions as a molecular sensor of warmth and normally activates a small set of warmth-activated anterior cell(AC) neurons in the *Drosophila* brain. AC neurons are activated at 24.9ºC(±0.6) (Hamada, 2008).

In our lines the DTRPA1 was driven by the GAL4 driver. The protein DTRPA1 was expressed in and activated the neurons were our NPs of interest are expressed

normally in the fly brain. This made possible the targeted release of NPs as soon as DTRPA1 was activated.

3.2.5. T-maze set-up

To study the avoidance or approach behavior of Drosophila the T-maze set-up was used. Flies were transferred into an elevator by tabbing and then moved to the choice point. At this point they can choose to walk into one of the two attached tubes: The one contains the putrescine odor, while the other contains H_2O. After 1 minute the choice point was blocked and the tubes with the trapped flies were moved out of the set-up.

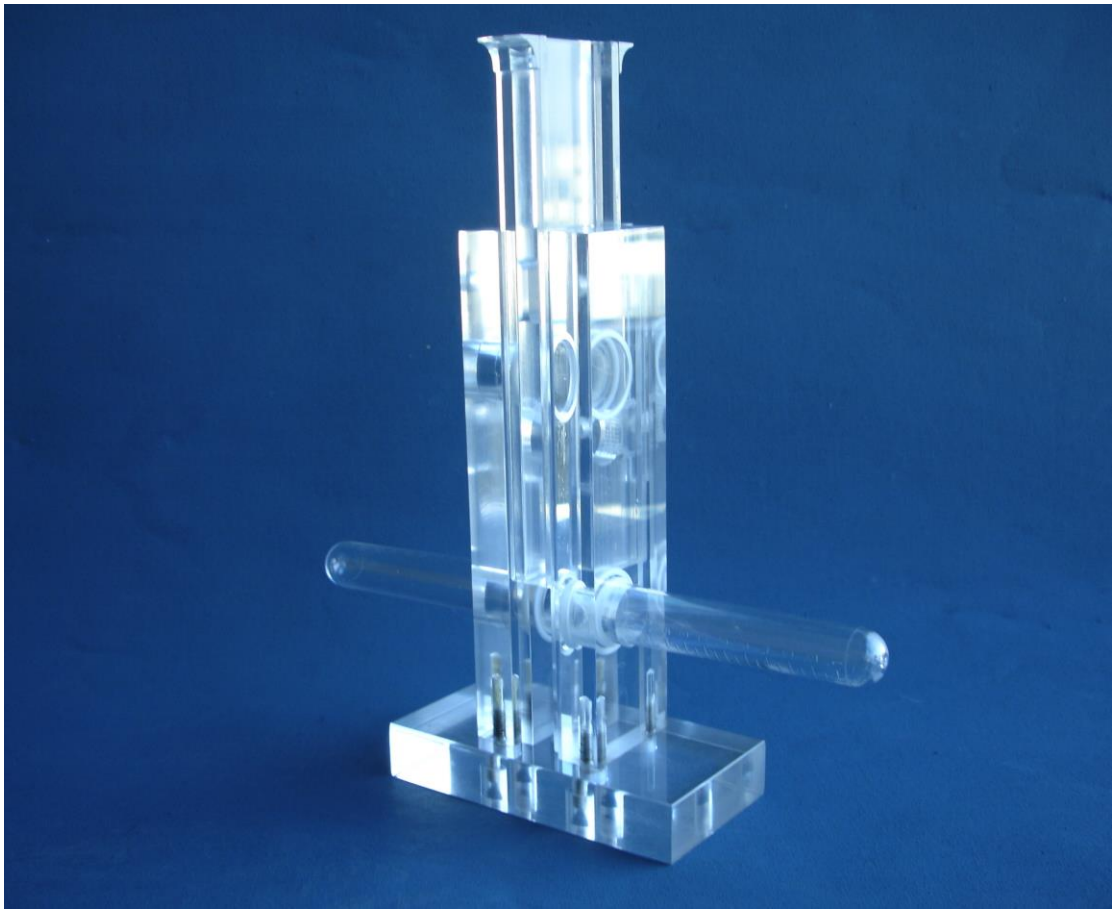

Figure 3-3 T-maze set-up

T-maze set-up similar to the one that was used for the behavioral essay.

Each line was tested on four different days, in reciprocal manner and each day the control line was tested in reciprocal manner as well.

3.2.6. Quantification and statistical analysis

Flies in both tubes are anesthetized with CO2 and counted. The performance index (PI) is determined by subtraction of the number of flies who chose the tube without any odor from the number of flies that chose the tube containing the putrescine odor and then dividing this number by the total number of flies.

PI= (#flies in tube with odor- #flies in tube without odor)/ total# of flies.

PI=0 shows no preference between H2O and putrescine, while PI=100 connotes the strongest possible attraction to putrescine, meaning that each one of the flies chose the putrescine including vial.

Table 3-3 Results of the T-maze essay of line Capa-GAL4.4F

	Control					51969-gal4;UAS-TRPA1			
Date	**Odors**	**#flies**	**total**	**P.I**	**Date**	**Odors**	**#flies**	**total**	**P.I**
04/06/14	PUT	20	24	0.66667	04/06/14	PUT	24	25	0.92000
	H2O	4				H2O	1		
	PUT	14	20	0.40000		PUT	21	38	0.10526
	H2O	6				H2O	17		
11/06/14	Put	39	63	0.23810	11/06/14	Put	33	50	0.32000
	H2O	24				H2O	17		
	Put	36	43	0.67442		Put	28	45	0.24444
	H2O	7				H2O	17		
17/06/2014	PUT	31	52	0.19231	17/06/2014	Put	22	42	0.04762
	H2O	21				H2O	20		
	PUT	36	65	0.10769		Put	16	30	0.06667
	H2O	29				H2O	14		
04/07/14	Put	24	37	0.29730	04/07/14	Put	27	41	0.31707
	H2O	13				H2O	14		
	Put	31	45	0.37778		Put	26	44	0.18182
	H2O	14				H2O	18		

Text: In the table are presented the data of the behavioral essay of the line Capa-GAL4.4F (stock line number 51969) after four repetitions in reciprocal manner. The control line tested on the same days is taken as reference. According to these results, the p value is p=0.46 and thus the line's response to putrescine doesn't differ significantly from the response of the control. First column (Date) of each box shows the date on which the essay took place. Second column (Odors) shows the odors that were contained in the vials. PUT=Putrescine. Third column shows the number of flies that were trapped in each vial after the essay. Fourh column (#total) shows the number of flies that were contained in both vials together. Fifth column shows the calculated preference index. All the lines were analyzed as presented here.

The graphs were made using Microsoft Excel and Adobe Ilustrator.

The standard error (SEM) in graph 2 was calculated by the gaussian error propagation.

ANOVA:

I used one-way ANOVA with Bonferroni correction, mean ± SEM, n = 8 in GraphPad to analyze the significance of the data.

24 of the lines could be grouped according to the days that they were tested and analyzed with ANOVA.

Each line was tested on four different days, in reciprocal manner and each day the control line was tested in reciprocal manner as well. The average w^{-}GAL4 P.I.-value is the average of the preference of the control line. This value was calculated for the requirements of a graph were all the lines are presented together and was not used in the calculation of the significance.

T-test

5 of the lines were analyzed with the t-test in Microsoft Excel and compared to control group.

4. Results

The flies of the crosses Proc-GAL4.TH}2M-UAS-TRPA1 and Proc-GAL4.TH}6M-UAS-TRPA1 couldn't be tested in the t-maze at 32ºC. When the flies were put in the 32ºC incubator, they fell on the bottom of the vial and were unable to move, possibly indicating a locomotor effect.

4.1. T-Maze behavioral essay

In order to figure out which neuropeptides (NPs) participate in the neural circuit that is responsible for the response to putrescine, 29 different NP-GAL4-UAS-TRPA1 fly lines were tested. To hyper-activate the neuropeptides, UAS-TRPA1 constructs were expressed by the driver NP-GAL4 and the flies were tested at 32ºC in a T-maze set-up. Flies are given a choice between a tube containing a filter paper with 50ml putrescine on it and one containing filter H_2O. In each of the testing lines one NP was hyper-activated.

The results show the average response (Preference Index) of each of the 29 GAL4-NP-lines in the T-maze essay (n=8).

From the T-maze screen of the 29 NPs result the data presented in Figure 4-1.

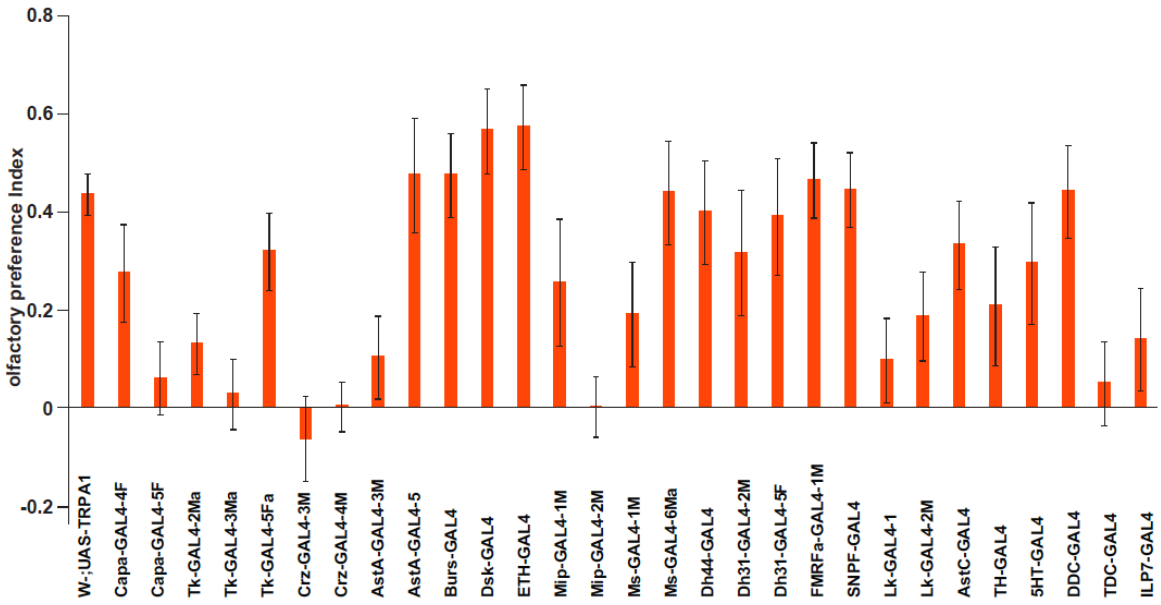

Figure 4-1 Preference to putrescine

Text: The graph shows the percent preference of the 29 tested lines to putrescine. x-

axis: Tested lines, crossed with UAS-TRPA1, y-axis: Preference Index(PI). The control shows an average preference of 0.45. Six following lines show a significant different preference than the control: Capa-GAL4-5F, Tk-GAL2-2Ma, Crz-GAL4-3M, Crz-GAL4-4M, Mip-GAL4-2M, Lk-GAL4-1

As control was used the line w-; UAS-TRPA1. In this line no GAL4 driver is included, thus no NP is hyper-activated, and as expected the flies are normal attracted by putrescine (P.I.=0.45). This P.I. represents the average of the P.I. of the 24 control tests that were made respectively to the experiments. This value was calculated for the requirements of a graph were all the lines are presented together and was not used in the calculation of the significance.

Flies from the line Crz-GAL4-3M;TRPA1 respond conversely comparing to control and avoid putrescine, the lines Crz-GAL4-4M;TRPA1 and Mip-GAL4-2M;TRPA1 show almost no preference between water and putrescine. The lines Capa-GAL4-5F;TRPA1 as well as Tk-GAL4-3Ma;TRPA1 show very low response according to the graph, while the lines Lk-GAL4;TRPA1 shows low to middle response compering to w^-GAL4.

ANOVA:

The ANOVA test was used to verify which of the tested lines show a significant different behavior comparing to the control w^-;GAL4.

Twenty three of the transgenic lines show no significant different behavior, while 6 of the lines show reduced attraction to putrescine, loss of attraction or avoidance. We can assume that these 6 NPs are involved in the neural circuit that is activated when the fly's olfactory receptors detect putrescine.

The 6 lines as well as their genotype and the significance are presented in Table 4-1 and in figure 5-1.

Table 4-1 Genotype and the significance

Tested NP	Fly Genotype	p-value	Significance
Capa	w[1118]; P{w[+mC]=Capa-GAL4.TH}5F	0.0024	**

Tk	w[1118]; P{w[+mC]=Tk-GAL4.TH}3Ma/TM 6B, Tb[1]	0.0222	*
Crz	w[1118]; P{w[+mC]=Crz-GAL4.391}3M	0.0021	**
Crz	w[1118]; P{w[+mC]=Crz-GAL4.391}4M	0.0062	**
Mip	w[1118]; P{w[+mC]=Mip-GAL4.TH}2M	0.0017	**
Lk	w[1118]; P{w[+mC]=Lk-GAL4.TH}1	0.0012	**

Lines that show significant different behavior compared to the control after analyzed with ANOVA. First column shows the Neuropeptide which is hyperactivated, the second column shows the exact genotype of the line, the third colmun the p-value resulted after ANOVA, while the fourth column shows the grade of significance. One star shows relative lows significance, two stars medium significance.

The lines modified for the NPs Lk, Mip, Crz and Capa show medium significance while the line modified for Tk shows low significance (Table 4-1).

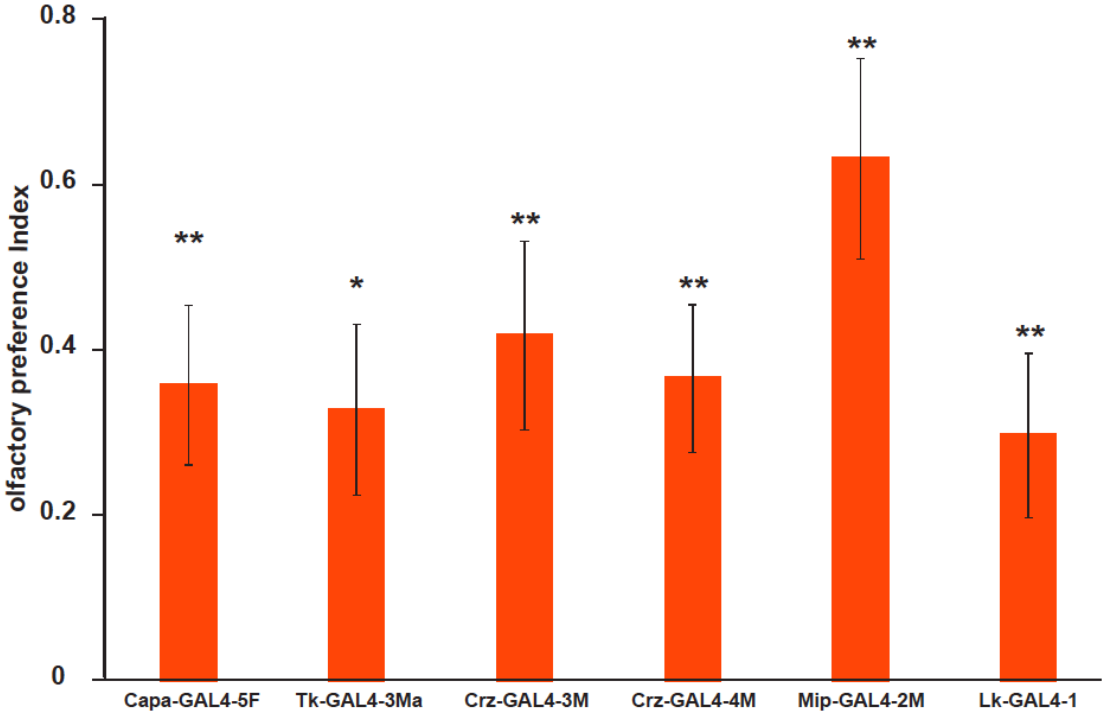

Figure 4-2 Olfactory preference index

Olfactory preference index of the lines that show significant different behavior to the control are representing the value [PI of control- PI of tested line]. MIP has the higher significance according to the graph, and thus it presents a very possible participant NP. Furthermore, both lines hyperactivating CRZ show a relative high significance.

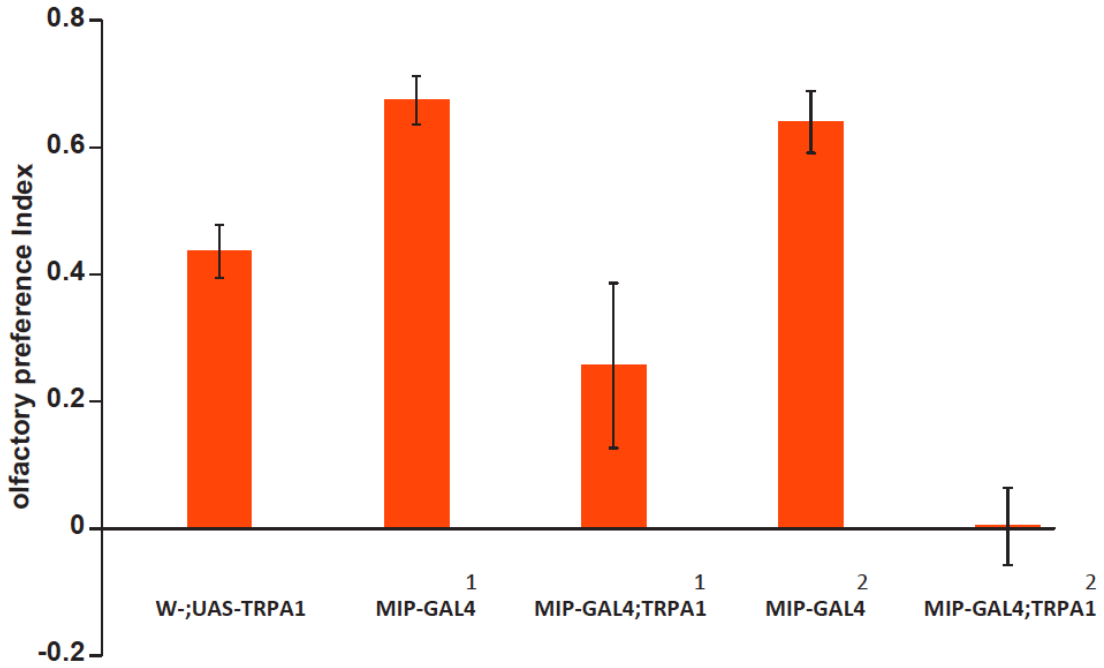

Figure 4-3: Preference Index of Drosophila after Hyperactivation of MIPs

Preference Index of MIP-GAL4; TRPA11 and MIP-GAL4; TRPA12 transgene flies compared to the control line w-; UAS-TRPA1 as well as to the corresponding MIP-GAL4. Both lines show a decrease in the attraction to putrescine.

Table 4-2: Final T-maze bahavioral essay data of the line Mip-GAL4; UAS-TRPA1.2M

Control						51984-gal4;UAS-TRPA1			
Date	**Odors**	**#flies**	**total**	**P.I**	**Date**	**Odors**	**#flies**	**total**	**P.I**
05/06/14	Put	25	29	0.72414	05/06/14	PUT	21	41	0.02439
	H2O	4				H2O	20		
	Put	11	17	0.29412		PUT	26	48	0.08333
	H2O	6				H2O	22		
23/6/2014	Put	44	52	0.69231	3.06.201	Put	23	51	-0.09804
	H2O	8				H2O	28		
	Put	38	41	0.85366		Put	33	59	0.11864
	H2O	3				H2O	26		
24/6/2014	Put	41	53	0.54717	4.06.201	Put	35	63	0.11111
	H2O	12				H2O	28		
	Put	46	55	0.67273		Put	34	66	0.03030
	H2O	9				H2O	32		
03/07/14	Put	33	41	0.60976	03/07/14	PUT	8	33	-0.51515
	H2O	8				H2O	25		
	Put	18	30	0.20000		PUT	24	43	0.11628
	H2O	12				H2O	19		

Table 4-3 Final T-maze bahavioral essay data of the line Mip-GAL4; UAS-TRPA1.1M

Control						51983-gal4;UAS-TRPA1			
Date	**Odors**	**#flies**	**total**	**P.I**	**Date**	**Odors**	**#flies**	**total**	**P.I**
05/06/14	Put	25	29	0.72414	05/06/14	PUT	11	11	1.00000
	H2O	4				H2O	0		
	Put	11	17	0.29412		PUT	3	3	1.00000
	H2O	6				H2O	0		
23/6/2014	Put	44	52	0.69231	3.06.201	Put	23	52	-0.11538
	H2O	8				H2O	29		
	Put	38	41	0.85366		Put	27	51	0.05882
	H2O	3				H2O	24		
24/6/2014	Put	41	53	0.54717	4.06.201	Put	34	61	0.11475
	H2O	12				H2O	27		
	Put	46	55	0.67273		Put	29	52	0.11538
		9				H2O	23		
02/07/14	PUT	33	52	0.26923	02/07/14	PUT	22	33	0.33333
	H2O	19				H2O	11		
	PUT	24	37	0.29730		PUT	27	51	0.05882
	H2O	13				H2O	24		

Hyperactivating the MIP releasing neurons results to a decreasing of flies' attraction to putrescine (p^1= 0.6518, p^2=0.0029). However, only the second line (Mip-GAL4.2M) shows a significant decreasing. The role of the MIP in this neural circuit could potentially reveal a function of this neuropeptide in the fly's olfactory response.

5. Discussion

Animals are surrounded by a myriad of chemicals in their environment. These chemical send specific signals associated with food search, finding a suitable mating partner and protection from predator. Like other organisms, *Drosophila melanogaster* uses olfactory system to sense these signals. The detection of chemicals through olfaction can lead to different behaviors, for instance attraction or avoidance of the source. These behaviors, however, also depend on the context of additional external and internal signals. For instance, according to different publications, the metabolic state of an animal influences its response to odors and the way they are perceived. Thus, the olfactory system and the underlying neuronal circuit are a great model to investigate how context-dependent decisions are taken.

The olfactory system of Drosophila melanogaster represents a manipulable model for understanding the olfaction. Despite being numerically simpler, the logic of the fly olfactory system is quite reminiscent of the one in vertebrates. The third segment of the antenna represents the main peripheral olfactory organ in the fly and houses the majority of ORNs (Vosshall and Stocker, 2007). These neurons are contained in groups of typically two or three in sensory hairs, so-called sensilla. In addition to neurons, sensilla contain support cells with functions in part similar to glia cells. ORs, as in mammals, are seven transmembrane proteins that function likely both as ionotropic channels and G-protein coupled receptors (Sato et al., 2008; Wicher et al., 2008). The receptors are localized predominantly in the dendritic extension of the sensory neuron (Benton et al., 2006). The mechanisms required for this localization are not well understood, but likely involve similar cellular transport systems as in cilia and neurites. The fly antenna expresses three types of olfactory receptors: the classical OR (Vosshall, 2000), the more recently discovered ionotropic receptors (IRs) (Benton et al., 2009), and the CO2 receptors GR21a and GR63a (Jones et al., 2007; Kwon et al., 2007). The ORN axons connect to the AL, the equivalent of the olfactory bulb, in the brain, where the olfactory information is further processed by local interneurons and transferred by projection neurons (PNs) to higher brain centers. The main higher brain centers involved in olfactory information processing are the mushroom body (MB) and the lateral horn (LH). Interestingly, dopamine and other neurotransmitters as well as neuropeptides modulate olfactory information processing starting already at the first central synapse in the antennal lobe (Fiala, 2007; Wang, 2012; Wilson, 2013). Genetic

techniques make it possible to selectively manipulate and look at all different neuron types involved in odor processing starting at ORNs in the antenna up to higher brain centers.

We were able to identify five different NPs- Mip, Crz, Capa, Dtk, Lk-expressed by six different GAL4 lines, that eventually participate in the neural circuit that is associated with the odor of putrescine. It is possible that some of these NPs influence the response to putrescine directly, while others indirectly. Nevertheless, further studies have to be done before we can say with certainty that these NPs are participants in the one or the other way.

Here I am going to discuss how these five NPs could be associated with the response to putrescine, according to the information that we already have by previous studies, and also suggest how the research on the particular neural circuit should go on for our questions to be answered.

There is very little data on functions of neuropeptides, although many of them have been mapped and we know at least partially where they are expressed and some of their functions.

It has been shown, that some of the NPs that we tested modulate functions as appetite, egg laying or digestion in distinct ways.

In antennal lobes only DTK and MIP from the NPs we tested have been reported in Drosophila. The other peptides don't seem to be present in cell bodies of the brain proper, but in subesophageal ganglia and/or thoracic/abdominal ganglia and have processes to the brain(Naessel and Winther, 2010). Capa and crz appear to be present in neurons with processes to neurohemal organs. Crz may be responsible for the release of hormones from the corpora cardiaca. In the adult brain there are sets of lateral neurosecretory cells(LNCs) and additional corazonin positive neurons can be seen near the LNCs and medially in the dorsal protocerebrum a pair of descending neurons are located (Cantera et al., 1994; Choi et al., 2005). The presence of corazonin in LNCs innervating the corpora cardiaca and anterior aorta of adult flies may suggest a hormonal role of the peptide. As an alternative, corazonin could be a releasing factor of AKH (Adipokinetic hormone) in the corpora cardiaca and involved in nutricial stress signaling. In fact it was shown that ablating the corazoninexpressing LNCs led to decreased trehalose levels (Choi et al., 2008,Veenstra, 2009). It can be noted that the

corazonin producing LNCs express receptors for the diuretic hormones DH31 and DH44 and the AstA receptor DAR-2.

DTK and MIP have been also identified in the endocrine cells of intestines.

It looks like the several NPs don't have a really specific region of expression and are probably pleiotropic. Many of them are likely to have hormonal action. The NPs that are not, according to previous studies, expressed in the CNS of the fly may have an indirect influence on the putrescine neural circuit. By that is meant that these NPs induce specific changes in the fly metabolism, and those changes make putrescine more attractive for the fly. We don't know yet, if satiation has an influence on the fly's putrescine preference. Further analysis of those NPs is needed to understand if they act directly on the fly's response to putrescine or by actually inducing other responses. To answer this questions, the exact action of these NPs should be studied and other elements of the downstream pathway should be studied, and also starved flies should be tested in comparison to fed ones.

Therefore, the action of the NP Tk has been shown to be the last part of a number of metabolic changes and thus directly regulating fly's behavior(Ignell et al).

DTKs were obtained before to have a role in olfaction, and according to our data the particular NP is participant in the putrescine olfactory response as well. In the previous study, when dtk expression was knocked down using RNAi, the flies responded more sensitive to odors. The researchers came up to the conclusion that DTK released by LNs modulate ORNs presinaptically. These DTK-expressing LNs appear to innervate all glomeruli and thus are able to transfer the DTK signal to the entire antennal lobe (Ignell et al., 2009; Winther et al., 2003). We can assume that DTK is in general participant in olfactory responses and thus it's not connected to the postmating increased attraction to putrescine. Therefore, that could be proved if virgin flies would be tested compared to mated ones.

MIPs as alternative ligands of the SPR are of specific interest. SPR is known for causing a number of postmating behaviors after bound by SP. Thus, it could be possible that MIP, as a lingand of SPR, also triggers responses associated with mating that are different from the previously described postmating-switch (Nilay et al.).

MIPs have been shown to be extremely active in the AL and thus it's very probable that they play a role in olfaction(Naessel and Winther, 2010). Taken together, MIPs may play a role in the postmating increased preference of the flies to putrescine.

Therefore, more experiments need to be made for us to understand the postmating increased attraction of *Drosophila* to putrescine. First, the here described experiment should be repeated using virgin flies to see if the overexpression of certain NPs has any influence in the virgin fly's attraction to putrescine. It would be of meaning, if the overexpression of NPs in virgins would cause attraction to the odor. That way we could figure out, whether the different response of mated flies is a result of the expression of certain NPs and not of lack of their expression.

Furthermore, two T-maze essays should be completed, for mated and for virgin flies, testing the response of the flies after deactivation of the NPs that were also tested here. For the deactivation either UAS-Kir2.1 or RNAi-lines could be used. We want to answer the question, if the inactivation of the NPs that were seen to be associated with the response to putrescine will show an opposite reaction of this of the UAS-TRPA1 lines.

Another way to identify the different expression pattern of the NP in virgin and mated flies is to observe the parts of the brain where they are expressed as well as the amount of the expression. The NP distribution could be observed through immunocytochemistry or through expression of membrane bound GFP (mcd8-GFP) driven by the GAL4/UAS system

Acknowledgments

I want to thank Dr. Ilona Kadow for giving me this great opportunity to do my thesis in her lab and for all the things I learned from her.

Thanks to Dr. Rüdiger Klein for being my academic supervisor and for his support to do my project in the Max Planck Institute for Neurobiology.

Many thanks to Ashiq Hussain for supervising me and letting me contribute in his project. I want to thank him for spending so much time on explaining things to me and teaching me how to work with flies. He was very patient and willing answering all my questions and also helping me whenever I needed it.

Special thanks to Laurence for helping me with my project by sharing his ideas with me and giving me his advices. I learned a lot through our discussions.

I wish to acknowledge all the lab members, Habibe, Christina, Siju, Sercan, Mo, Kacey, Laura and Anja for answering my questions and for the nice atmosphere in the lab.

Many thanks to my friend Marion for her help and tips. Also to my parents who were supporting me all this time.

References

Benton, Richard, et al. "Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in *Drosophila*." *Cell* 136.1 (2009): 149-162.

Benton, R. "On the ORigin of smell: odorant receptors in insects." *Cellular and molecular life sciences CMLS* 63.14 (2006): 1579-1585.

Bräcker, Lasse B., K.p. Siju, Nélia Varela, Yoshinori Aso, Mo Zhang, Irina Hein, Maria Luísa Vasconcelos, and Ilona C. Grunwald Kadow. "Essential Role of the Mushroom Body in Context-Dependent CO2 Avoidance in Drosophila." *Current Biology* 23.13 (2013): 1228-234. Print.

Bräcker, Lasse B., et al. "Essential Role of the Mushroom Body in Context-Dependent CO_2 Avoidance in *Drosophila*." *Current Biology* 23.13 (2013): 1228-1234.

Brand, Andrea H., and Norbert Perrimon. "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes." *development*118.2 (1993): 401-415.

Burger, B. V., et al. "Chemical characterization of territorial marking fluid of male Bengal tiger, Panthera tigris." *Journal of chemical ecology* 34.5 (2008): 659-671.

Burnstock, Geoffrey. "Cotransmission." *Current opinion in pharmacology* 4.1 (2004): 47-52.

Burnstock, Geoffrey. "Historical review: ATP as a neurotransmitter." *Trends in* pharmacological sciences 27.3 (2006): 166-176.

Cantera, Rafael, Dick R. Nässel, and Jan A. Veenstra. "Postembryonic development of corazonin-containing neurons and neurosecretory cells in the blowfly, Phormia terraenovae." *Journal of Comparative Neurology* 350.4 (1994): 559-572.

Chen, P. S., et al. "A male accessory gland peptide that regulates reproductive behavior of female D. melanogaster." *Cell* 54.3 (1988): 291-298.

Choi, Youn J., et al. "Comparative analysis of Corazonin-encoding genes (Crz's) in Drosophila species and functional insights into Crz-expressing neurons." *Journal of Comparative Neurology* 482.4 (2005): 372-385.

Chou, Ya-Hui, et al. "Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe." *Nature neuroscience* 13.4 (2010): 439-449.

Couto, Africa, Mattias Alenius, and Barry J. Dickson. "Molecular, Anatomical, and Functional Organization of the *Drosophila* Olfactory System."*Current Biology* 15.17 (2005): 1535-1547.

Cropper, Elizabeth C., et al. "Structure, bioactivity, and cellular localization of myomodulin B: A novel *Aplysia* peptide." *Peptides* 12.4 (1991): 683-690.

Cropper, Elizabeth C., Ferdinand S. Vilim, Alexandros Alevizos, Renata Tenenbaum, Mary Ann Gawinowicz Kolks, Steven Rosen, Irving Kupfermann, and Klaudiusz R. Weiss. "Structure, Bioactivity, and Cellular Localization of Myomodulin B: A Novel Aplysia Peptide." *Peptides* 12.4 (1991): 683-90. Print.

Cropper, Elizabeth C., et al. "Structure, bioactivity, and cellular localization of myomodulin B: A novel *Aplysia* peptide." *Peptides* 12.4 (1991): 683-690.

Das, Abhijit, et al. "Drosophila olfactory local interneurons and projection neurons derive from a common neuroblast lineage specified by the empty spiracles gene." *Neural Dev* 3 (2008): 33.

Duffy, Joseph B. "GAL4 system in Drosophila: a fly geneticist's Swiss army knife." *genesis* 34.1-2 (2002): 1-15.

Fiala, André. "Olfaction and olfactory learning in *Drosophila*: recent progress." *Current opinion in neurobiology* 17.6 (2007): 720-726.

Finger, Thomas E. *International Symposium on Olfaction and Taste*. Vol. 21. John Wiley & Sons, 2009.

Gioti, Anastasia, et al. "Sex peptide of Drosophila melanogaster males is a global regulator of reproductive processes in females." *Proceedings of the Royal Society B: Biological Sciences* 279.1746 (2012): 4423-4432.

Goodwin, Stephen F. *Genetic dissection of neural circuits and behavior*. Vol. 65. Academic Press, 2009.

Hamada, Fumika N., et al. "An internal thermal sensor controlling temperature preference in Drosophila." *Nature* 454.7201 (2008): 217-220.

Hazelrigg, Tulle, Robert Levis, and Gerald M. Rubin. "Transformation of *white* locus DNA in Drosophila: Dosage compensation, *zeste* interaction, and position effects." *Cell* 36.2 (1984): 469-481.

Heale, V. R., K. Petersen, and C. H. Vanderwolf. "Effect of colchicine-induced cell loss in the dentate gyrus and Ammon's horn on the olfactory control of feeding in rats." *Brain research* 712.2 (1996): 213-220.

Hergarden, Anne Christina, Timothy D. Tayler, and David J. Anderson. "Allatostatin-A neurons inhibit feeding behavior in adult Drosophila."*Proceedings of the National Academy of Sciences* 109.10 (2012): 3967-3972.

Huetteroth, Wolf, and Scott Waddell. "Hungry Flies Tune to Vinegar." *Cell* 145.1 (2011): 17-18.

Husch, A., M. Paehler, D. Fusca, L. Paeger, and P. Kloppenburg. "Distinct Electrophysiological Properties in Subtypes of Nonspiking Olfactory Local Interneurons Correlate With Their Cell Type-Specific Ca2 Current Profiles." *Journal of Neurophysiology* 102.5 (2009): 2834-845. Print.

Hussain, Ashiq, et al. "High-affinity olfactory receptor for the death-associated odor cadaverine." *Proceedings of the National Academy of Sciences* 110.48 (2013): 19579-19584.

Ignell, Rickard, et al. "Presynaptic peptidergic modulation of olfactory receptor neurons in Drosophila." *Proceedings of the National Academy of Sciences*106.31 (2009): 13070-13075.

Jefferis, Gregory SXE, and Thomas Hummel. "Wiring specificity in the olfactory system." *Seminars in cell & developmental biology*. Vol. 17. No. 1. Academic Press, 2006.

Jones, Walton D., et al. "Two chemosensory receptors together mediate carbon dioxide detection in Drosophila." *Nature* 445.7123 (2006): 86-90.

Joseph, Robert M., et al. "Why is visual search superior in autism spectrum disorder?." *Developmental science* 12.6 (2009): 1083-1096.

Kaupp, U. Benjamin. "Olfactory signalling in vertebrates and insects: differences and commonalities." *Nature Reviews Neuroscience* 11.3 (2010): 188-200.

Kim, Young-Joon, et al. "MIPs are ancestral ligands for the sex peptide receptor." *Proceedings of the National Academy of Sciences* 107.14 (2010): 6520-6525.

Kwon, Jae Young, et al. "The molecular basis of CO2 reception in Drosophila."*Proceedings of the National Academy of Sciences* 104.9 (2007): 3574-3578.

McGann, John P., et al. "Odorant representations are modulated by intra-but not interglomerular presynaptic inhibition of olfactory sensory neurons." *Neuron*48.6 (2005): 1039-1053.

Moss, Cynthia F., and V. G. Dethier. "Central nervous system regulation of finicky feeding by the blowfly." *Behavioral neuroscience* 97.4 (1983): 541.

Murphy, Gabe J., Daniel P. Darcy, and Jeffry S. Isaacson. "Intraglomerular inhibition: signaling mechanisms of an olfactory microcircuit." *Nature neuroscience* 8.3 (2005): 354-364.

Nässel, Dick R., and Åsa ME Winther. *Drosophila* neuropeptides in regulation of physiology and behavior." *Progress in neurobiology* 92.1 (2010): 42-104.

Nässel, Dick R., and Uwe Homberg. "Neuropeptides in interneurons of the insect brain." *Cell and tissue research* 326.1 (2006): 1-24.

Nusbaum, Michael P., et al. "The roles of co-transmission in neural network modulation." *Trends in neurosciences* 24.3 (2001): 146-154.

Olsen, Shawn R., Vikas Bhandawat, and Rachel I. Wilson. "Excitatory Interactions between Olfactory Processing Channels in the *Drosophila* Antennal Lobe." Neuron 54.1 (2007): 89-103.

Olsen, Shawn R., and Rachel I. Wilson. "Lateral presynaptic inhibition mediates gain control in an olfactory circuit." *Nature* 452.7190 (2008): 956-960.

Pinel, John PJ, Boris B. Gorzalka, and Ferial Ladak. "Cadaverine and putrescine initiate the burial of dead conspecifics by rats." *Physiology & behavior* 27.5 (1981): 819-824.

Riffell, Jeffrey A. "Neuroethology: Lemon-Fresh Scent Makes Flies Lay Eggs. "*Current Biology* 23.24 (2013): R1108-R1110.

Rodrigues, Veronica, and Thomas Hummel. "Development of the Drosophila olfactory system." *Brain development in Drosophila melanogaster*. Springer New York, 2008. 82-101.

Rolen, S. H., et al. "Differential localization of NT-3 and TrpM5 in glomeruli of the olfactory bulb of mice." *Journal of Comparative Neurology* 522.8 (2014): 1929-1940.

Rolls, Edmund T., Fabian Grabenhorst, and Benjamin A. Parris. "Neural systems underlying decisions about affective odors." *Journal of Cognitive Neuroscience* 22.5 (2010): 1069-1082.

Rolls, Edmund T., et al. "Selective attention to affective value alters how the brain processes olfactory stimuli." *Journal of cognitive neuroscience* 20.10 (2008): 1815-1826.

Root, Cory M., et al. "Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search." *Cell* 145.1 (2011): 133-144.

Sato, Koji, et al. "Insect olfactory receptors are heteromeric ligand-gated ion channels." *Nature* 452.7190 (2008): 1002-1006.

Schloegl, Haiko, et al. "Peptide hormones regulating appetite—focus on neuroimaging studies in humans." *Diabetes/metabolism research and reviews*27.2 (2011): 104-112.

Seki, Yoichi, et al. "Physiological and morphological characterization of local interneurons in the Drosophila antennal lobe." *Journal of neurophysiology* 104.2 (2010): 1007-1019.

Seki, Yoichi, and Ryohei Kanzaki. "Comprehensive morphological identification and GABA immunocytochemistry of antennal lobe local interneurons in Bombyx mori." *Journal of Comparative Neurology* 506.1 (2008): 93-107.

Siju, K. P., et al. "Influence of blood meal on the responsiveness of olfactory receptor neurons in antennal sensilla trichodea of the yellow fever mosquito, *Aedes aegypti*." *Journal of insect physiology* 56.6 (2010): 659-665.

Simpson, Julie H. "Mapping and manipulating neural circuits in the fly brain."*Advances in genetics* 65 (2009): 79-143.

Stocker, Reinhard F. "The organization of the chemosensory system in Drosophila melanogaster: a rewiew." *Cell and tissue research* 275.1 (1994): 3-26.

Tanimoto, Hiromu, Yoshinori Aso, Igor Siwanowicz, Lasse Braecker, Chang Liu, Kei Ito, and Toshihiro Kitamoto. "Differential Dopaminergic Circuits for the Formation of Olfactory Memory in Drosophila." *Neuroscience Research* 68 (2010): E26.

Tayler, Timothy D., et al. "A neuropeptide circuit that coordinates sperm transfer and copulation duration in Drosophila." *Proceedings of the National Academy of Sciences* 109.50 (2012): 20697-20702.

Veenstra, Jan A. "Does corazonin signal nutritional stress in insects?." *Insect biochemistry and molecular biology* 39.11 (2009): 755-762.

Vosshall, Leslie B., Allan M. Wong, and Richard Axel. "An olfactory sensory map in the fly brain." *Cell* 102.2 (2000): 147-159.

Vosshall, Leslie B., and Reinhard F. Stocker. "Molecular Architecture of Smell and Taste in." *Annual Review of Neuroscience* 30.1 (2007): 505-33. Print.

Wicher, Dieter, et al. "Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels." *Nature* 452.7190 (2008): 1007-1011.

Winther, Åsa ME, et al. "Neuronal expression of tachykinin-related peptides and gene transcript during postembryonic development of Drosophila." *Journal of Comparative Neurology* 464.2 (2003): 180-196.

Yang, Chung-hui, et al. "Drosophila egg-laying site selection as a system to study simple decision-making processes." *Science* 319.5870 (2008): 1679-1683.

Yapici, Nilay, et al. "A receptor that mediates the post-mating switch in Drosophila reproductive behaviour." *Nature* 451.7174 (2007): 33-37.

Yew, Joanne Y., et al. "Analysis of neuropeptide expression and localization in adult Drosophila melanogaster central nervous system by affinity cell-capture mass spectrometry." *Journal of proteome research* 8.3 (2009): 1271-1284.

Wang, Yonghua, Yuhan Pu, and Ping Shen. "Neuropeptide-Gated Perception of Appetitive Olfactory Inputs in Drosophila Larvae." *Cell Reports* 3.3 (2013): 820-30. Print.

Wilson, Rachel I. "Early olfactory processing in Drosophila: mechanisms and principles." *Annual review of neuroscience* 36 (2013): 217.

Name und Vorname: _____

Titel der Arbeit:_____

_____

Fachgebiet: _____

Institution, an der die Arbeit angefertigt wurde:

_____

Betreuer/in der Arbeit bzw. Gutachter/in bei externen Arbeiten:

_____

Unterschrift Betreuer/in: _____

Datum des Beginns der Arbeit:_____

Datum der Abgabe der Arbeit beim Prüfungsamt: _____

_____

Unterschrift des Vorsitzenden des Prüfungsausschusses oder
seines Bevollmächtigten