

Modulation of Olfactory Information in  
the Antennal Lobe of *Spodoptera*  
*littoralis*

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### Abstract

In order to respond appropriately to external stimuli, e.g. odours in the environment, insects have to evaluate these stimuli in a context of relevance and need. These decisions are, in turn, based on internal physiological status, such as mating status.

In the cotton leafworm, *Spodoptera littoralis*, mating induces profound physiological changes that alter the behavioural output to fit with the current needs of the animal. In female *S. littoralis*, mating switches the behavioural attraction from food sources to oviposition sites and concurrently down- and up-regulates olfactory neuron sensitivity. This switch in olfactory sensitivity is correlated with a transient increase in dopamine levels in the primary olfactory brain centres, the antennal lobes.

In male *S. littoralis*, mating induces a transient behavioural and olfactory inhibition towards female-emitted sex pheromone as well as to mating site-related odours. However, responses towards food sources remain constant. The shift in olfactory responsiveness is not correlated with changes in dopamine levels, but may be regulated by neuropeptides expressed in the olfactory system. Distribution patterns of 6 of the most abundant neuropeptides reveal distinct differential expressions within the antennal lobe glomerular array, providing the possibility for selective and state-dependent modulation of the olfactory system.

*Keywords:* *Spodoptera littoralis*, olfaction, modulation, mating, neuromodulators

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To my family...

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I A.M. Saveer, S.H. Kromann, G. Birgersson, M. Bengtsson, T. Lindblom, A. Balkenius, B.S. Hansson, P. Witzgall, P.G. Becher and R. Ignell (2012).  
Floral to green: mating switches moth olfactory coding and preference.  
*Proc. R. Soc. B* 279(1737), 2314-2322.
- II A-E. Félix, S.H. Kromann, J-B. Gramsbergen, B.S. Hansson, R. Ignell.  
Dopamine regulates the mating switch in olfactory coding and preference in a female moth (manuscript).
- III S.H. Kromann, A.M. Saveer, M. Binyameen, M. Bengtsson, G. Birgersson, B.S. Hansson, F. Schlyter, P. Witzgall, R. Ignell and P. G. Becher.  
Concurrent modulation of olfactory responses to sex and host plant cues in a male moth (manuscript).
- IV S.H. Kromann, B.S. Hansson and R. Ignell. Distribution of neuropeptides in the antennal lobes of male *Spodoptera littoralis* (manuscript).

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The contribution of Anne Sophie Haastrup Kromann to the papers included in this thesis was as follows:

- I Conducted and analysed the optical imaging experiments and contributed to the writing of the article. AM Saveer and ASH Kromann share joint first authorship.
- II Conducted and analysed the tissue preparation for HPLC, conducted and analysed the optical imaging experiments and co-wrote the manuscript. A-E Felix and ASH Kromann share joint first authorship.
- III Conducted and analysed the EAG and optical imaging experiments and wrote the manuscript. ASH Kromann and AM Saveer share joint first authorship.
- IV Conducted and analysed all the experiments and wrote the manuscript.







# 1 Introduction

Olfaction is an essential sensory modality for the survival and reproduction of many animals, especially insects. Food sources, mates and oviposition sites are located and evaluated through the use of the olfactory apparatus. Despite growing knowledge of insect olfactory detection and processing very little is known about the modulation and regulation of the olfactory system.

The objective of this thesis was to explore modulation of the peripheral and central olfactory systems in the Egyptian cotton leafworm, *Spodoptera littoralis*. This was achieved by comparing olfactory properties, such as sensitivity, between unmated and mated insects of both sexes. Through the use of behavioural, physiological, pharmacological and neuroanatomical techniques I have begun to elucidate the modulatory effects of internal factors, specifically change in mating status, on insect olfaction.

## 2 The Cotton Leafworm, *Spodoptera littoralis*

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera, Noctuidae), is distributed in the Mediterranean countries of northern Africa and the Middle East. As a highly polyphagous insect, with larvae feeding on over 80 plant species from 40 different families, *S. littoralis* is a serious pest on agricultural and economical important crops, e.g. cotton (Brown & Dewhurst, 1975; Salama *et al.*, 1971).

Both male and female *S. littoralis* depend on olfactory cues to locate relevant resources in the environment. Such resources include nectar sources as well as possible mating and oviposition sites. Furthermore, males detect mating partners by tracking the female produced sex pheromone, consisting of at least 6 individual components, (*Z,E*)-9,11-tetradecadienyl acetate (*Z9,E11*-14:Ac), (*Z,E*)-9,12-tetradecadienyl acetate (*Z9,E12*-14:Ac), (*E*)-11-tetradecenyl acetate (*E11*-14:Ac), (*Z*)-11-tetradecenyl acetate (*Z11*-14:Ac), (*Z*)-9-tetradecenyl acetate (*Z9*-14:Ac) and tetradecyl acetate (14:Ac) (Malo *et al.*, 2000).

Using behavioural relevant odours, olfaction in *S. littoralis* has been studied at the peripheral (Binyameen *et al.*, 2012; Guerrieri *et al.*, 2012; Anderson *et al.*, 1995; Ochieng *et al.*, 1995) and the central nervous level (Guerrieri *et al.*, 2012; Carlsson & Hansson, 2003; Carlsson *et al.*, 2002; Anton & Hansson, 1995; Anton & Hansson, 1994) as well as at the behavioural output level (Andersson *et al.*, 2011; Anderson *et al.*, 2003; Fan & Hansson, 2001; Fan *et al.*, 1997).

## 3 The Insect Olfactory System

The olfactory system of insects consists of the antennae, labial- and maxillary palps, as well as the olfactory brain centres, constituting the peripheral and the central parts, respectively. On the flagellum of the antenna, as well as on the labial- and maxillary palps, there are cuticular processes covering the surface. These cuticular formations, sensilla, contain the olfactory receptor neurons (ORNs) responsible for the detection of information about the odorous environment (Todd & Baker, 1999). The axons of the ORNs project into the primary olfactory centres of the brain, the antennal lobes (ALs), where they converge onto spherical neuropil structures, glomeruli (Hansson & Anton, 2000; Todd & Baker, 1999). Local interneurons (LNs) transform the olfactory information before it is conveyed by projection neurons (PNs) to higher olfactory centres, including the mushroom bodies (MB) and the lateral protocerebrum (Hansson & Anton, 2000; Anton & Homberg, 1999). The MBs and the lateral protocerebrum each comprise a second order brain centre where olfactory input is further processed and integrated with other sensory modalities to produce a behavioural output.

### 3.1 Peripheral Olfactory System

Insect antennae come in a variety of shapes and sizes but can generally be subdivided into 3 segments; the scapus, the pedicellus and the flagellum (Keil, 1999). The flagellum carries most of the olfactory sensilla yet other sensilla can be found on the labial- or maxillary palps (Keil, 1999). The number of antennal sensilla is dependent on the insects' lifestyle, where males of some insect species often have more sensilla compared to females reflecting the sexual dimorphic need to detect sex pheromone cues (Blaney & Simmonds, 1990). Irrespectively, adult insect antennae can carry tens of thousands of

sensilla ensuing high functional fidelity when “sieving” through the odorous environment (Keil, 1999).

### 3.1.1 Olfactory Sensilla

#### *Morphology*

Insect olfactory sensilla can be distinguished based on their external morphology. Although their morphology may vary greatly, the sensilla may be classified as either single walled, including e.g. *sensilla trichodea* and *s. basiconica*, or double walled, e.g. *s. coeloconica*, where all have pores along the shaft of their cuticular wall (Keil, 1999).

The internal morphology of the sensilla is characterised by a lymph-filled lumen, a varying number of bipolar ORNs and auxiliary cells (Keil, 1999). The ORNs are characterised by a soma that apically projects into the sensillum lymph where the outermost part of the dendrite forms a modified cilium (Keil, 1999). In certain sensilla, the cilium remains unbranched (e.g. *s. trichodea*) yet in other sensilla the cilium gives rise to several branches (e.g. *s. basiconica*) (Keil, 1999). The olfactory receptors (ORs), the proteins that selectively bind the odour molecules, are expressed in the membrane of the cilium, as well as other receptors and ion channels (Stengl, 2010; Keil, 1999). Surrounding the ORN somata are three auxiliary cells, including the thecogen, the tricogen and the tormogen cells. The auxiliary cells wrap tightly around the ORNs and ensure that the sensillum lumen is physically and chemically separated from the rest of the internal antennal environment (Keil, 1999; Keil & Steinbrecht, 1987; Keil, 1984). Furthermore, the auxiliary cells produce and excrete the sensillum lymph, which is characterised by a high concentration of  $K^+$  as well as a high concentration of Odour Binding Proteins (OBPs), Pheromone Binding Proteins (PBPs) and Odour Degrading Enzymes (ODEs) that are involved in the olfactory perireceptor events (Keil, 1999; Stengl *et al.*, 1999).

#### *Function - Perireceptor events*

The structure and internal organisation and environment of the olfactory sensilla make them high fidelity detectors of odours. Once through the pores in the sensillum cuticle, the odour molecules are hypothesised to bind to OBPs or PBPs making otherwise lipophilic molecules more hydrophilic (Stengl, 2010; Kaissling, 2009; Stengl *et al.*, 1999). This complex of odour molecule and OBP/PBP then traverses the sensillum lymph to the olfactory receptors located on the ORN cilium. At the receptor site, the odour molecule binds to the receptor and induces transduction (Vosshall & Hansson, 2011). It is currently

unclear whether the OBP/PBPs also binds to the receptor or dissociates from the odour molecule before receptor activation (Kaissling, 2009; Stengl *et al.*, 1999).

After receptor activation it is paramount to have a fast termination of the response and removal of the odour molecule to allow for subsequent activation by the next stimulus (Kaissling, 2009; Stengl *et al.*, 1999). However, it is not yet clear how this deactivation occurs; some experiments points to OBP/PBPs being involved in odour deactivation whereas other experiments suggest the involvement of esterases, the ODEs (Kaissling, 2009; Stengl *et al.*, 1999).

#### *Function - Transduction*

Insect ORs span the membrane with 7 transmembrane (7TM) domains but do not share sequence similarities with conventional 7TM receptors, other G-protein coupled receptors or vertebrate ORs (Silbering & Benton, 2010; Nakagawa & Vosshall, 2009). Furthermore, evidence suggests that insect ORs have an inverted topology so that the generic G-protein binding motif is located extracellularly (Benton *et al.*, 2006).

It is believed that each ORN only expresses one type of OR (Couto *et al.*, 2005; Nef *et al.*, 1992) alongside the ubiquitous chaperone co-receptor, “Orco” (Vosshall & Hansson, 2011; Nakagawa *et al.*, 2005; Larsson *et al.*, 2004; Pitts *et al.*, 2004; Krieger *et al.*, 2003). Together, Orco and the expressed OR form a heteromeric complex that encompasses both an ionotropic (direct ion channel) and a metabotropic (through second messenger systems) mechanisms for signal transduction. Upon odour stimulation of the Orco/OR complex, induction of ionotropic and metabotropic currents results in a graded receptor potential that, at the spike initiation zone of the ORN, either increases the spike activity (excitation) or decreases spike activity (inhibition) (Rospars *et al.*, 2010; Stengl, 2010; Nakagawa & Vosshall, 2009; Wicher *et al.*, 2008).

A common property of ORNs is that of selectivity and tuning. An ORN is said to be tuned to an odour molecule or a type of odour molecules when the ORN responds with the lowest threshold to that molecule (Todd & Baker, 1999). The tuning of an ORN is imposed by the particular OR type expressed on the cilium membrane and how strong an affinity the receptor has to different odour molecules. An odour identity is encoded by ORNs with overlapping tuning spectra responding to the odour with different intensities through a so-called “across fibre pattern” (Todd & Baker, 1999). The tuning of ORNs can vary greatly; pheromone-responding ORNs often respond only to one odour compound whereas certain plant-odour-responding ORNs often respond to several odours, e.g. alcohol or aldehydes (Todd & Baker, 1999).

Another aspect of ORN functionality is their sensitivity to odour concentrations. Insect ORNs have been suggested to function as flux detectors, reporting changes in abundance over time (Kaissling, 1998). But as with odour identity, odour concentration cannot be encoded by a single ORN; a decrease in firing activity could equally mean a lower concentration of an odour to which the receptor has a high affinity as well as a high concentration of an odour to which the receptor has a lower affinity (Todd & Baker, 1999). Rather, changes in concentrations are encoded by changes in spike frequencies between several ORNs where relative ratios of frequencies remain constant (Todd & Baker, 1999).

### 3.2 Central Olfactory System

The insect antennal lobes (ALs) receive input from the antennae and the labial- or maxillary labial palps (Anton & Homberg, 1999). The ALs are divided into spheroidal neuropilar structures, glomeruli, where the ORN axons make synaptic contacts with AL interneurons. The number of glomeruli is species-specific, where e.g. moths have been shown to have approximately 60 glomeruli (Hansson & Anton, 2000; Rospars & Hildebrand, 2000; Anton & Homberg, 1999). The glomeruli are arranged in one or more layers around a central fibrous core within the AL (Hansson & Anton, 2000; Anton & Homberg, 1999). In moths, as well as in some other insect taxa, one or several glomeruli are sexually dimorphic. The dimorphic enlarged glomeruli in male moths, located close to the antennal nerve entrance, constitute the macroglomerular complex (MGC) and have been shown to receive input from sex-pheromone sensitive ORNs (Hansson & Anton, 2000; Anton & Homberg, 1999). Below the MGC are the sexually isomorphic ordinary glomeruli (OG) that receive input from non-pheromonal, plant-related odour sensitive ORNs (Anton & Homberg, 1999; Hansson & Christensen, 1999). Hence, with specifically tuned ORNs innervating specific glomeruli, each glomerulus becomes a physical landmark of an odour molecule or class of molecules. Consequently, glomeruli collectively create a chemotopic map of the odour representation (Hansson & Christensen, 1999).

Surrounding the ALs are groups of cell bodies that in moths usually make up three clusters, including a lateral, a medio-dorsal and an antero-ventral cluster. However, in certain moth species, the lateral and the antero-ventral cell clusters are fused into one (Anton & Homberg, 1999). The cellular elements of the cell clusters comprise three different neuron types; PNs, LNs and centrifugal neurons (CNs) (Anton & Homberg, 1999). Of these three neuronal types, the antennal lobe interneurons (PNs and LNs) are responsible for



organising the olfactory information from the antennae by selectively integrating the synaptic input from the ORNs (Hansson & Christensen, 1999) and the PNs then convey the transformed neural representations of the odour to higher order brain centres (Anton & Homberg, 1999; de Belle & Kanzaki, 1999; Hansson & Christensen, 1999).

### 3.2.1 Antennal Lobe Local Interneurons

Local interneurons are confined to the ALs and have their cell bodies in cell clusters at the periphery of the AL (Anton & Homberg, 1999). In moths, 300-400 LN somata are situated mainly in the large lateral cell cluster (Anton & Homberg, 1999; Homberg *et al.*, 1988). Three morphologically different subtypes of LNs have been demonstrated in moths: (1) multiglomerular LNs with homogeneous arborisations throughout the AL; (2) multiglomerular LNs with heterogeneous, asymmetrical arborisations; and (3) oligoglomerular LNs with arborisations in only a few glomeruli (Anton & Hansson, 1994; Hansson *et al.*, 1994; Christensen *et al.*, 1993; Matsumoto & Hildebrand, 1981).

### 3.2.2 Antennal Lobe Projection Neurons

In moths, PNs have their somata located mainly in the medial cell cluster and have been shown to have uni- and multiglomerular dendritic arborisations within the AL (Anton & Homberg, 1999). The axons of PNs leave the ALs through one of several antenno-cerebral tracts, with the most prominent one being the inner antenna-cerebral tract (IACT) followed by the middle, the outer and the dorsal antenno-cerebral tracts (M-, O- and D-ACT, respectively) (Hansson & Anton, 2000; Anton & Homberg, 1999).

Several subtypes of PNs have been described for moths based on the projection pattern and pattern of arborisation within the ALs and the protocerebrum (Anton & Homberg, 1999). Projection neurons found in the IACT arborise in the calyces of the ipsilateral MB and/or the ipsilateral protocerebrum, whereas PNs found in the MACT project solely to areas in the ipsilateral protocerebrum. Outer-ACT PNs project to the calyces of the ipsilateral MB and to both the ipsi- and contralateral protocerebra (Anton & Homberg, 1999). Projection neurons found in the DACT have only been described for *M. sexta* and are characterised by having their somata in a protocerebral cell cluster near the AL but having dendritic arborisations in the contralateral AL (Kanzaki *et al.*, 1989; Homberg *et al.*, 1988).

### 3.2.3 Olfactory Processing in the Antennal Lobes

The major function of the antennal lobe circuitry is to receive odour information from the ORNs and re-shape this into meaningful neural representations that can be “read” and further processed by higher order brain centres (Hansson & Christensen, 1999). The ALs are the first brain centre where molecular information about odours is integrated and where spatio-temporal characteristics of this information can be accentuated or attenuated (Hansson & Anton, 2000; Hansson & Christensen, 1999). With ORNs converging on the AL glomeruli, interneurons are presented with input that varies in quality, quantity, space and time. These parameters relate to natural, intermittent odour plumes and where and which antennae they hit. The integration of odour signals then becomes a matter of combining this information into activity patterns that reflect odour identity and concentration as well as timing of detection (Hansson & Christensen, 1999).

Based on the ORN tuning specificity, two types of information can be relayed to the ALs: highly specific information regarding single compounds or more generalised information about classes of odour molecules. This has led to two hypothetical coding schemes: “labelled-line” and “across-fibre pattern” (Hansson & Christensen, 1999). In “labelled-line” coding, specifically tuned ORNs terminate in specific glomeruli. Hence, encoding and identification of an odour then becomes a matter of recognising which glomerulus is activated. Contrary, in “across-fibre pattern” coding, where ORNs with overlapping, broad tuning spectra result in activation of several glomeruli, the odour identity is encoded by the pattern of activation of ensembles of glomeruli (Hansson & Christensen, 1999). However, it should be noted that, based on empirical evidence, neither coding scheme alone can fully explain how information is represented in the ALs and a hybrid model, “across-label coding” for odour encoding has been suggested (Christensen & White, 2000).

Most LNs are GABAergic, i.e. produce and release  $\gamma$ -amino butyric acid to the synaptic cleft, thereby exerting an inhibitory action on the postsynaptic neurons (Christensen *et al.*, 1998b; Christensen *et al.*, 1998a; Christensen *et al.*, 1993; Waldrop *et al.*, 1987). A commonly accepted theory is that periods of inhibition, caused by the GABAergic LNs, in odour-evoked responses represent lateral interactions between AL neurons. This can lead to contrast-enhancement where lateral inhibition accentuates odour signals processed in one information stream while attenuating odour signals processed in a neighbouring stream (Sachse & Galizia, 2002; Hansson & Christensen, 1999). Yet, in the vinegar fly *Drosophila melanogaster*, cholinergic, excitatory LNs have recently been found (Bhandawat *et al.*, 2007; Olsen *et al.*, 2007; Shang *et al.*, 2007). The presence of both GABAergic and cholinergic LNs indicate that

these neurons may function as a neural substrates for gain control, to enhance weak signals, and to dampen strong signals to ensure high fidelity of the olfactory system (Olsen & Wilson, 2008; Bhandawat *et al.*, 2007; Olsen *et al.*, 2007; Shang *et al.*, 2007; Wilson & Laurent, 2005; Wilson *et al.*, 2004). Due to the oligo- and multiglomerular arborisation patterns, LNs have been suggested to act in cross-talk between glomeruli (Hansson & Christensen, 1999), where lateral interactions of excitatory and inhibitory interactions serves to broaden the tuning spectrum of the entire system (Wilson & Laurent, 2005; Friedrich *et al.*, 2004; Friedrich & Laurent, 2004; Stopfer *et al.*, 2003; Friedrich & Laurent, 2001).

### 3.2.4 Protocerebral Olfactory Centres

The protocerebrum consists mostly of seemingly unstructured neural networks except for the MBs and the central complex (CC) (de Belle & Kanzaki, 1999). Even so, these neuronal networks have on several occasions been shown to be composed of accurately arranged neurons both in relation to position of cells and the pattern of their arborisations (de Belle & Kanzaki, 1999; Kanzaki *et al.*, 1991). The protocerebral centres integrate input from different sensory centres, including but not limited to olfactory centres like the ALs, and coordinate motor and behavioural output (de Belle & Kanzaki, 1999).

#### *Morphology and function*

The prominent insect MBs consist of the cup-shaped calyces, the pedunculus and the  $\alpha/\alpha'$  and  $\beta/\beta'/\gamma$  lobes (de Belle & Kanzaki, 1999). The MBs receive chemosensory and mechanosensory input originating from e.g. the ALs, the suboesophageal ganglion (SOG) and thoracic ganglia. Furthermore, projections from the visual centres have been reported from flies and honeybees (de Belle & Kanzaki, 1999). The MB intrinsic cells, the Kenyon cells (KCs), have their somata directly on top of the calyces and project their dendrites into the calyces. The KC axons project through the calyces and into the pedunculus and terminate in either of the lobes. From the MB lobes, two distinct tracts of output neurons connect the MBs with the lateral protocerebrum (de Belle & Kanzaki, 1999).

The lateral protocerebrum (LPR) consists of a large number of unstructured neurons receiving extensive input from the MBs but also from other brain centres like the ALs, and the visual and mechanosensory centres (de Belle & Kanzaki, 1999). From the LPR, output neurons project mainly to motor centres and the thoracic ganglia but projections can be found throughout most of the brain as well (de Belle & Kanzaki, 1999).

Both the CC and the lateral accessory lobes (LAL) receive input from LPR, with the LAL receiving additional input from the CC. Both structures send axons into the thoracic ganglia and arborise within thoracic motor centres (de Belle & Kanzaki, 1999).

Based on connectivity, the protocerebral olfactory neurons are likely involved in the integration of olfactory information with information from other sensory modalities such as mechanosensation and vision. The integrated information from the protocerebral centres is then relayed to motor output centres in the thoracic ganglia producing a behavioural output (de Belle & Kanzaki, 1999).

Interestingly, protocerebral centres are not just relay stations for the integration of sensory inputs. The MBs have been demonstrated to be involved in olfactory memory formation and storage as well as olfactory learning (Blackiston *et al.*, 2008; Strausfeld *et al.*, 1998; Fan *et al.*, 1997; Mercer & Flanagan, 1988; Mercer & Menzel, 1982).

## 4 Modulation of the Olfactory System

For the insect to be able to accommodate constant changes in both the external and the internal environment, the nervous system has to be able to change accordingly. A fast and reversible way of achieving such changes is by modulating the olfactory system through the use of neuroactive chemicals, i.e. neuromodulators.

### 4.1 Neuromodulation

Alongside conventional neurotransmitters, such as acetylcholine and GABA, insect olfactory neurons are known to produce a large number of neuroactive substances (Nüssel & Homberg, 2006; Nüssel, 2002; Homberg & Müller, 1999). These neuromodulators, including biogenic amines and neuropeptides, are known to be widely distributed within the olfactory system, especially the ALs and the MBs (Heuer *et al.*, 2012; Nüssel & Winther, 2010; Nüssel & Homberg, 2006; Homberg & Müller, 1999). However, direct physiological function within the olfactory system has so far only been attributed to a small subset of these neuromodulators (Root *et al.*, 2011; Ignell *et al.*, 2009; Dacks *et al.*, 2008).

Biogenic amines and neuropeptides are thought to be co-released with the conventional neurotransmitters and involved in synaptic transmission of information. Both biogenic amines and neuropeptides are believed to act through G-protein coupled receptors (GPCRs / metabotropic receptors) with a second messenger system that involves up- or down-regulation of cytosolic cyclic adenosine monophosphate (cAMP) (Schlenstedt *et al.*, 2006; Johnson *et al.*, 2003; Hewes & Taghert, 2001; Osborne, 1996; Bodnaryk, 1982).

## 4.2 Biogenic Amines

Over the last couple of decades, increasing amounts of information about biogenic amines have emerged. Distribution, pharmacological and biochemical analyses have revealed the presence and functionality of several amines (Osborne, 1996; Roeder, 1994), many of which have also been suggested to function as neuromodulators in the ALs (Homberg & Müller, 1999). Through immunolabelling studies, the biogenic amines have been suggested to be expressed in both LNs and/or CNs (Homberg & Müller, 1999).

Strictly speaking, biogenic amines are any compound produced by life processes and contain one (or more) amine moiety, e.g. acetylcholine, melatonin, histamine, serotonin, dopamine, octopamine and tyramine. However, in the following part only serotonin, dopamine, octopamine and tyramine will be discussed.

### 4.2.1 Serotonin

5-hydroxytryptamine (serotonin) is widely spread throughout the insect nervous system (Nässel, 1988), and has been implicated in many physiological processes, e.g. development (Taylor *et al.*, 1992), peripheral and central olfactory modulation (Dacks *et al.*, 2008; Kloppenburg & Mercer, 2008; Siju *et al.*, 2008; Hill *et al.*, 2003; Hill *et al.*, 2002; Kloppenburg *et al.*, 1999; Macmillan & Mercer, 1987; Linn & Roelofs, 1986), olfactory learning (Wright *et al.*, 2010; Mercer & Flanagan, 1988; Mercer & Menzel, 1982) as well as hearing, vision and mechanosensation (Homberg, 2002; Osborne, 1996) (for further information see Table 1).

In the olfactory system, processes from serotonin-producing neurons have been found in the ALs across insect taxa although the arborisation pattern varies between individual species (Homberg & Müller, 1999). In certain species of cockroaches, beetles and locusts, these serotonergic neurons are ascending from the ALs into the protocerebrum (Homberg, 2002; Homberg & Müller, 1999; Breidbach, 1990). However, in moths and some cockroaches, these neurons have changed direction to become descending neurons from protocerebral centres and likely act as feedback circuits from higher order brain centres (Sun *et al.*, 1993; Salecker & Distler, 1990).

In the moths, serotonin has been shown to alter  $K^+$  conductances in AL neurons leading to spike broadening and increased input resistance of individual neurons (Kloppenburg & Mercer, 2008; Mercer *et al.*, 1996; Mercer *et al.*, 1995), as well as increased odour and concentration discrimination in ORNs (Dacks *et al.*, 2008; Grosmaître *et al.*, 2001; Kloppenburg *et al.*, 1999).

Table 1. Biogenic amines in insects

<b>Biogenic Amine</b>	<b>Species</b>	<b>Receptor type</b>	<b>Receptor effectuation</b>	<b>Tissue</b>	<b>Sensory modality</b>	<b>Physiological process</b>	<b>Reference</b>		
<b>Dopamine</b>	<i>Schistocerca gregaria</i>	Metabotropic	Activation of AC	CNS	Vision	Potentiation of flight motor pattern	Osborne 1996		
	<i>Apis mellifera</i>			PNS	Taste				
	<i>Locusta migratoria</i>			Salivary glands	Olfaction			Motor output	Homberg 2002
	<i>Drosophila melanogaster</i>			Flight muscle	Mechanosensation			Salivary secretion	
	<i>Rhodnius prolixus</i>			Visceral muscle				Gut contraction	
	<i>Blaberus giganteus</i>			Locomotion					
	<i>Culex pipiens</i>			AKH release					
<b>Octopamine</b>	<i>Leucophaea maderae</i>	Metabotropic	Activation of AC	CNS	Proprioception	Learning and memory	Roeder 1999		
	<i>Glossina morsitans</i>			Flight muscle	Hearing	Rhythmic behaviours	Osborne 1996		
	<i>L. migratoria</i>			Increase in $[Ca^{2+}]_{cytosol}$	Endocrine organs	Taste	Desensitisation	Farooqui 2007	
	<i>Manduca sexta</i>			Inactivation of AC in antennae and brain of <i>H. virescens</i> and <i>B. mori</i>	Air sacs	Vision	Motivation		
	<i>Periplaneta americana</i>			Visceral muscle	Olfaction	Locomotion			
	<i>S. gregaria</i>			Ovipositor	<i>Pheromone response</i>	Glycogenolysis			
	<i>Spodoptera littoralis</i>			Fat body	Mechanosensation	Sting response			
	<i>Heliothis virescens</i>			Hemocytes	Feeding behaviour				
	<i>Bombyx mori</i>			Compound eyes					
	<i>A. mellifera</i>			Ocelli					
	<i>D. melanogaster</i>			Chemosensory organs					
	<i>Phormia regina</i>			Chordotonal organs					
	<i>Acheta domesticus</i>								

Table 1 - continued.

<b>Biogenic Amine</b>	<b>Species</b>	<b>Receptor type</b>	<b>Receptor effectuation</b>	<b>Tissue</b>	<b>Sensory modality</b>	<b>Physiological process</b>	<b>Reference</b>
<b>Serotonin</b>	<i>S. gregaria</i>	Ionotropic	Potassium channels	CNS	Hearing	Suppression of flight motor pattern	Osborne 1996
	<i>L. migratoria</i>	Metabotropic	Sodium channels	PNS	Olfaction		
	<i>M. sexta</i>		Calcium channels	Chordotonal organs	<i>Pheromone response</i>	Motor output	Tierney 2001
	<i>Bombyx mori</i>		Activation of AC	Flight muscle	Vision	Salivary secretion	Homberg 2002
	<i>A. domesticus</i>		Inactivation of AC	Other muscles	Mechanosensation	Gut contraction	
	<i>D. melanogaster</i>		Activation of IP <sub>3</sub>	Visceral muscle			
	<i>P. americana</i>			Salivary glands			
	<i>R. prolixus</i>						
	<i>D. melanogaster</i>						
	<i>Teleogryllus commodus</i>						
<b>Tyramine</b>	<i>P. americana</i>	Metabotropic	Inactivation of AC	Fat body	Taste	Glycogenolysis	Osborne 1996
	<i>A. mellifera</i>		Increase in [Ca <sup>2+</sup> ] <sub>cytosol</sub>	Malpighian tubules	Vision	Diuresis	
	<i>B. mori</i>			Skeletal muscle	Olfaction	Locomotion	Lange 2009
	<i>L. migratoria</i>			Visceral muscle		♀ reproductive organ contraction	
	<i>D. melanogaster</i>			Pheromone gland		Pheromone production	
	<i>P. regina</i>			Flight muscle		Appetite	
	<i>Trichoplusia ni</i>			CNS		Flight	
				PNS			

AC, adenylate cyclase; AKH, adipokinetic hormone; [Ca<sup>2+</sup>]<sub>cytosol</sub>, cytosolic concentration of calcium; CNS, central nervous system; IP<sub>3</sub>, inositol triphosphate; PNS, peripheral nervous system



#### 4.2.2 Dopamine

Dopamine has been demonstrated in the PNS and CNS of several insect species and has been found to affect various sensory modalities, including vision, taste, mechanosensation and olfactory memory (Berry *et al.*, 2012; Homberg & Müller, 1999; Osborne, 1996; Mercer & Menzel, 1982). Furthermore, dopamine is known to regulate the metabolisms of the classic insect hormones, juvenile hormone and ecdysteroids (Rauschenbach *et al.*, 2011; Gruntenko & Rauschenbach, 2008; Gruntenko *et al.*, 2005a; Gruntenko *et al.*, 2005b) (for further information see Table 1).

The presence of dopaminergic neurons in the olfactory system of insects varies greatly among species (Homberg & Müller, 1999). However, in species where these neurons are found, evidence suggests that they function as wide-field CNs with dendritic arborisations in both the protocerebrum and the SOG and axonal terminations in the AL (Homberg & Müller, 1999; Schafer & Rehder, 1989).

Several dopamine-GPCRs have been found in the insect nervous system (Kahsai *et al.*, 2012; Blenau & Baumann, 2001; Vallone *et al.*, 2000; Kokay & Mercer, 1996; Roeder, 1994) and they appear to be connected to specific dopamine-sensitive adenylate cyclase (Bodnaryk, 1982; Bodnaryk, 1979a) regulating cAMP concentration and ion channel activity (Vallone *et al.*, 2000; Missale *et al.*, 1998).

#### 4.2.3 Octopamine

Octopamine is thought to act as a hormone, a modulator and a transmitter in many invertebrate species (Farooqui, 2007; Roeder, 1999). Octopamine has been found in both neuronal and non-neuronal tissue and is likely to modulate almost every physiological process in invertebrates (Farooqui, 2007; Roeder, 1999), including olfactory detection (Pophof, 2002; Grosmaître *et al.*, 2001; Pophof, 2000; Linn *et al.*, 1992; Linn & Roelofs, 1992; Roelofs & Linn, 1987; Linn & Roelofs, 1986) and learning and memory (Farooqui *et al.*, 2003; Menzel, 2001; Erber *et al.*, 1993; Menzel *et al.*, 1988). Octopamine is thought to act as a “stress-hormone” in the peripheral nervous system of insects, inducing increased muscle contraction, increased glycogenolysis and mobilization of lipids (Chapman, 1998; Orchard & Lange, 1984) (For further information see Table 1).

In the CNS, a small number of octopaminergic neurons, including ascending thoracic dorsal unpaired median (DUM) and ventral unpaired

median (VUM) neurons, supply most neuropils of the insect brain (Bräunig, 1991). These elaborate neurons send projections into the ALs, the MBs and in some insects also the lateral protocerebrum (Dacks *et al.*, 2005; Homberg & Müller, 1999; Kreissl *et al.*, 1994).

Octopaminergic GPCRs (Roeder, 1999) are found in the antennae as well as throughout the insect brain, especially in the MBs (Brigaud *et al.*, 2009; Han *et al.*, 1998; Roeder & Nathanson, 1993) and cause an increase of the intracellular second messenger cAMP and an activation of cAMP-dependent protein kinase A (Hildebrandt & Muller, 1995b; Hildebrandt & Muller, 1995a; Roelofs & Linn, 1987; Linn & Roelofs, 1986; Bodnaryk, 1982; Bodnaryk, 1979a; Bodnaryk, 1979b).

#### 4.2.4 Tyramine

Tyramine is a decarboxylation product of tyrosine and a precursor of octopamine (Roeder, 2005). Not much is known about the physiological function of tyramine in insects (Lange, 2009; Roeder, 2005). However, tyramine has been implicated in the muscle contraction of the locust oviduct (Lange, 2009; Donini & Lange, 2004). A mutant *Drosophila* line, *honoka*, with reduced tyramine receptor expression, shows few phenotypical differences compared with wild type flies. They are slightly hyperactive, impaired in certain olfactory tasks and show reduced tyramine-induced muscle contractions (Roeder, 2005; Nagaya *et al.*, 2002; Kutsukake *et al.*, 2000) (For further information see Table 1).

Tyraminergetic labelling has been found in the CNS, PNS and visceral muscle tissue, all stemming from a small set of DUM and VUM neurons located in the SOG and thoracic and abdominal ganglia (Lange, 2009; Downer *et al.*, 1993).

Tyramine receptor pharmacology suffers greatly from the lack of pharmacology tools and there are therefore only a few studies available (Roeder, 2005). The only pharmacological relevant antagonist, yohimbine, is a high-affinity antagonist of tyramine receptors and induces pharmacological effects similar to what is observed in flies with reduced expression of tyramine receptors (Roeder, 2005; Kutsukake *et al.*, 2000). Evidence suggests that, whereas octopamine induces an increase in cAMP and/or intracellular Ca<sup>2+</sup> concentration, tyramine induces a decrease in cAMP (Roeder, 2005; Roeder *et al.*, 2003).

### 4.3 Neuropeptides

Neuropeptides comprise a diverse group of signalling molecules and are thought to be involved in the regulation of several physiological processes. Other functions include inhibition of hormone production and myoinhibition (allatostatins), stimulation of hormone production (allatotropins) and myostimulatory (tachykinins) (Nässel & Winther, 2010) as well as functions as regulatory hormones in the heart, gut and reproductive tissue of insects (Spit *et al.*, 2012; Nässel & Winther, 2010; Clark *et al.*, 2008; Ejaz & Lange, 2008; Nässel & Homberg, 2006; Hill & Orchard, 2004; Clark & Lange, 2002; Lange, 2001; Fuse *et al.*, 1999; Kwok *et al.*, 1999). In addition, based on distribution studies, many neuropeptides have been implicated to be involved in olfaction (Nassel & Winther, 2010).

Neuropeptides with similar C-terminal ends from orthologous genes across species comprise a peptide family (Nässel & Winther, 2010; Nässel & Winther, 2002; Vanden Broeck, 2001), and the most prominent neuropeptide families characterised in insects are A-type allatostatins, allatotropins, FMRFamide-related peptides and tachykinin-related peptides (Nässel & Homberg, 2006; Schachtner *et al.*, 2005).

In the following section, a subset of the neuropeptide families, as relating to olfaction and olfactory processing, will be described.

#### 4.3.1 Tachykinins

Tachykinin has been shown to be expressed in AL LNs and a small subset of CNs in several insect species (Berg *et al.*, 2007; Neupert *et al.*, 2007; Nässel & Homberg, 2006; Schachtner *et al.*, 2005; Homberg & Müller, 1999). The high degree of similarity in tachykinin expression across taxa, especially amongst LNs, indicates an important function of tachykinin within the olfactory system (Nässel & Winther, 2010; Schachtner *et al.*, 2005).

Tachykinin is one of only two neuropeptides to have been functionally characterised within the olfactory system. Tachykinin signalling has been studied in *Drosophila* and tachykinin-releasing LNs have been shown to presynaptically inhibit ORNs and suppress synaptic transmission from ORNs. Furthermore, tachykinin-releasing LNs also modulate postsynaptic LN activity, possibly providing a release of inhibition in local circuits (Winther & Ignell, 2010). The combined action of tachykinin on ORNs and LNs has been shown to alter the olfactory-guided behavioural choices towards certain food-related odours (Winther & Ignell, 2010; Ignell *et al.*, 2009; Winther *et al.*, 2006).

#### 4.3.2 FMRFamide-Related Peptides

The FMRFamide-related peptide (FaRP) superfamily includes the extended FMRFamides, the myosuppressins, short neuropeptide F (sNPF) and the sulfakinins (Predel *et al.*, 2010; Marder *et al.*, 1987). Immunocytochemical analyses, using antibodies against this family, have revealed extensive labelling of LNs, PNs and/or CNs across several species (Berg *et al.*, 2007; Neupert *et al.*, 2007; Nässel & Homberg, 2006; Iwano & Kanzaki, 2005; Schachtner *et al.*, 2005; Homberg *et al.*, 1990). This evolutionary conserved feature of FaRPs indicates a significant role of this family of neuropeptides in olfactory processing and modulation (Schachtner *et al.*, 2005). However, no true olfactory function has yet been attributed any of these peptides, except for sNPF. Recently, sNPF signalling has been shown to presynaptically modulate ORNs, facilitating enhanced ORN activity (Root *et al.*, 2011). Short neuropeptide F and insulin were demonstrated, through the expression of the sNPF receptor, to modulate odour-driven food search in starvation/satiety dependent behaviours (Root *et al.*, 2011).

#### 4.3.3 Allatotropin

Allatotropin has only been studied in a few insect species, including cockroaches (Neupert *et al.*, 2012; Homberg & Müller, 1999), locusts (Homberg *et al.*, 2004; Ignell, 2001) and moths (Berg *et al.*, 2007; Schachtner *et al.*, 2005; Schachtner *et al.*, 2004). However, where allatotropin expression within the olfactory system has been reported, the distribution pattern looks strikingly similar, with only minor differences in the actual number of labelled cells (Schachtner *et al.*, 2005). So far, no olfactory function has been established.

#### 4.3.4 Allatostatin

Gene products of insect A-type allatostatins vary greatly in numbers between species; from 4 isoforms in *D. melanogaster* to 14 isoforms in cockroaches (Schachtner *et al.*, 2005). Yet, immunolabelling studies have shown pronounced similarities between several species where AL LNs, PN and/or CNs have been labelled (Nässel & Homberg, 2006; Schachtner *et al.*, 2005). Despite the large number of immunocytochemical studies, no olfactory function has been reported.

#### 4.3.5 SIFamide

Although only a few insect species have been examined in relation to SIFamide distribution, a striking pattern emerges; a very small number of SIFamide expressing neurons are present in the brain of these insects. These neurons have their somata in the pars intercerebralis and project branches to almost all centres of the brain (Neupert *et al.*, 2012; Carlsson *et al.*, 2010; Predel *et al.*, 2010; Terhzaz *et al.*, 2007; Verleyen *et al.*, 2004). The conserved SIFamide distribution in diverse species such as dipterans and lepidopterans suggests that SIFamide has a significant function, not just in the olfactory system but on a global CNS level (Schachtner *et al.*, 2005).

SIFamide has been implicated in the control of sexual behaviours of *D. melanogaster*, where ablation of SIFamidergic neurons in the brain results in males indiscriminately courting both conspecific females and males (Terhzaz *et al.*, 2007). This change in behaviour indicates that SIFamide modulation of olfaction is involved in the male-male pheromone recognition system that otherwise inhibits male-male courtship (Carlsson *et al.*, 2010; Terhzaz *et al.*, 2007).

#### 4.3.6 MIPamides

Myoinhibitor peptides (MIPamides) have until recently received very little attention and there are, therefore, not much known about this family. MIPamide distribution has, so far, only been reported for *D. melanogaster* (Carlsson *et al.*, 2010) and *P. americana* (Neupert *et al.*, 2012) where it seems to be confined to 10-15 LNs in *D. melanogaster* and 30-50 LNs in *P. americana* plus additional endocrine cells (Neupert *et al.*, 2012; Carlsson *et al.*, 2010).

## 5 Summary of Results

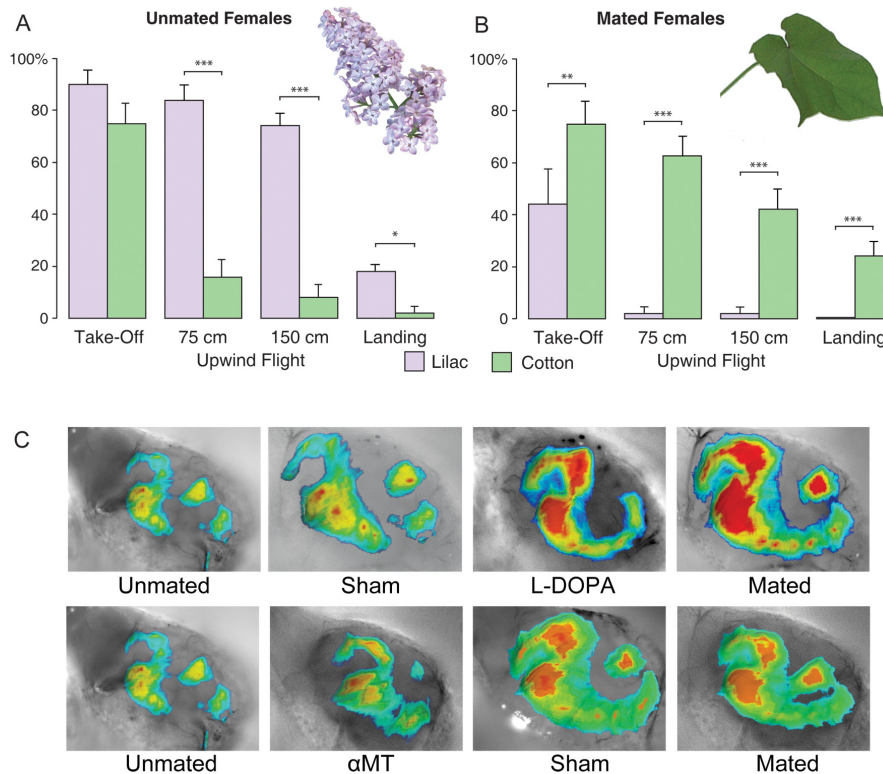
### 5.1 Modulation of Olfaction in Female *Spodoptera littoralis* (Papers I-II)

Mating induces profound changes in the olfactory system of female *S. littoralis*. Naïve, unmated females are highly attracted to nectar sources (flowers of lilac, *Syringa vulgaris*) but only partially attracted to oviposition sites (leaves of cotton, *Gossypium hirsutum*). However, after mating, behavioural responses are now switched to a strong attraction to cotton whereas the attraction to lilac is abolished (Fig. 1A-B). This behavioural switch is mirrored at the neuronal level, where the olfactory input to the ALs is significantly modulated by mating. Electroantennographic and optical imaging analyses of odour detection revealed that responses to lilac volatiles were greatly reduced after mating, whereas responses to cotton volatiles were significantly enhanced.

Through a series of high performance liquid chromatography analyses, we found that dopamine levels in the ALs changed as a consequence of mating. Shortly after mating a transient, but significant increase in dopamine levels in the olfactory system modulates the sensitivity of the system to both lilac and cotton volatiles. These changes could be mimicked by systemic injection of the biosynthetic dopamine precursor, L-DOPA<sup>1</sup>, into unmated females, which made them respond as if they were in fact mated. In contrast, removing dopamine from the system by injecting a depletion agent into mated females, made them respond as if they were unmated (Fig. 1C).

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1. Dopamine does not cross the blood-brain barrier, in contrast to L-DOPA. Once inside the brain, L-DOPA is converted to dopamine through the dopamine biosynthetic pathway (Lehman *et al.*, 2000; Livingstone & Tempel, 1983).



**Figure 1:** Mating-induced modulation of behavioural attraction of (a) unmated and (b) mated female *Spodoptera littoralis* to cotton and lilac in the wind tunnel. (c) Antennal lobe glomerular responses to cotton of female *S. littoralis* either unmated, sham injected, 24 h post-mated or unmated injected with L-DOPA. (d) Antennal lobe glomerular responses to cotton of female *S. littoralis* either unmated, sham injected, 3 h post-mated or unmated injected with  $\alpha$ MT. Results in (c) and (d) were obtained with amounts of volatiles as released from a cotton plant and a cluster of lilac flowers during 10 min.

The mechanism by which dopamine acts on the olfactory system is not known but dopamine may alter ORN excitability by regulating the production of second messengers (Bodnaryk, 1982; Bodnaryk, 1979a). In mammals, dopamine has been shown to depress olfactory input to the olfactory bulb in this way (Coronas *et al.*, 1999; Hsia *et al.*, 1999; Mania-Farnell *et al.*, 1993). Alternatively or in addition, dopamine may act through protein kinases and regulate gene expression for e.g. olfactory receptors (Sands & Palmer, 2008; Eisenhardt *et al.*, 2006; Vallone *et al.*, 2000; Missale *et al.*, 1998).

Our results indicate that dopamine primarily acts on the peripheral olfactory system of the *S. littoralis* and preliminary *in situ* hybridisation analysis have revealed the expression of dopamine receptors (SlitDAR-1 and SlitDAR-2) in

olfactory receptor neurons of female *S. littoralis* (unpublished data). Furthermore, a pair of dopaminergic centrifugal neurons innervate the ALs of *S. littoralis* (unpublished data) as seen in other moths (Klemm, 1976). It is plausible that these receptors and centrifugal neurons provide a substrate for peripheral and central dopaminergic olfactory modulation. Dopamine levels in the brain have previously been shown to be correlated with reproductive state in both insects and vertebrates (Serguera *et al.*, 2008; Sasaki *et al.*, 2007; Boulay *et al.*, 2001; Sasaki & Nagao, 2001; Harris & Woodring, 1995). Yet, direct modulation of the olfactory system has only been shown in mice, in which increased dopamine levels in the olfactory bulb reduce sensitivity to male-produced social cues that would otherwise inhibit pregnancy (Serguera *et al.*, 2008).

Selective dopaminergic modulation of AL neuronal circuits could be responsible for the up- and down-regulation of neuronal responses to cotton and lilac volatiles, respectively. Yet, it is still unclear how insects, like *S. littoralis*, can distinguish between different plants. Many of the volatiles found in lilac and cotton headspaces are ubiquitous compounds found in many plants. However, the attraction of *S. littoralis* might be because of a subset of essential compounds, in addition to otherwise redundant, interchangeable compounds (Cha *et al.*, 2011; Tasin *et al.*, 2007). In cotton and lilac, the ratio between the two enantiomers of linalool, (*R*)-(-)- and (*S*)-(+)-linalool, is different. Yet, it has been observed in *M. sexta* that females can differentiate between these two enantiomers and that one signifies feeding source whereas the other is indicative of oviposition site (Reisenman *et al.*, 2010). It is, therefore, highly plausible that female *S. littoralis* could be able to distinguish between the two enantiomers and, thus, utilize this information as a guide towards cotton versus lilac.

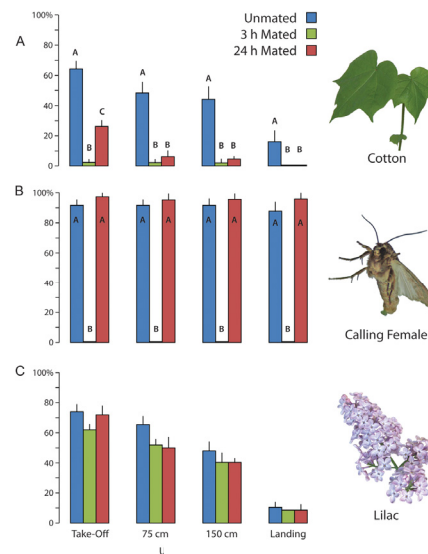


Figure 2: Mating-induced modulation of behavioural attraction of male *Spodoptera littoralis* towards (a) cotton foliage, (b) calling female *S. littoralis* and (c) lilac flowers in the wind tunnel (mean ± SEM, n = 50).



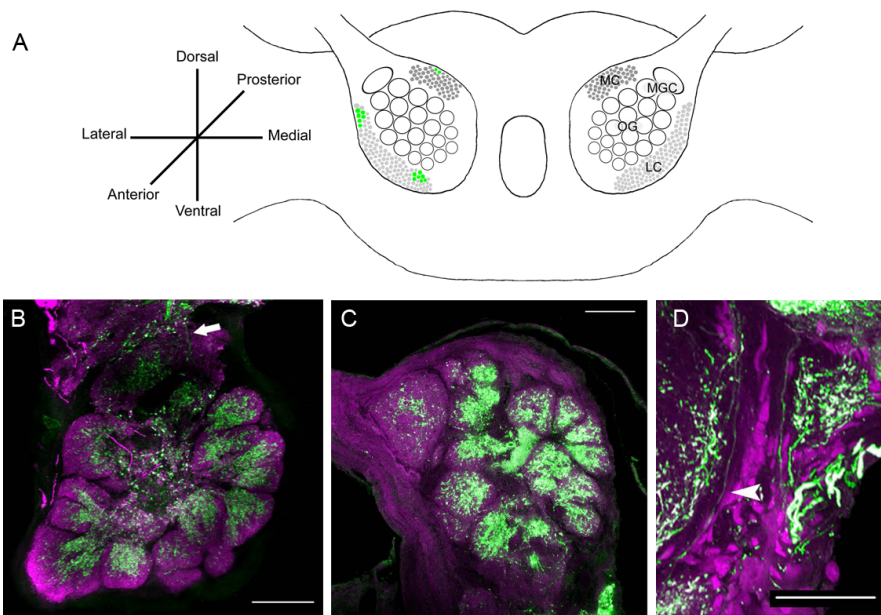
## 5.2 Modulation of Olfaction in Male *Spodoptera littoralis* (Paper III)

Mating also induces profound changes in the olfactory system of male *S. littoralis*. Naïve, unmated males are attracted to cotton (mating site) and lilac (food source) but even more so to pheromone-releasing females. However, directly after mating, behavioural responses to calling females and cotton are abolished. This inhibition of behavioural response is temporary and pre-mating response levels are restored by the following night (Fig 2). These mating-induced changes were analysed with electroantennographic, single sensillum and optical imaging analyses, and we found that the behavioural inhibition towards pheromone and cotton volatiles were correlated to a remarkable lowered sensitivity of the antennae and ORN input to the ALs. Twenty-four hours later, the responses at both neuronal levels had also reverted to that of unmated males. This transient inhibition of olfaction and olfactory-guided behaviour is most likely an adaptive value to avoid remating a now unreceptive female (Fischer & King, 2008). Furthermore, it could also be a necessary period for refilling the sex accessory glands and for production of new spermatophores as suggested for the moth *Agrotis ipsilon* (Barrozo *et al.*, 2011; Barrozo *et al.*, 2010a; Barrozo *et al.*, 2010b; Gadenne *et al.*, 2001; Lachmann, 2000; Duportets *et al.*, 1998).

Interestingly, mating does not alter the behavioural or the neuronal responses to lilac in male moths (Fig. 2) as it does in female *S. littoralis*. Because of this, we hypothesise that olfaction in males is characterised by two separate information streams; one for social cues, i.e. mate and mating sites, and one for food source-related odours. These two information streams are modulated differentially with the responses to social cues being regulated by internal factors such as mating status.

## 5.3 Neuropeptides in the Antennal Lobes of Male *Spodoptera littoralis* (Paper IV)

To elucidate the underlying mechanism of mating-induced olfactory modulation in males, we also conducted high performance liquid chromatography on excised male ALs. However, contrary to what we found in females, none of the biogenic amines changed in relation to mating in males, indicating separate modes of modulation between the two sexes. Another set of proposed neuromodulators are the neuropeptides. In view of the fact that only a small subset of neuropeptides has a characterised function in the olfactory system, we conducted a series of immunocytochemical experiments to identify prime candidates for future studies.



**Figure 3:** Distribution patterns of neuropeptides within the antennal lobes of male *Spodoptera littoralis*. (a) Schematic drawing of the brain of *S. littoralis* with LemTK immunoreactive cell bodies (green), (b) allatostatin distribution within the antennal lobe and an extrinsic fibre connecting the antennal lobe with higher order brain centres (arrow), (c) FMRFamide distribution within the antennal lobe and (d) FMRFamide immunoreactive extrinsic neurons in the inner antennocerebral tract (arrow). Scale bars = 50  $\mu$ m.

We found that five of the six neuropeptides screened for were expressed primarily in intrinsic AL LNs; tachykinin, FMRFamide-related peptides, allatotropin, allatostatin and MIPamide (Fig 3). However, a subset of the neuropeptides were also expressed in extrinsic, projection or centrifugal, neurons. These findings suggest that neuropeptide signalling occurs mainly within local circuits of the ALs but that crosstalk with other brain centres is also subject to peptidergic modulation.

Within the ALs, we found conspicuous distribution patterns of the tested neuropeptides. Tachykinin, SIFamide and MIPamide were expressed homogeneously throughout all glomeruli whereas allatotropin and allatostatin were only expressed in the basal part of the glomeruli. FMRFamide had a dense expression pattern amongst the ordinary glomeruli but was almost devoid in the major glomerulus of the macroglomerular complex, the cumulus. This differential expression of neuropeptides within and amongst the glomeruli indicates differential function in processing of odours, e.g. pheromones and plant-related odours. Furthermore, differential expression offers the possibility

of selective modulation of afferent and efferent neurons, i.e. ORNs and PNs, respectively.

Interestingly, SIFamide, which is only expressed in extrinsic neurons, was recently found to be involved in the regulation of courtship behaviour in the *D. melanogaster*. If SIFamide expressing neurons are genetically removed, male *D. melanogaster* started to court both females and males equally (Terhzaz *et al.*, 2007). It has, therefore, been suggested that SIFamide might be involved in the detection and processing of pheromones that normally prevent males from courting other males (Carlsson *et al.*, 2010).

Allatotropin and allatostatin are known to regulate the production and release of juvenile hormone, which stimulates filling of sex accessory gland and production of spermatophers. Filling of the sex accessory glands, in turn, stimulates release of allatotropin, creating a feedback loop (Stay & Tobe, 2007; McNeil & Tobe, 2001). It is, therefore, plausible that, after mating when males are depleted of spermatophores, an induction of the allatostatin-allatotropin-juvenile hormone system is also accountable for the observed decrease in olfactory responses of male *S. littoralis*.

The presence and distinctive distribution patterns of the neuropeptides and the possibility for selective and physiological state-dependent modulation of the olfactory system could ensure neuronal and behavioural plasticity in response to changing internal and external environments.

## 6 General Conclusions

In the four papers presented in this thesis, I have described how mating alters the olfactory system of female and male *Spodoptera littoralis*. Although we still only know relatively little about insect olfaction, I believe that our findings offer an important contribution to how internal states can modulate the olfactory system.

Mating affects the olfactory system of female *S. littoralis* (Papers I and II). This modulation is caused by an increase in dopamine levels in the olfactory system (Paper II) and results in a shift in olfactory preference and a concurrent change in olfactory sensitivity to food source and oviposition site related odours (Papers I and II). Similarly, in males, mating induces a change in the olfactory system, with a transient inhibition of behavioural and olfactory responses to pheromones and mating site related odours (Paper III).

Neuropeptide distribution in the antennal lobes of males was investigated to elucidate prime candidates for future studies on mating-induced modulation of olfaction. Distinct patterns were found for 6 neuropeptides and revealed a rich neural substrate for the modulation of olfactory integration (Paper IV).

Based on these findings, future studies of the olfactory system in *S. littoralis* will be aimed at elucidating additional modulatory mechanisms, how these affect mating-induced changes as well as other changes in the olfactory system and how this relates to the coding of odours. With the advancement in molecular techniques available for the study of insect chemical ecology, a multitude of facets can be explored. Characterisation of olfactory receptors as well as receptors involved in synaptic transmission will offer great advances in our understanding of how odours are processed. This can then be related to the behavioural output of physiological state and context-dependent choices.

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