

**VISUALIZATION OF CENTRAL OLFACTORY
PATHWAYS IN A MODEL BRAIN; UNIVERSAL
PRINCIPLES OF STRUCTURAL ORGANIZATION
ACROSS TAXA**

Graduate Thesis

Spring 2010

Student: Toke Knizek Vang

Supervisor: Associate professor Bente Gunnveig Berg



NTNU

Norwegian University of
Science and Technology

Abstract

This thesis in clinical psychology has two main parts, answering the two main research problems. This scientific project includes visualization of neural pathways connecting the primary olfactory centre to higher association regions in a small insect brain for the purpose of comparing anatomical organization of the olfactory system in species of different taxa. The noctuid moth *Heliothis virescens*, which is particularly well adapted to chemical communication, is used as model organism. By utilizing the technique of fluorescent staining combined with confocal microscopy, the three main antennocerebral tracts and their target areas, the lateral protocerebrum and calyces of mushroom bodies, are labeled and visualized. The second part of the thesis includes a relatively comprehensive theoretical discussion of universal principles of olfactory structural organization across taxa. This discussion is founded on the experimental investigation (using the anatomy of *Heliothis virescens* as a basis for comparison, and keeping its main focus on the olfactory structures identified by confocal microscopy), but is not limited to this. It is a general discussion of organizational principles in vertebrates and invertebrates, and includes research on other organisms and olfactory structures beyond what was identified in the experimental study. Clear evidence of universal principles of structural – and functional olfactory organization is found, and it is argued that the study of different taxa is a way for psychologists to gain further knowledge about human behavior.

Index

Abstract	1
Index	2 – 3
1. Introduction	4 – 8
<i>1.1. Theoretical background</i>	<i>4 – 7</i>
<i>1.2. Using Heliothis virescens</i>	<i>7</i>
<i>1.3. Research problem statement</i>	<i>8</i>
2. Materials and methods	9 – 11
<i>2.1. Materials</i>	<i>9</i>
<i>2.2. Preparation</i>	<i>9</i>
<i>2.3. Staining</i>	<i>9 – 10</i>
<i>2.4. Confocal imaging and reconstruction</i>	<i>10</i>
<i>2.5. Ethical considerations</i>	<i>11</i>
3. Results	12 – 21
<i>3.1. Antennocerebral pathways</i>	<i>12 – 13</i>
<i>3.2. Calyces of mushroom bodies</i>	<i>13</i>
4. Discussion	22 – 36
<i>4.1. Experimental findings and the main antennocerebral pathways</i>	<i>22 – 25</i>
<i>4.2. General anatomical similarities</i>	<i>25 – 26</i>

<i>4.3. Odor detection</i>	26 – 28
<i>4.4. Glomerular organization and functionality.</i>	28 – 35
<i>4.5. The calyces of mushroom bodies</i>	35 – 36
5. Conclusion	37 – 39
6. Acknowledgements	40
7. References	41 – 48
Appendix A	49 – 50

1. Introduction

1.1. *Theoretical background*

A common question when presenting the theme of this thesis has been: "why is this relevant for the study of psychology"? In this introduction, I hope to sum up why I believe that there might be a theoretical basis of relevance for this thesis. Whether or not this thesis indeed proves to be of relevance is for the reader to answer.

The basic process of investigating neural systems has been known for a long time. As early as 1669, the Danish anatomist Niels Steensen (better known by the Latin spelling of his name, Nicolaus Steno) described how one might in theory conduct studies of neural functioning and neural organization.

"There are two ways only of coming to know a machine: one is that the master who made it should show us its artifice; the other is to dismantle it and examine its most minute parts separately and as a combined unit [...] since the brain is a machine [...] we need not hope to discover its artifice by methods other than those that are used to find such for other machines [...] I mean the dismantling of all its components, piece by piece, and consideration of what they can do separately and as a whole",
(Steno, 1965, p. 139).

Although our current knowledge of the anatomy, organization and working of the brain is substantially larger, and our tools for research into these subjects are far more

advanced, the basic process of investigation as described by Steno is still well and truly alive.

The use of animal – and insect research in order to gain knowledge about human neural systems and human psychology is also a longstanding tradition. In fact, our initial knowledge of pheromones comes from the study of moths. In 1959, the German biochemist Butenandt and his colleagues managed to identify the pheromone bombykol, which is used by the silk moth *Bombyx mori*. Later, the importance of olfactory signals in reproductive behavior has been studied in several moth species, including *Heliothis virescens* (e.g. Berg, Tumlinson & Mustaparta, 1995). Studies such as this have established that male moths search for potential partners by following the pheromone trace of females. Several studies have shown that the pheromone system is of great importance for sexual behavior in mammals as well. Afrodosin, a protein with a pheromone function, which is secreted by the vagina of the female hamster, triggers sexual behavior in the male hamster by activating the vomeronasal organ (Singer, 1991). In mice, stimulation of the vomeronasal organ triggers neuroendocrine responses and mating behavior, which can only be somewhat modified by learning (Herrada & Dulac, 1997). In a similar way, studies show that sexual behavior and mating behavior among humans are affected by olfactory signals, although to a lesser degree than among insects and animals (Brodal, 2003). There are some indications that exposure to male pheromones increases the rate of ovulation in women (Veith, Buck, Getzlaf, Van Daltsen & Slade, 1983). Women who were exposed to androstenol, an active pheromone in pigs and possibly humans, seemed to be more willing to seek out the company of men than women who were not exposed to androstenol (Cowley & Brooksbank, 1991). The similarities in behavioral

responses to stimulation of the pheromone system suggest that studies of disparate taxa may yield valuable insight into human psychology.

Furthermore, several studies show that specific mental disorders are associated with olfactory dysfunction, including psychopathy (Lapierre, Braun & Hodgins, 1995), anorexia nervosa (Fedoroff, Stoner, Andersen, Doty & Rolls, 1995), attention deficit/hyperactivity disorder (Gansler, Fucetola, Krengel, Stetson, Zimering & Makary, 1998), and schizophrenia (e.g. Kopala, Good, Koczapski & Honer, 1998; Stedman & Clair, 1998; Striebel, Beyerstein, Remick, Kopala, Honer, 1999).

McCaffrey, Duff and Solomon (2000) found that a simple, standardized smell test (3-item Pocket Smell Test) discriminated better between people with Alzheimer's disease and major affective disorder than the commonly used Mini-mental state examination (MMSE). These studies not only show that a thorough understanding of the olfactory system may aid in psychological diagnostics (especially of neurodegenerative disorders), but may also be important in the study of the etiology of mental disorders. Furthermore, it demonstrates the importance of interdisciplinary research in clinical psychology and the need to expand the accepted boundaries of mental health research.

Like the study of neural organization itself, and the use of animal- and insect research, the study of neural organization in different taxa (phylogenetically different organisms) has been with us for quite a while (Zawarzin, 1925). The question has been whether similarities in structural organization are the result of evolutionary convergence or a sign of common origin. According to Strausfeld and Hildebrand (1999):

“It would be reasonable to assume that common selective pressures resulted in the convergent evolution, in those disparate taxa, of comparable structures that solve similar computational problems imposed by identical physicochemical constraints”,
(Strausfeld & Hildebrand, 1999, p. 634).

The sense of smell, arguably the oldest and best preserved sensory modality (Dethier, 1990), should be well suited for studies of this question.

1.2. *Using Heliothis virescens*

Heliothis virescens is in many ways a well-suited model organism for studies of the olfactory system. As with all insects, the relative simplicity and accessibility of their nervous system makes thorough studies of general principles of function and structure far more manageable than the complex systems of vertebrates. Furthermore, the extensive studies carried out on *Heliothis virescens* give a solid foundation for investigations of its overall structural principles. For instance, three-dimensional (3D) maps of the glomeruli of the antennal lobe (Berg, Galizia, Brandt & Mustaparta, 2002; Skiri, Rø, Berg & Mustaparta, 2005) and the antennal lobe projection neurons (Rø, Müller & Mustaparta, 2007) have been produced. In discussing possible structural similarities across taxa, and functional organization of the olfactory system, realistic and thorough maps of the underlying neural architecture are vital. Finally, the presence of significant expertise on *Heliothis virescens* at NTNU makes it possible to carry out more thorough research within the framework of a thesis than would be possible otherwise.

1.3. *Research problem statement*

In this thesis, I will attempt to:

- Visualize the major neural pathways between the primary olfactory centre and the higher associative areas in the brain of *Heliothis virescens*.
- Discuss, by comparing the anatomical organization of these tracts with that of corresponding tracts in vertebrates (humans included), whether there are similar basic principles.
- Discuss, by comparing other essential structures of the moth olfactory system with those of vertebrates, whether there are universal principles across different taxa.

2. Materials and methods

2.1. Materials

Female *Heliothis virescens* (Heliiothinae; Lepidoptera; Noctuidae) pupae from a laboratory culture at Syngenta (Basel, Switzerland) were kept in separate containers in an incubator on a phase-shifted LD 14:10 hours photoperiod at 22°C. When emerged, adults were transferred to new containers and given sucrose solution. The insects that were used in the experiments were 2 - 7 days old.

2.2. Preparation

The insect was restrained in a plastic tube with the head and the antennae exposed, and was immobilized using dental wax. Thereupon, the cuticle between the eyes was removed, which exposed the frontal and dorsal part of the brain, including the antennal lobes. Using forceps, the antennal lobe was nipped to facilitate penetration of the dye into the neurons. To prevent the brain tissue from drying out, Ringer solution (mM: 150 NaCl, 3 CaCl₂, 3 KCl, 25 sucrose and 10 TES buffer, pH 6,9) was applied as needed.

2.3. Staining

The antennal lobes were stained by inserting crystals of Dextran tetra methyl rhodamine (Molecular Probes; Invitrogen) using a small needle. The restrained insect was then kept in a humid environment at room temperature for 3 hours for transportation of the dye within the stained neurons. After staining the brains were dissected from the head capsule under saline solution and fixed in 4% paraformaldehyde fixative for at least 1 hour at room temperature or overnight at 4

°C. The brains were then dehydrated in an increasing ethanol series (30%, 50%, 70%, 90%, 96% each for 5 minutes, and 2 x 100% each for 10 minutes). Following dehydration, the whole brains were clarified, oriented frontally, and stored in methyl salicylate in double-sided custom slides. Each brain was then examined by use of a Zeiss bright-field microscope (Axiovision Z1) equipped with tools for detection of fluorescent staining.

2.4. *Confocal imaging and reconstruction*

Three brains were chosen for confocal imaging, based on the quality of their stainings. Serial optical images were acquired by using a laser scanning confocal microscope (LSM 510, META Zeiss, Jena, Germany) with a 20x (Plan-Neofluar 20x/0.5 I) and a 40x objective (C-Acrophlan 40 x/0.8W). The staining from the fluorescent dye (Ex_{max} 550 nm, Em_{max} 570 nm) was excited by the 543-nm line of a HeNe1 laser. The distance between each section was 2 μ m. The pinhole eye was 1 and the resolution 1024 x 1024 pixels. The data were stored on the university's secured intranet. Optical sections from confocal stacks were reconstructed by using the projection tool of LSM 510. The stereo images were made by using the stereo tool of LSM 510. PhotoShop CS2 (Adobe Systems, San Jose, CA) was used to rotate photographs so that all were similarly oriented, and to adjust for size, brightness, and contrast. Series of single scans as well as 3D-reconstructions were then edited in Adobe Illustrator CS2 (Adobe Systems, San Jose, CA).

2.5. *Ethical considerations*

All experiments were conducted in accordance with the Norwegian laws concerning animal welfare and protection. These laws do not protect invertebrates, and no ethical approval by the Regional Committee of Ethics (REK) was therefore required.

3. Results

3.1. Antennocerebral pathways

Eight brains were stained, and three of these preparations were chosen for confocal microscopy based on the quality of their stainings. The main criterion for exclusion was insufficient staining of the protocerebrum, which might have been caused by insufficient amounts of crystals or by inadequate penetration of dye into the antennal lobe. The processed scans show the general anatomy of the central antennocerebral pathways of the female *Heliothis virescens* (a full index of the scans presented in this thesis can be seen in appendix A, including placement of the chosen slices in the confocal stacks). The brains investigated by confocal microscopy showed a similar staining pattern including three main pathways connecting the antennal lobe to protocerebral regions. These antennocerebral tracts are visible in Figures 1, 2 and 3. Some other major anatomical structures are also visible and clearly illustrate the anatomical geography. These are the central body, the esophagus, and the optic lobe. The inner antennocerebral tract projects to the calyces of mushroom bodies and terminates in the lateral protocerebrum. This is the most prominent tract, which exits dorsomedially from the antennal lobe and runs posteriorly in the protocerebrum. It passes close to the lateral edge of the central body, before turning laterally and running along the anterior surface of the calyces of the mushroom bodies. The inner antennocerebral tract sends branches that innervate the calyces of the mushroom bodies, before terminating in the lateral part of the median protocerebrum. The middle antennocerebral tract and the outer antennocerebral tract both project directly from the antennal lobe to the lateral protocerebrum. The middle antennocerebral tract runs dorsomedially from the antennal lobe along with the inner antennocerebral tract, and

follows the same path as the inner antennocerebral tract for a while, before it turns laterally by the central body. It then divides into smaller branches, which terminate in different parts of the lateral protocerebrum. The outer antennocerebral tract exits the antennal lobe more ventrally than the inner antennocerebral tract and the middle antennocerebral tract. It then turns laterally, continuing to the lateral protocerebrum. In these illustrations, the inner antennocerebral tract is marked with a white arrow, the middle antennocerebral tract with a red arrow, and the outer antennocerebral tract with a blue arrow. The antennal lobe, the calyces of the mushroom bodies and the lateral protocerebrum are clearly visible in these confocal stacks. By viewing 3D-reconstructions of the stained brains, the antennocerebral tracts become more coherent. Figure 4 is a 3D-reconstruction of the same brain as shown in Figure 1 and Figure 5 is a 3D-reconstruction of the same brain as shown in Figure 3. Figure 7 is a 3D-reconstruction of the main antennocerebral pathways presented in stereo.

3.2. Calyces of the mushroom bodies and the lateral protocerebrum

One of the brains yielded a particularly clear illustration of the calyces of the mushroom bodies. This can be seen in Figure 6. As can be seen in the illustration, the calyces of the mushroom bodies are cup-shaped regions of neuropils with completely fused walls. What can be seen in the illustrations in Figure 6 are the terminals of antennal lobe projection neurons. Figure 8 presents the calyces of the mushroom bodies in stereo.

In Figures 1, 2, 3, 4 and 5, the lateral protocerebrum is clearly visible. The staining pattern in this brain region is quite distinctive and is characterized by a seemingly lack of organization. In this area, the terminals of the three antennocerebral tracts

cover partly overlapping areas. This is particularly visible in the 3D-reconstructions in Figures 4 and 5.

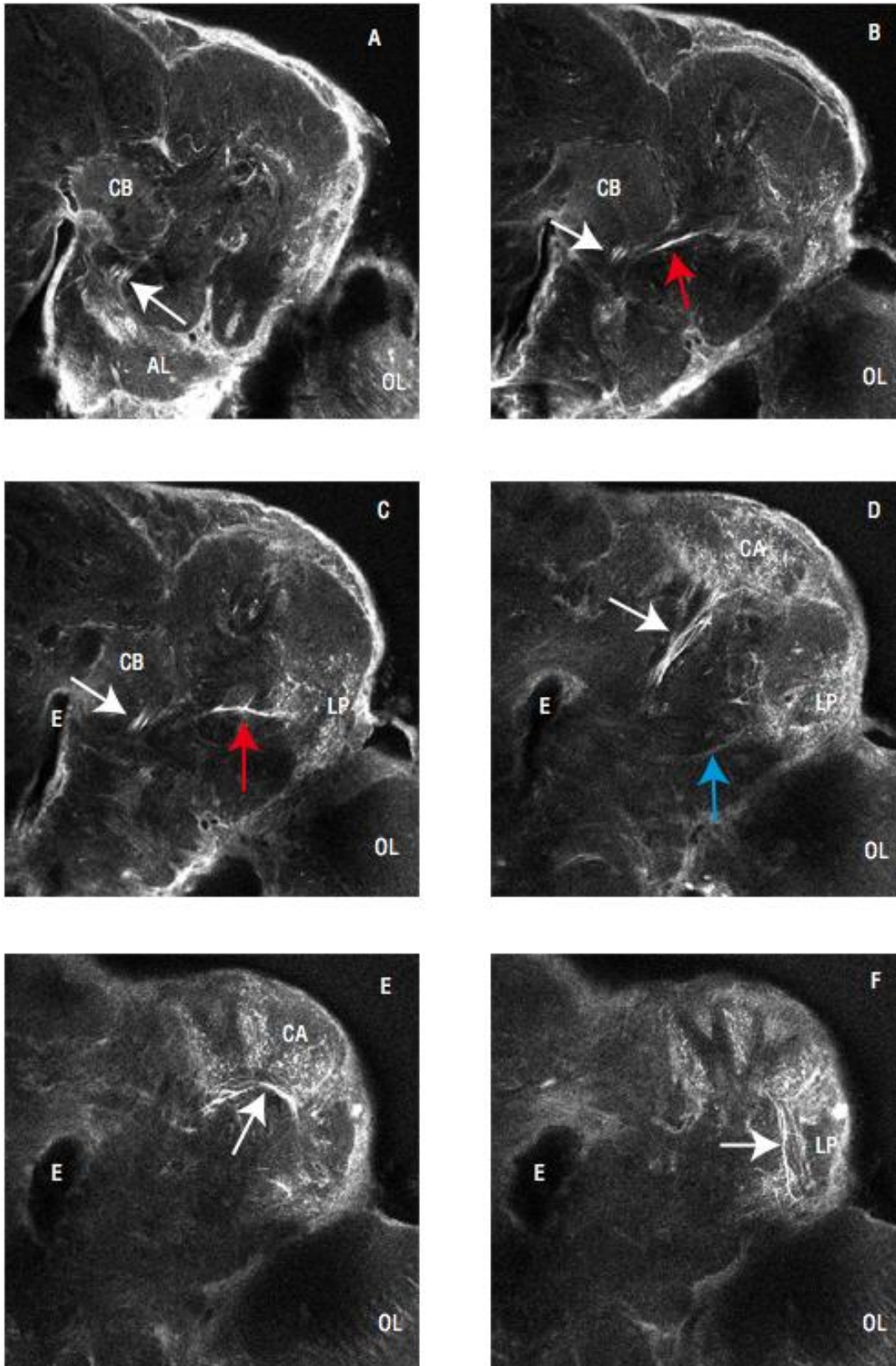


Figure 1: A selection of slices from a confocal stack. Presented in a frontal to posterior view (A →F). The inner antennocerebral tract (IACT) is marked with a white arrow (A →F), the middle antennocerebral tract (MACT) is marked with a red arrow (B →C), and the outer antennocerebral tract (OACT) is marked with a blue arrow (D). Also visible are the antennal lobe (AL) (A), the central body (CB) (A →C), the esophagus (E) (C →F), the optic lobe (OL) (A →F), the calyces of the mushroom bodies (CA) (D →E), and the lateral protocerebrum (LP) (C, D, F).

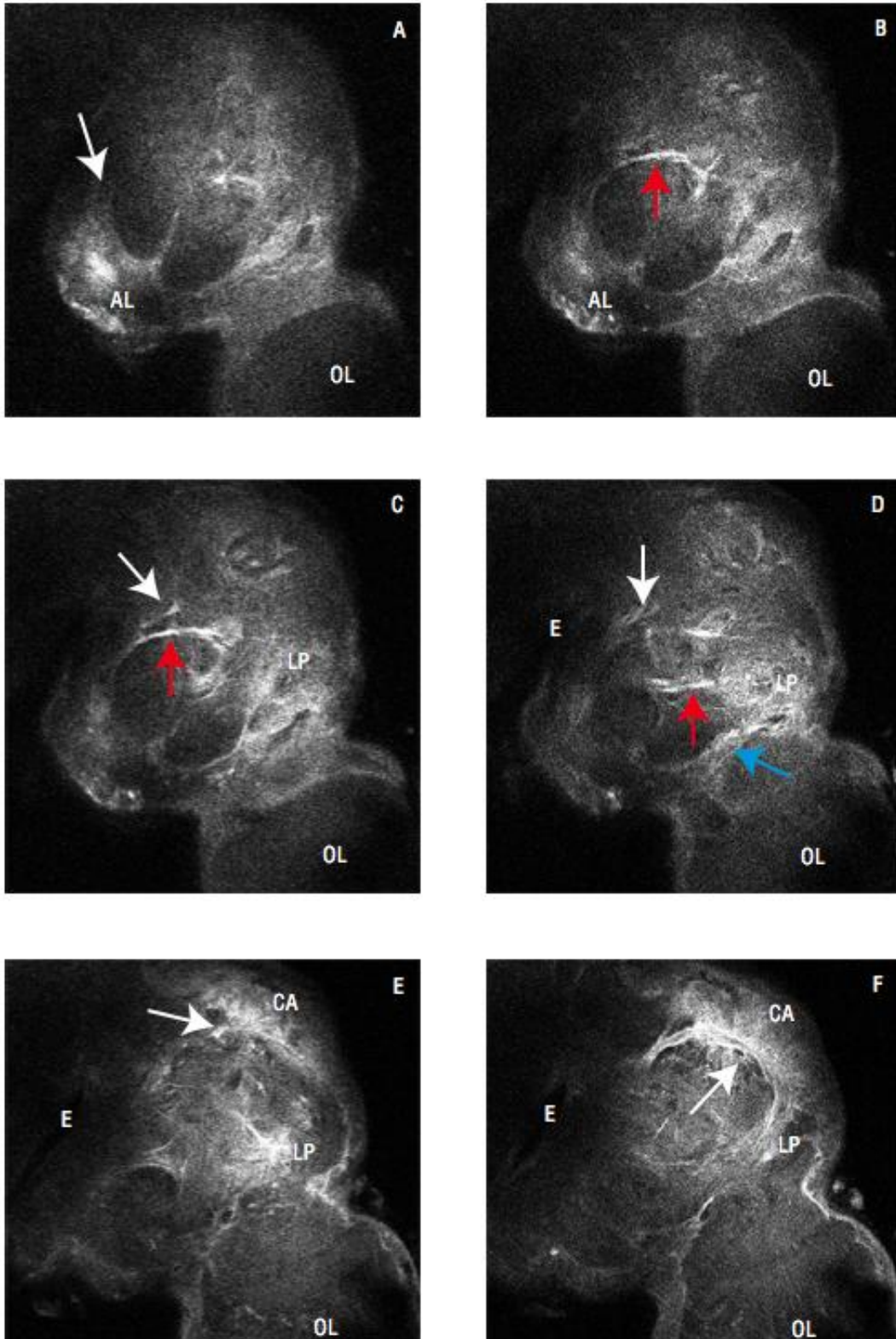


Figure 2: A selection of slices from a confocal stack. Presented in a frontal to posterior view (A \rightarrow F). The inner antennocerebral tract (IACT) is marked with a white arrow (A, C \rightarrow F), the middle antennocerebral tract (MACT) is marked with a red arrow (B \rightarrow D), and the outer antennocerebral tract (OACT) is marked with a blue arrow (D). Also visible are the antennal lobe (AL) (A \rightarrow B), the esophagus (E) (D \rightarrow F), the optic lobe (OL) (A \rightarrow F), the calyces of the mushroom bodies (CA) (E \rightarrow E), and the lateral protocerebrum (LP) (C \rightarrow F).

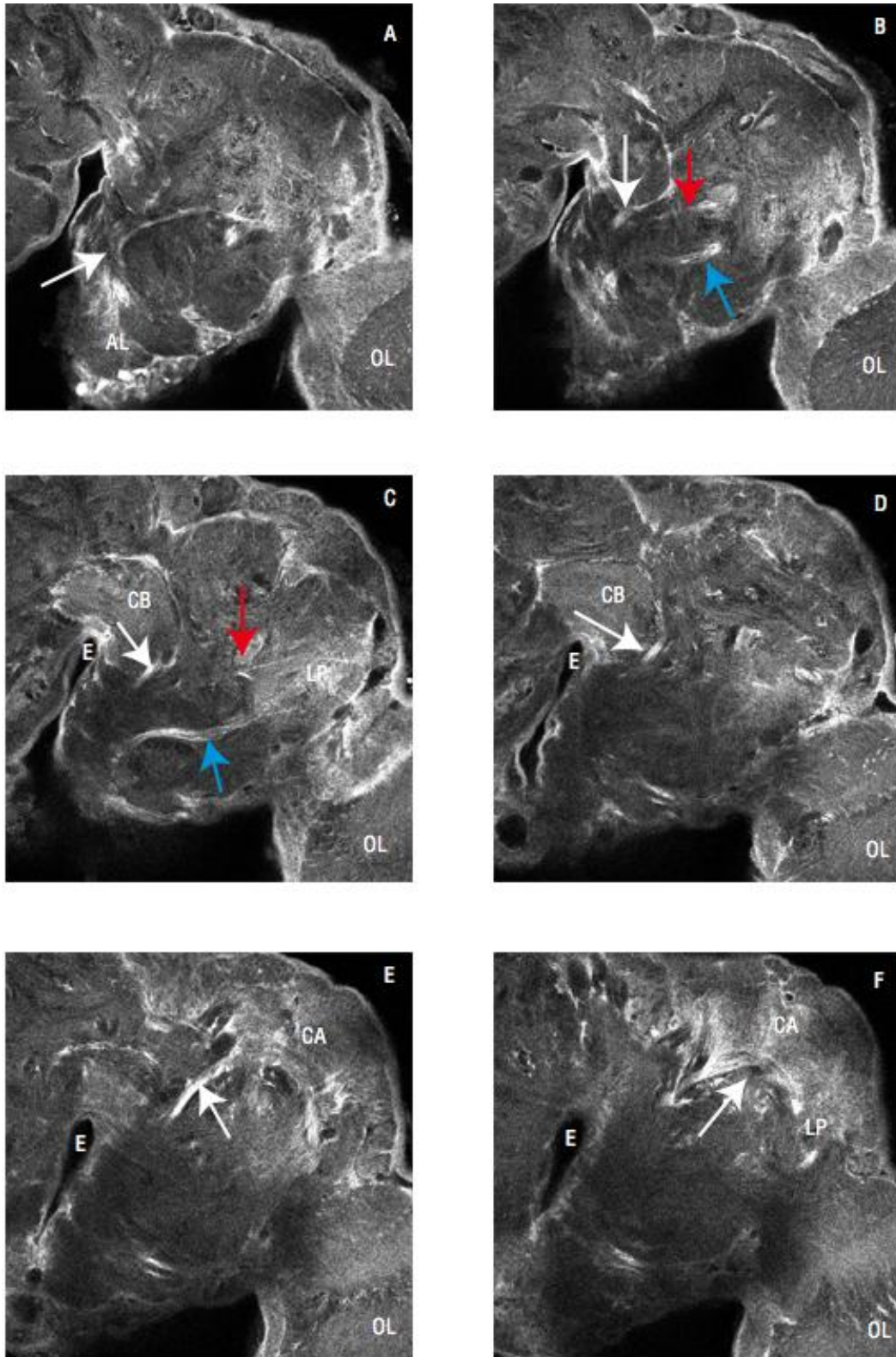


Figure 3: A selection of slices from a confocal stack. Presented in a frontal to posterior view (A → F). The inner antennocerebral tract (IACT) is marked with a white arrow (A → F), the middle antennocerebral tract (MACT) is marked with a red arrow (B → C), and the outer antennocerebral tract (OACT) is marked with a blue arrow (B → C). Also visible are the antennal lobe (AL) (A), the central body (CB) (C → D), the esophagus (E) (C → F), the optic lobe (OL) (A → F), the calyces of the mushroom bodies (CA) (E → F), and the lateral protocerebrum (LP) (C, F).



Figure 4 (above) and Figure 5 (below): 3D-reconstructions of confocal stacks. Presented in a frontal view. Figure 4 is based on the same confocal stack as Figure 1, and Figure 5 is based on the same confocal stack as figure 3. Both figures clearly show the three main antennocerebral pathways (the inner antennocerebral tract, the middle antennocerebral tract, and the outer antennocerebral tract). Very clearly visible in Figure 4 are also the calyces of the mushroom bodies and the lateral protocerebrum. These are also visible in Figure 5, albeit less clearly. In Figure 5, one can also see the antennal lobe.

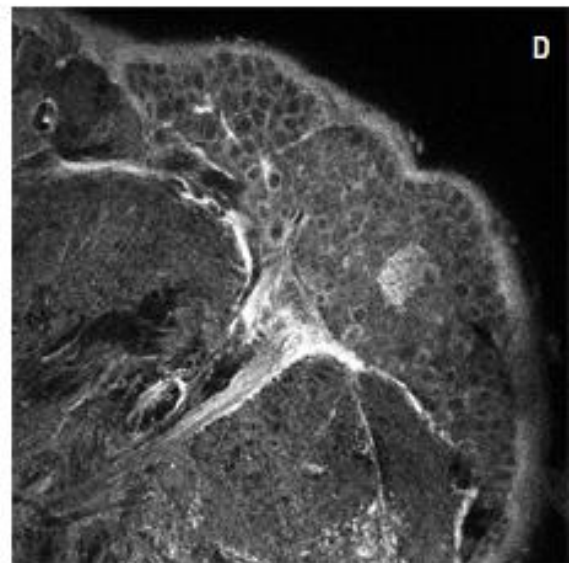
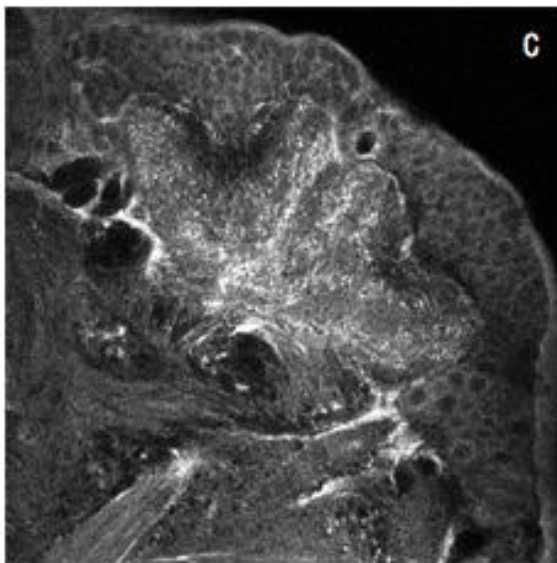
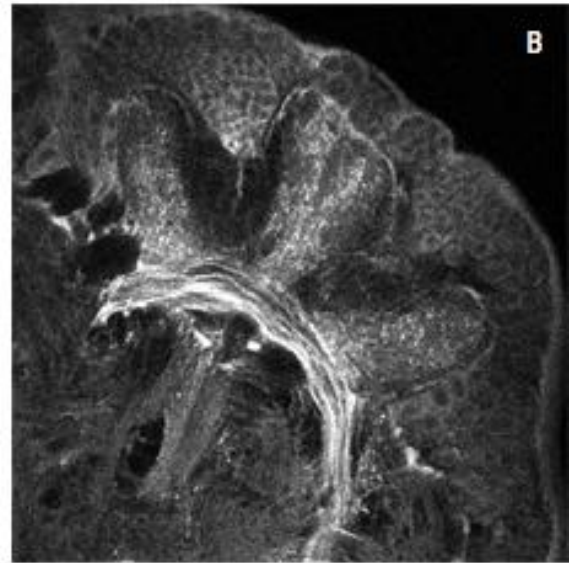
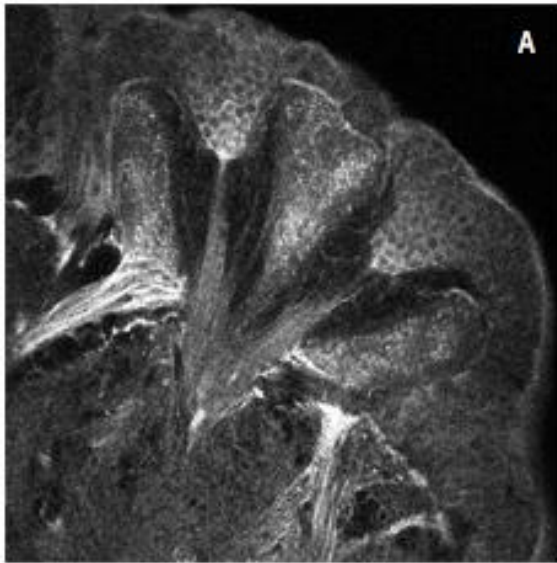


Figure 6: The calyces of the mushroom bodies. Presented in a frontal to posterior view (A →D). What can be seen in these illustrations are the terminals of antennal lobe projection neurons. Also clearly visible is the distinctive cup-shape of the calyces of the mushroom bodies.

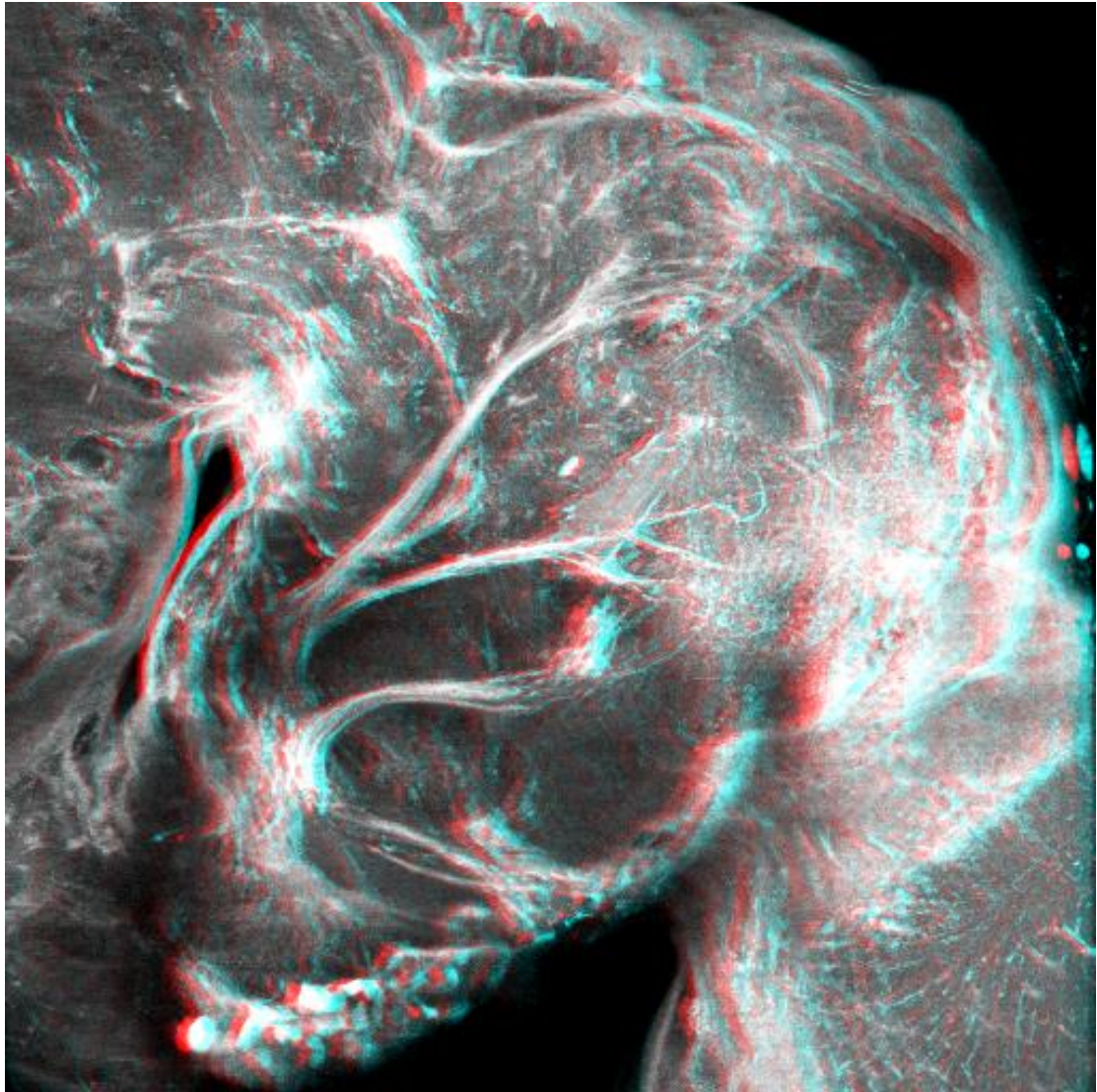


Figure 7: Three-dimensional (3D) reconstruction of a confocal stack. Presented in a frontal view. The three main antennocerebral tracts are clearly visible in this illustration. Also visible are the calyces of mushroom bodies and the lateral protocerebrum, albeit somewhat less clearly.

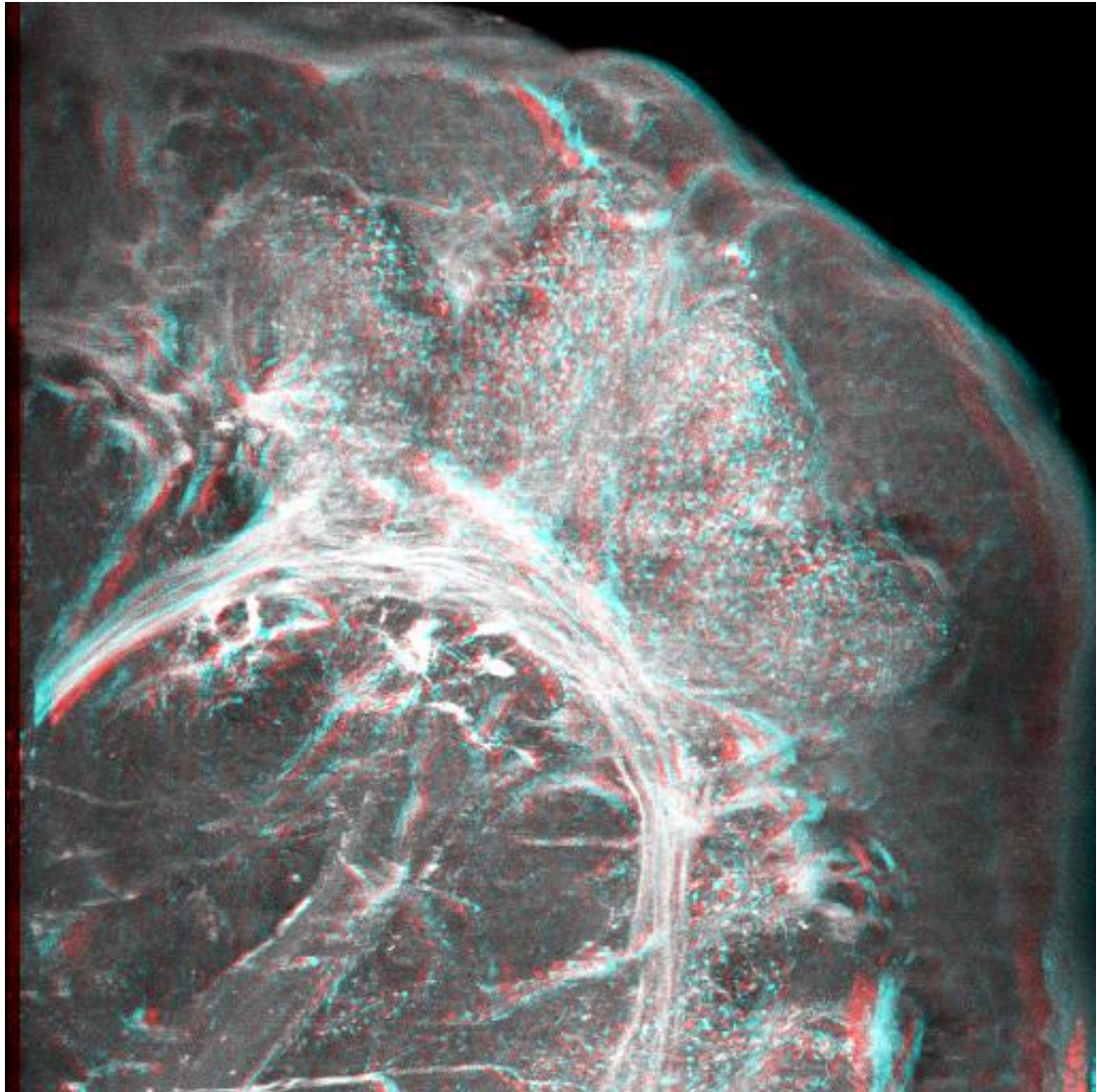


Figure 8: A three-dimensional (3D) reconstruction of the calyces of the mushroom bodies. This is the same confocal stack as was presented in Figure 6.

4. Discussion

4.1. *Experimental findings and the main antennocerebral pathways*

By use of the classic labeling technique involving axonal transportation of the dye, in this case retrograde tracing, the main antennocerebral tracts in the moth brain were visualized. Furthermore, the quality of the stainings made possible reconstructions of the optical stacks so that the neural pathways could be visualized in images of the raw data (Figures 4 and 5), and stereo images (Figures 7 and 8). In particular, the stereo images, made by utilizing a specific tool in the LSM510 software, provide a unique kind of information.

The anatomy of antennocerebral pathways in insects has previously been thoroughly described (e.g. Strausfeld, 1976; Mobbs, 1982; Homberg, Montague & Hildebrand, 1988). A more specific mapping of the antennocerebral pathways of *Heliothis virescens* has also been carried out (Rø, Müller & Mustaparta, 2007). The experimental data presented in this thesis correspond with the findings of these researchers. This indicates that a general consensus concerning the anatomy of antennocerebral pathways in insects has been reached. Such a consensus is of great benefit if one is to use these data as a basis for comparisons across taxa. As described in the introduction, the purpose of working in such a way is to gain valuable insight into the logic of complex neural systems (such as the human neural system) by transferring knowledge and findings about less complex neural systems (such as the neural system of *Heliothis virescens*). Consensus about the anatomy of the less complex neural system makes comparative studies across taxa far more manageable and allows one to draw conclusions and formulate hypotheses that are more accurate.

While the experimental findings correspond with previous research, some features of the structural organization are not visible in the illustrations. For instance, some fibers of the outer antennocerebral tract turn dorsomedially, before terminating in the calyces of the mushroom bodies (Rø et al, 2006), but this is not clearly visible in these illustrations. The illustrations of the calyces of the mushroom bodies in Figure 6 do not show, that from the base of the cup-shaped neuropil region, two tracts emerge and fuse to become the pedunculus. This runs through the protocerebral neuropil, terminating in different parts of the brain. Two smaller tracts emerging from the calyces of the mushroom bodies form the Y lobe and also terminate in different areas of the brain. The terminals of these tracts are not visible in the illustration. Rø, Müller and Mustaparta (2007) have mapped and labeled them, and provide a map of these tracts. Furthermore, the anatomy of the calyces of the mushroom bodies is not clearly visible in these illustrations. The calyces of the mushroom bodies are paired neuropils consisting of Kenyon cells arranged approximately parallel to each other. Their somata are on the dorsoposterior surface of the protocerebrum, and they have their dendrites directed inward, thus forming the calyces of the mushroom bodies.

Principles guiding olfactory information processing have been studied in a number of different species for quite some time, and include contributions from various disciplinary approaches (Hildebrand & Shepherd, 1997). However, only relatively recently did it become clear that general principles of functional organization across taxa were being studied. Hildebrand and Shepherd for instance made the assumption that: “[...] we postulate that a common set of neural mechanisms has evolved across taxa for detecting and discriminating among olfactory stimuli”. And indeed, many

similarities between the structural – and functional organization of the olfactory system seem to exist across taxa. The experimental data in this thesis focuses on the olfactory pathways from the main integration center to higher-order processing centers.

There are remarkable similarities between the organizations of the olfactory neural pathways in vertebrates and invertebrates. Because of the simplicity of the invertebrate olfactory structure, it is far more manageable to study the cellular organization than in vertebrates. As can be seen in Figure 1 – 5, *Heliothis virescens* has three main antennocerebral tracts (or pathways) (the IACT, the MACT and the OACT) projecting to two main areas of higher associative learning (the calyces of mushroom bodies and the lateral protocerebrum). In vertebrates, information from the glomeruli is projected to many more parts of the cortex and the neural pathways are far more numerous. But as we shall see, the principle of cellular organization of these pathways appears to be relatively common among these disparate taxa.

In the synaptic organization of the invertebrate antennal lobes, we find three basic cell types (Kanzaki, Arbas, Strausfeld & Hildebrand, 1989). Projection neurons (PN) receive information from olfactory receptor cells (ORC) directly. The dendrites of the projection neurons are normally located within a single glomerulus. In addition to the direct input from the olfactory receptor cells, projection neurons receive input from local interneurons (Flanagan & Mercer, 1989). The projection neurons then form the antennocerebral tracts and project to the lateral protocerebrum and the calyces of mushroom bodies. Finally, the anaxonal local neurons (ALN) connect the projection neurons' subglomerular dendrites. All these basic cell types have their equivalents in

vertebrates, including humans. The invertebrate projection neurons are similar to the mitral and tufted cells of the vertebrate olfactory system. These cells receive input from the olfactory bulb and project directly to the primary olfactory cortex (which encompasses: the anterior olfactory nucleus, the prepiriform cortex, the lateral entorhinal cortex, the periamygdaloid cortex, and the cortical nucleus of the amygdala) (Doty, 2001). Both the insect projection neurons and mammalian projection neurons (the mitral and tufted cells) can be seen in Figure 8. The projections of the mitral and tufted cells are commonly known as the olfactory tract, including the lateral – and medial tracts in humans, but this is a misnomer, as primates have no medial tract (Price, 1990). The mitral and tufted cells receive input both directly from the olfactory receptor cells and from periglomerular cells, which are similar to the local interneurons in the invertebrate AL (Pinching & Powell, 1971).

The similarities in cellular structure of the primary neural pathways seem to be echoed in similarities in olfactory coding mechanisms. Christensen, Heinbockel and Hildebrand (1996) showed that when a vertebrate mitral cell or a insect PN elicits an excitatory response, this is often preceded and followed by hyperpolarization of the cell membrane. Thus, this response pattern applies both to vertebrates and invertebrates, in this case salamanders and moths.

4.2. *General anatomical similarities*

Before a more in-depth discussion of similarities in the olfactory systems of vertebrates and invertebrates, it is worth briefly mentioning some general similarities in anatomical organization. Firstly, in both vertebrates (e.g. Brodal, 2003) and in

invertebrates (e.g. Rø, Müller & Mustaparta, 2007), the main olfactory pathways are made up of neurons projecting directly from the main olfactory centre (antennal lobe in *Heliothis virescens* and olfactory bulb in humans) to areas of higher-order processing centers. This “simple” organization means that primary olfactory centers of both vertebrates and invertebrates are merely “one neuron removed” from the surrounding world, which is quite unique in the sensory modalities. As could be seen in Figures 1 – 5, these pathways mainly project ipsilaterally in *Heliothis virescens*. This is also the case in humans (Brodal, 2003), in contrast to most sensory modalities. Finally, there is some similarity in the anatomical organization of the terminal areas of the projections from the main olfactory centers. As can be seen in Figures 1 – 5, the olfactory pathways terminate in a multitude of areas in the brain, without any strict anatomical organization. This is also the case in humans (Brodal, 2003). More specific similarities in structural – and functional organization will be discussed in the remainder of the discussion.

4.3. *Odor detection*

Even though the olfactory organs of vertebrates (nasal chamber with sensory epithelia) and invertebrates (appendages, for instance antennae) are morphologically different, odor molecules access them by quite similar mechanisms (Pelosi, 1996; Hildebrand & Shepherd, 1997). Figure 9 shows a schematic representation of olfactory receptor cells in insects and vertebrates.

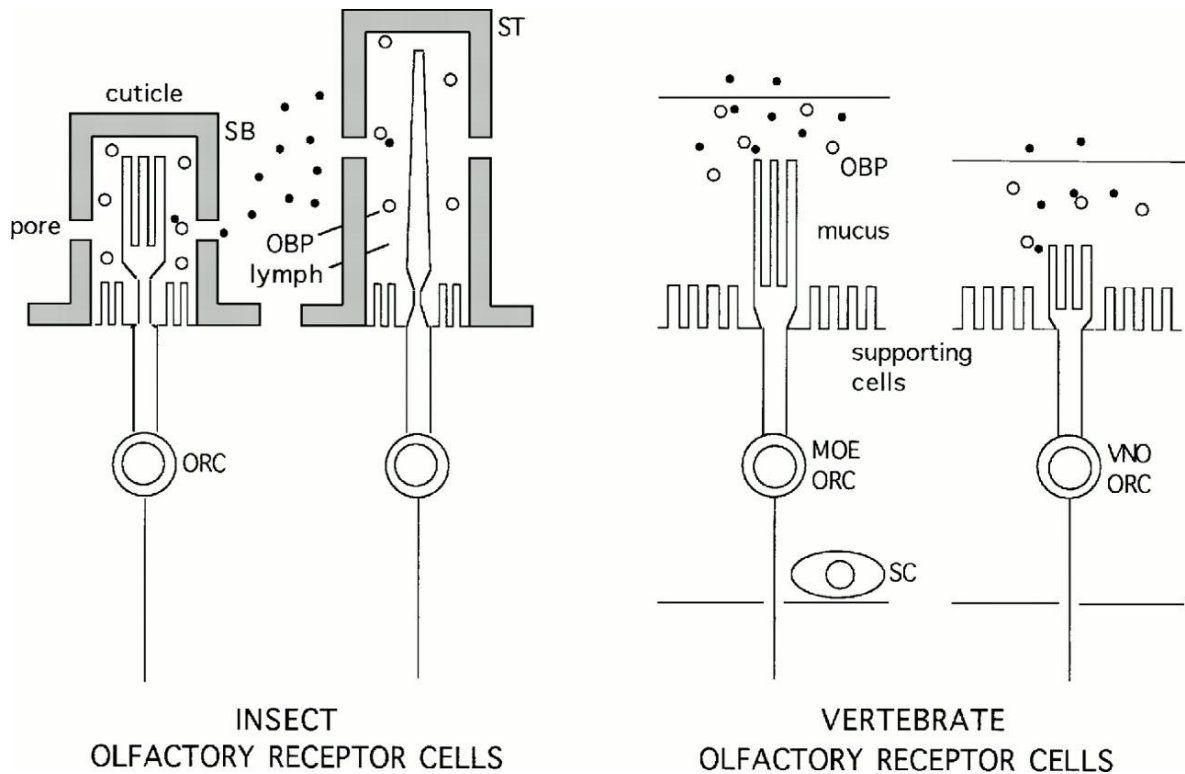


Figure 9 (from Hildebrand & Shepherd, 1997): In the insect (invertebrate) ORC, we see the basiconic sensillum (SB) and the trichoid sensillum (ST) and the odorant binding protein (OBP). In the vertebrate ORC, we see the ORC of the main olfactory epithelium (MOE) and of the vomeronasal organ (VNO). We also see the stem cells (SC), which continually regenerate MOE ORCs in vertebrates.

The simplification of this figure makes the structural similarity readily apparent. Such a similarity across taxa suggests an evolutionary value of this structural organization.

There appears to be some further similarity between vertebrates and invertebrates, in that odorant binding proteins have been found in both (in the antennae of moths (including *Heliothis virescens*, Krieger et al., 1993) and in the mucus of the vertebrate nasal epithelium (Pevsner & Snyder, 1990)). The function of these proteins in invertebrates seems to be transportation of hydrophobic odor molecules through the sensillum lymph. However, some important differences have emerged (Hildebrand & Shepherd, 1997). Vertebrate odorant binding proteins have an eight-stranded structure, which differs in helical structure to invertebrate odorant binding proteins. They also have broad ligand-ligand affinities, as compared to the narrow binding

affinities of invertebrate odorant binding proteins. Little is known of the role of the odorant binding proteins in vertebrates, but it has been speculated that they enhance odor responses by concentrating odor molecules in the mucus (Hildebrand & Shepherd, 1997). A further important difference between vertebrate – and invertebrate olfactory receptor cell structural organization, is the lack of regeneration through stem cells in invertebrates. Nevertheless, this structural (if not functional) similarity further clarifies the common olfactory structural organizational principles.

4.4. *Glomerular organization and functionality*

In *Heliothis virescens*, as in all invertebrates (Strausfeld & Hildebrand, 1999), the primary olfactory centre, the antennal lobe, is made up of structures called glomeruli. The fact that this organization is common to insects and humans, divided by 600 million years of evolution (Axel, 2005), is a clear demonstration of the evolutionary efficiency of such an organization. The glomeruli of the antennal lobes in insects are where the terminals of the projections from the sensory neurons of the sensilla make synapses with second-order neurons (Boeckh & Tolbert, 1993). The spheroidal structures in which this happens are the glomeruli. A glomerulus has been defined as “a synaptic complex enclosed in glial membranes or otherwise set apart” (Shepherd, 1974). This is a definition, which also applies to vertebrates (Berg et al., 2002). The structural organization of the antennal lobes is strikingly similar to that of humans. In humans, the olfactory nerves from the olfactory epithelium terminate in glomeruli in the olfactory bulb (e.g. Leise, 1990; Axel, 1995; Kandel, Schwartz & Jessell, 2000). Knowledge about this structural organization in humans is quite old (Retzius, 1892). An illustration of the organization of glomeruli in vertebrates and invertebrates can be seen in Figure 10.

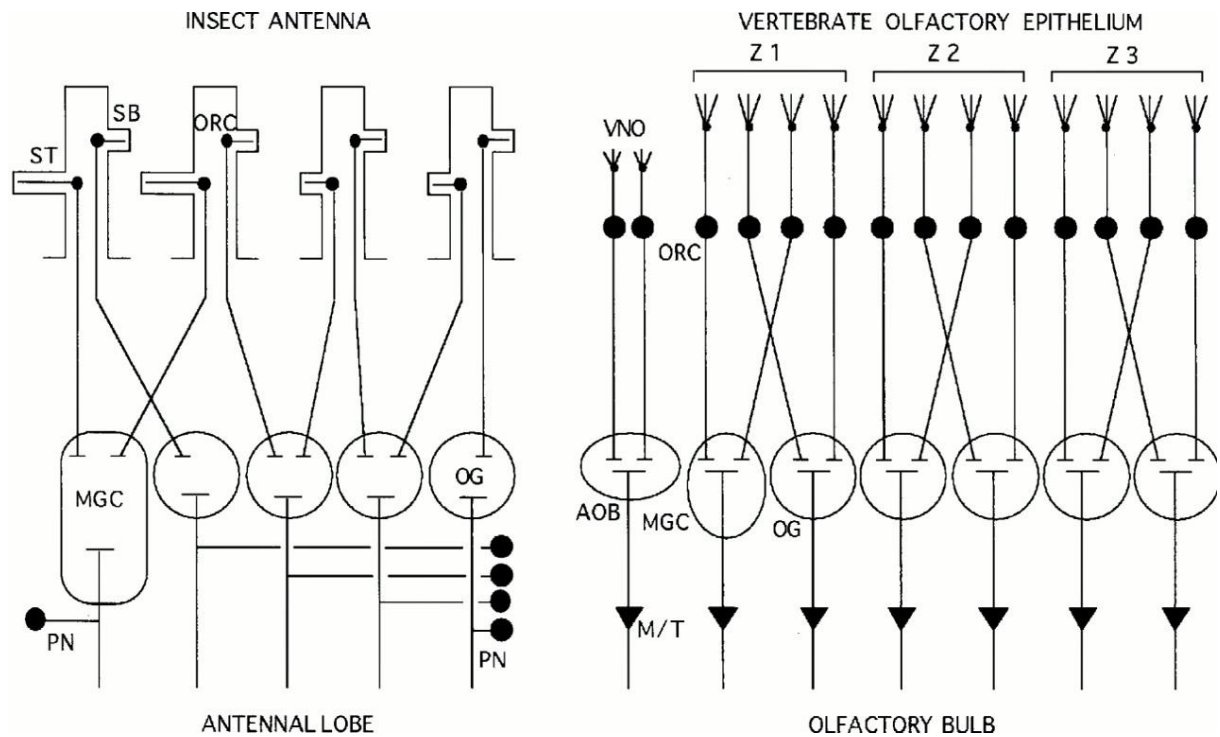


Figure 10 (from Hildebrand and Shepherd, 1997): On the left, we see the invertebrate (insect) glomeruli. Olfactory receptor cells (ORC) from the basiconic sensillum (SB) and the trichoid sensillum (ST) converge on the glomeruli. In this illustration we both see the ordinary glomeruli (OG) and the male-specific macroglomerular complex (MGC). From the glomeruli, projection neurons (PN) send information via the antennocerebral tracts to areas of higher olfactory processing (not shown). On the right, we see the vertebrate glomeruli. Z1-3 express different olfactory receptor proteins and project to the olfactory glomeruli (OG) and the modified glomerular complex (MGC) (in mammals). The ORCs of the vomeronasal organ (VNO) have their axons in the accessory olfactory bulb. From the AOB, the MGC and the OGs, mitral and tufted cells project to areas of higher olfactory processing (not shown).

In fact, this organization into glomeruli seems to be common to all organisms with a sense of smell (e.g. Boeckh & Tolbert, 1993; Hildebrand & Shepherd, 1997; Strausfeld & Hildebrand, 1999). The antennal lobe, containing the glomeruli of *Heliothis virescens*, is visible in Figure 1, 2, 3, 4 and 5, but the structural organization of the individual glomeruli does not appear in these illustrations. However, a digital atlas of the antennal lobe of *Heliothis virescens* has been developed, which clearly illustrates the glomerular organization. This atlas can be seen in figure 11. Because of the comparatively small number of glomeruli in the insect antennal lobe, the production of a digital atlas is a far more manageable task than a similar mapping of the human bulb. Complete organizational maps make it possible to investigate the

function of individual glomeruli. It also makes comparative investigations across species and gender possible. Thus, one might gain knowledge about the behavioral consequences of neural organization and the evolutionary role of sensory modalities. Such research offers a unique opportunity of achieving insight into the biological and evolutionary influences on human behavior.

In all organisms, including *Heliothis virescens* and humans, glomeruli seem to be organized according to the chemotopic principle, what Richard Axel (2004) calls “a molecular logic of olfactory perception”. Buck and Axel (1991) showed that sensory neurons expressing the same odor receptors project to the same glomerulus, and that each neuron seems to express only one odor receptor type. In humans, the gene family expressing odor receptors is made up of about 1000 different genes, roughly 1 % of the human genome (Axel, 1995). It is clear that such a vast gene family and the resulting number of glomeruli is difficult to study in any meaningful manner if one has no inkling on where to start. Therefore, studies of insects, with their much less complicated genome and glomerular organization, provide important backdrops for trying to understand the role of the human olfactory neural organization.

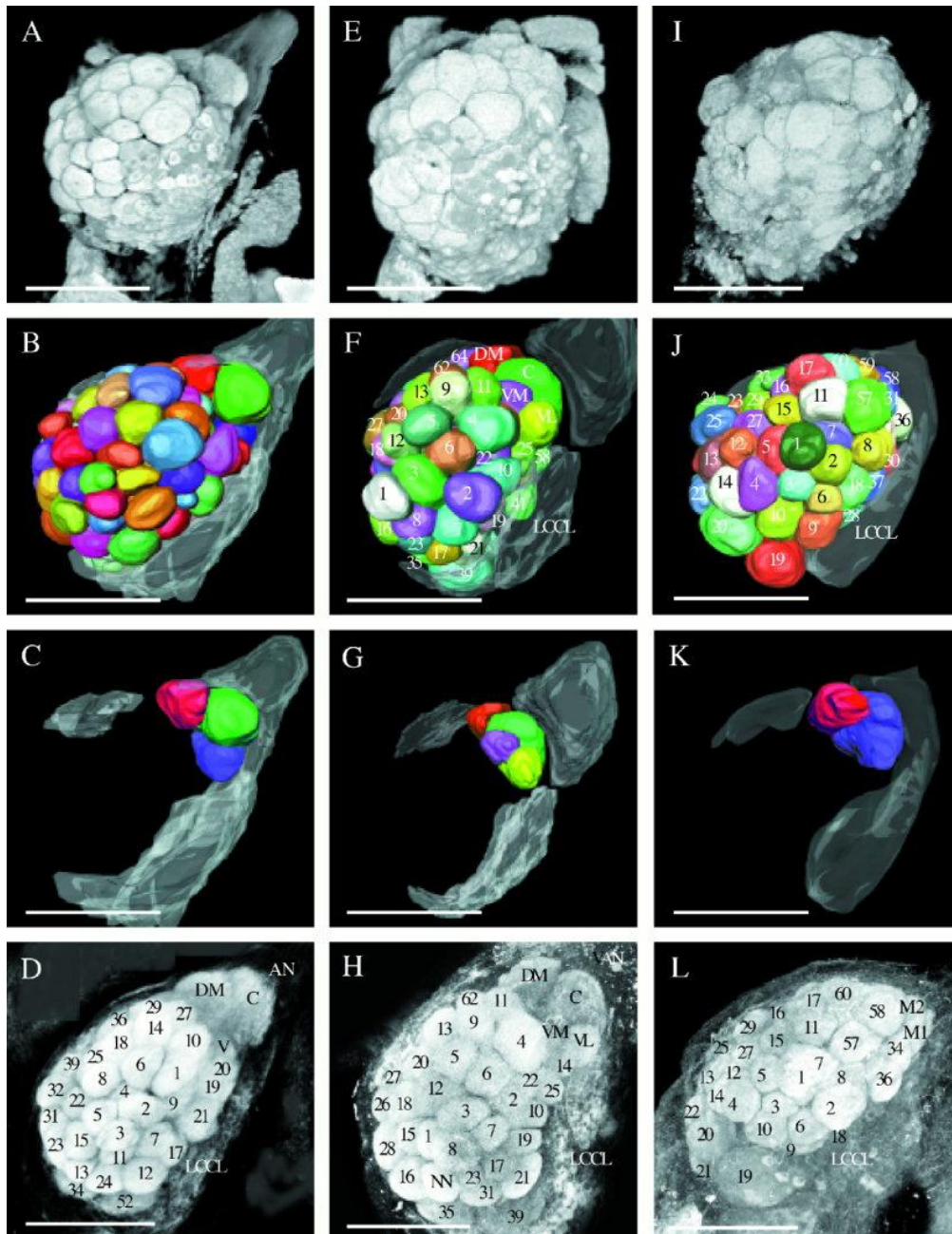


Figure 11 (from Berg et al., 2002): The left column shows a 3D reconstruction of the *Helicoverpa assulta*, the middle column shows the male *Heliiothis virescens* and the right column shows the female *Heliiothis virescens*. The first row shows data from the confocal stacks, the second row shows the digital atlases, the third row shows the male MCG and the large female glomeruli (LFG) and the fourth row shows data from a second specimen. The authors have identified and numbered the individual glomeruli.

With the knowledge that there are significant similarities between the invertebrate antennal lobes and the human olfactory bulb, it is possible to use invertebrate research to investigate whether the structural organization of the glomeruli translates into behavioral output. Although one cannot simply assume that findings in invertebrate research can be directly applied to the behavior of vertebrates, the structural similarities make it seem likely that they are evolutionarily well adapted. If the functional organization differed substantially across taxa, it would seem likely that the structural organization would differ accordingly. Furthermore, the similarities in behavioral responses to pheromone stimulation discussed in the introduction of this thesis are a strong indication of shared behavioral responses to odorant stimulation across taxa.

Indeed, there seem to be strong indications of glomerular organization as functional - / behavioral units as well as structural units. As the first relay station of olfactory information in both vertebrates and invertebrates, it seems that olfactory processing concerning the odor quality begins in the glomeruli before information is processed in higher olfactory centers (Axel, 2005). Wang, Wong, Flores, Vosshall and Axel (2003), working with the fruit fly, *Drosophila*, showed that specific odor stimulations activate specific patterns of glomeruli. They furthermore showed that these patterns are conserved among different organisms. An illustration of this can be seen in Figure 12.

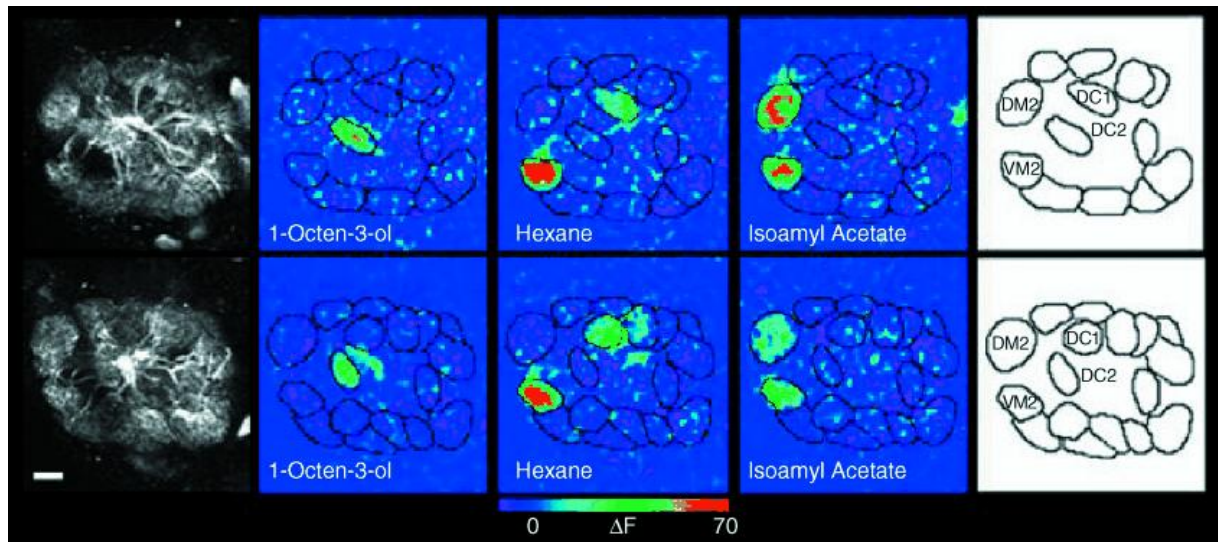


Figure 12 (from Axel, 2005): Illustration showing similar patterns of glomerular activation in response to the same odor stimulation in two different organisms. The column on the left shows the glomerular structure before odor stimulation, and the column on the right is a schematic representation of the glomerular organization.

This is a clear illustration of common principles of olfactory functional organization. Furthermore, there is emerging evidence for a similar principle being present in vertebrates, including mammals (e.g. Rubin & Katz, 1999; Uchida, Takahashi, Tanifuji & Mori, 2000; Belluscio, Lodovichi, Feinstein, Mombaerts & Katz, 2002). However, when studying patterns of glomerular activation in mammals, one normally utilizes electrical recordings. While these are sensitive imaging techniques, they have some important drawbacks for an overall understanding of organizational principles. Mainly, there is poor spatial resolution, limiting the results to the activation of either a few cells or small areas of the olfactory bulb. The staining and imaging techniques possible with invertebrates still give us the most comprehensive knowledge of overall principles of glomerular activation. But there are clear indications of similar principles of functional -, as well as structural organization across taxa.

The question then becomes whether one can relate these patterns of glomerular activation to specific behavioral responses. And this does indeed seem to be the case. It is well known that: “All animal species exhibit innate behaviors in response to specific sensory stimuli that are likely to result from the activation of developmentally programmed circuits”, (Axel, 2005). Suh, Wong, Hergarden, Wang, Simon, Benzer, Axel and Anderson (2004) demonstrated this in studies of *Drosophila*. They found that *Drosophila* exhibits clear avoidance behavior when encountering odors released by stressed flies. Identifying CO₂ as an active component of the odor released by stressed flies, they found that a single glomerulus (and a single family of olfactory receptor cells) is involved in detecting and avoiding it. Behavioral principles in glomerular organization and activation have also been found in *Heliothis virescens* (Hansson, Almaas & Anton, 1995; Berg, Galizia, Brandt & Mustaparta, 2002). The male macro glomerular complex with its relatively simple organization into four compartments provides an excellent opportunity for the link between structure and behavior. Two of the compartments receive neural input stimulating behavioral attraction, while two compartments receive neural input inhibiting behavioral attraction. Thus, the behavioral organization of the olfactory information starts already at the first relay station. Although human behavior is far more complex than that of *Drosophila* or *Heliothis virescens*, based on the indications of common principles of structural – and functional organization, this might well be an area of greater future interest to psychologists interested in the biological influences on human behavior.

Vertebrates seem to have an olfactory equivalent to the visual “centre-surround” system (Mori, Nagao & Yoshihara, 1999). In rabbits, aliphatic aldehydes activate a

collection of neighboring glomeruli (Mori, Nagao & Yoshihara, 1999). This offers the opportunity of lateral inhibition at the glomerular level. It was thought that such processing did not occur in invertebrates, but emerging evidence seems to suggest that this may also be found in invertebrates (Sachse & Galizia, 2002), suggesting a more advanced level of processing in invertebrates than previously considered

Finally, there seems to be a great degree of commonality in the events underlying the development of glomeruli in vertebrates and invertebrates (Strausfeld & Hildebrand, 1999). Both in vertebrates (Valverde, Santanaca & Heredia, 1992) and in invertebrates (Oland & Tolbert, 1996), glial cells guide the axons of the olfactory receptor cells to glomeruli according to the chemotopic principle. This similarity poses quite a few questions, expressed by Strausfeld and Hildebrand (1999): “Do such commonalities suggest that the glomerulus [...] originated in a common bilaterian ancestor to protostomes and deuterostomes? Or, is common design and physiology the consequence of convergent evolution? Is there a uniquely logical response to common selective pressures such that to construct a glomerulus requires the same set of rules?”.

4.5. *The calyces of mushroom bodies*

As previously discussed, the axons of the projection neurons in invertebrates and the mitral and tufted cells in vertebrates project to various locations in their respective brains. Although the degree of divergence in areas of higher olfactory learning of vertebrates and invertebrates remains unclear, similarities have been proposed (Farris, 2008). The calyces of mushroom bodies (seen in Figures 6 and 8) have been the focus of much research and discussion regarding this topic. There seem to be many

functions performed by the mushroom bodies. Yasuyama, Meinertzhagen and Schürmann (2002) described the mushroom bodies as: “[...] a high-order integrative center required for olfactory learning and memory [...]”. There is evidence that they are implicated in olfactory learning and memory (Heisenberg, 1998) and context-dependent stimulus recognition (Li & Strausfeld, 1999). This corresponds with the functions of the hippocampus in mammals (Morris, Garru, Raulins & O’Keefe, 1982), which also receives input from the olfactory bulb via the entorhinal cortex (Strausfeld & Hildebrand, 1999).

Comparisons have also been made between the calyces of the mushroom bodies and the cerebellum of mammals (Schürmann, 1974; Yasuyama, Meinertzhagen & Schürmann, 2002). The terminals of the antennal lobe projection neurons in the calyces seem to share many features of the mossy fiber rosettes of the granular layer of the cerebellum. Antennal lobe projection neurons have an excitatory effect on Kenyon cells, and the mossy fiber rosettes of the cerebellum have an excitatory effect on granule cells. Kenyon cells and granule cells also both receive inhibitory input from GABAergic cells. Thus, it is speculated that the calyces mediate inhibitory feedback in a manner similar to cerebellar Golgi cells. At the present time though, this is no more than speculation based on indications. It does however demonstrate how one might use knowledge of different taxa to gain further insight into structural – and functional organization.

5. Conclusion

This thesis has discussed findings and literature concerning the two research problems formulated in chapter 1.3. The experimental research carried out clearly showed the main neural pathways from the primary olfactory centre (antennal lobe) to areas of higher olfactory learning (mushroom bodies and lateral protocerebrum). Overall, the illustrations did not provide any surprises, but rather served to support the general anatomical architecture found by other researchers (e.g. Berg, Galizia, Brandt & Mustaparta; 2002; Skiri, Rø, berg & Mustaparta, 2005; Rø, Müller & Mustaparta, 2007). While one may always have a desire to discover something new and push the boundaries of knowledge, findings that support a general consensus are no less important. Indeed, in order to study structural – and functional organization across taxa, significant certainty regarding organization in each taxon is needed. One might liken it to trying to explain the general mechanics of a clock, if there is no agreement as to the function of its individual parts.

The second part of the thesis discussed whether there might be similarities in the organization of primary olfactory structures in the neural pathways between the primary olfactory centre (antennal lobe in invertebrates and olfactory bulb in vertebrates) and areas of higher olfactory learning. The main olfactory structures found in the experimental research provided the framework for this discussion, i.e. focusing on the antennal lobe, the antennocerebral tracts and the mushroom bodies and the lateral protocerebrum. Significant similarities were found both in the mechanisms of odor detection; the structure and function of the glomeruli; the cellular structure and olfactory coding in neural pathways; and the functional profile of the

first areas of higher olfactory learning, the mushroom bodies and the hippocampus primarily. Quite a few significant differences were also found, but overall, this discussion supports the notion that there are many universal principles exist in the olfactory structural organization across taxa.

Of course, this thesis has its limitations. The area of research covers mainly a small (but significant) part of the olfactory system, namely the structures involved in the first steps of olfactory processing. Studies of higher areas of olfactory processing may show more clearly the evolutionary differences between vertebrates and invertebrates. Furthermore, this discussion has not focused on the accessory olfactory system. It is quite possible that this may be a fruitful area for future psychological research, as suggested by research cited in the introduction to this thesis (e.g. Veith, Buck, Getzlaf, Van Dalfsen & Slade, 1983; Cowley & Brooksbank, 1991; Herrada & Dulac, 1997; Brodal, 2003). However, at the present time, much less is known about the accessory olfactory system in humans, and, as was previously argued, any meaningful comparison across taxa requires a fair amount of knowledge about the individual taxa and organisms. Finally, this thesis has focused on possible universal principles of current olfactory organization, rather than universal principles of the evolutionary development of the olfactory organization. Although this has been touched upon a few times, it has been left out to create a realistic framework for this thesis. Research into this field might however enlighten us further as to the function of our existing olfactory organization and the general process of evolution.

Overall, the thesis supports the notion that psychological research (including clinical research) should look beyond the boundaries of our own taxon in order to gain a more

profound and rich understanding of the human experience. This applies to both research into normal, human behavior and psychopathology.

6. Acknowledgements

There is one person above all I would like to thank – my supervisor and mentor, Bente Gunnveig Berg. Her never-ending patience, indomitable optimism and remarkable belief in the beauty and logic of nature and of man gave me the inspiration to explore the outer limits of psychology and perseverance in the face of discouragement.

The rest of the staff at MTFS was also an encouragement through their genuine interest, willingness to share their knowledge and overall kindness.

And finally, the interest and encouragement of my colleagues at Blå Kors Senter supported my belief that clinical psychologists are willing to look beyond their zone of comfort in order to further our understanding of the complex interplay behind human behavior.

Oslo, January 2010

Toke Knizek Vang

7. References

All references in the citation style of the American Psychological Association

Axel, R. (1995). The molecular logic of smell. *Scientific American*, 273(4), 154-159.

Axel, R. (2005). Scents and sensibility: A molecular logic of olfactory perception (Nobel lecture). *Angewandte Chemie International Edition*, 44(38), 6110-6127.

Belluscio, L., Lodovichi, C., Feinstein, P., Mombaerts, P. & Katz, L. C. (2002). Odorant receptors instruct functional circuitry in the mouse olfactory bulb. *Nature*, 419, 296-300.

Berg, B. G., Galizia, C. G., Brandt, R. & Mustaparta, H. (2002). Digital atlases of the antennal lobe in two species of tobacco budworm moths, the Oriental *Helicoverpa assulta* (male) and the American *Heliothis virescens* (male and female). *The Journal of Comparative Neurology*, 446, 123-134.

Berg, B. G., Tumlinson, J. H. & Mustaparta, H. (1995). Chemical communication in heliothine moths IV. Receptor neuron responses to pheromone compounds and formate analogues in the male tobacco budworm moth *Heliothis virescens*. *Journal of Comparative Physiology*, 177, 527-534.

Bjering, S., Deinboll, I. I. & Mæhlen, J. (2000). Luktesansen. *Tidsskrift for den Norske Lægeforening*, 120, 3719-3725.

Boeckh, J. & Tolpert, L. P. (1993). Synaptic organization and development of the antennal lobe in insects. *Microscopy Research and Technique*, 24, 260-280.

Brodal, P. (2003). *Sentralnervesystemet*. Oslo, Norway: Universitetsforlaget.

Buck, L. & Axel, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell*, 65, 175-187.

Butenandt, A., Beckmann, R., Stamm, D. & Hecker, E. (1959). Über den Sexuallockstoff den Seidenspinners *Bombyx mori*. Reindarstellung und Konstitution. *Zeitschrift der Naturforschung*, 14(b), 283-284.

Christensen, T. A., Heinbockel, T. & Hildebrand, J. G. (1996). Olfactory information processing in the brain: encoding the chemical and temporal features of odors. *Journal of Neurobiology*, 30, 82-91.

Christensen, T. A., Waldrop, B. R., Harrow, I. D. & Hildebrand, J. G. (1993). Local interneurons and information processing in the olfactory glomeruli of the moth *Manduca sexta*. *Journal of Comparative Physiology*, 173, 385-399.

Cowley, J. J. & Brooksbank, B. W. L. (1991). Human exposure to putative pheromones and changes in aspects of social behavior. *The Journal of Steroid Biochemistry and Molecular Biology*, 39, 647-659.

Dethier, V. G. (1990). Five hundred million years of olfaction. In K. Colbow (Ed.), *Frank Ellison Linville's RH Wright Lectures on Olfactory Research*, (pp. 1-37). Burnaby, Canada: Simon Fraser University.

Doty, R. (2001). Olfaction. *Annual Review of Psychology*, 52, 423-452.

Farris, S. M. (2008). Structural, functional and developmental convergence of the insect mushroom bodies with higher brain centers of vertebrates. *Brain, Behavior and Evolution*, 72, 1-15.

Fedoroff, I. C., Stoner, S. A., Andersen, A. E., Doty, R. L. & Rolls, B. J. (1995). Olfactory dysfunction in anorexia and bulimia nervosa. *International Journal of Eating Disorders*, 18, 71-77.

Flanagan, D. & Mercer, A. R. (1989). Morphology and response characteristics of neurones in the deutocerebrum of the brain in the honeybee *Apis mellifera*. *Journal of Comparative Physiology*, 164, 483-494.

Gansler, D. A., Fucetola, R., Kregel, M., Stetson, S., Zimering, R. & Makary, C. (1998). Are there cognitive subtypes in adult attention deficit/hyperactivity disorder? *The Journal of Nervous and Mental Disease*, 186, 776-781.

Hansson, B. S., Almaas, T. J. & Anton, S. (1995). Chemical communication in heliothine moths V. Antennal lobe projection patterns of pheromone-detecting

olfactory receptor neurons in the male *Heliothis virescens* (Lepidoptera: Noctuidae). *Journal of Comparative Physiology*, 177(A), 535-543.

Heisenberg, M. (1998). What do mushroom bodies do for the insect brain? *Learning Memory*, 5, 1-10.

Herrada, G. & Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell*, 90, 763-773.

Hildebrand, J. G. & Shepherd, G. M. (1997). Mechanisms of olfactory discrimination: Converging evidence for common principles across taxa. *Annual Review of Neuroscience*, 20, 595-631.

Homberg, U., Montague, R. A. & Hildebrand, J. G. (1988). Anatomy of antennocerebral pathways in the brain of the sphinx moth *Manduca sexta*. *Cell and Tissue Research*, 254, 255-281.

Kandel, E., Schwartz, J. & Jessell, T. (2000). *Principles of neural science*, 4th. Ed. New York, NY: McGraw-Hill.

Kanzaki, R., Arbas, E. A., Strausfeld, N. J. & Hildebrand, J. G. (1989). Physiology and morphology of projection neurons in the antennal lobe of the male moth *Manduca sexta*. *Journal of Comparative Physiology*, 165, 427-453.

- Kopala, L. C., Good, K. P., Koczapski, A. B. & Honer, W. G. (1998). Olfactory deficits in patients with schizophrenia and severe polydipsia. *Biological Psychiatry*, *43*, 497-502.
- Lapierre, D., Braun, C. M. & Hodgins, S. (1995). Ventral frontal deficits in psychopathy: neuropsychological test findings. *Neuropsychologia*, *33*, 139-151.
- Leise, E. M. (1990). Modular construction of nervous systems: a basic principle of design for invertebrates and vertebrates. *Brain Research Reviews*, *15*(1), 1-23.
- Li, Y. S. & Strausfeld, N. J. (1999). Multimodal efferent and recurrent neurons in the medial lobes of cockroach mushroom bodies. *Journal of Comparative Neurology*, *409*, 647-663.
- McCaffrey, R. J., Duff, K. & Solomon, G. S. (2000). Olfactory dysfunction discriminates probable Alzheimer's dementia from major depression: a cross validation and extension. *The Journal of Neuropsychiatry and the Clinical Neurosciences*, *12*, 29-33.
- Mobbs, P. G. (1982). The brain of the honeybee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *298*, 309-354.
- Mori, K., Nagao, H. & Yoshihara, Y. (1999). The olfactory bulb: coding and processing of odor molecule information. *Science*, *286*, 711-715.

Morris, R. G. M., Garru, P., Raulins, J. N. P. & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, 297, 681-683.

Oland, L. A. & Tolbert, L. P. (1996). Multiple factors shape development of olfactory glomeruli: insights from an insect model system. *Journal of Neurobiology*, 30, 92-109.

Pinching, A. J. & Powell, T. P. S. (1971). The neuropil of the glomeruli of the olfactory bulb. *Journal of Cell Science*, 9(2), 347-377.

Price, J. L. (1990). Olfactory system. In G. Paxinos (Ed.), *The Human Nervous System*, (pp. 979-998). San Diego, CA: Academic.

Retzius, G. (1892). Die Endungsweise der Riechnerven. *Biologische Untersuchungen Neue Folge*, 3, 25-27.

Rø, H., Müller, D. & Mustaparta, H. (2007). Anatomical organization of antennal lobe projection neurons in the moth *Heliothis virescens*. *The Journal of Comparative Neurology*, 500, 658-675.

Rubin, B. D. & Katz, L. C. (1999). Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron*, 23, 499-511.

Sachse, S. & Galizia, C. G. (2002). Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. *Journal of Neurophysiology*, 87, 1106-1117.

Schürmann, F.-W. (1974). Bemerkungen zur Funktion der Corpora pedunculata im Gehirn der Insekten aus morphologischer Sicht. *Experimental Brain Research*, 19, 406-432.

Singer, A. G. (1991). A chemistry of mammalian pheromones. *The Journal of Steroid Biochemistry and Molecular Biology*, 39, 627-632.

Stedman, T. J. & Clair, A. L. (1998). Neuropsychological, neurological and symptom correlates of impaired olfactory identification in schizophrenia. *Schizophrenia Research*, 32, 23-30.

Steno, N. (1965). *Lecture on the anatomy of the brain. Introduction by G. Scherz.* Copenhagen, Denmark: A. Brusck.

Strausfeld, N. J. & Hildebrand, J. G. (1999). Olfactory systems: common design, uncommon origins? *Current opinion in neurobiology*, 9, 634-639.

Striebel, K. M., Beyerstein, B., Remick, R. A., Kopala, L. & Honer, W. G. (1999). Olfactory identification and psychosis. *Biological Psychiatry*, 45, 1419-1425.

Uchida, N., Takahashi, Y. K., Tanifuji, M. & Mori, K. (2000). Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. *Nature Neuroscience*, 3, 1035-1043.

Valverde, F., Santacana, M. & Heredia, M. (1992). Formation of an olfactory glomerulus: morphological aspects of development and organization. *Neuroscience*, 49, 255-275.

Veith, J. L., Buck, M., Getzlaf, S., Van Dalfsen, P. & Slade, S. (1983). Exposure to men influences the occurrence of ovulation in women. *Physiology & Behavior*, 31, 313-315.

Wang, J. W., Wong, A. M., Flores, J., Vosshall, L. B. & Axel, R. (2003). Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell*, 112(2), 271-282.

Yasuyama, K., Meinertzhagen, I. A. & Schürmann, F.-W. (2002). Synaptic organization of the mushroom body calyx in *Drosophila melanogaster*. *The Journal of Comparative Neurology*, 445(3), 211-226.

Zawarzin, A. A. (1925). Der Parallismus der Strukturen als ein Grundprinzip der Morphologie. *Zeitschrift für wissenschaftliche Zoologie*, 124, 118-212.

Appendix A

Placement of individual slices in confocal stacks

Figure 1

A: Slice: 17/74

B: Slice: 26/74

C: Slice: 31/74

D: Slice: 46/74

E: Slice: 54/74

F: Slice: 58/74

Figure 2

A: Slice: 56/122

B: Slice: 64/122

C: Slice: 76/122

D: Slice: 83/122

E: Slice: 88/122

F: Slice: 96/122

Figure 3

A: Slice: 50/135

B: Slice: 58/135

C: Slice: 68/135

D: Slice: 77/135

E: Slice: 85/135

F: Slice: 94/135

Figure 4

Slice: 1/10

Figure 5

Slice: 32/32

Figure 6

A: Slice: 35/99

B: Slice: 64/99

C: Slice: 74/99

D: Slice: 81/99