Characterization of octopaminergic descending unpaired median neurons of the suboesophageal ganglion in *Manduca sexta*

Dissertation
zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie und Pharmazie
der Freien Universität Berlin

vorgelegt von

Jessika Erdmann
aus Berlin

2014

Die Arbeit wurde von Mai 2010 bis Juli 2014 unter der Leitung von Prof. Dr. Hans-Joachim Pflüger am Institut für Neurobiologie angefertigt.

1. Gutachter: Prof. Dr. Hans-Joachim Pflüger

2. Gutachter: Prof. Dr. Jens Rolff

Datum der Disputation: 30.09.2014

CONTENTS

1 INTRODUCTION

1.1 Neuromodulation

Neuromodulation enables the flexibility of an animal's behavior to adapt to internal and external environmental changes. Unlike neurotransmitters, neuromodulators do not necessarily transfer excitation or inhibition from one neuron to the other. Instead, they alter the efficacy of synaptic transmission, or the cellular and/or synaptic properties (Klaassen and Kammer, 1985; Lopez and Brown, 1992). By these alterations they are able to reconfigure a neural network leading to multiple different output patterns and therefore making it multifunctional (Hooper and Marder, 1984, 1987; Katz and Harris-Warrick, 1990). Among substances causing such effects is the group of biogenic amines which serve not only as neuromodulators, but also as neurotransmitters and neurohormones (Evans, 1980). While neurotransmitters act fast and directly on a postsynaptic neuron and neurohormones, on the other hand, are released into the haemolymph and affect distant peripheral targets, biogenic amines as neuromodulators cause slow but long lasting effects on a group of neurons by its local release in the central nervous system or periphery. The latter induce their effects by binding to G-protein coupled receptors which are coupled, depending on the receptor type, to different second messenger pathways, including e.g. inositol 1,4,5-trisphosphate (IP_3), adenosine 3´,5´-cyclic monophosphate (cAMP), calcium and diacylglycerol (DAG). Biogenic amines are found in invertebrates as well as in vertebrates where they control and modulate a variety of processes like sexual behavior, cardiovascular homeostasis, learning and memory and thermoregulation (Blenau and Baumann, 2001). In vertebrates, several neurological and neuropsychiatric disorders are linked with an impairment of biogenic amine neurotransmission, such as that of the catecholamines dopamine and noradrenline (Rubin et al., 1985; Davidson et al., 1987; Oades, 1987; Dailly et al., 2004).

1.2 Octopamine

Octopamine, p-hydroxyethanolamine (OA), is the most abundant biogenic monoamine in the nervous system of invertebrates where it modulates almost every physiological process (Roeder, 1999; Pflüger and Stevenson, 2005; Verlinden et al., 2010). It was first discovered in

the salivary glands of the *Octopus vulgaris* (Erspamer and Boretti, 1951) and its actions appear to be universal for neuromodulatory processes in the nervous system in general. Therefore and because of the advantage of a less complex invertebrate central nervous system and a relatively small number of neurons involved in generating a certain behavior compared to vertebrates, invertebrate's octopamine is a well suited model to investigate general features of neuromodulation.

Octopamine is the decarboxylation product of the single amino acid tyrosine. In a two-step synthesis tyrosine is first decarboxylated by tyrosine decarboxylase to tyramine, which acts as an independent neurotransmitter as well. In a second step tyramine is hydroxylated to octopamine by tyramine β-hydroxylase. Interestingly, the catecholamine noradrenaline, one of the principal neuromodulators of the vertebrate's nervous system and octopamine reveal an analog chemical structure. Additionally, the invertebrate's octopaminergic and vertebrate's noradrenergic systems appear to be homologous as both neuromodulators reveal similar central nervous and peripheral functional roles (Roeder, 1999). In the mammalian nervous system octopamine is present only in trace levels where its function remains speculative (Borowsky et al., 2001).

1.3 Peripheral effects of octopamine

Octopamine is involved in a variety of physiological processes in insects such as in the immune response where it not only modulates the haemocyte activity and mediates the bacterial attachment to haemocytes (Dunphy and Downers, 1994) but also elevates phagocytosis and nodule formation (Baines et al., 1992). In addition, octopamine has been assigned to aggressive behavior in different insects species, such as *Drosophila,* crickets and lobsters (Livingstone et al., 1980; Stevenson et al., 2005; Hoyer et al., 2008; Zhou et al., 2008). In 2012, Stevenson and Rillich postulated that octopamine is not essential for the initiation of aggressive behavior in crickets but rather promotes the tendency of animals to fight. Further behaviors that are modulated by octopamine are sleep in *Drosophila* (Crocker et al., 2010) and various social behaviors such as nestmate recognition (Robinson et al., 1999) and division of labor in honeybees *Apis mellifera* (Schulz et al., 2002).

The homology of the vertebrate's noradrenergic and invertebrate's octopaminergic system is most obvious in their involvement in the fight or flight response, the fast adaptation to energy demanding situations (Roeder, 2005). In response to stressful stimuli, octopamine is

released into the haemolymph and acts as a neurohormone to liberate lipids from the fat body by either effecting the fat body directly (Orchard et al., 1982) or by inducing the release of adipokinetic hormones (AKH I and AKH II) from the corpora cardiaca (Pannabecker and Orchard, 1986). A further metabolic adaptation within the fight or flight response is the enhancement of fructose-2,6-bisphosphate, a substance stimulating the glycolysis, in the locust flight muscle (Blau and Wegener, 1994). Additional behavioral adaptations as part of the fight or flight responses due to octopamine is an enhanced heart rate (Prier et al., 1994) and increased ventilation rate (Bellah et al., 1984).

Similar to the vertebrate's noradrenaline, octopamine has opposite effects on skeletal and visceral muscles. In general, it positively modulates skeletal and negatively visceral muscles. In skeletal muscles such as the locust extensor tibiae of the hind leg or flight muscles, octopamine leads to an increased tension (Evans and O'Shea, 1977), increased relaxation rate (Evans and Siegler, 1982) and increased amplitude of twitch tension (Whim and Evans, 1988). In contrast, in visceral muscles, like the locust oviduct, octopamine decreases the basal tonus and inhibits myogenic contractions (Lange and Tsang, 1993). Further peripheral effects of octopamine include the modulation of sensory input. Almost every sense organ of invertebrates is modulated by octopamine, including those for acoustical, visual, olfactory, tactile, gustatory and proprioception (Roeder, 2005). This modulation can take place directly at the receptor site and is not characterized by a general increase in sensitivity which could be shown for various proprioceptors in different insect species (Pasztor and Bush, 1989; Ramirez and Orchard, 1990; Ramirez et al., 1993).

1.4 Central effects of octopamine

Additionally, octopamine is involved in various processes within the central nervous system. Among these central effects of this biogenic amine is the initiation and maintenance of motor programs (Roeder, 2005). In different invertebrate species, octopamine, depending on its injection site, is able to induce several different motor patterns, including flight or walking in locusts (Sombati and Hoyle, 1984a), flight in *Manduca sexta* (Claassen and Kammer, 1986), chewing in the lobster (Heinzel, 1988) and swimming in the medicinal leech (Hashemzadeh-Gargari and Friesen, 1989). Further central effects that can be assigned to octopamine are sensitization and dishabituation as shown in studies of Sombati and Hoyle (1984b) in which a habituated resistance-reflex-to-extension of the locust hind leg could be fully dishabituated by

iontophoretically released octopamine. Furthermore, dishabituation and sensitization effects by octopamine could be also observed in the visual system of the locust (Stern, 2009) and proboscis extension reflex (PER) of the honeybee (Mercer and Menzel, 1982; Braun and Bicker, 1992), respectively.

The involvement of octopamine in learning and memory is well investigated in the honeybee, where its injection into the mushroom body calyces and antennal lobes leads to an enhancement of the PER in classical conditioning experiments (Hammer and Menzel, 1998). The involvement of octopamine in the formation of appetitive olfactory memory in *Drosophila* could be proven in various studies (Schwaerzel et al., 2003; Schroll et al., 2006; Burke et al., 2012).

1.5 Octopaminergic neurons

Interestingly, only a small number of neurons mediate the modulatory effects of octopamine in the insect nervous system, varying among different species. The larval *Drosophila* central nervous system for example exhibits 40, the adult 70 octopaminergic neurons (Monastirioti et al., 1995). In contrast, over 100 octopaminergic neurons could be found in the head ganglia of the honeybee (Kreissl et al., 1994) and 160 octopamine-immunoreactive somata in the whole central nervous system of the cricket (Spörhase-Eichmann et al., 1992). Apparently, a correlation of the insect body size and number of octopaminergic neurons within the central nervous system exists. According to Farooqui (2012) the approximate number of octopaminergic neurons in all ganglia of large insects is 108 while small insects possess only approximately 40 to 50 neurons containing octopamine. In general, in the central nervous system two main populations of octopaminergic neurons exist: paired neurons that occur in the brain and some segmental ganglia and unpaired median (UM) neurons that can be found in all segmental ganglia except in the brain. The group of UM neurons are the best characterized neuromodulatory neurons in insects, especially in locusts (Bräunig and Pflüger, 2001).

This type of neurons was first described by Plotnikova in 1969 in locusts and shown to be octopaminergic by Hoyle in 1975. They exhibit large, pear-shaped somata along the midline and anteriorly running primary neurites which bifurcate into two bilaterally symmetrical axons. According to the position of their soma just beneath the dorsal or ventral surface of a ganglion they are called dorsal or ventral unpaired median (DUM/VUM) neurons.

They derive from the unpaired median neuroblasts which persist and remain active during metamorphosis in holometabolous insects (Bräunig and Pflüger, 2001). Octopaminergic UM neurons can be classified into two main populations on the basis of their morphology: in segmental and intersegmental UM neurons. Segmental UM neurons can be found in all thoracic and abdominal ganglia and exhibit axons projecting into the peripheral nerves of their own ganglion to innervate peripheral targets, in this study referred to as efferent UM neurons of the thoracic and abdominal ganglia. Among the peripheral targets of locust UM neurons are skeletal (Bräunig, 1997) and visceral muscles (Lange and Orchard, 1984), the heart (Ferber and Pflüger, 1990) and sense organs like a special class of proprioreceptors of the locust hindleg (Bräunig and Eder, 1998). In addition, efferent UM neurons of the thoracic and abdominal ganglia innervate glands like the salivary gland and form neurohaemal release sites on the surface of peripheral nerves and are therefore a potential source of octopamine acting as a neurohormone (Bräunig et al., 1994). They possess multiple spike initiation zones and an excitable soma that is able to form characteristic large overshooting spontaneous action potentials (Grolleau and Lapied, 2000). Interestingly, the thoracic efferent DUM neurons of the locust form morphological subpopulations based on the peripheral nerves through which they exit to innervate their peripheral targets (Watson, 1984; Duch et al., 1999). Furthermore, functional subpopulations of the locust thoracic DUM neurons exist as they are recruited or inhibited differentially during specific motor behaviors (Burrows and Pflüger, 1995; Baudoux et al., 1998; Duch et al., 1999).

In contrast to the segmental UM neurons, axons of the intersegmental UM neurons do not exit the nerves of their own ganglia. Instead, they send their axons into the connectives towards the adjacent ganglia. A morphological study of Bräunig in 1988 revealed that the mouthpart segments of the locust are not innervated by segmental efferent DUM neurons of the suboesophageal ganglion (SOG). Nevertheless, axons of a single, intersegmentally projecting prothoracic DUM neuron could be found in the maxillary and labial nerves. Interestingly, the SOG of insects, a higher center for motor coordination involved in the initiation of walking (Roeder, 1937; Ridgel and Ritzmann, 2005; Gal and Libersat, 2010), stridulation and ventilation (Otto and Henning, 1993), appears to lack segmental UM neurons unlike the thoracic and abdominal ganglia. Instead, it possesses two main populations of intersegmental UM neurons: ascending UM neurons projecting towards the brain and descending UM neurons projecting towards the thoracic ganglia (Bräunig, 1991; Dacks et al., 2005; Schröter et al., 2007; Cholewa and Pflüger, 2009). Compared to the thoracic efferent UM neurons of insects, relatively little is known about the intersegmental UM neurons of the

SOG. Their morphology was first described in great detail in the locust by Bräunig in 1991 and later in the honeybee (Schröter et al., 2007) and *Manduca sexta* (Dacks et al., 2005; Cholewa and Pflüger, 2009). Ascending UM neurons of the SOG are either central and innervate neuropile regions of the brain and SOG exclusively including the antennal lobes, calyces and pedunculi of the mushroom bodies, protocerebral lobes, central complex and lobula of the optic lobes or peripheral with axons in peripheral nerves of the brain. The latter project into the nervus corporis cardiaci III (NCC III) and appear to be associated with the antennal heart and the retrocerebral glandular complex in locusts and cockroaches (Pass et al., 1988; Bräunig, 1990). Additionally, some of the ascending peripheral UM neurons project into the antennal nerves as shown in crickets, locusts and honeybees (Bräunig et al., 1990; Schröter et al., 2007). So far, probably the most famous and best described ascending central UM neuron of the insect SOG is the VUMmx1 neuron of the honeybee (Hammer, 1993). Its soma is located in the maxillary neuromere and arborizations could be found in the glomeruli of the antennal lobes, lateral protocerebrum and lips and basal rings of the mushroom bodies. Its electrical stimulation is able to substitute the sugar reward in associative olfactory learning.

Descending UM neurons of the SOG possess bilaterally symmetrical descending axons that project via the neck connective towards the thoracic ganglia where they exhibit broad arborizations (Bräunig, 1991; Bräunig and Burrows, 2004). In the adult tobacco hawkmoth *Manduca sexta*, these types of neurons were first described by Dacks et al. in 2005. Their immunohistochemical study revealed a cluster of nine octopaminergic VUM neurons in the labial neuromere of the SOG that are therefore referred to as VUMlb neurons. Two of them ascend towards the brain while seven VUMlb neurons (VUMlb3-9) exhibit descending axons that project towards the thoracic ganglia. In contrast, the larval SOG of *Manduca sexta* contains three descending VUM (desVUM) neurons with somata in the labial neuromere (Cholewa and Pflüger, 2009).

Many of the described peripheral effects of octopamine could be assigned to the segmental efferent UM neurons of the thoracic and abdominal ganglia (Evans and O'Shea, 1978; Bräunig and Eder, 1998; Mentel et al., 2003) which are thought to be the source of the peripheral octopamine (Roeder, 2005). Surprisingly, output synapses of thoracic and abdominal segmental efferent UM neurons were rarely observed within a ganglion. Therefore, it appears unlikely that these neurons are responsible for the central effects of octopamine (Watson, 1984; Pflüger and Watson, 1995). As the intersegmental descending UM neurons seem to be restricted to CNS and exhibit broad arborizations at least in the thoracic ganglia as

shown in locusts (Bräunig and Burrows, 2004), they are good candidates to play an essential role in octopaminergic actions in central processes that were described above. To give further evidence to this hypothesis this thesis deals with the characterization of the three larval desVUM neurons of the SOG of *Manduca sexta* with its main focus on their morphology and sensory input.

The first part of the thesis describes the morphology of the larval desVUM neurons in detail. It addresses the question whether these neurons are a homogeneous or heterogeneous group in a morphological sense and if they are restricted to the CNS similar to the locust descending DUM neurons and therefore are potential candidates to be involved in the central effects of octopamine. Furthermore, with electrophysiological and histological approaches their posterior projections within the ventral nerve cord were investigated. Additionally, the morphology of adult desVUM neurons was compared with those of the larva. The second part of this thesis focuses on the question whether these neurons are a homogeneous or heterogeneous group in a functional sense, thus receive common or different sensory input and are recruited collectively or differentially during fictive crawling.

In isolated nerve cord preparations of invertebrates, fictive motor patterns, thus motor patterns that would drive muscle movements in intact animals, can be induced by various substances (Marder and Bucher, 2001). Among these substances is the acetylcholine receptor agonist pilocarpine that activates certain central pattern generators (CPGs) to produce an appropriate rhythmic motor output (Ryckebusch and Laurent, 1993; Büschges et al., 1995). Application of pilocarpine in *Manduca sexta* larvae induces fictive crawling which is characterized by alternating rhythmic activity in motoneurons that supply the thoracic leg. This rhythmic motor pattern is similar to what can be observed in intact animals (Johnston and Levine, 1996).

In this study, projections of *Manduca sexta* larval desVUM neurons are shown for the first time in the thoracic ganglia in addition to their processes within the SOG. Additionally, it provides a detailed description of their sensory input from hairlike sensilla of the thoracic segments and receptors of the mouthparts and thoracic legs.

2 MATERIALS AND METHODS

2.1 Animals

Manduca sexta of both sexes obtained from a laboratory colony at the Freie Universität Berlin were reared on an artificial diet based on Bell and Joachim (1976).

2.2 Preparation

Larval preparation

Manduca larvae (5^{th} instar) were anesthetized on ice for at least 30 min and a maximum of 60 min before dissection. Animals were pinned dorsal side up in a Sylgard dish and opened by a dorsal incision from the last abdominal segment to the head. To expose the ventral nerve cord the gut was removed. Nerves of the SOG and the thoracic ganglia were dissected free from surrounding muscles and fat tissue. The ventral nerve cord from the brain to at least the second abdominal ganglion was dissected from the body and pinned ventral side up in a Sylgard dish filled with saline. All dissections and recordings were performed under chilled saline consisting of 140 mM NaCl, 5 mM KCl, 28 mM Glucose, 5 mM Hepes and 4 mM $CaCl_2$, pH=7.4.

Adult preparation

Manduca adults of either sex were anesthetized at 4°C for at least 20 min. Hairs on the head and ventral side of the thorax were removed using the blade of a scissor. Wings, proboscis and labial palp were removed. The animal was put ventral side up on a wax platform with the head capsule fixed with wax. The legs were stretched to the side and fixed at the tarsi with tape. To dissect free the SOG, labium, maxillary palp and the cuticular structures of the compound eyes were removed. In some experiments ice was constantly put on the animal to minimize movements.

Backfill techniques

Retrograde axonal fills of peripheral nerves of the thoracic ganglia and SOG were performed. Therefore, nerve trunks of the respective ganglia were cut with a fine scissor and placed in a Vaseline pool filled with distilled water. After approximately 5 min crystals of dextran tetramethylrhodamine (dextran-TRITC, Molecular Probes) were dissolved within this pool until the solution became dark red. Preparations were left overnight at 4°C in saline. In some preparations 5% neurobiotin (Neurobiotin tracer, Vector Laboratories Inc.) was used instead of dextran-TRITC.

Additionally, neck connectives were backfilled in 2nd and 3rd larval instars to examine the posterior projections of desVUM neurons and the location of their terminals. One of the two neck connectives of the isolated nervous system (brain to terminal ganglion) were carefully cut most posterior near the prothoracic ganglion and placed in a Vaseline pool filled with distilled water. After 5 min the water was replaced with 1.5% or 3% neurobiotin in distilled water. Preparations were left in saline for 3, 4 or 5 days at 4°C. For fixation and further proceeding see 2.4.

2.3 Electrophysiological techniques

Intracellular recordings and stainings

For intracellular recordings the ventral side of the respective ganglion was treated with Collagenase/Dispase (Roche) dissolved in 1 M phosphate-buffer (PB) and desheated with a pair of fine forceps to facilitate the penetration with a glass microelectrode. Their tips were filled with 2.5% or 5% neurobiotin in either 1 or 2 M potassium chloride or 2 M potassium acetate. Alternatively, tips of electrodes were filled with 1 M lithium chloride containing 3% lucifer yellow (Dilithium salt, Sigma). Resistances of electrodes varied from 40 MΩ to 120 MΩ.

DesVUM neurons of the SOG were identified by their characteristic soma spikes, prominent postsynaptic potentials and their position of their soma within the ganglion. Electrophysiological signals were digitalized with an A/D converter (Micro 1401 mkII, Cambridge Electronic Design) and recorded with Spike 2 (Version 7.00, Cambridge Electronic Design).

After successful experiments intracellular recorded neurons were filled iontophoretically with neurobiotin by applying depolarizing voltage pulses of 2-5 nA, at a rate of 2 or 4 Hz and a duration of 200 ms for up to 60 min with a stimulator (SD9 Stimulator, Grass Technologies, W. Warwick, RI, U.S.A.). Alternatively, neurons were filled with lucifer yellow by application of pulses of alternating polarity consisting of the same properties as described above. To allow diffusion of the dye within the neuron preparations were kept overnight at 4°C. Intracellular recordings in adult preparations were performed under constant saline flow.

Nerve stimulation

To test possible sensory inputs of the desVUM neurons of the SOG, intracellular recordings were combined with electrical stimulation of peripheral nerves of either the SOG or thoracic ganglia (Fig. 1) in an isolated nervous system preparation.

Nerves were carefully cut with a fine scissor and taken up with a glass-tipped suction electrode. Different stimuli were applied by a Grass S48 stimulator, either a single stimulus every 10 to 20 s with a duration of 0.1 ms or trains of 500 ms duration and a stimulus rate of 20 Hz or 30 Hz (single stimulus duration=0.1 ms). Voltages of the stimuli varied from 3 to 10 V. To test whether larval desVUM neurons project as far as the terminal ganglion, the connective right in front of this ganglion was electrically stimulated.

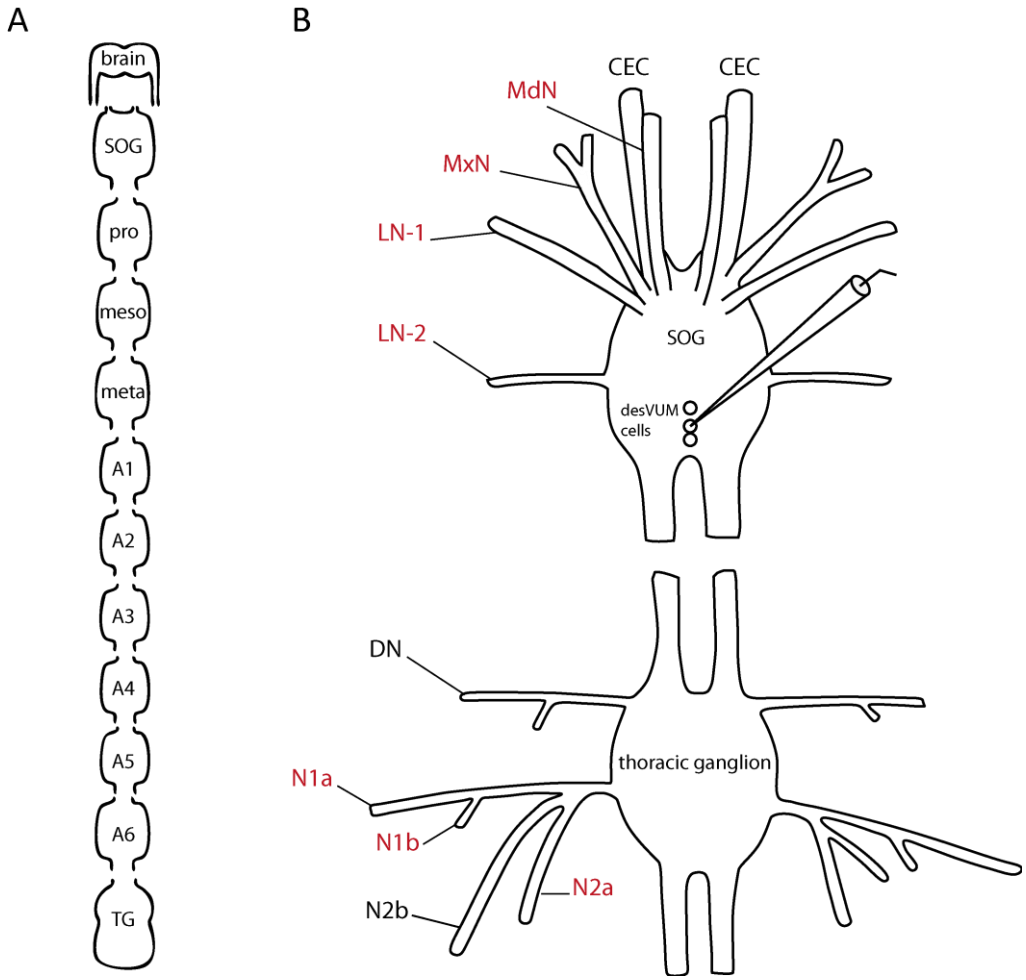

Fig. 1: Schematic illustration of (**A**) the larval ventral nerve cord of *Manduca sexta* consisting of the brain, suboesophageal ganglion (SOG), three thoracic ganglia (pro-, meso-, methoracic ganglion), six abdominal ganglia (A1 – A6) and the terminal ganglion (TG) and of (**B**) the stimulation and recording sites at the SOG and thoracic ganglia. Nerves labeled red were electrically stimulated via a suction electrode. For intracellular recordings of desVUM neurons the microelectrode were impaled in the posterior part of the midline of the SOG. Abbr.: CEC, circumoesophageal connective; DN, dorsal nerve; LN-1, first labial nerve; LN-2, second labial nerve; MdN, mandibular nerve; meso, mesothoracic ganglion; meta, metathoracic ganglion; MxN, maxillary nerve; pro, prothoracic ganglion; SOG, suboesophageal ganglion.

Intact head preparations

In additional experiments, thoracic and abdominal ganglia were isolated by removing the body wall and tissue. The head capsule including mouthparts were left intact. The SOG was placed on a wax coated platform to stabilize the ganglion for intracellular recordings. Thoracic and abdominal ganglia were surrounded by a Vaseline wall and covered with saline. Therefore, the intact head capsule was kept dry for the whole experiment. Protease (Sigma)

was placed on the SOG to enable the penetration with the microelectrode for a few seconds before rinsed with saline. Head and mouthparts were stimulated by touching with a brush or by gently blowing at the head for 5 s each during intracellular recording.

Fictive crawling experiments

Additionally, in some experiments possible changes in the activity of desVUM neurons of the SOG due to pilocarpine induced fictive crawling were tested in isolated larval nerve cord preparations. After dissecting the nervous system (from brain up to the second abdominal ganglion), it was pinned ventral side up in a Sylgard dish separated into two chambers by a plastic stripe. A thin layer of Vaseline was put on top of the stripe before the connectives between pro- and mesothoracic ganglia were placed on it. With this approach the brain, SOG and prothoracic ganglion were separated spatially from the rest of the nervous system and fluid fluctuation between both chambers and parts of the nervous system could be avoided. Gaps between the wall of the Sylgard dish and plastic stripe and surface of connectives located on the plastic stripe were covered with Vaseline. In these preparations both the SOG and mesothoracic ganglion were gently desheated as described above.

To monitor fictive crawling the motor activity of the two main branches of the larval leg of the mesothoracic ganglion N1b and N2a were recorded extracellulary with saline filled glass-tipped electrodes. N1b carries axons of motoneurons innervating femoral, tibial and pleurocoxal extensor muscles, referred here as levator N1b. The main leg nerve N2a innervates flexor muscles in each leg segment, referred here as depressor N2a (Kent and Levine, 1988a; Johnston and Levine, 1996). Extracellular signals were preamplified with a Grass P55 A.C. pre-amplifier (Grass Technologies). For intracellular recordings of desVUM neurons of the SOG see 2.3.

Motor activity and intracellular signals were recorded for at least 5 min in saline before application of the muscarinic acetylcholine receptor agonist pilocarpine. Saline was manually exchanged for 10^{-3} M pilocarpine (Johnston and Levine, 1996) with a pipette in the chamber of meso-, meta- and first two abdominal ganglia. Thus, brain, SOG and prothoracic ganglia were located exclusively in saline throughout the whole experiment.

2.4 Immunocytochemistry

Neurobiotin protocol

Iontophoretically neurobiotin-filled preparations were incubated in 4% paraformaldehyde (in 0.1 M phosphate buffer, pH= 7.4, PB) at room temperature for at least 2 h after kept overnight in saline at 4°C. After fixation ganglia were rinsed three times, 10 min each, in PB followed by an ascending ethanol-series (50, 70, 90, 100%, 10 min each) for dehydration. After cleared in methylsalicylate for 10 min ganglia were rehydrated by means of a descending ethanol-series (100, 90, 70, 50%, 10 min each). For better penetration of the subsequently applied streptavidin preparations were washed in 1% Triton X-100 (TX, Sigma) in 0.1 M PB for three times, 20 min each. To block unspecific bindings ganglia were preincubated in 10% normal goat serum (NGS, ICN Biomedicals) in 1% TX in 0.1 M PB for 1 h followed by an incubation overnight with streptavidin conjugated with Cy2 (Dianova, 1:200 in 0.1 M PB + 1% TX). After washing in PB three times for 10 min each preparations were dehydrated by an ascending ethanol-series followed by clearing in a 1:1 mixture of 100% ethanol and methylsalicylate and finally cleared and mounted in methylsalicylate.

Lucifer yellow protocol

Preparations filled with lucifer yellow were treated and fixed as described above. Ganglia were rinsed three times in PB for 15 min each before dehydrated by an ascending ethanol series (50, 70, 90, 100%, 10 min each) and subsequently rehydrated (100, 90, 70, 50%, 10 min each). After washing in PB for 10 min at 37°C, preparations were treated with 0.1% Collagenase/Dispase + 0.1% hyaluronidase (Sigma) in 0.1 M PB at 37°C for 30 min to improve permeability of the antibody. Ganglia then were washed in PB for 15 min, treated with 1% TX in PB (3 times, 15 min each), followed by a preincubation in 10% NGS in 1% TX + 0.1 M PB for 1 h. The primary antibody anti-lucifer yellow (rabbit, Life technologies) were applied in a concentration of 1:500 in 0.1 M PB + 1% TX + 1% NGS. Preparations were left overnight in primary antibody solution and then washed six times in 1% TX in PB, 20 min each before incubated with the secondary antibody, Alexa 488-conjugated goat anti-rabbit (1:200 in 0.1 M PB + 1% TX, Dianova) overnight at room temperature. Then washed

for 1 h in PB preparations were dehydrated (50, 70, 90, 100%), cleared in 1:1 methylsalicylate/100% ethanol and mounted in methylsalicylate.

Protocol neurobiotin backfills

After removal of the Vaseline pool, preparations were treated as described in the lucifer yellow protocol with slightly different washing durations. To visualize neurobiotin Cy2-conjugated streptavidin in a concentration of 1:200 in 0.1 M PB + 1% TX was used (overnight, at room temperature). Then preparations were washed three times for 20 min in PB, de- and rehydrated and finally cleared and mounted as described above.

2.5 Data acquisition

Image acquisition

Fluorescent dyes were visualized and scanned with a Leica TCS SP2 and SP8 confocal laser scanning microscope using a Leica HCX APO LU-V-I 20x0.50 W and HC PL 10x0.40 imm UV objective and a zoom factor ranging from 1.0 to 1.4. Step size of optical sections of an image stack was set at 2.2 µm for the 20x water objective and 2.0 or 0.98 µm for the 10x immersion objective at an image resolution of 1024x1024 pixels. Cy2 and Alexa 488 were excited at 488 nm by using an Ar/Kr-laser. In contrast, excitation of dextran-TRITC at 543 nm was enabled by using a He/Ne-laser. Image stacks were processed in AMIRA 5.2.2 (Mercury Computer Systems, Merignac, France).

Analysis of fictive crawling data

Cycle period was defined as time interval between onset of two subsequent levator bursts. In experiments lacking extracellular recordings of levator activity, cycle period was defined as time between onset of subsequent depressor bursts. Burst duration was defined as time from onset to termination of burst motor activity in the respective nerve. In Spike 2 (Version 7.00 Cambridge Electronic Design) the cycle period was divided into 20 bins (therefore, each bin

representing 5% of cycle). The number of spikes per bin was counted from the intracellular recording for ten cycles.

Statistical analysis

All statistical tests were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Data sets were tested for normality using the non-parametric Kolmogorov-Smirnov-test. Differences between two data sets were assessed using the Mann-Whitney U test. The Chi-squared test was applied to compare latencies. Responses of desVUM neurons to nerve stimulation were superimposed using MATLAB (R2013a, Mathworks).

Data are represented in boxplots. The bar indicates the median, box the first and third quartiles (interquartile ranges, IQR) and whiskers data within 1.5*IQR. Outliers are defined as data outside the 1.5*IQR and illustrated as black dots.

3 RESULTS

3.1 Morphology and electrophysiological properties of desVUM neurons in the SOG of *Manduca sexta* larva

General remarks

The larval SOG of *Manduca sexta* is formed by fusion of the labial, maxillary and mandibular neuromeres (Davis et al., 1996) and contains three octopaminergic descending ventral unpaired median neurons (VUM) in the most posterior part, the labial neuromere. Based on the relative position of their somata along the anterior-posterior axis these neurons are referred to as anterior, middle and posterior desVUM neurons (Cholewa and Pflüger, 2009). Still little is known about their morphology regarding their projection patterns, especially within the thoracic ganglion. Therefore, intracellular dye fillings of these neurons were performed.

Comparison of intracellular dyes

Neurobiotin or lucifer yellow was used in experiments of this study. Interestingly, lucifer yellow proved to be a more potent dye for intracellular labeling of neurons in *Manduca sexta* larval nerve cord preparations. While only 38% of intracellular fillings using neurobiotin as dye resulted in a successful staining of at least the soma of a neuron (n=171), this is true for 75% of iontophoresis procedures using lucifer yellow (n=36). Nevertheless, anterograde diffusion of neurobiotin within a neuron seemed to be better than diffusion of lucifer yellow resulting in stained projections further than the SOG.

Common morphological features of the three larval desVUM neurons in the SOG

In total, 76 desVUM neurons were successfully stained displaying fine ramifications within the SOG. In a few exceptionally well stained preparations branches within the prothoracic or even mesothoracic ganglia were visible (Fig. 2). DesVUM neurons possess somata located on

the ventral midline in the labial neuromere (Fig. 3) with a size varying from 55-75 µm (based on six measured somata of 5th instar larvae).

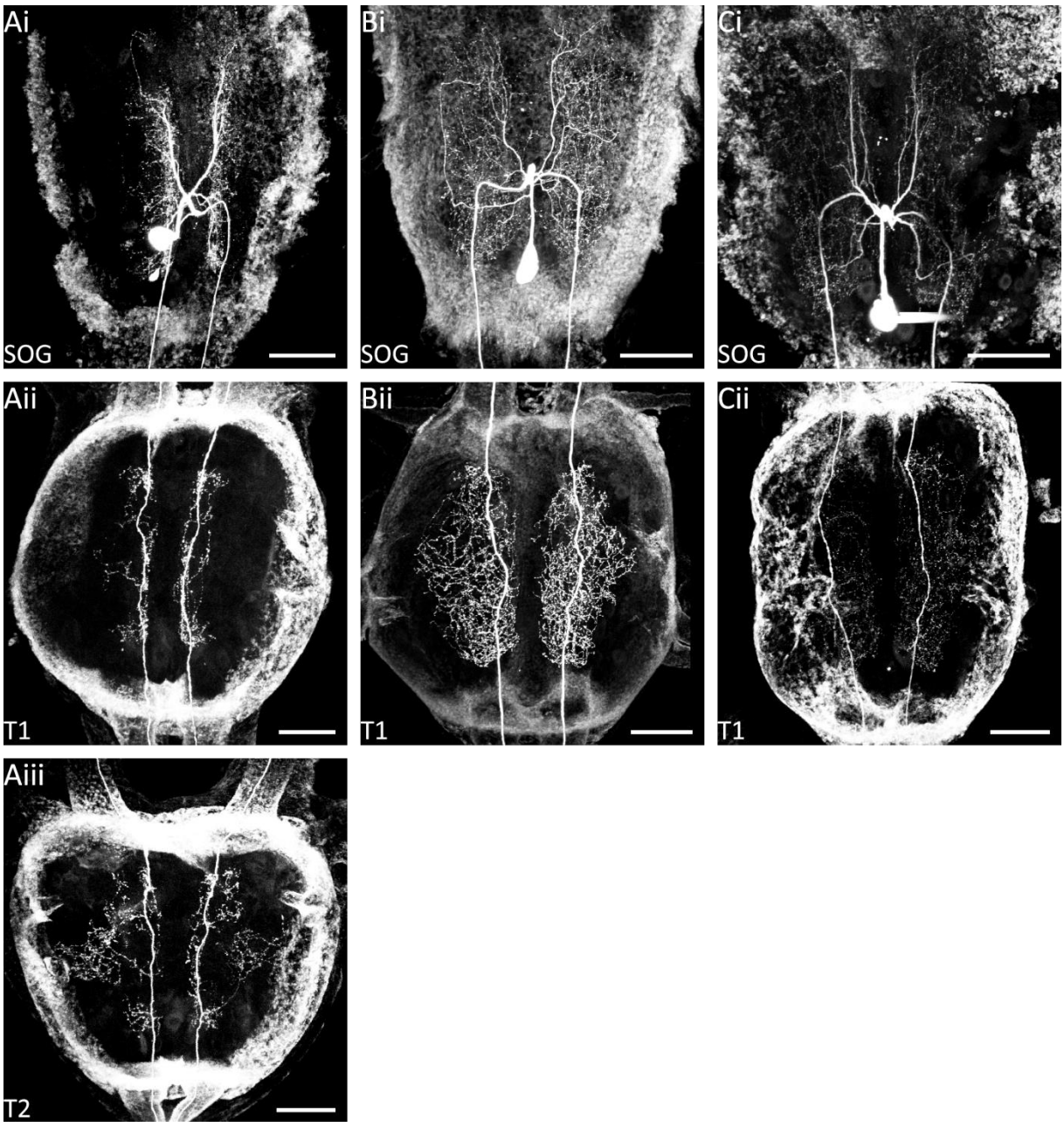

Fig. 2: Morphology of an anterior (**Ai-Aiii**), middle (**Bi-Bii**) and posterior (**Ci-Cii**) desVUM neuron of the SOG in *Manduca sexta* larva. Projection views of intracellular stainings in whole mounts of the suboesophageal (SOG), prothoracic (T1) and mesothoracic ganglion (T2) revealing their restriction to the CNS. Scale bars 100 µm.

All three desVUM neuron types exhibit an X-shaped morphological structure due to the dendritic branching pattern within the SOG. Their primary neurite runs anteriorly, then turns

dorsally before bifurcating at the T-junction into bilaterally symmetrical ascending and descending branches that form further second order branches. Fine ramifications of those branches are concentrated in the dorsolateral labial and maxillary neuromere while the midline comprises only sparse dendritic processes. Strikingly, the position of the T-junction among all three desVUM neurons is very similar. At this site two bilaterally symmetrical axons originate from the primary neurite and descend towards the segmental ganglia via both neck connectives. A lateral view on the SOG reveals that the descending axons are located just underneath the dorsal neurolemma (Fig. 3). In all ganglia, branches and their ramifications are bilaterally symmetrical. In the prothoracic ganglion, the axons form fine processes that are distributed over the anterior-posterior axis mostly running ventrally and laterally. One anterior desVUM neuron could be filled as far as the mesothoracic ganglion, where three clusters of fine processes of the axons are visible (Fig. 2Aiii). Furthermore, processes within peripheral nerves of the SOG, pro- or mesothoracic ganglion could not be found in any staining of the three desVUM neurons. Hence, these neurons are likely to be restricted to the CNS and therefore are considered to be interneurons.

Differences in the morphology of the three larval desVUM neurons in the SOG

In general, a distinction between the three desVUM neurons of the SOG on the basis of their morphology is quite difficult because of their similar projection patterns within the SOG and prothoracic ganglion. Their most obvious differences are present in the position of their soma and length of their primary neurite while the anterior desVUM exhibits a soma in the most anterior position and the shortest primary neurite (Fig. 2 and 3). The reverse is true for the posterior desVUM neuron. As these features are very similar for the middle and posterior desVUM neuron it is especially hard to distinguish them from each other. The most striking difference between those two types is the number of branches originating from the primary neurite. In the anterior and posterior desVUM neuron the primary neurite gives rise to two main ascending and two descending branches. The main ascending branches possess a minimum of three further branches each (Fig. 2Ai and Ci). In contrast, the middle desVUM neuron exhibits three branches each hemisphere originating from the primary neurite at the T-junction. Two of them ascend anteriorly and form further ramifications projecting anteriorly and laterally. A third branch with shorter processes runs anteriorly and reveals dense arborizations directed laterally as well (Fig. 2Bi). Interestingly, axons of the anterior desVUM neuron process laterally within the neck connectives and those between pro- and mesothoracic

ganglia. In contrast, middle and posterior desVUM neurons exhibit axons projecting to the prothoracic ganglion via the middle region of the neck connectives.

In this study, identification as an anterior, middle or posterior desVUM neuron results from a comparison of the location of the relative position of their somata, number of branches originating from the primary neurite and its length.

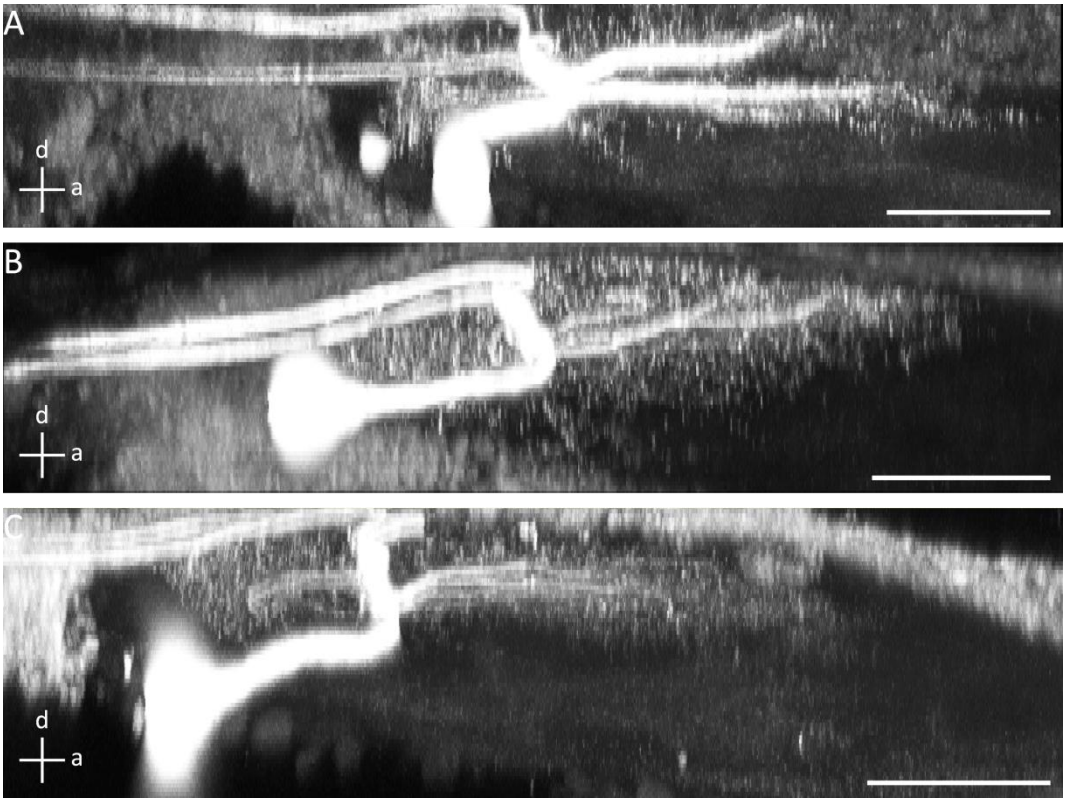

Fig. 3: Lateral view of an anterior (**A**), middle (**B**) and posterior (**C**) desVUM neuron of the SOG in *Manduca sexta* larva. Their soma is located under the ventral surface and their dendritic ramifications and axons are restricted to the dorsal region of the SOG (a, anterior; d, dorsal). Scale bars 100 µm.

Posterior projections of the axons of desVUM neurons

In a few exceptional well stained neurons their axons could be followed to the mesothoracic ganglion but faded before entering the metathoracic ganglion. To investigate potential further projections within the ventral nerve cord, different electrophysiological and histological approaches were implemented. In a first attempt, intracellular recordings of desVUM neurons were combined with electrical stimulation of the connective right in front of the terminal ganglion (n=9). In none of these experiments antidromic spikes in desVUM neurons somata could be observed suggesting that neurons of these recordings do not project to the terminal

ganglion. To determine how far they project within the ventral nerve cord, backfills of connectives at various levels were performed but staining of their somata in the SOG failed. In general, retrograde backfills of their axons proved to be difficult. Backfills of either the right or left neck connective were performed in over 27 isolated nerve cord preparations (Fig. 4). As the desVUM neurons are considered to be the only cells in the SOG with bilaterally symmetrical descending axons, stained projections within the contralateral neuropile of the subsequent ganglia (related to site of backfilled neck connectives) should belong to them. In 2 out of 27 preparations projections in the thoracic and abdominal ganglia could be successfully stained (Fig. 4Bi-Cv). In the subsequent ganglion more than three axons are clearly visible, also a few projections in the meso-, meta- and first abdominal ganglia (Fig. 4Bii-Bv and Cii). This fact might be due to degeneration processes of the cellular tissue resulting from a long incubation time and therefore leakage of neurobiotin. However, in the first and second abdominal ganglia axons and their projections are clearly visible resembling those of desVUM neurons (Fig. 4Biv-Bv and Civ-Cv, for comparison Fig. 2). Even though 2nd and 3rd larval instars were used for these backfill experiments and neurobiotin was allowed to diffuse for a maximum of five days, projections could be followed only to the third abdominal ganglion where they appeared in a weak and diffuse staining (Fig. 4Bvi and Cvi). Overall, these results indicate that the desVUM neurons of the SOG project at least to the second abdominal ganglion in *Manduca* larva.

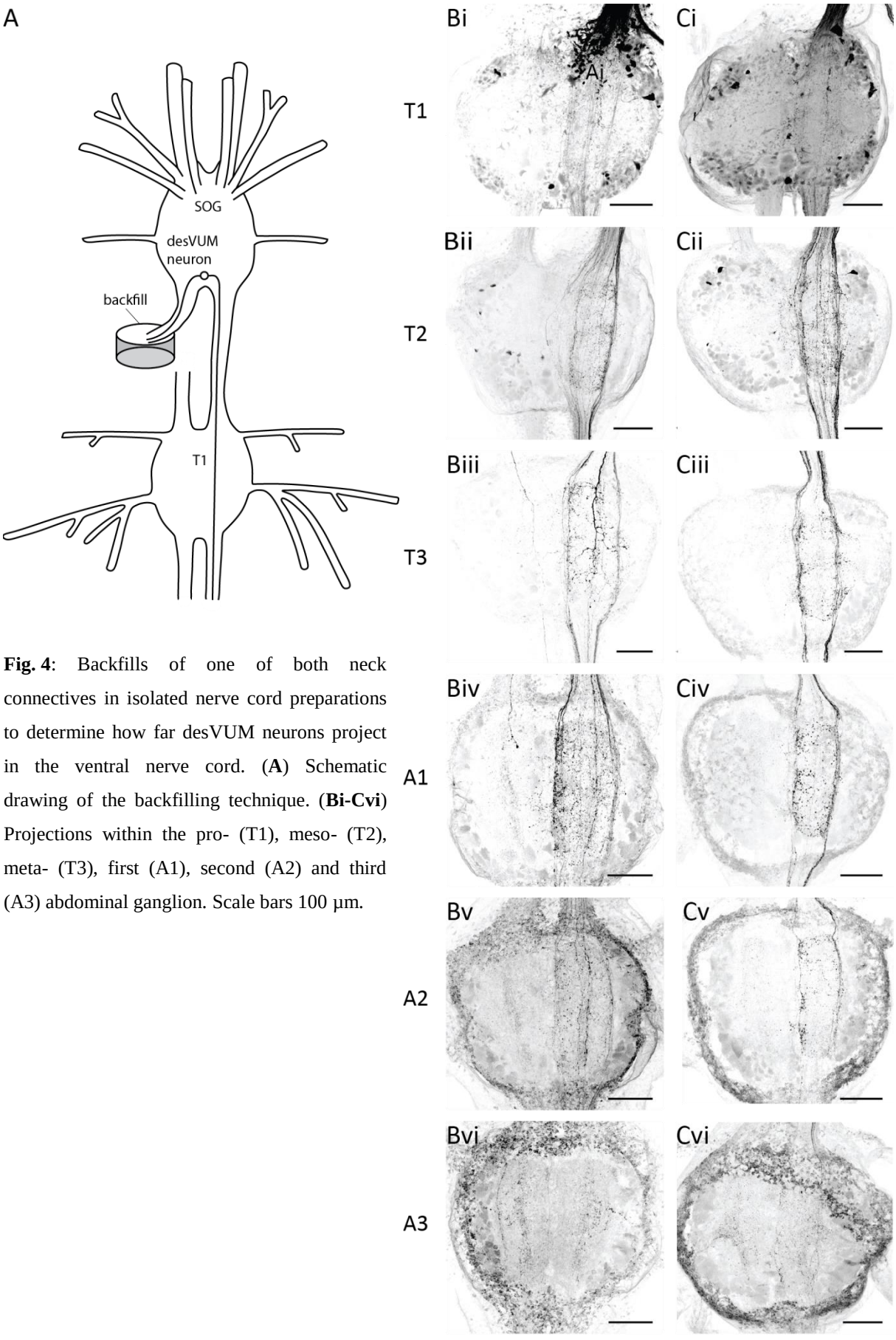

Fig. 4: Backfills of one of both neck connectives in isolated nerve cord preparations to determine how far desVUM neurons project in the ventral nerve cord. (**A**) Schematic drawing of the backfilling technique. (**Bi-Cvi**) Projections within the pro- (T1), meso- (T2), meta- (T3), first (A1), second (A2) and third (A3) abdominal ganglion. Scale bars 100 µm.

Comparison of intersegmental desVUM neurons of the SOG and efferent VUM neurons of the thoracic ganglia

DesVUM neurons of the SOG and VUM neurons of the thoracic ganglia reveal some similar features in their morphology (Fig. 5A-B). One of them is the large soma located on the ventral midline in the posterior part of the respective ganglion. Additionally, both neurons exhibit a prominent primary neurite projecting anteriorly before giving rise to two bilaterally symmetrical axons. The projection pattern of their axons is one of the most striking morphological differences between VUM neurons of the SOG and those of the thoracic ganglia. The metathoracic VUM neuron (Fig. 5B) sends its axons into the periphery via the dorsal nerve to innervate body wall muscles and therefore being an efferent neuron. In contrast, the desVUM neuron does not project into the periphery of their own segment to supply peripheral targets like the mouthparts. Instead, it possesses descending axons projecting towards the segmental ganglia. As the results of this thesis suggest, the desVUM neurons are restricted to the CNS and therefore are considered to be interneurons. Additional to their morphological differences both VUM neuron types possess different electrophysiological properties. Both types exhibit distinct spontaneous soma spikes (Fig. 5D) that differ significantly in their amplitude, duration and spontaneous frequency (Fig. 5E-G). Thoracic efferent VUM neurons form large overshooting action potentials with a median amplitude of 69.31 mV and an interquartile range (IQR) between 56.87-79.04 mV, a median duration of 89.72 ms (IQR=85.59-111.5 ms) and a median frequency of 0.12 Hz (IQR=0.11-0.16 Hz). In contrast, desVUM neurons of the SOG exhibit significant smaller spikes with a median amplitude of 17.09 mV (IQR=10.76-20.36 mV, $p < 0.0001$) that are significantly faster ($\tilde{x}_{duration}$=7.78 ms, IQR=7.62-8.3 ms, $p < 0.0001$). Furthermore, the spontaneous frequency of both VUM neuron types differ significantly ($p < 0.0001$) with desVUM neurons showing a 23 times higher frequency than efferent VUM neurons ($\tilde{x}_{frequency}$=2.76 Hz, IQR=2.05-4.03 Hz, n_{desVUM}=10 spikes of 8 neurons, $n_{efferent}$=10 spikes of 3 neurons). Additionally, desVUM neurons exhibit pronounced inhibitory (IPSP) and excitatory potentials (EPSP).

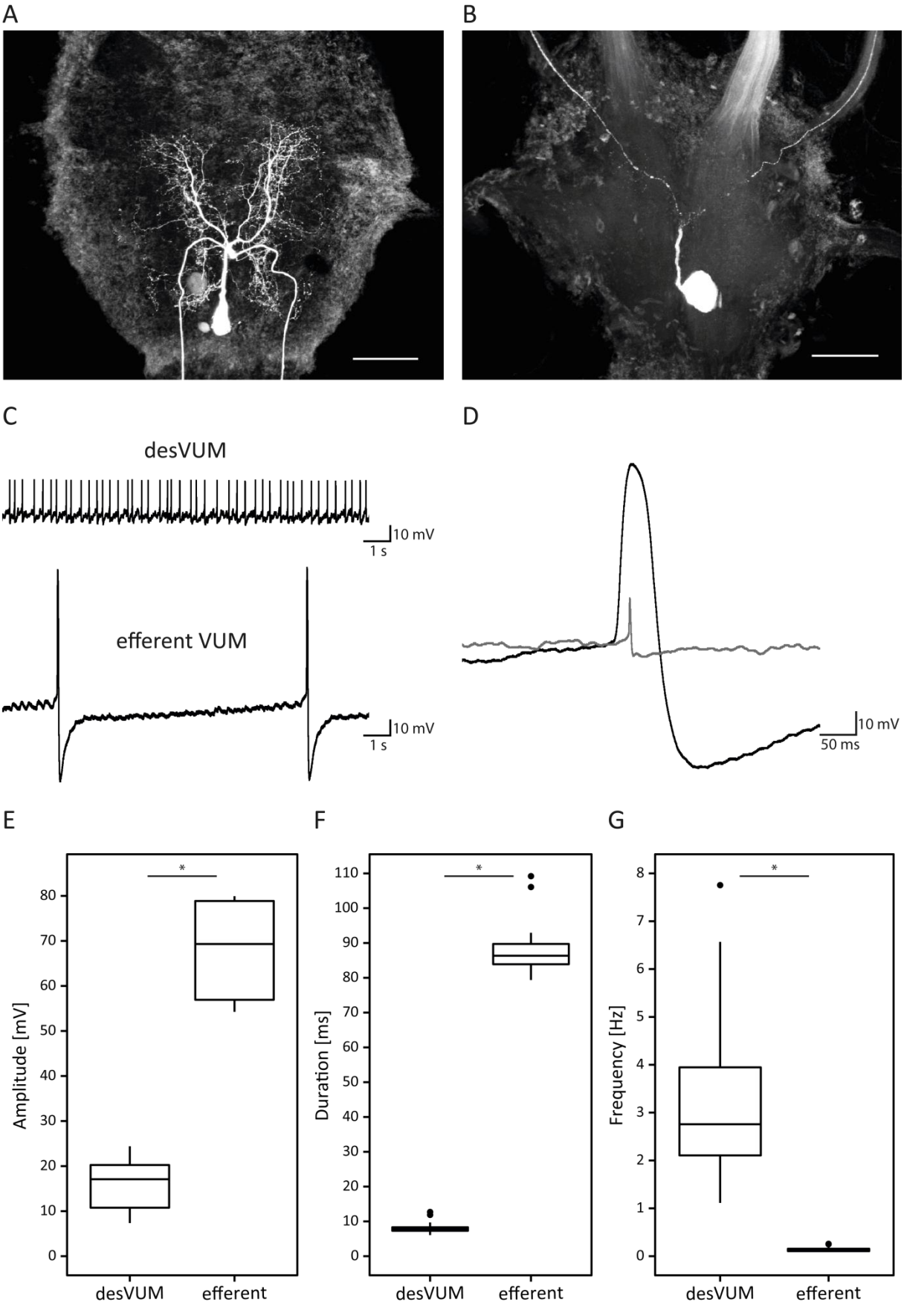
A
B
C
desVUM
10 mV
1 s
efferent VUM
10 mV
1 s
D
10 mV
50 ms
E
Amplitude [mV]
desVUM
efferent
F
Duration [ms]
desVUM
efferent
G
Frequency [Hz]
desVUM
efferent

Fig. 5: Comparison of intersegmental desVUM neurons in the SOG and efferent VUM neurons in the thoracic ganglia regarding their morphology and electrophysiological properties. Intracellular stainings of (**A**) a posterior desVUM neuron in the SOG and (**B**) an efferent VUM neuron in the metathoracic ganglion reveal different projections of their bilaterally symmetrical axons (scale bars 100 µm). (**C**) Intracellular recordings of their spontaneous soma spikes and (**D**) overlay of representative spikes of a desVUM neuron (grey) and efferent VUM neuron (black). (**E**) Amplitude, (**F**) duration and (**G**) frequency are illustrated in boxplots (n_{desVUM}=10 spikes of 8 neurons, $n_{efferent}$=10 spikes of 3 neurons). Bars represent the median, boxes the first-third quartiles, whiskers the data within the 1.5*IQR and dots outliers outside the 1.5*IQR. Significant differences between desVUM and efferent VUM neurons are indicated by asterisks (Mann-Whitney U test, $p < 0.05$).

Other neurons of the SOG

Despite desVUM neurons two other types of neurons in the SOG could be successfully recorded and stained (Fig. 6, n=8). An interneuron, that was recorded and stained, possesses a soma located laterally in the labial neuromere and exhibits a contralateral descending axon leaving the SOG via the neck connective and therefore this neuron is called descending interneuron (Fig. 6Ai, desIN). Within the prothoracic ganglion three clusters of fine ramifications are visible projecting medially and laterally being restricted to the contralateral hemisphere (in reference to its soma, Fig. 6Aii). A similar projection pattern can be observed in the meso- and metathoracic ganglion (Fig. 6Aiii-Aiv). The other neuron that could be successfully stained in the SOG exhibit a soma near the midline in the labial neuromere of the SOG and a primary neurite running anteriorly similar to desVUM neurons (Fig. 6Cii and E). Two bilaterally symmetrical ascending branches originate from the primary neurite and form fine branches in the mandibular neuropile. One of both branches gives rise to an axon projecting laterally and then turning orthogonally before ascending towards the brain. Within the brain the axon runs laterally and projects to the periphery via the NCC-III (third nerve of corpus cardiacum) (Fig. 6Ci). This neuron could be identified as the median neuroendocrine cell of the labial neuromere (M_{lb}, Davis et al., 1996). M_{lb} neurons and desINs exhibit spontaneous spikes that can be well distinguished from those of desVUM neurons (Fig. 6B and D). Spikes of Mlb neurons are significantly slower ($\tilde{x}_{duration}$=40.34 ms, IQR=37.76-47.38 ms, $p < 0.0001$) and possess a significantly taller amplitude ($\tilde{x}_{amplitude}$=32.8 mV, IQR=26.17-38.4 ms, $p < 0.0001$) than desVUM neurons. Additionally, they spike spontaneously less frequently ($\tilde{x}_{frequency}$=0.62 Hz, IQR=0.38-1.32 Hz, $p < 0.0001$, Fig. 6F-H).

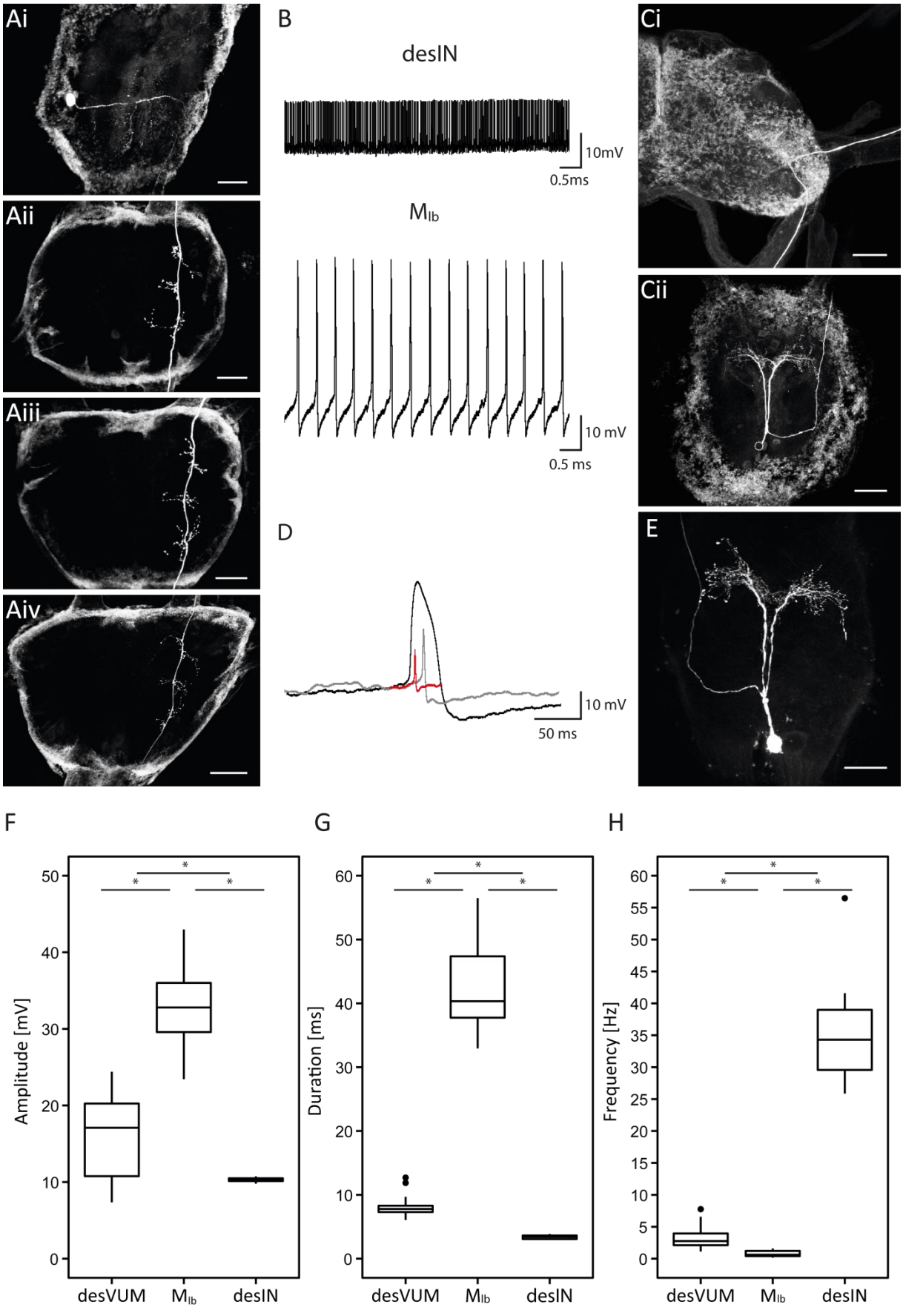
Ai
Aii
Aiii
Aiv
B
desIN
10mV
0.5ms
MIb
10 mV
0.5 ms
D
10 mV
50 ms
Ci
Cii
E
F
Amplitude [mV]
desVUM
MIb
desIN
G
Duration [ms]
desVUM
MIb
desIN
H
Frequency [Hz]
desVUM
MIb
desIN

Fig. 6: Morphological and electrophysiological characteristics of a descending interneuron (desIN) and a median neuroendocrine cell of the labial neuromere (M_{lb}) of the SOG. Projection views of an intracellular labeled desIN in the SOG (**Ai**), pro- (**Aii**), meso- (**Aiii**) and metathoracic ganglion (**Aiv**). Intracellular filling of a M_{lb} neuron in the SOG (**Ci** and **E**) and its projections in the prothoracic ganglion (**Cii**). Dashed circle in Cii indicates the position of its soma (scale bars 100 µm). (**B**) Intracellular recordings of their spontaneous soma spikes and (**D**) overlay of representative spikes of a M_{lb} neuron (black), desVUM (grey) and desIN (red). (**F**) Amplitude, (**G**) duration and (**H**) frequency of these neurons are illustrated in boxplots (n_{desVUM}=10 spikes of 8 neurons, n_{Mlb}=10 spikes of 4 neurons, n_{desIN}=10 spikes of 4 neurons). Significant differences between individual groups are indicated by asterisks (Mann-Whitney U test, * $p < 0.05$).

DesINs exhibit spikes distinct from those of desVUM neurons as they are significantly smaller ($\tilde{x}_{amplitude}$=10.23 mV, IQR=10.1-10.5 mV, p=0.0006), faster ($\tilde{x}_{duration}$=3.64 ms, IQR=3.01-3.64 ms, $p < 0.0001$) and occur significantly more frequently ($\tilde{x}_{frequency}$=34.3 Hz, IQR=28.51-39.8 Hz, $p < 0.0001$, n_{desVUM}=10 spikes of 8 neurons, n_{Mlb}=10 spikes of 4 neurons, n_{desIN}=10 spikes of 4 neurons). In summary, desVUM neurons form characteristic soma spikes that can be well distinguished form other randomly recorded neurons of the SOG. Therefore, even though intracellular labeling procedures were not successful, neurons in this study could be identified as desVUM neurons in general by their characteristic soma spike shape and pronounced postsynaptical potentials (PSP). In the following, these neurons are referred to as unidentified desVUM neurons.

Morphology of adult desVUM neurons in the SOG

Intracellular labeling of desVUM neurons in adult *Manduca* seemed to be of great interest as little is known about their morphology. Therefore, 20 intracellular recordings and subsequent fillings of neurons in semi-intact adult preparations were performed, succeeding in one labeled desVUM neuron (Fig. 7). Unfortunately, the neck connectives and part of the posterior region of the SOG were torn apart during preparation and histological procedures so that the soma and descending axons are not displayed in this staining. Nevertheless, morphological characteristics similar to larval desVUM neurons are visible. One striking common feature is the X-shaped structure of their dendritic trees. As in larval desVUM neurons the primary neurite runs anteriorly and gives rise to two main bilaterally symmetrical ascending branches forming dense ramifications that project mainly to the lateral region of the SOG. Another common morphological characteristic is the occurrence of only sparse

dendritic processes on the midline in the more anterior region of the SOG. But in contrast to larval desVUM neurons, the adult one exhibits densely packed ramifications on the midline in the posterior part of the SOG.

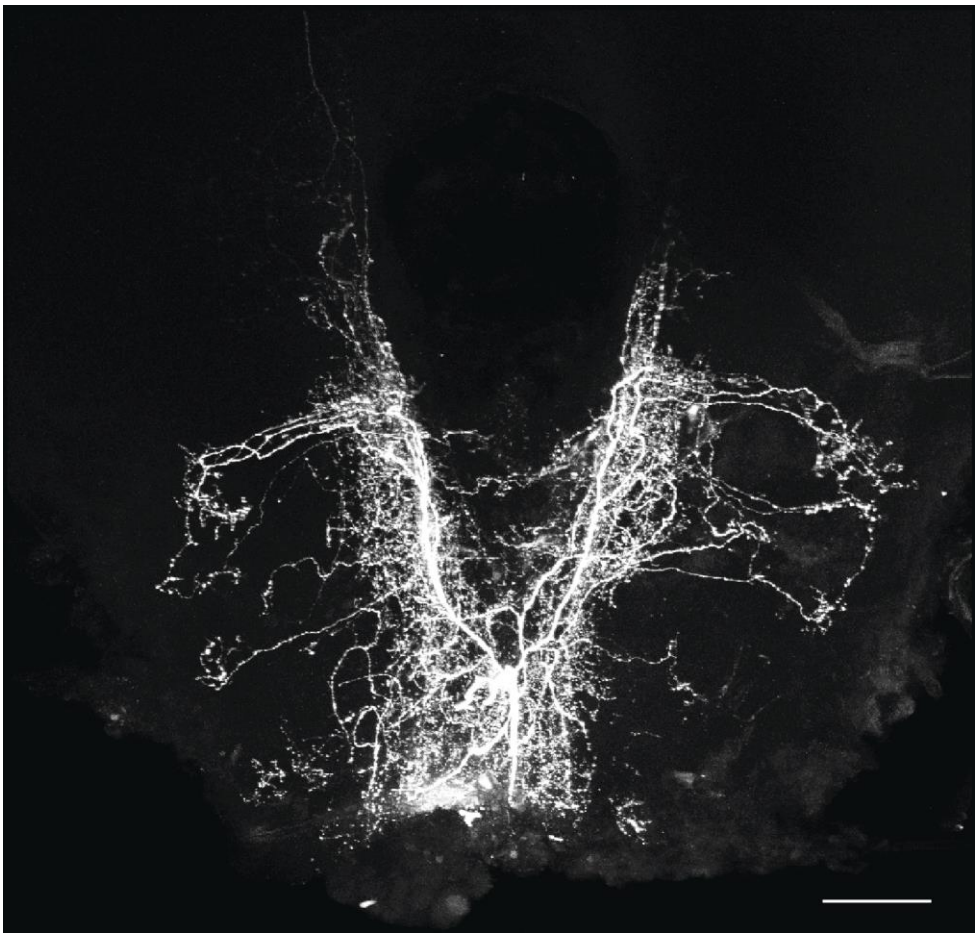

Fig. 7: Projection view of an intracellular labeled desVUM neuron in the adult SOG of *Manduca sexta* revealing morphological features similar to larval desVUM neurons. Scale bar 100 µm.

In comparison to larval desVUM neurons, spikes of this adult desVUM neuron are significantly smaller in amplitude ($\tilde{x}_{amplitude}$=6.61 mV, IQR=6.38-6.68 mV, $p < 0.0001$) and longer in duration ($\tilde{x}_{duration}$=14.52 ms, IQR=14.14-14.99 ms, $p < 0.0001$, n_{adult}=10 spikes of 1 neuron, n_{larval}=10 spikes of 8 neurons). As these are very preliminary data, influences of the recording quality of the adult desVUM neuron on its spike shape cannot be fully excluded.

3.2 Descending unpaired median neurons and their sensory input

In contrast to the well described thoracic efferent DUM neurons of the locust (e.g. Duch et al., 1999; Field et al., 2008), little is known about the sensory input of the intersegmental desDUM neurons of the SOG (Bräunig et al., 2004) and nothing about the desVUM neurons of SOG of *Manduca sexta*. Therefore, intracellular recordings were combined with electrical stimulation of nerves of the SOG and thoracic ganglia in larval isolated nerve cord preparations. Additionally, after a successful experiment, the electrically stimulated nerves were filled by using the retrograde backfill technique to show their afferent projections.

Backfills of stimulated nerves

In this study, three of the four pairs of peripheral nerves of the SOG could be successfully labeled in *Manduca sexta* larvae (Fig. 8). None of these nerve stainings labeled desVUM neurons, thus these nerves do not contain peripheral axonal projections of these neurons, supporting the idea that desVUM neurons do not have efferent output functions. Instead different clusters of motor neurons innervating muscles of the larval mouthparts could be stained (Tab. 1). Backfilling of the left first labial nerve (Fig. 8A) resulted in a cluster containing nine ipsilateral somata in the anterior part of the SOG. Additionally, dense fibers can be seen in this region. One prominent fiber projects posteriorly along the midline before turning laterally. A few fine afferents project towards the thoracic ganglia but their staining faded before reaching the prothoracic ganglion. Three contralateral somata can be observed after filling of the maxillary nerve (Fig. 8B). Furthermore, a cluster of 16 efferent somata and dense packed branches are located in the ipsilateral maxillary neuromere. Some fibers turn and then run medially before branching near the midline of the SOG. A few branches of these fibers pass beyond the midline. Some afferent projections run posteriorly to the prothoracic ganglion. After staining of the mandibular nerve 15 somata of efferent neurons could be found which are concentrated in the ipisilateral mandibular neuromere (Fig. 8C). A few fibers project to the midline and similar to the first labial and maxillary nerve stainings some afferents descend to the thoracic ganglia. Unfortunately, staining of these afferent fibers faded in all preparations before reaching the prothoracic ganglion due to the short retrograde backfilling period (approximately 15-20 h).

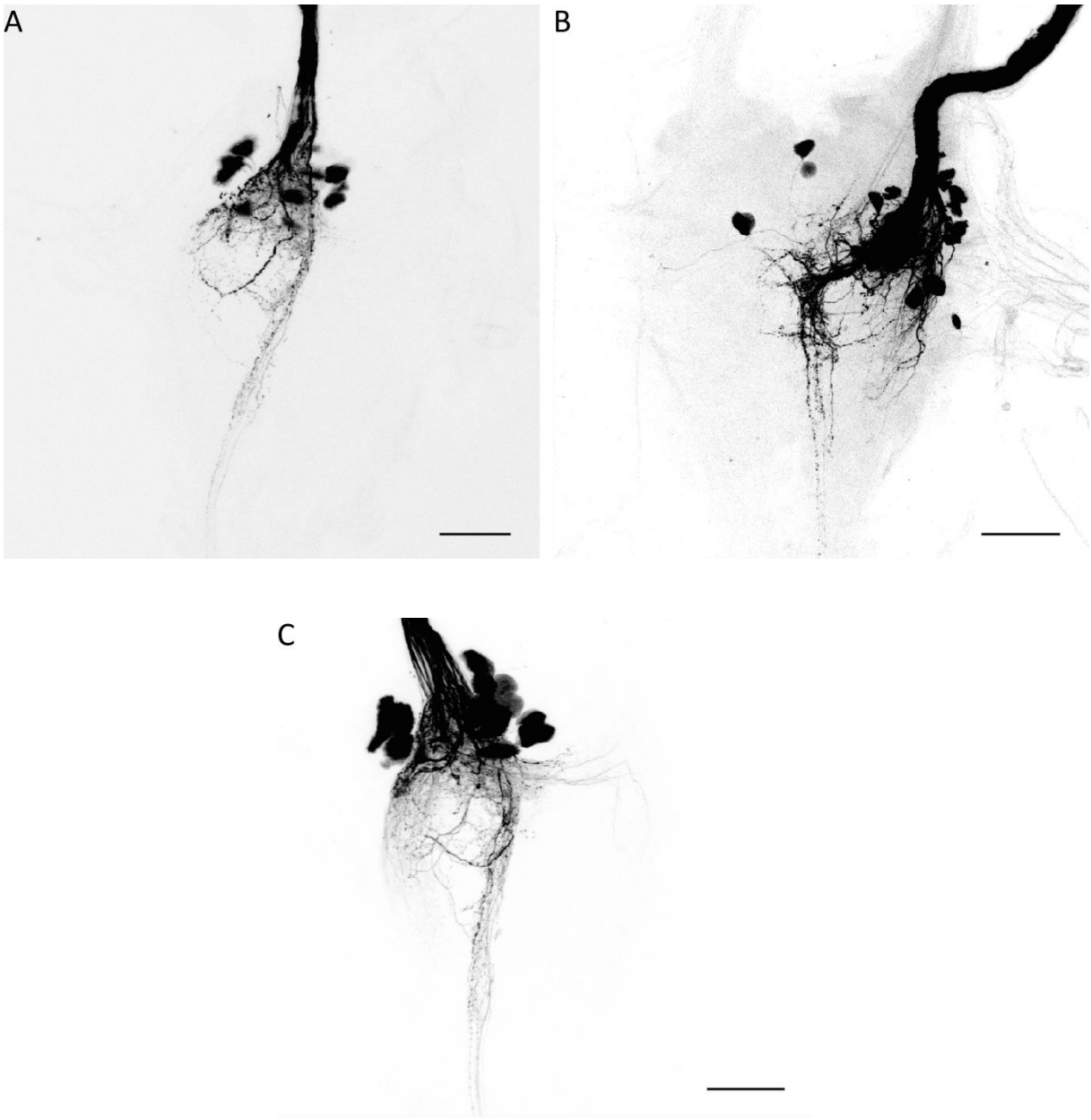

Fig. 8: Retrograde stainings of (**A**) first labial nerve, (**B**) maxillary nerve and (**C**) mandibular nerve of the SOG in *Manduca sexta* larva reveal somata of efferent neurons and fine afferent fibers mainly in the maxillary and mandibular neuromere. Scale bar 100 µm.

Manduca larvae have three pairs of thoracic segmented legs with a simple structure. They are capable of simple extension and flexion movements to grasp the substrate during crawling. The legs are innervated by two main nerves. N1b is a motor nerve carrying axons innervating femoral, tibial and pleurocoxal extensor muscles (Kent and Levine, 1988b). In contrast, N2a innervates flexor muscles in each leg segment and the larval legs proprioceptors like campaniform sensilla, femoral and tibial chordotonal organs and hair sensilla (Johnston and Levine, 1996 and Consoulas, 2000).

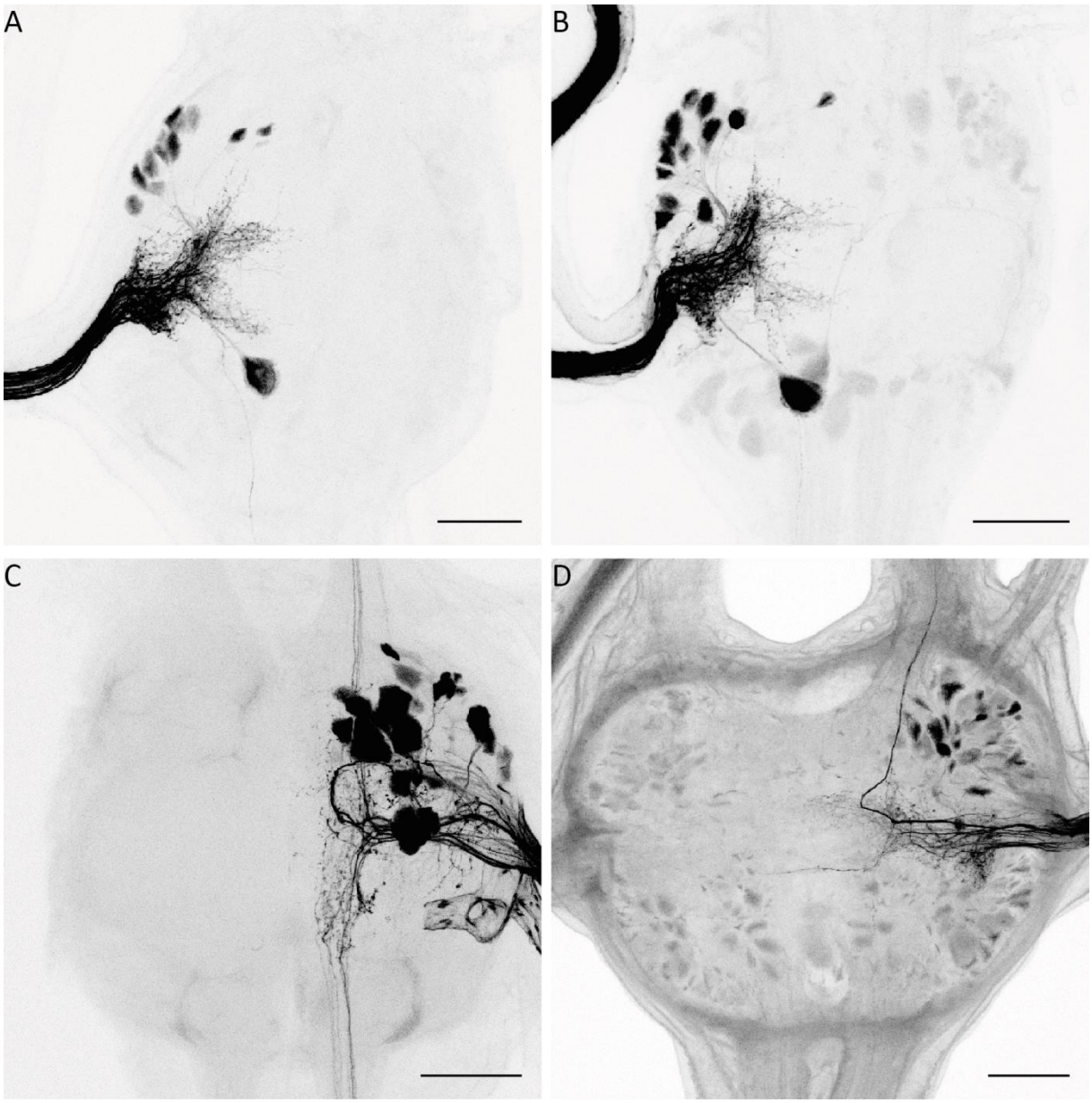

Fig. 9: Retrograde stainings of the two main nerves of the larval thoracic leg in *Manduca sexta* with dextran tetramethylrhodamine. (**A**) and (**B**) retrograde backfill of N2a, (**C**) N1b of the prothoracic ganglion and (**D**) N2a of the mesothoracic ganglion reveals the distribution of somata of efferent neurons and fine afferent processes within the respective ganglion. An efferent VUM neuron could be successfully stained in B. Scale bars 100 µm.

Backfilling the leg nerve N2a of the prothoracic ganglion revealed 14-15 somata in the prothoracic ganglion (Fig. 9A, Tab. 1). The majority of these cell bodies reside in a cluster in the anterior ipsilateral region of this ganglion. Conspicuously, one large soma is located near the midline in the posterior region. In one preparation (Fig. 9B) a large soma on the ventral midline could be successfully stained sending its axons into the ipsilateral and contralateral leg nerve N2a. This neuron can be identified as an efferent VUM neuron due to its position of

soma and bilaterally symmetrical axons. Only weak stainings of afferent processes descending towards the mesothoracic ganglion could be observed. Labeling N2a of the mesothoracic ganglion resulted only in a weak staining (Fig. 9D). Nevertheless, a cluster of approximately 21 efferent somata located in the ipsilateral anterior part of this ganglion can be seen. Additionally, one thick afferent fiber runs anteriorly and leaves the ganglion via the ipsilateral connective.

Labeling of N1b of the prothoracic ganglion revealed 19 somata exclusively located in the ipsilateral anterior part (Fig. 9C). Interestingly, in this preparation afferent projections could be stained running anteriorly and posteriorly leaving the ganglion via the ipsilateral connectives. But again, staining of these projections faded before reaching the prior or subsequent ganglion suggesting that a longer backfilling period is required. Dense packed arborizations are concentrated in the medial ipsilateral hemisphere. This observation also applies for staining of N2a of either the pro- or mesothoracic ganglion. Interestingly, main afferent projections in nerve stainings of the pro- and mesothoracic ganglia remain in the ganglion of its backfilled nerve. Nevertheless, some afferent fibers projecting anteriorly were labeled. Therefore, monosynaptic connections between afferents and desVUM neurons cannot be excluded.

Tab. 1: Numbers of motoneurons, descending and efferent VUM neurons labeled by retrograde filling of nerves of the SOG, pro- (T1) and mesothoracic (T2) ganglia and number of expected motoneurons found in literature. Numbers of experiments are written in brackets.

Ganglion	Nerve	DesVUM	Motoneurons	Motoneurons expected	Efferent VUM
SOG	LN-1	0	9 (n=1)	5 (Cholewa and Pflüger, 2009)	0
SOG	MxN	0	19 (n=1)	16 (Cholewa and Pflüger, 2009)	0
SOG	MdN	0	15 (n=1)	20 (Griss, 1990)	0
SOG	LN-1	-	-	2 (Cholewa and Pflüger, 2009)	0
T1	N2a	-	14-15 (n=2)	23-25 (Kent and Levine, 1988b)	1
T1	N1b	0	19 (n=1)	?	0
T2	N2a	1	21 (n=1)	?	(0)

General remarks

Backfills of stimulated nerves labeled a multitude of stained afferent axons. Therefore, electrical stimulation of a main nerve of a certain ganglion always results in a multisensory stimulation. Additionally, responses of desVUM neurons to electrical nerve stimulation are integrated in their spontaneous activity. In some cases, when their spontaneous frequencies were too high to be able to observe a possible excitation to stimulation, desVUM neurons were hyperpolarized.

In none of the nerve stimulation experiments antidromic spikes in somata of desVUM neurons could be observed supporting the hypothesis that desVUM neurons are restricted to the central nervous system and thus are interneurons.

Descending VUM neurons receive input from receptors of the mouthparts

Responses of desVUM neurons to stimulation of the first labial nerve

In a first set of experiments possible connections between receptors of the mouthparts and desVUM neurons of the SOG were tested. The SOG gives rise to four pairs of peripheral nerves. The first labial (LN-1), maxillary (MxN) and mandibular nerve (MdN) arise from the anteroventral part of the ganglion. The fourth pair of peripheral nerves, the second labial nerve (LN-2), leaves the SOG in the posterodorsal region.

LN-1 innervates labial muscles and sensory sensilla of the maxillary-labial complex, including tactile hairs on the labium and sensilla on the labial-palp spinneret (Eaton, 1988; Kent and Hildebrand, 1987). This nerve was stimulated in 12 isolated nerve cord preparations. In one recording excitation of a posterior desVUM neuron to train stimulation could be observed (Fig. 10A). This neuron could be successfully stained and identified as a posterior desVUM neuron by its characteristic soma position and primary neurite length (Fig. 10B). High frequency train stimulation with 20 Hz (14 trains per stimulation) of LN-1 resulted in excitation, thus, increase in firing rate of this neuron. It shows a spontaneous instantaneous frequency in the range of 2.3 to 8.4 Hz (Fig. 10A, upper trace) and firing rate increases during stimulation to a maximum 14.4 Hz after first given train during the first stimulation sequence. This maximum in frequency is followed by a rapid decrease to almost spontaneous frequency level indicating a rapid habituation of this posterior desVUM neuron. During the second stimulation sequence the increase in firing rate is lower than in the first sequence reaching a

maximum of 9.8 Hz. Additionally, a rapid decrease in spike frequency like in the first sequence could not be observed.

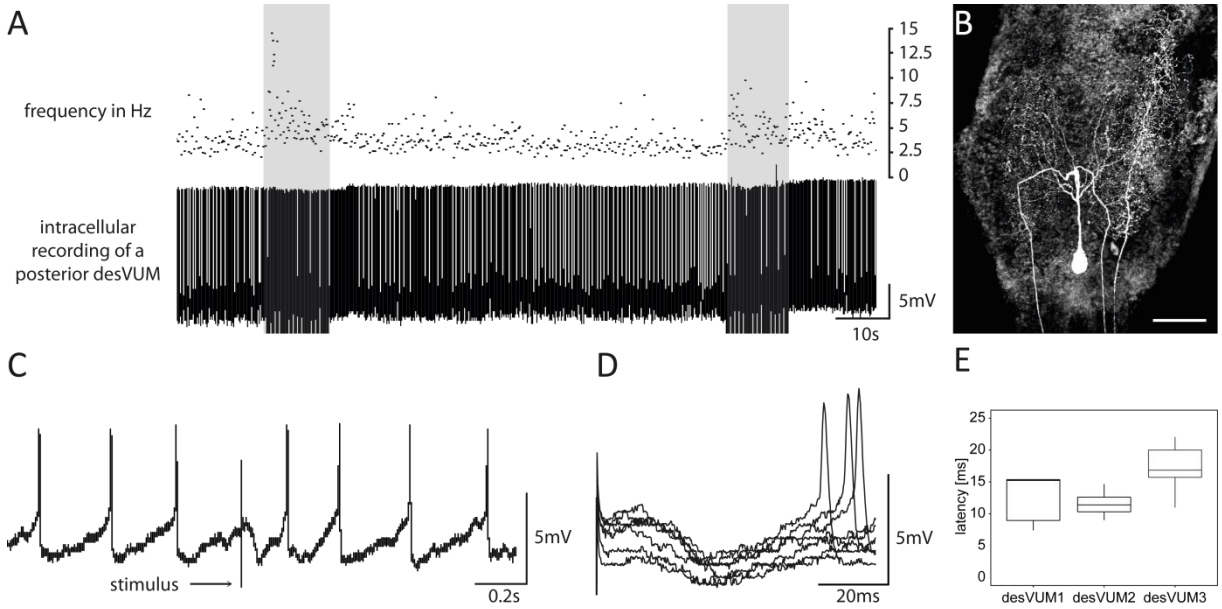

Fig. 10: DesVUM neurons respond either with excitation or inhibition to first labial nerve stimulation. (**A**) High frequency train stimulation (20 Hz) of a posterior desVUM neuron leads to an increased spike-frequency (instantaneous frequency in upper trace). Grey shaded areas denote sequence of stimulation. (**B**) Intracellular labeling of this neuron displays a posterior desVUM neuron identifiable of the position of its soma (scale bar 100 µm). (**C**) Representative section of an intracellular recording of a desVUM neuron that responded with an IPSP to a single stimulus. (**D**) Overlay of seven IPSPs of the same recording as in D. (**E**) Boxplots comparing latencies of inhibitory responses of three desVUM neurons to stimulation of first labial nerve (n=5 latencies per neuron).

In another four experiments, neurons could be recorded that can be identified as desVUM neurons by their characteristic spike shape (Fig. 5 and 6) that responded with inhibition to single stimulation of LN-1. Stimulation led to pronounced IPSPs in these neurons (Fig. 10C, D). Unfortunately, intracellular labeling of these neurons failed and an exact identification as an anterior, middle or posterior desVUM neuron is not possible. Latencies (time interval between onset of an applied stimulus and observed response in intracellular recording) varied widely not only among preparations but also within an experiment. The median latencies of three unidentified desVUM neurons (five measured latencies each neuron, this applies to all other experiments if not stated otherwise, desVUM1, 2, 3) responding with an IPSP to LN-1 stimulation were calculated (Fig. 10E). The fastest response could be observed in desVUM2 with a median latency of 11.38 ms and ($IQR_{desVUM2}$=10.31-12.6 ms). DesVUM1 and desVUM3 showed a median latency of 15.21 ms ($IQR_{desVUM1}$=8.95-15.37 ms)

and 16.84 ms ($IQR_{desVUM3}$=15.71-19.99 ms), respectively. Variation of measured latencies within an experiment due to habituation of desVUM neuron responses to stimulation can be excluded as interstimulus-intervals were set 10 s. This applies to all single stimulation experiments in this thesis.

In literature, different conduction velocities for different neurons in *Manduca sexta* larva can be found. Novicki and Weeks (1995) state a conduction velocity for an interneuron of the terminal ganglion of 0.54 m/s. In contrast, in sensory afferents and motoneurons involved in the larval proleg withdrawl reflex a conduction velocity of 0.81 m/s has been described (Weeks and Jacobs, 1987). Cholewa and Pflüger (2009) calculated a conduction velocity for desVUM neurons of the SOG of approximately 1 m/s. Furthermore, the synaptic delay at a chemical synapse described in these studies varied from 0.55 ms to 1.57 ms. Taken the minimal conduction velocity and longest synaptic delay that has been calculated in these publications and a distance between stimulation and recording site of 450 µm minimum a monosynaptic afferent pathway can be excluded. The longest assumed latency for a monosynaptic connection over this distance would be 2.13 ms. It is rather likely that at least one interneuron is intercalated in the inhibitory pathway between receptors of the labium and the labial-palp spinneret and desVUM neurons.

Responses of desVUM neurons to stimulation of the maxillary nerve

The maxillary nerve (MxN) innervates maxillary muscles and sensory sensilla of the maxillary-labial complex like tactile hairs and campaniform sensilla (Eaton, 1988; Kent and Hildebrand, 1987). This nerve was stimulated in 16 experiments with simultaneous intracellular recordings of desVUM neurons that could be either identified as a desVUM neuron in general by their characteristic soma spikes or as a certain desVUM type with the help of successful intracellular fillings. Eight desVUM neurons responded with inhibition to stimulation of MxN. Two out of them could be identified as middle desVUM neurons. Figure 11Bi shows a representative section of an intracellular recording of a middle desVUM neuron exhibiting an IPSP to stimulation of MxN. This inhibition is more obvious in the overlay of IPSPs as a response to six successive stimuli (Fig. 11Bii). The median latency of these inhibitory responses is 10.38 ms ($IQR_{middle1}$=10.06-11.27 ms, middle1 in Fig. 11D). Identification of this neuron as a middle desVUM neuron could be ensured with the help of a successful labeling of its soma with its typical location in the SOG (Fig. 11Biii). The second middle desVUM neuron that showed IPSPs to MxN stimulation (middle2 in Fig. 11D)

showed a faster response to stimulation than the first middle desVUM neuron ($\tilde{x}_{latency}$=15.15 ms, $IQR_{middle2}$=14.82-16 ms). In another experiment, trains of 20 Hz were applied resulting in inhibition of an unidentified desVUM neuron (Fig. 11A). During 30 successive trains this desVUM was silent, except for showing four action potentials (APs) after 14 applied trains before being silent again. After the sequence of MxN stimulation this neuron showed postinhibitory rebound spikes typical for long lasting inhibition.

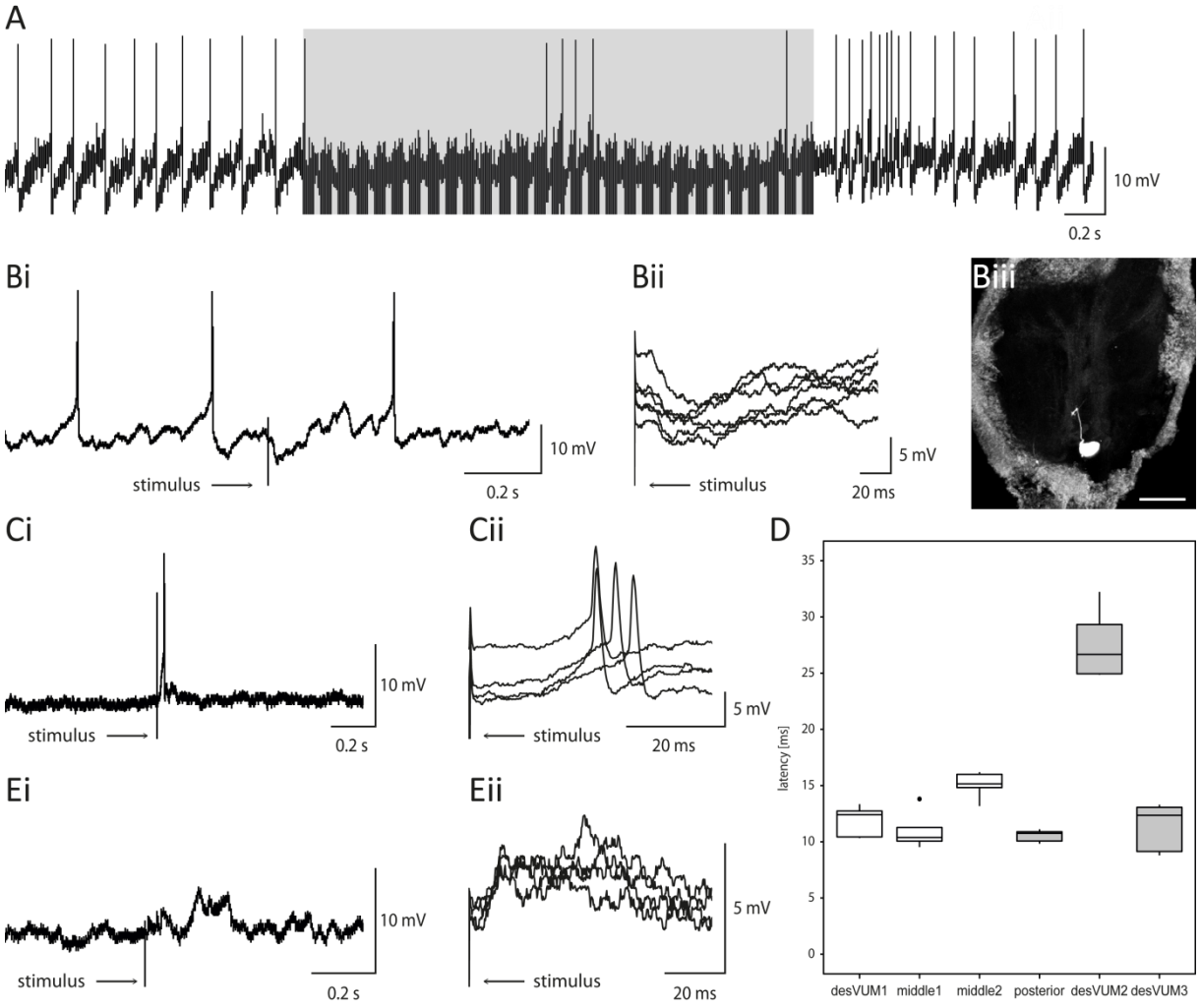

Fig. 11: Stimulation of the maxillary nerve leads to inhibition or excitation in desVUM neurons. (**A**) Train stimulation (20 Hz) led to inhibition of a desVUM neuron. Grey shaded areas denote sequence of stimulation. (**Bi**) Section of an intracellular recording of a middle desVUM neuron responding with an IPSP to maxillary nerve stimulation. (**Bii**) Overlay of six IPSPs to stimulation of the same recording as in Bi. (**Biii**) Identification as a middle desVUM neuron was enabled by a successfully intracellular staining (scale bar 100 µm). (**Ci**) Stimulation of another desVUM neuron led to APs. (**Cii**) Overlay of four responses taken from the same recording as in Ci. (**D**) Boxplots comparing the latencies of different desVUM neurons to stimulation of the maxillary nerve. Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes. (**Ei**) In a posterior desVUM neuron stimulation of this nerve led to EPSPs. (**Eii**) Overlay of responses to four successive stimuli of the same recording as in Ei.

In contrast to the middle desVUM neuron, the posterior desVUM neuron responded with EPSPs to MxN stimulation (n=1) with a median latency of 10.75 ms (IQR=10.07-10.89 ms, posterior in Fig. 11D and Ei-Eii). In total, excitation could be observed in four out of 16 experiments. Two unidentified desVUM neurons showed APs as a response to single MxN stimulation. A representative recording and an overlay of excitatory responses to four successive stimuli is shown in Figure 11Ci and Cii, respectively. Again, latencies varied not only among preparations but also within an experiment (Fig. 11D). The fastest responses were measured in a middle desVUM neuron exhibiting IPSPs to MxN stimulation (middle1, see above). In contrast, the slowest response could be observed in an unidentified desVUM neuron showing APs to stimulation (Fig. 11D, desVUM2). The median latency of the latter was 2.5 times slower than that of the fastest responding neuron ($\tilde{x}_{\text{latency}}$=26.67 ms, $IQR_{desVUM2}$=24.94-29.33 ms, n=latencies of 4 responses). As described for LN-1 stimulation monosynaptic pathways can also be excluded for MxN stimulation as latencies and distances between stimulation and recording sites are very similar.

Responses of desVUM neurons to stimulation of the mandibular nerve

A branch of the mandibular nerve (MdN) innervates the retractors of the hypopharynx. Its main branch projects to the mandibular adductor and abductor muscles and sensory sensilla on the mandible like tactile hairs (Eaton, 1988; Kent and Hildebrand, 1987). MdN was stimulated in 19 experiments while desVUM neurons were recorded. One middle desVUM neuron could be successfully stained (Fig. 12Aiv) that responded with excitation to MdN stimulation in the meaning of formation of either EPSPs or APs (Fig. 12Ai-Aiii). These excitatory responses were the slowest measured for MdN stimulation with a median latency of 107.8 ms (IQR_{middle}=104.56-108.16 ms, middle in Fig. 12C). Furthermore, excitation to stimulation of MdN could be observed in four further unidentified desVUM neurons (see desVUM4, 5 and 6 in Fig. 12C). Median latencies varied from 39.42 ms ($IQR_{desVUM6}$=34.04-50.01 ms, n=latencies of 3 responses, desVUM6) to 8.17 ms ($IQR_{desVUM5}$=8.15-9.92 ms, desVUM5).

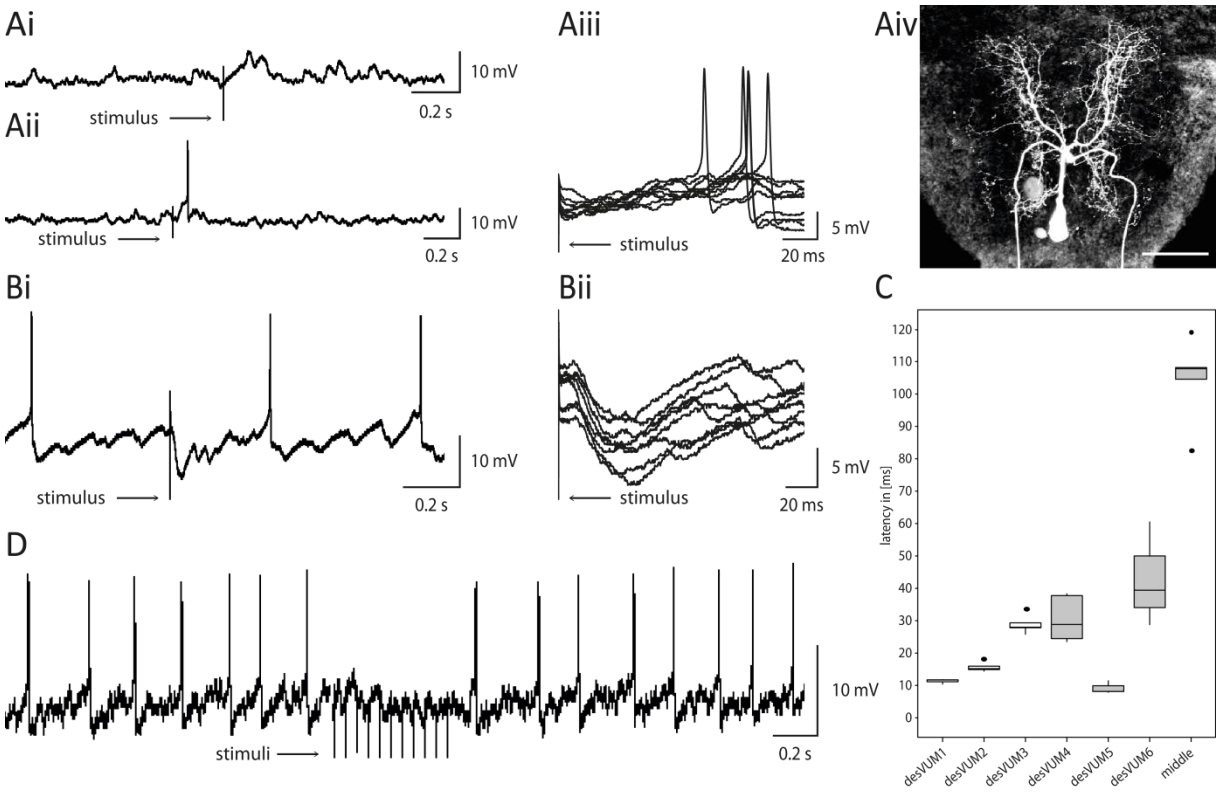

Fig. 12: DesVUM neurons respond either with excitation or inhibition to stimulation of the mandibular nerve. (**Ai** and **Aii**) Representative section of an intracellular recording of a middle desVUM neuron responding either with EPSPs or APs to stimulation while hyperpolarized simultaneously. (**Aiii**) Overlay of responses of the same neuron as in Ai and Aii to nine successive stimuli. (**Aiv**) Intracellular staining of this neuron revealed its type as a middle desVUM neuron (scale bar 100µm). (**Bi**) Representative section of an intracellular recording of a desVUM neuron showing IPSPs to mandibular nerve stimulation. (**Bii**) Overlay of responses to nine successive stimuli of the same neuron as in Bi. (**C**) Boxplots comparing the latencies of responses to stimulation of different desVUM neurons. Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes. (**D**) Section of an intracellular recording of a desVUM neuron that was silent during application of one train with 20 Hz.

In another five experiments MdN stimulation led to inhibition of unidentified desVUM neurons. This inhibition becomes clear when applying a train of 20 Hz which silenced the neuron as shown in Figure 12D. In other cases, single stimuli were applied that led to IPSPs. A representative recording of a desVUM neuron responding with IPSPs to a single stimulus is shown in Figure 12Bi-Bii. The median latency of responses of this neuron was the second fastest in all MdN stimulation experiments ($\tilde{x}_{\text{latency}}$=11.7 ms, $\text{IQR}_{\text{desVUM1}}$=11.14-11.75 ms, desVUM1, Fig. 12C). Again, latencies of the other unidentified desVUM neurons responding with IPSPs to stimulation varied widely. DesVUM2 responded with a median latency of

15.2 ms ($IQR_{desVUM2}$=11.14-11.75 ms, n=5 latencies) almost two times faster than desVUM3 ($\tilde{x}_{latency}$=28 ms, $IQR_{desVUM3}$=27.83-29.35 ms, n=5 latencies).

Responses of desVUM neurons to stimulation of the second labial nerve

The second labial nerve (LN-2) innervates the retractor muscles of the hypostome and certain prothoracic dorsolongitudinal muscles (Eaton, 1988).

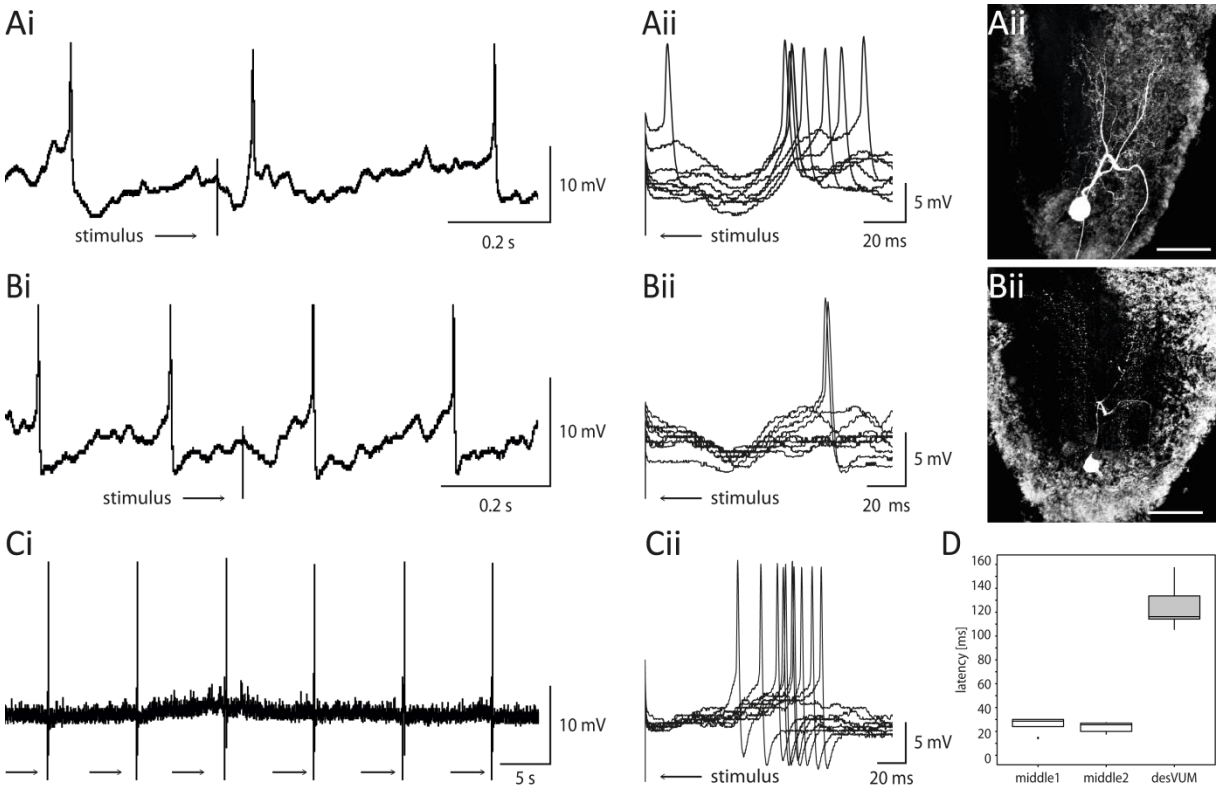

Fig. 13: Responses of desVUM neurons to second labial nerve stimulation. (**Ai** and **Bi**) Sections of intracellular recordings of middle desVUM neurons responding with IPSPs to second labial nerve stimulation. (**Bii** and **Cii**) Overlay of 8 and 10 IPSPs of the same recordings as in Ai and Bi, respectively. (**Aiii** and **Biii**) Intracellular filling indentified these neurons as middle desVUM neurons. Scale bars indicate 100 µm. (**Ci**) Representative section of an intracellular recording of a desVUM neuron responding with APs to second labial nerve stimulation while this neuron was hyperpolarized simultaneously. (**Cii**) Overlay of 10 APs formed as a response to stimulation of the same recording as in Ci. (**D**) Boxplots comparing latencies of responses of two middle desVUM neurons responding with IPSPs and a desVUM neuron responding with APs (middle 1=neuron from Ai-Aiii, middle 2=neuron from Bi-Biii, desVUM=neuron from Ci-Ciii; n=5 latencies per neuron). Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes.

In total, four desVUM neurons were recorded while LN-2 was stimulated. Two middle desVUM neurons could be successfully stained that responded with IPSPs to stimulation (Fig. 13Ai-Biii), both revealing similar latencies ($\tilde{x}_{\text{latency middle1}}$=28.59 ms, $IQR_{middle1}$=24.02-30.05 ms; $\tilde{x}_{\text{latency middle2}}$=25.57 ms, $IQR_{middle2}$=20-26.88 ms; Fig. 13D). In one experiment a single stimulus led to excitation in the meaning of AP formation in an unidentified desVUM neuron (Fig. 13Ci-Cii). The median latency of these excitatory responses ($\tilde{x}_{\text{latency}}$=116.25 ms, IQR_{desVUM}=114.25-133.6 ms) is 4.5 times slower than the fastest inhibitory response to LN-2 stimulation that could be observed in a middle desVUM neuron (middle2, Fig. 13D).

As described for LN-1 stimulation in detail, monosynaptic afferent pathways between receptors of the mouthparts and desVUM neurons of the SOG can be excluded. Assuming a distance of recording and stimulation site between 450-800 µm and the minimal conduction velocity and longest synaptic delay that have been described, latencies in these experiments should have been between 2.13-2.56 ms for monosynaptic connections. This leads to the conclusion that at least one interneuron is intercalated in these sensory pathways.

Descending VUM neurons receive input from receptors of the larval leg

Responses of desVUM neurons to stimulation of the main leg nerve N2a of the prothoracic ganglion

The larval legs carry many proprioceptors like the femoral and tibial chordotonal organs, hair sensilla, multiterminal junctional and tension receptors (Consoulas, 2000). To test if the desVUM neurons of the SOG receive any sensory input from the larval leg, its main nerve N2a, innervating the leg sense organs, was electrically stimulated in isolated nerve cord preparations.

N2a of the prothoracic ganglion (N2aT1) was stimulated in 16 experiments leading to either excitatory or inhibitory responses in desVUM neurons. One middle desVUM neuron could be identified by a successful intracellular staining that received excitatory input from sense organs of the prothoracic leg in the meaning of EPSP formation. The median latency of these excitatory responses to N2aT1 stimulation is 31.87 ms (IQR_{middle}=30.8-32.37 ms, Fig. 15B). In contrast, stimulation led to inhibition (formation of IPSPs) of a posterior desVUM neuron (Fig. 15Ai-Aiii). This posterior desVUM neuron revealed the second fastest measured response to N2aT1 stimulation with a median latency of 16.04 ms

($IQR_{posterior}$=14.87-16.68 ms, posterior in Fig. 15B). The neuron that showed the fastest response also responded with IPSPs to stimulation of N2aT1 ($\tilde{x}_{latency}$=13.99 ms, $IQR_{desVUM3}$=13.84-14.32 ms, Fig. 15B).

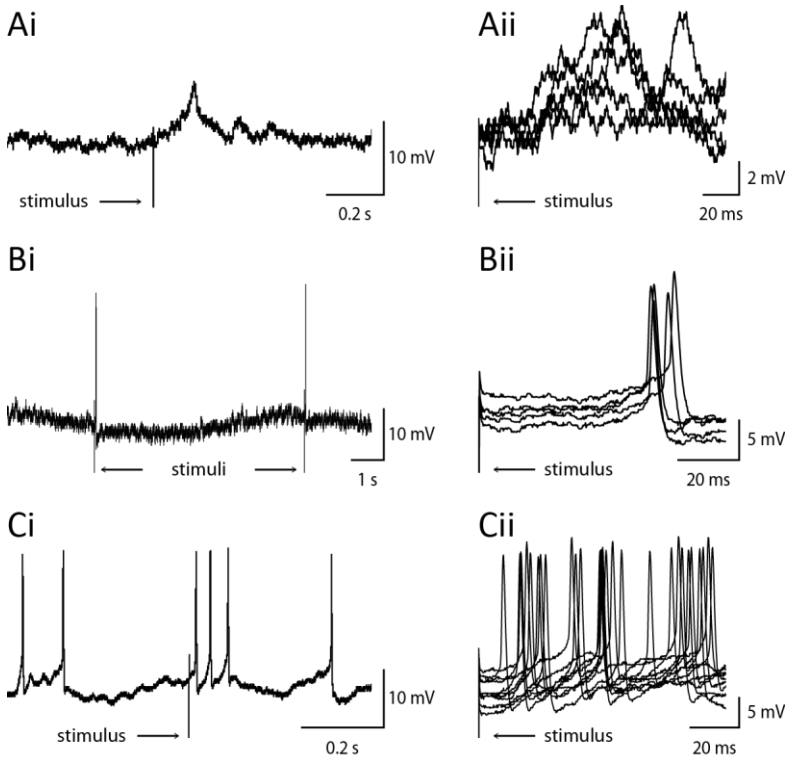

Fig. 14: Excitatory responses to stimulation of the main leg nerve N2a of the prothoracic ganglion. (**Ai**) Representative section of an intracellular recording of a desVUM neuron that responded with an EPSP to stimulation. (**Aii**) Overlay of the same neuron as in Ai to six successive stimuli. (**Bi**) Section of an intracellular recording of a desVUM neuron responding with APs to stimulation. (**Bii**) Overlay of five APs as response to stimulation of the same recording as in Bi. (**Ci**) Representative intracellular recording of desVUM neuron responding with a salvo of three APs (**Cii**) Overlay of nine responses to stimulation of N2a of the same neuron as in Ci.

In total, stimulation of N2aT1 led to excitatory responses in eight neurons in the meaning of formation of either EPSPs (n=3, desVUM7, 8, 9 and middle in Fig. 15B) or APs (n=5, desVUM4, 5 and 6 in Fig. 15B, also see Fig. 14). In four experiments, inhibition of desVUM neurons to N2aT1 could be observed (desVUM1, 2, 3 in Fig. 15B). Strikingly, median latencies of desVUM neurons responding with IPSPs seem to be faster than those of desVUM neurons revealing excitation to N2aT1 stimulation. But median latencies also showed a high variation, especially among the group of desVUM neurons showing excitation to stimulation.

The fastest excitatory responses were measured in an unidentified desVUM neuron forming salvos of APs to N2aT1 stimulation (Fig. 14Ci-Cii) with a median latency of 22.98 ms ($IQR_{desVUM4}$=19.9-23.72 ms, desVUM4 in Fig. 15B). In contrast, an unidentified desVUM neuron responding with a single AP to stimulation of main nerve of the prothoracic leg (Fig. 14Ci-Cii) revealed a 2.4 times slower median latency than that in the fastest excitatory responding desVUM neuron ($\tilde{x}_{latnecy}$=55.37 ms, $IQR_{desVUM6}$=55.21-60.05 ms, desVUM6).

Considering the minimal conduction velocity and longest synaptic delay found in literature plus a distance of 1-1.5 mm between stimulation and recording site, latencies should be around 2.8-3.4 ms for monosynaptic connections. Median latencies in N2aT1 stimulation experiments varied from 13.99 ms ($IQR_{desVUM3}$=13.84-14.32 ms, desVUM3) to a maximum of 62.89 ms ($IQR_{desVUM8}$=62.39-73.2 ms, desVUM8 in Fig. 15B). Therefore, the afferent pathway between sense organs of the larval prothoracic leg and desVUM neurons of the SOG has to be polysynaptic with a few interneurons conveying the signal.

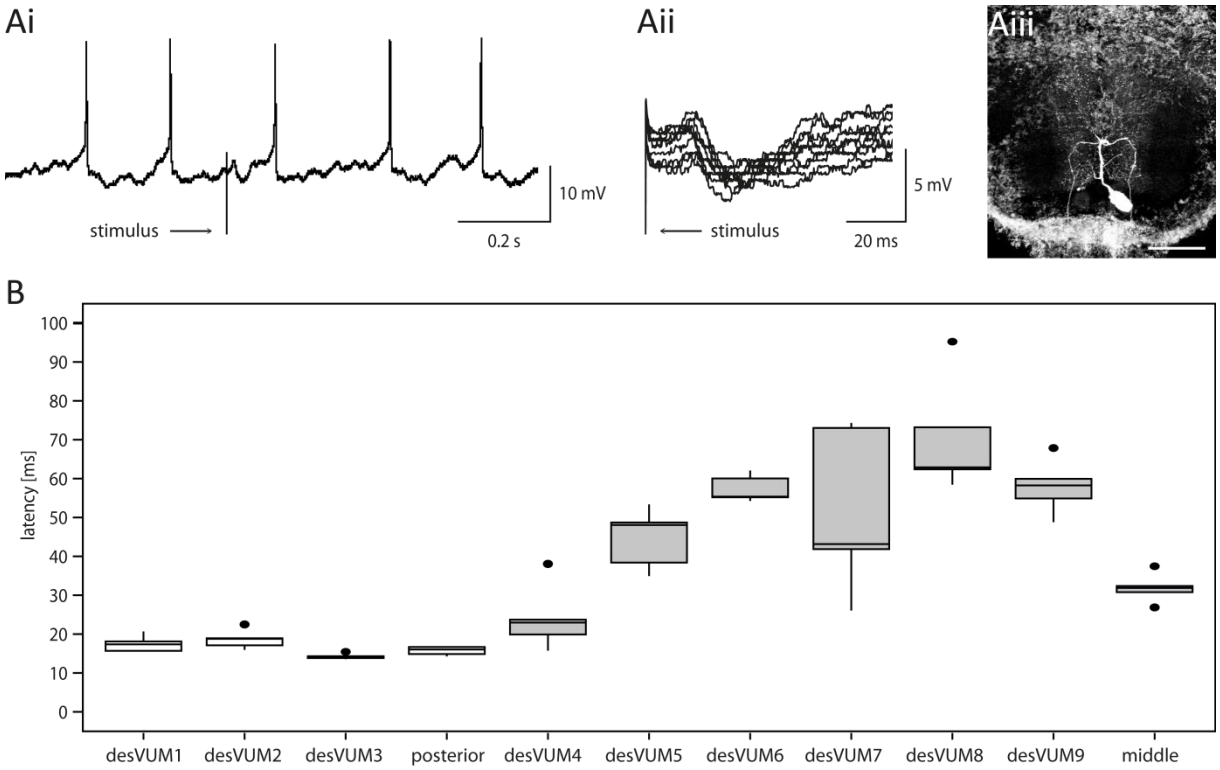

Fig. 15: Stimulation of the prothoracic N2a led to formation of IPSPs in a posterior desVUM neuron. (**Ai**) Representative section of an intracellular recording of a posterior desVUM neuron responding with an IPSP to stimulation. (**Aii**) Overlay of responses of the same neuron as in Ai to 10 stimuli. (**Aiii**) Projection view of the intracellular staining of this neuron revealed its type as a posterior desVUM neuron. Scale bar 100 µm. (**B**) Boxplots comparing latencies of responses to prothoracic N2a stimulation of 11 desVUM neurons. (n=5 latencies per neuron). Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes.

Responses of desVUM neurons to stimulation of the main leg nerve N2a of the mesothoracic ganglion

The mesothoracic N2a (N2aT2) was stimulated in five experiments. The middle desVUM neuron receives inhibitory input from sense organs of the larval mesothoracic leg (n=1). As seen in Figure 16Ai and Aii, single stimuli lead to the formation of pronounced IPSPs in a middle desVUM neuron with a median latency of 17.51 ms (IQR_{middle}=14.64-17.66 ms, middle, Fig. 16D). In contrast, in posterior desVUM neurons this stimulation leads to excitation (Fig. 16Bi-Ciii, n=2). One posterior desVUM neuron displayed salvos of three APs (Fig. 16Bi-Bii) with a median latency of 26.53 ms ($IQR_{posterior1}$=25-28.95 ms, posterior1, Fig. 16D). The second posterior desVUM neuron that could be identified with the help of a successful intracellular filling responded with the formation of one AP to a single stimulus (Fig. 16Ci-Cii). Here, the median latency of these excitatory responses was almost three times longer ($\tilde{x}_{latency}$=78.15 ms, $IQR_{posterior2}$=71.07-86.14 ms, posterior2, Fig. 16D) than that measured in the first described posterior desVUM neuron.

Interestingly, this response pattern to stimulation of N2aT2 of middle and posterior desVUM neurons is the exact opposite what has been observed in N2aT1 stimulation experiments. While the middle desVUM neuron receives excitatory input from sense organs of the prothoracic but inhibitory input from those of the mesothoracic leg, the posterior desVUM responds just in the opposite manner.

Monosynaptic pathways between sense organs of the mesothoracic leg and desVUM neurons of the SOG can be excluded. Considering a minimal distance between recording and stimulation site of 1800 µm and the slowest conduction velocity and longest synaptic delay that has been published, the latencies should be around 3.02 ms to indicate a monosynaptic connection. It is rather likely that at least one or more interneurons convey the signal of the receptors of the leg to the desVUM neurons of the SOG.

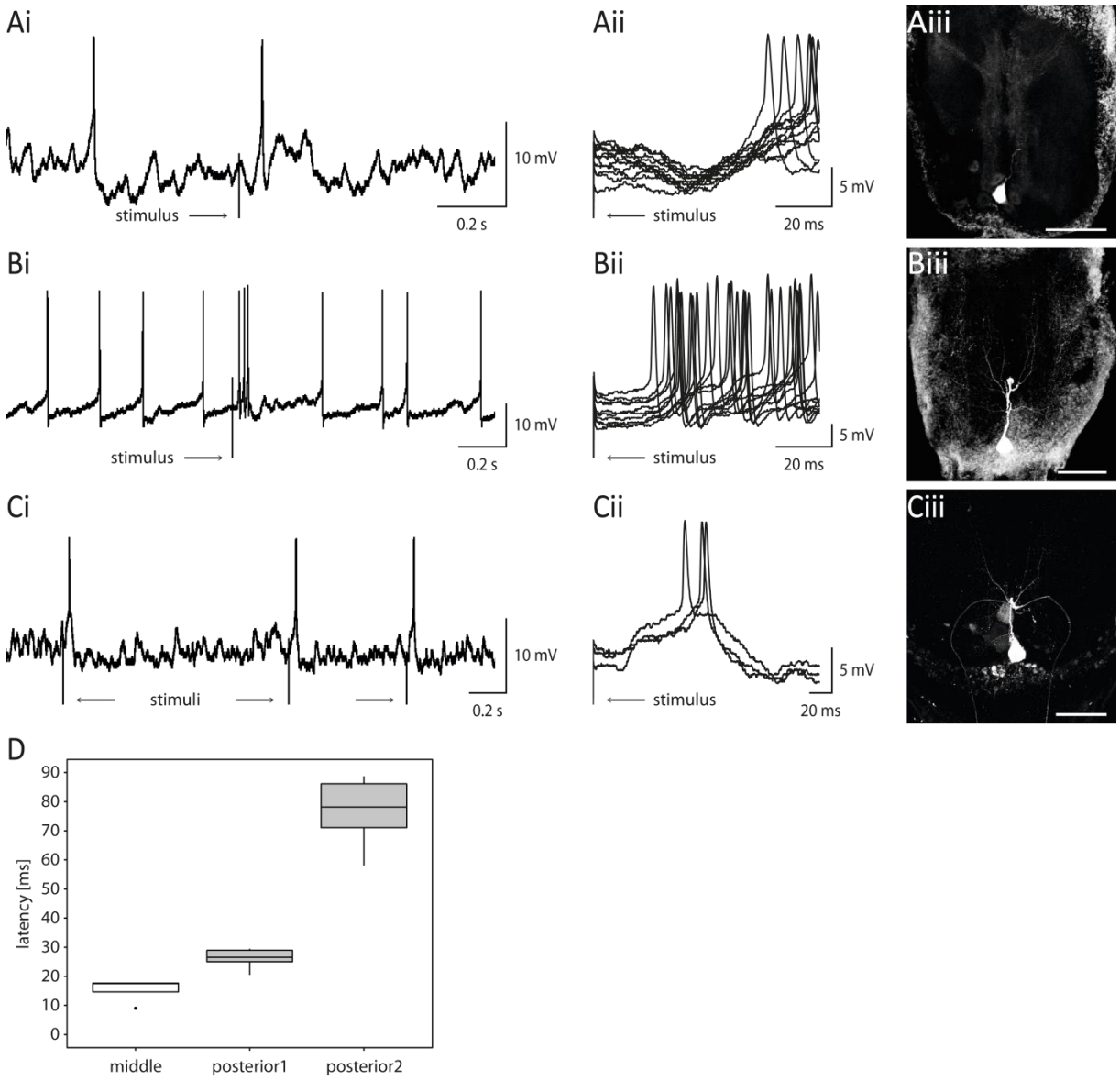

Fig. 16: Responses of desVUM neurons to stimulation of the main leg nerve N2a of the mesothoracic leg. (**Ai**) Intracellular recording of a middle desVUM neuron responding with an IPSP to stimulation. (**Aii**) Overlay of responses of the same neuron as in Ai to 12 stimuli. (**Aiii**) Intracellular labeling of this neuron revealed its type as middle desVUM neuron. (**Bi**) Section of an intracellular recording of a posterior desVUM neuron responding with a salvo of three APs. (**Bii**) Overlay of responses to 10 stimuli of the same recording as in Bi. (**Biii**) Projection view of the intracellular staining of this neuron. (**Ci**) Intracellular recording of posterior desVUM neuron where stimulation led to formation of an AP. (**Cii**) Overlay of responses to three stimuli. (**Ciii**) Projection view of the intracellular labeling of this neuron. (**D**) Boxplots comparing latencies of responses to mesothoracic N2a stimulation (middle=neuron of Ai-Aiii, posterior1=neuron of Bi-Biii, posterior2=neuron of Ci-Ciii). Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes. Scale bars 100 µm.

Responses of desVUM neurons to stimulation of the prothoracic leg nerve N1b

N1b, the second main leg nerve of *Manduca sexta* larvae, innervates femoral, tibial and pleurocoxal extensor muscles (Kent and Levine, 1988b) and was stimulated in five desVUM neuron recordings leading to either inhibition (n=3) or excitation (n=1). A middle desVUM neuron, identified by successful intracellular labeling, responded with formation of IPSPs (Fig. 17Ai-Aiii) with a median latency of 30.78 ms (IQR_{middle}=29.07-30.78 ms, middle in Fig. 17D). One recorded unidentified desVUM neuron showed excitation to prothoracic N1b stimulation by forming an AP as a response to a single stimulus (Fig. 17Bi-Bii). The median latency of these excitatory responses is 2.2 times longer than the shortest median latency measured in an unidentified desVUM neuron that responded with IPSPs to stimulation (excitatory: $\tilde{x}_{latency}$=51.47 ms, IQR=49.21-51.7 ms, desVUM3; inhibitory: $\tilde{x}_{latency}$=23.31 ms, $IQR_{desVUM1}$=22.07-23.98 ms; desVUM1, Fig. 17D).

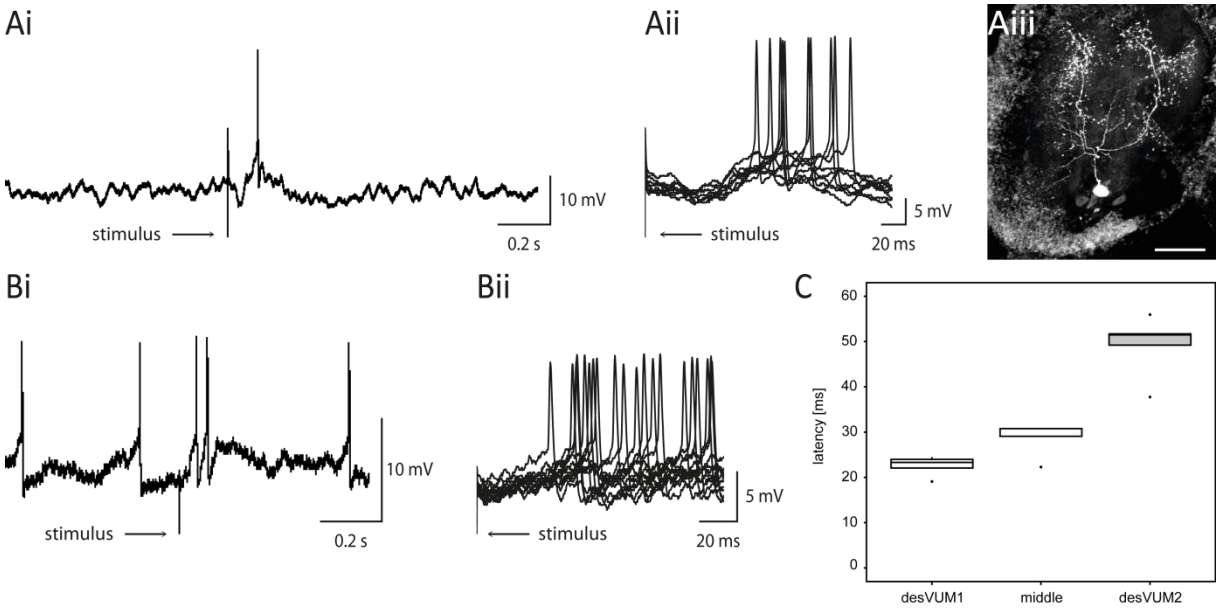

Fig. 17: Responses of desVUM neurons to prothoracic N1b stimulation. (**Ai**) Section of an intracellular recording of a middle desVUM neuron responding with the formation of an IPSP to single stimulation. (**Aii**) Overlay of responses to nine successive stimuli of the same neuron as in Ai. (**Aiii**) Projection view of the neuron of Ai revealing its type as a middle desVUM neuron. Scale bar indicates 100 µm. (**Bi**) Section of an intracellular recording of a desVUM responding with APs to stimulation. (**Bi**) Overlay of responses to 12 successive responses. (**C**) Boxplots comparing latencies of responses to prothoracic N1b stimulation of three desVUM neurons (n=5 latencies per neuron). Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes.

Descending VUM neurons receive input from hairlike sensilla on the dorsal surface of the prothoracic segment

The thoracic and abdominal segments support many hairlike sensilla sitting in tight-fitting sockets and being innervated each by a single sensory neuron (Kent and Levine, 1988; Levine et al., 1985). Sensory neurons innervating the hairlike sensilla on the dorsal surface of the prothoracic segment sent their axons into the CNS via N1a. To test whether the desVUM neurons of the SOG receive sensory input from these "bristle" sensilla N1a was electrically stimulated.

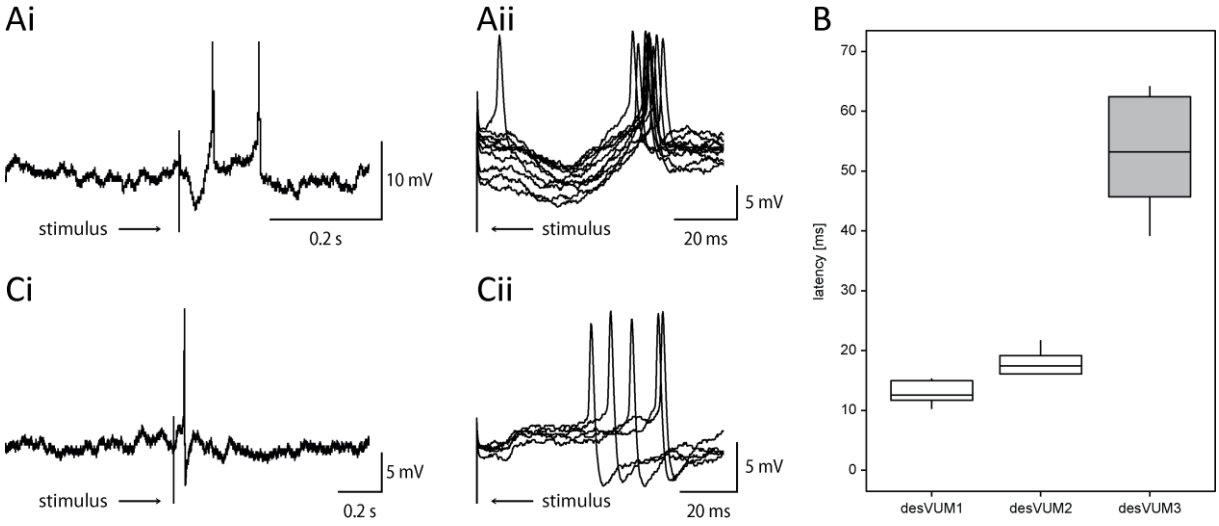

Fig. 18: Stimulation of the prothoracic N1a innervating hairlike sensilla on the dorsal surface of the prothoracic segment led to either inhibition or excitation of desVUM neurons. (**Ai**) Section of an intracellular recording of a desVUM neuron responding with an IPSP to N1a stimulation. (**Aii**) Overlay of responses to ten successive stimuli of the same neuron as in Ai. (**B**) Boxplots comparing latencies of inhibitory responses to prothoracic N1a stimulation of three desVUM neurons (n=5 latencies per neuron). Latencies of inhibitory responses are illustrated in white, of excitatory responses in grey boxes. (**Ci**) Representative section of an intracellular recording of a desVUM neuron responding with an AP to stimulation. (**Cii**) Overlay of responses to five successive stimuli of the same neuron as in Ci.

Three out of five recorded desVUM neurons showed inhibition, one excitation when N1a of the prothoracic ganglion was stimulated. Unfortunately, none of these neurons could be assigned to a specific desVUM neuron type as their intracellular fillings failed. A representative intracellular recording of an unidentified desVUM neuron that responded with the formation of IPSPs to stimulation is shown in Figure 18Ai-Aii. Its latencies were the fastest measured in desVUM neurons responding to N1a stimulation ($\tilde{x}_{\text{latency}}$=12.56 ms,

$IQR_{desVUM1}$=11.71-14.97 ms, desVUM1 in Fig. 18B). In contrast, an unidentified desVUM neuron that responded with APs (Fig. 18Ci-Cii) displayed a 4.2 times slower median latency ($\tilde{x}_{latency}$=53.21 ms, $IQR_{desVUM3}$=45.7-62.45 ms, desVUM3 in Fig. 18B).

As distances between recording stimulation sites in N1bT1 and N1aT1 stimulation experiments are similar to that described for the experiments stimulating N2aT1 in detail, latencies for monosynaptic connections should be between 2.8-3.4 ms as well. Short latencies like this could not be observed in N1bT1 and N1aT1 stimulation experiments. This indicates that pathways between hairlike sensilla on the dorsal surface of the prothoracic segment and desVUM neurons of the SOG are rather polysynaptic. This also applies for N1bT1 stimulation experiments.

Summary nerve stimulation experiments

Electrical stimulation of a nerve evoked either excitatory or inhibitory responses in larval desVUM neurons indicating their different sensory input (Tab. 2). The middle desVUM neuron is the best characterized neuron of all three desVUM neurons as responses to stimulation of all nerves except the LN-1 and N1a could assigned to it. Conspicuously, stimulation of mouthparts nerves did not evoke consistent responses in one desVUM neuron type. The middle desVUM neuron is inhibited during MxN and LN-2 stimulation but excited when stimulating MdN. Interestingly, in all experiments where both the middle and posterior desVUM neuron could be identified, opposite responses in both neurons to stimulation of the same nerve could be observed. Another striking observation is the response pattern of both neuron types to N2a stimulation. The posterior desVUM neuron is inhibited when stimulating the prothoracic N2a but excited when stimulating the same nerve of the mesothoracic ganglion. The opposite is true for the middle desVUM neuron.

Table 3 summarizes the median latencies of desVUM neurons responses of all stimulation experiments of this study. 71.43% of median latencies of all inhibitory responses are shorter than 20 ms, whereas this is true for only 16.76% of median latencies of all excitatory responses. Therefore, regardless of stimulation sites, inhibitory responses are significantly more often shorter than excitatory responses (p=0.0006, χ^2= 11.69). In addition, differences of the third and first quartiles (Q3-Q1= interquartile range, IQR) of latencies per preparation were calculated and stated in Table 3. The interquartile range is a common and robust measure of statistical variability (Sachs and Hedderich, 2006) and indicates the degree of robustness of either inhibitory or excitatory pathways, whereby a lower IQR means a

greater degree of robustness. Variabilities of latencies of inhibitory and excitatory responses were compared by calculation of the median of their interquartile ranges and applying the Mann-Whitney U test. Interestingly, inhibitory responses do not only reveal more often shorter latencies, but they are also significantly less variable (inhibitory responses: $\tilde{x}$=2.29 ms, IQR=1.18-3.77 ms; excitatory responses: $\tilde{x}$=4.62 ms, IQR=2.31-13.74 ms). These results indicate a more robust pathway for inhibitory responses.

Tab. 2: Summary of responses of the larval desVUM neurons in the SOG to nerve stimulation. Excitation of a neuron is indicated by a plus, inhibition by a minus. Numbers of recorded neurons are written in brackets.

Ganglion	Nerve	Anterior desVUM	Middle desVUM	Posterior desVUM	Unidentified desVUM
SOG	LN-1			+ (1)	− (4)
SOG	MxN		− (2)	+ (1)	− (6) + (3)
SOG	MdN		+ (1)		− (5) + (4)
SOG	LN-2		− (2)		+ (1)
T1	N2a		+ (1)	− (1)	− (4) + (7)
T1	N1b		− (1)		− (2) + (1)
T1	N1a				− (3) + (1)
T2	N2a		− (1)	+ (2)	

Tab. 3: Median latencies of inhibitory (red) and excitatory (green) responses of larval desVUM neurons of the SOG to electrical nerve stimulation. Medians are illustrated in bold, differences of interquartile ranges in brackets.

Ganglion	Nerve	Anterior desVUM (ms)	Middle desVUM (ms)	Posterior desVUM (ms)	Unidentified desVUM (ms)
SOG	LN-1				**11.38** (2.29) **15.21** (6.42) **16.84** (4.28)
SOG	MxN		**10.38** (1.21) **15.15** (1.18)	**10.75** (0.82)	**12.41** (2.31) **12.36** (3.93) **26.67** (4.39)
SOG	MdN		**107.8** (3.6)		**11.7** (0.61) **15.2** (1.07) **28** (1.52) **8.17** (1.77) **28.83** (13.3) **39.42** (15.97)
SOG	LN-2		**25.57** (6.88) **28.59** (6.03)		**116.25** (19.35)
T1	N2a		**31.87** (1.57)	**16.04** (1.18)	**13.99** (0.48) **17.36** (2.41) **18.79** (1.82) **22.98** (3.82) **43.15** (31.18) **48.06** (10.31) **55.37** (4.84) **62.89** (10.81) **58.25** (5.05)
T1	N1b		**30.78** (1.17)		**23.31** (1.91) **51.47** (2.49)
T1	N1a				**12.56** (3.26) **17.45** (3.05) **53.21** (16.75)
T2	N2a		**17.51** (3.02)	**26.53** (0.95) **78.15** (15.07)	

Intact head preparations

Recordings of desVUM neurons in a semi intact preparation seemed to be a method with a variety of opportunities for further characterization of these neurons, e.g. investigating their responses to external mechanical stimulation or their electrophysiological properties during certain behaviors like feeding. Intracellular recordings in a semi intact preparation of *Manduca* proved to be difficult because of its mostly non-sclerotized body. Several problems occurred during preparation procedure such as removal or maintenance of the large gut. Latter was impossible due to the animal's internal pressure and egression of the gut in consequence of an incision in the animal's body. Despite these preparation difficulties the biggest

challenge appeared to be contractions of the body wall musculature which not only result in movements of the whole soft-bodied larva but also directly translates on the ventral nerve cord which makes intracellular recordings almost impossible. Therefore, I established a preparation with an intact head capsule while the segmental ganglia were isolated. Stability of an intracellular recording was guaranteed by placing the SOG on a wax coated platform. With this approach the head could be either stimulated by blowing at the head capsule or by touching with a brush. In desVUM neurons wind stimuli resulted in an increase in spike frequency during stimulus onset (n=3). An example of this excitatory response pattern to two wind stimuli is displayed in Figure 19. In this case, the desVUM neuron exhibits a spontaneous firing-frequency of 4-5 Hz before presentation of a first wind puff and 5-6 Hz between both stimuli. Application of wind puffs led to a long lasting increase of frequency reaching a maximum of 8 Hz 0.5 s after stimulus onset. This is followed by a decrease to a minimum of 6 Hz during wind puff. But again, after 3.5 s frequency rises to 7 Hz before declining to almost spontaneous frequency level. Between both stimuli firing-frequency is still elevated, representing an off-response. Those observations apply for both wind stimuli.

Unfortunately, in none of the intact head preparations the recorded desVUM neurons could be successfully filled with dye and therefore an accurate classification as a specific desVUM neuron type was not possible.

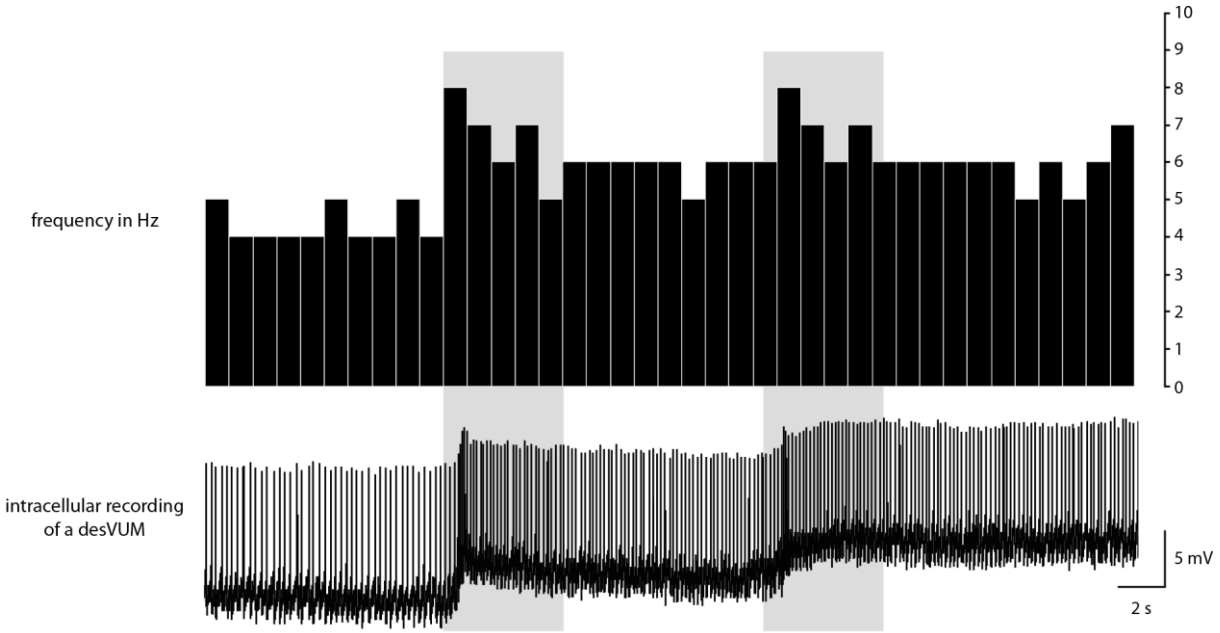

Fig. 19: Responses of a desVUM neuron to wind stimuli in an intact head preparation. Gently blowing at the head leads to increase in spike-frequency during stimulus onset. Grey shaded areas denote time of wind puff application.

3.3 Recruitment of desVUM neurons during fictive crawling in *Manduca* larvae

The desVUM neurons of the SOG are restricted to the CNS and therefore are good candidates for central effects of octopamine as described for the locust desDUM neurons of the SOG by Bräunig and Burrows (2004). Among these central effects is the modulation of circuits that organize motor patterns, e.g. CPGs. Therefore it seems to be of great interest to investigate the recruitment of desVUM neurons during patterned motor activity, like fictive crawling.

Intracellular recordings of desVUM neurons were combined with extracellular recordings of mesothoracic N1b that carries axons of motoneurons innervating femoral, tibial and pleurocoxal extensor muscles of the larval leg, referred here as levator, to monitor fictive crawling. Additionally, the activity of the mesothoracic N2a that carries axons of motoneurons innervating flexor muscles of the larval leg, referred here as depressor, was recorded. In *Manduca* larva fictive crawling is characterized by alternating bursts of levator and depressor activity of each larval leg. In 13 out of 23 isolated nerve cord preparations the muscarinic acetylcholine receptor agonist pilocarpine (10^{-3} M) elicited patterned activity of the depressor and/or levator that could be identified as fictive crawling (Fig. 20A). Note, pilocarpine was exclusively applied to the meso-, metathoracic and abdominal ganglia, while the brain, SOG and prothoracic ganglion were placed in saline throughout the experiment.

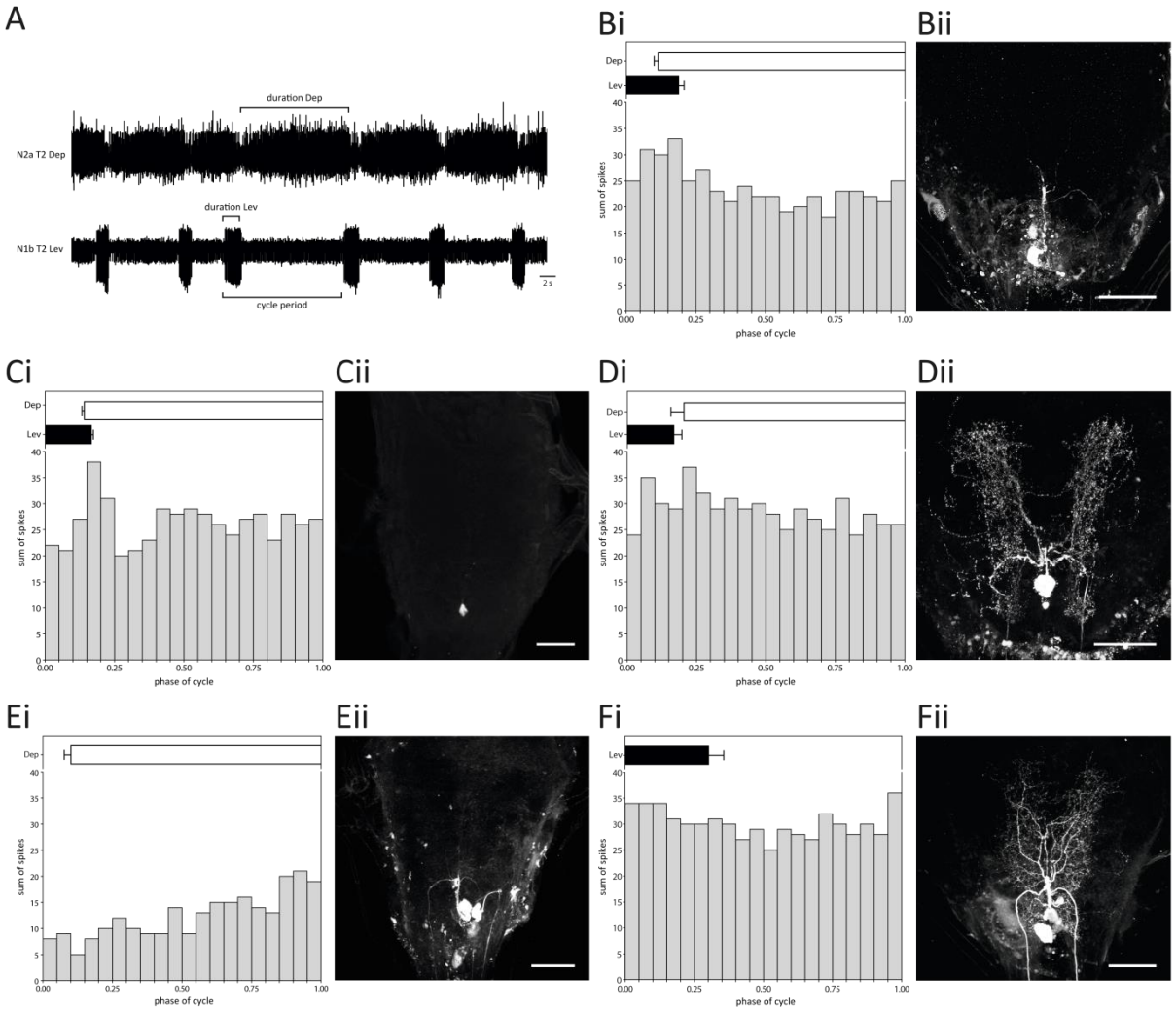

Fig. 20: Phase histograms displaying the spiking behavior of individual desVUM neurons of the SOG during fictive crawling cycle. Horizontal black and white bars show the mean relative levator (Lev) and depressor (Dep) duration ± SEM, respectively. Cycle period was divided into 20 bins and the sum of spikes per bin was calculated for cycles and is represented in vertical grey bars. Cycle period is defined as time interval between onset of two subsequent levator bursts. For experiments lacking extracellular recordings of levator activity, cycle period is defined as time interval between onset of two subsequent depressor bursts. (**A**) Extracellular recording of mesothoracic nerve 2a (N2a T2) innervating depressor muscles and mesothoracic nerve 1b (N1b T2) innervating levator muscles of the mesothoracic leg after 10^{-3} M pilocarpine application. Fictive crawling is characterized by alternating bursts of depressor and levator activity (**Bi-Bii**) A posterior desVUM neurons showed an increase in number of observed spikes during levator phase and decrease during depressor phase. (**Ci-Cii**) In a middle desVUM neuron the highest number of spikes occur during transition from levator to depressor phase. (**Di-Dii**) The highest number of spikes in an anterior desVUM neuron could be observed during levator phase and during transition from levator to depressor phase. During depressor phase an decrease in spikes can be seen. (**Ei-Eii**) A transient increase in spike occurrence during depressor phase was found in a presumable middle desVUM neuron. (**Fi-Fii**) In another middle desVUM neuron a similar spiking behavior as in Bi can be observed. Scale bars 100 µm.

In these experiments, cycle period is defined as time interval between the onset of two subsequent levator bursts. In experiments lacking recordings of levator N1b, cycle period is defined as time between the onset of contiguous bursts of motor activity in depressor N2a. Isolated nerve cord preparations revealed fictive crawling patterns with a mean cycle period of 10.33 s±0.64 s (SEM, n=10 cycles of 4 preparations with recorded levator and depressor activity). Furthermore they show a mean relative depressor duration (depressor phase, ratio of depressor duration/cycle period) of 0.85±0.01 and a mean relative levator duration of 0.21±0.01 (levator phase, ratio of levator duration/cycle period, n=10 cycles of 4 preparations with recorded levator and depressor activity).

In general, when fictive crawling was induced by pilocarpine the spike frequency of desVUM neurons increased significantly (n=8, $p < 0.0001$, Mann-Whitney U). This increase ranged from 53.22% in a desVUM neuron exhibiting a mean spike frequency of 5.34±0.78 Hz in saline to an almost 10-fold increase in a preparation where a desVUM neuron spiked rarely under saline conditions (saline: $\bar{x}$=0.32±0.47 Hz, pilocarpine: $\bar{x}$=3.06±0.81 Hz). In five experiments in which a robust fictive crawling pattern could be induced by pilocarpine, intracellular recordings of desVUM neurons revealed individual patterned activity as well. This patterned activity did not only vary among the three different desVUM neuron types, they spiking behavior during fictive crawling also differed within groups (Fig. 20B-F). At least two middle desVUM neurons could be identified with the help of an intracellular filling (Fig. 20Cii, Fii) that revealed a different spiking behavior during fictive crawling cycle. One middle desVUM neuron exhibits an increase in spike activity during levator phase reaching a maximum of 38 spikes (at 0.15 cycle phase) during transition from levator to depressor phase (Fig. 20Ci). After this transition phase a decrease in spiking could be observed in the beginning phase of depressor activity and then neuron spiked almost steadily during depressor phase. Similar to that, one anterior desVUM neuron showed a maximum of spike activity during transition from levator to depressor phase during fictive crawling cycle of 37 spikes at 0.2 cycle phase (Fig. 20Di). This neuron showed an increase of spiking during levator as well. The maximum of spike activity during transition is followed by a transient decrease in spike occurrence during depressor phase. A similar spiking behavior could be also observed in a posterior desVUM neuron (Fig. 20Bi). During levator phase spiking increases and reaches a maximum of 33 spikes at 0.15 cycle phase which coincides with the transition from levator to depressor phase. During depressor activity this neuron fires less than during levator phase. The second successfully stained middle desVUM neuron of these experiments showed a steady spiking behavior during the first half of levator phase (0.00-0.1 cycle phase) before

exhibiting a decrease in spiking reaching a minimum of 25 spikes at 0.5 cycle phase, thus during depressor phase. This is followed by an increase in spike activity. Overall, this neuron showed inhibition, thus decrease in spike activity in the second half of levator phase and first half of depressor phase of fictive crawling cycle (Fig. 20Fi). In one recording of a desVUM neuron a transient increase of spike activity over the whole cycle phase could be observed (Fig. 20Ei). Intracellular labeling of this neuron resulted in two not well stained neurons. The soma position of one of those neurons resembles the one of the middle desVUM neurons. Therefore, the neuron recorded in this experiment is presumably a middle desVUM neuron.

These results suggest that desVUM neurons of the SOG are centrally coupled to the CPG producing fictive crawling in larvae. But the preliminary data of these experiments do not allow a coherent conclusion if the three larval desVUM neurons of the SOG are activated differentially or collectively during fictive crawling motor pattern.

4 DISCUSSION

This study offers for the first time a detailed description of the morphology and sensory input of the three desVUM neurons of the larval tobacco hawkmoth *Manduca sexta*. Electrophysiological and histological approaches revealed that they are not just a homogeneous cluster of neurons but rather a heterogeneous group in a functional and morphological sense as they are activated differentially when stimulating nerves innervating sense organs of the mouthparts and thoracic legs as well as hairlike sensilla on the dorsal surface of the prothoracic segment and are distinguishable by their characteristic morphological features. This heterogeneity seems to be a common feature of suboesophageal descending UM neurons in insects (Bräunig and Burrows, 2004).

4.1 Morphology of larval desVUM neurons

The larval SOG of *Manduca sexta* contains three desVUM neurons in the labial neuromere that are shown to be octopaminergic (Cholewa and Pflüger, 2009) and exhibit common morphological features with the segmental efferent VUM neurons of the thoracic ganglia, such as a large pear-shaped soma located on the ventral midline and an anteriorly running primary neurite that bifurcates into two bilaterally symmetrical axons (Fig. 5). But in contrast to the thoracic and abdominal efferent VUM neurons, desVUM neurons of the SOG possess axons that do not project into nerves of their own ganglia as shown in intracellular stainings and indicated by experiments in which these nerves where stimulated. In none of these experiments antidromic spikes in desVUM neuron somata could be observed. Instead, their axons descend via both neck connectives towards the thoracic ganglia. The most obvious differences between the three larval desVUM neurons are the position of their soma along the midline and the length of their primary neurite. Therefore, they were called anterior, middle and posterior desVUM neurons (Cholewa and Pflüger, 2009). These features are very similar between the middle and posterior desVUM neurons which often made them difficult to distinguish. But the morphological characterization in the scope of this thesis allows a better identification of these neurons as it revealed that desVUM neurons exhibit different projection patterns within the SOG (Fig. 2). While anterior and posterior desVUM neurons reveal a similar number of branches originating from their primary neurites, the middle desVUM

neuron exhibit a unique projection pattern within the SOG with the highest number of branches originating from its primary neurite. These differences in their projection patterns within the SOG are not based on variability among stainings and morphologies of identified individual desVUM neurons as these differences were also clearly visible in the study of Cholewa and Pflüger (2009). Additionally to their morphology within the SOG, this thesis provides for the first time a detailed description of their projections within the prothoracic ganglion and for the posterior desVUM neuron even within the mesothoracic ganglion. In each of these thoracic ganglia, the desVUM neurons possess extensive branches concentrated in the ventral and lateral regions of the ganglia that are distributed over the anterior-posterior axis and are bilaterally symmetrical in both the left and right hemisphere.

4.2 Posterior projections of larval desVUM neurons

Intracellular fillings with either neurobiotin or lucifer yellow allowed labeling of desVUM neuron projections in exceptional cases as far as the mesothoracic ganglion. *Manduca* desVUM neurons appear to be restricted to the central nervous system and therefore are interneurons as projections into peripheral nerves of the SOG, pro- and mesothoracic ganglia could not be observed in any staining. Furthermore, this hypothesis is supported by the absence of antidromic spikes in desVUM somata when stimulating peripheral nerves of the SOG and thoracic ganglia. This is in accordance with findings in locusts where stimulation of peripheral nerves of the thoracic and abdominal ganglia did not elicit antidromic spikes in any descending DUM neuron which therefore are considered to be interneurons (Bräunig and Burrows, 2004).

To determine posterior projections of desVUM neurons further than the mesothoracic ganglion different electrophysiological and histological approaches were implemented that indicate that larval desVUM neurons project at least to the second abdominal ganglion. Backfills of one connective at various levels proved to be difficult as desVUM neurons exhibit thin axons impeding the entry of dye. Backfills of either the right or left neck connective indicate that desVUM neurons project at least to the second abdominal ganglia (Fig. 4). With this method axons of desVUM neurons should be stained exclusively as they are supposed to be the only cells in the SOG with bilaterally symmetrical descending axons. Nevertheless, in thoracic and abdominal ganglia more than three axons are clearly visible. As isolated nerve cord preparations were left in saline for a maximum of five days to guarantee

diffusion of the dye, the occurrence of more than three axons in each ganglion might be due to degeneration processes of neural tissue and therefore leakage of dye. Nevertheless, some of these projections clearly resemble those of the desVUM neurons. It also cannot be fully excluded that other neurons within SOG possess bilaterally symmetrical descending axons. In addition to these histological techniques, electrical stimulation experiments of connectives right in front of the terminal ganglion were performed but failed to elicit antidromic spikes in somata of desVUM neurons indicating that they do not project through the entire ventral nerve cord. This observation is in contrast to findings of Cholewa and Pflüger (2009) that observed antidromic spikes in the posterior desVUM neuron. Their results indicate that all three desVUM neurons project through the thoracic and first abdominal ganglia. The posterior desVUM neurons sends its axons even as far as the terminal ganglion. Similar to that, stimulation experiments in the locust revealed that four of six descending DUM neurons project through the entire ventral nerve cord (Bräunig and Burrows, 2004).

4.3 Morphology of adult desVUM neurons

In contrast to the larva, where the SOG is a single unit that is connected to the brain via the circumoesophageal connectives, the SOG of the adult *Manduca* is fused to the brain. Interestingly, the number of desVUM neurons in the SOG increases during metamorphosis similar to what can be observed for *Manduca* mesothoracic (Vierk, 2009) and abdominal (Thorn and Truman, 1994) VUM neurons. In the adult hawkmoth, seven octopaminergic desVUM neurons could be found with somata located in the labial neuromere of the SOG (Dacks et al., 2005) suggesting that the three larval desVUM neurons persist metamorphosis like the larval VUM neurons of the abdominal ganglia (Pflüger et al., 1993) while four desVUM neurons develop postembryonically. In holometabolous insects, such as *Manduca* (Booker and Truman, 1987; Witten and Truman, 1991) and *Drosophila*, (Truman and Bate, 1988) many neuroblasts remain active during larval life allowing postembryonic neurogenesis which contributes to adding new adult specific neurons during metamorphosis. A developmental study in *Drosophila* indicates that all suboesophageal UM neurons are the progeny of a median neuroblast (Busch and Tanimoto, 2010), an unpaired median stem cell that arises posteriorly at the midline of each embryonic neuromere. Such a median neuroblast is also shown to be the precursor cell of locust segmental efferent DUM neurons (Goodman and Spitzer, 1979).

So far, the morphology of *Manduca* adult desVUM neurons were illustrated with the help of immunohistochemical techniques (Dacks et al., 2005) that did not allow a detailed illustration of the dendritic processes within the SOG. Therefore, 20 intracellular recordings and fillings were performed in semi-intact adult preparations succeeding in one labeled desVUM neuron (Fig. 7) which revealed common morphological features with the larval desVUM neurons, such as the X-shaped structure of their dendritic trees and an anteriorly running primary neurite that bifurcates on the midline into two main ascending bilaterally symmetrical branches. While the larval desVUM neurons exhibit only sparse dendritic processes on the midline in the SOG, the adult desVUM neuron labeled in this thesis revealed densely packed processes in the posterior part of the SOG.

4.4 Electrophysiological properties

Similar to the efferent VUM neurons of the thoracic ganglia, the somata of desVUM neurons are excitable and thus able to generate spontaneous action potentials. Interestingly, these spikes are smaller in amplitude, shorter in duration, occur more frequently and exhibit a less pronounced hyperpolarization compared to the thoracic efferent VUM neurons (Fig. 5). In the locust, soma action potentials of efferent DUM neurons are carried out by Na^+ and Ca^{2+} (Grolleau and Lapied, 2000) which most likely also applies for the efferent VUM neurons of *Manduca*. In contrast, the somata of desVUM neurons appear to exhibit a different composition of ion channels.

Conspicuously, spikes recorded from the soma of the adult desVUM neuron labeled in this thesis posses a lower amplitude and brief duration compared to larval desVUM neurons. Nevertheless, these very preliminary results should be viewed with caution as influences of the recording quality on adult desVUM neuron spike shape cannot be fully excluded.

4.5 UM neurons of the SOG in other insect species

Suboesophageal UM neurons were described in various hemi- and holometabolous insects, such as cockroaches (Sinakevitch et al., 2005), crickets (Honegger et al., 1990), locusts (Bräunig, 1990, 1991; Bräunig et al., 1990, 2004; Bräunig and Burrows, 2004), *Drosophila* (Busch et al., 2009; Busch and Tanimoto, 2010; Selcho et al., 2014), honeybees (Kreissl et al., 1994; Sinakevitch et al., 2005; Schröter et al., 2007) and recently in stick insects (Thomas

Stolz, personal communication). Most of these studies focus on the ascending UM neurons that innervate principal brain neuropiles and/or project into peripheral nerves of the brain while little is known about descending UM neurons. In the cockroach *Periplaneta americana* 17 octopaminergic DUM somata were found in the SOG that are concentrated in three clusters, one in each neuromere (Sinakevitch et al., 2005). DUM neurons of the mandibular and maxillary exhibit ascending bilaterally symmetrical axons with processes within the trito-, deuto- and protocerebrum and NCCIII, while those of the labial neuromere descend towards the thoracic ganglia. The arrangement of ascending UM neurons within the mandibular and maxillary neuromeres and descending UM neurons within the labial neuromere seems to be a common feature in insects. So far, the exact number of cockroach descending DUM neurons of the SOG is not known, but most likely ranges between four to six DUM neurons in the labial neuromere.

Similar to the cockroach, the accurate number of octopaminergic VUM neurons within the SOG of honeybees is not yet known, but suggested to be fourteen in total, whereby three VUM neurons are still unreported (Schröter et al., 2007). Six VUM neurons ascend towards the brain and innervate brain neuropiles exclusively, including the antennal lobes, lip, basal ring and α-lobe of the mushroom body, lateral horn and parts of the deuto- and tritocerebrum. On the other hand, four ascending VUM neurons could be found that project into the peripheral nerves with two possessing axons in the antennal nerves. Surprisingly, two of these ascending VUM neurons appear to project into the mandibular nerves of the SOG. Certainly, it must be noted that these findings are based on one intracellular recording of an ascending VUM neuron within the maxillary neuromere that was stained together with at least the VUMmx1 neuron and on one backfill of the mandibular nerve that may have caused artifacts due to leakage. Thus, these observations are based on stainings that are rather unspecific. These findings are also in contrast with studies in locusts where electrophysiological approaches, intracellular stainings and backfills of nerves of the mouthparts also imply that UM neurons of the SOG do not innervate segments of the mouthparts (Bräunig, 1988; Bräunig and Burrows, 2004). Instead, an ascending prothoracic DUM neuron sends its axon into the labial and maxillary nerve (Bräunig, 1988). In the honeybee at least one descending VUM neuron exist that projects towards the prothoracic ganglion (Hammer, 1987).

In *Drosophila* larva six VUM neuron types exist in the SOG, three types in the maxillary and mandibular neuromere exhibit ascending axons, while the three types of the labial neuromere possess descending axons (Selcho et al., 2014). A similar number of VUM neuron types in the SOG were reported for adult *Drosophila*, eight types ascending towards

the brain (Busch and Tanimoto, 2010) and four types possessing descending axons (Busch et al., 2009). As methods used in these studies do not allow quantitative analyses in *Drosophila*, the exact number of VUM neurons in the SOG of larval and adult fruit flies remains unknown.

The distribution and projection patterns of UM neurons of the SOG are best described in locusts (Bräunig, 1990, 1991; Bräunig et al., 1990, 2004; Bräunig and Burrows, 2004). The locust SOG contains 19 octopaminergic DUM neurons, six of them with descending axons projecting towards the thoracic ganglia. Similar to *Manduca* desVUM neurons, this group is a morphological heterogeneous group as three types of descending DUM neurons can be distinguished by morphological criteria. Among the ascending DUM neurons in the maxillary and labial neuromere remain five centrally, thus are restricted to the SOG and brain, innervating principal brain neuropiles such as the antennal lobes, central complex, mushroom bodies and lobula of the optic lobe. Six further ascending DUM neurons send their axons into peripheral nerves of the brain with all of them innervating the NCCIII; two additionally into the frontal connectives, antennal nerves and tritocerebral ventral nerves. One additional ascending DUM neuron was reported with axons only in the frontal connectives. Interestingly, one DUM neuron exhibits both ascending and descending axons (Bräunig, 1991). Surprisingly, in *Manduca sexta* larva, ascending VUM neurons have not been reported, yet. An immunohistochemical study using an antibody against octopamine revealed three posterior somata, that could be identified as the three larval desVUM neurons, and two anterior somata (Cholewa and Pflüger, 2009). As the latter appear in a paired arrangement within the mandibular neuromere it is rather likely that these neurons belong to the group of octopaminergic paired neurons than unpaired median neurons. The SOG of adult *Manduca* contains two ascending VUM neurons projecting towards the brain (Dacks et al., 2005). In contrast to the locust, honeybee, *Drosophila* and cockroach, somata of ascending VUM neurons in adult *Manduca* are located in the same cluster as desVUM neurons, i.e. within the labial neuromere. As it is not clear if those ascending VUM neurons develop postembryonically or are already present in the larva, further immunohistochemical and electrophysiological experiments have to be performed.

The projection patterns of the six descending DUM neurons of the locust SOG with somata in the labial neuromere are described in great detail by Bräunig and Burrows (2004). Similar to the desVUM neurons in this thesis, they could label the descending DUM neurons intracellularly as far as the mesothoracic ganglia. Locust descending DUM neurons and larval *Manduca* desVUM neurons share many morphological and electrophysiological

characteristics. Neurons of both species possess spikes similar in shape that clearly differ from those of the efferent UM neuron in the thoracic ganglia. Additionally, descending UM neurons of both species reveal broad arborizations not only in the SOG but also in the pro- and mesothoracic ganglia.

UM neurons of the SOG of different insect species might be considered as homologous as they show a very similar morphology among species and are rather likely to be the progeny of a median neuroblast (Busch and Tanimoto, 2010), similar to what is true for insect efferent UM neurons of the thoracic and abdominal ganglia (for review see Bräunig and Pflüger, 2001).

4.6 Sensory input of larval desVUM neurons

Stimulation experiments of peripheral nerves of the SOG and thoracic ganglia revealed that the three larval desVUM neurons can be regarded as a heterogenous group in a functional sense. They receive different sensory input from receptors of the mouthparts, larval legs and hairlike sensilla on the dorsal surface of the prothoracic segment. Similar observations have been made for the thoracic efferent DUM neurons that form morphological groups on basis of the peripheral nerves through which they exit (Watson, 1984). Depending on their affiliation to either the “wing” or “leg group”, thus on their innervation of either wing or leg muscles, they respond differentially to various stimulations, such as mechanical stimulation of the legs, antenna, electrical stimulation of tegula or thoracic chordotonal organ afferents and to wind stimuli (Baudoux and Burrows, 1998; Duch and Pflüger, 1999; Morris et al., 1999; Field et al., 2008; Stocker, 2011).

Whereas this thesis provides a detailed description of sensory input of desVUM neurons in *Manduca* larva, especially of the middle desVUM neuron, little is known about the sensory input of descending UM neurons of the SOG in other insect species. For instance, the locust descending DUM neurons of the SOG receive excitatory input when stimulating certain leg nerves (Bräunig et al., 2004). Interestingly, the prominent proprioceptors of the leg, as the femoral chordotonal organ or the subgenual organ, do not evoke these excitatory responses in descending DUM neurons. Recently, it was shown that electrical stimulation of tarsal afferents lead to excitation of these neurons (Bräunig, personal communication). In contrast, in *Manduca* larva stimulation of the main leg nerve N2a innervating various proprioceptors of the thoracic legs, like campaniform sensilla, femoral and tibial chordotonal organs, hair

sensilla, multiterminal junctional and tension receptors (Consoulas, 2000), leads to synaptic responses with different polarities in the middle and posterior desVUM neuron (Fig. 14-15). While stimulation of afferents of prothoracic leg sense organs evoked excitatory responses but stimulation of those of the mesothoracic leg inhibitory responses in the middle desVUM neuron, the posterior desVUM neuron revealed an opposite response pattern to main leg nerve N2a stimulation. Conspicuously, latencies of excitatory responses in all nerve stimulation experiments are more often longer than inhibitory responses indicating that more interneurons are intercalated in the excitatory than in the inhibitory pathways. Similar observations have been made for responses of thoracic efferent DUM neurons to chordotonal organ afferents stimulation experiments in the locust (Morris et al., 1999). As stimulation of a main nerve containing several afferents of different proprioceptors results in a multisensory stimulation, it remains unclear if individual proprioceptor types of the larval leg might cause different responses, excitatory or inhibitory, in a single desVUM neuron. To investigate the exact source of desVUM neuron responses observed while stimulating the main leg nerve N2a further experiments have to be performed where further branches of this nerve are stimulated.

Stimulation of the second main leg nerve N1b innervating femoral, tibial and pleurocoxal extensor muscles (Kent and Levine, 1988b) and therefore most likely proprioceptors leads to inhibition in the middle desVUM neuron. In unidentified desVUM neurons either inhibitory or excitatory responses to N1b stimulation could be observed indicating that desVUM neurons are activated differentially when this nerve is stimulated (Fig. 17). Again, both inhibitory and excitatory pathways are polysynaptic whereat shorter latencies of inhibitory responses indicate that less synapses are involved in the inhibitory than in the excitatory pathway.

Stimulation of N1a of the prothoracic ganglion leads to inhibitory and excitatory synaptic responses in desVUM neurons (Fig. 18). As this nerve contains afferents of hairlike sensilla (Kent and Levine, 1988; Levine et al., 1985) it is likely that their stimulation evoke the excitation or inhibition in desVUM neurons.

Manduca larva mouthparts are covered with various receptors, such as olfactory sensilla on the maxillary palp and galea that help the animal to orient towards the food source and might facilitate feeding. Gustatory sensilla can be found on the epipharyngeal surface and on maxillary palps and galea that give the animal information about the presence and concentration of nutrients. Mechanosensilla are distributed over all mouthparts that help to coordinate their movements and inform the animal about texture and consistency of the food (Kent and Hildebrand, 1987). Stimulation of nerves of the mouthparts does not lead to

consistent responses in the middle desVUM neuron (Fig. 10-13). This neuron responds with inhibition to maxillary and second labial nerve stimulation, but with excitation to mandibular stimulation. In the posterior desVUM neuron excitatory responses to first labial and maxillary nerve stimulation could be observed. Latencies of these responses indicate a polysynaptic excitatory and inhibitory pathway from receptors of the mouthparts to desVUM neurons. Again stimulation of the maxillary, mandibular, first or second labial nerves results in a multisensory stimulation. Therefore, the exact sources of the observed responses remain unclear and have to be investigated in further studies. A preparation with an intact head capsule that was established within the scope of this thesis allows to investigate responses of desVUM neurons to different sensory modalities. While desVUM neurons are recorded mechano-, gustatory or olfactory sensilla on *Manduca* larva mouthparts can be activated by applying an odour, a food sample or by mechanical stimulation of the mouthparts.

In summary, desVUM neurons are activated differentially when stimulating nerves of the SOG and thoracic ganglia, i.e. they receive various sensory information from different parts of the body. Their differential activation to nerve stimulation suggest that different desVUM neurons have specific functions during behavior similar to the efferent DUM neurons of the locust (Stevenson and Meuser, 1997; Duch and Pflüger, 1999; Mentel et al., 2003). Latencies of all nerve stimulation experiments indicate polysynaptic pathways from afferents to desVUM neurons whereat more synapses seem to be involved in excitatory pathways. These synaptic pathways appear to be strong and robust as a single electrical stimulus is sufficient to evoke responses in a desVUM neuron.

4.7 Recruitment of desVUM neurons during fictive crawling

Results of this thesis reveal that desVUM neurons of the SOG are centrally coupled to the CPG producing fictive crawling in larvae. Pilocarpine induced fictive crawling leads to a general increase in desVUM neuron spike activity. As the spontaneous frequency of desVUM neurons is generally high only slight changes in their activity due to coupling to fictive crawling motor rhythm could be observed. Nevertheless, in three out of five preparations a peak in spike activity could be observed during transition from levator to depressor phase during crawling cycle (Fig. 20). It is possible that the manual application of pilocarpine to the meso-, metathoracic and abdominal ganglia decreases the quality of the intracellular recording and thereby influences the spike frequency. Furthermore, it cannot be excluded that the

increase in spike activity is induced by muscarinic receptors on desVUM neurons but seems unlikely as the SOG was not perfused with pilocarpine and as the anatomically descending VUM neurons are likely to be descending in a functional sense as well, i.e. their input regions appear to be located within the SOG while their output zones are located downstream within the thoracic and abdominal ganglia. This assumption has to be proven in future experiments and ultrastructural studies but seems likely as many other identified interneurons of insects have their main input areas near their somata in the same segment (O'Shea et al., 1974; Rehbein, 1976; Bacon and Tyrer, 1978). As results of fictive crawling experiments are preliminary it remains unknown whether the three larval desVUM neurons are activated differentially or collectively during fictive crawling motor pattern. The data of these experiments did not allow a coherent conclusion as the patterned activity of desVUM neurons during fictive crawling varied not only among the three different desVUM neuron types, but also differed within a type. For example in one experiment, the middle desVUM neuron revealed a peak in activity during transition phase of levator and depressor activity. In another recording it exhibits a decrease in spike activity in the second half of levator phase and during depressor phase of fictive crawling cycle. The observed coupling of individual desVUM neurons in an isolated nerve cord preparation might be different from what is true in an intact animal. Sensory input and feedback might lead to a clear phase relationship of desVUM neuron activity with motoneurons innervating levator and depressor. Therefore, further experiments have to be performed to test whether desVUM neurons of the SOG are activated differentially during crawling motor pattern similar to what is known for locust thoracic efferent DUM neurons during pilocarpine induced walking (Baudoux et al., 1998), kicking (Burrows and Pflüger, 1995) and flight (Duch and Pflüger, 1999), or collectively like efferent DUM neurons during stepping in stick insects (Mentel et al., 2008) and larval efferent VUM neurons of the thoracic and abdominal ganglia during fictive crawling in *Manduca* (Johnston et al., 1999). The latter study revealed that these neurons are synchronously active during fictive crawling regardless of the muscles they innervate. They are depolarized and produce action potentials during levator activity. A differential activation of larval *Manduca* desVUM neurons during motor programs is indicated by experiments by Cholewa and Pflüger (2009) in which only the anterior desVUM neuron revealed rhythmic activity during feeding motor pattern while the middle desVUM neuron did not show any coupling but continued to fire tonically.

4.8 Physiological relevance of desVUM neurons of the SOG

Not much is known about the physiological relevance of suboesophageal descending UM neurons in insects and mostly remains speculative. As efferent thoracic and abdominal ganglia rarely exhibit output synapses within the CNS (Watson, 1984; Pflüger and Watson, 1995) it appears unlikely that these neurons are responsible for octopaminergic central effects. Instead, the descending UM neurons of the SOG that are restricted to the CNS, as shown in this thesis or in a study of Bräunig and Burrows (2004), are good candidates for central actions of octopamine.

Results of this thesis suggest that *Manduca* larval desVUM neurons are involved in a locomotory pathway as they are rhythmically activated during fictive crawling in which larval legs are involved, thus are coupled to the CPG producing crawling pattern. Furthermore, they receive input of both polarities from the legs. A similar assumption was made for locust descending DUM neurons of the SOG, that might represent intergral but extended loops of locomotory circuits (Bräunig and Burrows, 2004; Bräunig et al., 2004). Thus, they are expected to be integral parts of feedback loops from the thoracic ganglia to the SOG and thought to be activated by motor commands or sensory feedback from motor patterns. They are regarded as a potential source for octopaminergic modulation of central circuits such as the modulation of processing of mechanosensory information from the legs. This modulation is expected to take place in the ventral sensory neuropiles of the locust thoracic ganglia which contain octopaminergic processes which do not belong to thoracic efferent DUM neurons (Stevenson et al., 1992). Interestingly, some of the locust descending DUM neurons have extensive axonal arborizations within these neuropiles, near ventral association centers, and therefore are strong candidates for this kind of modulation. Preliminary experiments indicate that locust descending DUM neurons modulate central synaptic connections (Bräunig et al., 2004). Antidromical activation of these neurons by stimulation of either one of both neck connectives leads to a reversible depression of the monosynaptic connection between the fast extensor tibiae (FETi) motoneuron and flexor motoneurons in the metathoracic ganglion, thus leads to a decrease in the amplitude of EPSPs in flexor motoneurons. Similar results can be obtained by bath application of octopamine (Parker, 1996). The fictive crawling data and results of leg afferents stimulation experiments indicate that *Manduca* desVUM neurons might be integral parts of feedback loops from the thoracic ganglia to the SOG similar to locust descending DUM neurons.

This thesis reveals that larval desVUM neurons exhibit broad arborizations within the pro- and mesothoracic ganglia and project at least to the second abdominal ganglion, one of them even as far as the terminal ganglion (Cholewa and Pflüger, 2009). Therefore, it can be assumed that these neurons also possess extensive branches within the metathoracic and abdominal ganglia. In adult *Manduca* and locusts the thoracic ganglia coordinate locomotory behaviors like walking and flight. These neurons are not restricted to these ganglia and therefore might have more general effects on neuronal networks, including locomotory networks (Bräunig et al., 2004). These general effects might include the maintenance of motor programs. If the desVUM neurons are part of a feedback loop of the CPG for fictive crawling, thus are activated during this motor pattern, it will lead to the release of octopamine into the CNS were it might modulate neuronal connections that are integrated in or associated with the CPG for fictive crawling and thus ensure its maintenance. Such a role of octopamine in the maintenance of motor programs could be shown for *Drosophila* where flies lacking octopamine exhibit shorter initial and total flight durations (Brembs et al., 2007). That centrally released octopamine act on various CPGs in different insect species is well established (Sombati and Hoyle, 1984a; Claassen and Kammer, 1986; Buhl et al., 2008; Rillich et al., 2013). To test a possible role of desVUM neurons in the maintenance of motor programs further experiments have to be performed. This could include studies where desVUM neurons are either stimulated or hyperpolarized during fictive crawling while possible changes in the rhythm are monitored. Furthermore, octopaminergic effects of desVUM neurons on CPGs for crawling can be abolished by cutting both connectives, or by epinastine application, an octopamine receptor antagonist.

Central release of octopamine by descending UM neurons could also be part of a general arousal mechanism that transforms neuronal circuits from a passive into a dynamic state (for review see Orchard et al., 1993). It could also facilitate the initiation of motor programs by acting on CPGs as shown for central octopamine in locust flight pattern (Stevenson and Kutsch, 1987; Buhl et al., 2008). Octopamine mediated facilitation of the initiation of motor programs and their maintenance can be induced by modulating membrane properties of central neurons including alteration of ionic conductances. The octopamine induced active membrane properties could lead to an increased excitability of neurons, elicitation of plateau potentials and endogenous bursting in central neurons (Flamm and Harris-Warrick, 1986; Ramirez and Pearson, 1991a, 1991b). Furthermore, they could lead to the modulation of synaptic transmission efficacy of neuronal connections (Kinnamon et al., 1984; Casagrand and Ritzrnann, 1989) and increase of responsiveness to sensory stimulation

(Kinnamon et al., 1984; Stevenson and Kutsch, 1987). These effects of octopamine would lead to an enhanced arousal thus excitability of an animal. The three different desVUM neurons of the SOG might have different effects indicated by their different sensory input they receive from different parts of the body.

5 ABSTRACT

Octopamine is a major biogenic amine in the insect nervous system where it functions as a neuromodulator acting on glands, sense organs, neuronal networks or muscles, as a neurohoromone mobilizing carbohydrates or lipids, or as a neurotransmitter. It is considered to play an important role in the initiation and maintenance of motor programs. It is released by two main populations of octopaminergic neurons: paired neurons that occur in the brain and some segmental ganglia and unpaired median (UM) neurons that can be found in all segmental ganglia except the brain. UM neurons exhibit dorsally or ventrally located somata and bilaterally symmetrical axons.

In contrast to the thoracic and abdominal ganglia, the suboesophageal ganglion (SOG) of insects lacks segmental UM neurons with peripheral axons. Instead, it possesses two main populations of intersegmental UM neurons: ascending UM neurons projecting towards the brain and descending UM neurons sending their axons towards the thoracic ganglia via both neck connectives.

The larval SOG of *Manduca sexta* contains three octopaminergic intersegmental descending ventral unpaired median (desVUM) neurons with somata in the labial neuromere. Electrophysiological and histological experiments of this thesis indicate that none of these neurons has peripheral axons. Therefore, they are considered to be interneurons and thus are strong candidates for the central release of octopamine. DesVUM neuron somata are able to generate spontaneous action potentials that are smaller in amplitude, shorter in duration and occur more frequently compared to the thoracic efferent VUM neurons. Experiments of this thesis reveal that desVUM neurons are not just a homogeneous cluster of neurons but rather a heterogeneous group in a functional and morphological sense as they are activated differentially when stimulating nerves innervating sense organs of the mouthparts and thoracic legs as well as hairlike sensilla on the dorsal surface of the prothoracic segment and are distinguishable by their characteristic morphological features. In addition to their morphology within the SOG, this thesis provides for the first time a detailed description of their projections within the prothoracic ganglion and for the posterior desVUM neuron even in the mesothoracic ganglion. In each of these thoracic ganglia, desVUM neurons possess extensive bilaterally symmetrical branches concentrated in the ventral and lateral regions of the ganglia that are distributed over the anterior-posterior axis. Histological experiments indicate that these neurons project as far as the second abdominal ganglia.

Pharmacological experiments reveal that desVUM neurons are coupled to central pattern generators producing fictive crawling in larva. Their central released octopamine might be involved in the facilitation of initiation of motor programs and its maintenance. Central release of octopamine by suboesophageal desVUM neurons could also be part of a general arousal mechanism transforming neuronal circuits from a passive into a more dynamic state.

6 ZUSAMMENFASSUNG

Octopamin ist ein wichtiges biogenes Amin im Nervensystem von Insekten, das in einer Vielzahl von physiologischen Prozessen involviert ist. So kann es als Neuromodulator Drüsen, Sinnesorgane, neuronale Netzwerke oder Muskeln modulieren, als Neurohormon Kohlenhydrate und Lipide mobilisieren oder auch als Neurotransmitter agieren. Dieses biogene Amin spielt eine wichtige Rolle in der Initiierung und Aufrechterhaltung von Motorprogrammen. Es wird von zwei Hauptgruppen von octopaminergen Neuronen freigesetzt: gepaarten Neuronen, die im Gehirn und einigen segmentalen Ganglien auftreten und ungepaart medianen (UM) Neuronen, die in allen segmentalen Ganglien, aber nicht im Gehirn, vorkommen. UM Neurone besitzen dorsal oder ventral liegende Somata und bilateral symmetrische Axone.

Im Gegensatz zu den thorakalen und abdominalen Ganglien befinden sich im Unterschlundganglion (USG) keine segmentalen UM Neurone mit peripheren Axonen. Vielmehr beinhaltet es zwei Hauptgruppen von intersegmentalen Neuronen: aufsteigende UM Neurone, die Richtung Gehirn projizieren und absteigende UM Neurone, die ihre Axone über die beiden Halskonnektive in die thorakalen Ganglien senden.

Das larvale USG des Tabakschwärmers *Manduca sexta* beinhaltet drei octopaminerge intersegmentale absteigende ventral ungepaart mediane (desVUM) Neurone mit Somata im labialen Neuromer. Elektrophysiologische und histologische Untersuchungen dieser Arbeit deuten an, dass diese Neurone keine Axone in peripheren Nerven besitzen. Demnach können desVUM Neurone als Interneurone angesehen werden und sind somit gute Kandidaten für die zentrale Freisetzung von Octopamin. Somata dieser Neurone sind in der Lage spontane Aktionspotentiale zu generieren, die im Vergleich zu denen der efferenten thorakalen VUM Neurone eine kleinere Amplitude und kürzere Dauer aufweisen sowie in einer höheren Frequenz auftreten. Untersuchungen dieser Arbeit zeigen, dass desVUM Neurone kein homogenes Cluster von Neuronen darstellen, sondern vielmehr eine heterogene Gruppe im funktionellen sowie morphologischen Sinne. Dies wird belegt durch ihre differentielle Aktivierung während der Stimulation von Nerven, die Sinnesorgane der Mundwerkzeuge und thorakalen Beine sowie Sensillen auf der dorsalen Oberfläche des prothorakalen Segments innervieren. Des Weiteren sind die drei desVUM Neurone anhand ihrer charakteristischen morphologischen Eigenschaften zu unterscheiden. Zusätzlich zu ihrer Morphologie innerhalb des USG zeigt diese Arbeit erstmals deren Projektionen im prothorakalen und für das

posteriore desVUM Neuron gar innerhalb des mesothorakalen Ganglions. In diesen Ganglien weisen desVUM Neurone extensive bilateral symmetrische Verzweigungen auf, die in den ventralen und lateralen Regionen des jeweiligen Ganglions konzentriert und über die anterior-posteriore Achse verteilt sind. Histologische Untersuchungen deuten an, dass diese Neurone zumindest bis ins zweite Abdominalganglion ziehen. Pharmakologische Untersuchungen zeigen, dass desVUM Neurone an zentrale Mustergeneratoren gekoppelt sind, die fiktives Kriechen in der Larve generieren. Ihr zentral freigesetztes Octopamin ist möglicherweise an der Erleichterung der Initiierung von Motorprogrammen und ihrer Aufrechterhaltung beteiligt. Die zentrale Freisetzung von Octopamin durch desVUM Neurone könnte auch Teil eines generellen Erregungsmechanismus sein, der neuronale Verbindungen von einem passiven in einen dynamischen Zustand transformiert.

REFERENCES

Bacon J, Tyrer M (1978) The tritocerebral commissure giant (tcg): a bimodal interneurone in the locust, *Schistocerca gregaria*. *J Comp Physiol A* 126:317–325.

Baines D, Desantis T, Downer RGH (1992) Octopamine and 5-hydroxytryptamine enhance the phagocytic and nodule formation activities of cockroach (*Periplaneta americana*) haemocytes. *J Insect Physiol* 38:905–914.

Baudoux S, Burrows M (1998) Synaptic activation of efferent neuromodulatory neurones in the locust *Schistocerca gregaria*. *J Exp Biol* 201:3339–3354.

Baudoux S, Duch C, Morris OT (1998) Coupling of efferent neuromodulatory neurons to rhythmical leg motor activity in the locust. *Jorunal Neurophysiol* 79:361–370.

Bell RA, Joachim FG (1976) Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann Entomol Soc Am* 69:365–373.

Bellah KL, Fitch GK, Kammer AE (1984) A central action of octopamine on ventilation frequency in *Corydalus cornutus*. *J Exp Biol* 292:289–292.

Blau C, Wegener G (1994) Metabolic integration in locust flight : the effect of octopamine on fructose 2,6-bisphosphate content of flight muscle in vivo. *J Comp Physiol B* 164:11–15.

Blenau W, Baumann a (2001) Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arch Insect Biochem Physiol* 48:13–38.

Booker R, Truman JW (1987) Postembryonic neurogenesis in the CNS of the tobacco hornworm, *Manduca sexta*. I. Neuroblast arrays and the fate of their progeny during metamorphosis. *J Comp Neurol* 255:548–559.

Borowsky B, Adham N, Jones K a, Raddatz R, Artymyshyn R, Ogozalek KL, Durkin MM, Lakhlani PP, Bonini JA, Pathirana S, Boyle N, Pu X, Kouranova E, Lichtblau H, Ochoa FY, Branchek TA, Gerald C (2001) Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proc Natl Acad Sci U S A* 98:8966–8971.

Braun G, Bicker G (1992) Habituation of an appetitive reflex in the honeybee. *J Neurophysiol* 67:588–598.

Bräunig P (1988) Identification of a single prothoracic “dorsal unpaired median” (DUM) neuron supplying locust mouthpart nerves. *J Comp Physiol A* 163:835–840.

Bräunig P (1990) The morphology of suboesophageal ganglion ceils innervating the nervus corporis cardiaci III of the locust. *Cell Tissue Res* 260:95–108.

Bräunig P (1991) Suboesophageal dum neurons innervate the principal neuropiles of the locust brain. *Philos Trans R Soc B Biol Sci* 332:221–240.

Bräunig P (1997) The peripheral branching pattern of identified dorsal unpaired median (DUM) neurones of the locust. *Cell Tissue Res* 290:641–654.

Bräunig P, Allgäuer C, Honegger HW (1990) Suboesophageal DUM neurones are part of the antennal motor system of locusts and crickets. *Experientia* 46:259–261.

Bräunig P, Burrows M (2004) Projection patterns of posterior dorsal unpaired median neurons of the locust subesophageal ganglion. *J Comp Neurol* 478:164–175.

Bräunig P, Burrows M, Morris OT (2004) Properties of descending dorsal unpaired median (DUM) neurons of the locust suboesophageal ganglion. *Acta Biol Hung* 55:13–19.

Bräunig P, Eder M (1998) Locust dorsal unpaired median (DUM) neurones directly innervate and modulate hindleg proprioceptors. *J Exp Biol* 201:3333–3338.

Bräunig P, Pflüger H-J (2001) The unpaired median neurons of insects. *Adv In Insect Phys* 28:185–266.

Bräunig P, Stevenson PA, Evans PD (1994) A locust octopamine-immunoreactive dorsal unpaired median neurone forming terminal networks on sympathetic nerves. *J Exp Biol* 192:225–238.

Brembs B, Christiansen F, Pflüger H-J, Duch C (2007) Flight initiation and maintenance deficits in flies with genetically altered biogenic amine levels. *J Neurosci* 27:11122–11131.

Buhl E, Schildberger K, Stevenson PA (2008) A muscarinic cholinergic mechanism underlies activation of the central pattern generator for locust flight. *J Exp Biol* 211:2346–2357.

Burke CJ, Huetteroth W, Owald D, Perisse E, Krashes MJ, Das G, Gohl D, Silies M, Certel S, Waddell S (2012) Layered reward signalling through octopamine and dopamine in *Drosophila*. *Nature* 492:433–437.

Burrows M, Pflüger HJ (1995) Action of locust neuromodulatory neurons is coupled to specific motor patterns. *J Neurophysiol* 74:347–357.

Busch S, Selcho M, Ito K, Tanimoto H (2009) A map of octopaminergic neurons in the *Drosophila* brain. *J Comp Neurol* 513:643–667.

Busch S, Tanimoto H (2010) Cellular configuration of single octopamine neurons in *Drosophila*. *J Comp Neurol* 518:2355–2364.

Büschges A, Schmitz J, Bässler U (1995) Rhythmic patterns in the thoracic nerve cord of the stick insect induced by pilocarpine. *J Exp Biol* 198:435–456.

Casagrand JL, Ritzrnann RE (1989) Biogenic amines modulate synaptic transmission between identified giant interneurons and thoracic interneurons in the escape system of the cockroach. *J Neurobiol* 23:644–655.

Cholewa J, Pflüger H-J (2009) Descending unpaired median neurons with bilaterally symmetrical axons in the suboesophageal ganglion of *Manduca sexta* larvae. *Zoology* 112:251–262.

Claassen DE, Kammer AE (1986) Effects of octopamine, dopamine, and serotonin on production of flight motor output by thoracic ganglia of *Manduca sexta*. *J Neurobiol* 17:1–14.

Consoulas C (2000) Remodeling of the leg sensory system during metamorphosis of the hawkmoth, *Manduca sexta*. *J Comp Neurol* 419:154–174.

Crocker A, Shahidullah M, Levitan IB, Sehgal A (2010) Identification of a neural circuit that underlies the effects of octopamine on sleep:wake behavior. *Neuron* 65:670–681.

Dacks AM, Christensen TA, Agricola H-J, Wollweber L, Hildebrand JG (2005) Octopamine-immunoreactive neurons in the brain and subesophageal ganglion of the hawkmoth *Manduca sexta*. *J Comp Neurol* 488:255–268.

Dailly E, Chenu F, Renard CE, Bourin M (2004) Dopamine, depression and antidepressants. *Fundam Clin Pharmacol* 18:601–607.

Davidson M, Keefe RS, Mohs RC, Siever LJ, Losonczy MF, Horvath TB, Davis KL (1987) {l}-Dopa challenge and relapse in schizophrenia. Am J Psychiatry 144:934–938.

Davis NT, Homberg U, Teal PEA, Altstein M, Agricola H-J, Hildebrand JG (1996) Neuroanatomy and lmmunocytochemistry of the median neuroendocrine cells of the subesophageal ganglion of the tobacco hawkmoth, *Manduca sexta*; immunoreactivities to PBAN and other neuropeptides. *Microsc Res Tech* 35:201–229.

Duch C, Mentel T, Pflüger HJ (1999) Distribution and activation of different types of octopaminergic DUM neurons in the locust. *J Comp Neurol* 403:119–134.

Duch C, Pflüger H-J (1999) DUM neurons in locust flight: a model system for amine-mediated peripheral adjustments to the requirements of a central motor program. *J Comp Physiol A* 184:489–499.

Dunphy GB, Downers RGH (1994) Octopamine , a modulator of the haemocytic nodulation response of non-immune *Galleria mellonella* larvae. *J Insect Physiol* 40:267–272.

Eaton JL (1988) Lepidopteran anatomy. Wiley, New York

Erspamer V, Boretti G (1951) Identification and characterization, by paper chromatography, of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of octopoda and in other tissue extracts of vertebrates and invertebrates. *Arch Int Pharmacodyn Therap* 88:296–332.

Evans P (1980) Biogenic amines in the insect nervous system. *Adv In Insect Phys* 15:317–473.

Evans PD, O'Shea M (1977) An octopaminergic neuron modulates neuromuscular transmission in the locust. *Nature* 270:257–259.

Evans PD, O'Shea M (1978) The identification of an octopaminergic neurone and the modulation of a myogenic rhythm in the locust. *J Exp Biol* 73:235–260.

Evans PD, Siegler MVS (1982) Octopamine mediated relaxation of maintained and catch tension in locust skeletal muscle. *J Physiol* 324:93–112.

Farooqui T (2012) Review of octopamine in insect nervous systems. *Open Acess Insect Physiol*:1–17.

Ferber M, Pflüger H-J (1990) Bilaterally projecting neurones in pregintal abdominal ganglia of the locust: anatomy and peripheral targets. *J Comp Neurol* 302:447–460.

Field LH, Duch C, Pflüger H-J (2008) Responses of efferent octopaminergic thoracic unpaired median neurons in the locust to visual and mechanosensory signals. *J Insect Physiol* 54:240–254.

Flamm RE, Harris-Warrick RM (1986) Aminergic modulation in lobster stomatogastric ganglion. ii. target neurons of dopamine, octopamine, and serotonin within the pyloric circuit. *J Neurocytol* 55:866–881.

Gal R, Libersat F (2010) A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in its cockroach prey. *PLoS One* 5:1–10.

Goodman CS, Spitzer NC (1979) Embryonic development of identified neurones: differentiation from neuroblast to neurone. *Nature* 280:208–214.

Griss C (1990) Mandibular motor neurons of the caterpillar of the hawk moth *Manduca sexta*. *J Comp Neurol* 296:393–402.

Grolleau F, Lapied B (2000) Dorsal unpaired median neurones in the insect central nervous system : towards a better understanding of the ionic mechanisms underlying spontaneous electrical activity. *J Exp Biol* 203:1633–1648.

Hammer M (1987) Elektrophysiologische und anatomische Charakterisierung von Motoneuronen und Interneuronen im Unterschlundganglion der Honigbiene, *Apis mellifera*. Diploma thesis, FU Berlin

Hammer M (1993) An identified neuron mediates the unconditioned stimulus in associative olfacoty learning in honeybees. *Nature* 366:59–63.

Hammer M, Menzel R (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn Mem* 5:146–156.

Hashemzadeh-Gargari H, Friesen WO (1989) Modulation of swimming activity in the medicinal leech by serotonin and octopamine. *Comp Biochem Physiol* 94C:295–302.

Heinzel HG (1988) Gastric mill activity in the lobster. II. Proctolin and octopamine initiate and modulate chewing. *J Neurophysiol* 59:551–565.

Honegger HW, Allgäuer C, Klepsch U, Welker J (1990) Morphology of antennal motoneurons in the brains of two crickets, *Gryllus bimaculatus* and *Gryllus campestris*. *J Comp Neurol* 291:256–268.

Hooper SL, Marder E (1984) Modulation of a central pattern generator by two neuropeptides, proctolin and FMRFamide. *Brain Res* 305:186–191.

Hooper SL, Marder E (1987) Modulation of the lobster pyloric rhythm by the peptide proctolin. *J Neurosci* 7:2097–2112.

Hoyer SC, Eckart A, Herrel A, Zars T, Fischer S a, Hardie SL, Heisenberg M (2008) Octopamine in male aggression of *Drosophila*. *Curr Biol* 18:159–167.

Hoyle G (1975) Evidence that insect dorsal unpaired medican (DUM) neurons are octopaminergic. *J Exp Biol* 9:425–431.

Johnston RM, Consoulas C, Pflüger H-J, Levine RB (1999) Patterned activation of unpaired median neurons during fictive crawling in *Manduca sexta* larvae. *J Exp Biol* 202:103–113.

Johnston RM, Levine RB (1996) Crawling motor patterns induced by pilocarpine in isolated larval nerve cords of *Manduca sexta*. *J Neurophysiol* 76:3178–3195.

Katz PS, Harris-Warrick RM (1990) Neuromodulation of the crab pyloric central pattern generator by serotonergic/cholinergic propioceptive afferents. *J Neurosci* 10:1495–1512.

Kent KS, Hildebrand JG (1987) Cephalic sensory pathways in the central nervous system of larval *Manduca sexta* (*Lepidoptera*: *Sphingidae*). *Philos Trans R Soc B Biol Sci* 315:1–36.

Kent KS, Levine RB (1988a) Neural control of leg movements in a metamorphic insect: persistence of larval leg motor neurons to innervate the adult legs of *Manduca sexta*. *J Comp Neurol* 276:30–43.

Kent KS, Levine RB (1988b) Neural control of leg movements in a metamorphic insect: sensory and motor elements of the larval thoracic legs in *Manduca sexta*. *J Comp Neurol* 271:559–576.

Kinnamon SC, Klaassen LW, Kammer AE, Claassen D (1984) Octopamine and chlordimeform enhance sensory responsiveness and production of the flight motor pattern in developing and adult moths. *J Neurobiol* 15:283–293.

Klaassen LW, Kammer AE (1985) Octopamine enhances neuromuscular transmission in developing and adult moths, *Manduca sexta*. *J Neurobiol* 16:227–243.

Kreissl S, Eichmüller S, Bicker G, Rapus J, Eckert M (1994) Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. *J Comp Neurol* 348:583–595.

Lange AB, Orchard I (1984) Dorsal unpaired median neurons, and ventral bilaterally paired neurons, project to a visceral muscle in an insect. *J Neurobiol* 15:441–453.

Lange AB, Tsang PKC (1993) Biochemical and physiological effects of octopamine and selected octopamine agonists on the oviducts of *Locusta migratoria*. *J Insect Physiol* 39:393–400.

Levine RB, Pak C, Linn D (1985) The structure , function and metamorphic reorganization of somatotopically projecting sensory neurons in *Manduca sexta* larvae. *J Comp Physiol A* 157:1–13.

Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce opposite postures in lobsters. *Science* 208:76–79.

Lopez HS, Brown a M (1992) Neuromodulation. *Curr Opin Neurobiol* 2:317–322.

Marder E, Bucher D (2001) Central pattern generators and the control of rhythmic movements. *Curr Biol* 11:R986–96.

Mentel T, Duch C, Stypa H, Wegener G, Müller U, Pflüger H-J (2003) Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J Neurosci* 23:1109–1113.

Mentel T, Weiler V, Büschges A, Pflüger H-J (2008) Activity of neuromodulatory neurones during stepping of a single insect leg. *J Insect Physiol* 54:51–61.

Mercer AR, Menzel R (1982) The effects of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honeybee *Apis melifera*. *J Comp Physiol A* 145:363–368.

Monastirioti M, Gorczyca M, Rapus J, Eckert M, White K, Budnik V (1995) Octopamine immunoreactivity in the fruit fly *Drosophila melanogaster*. *J Comp Neurol* 356:275–287.

Morris O, Duch C, Stevenson PA (1999) Differential activation of octopaminergic (DUM) neurones via proprioceptors responding to flight muscle contractions in the locust. *J Exp Biol* 202 Pt 24:3555–3564.

Novicki A, Weeks JC (1995) A single pair of interneurons controls motor neuron activity during pre-ecdysis compression behavior in larval *Manduca sexta*. *J Comp Physiol A* 176:45–54.

O'Shea M, Rowell CHF, Williams JLD (1974) The anatomy of a locust visual interneurone; the descending contralateral movement detector. *J Exp Biol* 60:1–12.

Oades RD (1987) Attention deficit disorder with hyperactivity (ADDH): the contribution of catecholaminergic activity. *Prog Neurobiol* 29:365–391.

Orchard I, Carlisle JA, Loughton BG, Gole JW, Downer RG (1982) In vitro studies on the effects of octopamine on locust fat body. *Gen Comp Endocrinol* 48:7–13.

Orchard I, Ramirez J-M, Lange AB (1993) A multifunctional role for octopamine in locust flight. *Annu Rev Entomol* 38:227–249.

Otto D, Henning RM (1993) Interneurons descending from the cricket subesophageal ganglion control stridulation and ventilation. *Naturwissenschaften* 80:10–12.

Pannabecker T, Orchard I (1986) Octopamine-2 receptors in locust neuroendocrine tissue. *J Insect Physiol* 32:909–915.

Parker D (1996) Octopaminergic modulation of locust motor neurones. *J Comp Physiol A* 178:243–252.

Pass G, Agricola H, Birkenbeil H, Penzlin H (1988) Morphology of neurones associated with the antennal heart of *Periplaneta americana* (*Blattodea, Insecta*). *Cell Tissue Res* 253:319–326.

Pasztor VM, Bush BM (1989) Primary afferent responses of a crustacean mechanoreceptor are modulated by proctolin, octopamine, and serotonin. *J Neurobio*l 20:234–254.

Pflüger H-J, Stevenson PA. (2005) Evolutionary aspects of octopaminergic systems with emphasis on arthropods. *Arthropod Struct Dev* 34:379–396.

Pflüger H-J, Watson AHD (1995) GABA and glutamate-like immunoreactivity at synapses received by dorsal unpaired median neurons in the abdominal nerve cord of the locust. *Cell Tissue Res* 280:325–333.

Pflüger H-J, Witten JL, Levine RB (1993) Fate of abdominal ventral unpaired median cells during metamorphosis of the hawkmoth, *Manduca sexta*. *J Comp Neurol* 335:508–522.

Plotnikova SI (1969) Effector neurons with several axons in the ventral nerve cord of the Asian grasshopper *Locusta migratoria*. *J Evol Biochem Physiol* 5:276–277.

Prier KR, Beckman OH, Tublitz NJ (1994) Modulating a modulator: biogenic amines at subthreshold levels potentiate peptide-mediated cardioexcitation of the heart of the tobacco hawkmoth *Manduca sexta*. *J Exp Biol* 197:377–391.

Ramirez J, Büchges A, Kittmann R (1993) Octopaminergic modulation of the femoral chordotonal organ in the stick insect. *J Comp Physiol A* 173:209–219.

Ramirez J-M, Orchard I (1990) Octopaminergic modulation of the forewing stretch receptor in the locust *Locusta migratoria*. *J Exp Biol* 149:255–279.

Ramirez JM, Pearson KG (1991a) Octopamine induces bursting and plateau potentials in insect neurones. *Brain Res* 549:332–337.

Ramirez JM, Pearson KG (1991b) Octopaminergic modulation of interneurons in the flight system of the locust. *J Neurophysiol* 66:1522–1537.

Rehbein H (1976) Auditory neurons in the ventral cord of the locust: morphological and functional properties. *J Comp Physiol A* 110:233–250.

Ridgel AL, Ritzmann RE (2005) Effects of neck and circumoesophageal connective lesions on posture and locomotion in the cockroach. *J Comp Physiol A* 191:559–573.

Rillich J, Stevenson PA, Pflüger H-J (2013) Flight and walking in locusts-cholinergic co-activation, temporal coupling and its modulation by biogenic amines. *PLoS One* 8:1–11.

Robinson GE, Heuser LM, LeConte Y, Lenquette F, Hollingworth RM (1999) Neurochemicals aid bee nestmate recognition. *Nature* 399:534–535.

Roeder KD (1937) The control of tonus and locomotor activity in the praying mantis (*Mantis Religiosa L.*). *J Exp Zool* 76:353–374.

Roeder T (1999) Octopamine in invertebrates. *Prog Neurobiol* 59:533–561.

Roeder T (2005) Tyramine and octopamine: ruling behavior and metabolism. *Annu Rev Entomol* 50:447–477.

Rubin AL, Price LH, CHarney DS, Heninger GR (1985) Noradrenergic function and the cortisol response to dexamethasone in depression. *Psychiatry Res* 15:5–15.

Ryckebusch S, Laurent G (1993) Rhythmic patterns evoked in locust leg motor neurons by the muscarinic agonist pilocarpine. *J Neurophysio*l 69:1583–1595.

Sachs L, Hedderich J (2006) Angewandte Statistik: Methodensammlung mit R. Springer Verlag Berlin.

Schroll C, Riemensperger T, Bucher D, Ehmer J, Völler T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, Fiala A (2006) Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr Biol* 16:1741–1747.

Schröter U, Malun D, Menzel R (2007) Innervation pattern of suboesophageal ventral unpaired median neurones in the honeybee brain. *Cell Tissue Res* 327:647–667.

Schulz DJ, Sullivan JP, Robinson GE (2002) Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. *Horm Behav* 42:222–231.

Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M (2003) Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J Neurosci* 23:10495–10502.

Selcho M, Pauls D, Huser A, Stocker RF (2014) Characterization of the octopaminergic and tyraminergic neurons in the central brain of *Drosophila* larvae. *J Comp Neurol*.

Sinakevitch I, Niwa M, Strausfeld NJ (2005) Octopamine-like immunoreactivity in the honey bee and cockroach: comparable organization in the brain and subesophageal ganglion. *J Comp Neurol* 488:233–254.

Sombati S, Hoyle G (1984a) Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. *J Neurobiol* 15:481–506.

Sombati S, Hoyle G (1984b) Central nervous sensitization and dishabituation of reflex action in an insect by the neuromodulator octopamine. *J Neurobiol* 15:455–480.

Spörhase-Eichmann U, Vullings HGB, Buijs RM, Horner M, Schürmann F (1992) Octopamine-immunoreactive neurons in the central nervous system of the cricket, *Gryllus bimaculatus*. *Cell Tissue Res* 268:287–304.

Stern M (2009) The PM1 neurons, movement sensitive centrifugal visual brain neurons in the locust: anatomy, physiology, and modulation by identified octopaminergic neurons. *J Comp Physiol* 195:123–137.

Stevenson PA, Dyakonova V, Rillich J, Schildberger K (2005) Octopamine and experience-dependent modulation of aggression in crickets. *J Neurosci* 25:1431–1441.

Stevenson PA, Pflüger H-J, Eckert M, Rapus J (1992) Octopamine immunoreactive cell populations in the locust thoracic-abdominal nervous system. *J Comp Neurol* 315:382–397.

Stevenson PA, Rillich J (2012) The decision to fight or flee - insights into underlying mechanism in crickets. *Front Neurosci* 6:1–12.

Stevenson PA, Meuser S (1997) Octopaminergic innervation and modulation of a locust flight steering muscle. *J Exp Biol* 200:633–642.

Stevenson PA, Kutsch W (1987) A reconsideration of the central pattern generator concept for locust flight. *J Comp Physiol A* 161:115–129.

Stocker B (2011) Locust thoracic dorsal unpaired median (DUM) neurons: Differential activation and peripheral distribution of octopamine and tyramine. Phd Thesis, Freie Universität Berlin -

Thorn RS, Truman JW (1994) Sexual differentiation in the CNS of the moth, *Manduca sexta*. I. Sex and segment-specifcity in production, differentiation, and survival of the imaginal midline neurons. *J Neurobiol* 25 (9):1039–1053.

Truman JW, Bate M (1988) Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Dev Biol* 125:145–157.

Verlinden H, Vleugels R, Marchal E, Badisco L, Pflüger H-J, Blenau W, Broeck J Vanden (2010) The role of octopamine in locusts and other arthropods. *J Insect Physiol* 56:854–867.

Vierk R (2009) Postembryonic maturation and putative modulation of the central pattern generator for flight in *Manduca sexta*. Phd Thesis, Freie Univ Berlin.

Watson AHD (1984) The dorsal unpaired median neurons of the locust metathoracic ganglion: neuronal structure and diversity, and synapse distribution. *J Neurocytol* 13:303–327.

Weeks JC, Jacobs GA (1987) A reflex behavior mediated by monosynaptic connections between hair afferents and motoneurons in the larval tobacco hornworm, *Manduca sexta*. *J Comp Physiol A* 160:315–329.

Whim MD, Evans PD (1988) Octopaminergic modulation of flight muscle in the locust. *J Exp Biol* 134:247–266.

Witten JL, Truman JW (1991) The regulation of transmitter expression in postembryonic lineages in the moth *Manduca sexta*. I. Transmitter identification and developmental acquisition of expression. *J Neurosci* 11:1980–11989.

Zhou C, Rao Y, Rao Y (2008) A subset of octopaminergic neurons are important for *Drosophila* aggression. *Nat Neurosci* 11:1059–1067.

DANKSAGUNG

Zunächst möchte ich Prof. Dr. Hans-Joachim Pflüger danken, der es mir ermöglichte meine Doktorarbeit über ein spannendes Thema in seiner Arbeitsgruppe anzufertigen.
Prof. Dr. Jens Rolff möchte ich für seine Bereitschaft zur Begutachtung meiner Arbeit danken.
Mein besonderer Dank gilt Dr. Jan Rillich, der sich nicht nur meiner musikalischen Bildung annahm, sondern auch stets ein offenes Ohr während meiner experimentellen und vor allem auch während des Schreibprozesses für mich hatte.
Bettina Stocker danke ich für ihre Geduld und Ruhe, die sie mir bei der Einführung in die Techniken der Elektrophysiologie entgegenbrachte.
Heike Wolfenberg, die maßgeblich zur angenehmen und freundlichen Atmosphäre in der Arbeitsgruppe beitrug, sei gedankt für ihre kompetente Unterstützung im Labor. Vielen Dank auch an Doreen Johannes für die Betreuung der Manducazucht.
Sergej Hartfil, Konstantin Lehmann und Leonard Nadler danke ich für ihre stete Hilfsbereitschaft, die Weitergabe von Erfahrungen, das Teilen von Erfolgen, aber auch Misserfolgen, und die freundschaftliche Arbeitsatmosphäre in unserer Gruppe. Konstantin Lehmann danke ich auch für das Erstellen des MATLAB Skripts. Auch Dr. Marco Schubert sei gedankt für die ein oder andere spannende Kickerpartie.
Ganz besonders danke ich auch Christine Damrau für viele aufmunternde Gespräche, Ablenkungen vom Arbeitsalltag, fachliche Diskussionen und für das unermüdliche Korrekturlesen.
Für das Teilen von Erfahrungen danke ich auch den Doktoranden der AG Menzel.
Danke auch an meine Freunde und meiner Familie für ihre stete Unterstützung und das nötige Durchhaltevermögen, dass sie mir gaben.