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Neuronal background of positioning of the posterior tentacles in the snail *Helix pomatia*

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Running head:
Positioning of the olfactory organs in the snail

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Abstract

The location of cerebral neurons innervating the three recently described flexor muscles involved in the orientation of the posterior tentacles as well as their innervation patterns were investigated, applying parallel retrograde Co- and Ni-lysine as well as anterograde neurobiotin tracings via the olfactory and the peritentacular nerves. The neurons are clustered in eight groups in the cerebral ganglion and they send a common innervation pathway via the olfactory nerve to the flexor and the tegumental muscles as well as the tentacular retractor muscle and distinct pathways via the internal and the external peritentacular nerves to these muscles except the retractor muscle. The three anchoring points of the three flexor muscles at the base of the tentacle outline the directions of three force vectors generated by the contraction of the muscles along which they can pull or move the protracted tentacle which enable the protracted tentacle to bend around a basal pivot. In the light of earlier physiological and the present anatomical findings we suggest that the common innervation pathway to the muscles is required to the tentacle withdrawal mechanism whereas the distinct pathways serve first of all the bending of the protracted posterior tentacles during foraging.

Keywords cerebral motoneurons, food conditioning, innervation patterns, olfactory orientation, ommatophore

Introductions

Olfaction in terrestrial snails is an essential sensory modality and plays a crucial role in orientation and foraging (for rev. Chase 2002). In Stylommatophora snails including *Helix pomatia*, although the entire head region is abundantly supplied with chemoreceptors and mechanoreceptors the posterior tentacles are the most important for olfaction involved principally in the orientation towards a distant food source (Croll and Chase 1980, Chase and Croll 1981, Croll 1983, Friedrich and Teyke 1998, Teyke 1995, for rev. Chase 2002). The posterior tentacles held upright display external lateral movements during exploring the environment and this active scanning behavior is likely to be involved in obtaining information on the occurrence and spatial distribution of actual odors. The tentacle movements serve to bring the olfactory receptors in an appropriate position for the perception of odor stimuli that help adjust the direction of locomotion in maintaining the perception of increasing odor concentration which then help the animal find the source of the odor during foraging (Peschel et al. 1996, Friedrich and Teyke 1998, Nikitin et al. 2008, for rev. Chase 2002).

Studying the role of odor memory in feeding behavior Peschel et al. (1996) observed that during normal locomotion both naïve and food conditioned snails kept the posterior tentacles upright, while after the introduction of the conditioned food (odor) the conditioned snails but not the naïve ones turned into the direction of the food and lowered the ipsilateral tentacle pointing at the direction of food. At the same time the contralateral tentacle remained in upright position. During the final approach to the food both tentacles were oriented horizontally pointing directly at the food. This type of tentacle movements is characterized by

bending the tentacle around a basal pivot in different directions without curving the stem. Consequently it can easily be distinguished from other tentacle movements, such as tentacle withdrawal (Prescott et al. 1997, for rev. Chase 2002) and local movements of the tentacle tip (Lemair and Chase 1998, Nikitin et al. 2005, 2008). Therefore the bearing of the posterior tentacles was considered as indicative of the feeding history of the snail at the level of behavior (Peschel et al. 1996).

The posterior tentacles are innervated by the olfactory nerve as well as by the external peritentacular (ePT) and the internal peritentacular (iPT) nerves (Kerkut and Walker 1975). In isolated tentacle preparation the electrical stimulation of the PT nerves, which innervate the tegumental muscle in the stem of the tentacle (Peschel et al. 1996), evoked the lateral bending of the protruded tentacle. The electrical stimulation of the ePT nerve evoked external lateral whereas the stimulation of the iPT nerve evoked the medial downward bending of the protruded tentacle. It resembled the tentacle movements carried out by conditioned snails during food approach. Contrary, the electrical stimulation of the olfactory nerve evoked no bending but only local movements of the tip of tentacle (Peschel et al. 1996). In semi-intact preparation obtained from odor conditioned animal the stimulation of the olfactory epithelium evoked activity discharge in the PT nerves only when applying the conditioned odor. On the contrary the conditioned odor failed to evoke nerve activity in the PT nerves in preparation obtained from naïve animal (Peschel et al. 1996). Therefore Peschel et al. (1996) suggested that the increased activity in the iPT nerve is responsible for the medial whereas in the ePT nerve for the lateral downward bending of the tentacle and the evoked electrical activity in PT nerves in the presence of an odor are indicative of the feeding history of the snail. Labeled axons have been shown in distinct areas of the tegumental muscle following anterograde Co-

lysine tracing via the different PT nerves, therefore Peschel et al. (1996) suggested that the musculature responsible for these movements is located inside the posterior tentacles (tegumental muscle) and the contractions of the different parts of the tegumental muscle evoked via the different PT nerves are sufficient to generate the full complement of tentacle movements, that is bending of the protracted tentacles around a basal pivot, associated with olfactory orientation in conditioned snail.

Snails which are supplied with a hydro skeleton have no extensor muscles, instead the haemolymph pressure plays an extensor function (for rev Chase 2002). Therefore when the posterior tentacles are fully protracted and stretched by the hydrostatic pressure of the haemolymph during olfactory orientation, the contraction of the different parts of the tegumental musculature alone cannot be able to execute the bending of the protracted tentacles around a basal pivot. We supposed that a still unknown separated flexor muscle or muscles exist which counterwork the hydrostatic pressure and execute the bending of the protracted tentacle around a basal pivot. Recently we have demonstrated the presence of three novel thin string-like muscles in each posterior tentacle spanning the entire length of the tentacle from the tip to the base of the tentacle (Hernádi and Teyke 2012). Their gross anatomical, ultrastructural and physiological properties described (Hernádi and Teyke 2012, Krajcs et al. 2012) fulfill the criteria of a flexor muscle.

The aim of the present study was to identify the cerebral neurons and their innervation patterns in these novel flexor muscles. Applying anterograde neurobiotin tracing via the olfactory and PT nerves we determined the nerves belonging to the different flexor muscles and visualized their projections in these muscles. Applying paired retrograde cobalt and nickel lysine tracing via the olfactory and the different peritentacular nerves the location of cerebral

neurons innervating the flexor muscles was demonstrated in order to outline a neuroanatomical background of a regulatory system which might be responsible for the generation of motor output to the space positioning of the olfactory organ during foraging.

Materials and Methods

Adult specimens of the snail *Helix pomatia* were collected locally, kept in an outdoor cage and fed on cucumber before using them for the experiments.

Anterograde neurobiotin tracing

Posterior tentacles with the flexor muscles and the CNS interconnected via the olfactory and the different peritentacular (PT) nerves were dissected and pinned out in Sylgard coated plates containing *Helix* saline (Vehovszky et al. 1992). One of the three nerves was cut and placed in a Vaseline cup containing 5% neurobiotin (Vector) diluted in distilled water. The cup was sealed with Vaseline thus anterograde tracing via the distal segment of the selected nerve was excluded. The preparation was covered with saline and kept at room temperature for one day. The preparations were then fixed in 4% paraformaldehyde (Reanal, Budapest, Hungary) buffered with 0.1 M phosphate buffer (pH 7.4) for 6 h at 4°C. The neurobiotin labeled axons were visualized in whole-mount preparations by applying avidine conjugated Alexa-fluor 488 (Molecular Probes, London, England) diluted 1:1000 in PBS-TX for 1 hour at room temperature. After washing in PBS-TX the samples were mounted in PBS-glycerol (2:1) and viewed under a fluorescence microscope equipped the appropriate filters.

Paired retrograde cobalt and nickel-lysine tracing

The CNS with all the cerebral nerves was dissected and pinned out in Sylgard coated plates containing *Helix* saline (Vehovszky et al. 1992). The olfactory nerve and one of the peritentacular nerves or the external and internal peritentacular nerves were cut and placed in a vaseline cups containing cobalt-lysine vs. nickel lysine complex. Then the cups were closed with Vaseline, covered with physiological saline and kept at room temperature for one day. Thereafter the cobalt and nickel ions in the cerebral neurons were visualized by applying rubeanic acid (Hernádi 1991). The whole-mount preparations were embedded into Cytomation matrix (Dako, Denmark). The cobalt containing (orange color) and nickel containing (blue color) neurons were studied in a light microscope equipped with a camera lucida apparatus.

Results

Anterograde neurobiotin tracing via the olfactory and the peritentacular nerves

Neurobiotin tracing via the olfactory nerve revealed that labeled fibers run through the tentacular ganglion and leave it via the digits of the ganglion at the sites where the flexor muscles are attached to the ventral sensory pad and innervate each flexor muscle (M1, M2, M3) from the tip to the base where the flexor muscles are anchored to the body wall (Fig.1). Labeled trunks of fibers run inside the muscles and give off collaterals which innervate the muscle fibers (Fig.2). Additionally parallel labeled fibers run through the digits of the tentacular ganglion and innervate the tegumental musculature in the wall of the stem from the tip to the base (Fig.1). The labeled axons give off varicose side branches which innervate the

tegumental muscle fibers (Fig.2 D). Labeled fibers also supply the tentacular retractor muscle (Fig.1).

Neurobiotin tracing via the internal peritentacular (iPT) nerve, which reaches the base of the stem ventromedially (Fig.1), revealed labeled axons in the M1 flexor muscle (Fig.3) which is anchored to the base of the stem also ventromedially (Fig.1). Labeled fibers run inside the M1 flexor muscle and give off varicose side branches which innervate the muscle fibers (Fig.3 A). Additionally labeled fiber trunks can be seen in the ventromedial part of the stem (Fig.3 B) which fibers innervate a stripe of the tegumental muscle from the base to the tip of the stem (Fig.1).

Neurobiotin tracing via the external peritentacular (ePT) nerve, (bifurcates before reaching the base of the stem and joins to it ventrolaterally and dorsolaterally as well (Fig.1) revealed labeled fibers in both the M2 (Fig.3 C) and the M3 (Fig.3 D) flexor muscles. The labeled fibers give off side branches which innervate muscle fibers. Additionally two large trunks of labeled axons can be observed ventrolaterally and dorsomedially at the base of the stem (Fig.1). They send parallel fibers towards the tip of the tentacle and innervate the ventrolateral and the dorsal parts of the tegumental musculature (Fig.3 E).

Location of cerebral neurons projecting to the flexor muscles via the olfactory and the peritentacular nerves

Parallel Co- and Ni-lysine tracing via the olfactory and the external peritentacular (ePT) nerves revealed that the neurons labeled via the two nerves are clustered in seven distinct groups (g1-g7). One of them is found in the procerebrum, one in the mesocerebrum and five in the metacerebrum, all located mostly on the ventral surface of the cerebral ganglion. (Fig.4

E). In the majority of the labeled groups both the olfactory and the ePT nerves are represented. However, in the g2 group only the olfactory nerve whereas in the g5 group only the ePT nerve is represented (Fig.4 E). Additionally on the dorsal surface of the metacerebrum a solitary double labeled neuron corresponding to the MtC3 neuron (Cottrell et al. 1983) could also be observed (Fig. 4 D, E). In the largest metacerebral group (g4) the neurons labeled via the ePT nerve are located under the neurons labeled via the olfactory nerve and they are partly intermingled. Among them a large (50-60 µm) neuron labeled via the ePT nerve can be seen located in the external part of the labeled group (Fig.4 A, E). A small group of neurons (g5) labeled via the external peritentacular nerve is located externally from the main labeled group (g4). Among them a large (50-60 µm) neuron can be distinguished (Fig. 4A, E). In the pleural lobe of the metacerebrum the neurons labeled via the ePT and the olfactory nerve are intermingled and can be separated in two loose groups (g6-g7) consisting of medium diameter (30-50 µm) neurons (Fig.4 E). In the mesocerebrum a few of neurons (g3) labeled via the ePT nerve (60-70 µm) are scattered in the medial part of the mesocerebrum (Fig.4 E).

The parallel tracing via the olfactory and the internal peritentacular nerves revealed eight groups of labeled neuron (Fig.4 E). In the majority of them both the olfactory and the iPT nerve are represented (g1, g5-g7). In the g3, g5 groups only the ePT and iPT nerves whereas in the g8 group only the iPT nerve is represented (Fig.4 E). In the largest metacerebral group of neurons (g4) a large (40-60 µm) neuron labeled via the iPT nerve can be seen among them (Fig.4 B,E). A large (40-60 µm) neuron and a few of small diameter neuron labeled via the iPT nerve form a small group (g5) located externally from the g4 labeled group (Fig.4 B,E).

In the pleural lobe the neurons labeled via the olfactory nerve are separated in two loose groups (g6-g7) and only a few of neurons labeled via the iPT nerve can be observed among them (Fig.4 E). In the mesocerebrum neurons are labeled only via the iPT nerve (g3), located dominantly on the dorsomedial part of the mesocerebrum (Fig.4 E). The identified large MtC3 neuron labeled via the olfactory and the ePT nerves is also labeled via the iPT nerve (Fig.4 E). Additionally, close to the MtC3 neuron a medium (30-40 µm) diameter neuron was labeled via the iPT nerve (Fig.4 E).

The parallel tracing via the external and the internal peritentacular nerves revealed that neurons labeled via the iPT nerve are located close to that labeled via the ePT nerve, however, double labeled neurons can rarely be seen. The large neuron in the g4 group was labeled dominantly via the iPT nerve whereas in the g5 group was labeled dominantly via the ePT nerve (Fig.4 C). They were only rarely double labeled. Caudally from the g4 group a small group of neuron (g8) consisting of small diameter (15-20 µm) neurons is labeled exclusively via the iPT nerve (Fig.4 C, E). In the pleural lobe only e few neurons are labeled via the iPT nerve and they are located in the groups of neurons (g6-g7) labeled via the ePT nerve (Fig.4 C, E). In the mesocerebrum the majority of the labeled neurons (g3) are labeled via the iPT nerve. Among them a few of double labeled neurons can also be detected (Fig.4 D, E).

The results of the retrograde Co and Ni-lyine tracing via the olfactory nerve and the different PT nerves outlined eight groups of labeled neurons (g1-g8) in the cerebral ganglion. Each nerve is represented in the g1, g4, g6, g7 groups. In the g2 group only the olfactory nerve, in

the g8 group only the iPT nerve whereas in the g3 and g4 group both the ePT and the iPT nerves are represented (Fig.4 E).

Discussion

The flexor muscles and the tegumental musculature receive both common and distinct innervation pathways

The anterograde neurobiotin tracing revealed that the flexor muscles receive labeled fibers from cerebral neurons via the olfactory as well as the external peritentacular (ePT) and internal peritentacular (iPT) nerves. The fibers labeled via the olfactory nerve innervate each flexor muscle (M1, M2, M3) and additionally each separable musculature of the tentacle as the tegumental musculature of the stem and the tentacular retractor muscle. The labeled fiber trunks in the olfactory nerve reach these muscles via the digits of the tentacular ganglion and innervate them from the tip to the base of the tentacle. Via the PT nerves distinct separated labeled pathways reach these muscles (except the tentacular retractor muscle) and innervate them from the base of the stem to the tip of the tentacle. The iPT nerve innervates the M1 flexor muscle as well as a ventromedial stripe of the tegumental musculature located close to the anchoring points of the M1 muscle. The ventro-lateral branch of the ePT nerve innervates the M2 muscle as well as the external ventro-lateral area of the tegumental musculature, whereas the dorso-lateral branch of the ePT nerve innervates the M3 flexor muscle as well as the dorsal area of the tegumental musculature. Contrary to the observations of Peschel et al (1996) we found that the tegumental musculature is innervated not only by the PT nerves but also through

the olfactory nerve. Our observations show that the flexor muscles and the stem of the tegumental musculature receive both common and distinct innervation pathways. The common pathway via the olfactory nerve innervates them from the tip to the base whereas the distinct pathways via the PT nerves innervate them from the base to the tip of the tentacle.

The cerebral neurons innervating the flexor muscles are clustered into eight groups

According to the paired cobalt and nickel lysine tracing via the olfactory and the external peritentacular (ePT) and the internal peritentacular (iPT) nerves the neurons sending axons to the tentacle and the flexor muscles are clustered into eight groups (g1-g8). In the majority of labeled groups (g1, g4, g6, g7) all the three nerves are represented. In the g3 and g5 groups only the PT nerves whereas in the g2 group only the olfactory nerve while in the g8 group only the ePT nerve is represented. Topographic arrangement of the labeled neurons could not be observed in the groups, except the g1 and g5 groups in which a limited topography was found.

Earlier findings have shown that the efferent neurons located in the cerebral ganglia which project to the different head areas of *Helix pomatia* via the olfactory and the different lip nerves were grouped in several loci and the neurons labeled via the different nerves showed a limited topographic arrangement in the loci (Hernádi 1992). The main neurochemical characters of the loci that is in their transmitter and modulator contents showed differences (Hernádi and Elekes 1993, 1999, Hernádi 2000). The location of the presently described groups (g1-g8) labeled via the olfactory and the different PT nerves fall into the areas of the loci sending axons to the different head areas. Therefore we

suggest that the different groups of labeled neurons which innervate the flexor and tegumental muscles send neurochemically different pathways to these muscles.
Only a solitary labeled neuron the MtC3 cell was identified functionally, taking part both in the tentacle withdrawal reflex and the graded tentacle retraction (Cottrell et al. 1983, Chase and Hall 1996, Prescott et al. 1997, Nikitin et al. 2005). Therefore it is difficult to render exact function (sensory, motor or modulator) to any group of the labeled neurons. In the g3 and g5 groups only the PT nerves are represented, therefore it cannot be excluded that the neurons in these two groups generate motor outputs to the flexor muscles.

The possible mechanism by which the labeled cerebral neurons regulate the movement of the protracted tentacle (olfactory organ)

To bring the olfactory receptors in an appropriate position for the perception of an odor stimulus requires complex movements by the protracted tentacle (for rev. Chase 2002). Our observations outline the anatomical and neuroanatomical basis (see Fig. 5) enabling the tentacles to execute the precise movements observed during foraging in food conditioned animals (Peschel et al. 1996).
It was suggested that the motor activity in PT nerves and the evoked muscular contraction in the stem of tentacle (tegumental musculature) are sufficient to generate the full complement of tentacle movements associated with olfactory orientation *in vivo* (Peschel et al. 1996). However our recent anatomical findings demonstrating the existence of the three flexor muscles (Hernádi and Teyke, 2012) suggest that the bending of the protracted posterior tentacle in different directions around a basal pivot needs first of all the contraction of the

flexor muscles which opposes the hydrostatic pressure in the protracted posterior tentacles and thus the tentacle can be pulled in different directions. Each flexor muscle and the tegumental musculature receive both distinct (via the iPT and the ePT nerves) and common innervation (via the olfactory nerve) (see Fig. 5). Therefore when the conditioned odor induces distinct motor output via a PT nerve (Peschel et al. 1996) and evokes the contraction of a flexor muscle it also induces contraction in a given stripe of the tegumental musculature which is innervated by the corresponding PT nerve. The sites where the muscles are anchored to the base of the stem outline three space axes and force vectors along which the contraction of a flexor muscle can pull the tentacle (Fig. 5). Therefore, the position of the olfactory organ located on the tip of the protruded tentacle is determined as a result of the three force vectors generated by the contraction of the three flexor muscles. Parallel, the evoked contractions of the different stripes of the tegumental muscle cooperate with the contractions of the flexor muscles helping the bending of the protruded tentacle. The food conditioned snail displays four basic tentacle position during foraging (Peschel et al. 1996): i) the upright position during locomotion ii) external lateral movements of the upright tentacle during exploration, iii) external lateral downward position during the perception of the conditioned odor, iv) the forward horizontal position during approaching the conditioned food. Considering the possible force vectors along which the flexor muscles can move the tentacle, the upright position is generated by the contraction of the M3 flexor muscle, the upright lateral position is generated by the contraction of the M2 and M3 muscles, the lateral downward position is generated by the contraction of the M2 muscle and the relaxation of the M3 muscle whereas the horizontal position is generated by the contraction of the M1 and M2 muscles and is maintained by the relaxation of M3 and M2 muscles.

The conditioned odor induced horizontal positioning of the posterior tentacles was considered as an odor memory driven motor response (Peschel et al. 1996). The M1 muscle receives distinct motor command via the iPT nerve, therefore the activity of motor neurons sending axons via the iPT nerve are probable commanded by the olfactory memory during the generation of horizontal positioning. However, the location of the odor memory and how it induces or drives the motor output via the iPT nerve is not known. The procerebrum of the cerebral ganglion which takes part in the odor information processing (for rev. see Chase 2002) was shown to be involved in odor discrimination but not in odor identification in *Limax* (Teyke and Gelperin 1999). Therefore the odor identification or the decision making center should be located in other yet unknown areas of the cerebral ganglion. It was shown that during odor or food conditioning the formation of odor memory requires additional feeding related stimuli, as sensory inputs from the anterior tentacles and the oral cavity, which inputs terminate on yet unknown cerebral neurons (Teyke 1995, Friedrich and Teyke 1998). Therefore it cannot be excluded that neurons in the presently labeled groups of the cerebral ganglion which project via the iPT nerves can be odor conditioned and respond directly to the conditioned odor.

Contrary to the distinct motor pathways, the common pathway via the olfactory nerve which innervate the tegumental, flexor and retractor muscles is probably responsible for the withdrawal reflex and the graded shortening of the tentacles when all muscles of the tentacle are contracted (Prescott et al. 1997, for rev. Chase 2002). This is supported by earlier findings according to which the electrical stimulation of the olfactory nerve failed to induce bending of the isolated tentacle preparation but evoked contractions on the tip of the tentacle (Peschel et

al. 1996). In the isolated tentacle preparation the tentacular retractor muscle is cut from the columellar muscle (Peschel et al. 1996) thus the contraction of the retractor muscle is not able to withdraw the tentacle although the stimulation of the olfactory nerve evoked the contraction of the retractor and tegumental musculature. Therefore we suggest that motor outputs via the olfactory nerve play a role first of all in the withdrawal mechanism rather than bending of the tentacle.

In conclusion our gross anatomical and neuroanatomical findings provide a basis to explain how the motor commands sent to the flexor and tegumental muscles via the PT nerves, generated by cerebral neurons and modulated by olfactory memory, can direct the movement of the olfactory organ to adjust the direction of locomotion in maintaining the perception of increasing concentration of conditioned odor during foraging.

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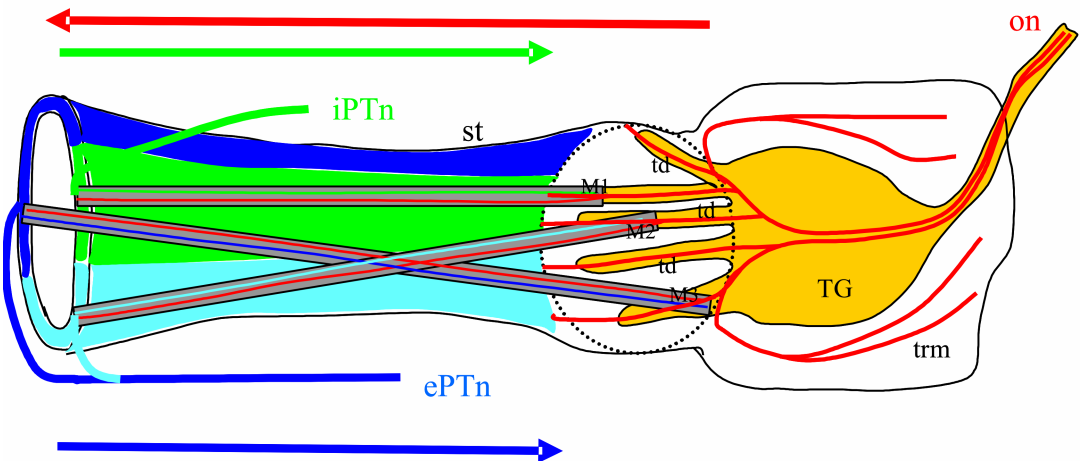

Fig.1. Schematic drawing of a stretched, turned from inside out preparation of a posterior tentacle, the flexor muscles (**M1, M2, M3**) and their innervation. Retrograde neurobiotin tracing via the olfactory nerve (**on**), as well as the external (**ePT**) and internal (**iPT**) peritentacular nerves. Fibers labeled via the **on** (red) run through the tentacular ganglion (TG) and its digit (td) and form a common innervation pathway (red dotted areas) for the retractor muscle (trm), the tegumental muscle of the stem (st) as well as for the three flexor muscles (M1, M2, M3). Fibers labeled via the iPT nerve (green) reach the tentacle at its base and innervate the M1 flexor muscle and a ventromedial stripe of the stem (green area). Fibers labeled via the ePT nerve bifurcate before reaching the base of the tentacle. The ventrolateral branch (light blue) innervates the M2 muscle and the ventrolateral stripe of the stem (light blue area), whereas the dorsolateral branch (dark blue) innervates the M3 muscle and the dorsal stripe of the stem (dark blue area). Arrows indicate the direction of the innervation by the given nerves.

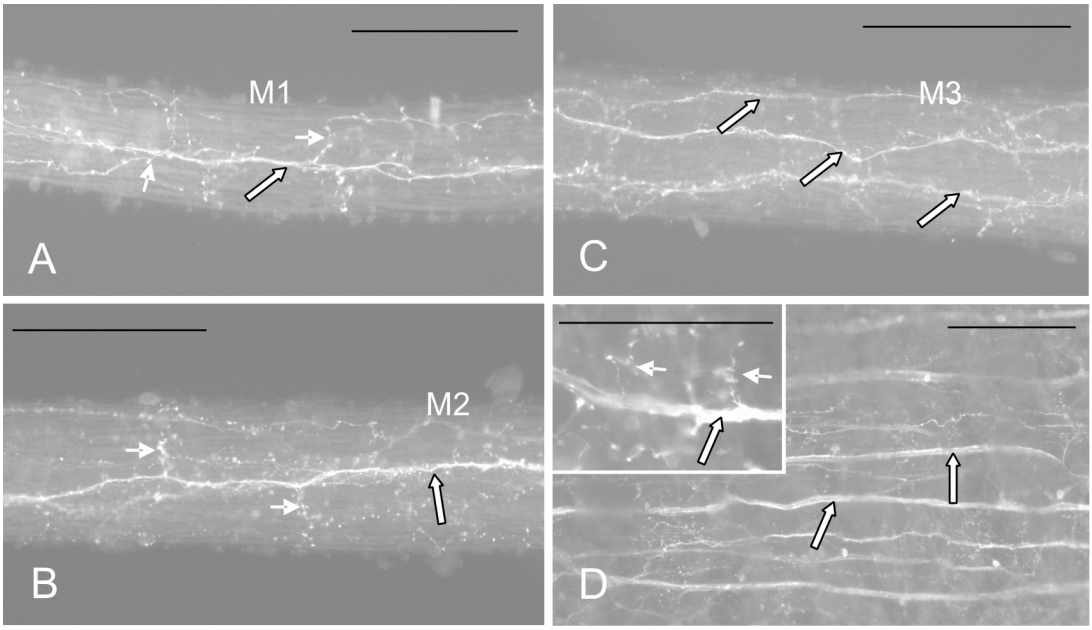

Fig.2. Representative examples of the innervation of the M1 (A), M2 (B) and M3 (C) flexor muscles as well as the tegumental muscle (D) via the olfactory nerve. Thick neurobiotin labeled fibers (long arrows) run in the muscles giving off side branches (short arrows) which innervate the muscles. Calibration bars 100 µm.

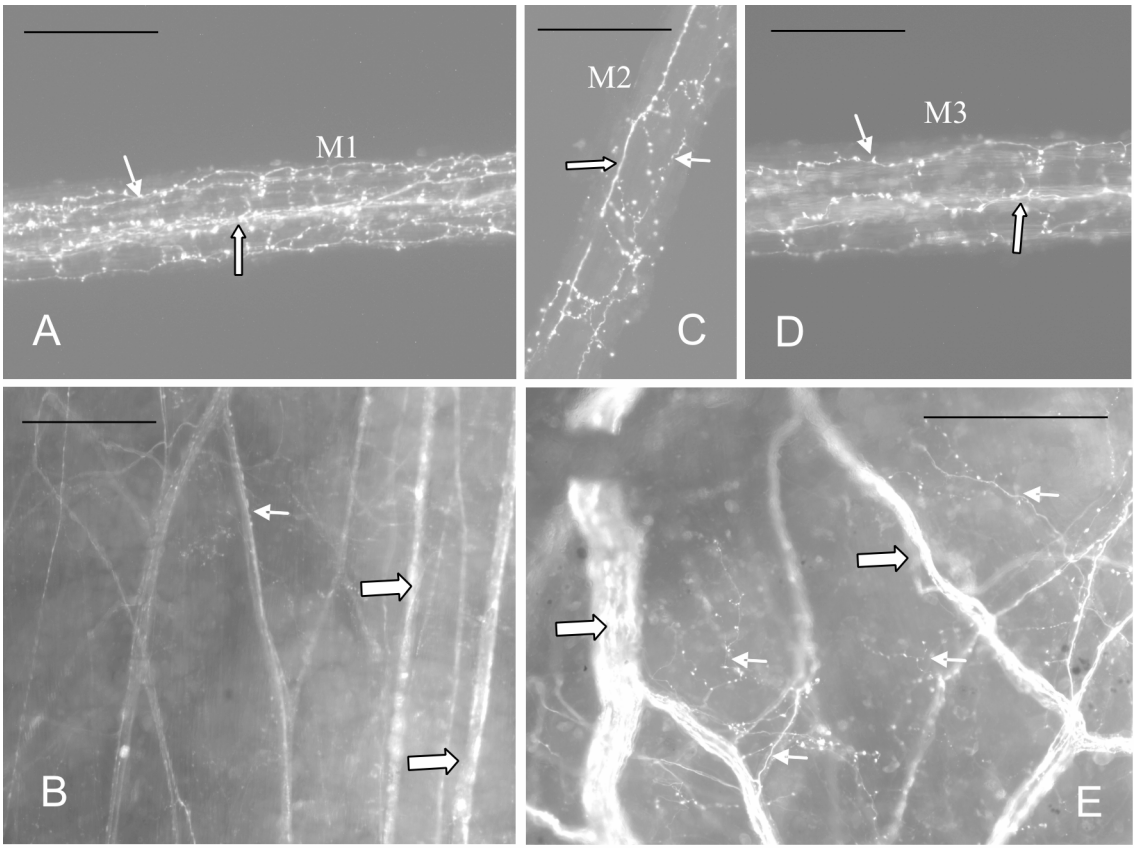

Fig.3. Representative examples of the innervations of flexor and tegumental muscles via the peritentacular (PT) nerves. Neurobiotin labeled fibers (long arrows) innervate (short arrows) via the iPT nerve the M1 flexor muscle (A) as well as a stripe of tegumental muscle (B). Labeled fibers (long arrows) innervate (short arrows) via the ePT nerve the M2 (C) and the M3 flexor muscles (D) as well as a lateral and a dorsal stripe of the tegumental muscle (E).

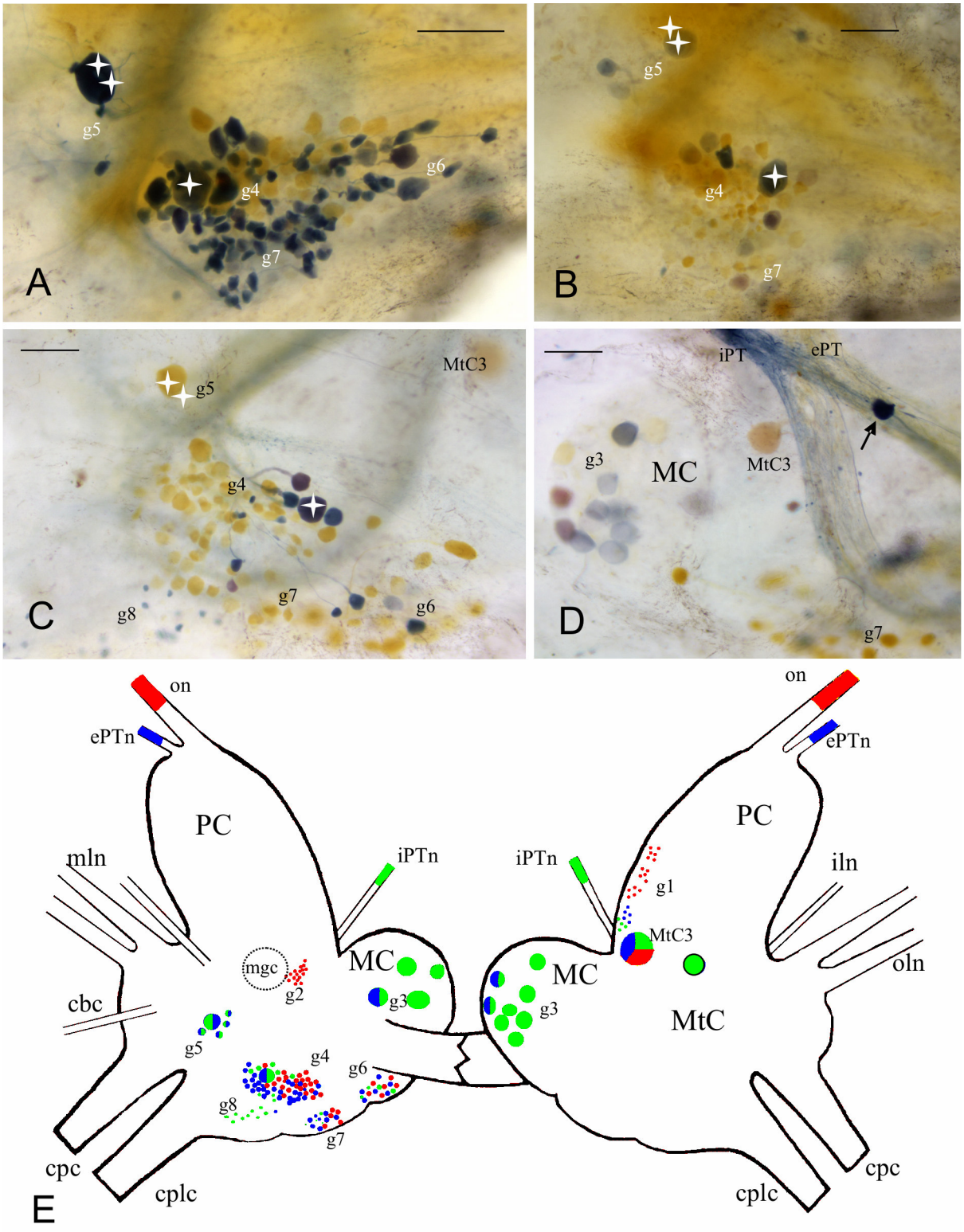

Fig.4. Representative examples of parallel retrograde Co-lysine (orange) and Ni-lysine (blue) tracings via pairs of nerves innervating the flexor muscles. **A** Parallel tracing via the olfactory (orange) and the external peritentacular nerves (blue) revealed several labeled groups. Among

them in the g4 group the neurons labeled via the ePT nerve (blue) are located under the neurons labeled via the olfactory nerve. In the g 4 and g5 groups one-one large neuron (stars) labeled via the ePT nerve can be seen. **B** Parallel tracing via the olfactory (orange) and the internal peritentacular nerves (blue) shows that neurons labeled via the iPT nerve are located among the neurons labeled via the olfactory nerve. The large neurons (stars) in the g4 and g5 groups are labeled also by the iPT nerve. **C** Parallel tracing via the ePT (orange) and the iPT nerves (blue) shows that in the g5 group the Ni-lysine labeled neurons (blue) are separated and located over the Co-lysine labeled neurons (orange). **D** On the dorsal surface of the mesocerebrum (MC) in the g3 group neurons (pink) are labeled via both the iPT (blue) and ePT (orange) nerves. MtC3 and the arrow show solitary labeled neurons. **E** Schematic drawing shows that neurons labeled via the olfactory (red), the internal (green) peritentacular (iPTn) and the external (blue) peritentacular nerve (ePTn) are clustered into eight groups (g1-g8) in the procerebral (PC) mesocrebral (MC) and metacerebral (MtC) lobes of the cerebral ganglion. Left side shows the ventral surface whereas the right side shows the dorsal surface of the right cerebral ganglion. *cbc* cerebro-buccal connective, *cpc* cerebro-pedal connective, *cplc* cerebro-pleural connective *iln* inner lip nerve, *mgc* metacerebral giant cell, *mln* medial lip nerve, *MtC3* identified neuron (Cottrel et al. 1983) *oln* outer lip nerve,

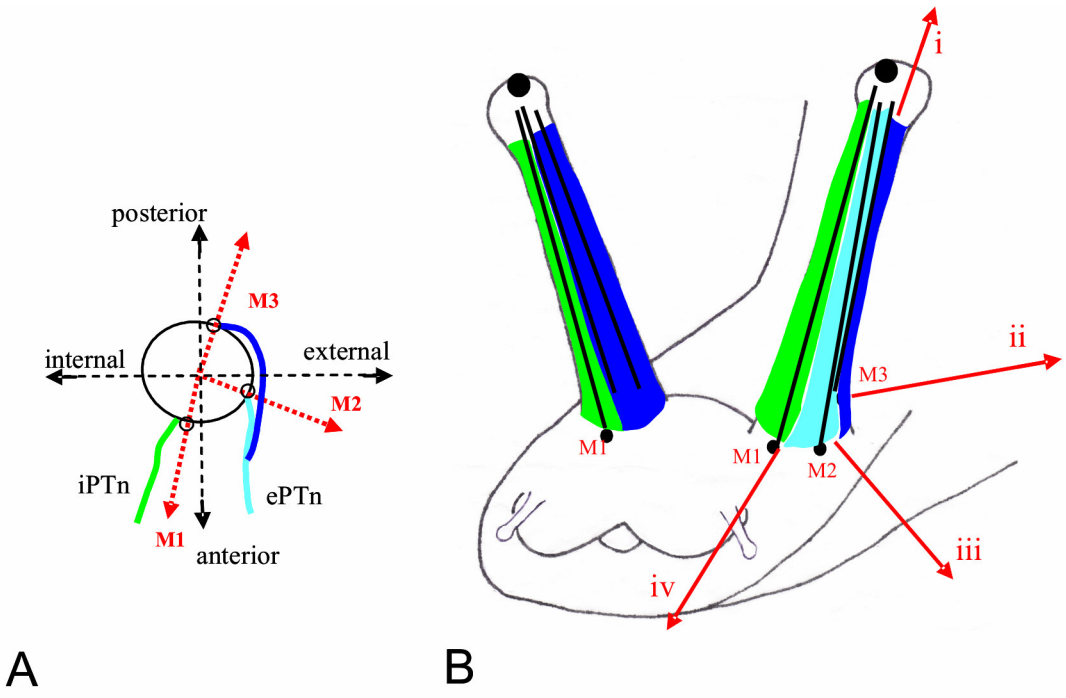

Fig.5. A Schematic drawing showing the three flexor muscles (M1, M2, M3) anchored to different sites at the base of the stem (circles). The M1 is fixed to the base of stem at ventromedial position and is innervated by the iPT nerve (green). The M2 muscle is anchored at external ventrolateral position and is innervated by the ventral branch of the ePT nerve (light blue). The M3 muscle is anchored at dorso-lateral position and is innervated by the dorsal branch (dark blue) of the ePT. The anchoring points of the flexor muscles outline the directions of three force vectors (red dotted arrows) generated by the contraction of the flexor muscles. **B** Schematic drawing showing the innervation of the protracted tentacles by the iPT nerve (green) as well as the ventral (light blue) and the dorsal (dark blue) branch of the ePT nerve. The posterior tentacles are in the basic upright position. Red arrows shows the typical positions of the tentacle generated by the contraction of M3, M2 and M1 flexor muscles during foraging behavior which includes locomotion (i), exploration (ii), perception of conditioned odor (iii), and food approach (iv).

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