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Novel peripheral motor neurons in the posterior tentacles of the snail responsible for local tentacle movements

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Abstract

Three flexor muscles of the posterior tentacles of the snail *Helix pomatia* have recently been described. Here we identify their local motor neurons by following the retrograde transport of neurobiotin injected into these muscles. The mostly unipolar motor neurons (15-35 μm) are confined to the tentacle digits and send motor axons to the M2 and M3 muscles. Electron microscopy revealed small dark neurons (5-7 μm diameter) and light neurons with 12-18 μm (T1 type) and 18-30 μm diameters (T2 type) in the digits. The diameters of the neurobiotin labeled neurons corresponded to the T1 type light neurons. The neuronal processes of T1 type motor neurons arborize extensively in the neuropil area of the digits and receive synaptic inputs from local neuronal elements involved in peripheral olfactory information processing. These findings support the existence of a peripheral stimulus-response pathway, consisting of olfactory stimulus - local motor neuron - motor response components, to generate local lateral movements of the tentacle tip ("quiver"). In addition, physiological results showed that each flexor muscle receives distinct central motor commands via different peritentacular nerves, and common central motor commands via tentacle digits, respectively. The distal axonal segments of the common pathway can receive inputs from local interneurons in the digits modulating the motor axon activity peripherally without soma excitation. These elements constitute a local microcircuit consisting of olfactory stimulus - distal segments of central motor axons - motor response components, to induce patterned contraction movements of the tentacle. The two local microcircuits described above provide a comprehensive neuroanatomical basis of tentacle movements without the involvement of the CNS.

Introduction

The main olfactory organs of terrestrial molluscs are located on the tip of the posterior tentacles, which display complex movements when exploring a new environment.

Snails scan the environment with protracted posterior tentacles executing large scale lateral movements (bending) by slowly rotating the tentacle from side to side around the basal pivot (Peschel et al. 1996; Nikitin et al. 2005, 2008). Odor or food conditioned snails raise their protracted tentacles when they perceive an unknown odor, but lower their tentacles after perceiving a conditioned odor (Peschel et al. 1996).

These large scale tentacle movements are frequently interrupted by local movements such as twitches, contractions and quivers. The local tentacle movements are superimposed on the larger scale tentacle movements and are always performed unilaterally (Wondrak (1977; Lemair and Chase 1998; Nikitin et al. 2005). A “twitch” is a brief retraction of the protracted tentacle that inverts the olfactory epithelium, and has been suggested to perform the function of removing odor molecules from the surface of sensory epithelium (Lemair and Chase 1998). “Contraction” describes a shortening of the protracted tentacle to reduce the scanning area in a hostile environment (Nikitin et al. 2005, 2008), while “quiver” is a rapid external lateral flexion of the tentacle tip (often consisting of two or more consecutive movements without retraction) to increase the access of odor molecules to receptors (Wondrak 1977; Lemair and Chase 1998). At the level of behavior, the large scale tentacle movements are considered as indicative of the feeding history of the snail (Peschel et al. 1996), whereas the local tentacle movements are considered to reflect

the active sensing of the actual environment (Lemair and Chase 1998; Nikitin et al. 2005).

All forms of tentacle movement serve to bring the olfactory receptors into position for better odor perception in different quality odor environments (naïve, aversive or attractive odors) (Croll and Chase 1980; Chase and Croll 1981; Chase and Tolloczko 1993; Friedrich and Teyke 1998; Nikitin et al. 2008; for rev Chase 2002). The execution of the different forms of posterior tentacle movements described above requires the activity of different effector muscles of the tentacle. The large scale tentacle movements are suggested to be executed by the coordinated contraction of the recently described tentacle flexor muscles (Hernádi and Teyke 2012; Krajcs et al. 2012) and the appropriate band of the tegumental musculature (Hernádi and Teyke 2013). It has been shown that each of these effector muscles receives dual innervation, both distinct and common, from cerebral neurons. The distinct innervation pathways arise from separated clusters of cerebral neurons, projecting motor axons via the different peritentacular nerves to generate contraction in these muscles. The common innervation pathway sends motor axons from clusters of cerebral neurons to the tentacle muscles via the olfactory nerve. This type of innervation is suggested to play a role in tentacle withdrawal and retraction (Hernádi and Teyke 2013).

Lesion experiments have shown that local tentacle movements such as quiver and brief contraction persists in isolated tentacle-nose preparations without any CNS control; therefore it has been proposed that quiver is under peripheral neuronal control (Nikitin et al 2005). However, the peripheral or local motor neurons in the tentacle that may constitute this peripheral stimulus-motor response pathway have not been identified.

In the present study we applied the retrograde tracer neurobiotin via the tentacle flexor muscles, and investigated whether labeled local neurons in the tentacle ganglion and digits fulfilled the criteria of putative motor neurons. We investigated whether local putative motor neurons in the tentacle digits could be differentiated on the basis of their fine structure and synaptology, using electron microscopy. Furthermore, we applied electrical stimulations via the peritentacular and olfactory nerves to test our earlier suggestion that each flexor muscle receives distinct motor commands via a distinct peritentacular nerve, and that all muscles receive common motor commands via the olfactory nerve (Hernádi and Teyke 2013).

Materials and Methods

Adult specimens of the snail *Helix pomatia* were kept in an outdoor cage and fed with cucumber.

Neurobiotin tracing via the tentacle flexor muscles

For neurobiotin tract tracing experiments, the withdrawn inverted (inside out) posterior tentacles were used (Fig.1). The inverted tentacles were pinned out on Sylgard coated plates. One of the flexor muscles was selected and cut at the base of the tentacle where it is anchored to the body wall (see Fig.1). The 5-6 mm long muscle was placed over a Vaseline cup containing 5% neurobiotin (VECTOR) diluted in distilled water. Thereafter the cup was sealed with Vaseline and covered with physiological saline. The preparations were kept at room temperature for one day. All preparations were then fixed in 4% paraformaldehyde (REANAL, Budapest, Hungary) buffered with 0.1 M phosphate buffer (pH 7.4) for 6 hours

at 4°C. After fixation, the samples were transferred into phosphate buffered saline (PBS) containing 20% sucrose. Cryostate sections (30 μm thick) were made from the preparations. The neurobiotin in the neuronal elements were visualized by incubation with fluorescent-conjugated Streptavidine 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 into PBS-glycerol (2:1).

Muscles displayed spontaneous contraction during incubation and therefore they could frequently pull themselves out from the Vaseline cups, causing neurobiotin leakage. Therefore, retrograde tracing was considered successful and used for evaluation if no leakage of neurobiotin was seen in the preparation and labeled fibers could be detected in the tentacle ganglion. Only 50% of the retrograde labeled preparations could be used for evaluation. The neurobiotin labeled neuronal elements were photographed under a fluorescence microscope equipped with appropriate filters. For counting labeled cell bodies, 5-8 preparations of each flexor muscle were used. For electron microscopy, the tentacles were fixed in a mixture of 2% OsO4 (SIGMA) and 2.5 % glutaraldehyde (SIGMA) buffered with 0.1 M S-Collidine (pH 7.2) for three hours at 4 ºC. Following a brief rinsing in buffer the samples were post fixed in 1% OsO4 solution buffered with 0.1 M S-Collidine for 4 hours. The fixed preparations were dehydrated in graded ethanol. Block staining was performed in 70% ethanol saturated with uranyl acetate (SIGMA). The fixed tentacle was separated into flexor muscles, tegumental muscle and tentacle tip, containing the tentacle ganglion and the tentacle digits. The separated parts were embedded in Durcupan ACM . Ultrathin sections were cut on an LKB Ultratome, stained with lead citrate (SIGMA) and examined with a TESLA BS 500 electron microscope.

Semi-thin (1µm) section series were cut from the tip of the tentacle on the LKB Ultratome and collected on slides. After tolouidin blue staining, the distributions of light and dark neurons were studied under a light microscope.

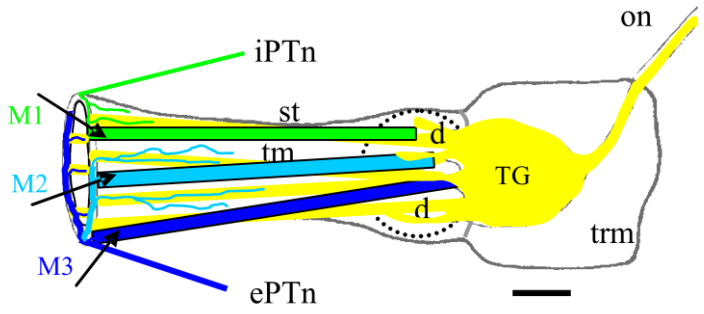

Fig.1 Schematic drawing shows the different parts of the inverted tentacle (inside out) including the stem of tentacle (*st*) with the tegumental muscle (*tm*), the flexor muscles (*M1, M2, M3*), the tentacle retractor muscle (*trm*) and the tentacle ganglion (*TG*) with the tentacle digits (*d*). Tentacle is innervated by the olfactory nerve (*on*) as well as by the internal (*iPTn*) and external (*ePTn*) peritentacular nerves attaching to the base of the stem (*st*). PT nerves (*iPTn* green; internal branch of *ePTn* light blue; external branch of *ePTn* dark blue) innervate the tentacle and the flexor muscles from the base of the stem to the tip. Arrows show the sites where the flexor muscles were cut and the cut ends were used for neurobiotin tracing. Bar represents 500 µm

Recording of electrically evoked muscle contractions

In isometric experiments both ends of the muscle were fixed in a perfusion chamber. One end of the muscle was hooked to a force transducer (WPI, FORT 1000) and the other end was fixed at the bottom of the chamber. For the electrical stimulation of innervated muscles, a pair of silver hooked electrodes was placed under the nerve. The ganglion was placed in a pit of the recording chamber and separated from the muscle by a Vaseline gap. 10 ms electrical pulses of 5-10 V were applied at 1-1.5 Hz.

Results

Neurobiotin tracing via the flexor muscles

Neurobiotin tracing via the M1 flexor muscle (n=5) revealed labeled neuronal fibers in both the tentacle digits and ganglion, whereas labeled neuronal cell bodies were only rarely observed (1-2). Additionally a few labeled fibers were observed in areas of the tentacle retractor and tegumental muscle proximal to the M1 muscle (Fig.8A).

Tracing via the M2 flexor muscle (n=8) outlined 10-15 labeled cell bodies in the ventro-medial and lateral digits of the tentacle ganglion. The labeled neurons had diameters of 20-35 μm and a mostly unipolar appearance (Fig. 2). Labeled fibers were seen in the tentacle ganglion and digits as well as in the olfactory nerve. Labeled fibers could also be observed in the retractor and tegumental muscles (Fig. 8A).

Neurobiotin tracing via the M3 flexor muscle (n=6) revealed the presence of 20-35 μm diameter labeled cell bodies in the ventral and lateral digits of the tentacle ganglion. These neurons displayed a mostly unipolar appearance (Fig. 3, 8A). Labeled neurons could also be seen in the distal segment of the stem, housing the tegumental musculature (Fig.3F). Labeled fibers were present in the tentacle ganglion, digits, and olfactory nerve as well as in the distal parts of the retractor and tegumental muscles (Fig.3, 8A).

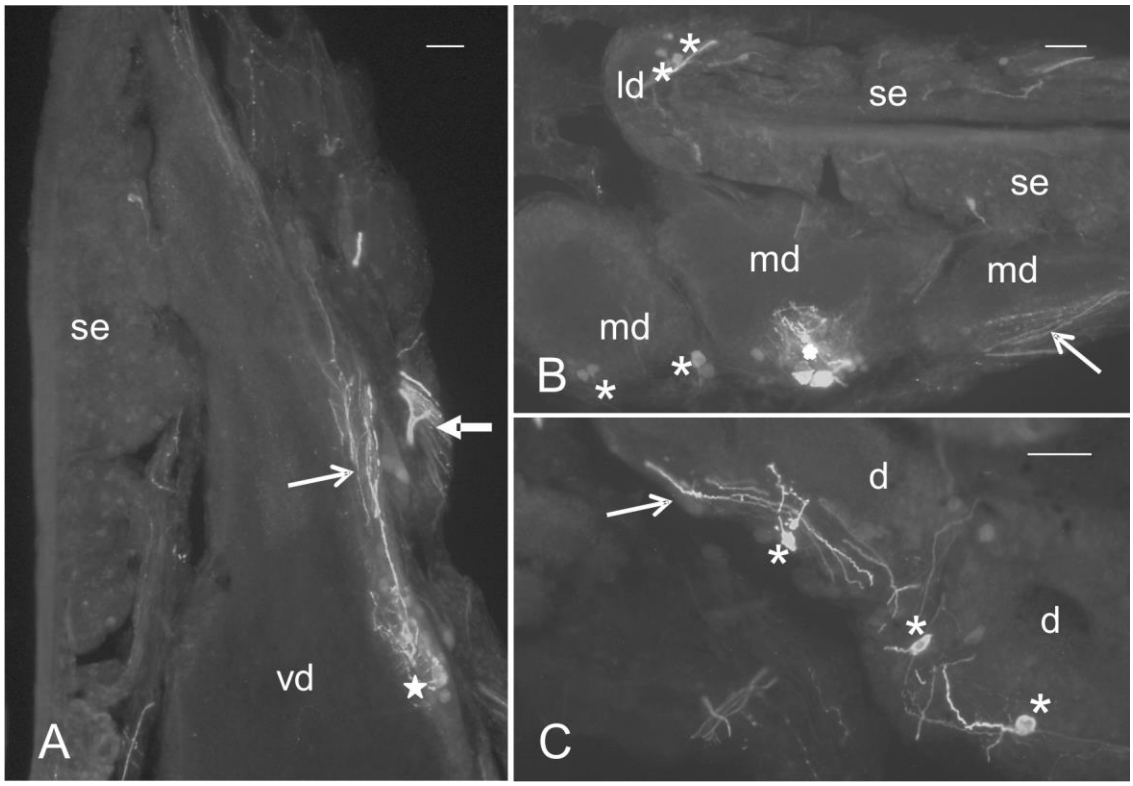

Fig.2 A Both neuronal cell bodies (*asterisk*) and fibers (*thin arrow*) are labeled in the ventral digits after neurobiotin tracing via the M2 flexor muscle. The M2 muscle joins (*thick arrow*) to the ventro-medial part of the sensory epithelium (*se*) (sagittal section). **B** Labeled cell groups (*large asterisk*) and solitary labeled neurons (*small asterisk*) can be seen in both the ventromedial (*md*) and lateral (*ld*) digits (sagittal section). **C** The labeled neurons (*small asterisks*) in the digits (*d*) are mostly unipolar and they send off side branches at their proximal segments (sagittal section). *Bars* 50 µm.

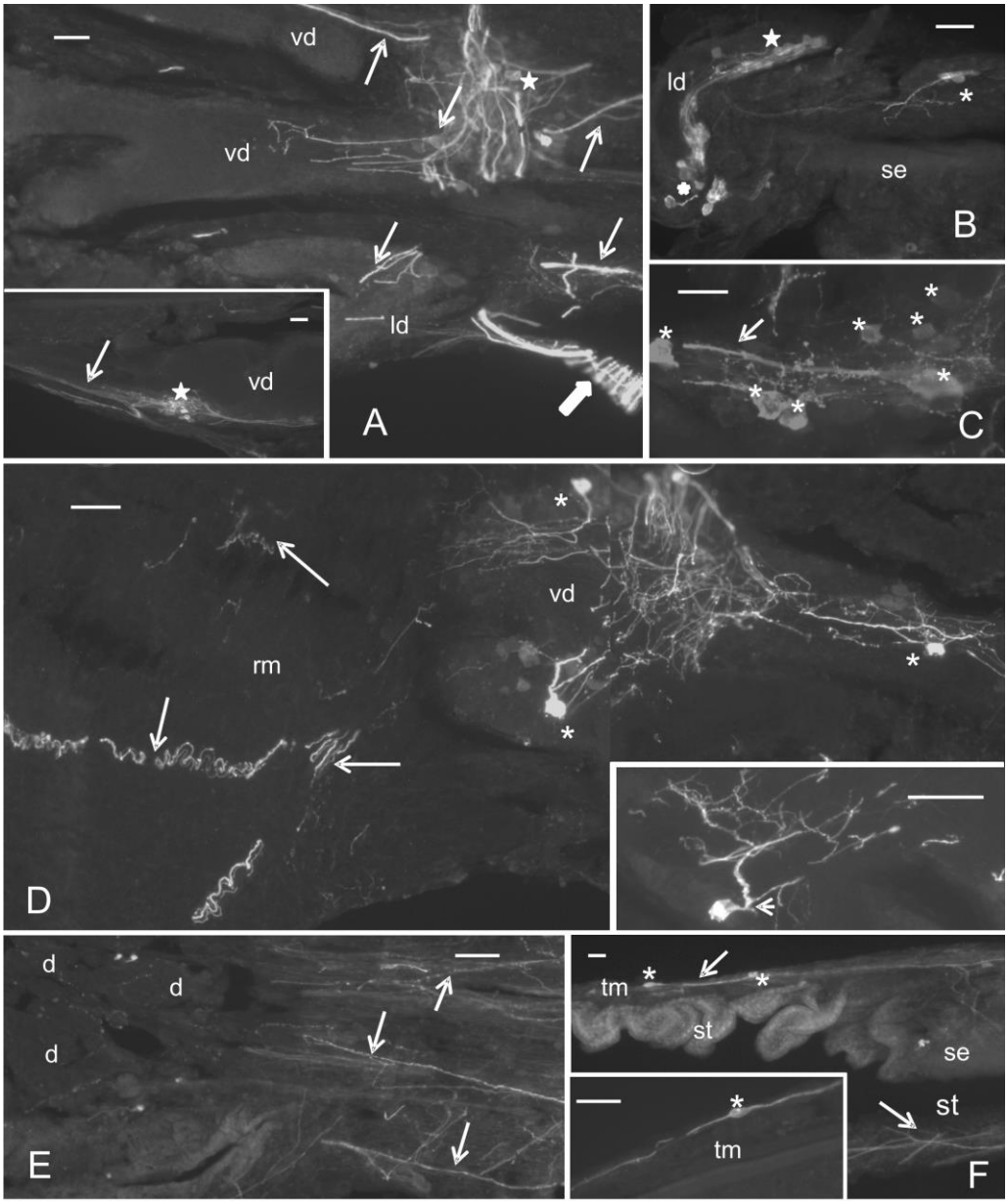

Fig.3A Neurobiotin tracing via the M3 flexor muscles (*large arrow*) labeled both groups of cell bodies (*asterisk*) and fibers (*thin arrows*) in the ventral (*vd*) and lateral (*ld*) digits of the ganglion (horizontal section). **Insert** The area of the same ventral digit (*asterisk*) in sagittal section. **B** Groups (*large asterisk*) of labeled neurons can be seen in the lateral digit (*ld*) of the tentacle ganglion. **C** Higher magnification micrograph shows the inner part of the lateral digit displaying labeled unipolar (*white asterisks*) neurons and fibers (*thin arrow*) (horizontal section). **D** Horizontal section shows one of the ventral digits (*vd*) and the tentacle retractor musculature (*rm*). In the digit unipolar labeled neurons (*small asterisks*) and numerous labeled fibers can be seen after tracing. Groups of labeled fibers (*thin arrows*) leave the digit and innervate the retractor musculature (*rm*). **Insert** Higher magnification micrograph shows a solitary labeled unipolar neuron (*small asterisk*) which displays a branching point (*arrow head*) close to its soma and arborize in the neuropil area of the digit. **E** Groups of labeled fibers (*thin arrows*) run on the ventral surface of the sensory pad towards the stem of the tentacle. **F** On the internal surface of the stem (*st*), close to the tegumental muscle (*tm*), bipolar neurons (*small asterisks*) and fibers (*thin arrow*) are labeled via the M3 muscle. **Insert** Higher magnification micrograph shows a bipolar neuron (*small asterisk*) on the surface of the tegumental muscle. *Bars* 50 μm.

Ultrastructural analysis of neurons in the tentacle digits

In semi-thin sections from the tentacle tip, two forms of neurons could be separated in the digits: the small dark and the larger light neurons. The small diameter (5-8 μm) dark neurons constituted the overwhelming majority of neurons in the digits (Fig.4A). The light neurons were located dominantly in the ventral digits, which are separated by a thin connective tissue sheath (Fig.4A). Based on their diameter and location, the light neurons could be distinguished as two types of neurons, Type 1 (T1) and Type 2 (T2) (Fig.4A). The T1 light neurons were located in the digits close to the dark neurons and had a diameter of 15-30 μm, whereas the T2 light neurons were located close outside the digit and had a 25-45 μm diameter (Fig.4A).

Ultrastructural analysis showed that the small diameter dark neurons had a thin, medium-dense pericaryon, with cellular organelles homogenously dispersed within the cytoplasm (Fig.4B). Both the T1 (Fig. 4B, D) and T2 type (Fig. 4C) light neurons had a light cytoplasm and the majority of cell organelles were gathered in a ring around the nucleus. Neither dense-core nor empty vesicles could be found in the cytoplasm or the proximal segments of the neuronal processes. In the light pericaryon and the light neuronal processes, fine dense filaments were seen, giving them a characteristic ultrastructural appearance (Fig.4D, E). The neuronal processes of the mostly unipolar light neurons displayed arborization in the neuropil area of the digits. Their side branches joined to other light processes, forming a separated pathway in the neuropil area of the digit (Fig.4D). In the neuropil area of both the ventral and lateral digits, the light type neuronal processes were observed in close contact and synapses were present (Fig.4 D,E).

Light type neurons could also be seen in the distal segment of the stem, usually displaying a bipolar appearance (Fig. 5A). Neuronal processes of the light type neurons received synapses in the neuropil area of the digit (Fig.5B). Muscle fibers in both the tegumental (Fig. 5C) and flexor (Fig. 5D) muscles received neuro-muscular contacts from axons displaying ultrastructure corresponding to the neuronal processes of the light neurons. At the neuro-muscular contacts, small diameter empty vesicles could be seen in the axon terminals (Fig.5C, D inserts). Based on these neuroanatomical observations it was supposed that part of the population of light type neurons fulfill motor neuron function in the ventral and lateral digits.

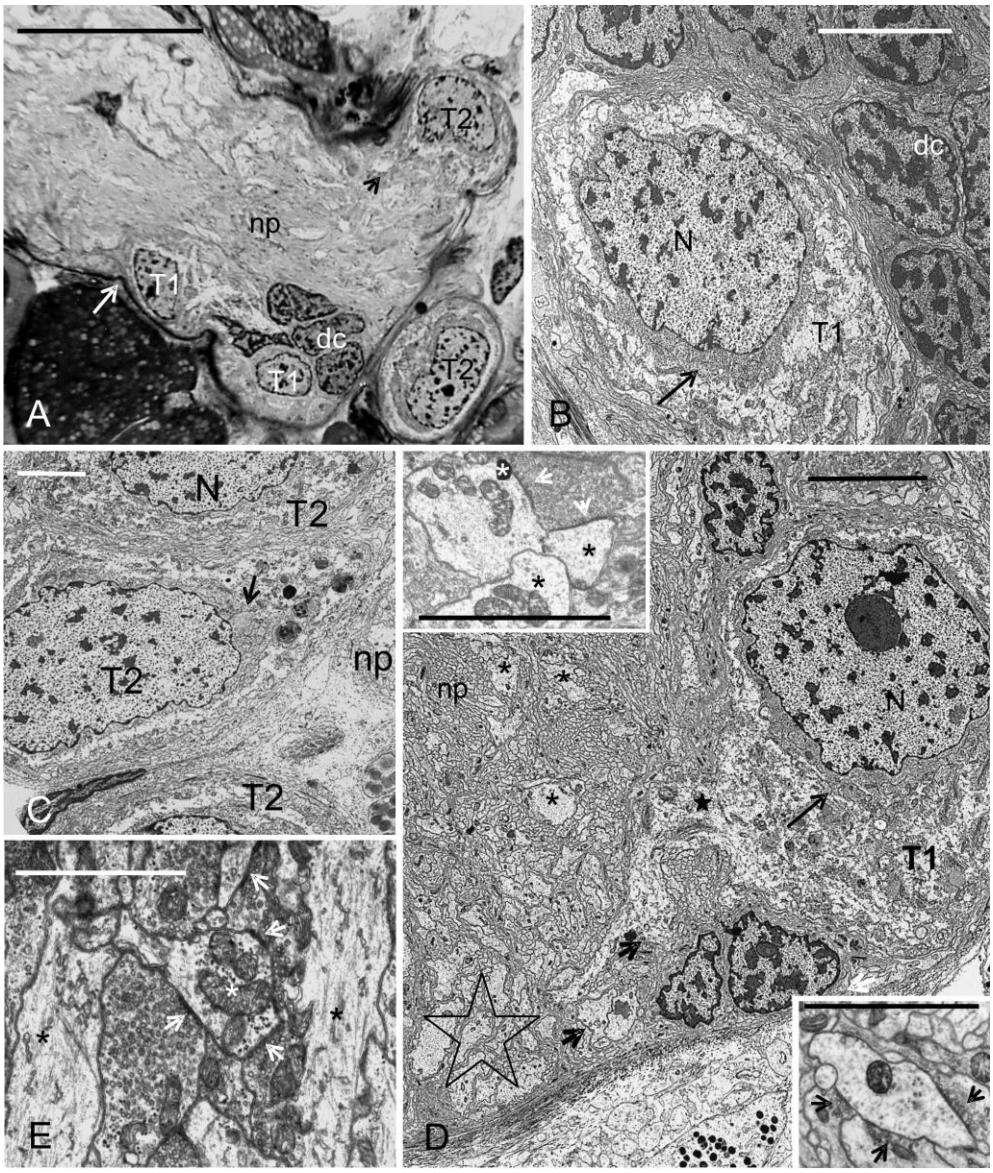

Fig.4A Semithin section shows the organization of neuronal elements in a ventral tentacle digit. Small sized dark (*dc*) and the T1 type light cells (*T1*) can be seen inside the digit which is limited by a thin connective tissue layer (*arrow*). T2 type light cells are located outside the digit and they join to the neuropil area by thick neuronal processes (*arrow head*). *Bar* 50 µm. **B** Electron micrograph of a ventral digit shows dark cells (*dc*) and a T1 type light neuron (*T1*). Arrow shows the dense ring around the nucleus (*N*) of T1 neuron. *Bar* 5 µm. **C** T2 type light neurons (*T2*) located outside the digit (*d*). The cell organelles are gathered in a ring (*arrows*) around the nucleus (*N*). *Bar* 5 µm. **D** Part of a ventral digit shows a T1 type light neuron (*T1*) and the neuropil area of the digit (*np*). The neuronal process of the light neuron (*solid asterisk*) sends off side branches (*arrow heads*) which join to other fibers with similar ultrastructure forming a separated pathway (*large empty asterisk*). In the neuropil area light type neuronal processes are in close apposition (*small asterisks*). *Bar* 5 µm. **Insert upper** Light profiles (*small asterisks*) are in close apposition and two of them receive synaptic connections (*arrow heads*) with unknown origin. *Bar* 1 µm. **Insert lower** One light profile may receive more synaptic connections (*arrow heads*). *Bar* 1 µm. **E** Light type neuronal processes (*small asterisks*) have numerous neurofilaments and receive synapses (*arrow heads*) with unknown origin in the neuropil area. *Bar* 1 µm.

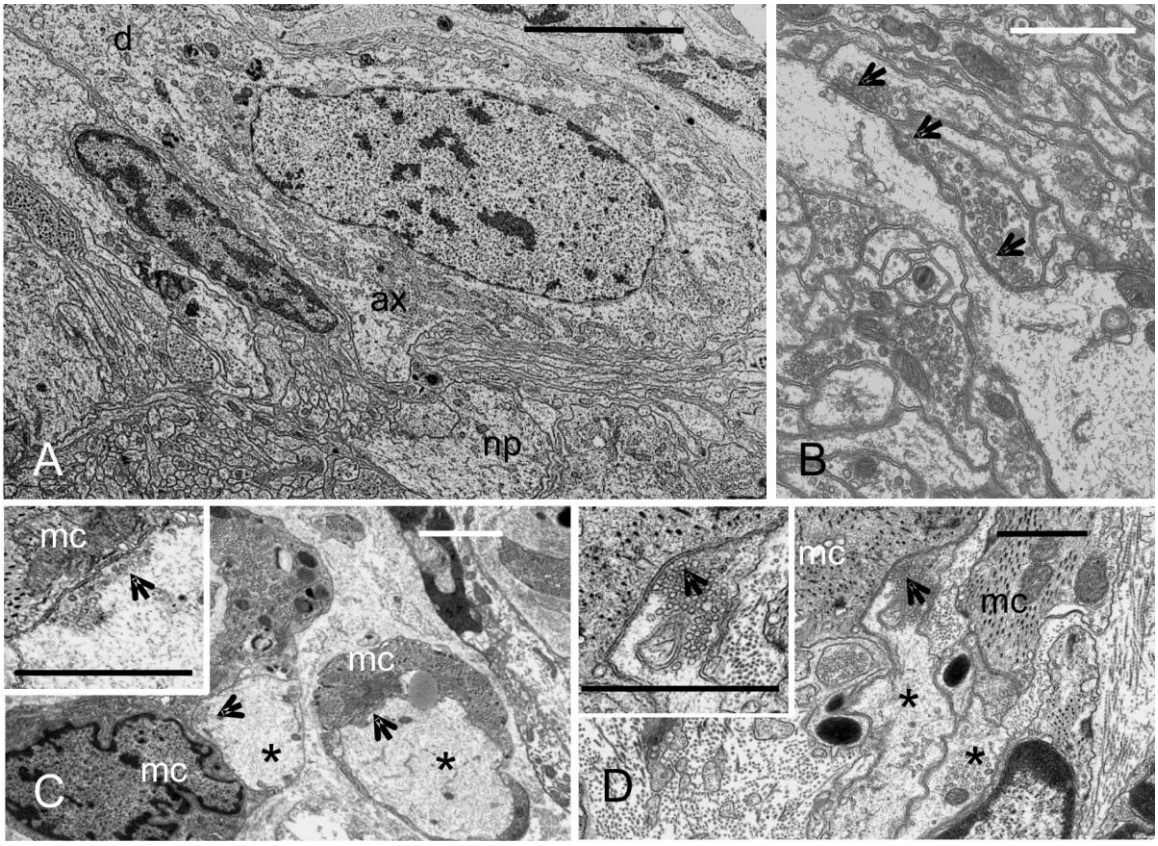

Fig.5A Bipolar T1 type light neuron (*T1*) located along a nerve trunk in the stem of the tentacle. The neuron gives off a dendritic (*d*) and an axonal (*ax*) process which run into the nerve trunk (*np*). *Bar* 5 µm. **B** In the neuropil area of the nerve trunk a light type neuronal process receives synaptic contacts (*arrows*) with unknown origin. *Bar* 1 µm. **C** In the distal segment of the tegumental muscle light type axon profiles (*small asterisks*) make neuromuscular contact (*arrow heads*) with muscle cells (*mc*). *Bar* 1 µm. **Insert** Higher magnification of the neuromuscular contact shows small clear vesicles at the active zone (*arrow head*). *Bar* 1 µm. **D** In the flexor muscle light type axon profiles (*small asterisks*) make neuromuscular contact (*arrow*) with muscular elements (*mc*). *Bar* 1 µm. **Insert** Higher magnification micrograph shows small clear vesicles at the active zone of the neuromuscular contact (*arrow head*). *Bar* 1 µm.

Electrically evoked contraction of flexor muscles via the olfactory and different peritentacular nerves

Different nerves were stimulated with identical square wave pulses in order to record and compare contractions of the M1, M2 and M3 flexor muscles. Stimulation of the olfactory

nerve by a single stimulus elicited a tension response with the same efficiency in all of the muscles (Fig.6). Stimulation of the external peritentacular nerve (ePTn) by a single stimulus elicited a tension response in the M3 similar to that evoked via the olfactory nerve (Fig. 6). Stimulation of ePTn evoked no contraction of the M1 and only a weak contraction of the M2 (Fig. 6). Stimulation of the internal peritentacular nerve (iPTn) elicited tension effectively in the M1 muscle, but with less efficiency in the M2 and M3 muscles (Fig.6). The time course of the contraction elicited by iPTn was shorter than that elicited by stimulating the olfactory nerve (Fig. 6)

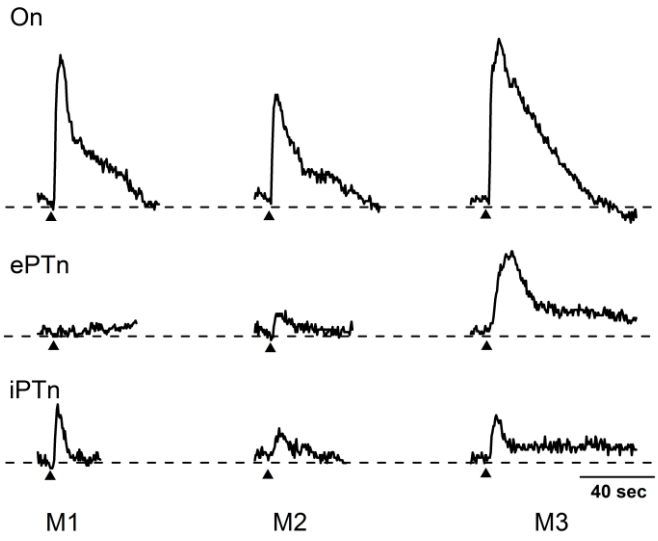

Fig.6 The time course of isometric tension responses of flexor muscles (*M1*, *M2*, *M3*) elicited by electrical stimulation of the olfactory and peritentacular nerves. Stimulation via the olfactory nerve (*On*) induced contraction in each muscle whereas stimulation via the external (*ePTn*) and internal (*iPTn*) peritentacular nerves induced contraction only in the muscle they innervate. Triangles show the delivery of single 100 ms and 5 V square wave pulse.

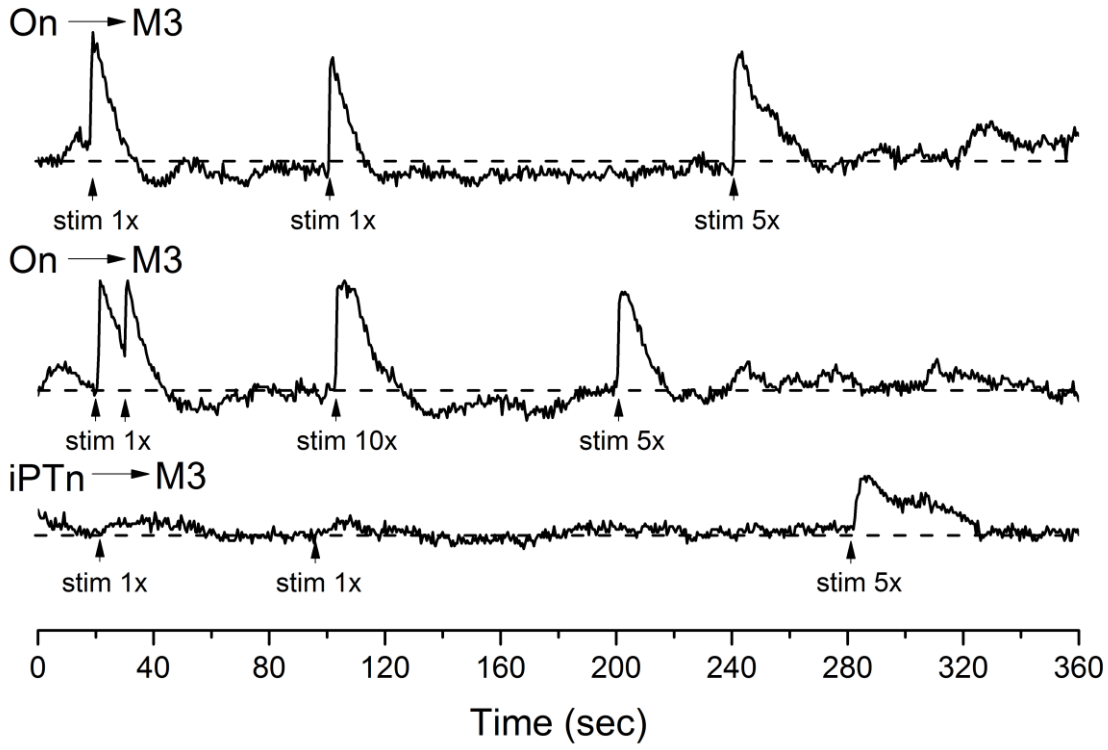

Fig.7 The M3 was stimulated repetitively through different nerves. Increasing the frequency of the stimuli via the olfactory nerve (*On*) the muscle response was prolonged (1 and 2 rows). Increasing the stimulus frequency delivered through the internal peritentacular nerve (*iPTn*) indirectly stimulated the M3 probably sensitizing the pathway (3rd row).

In the next experiment the M3 was stimulated repetitively through different nerves by a single stimulus or by a train of impulses (Fig.7). When the frequency of the stimulus applied via the olfactory nerve was increased, the response of M3 was prolonged. Interestingly the amplitude of the evoked response remained unchanged and the muscle could not be tetanized (Fig.7). Stimulation of the M3 through the nerve innervating the muscle indirectly (iPTn) was effective only after repeated stimulation, which seemingly facilitated the tension development (Fig.7).

Discussion

Putative motor neurons in the tentacle digits

Neurobiotin tracing via the flexor muscles (M1, M2, and M3) revealed mostly unipolar (15-35 μm) labeled neurons located in the digits of the tentacle ganglion but not in the body of the ganglion. The majority of these could be labeled via the M3 muscle, whereas neurons could only rarely be labeled via the M1 muscle. These findings indicated that the labeled neurons innervated the M3 and M2. Since no labeled olfactory receptor cells were observed in the sensory pad, the labeled neurons were considered to be motor neurons local to these muscles. It cannot be excluded, however, that some of the labeled neurons may function as stretch receptors.

Tracing via the M1 muscle did not reveal labeled cell bodies; only fibers were observed in the tentacle ganglion and retractor muscle. Therefore, fibers running in the ganglion and retractor muscle labeled via all the flexor muscles appear to belong to the common innervation pathway originating from central neurons (see Fig.8).

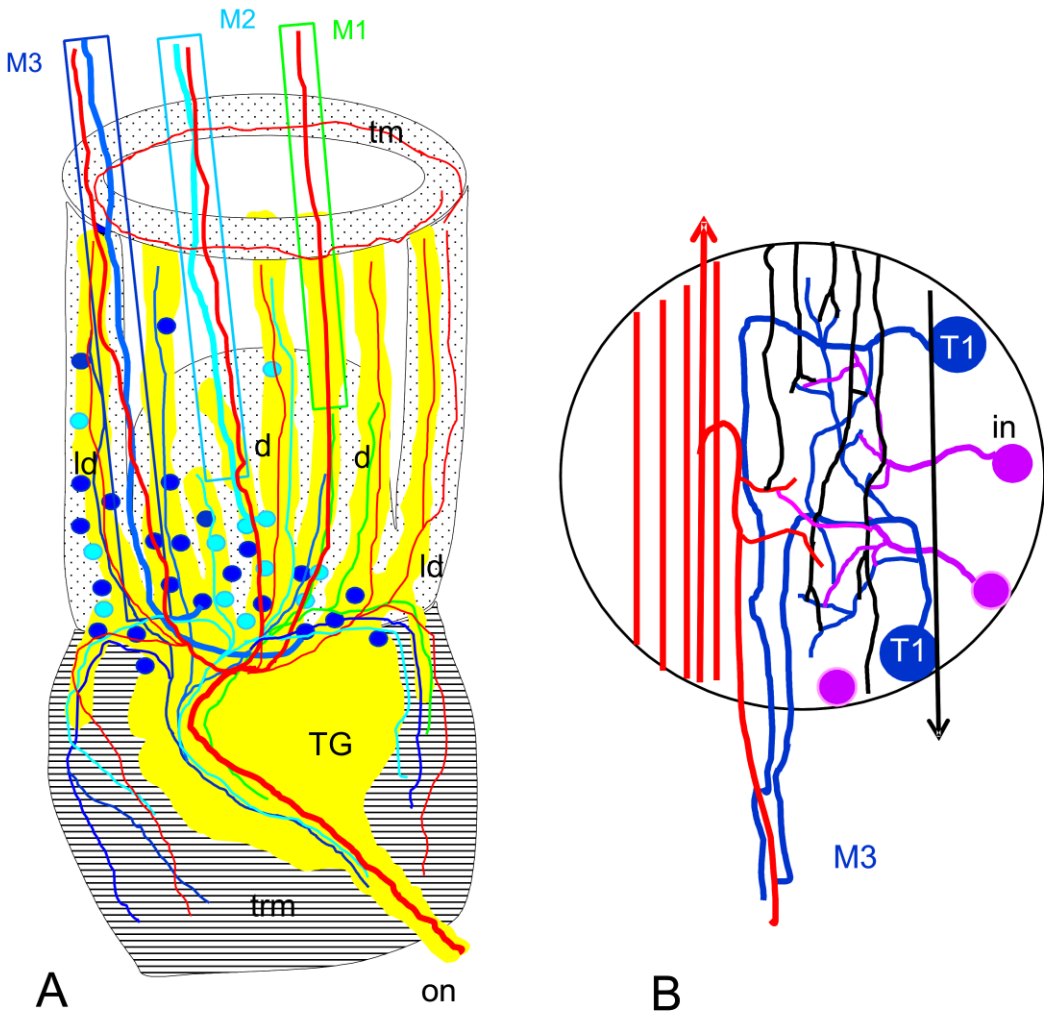

Fig.8A Schematic drawing summarizes the distribution of labeled neurons and fibers in the tentacle after neurobiotin tracing via the M1 (*green*), M2 (*light blue*), and M3 (*dark blue*) flexor muscles. Cell bodies (*circles*) are located along the ventral (*d*) and lateral (*ld*) tentacle digits and send axons to the M2 and M3 flexor muscles. Labeled nerve fibers can be seen in the digits, tentacle ganglion (*TG*), the tentacle retractor (*trm*) and tegumental (*tm*) muscles. *Red lines* represent the common innervation pathway from the cerebral ganglion to the tentacle via the olfactory nerve (*on*) (for details see Hernádi and Teyke 2013). The number of cell bodies on the drawing does not correspond to the real number of labeled neurons.
B Schematic drawing shows the possible inputs to the T1 type local motor neurons (*dark blue*) in a ventral tentacle digit. They may receive synaptic inputs from the neighbor local interneurons (*pink*) as well as from descending neuronal processes (*black*) which may originate from receptor cells and remote local interneurons. The distal axonal segments of the ascending common motor pathway (*red*) before reaching the flexor muscle may receive synaptic inputs from local neuronal elements.

On the basis of ultrastructural characteristics, two types of neuronal cell bodies, the small dark and the larger light type neurons, were differentiated in the digits of the tentacle ganglion. The small unipolar dark neurons have a 5-7 μm diameter and a thin medium dense pericaryon, whereas the mostly unipolar light neurons have a light pericaryon and a uniform ultrastructure. Based on their diameter the light neurons could be referred to as T1 (15-25 μm) and T2 (20-35 μm) types. Considering the diameters of the neurobiotin labeled as well as the dark and the light neurons, the labeled neurons in the digits corresponded to the T1 type light neurons. The ultrastructural analysis showed that the neuronal processes of the light neurons arborized extensively in the neuropil area of the digits and received numerous synaptic contacts from non-identified neuronal elements. These synaptic contacts possibly originated from local interneurons, as well as from axons of olfactory receptor cells and central neurons. Thus it is possible that the electrical activities of the light neurons were modulated locally by different synaptic inputs. These neuroanatomical features of the T1 neurons make them suitable candidates for peripheral or local motor neurons. The finding that a type of axon terminal establishing neuro-muscular contacts in both the tegumental and the flexor muscle displayed an ultrastructure identical to that of the neuronal processes of the light neurons, supports this suggestion.

The organization of peripheral stimulus-response pathways mediating local tentacle movements

Until recently, peripheral motor neurons had not been identified in the tentacles; therefore the motor control of local tentacle movements without CNS control could not be

described. Because the external lateral flexion of the tentacle tip or quiver can be induced in isolated tentacle preparations, the motor commands to the effector muscles were thought to be generated by unknown local neuronal elements (Nikitin et al. 2005). The work presented here is the first description of the neuroanatomy of putative local motor neurons in the tentacle digits innervating the M3 and M2 flexor muscles, which are required to perform the external lateral bending of the tentacle (Hernádi and Teyke 2013). These local motor neurons provide the neuroanatomical background underlying the execution of the external lateral flexion of the tentacle tip, or quiver. It is suggested that the T1 type local motor neurons, in the tentacle digits, receive synaptic inputs from local neuronal elements such as olfactory receptor axons and local interneurons, which take part in olfactory information processing (Chase and Tolloczko 1986, 1993). Therefore olfactory stimuli may also modulate the electrical activity of the local motor neurons and generate motor commands to the distal segments of the flexor and tegumental muscles, leading to the external lateral flexion of the tentacle tip. The neuronal elements outlined here build up a peripheral stimulus-response pathway to generate local tentacle movements, including quiver, in response to olfactory stimuli (see Figs.8, 9).

The flexor and the tegumental muscles also receive central motor axons both via PT and via the olfactory nerve, forming the distinct and common innervation pathways, respectively (Hernádi and Teyke 2013). The common innervation pathway reaches flexor muscles through the tentacle digits (Hernádi and Teyke 2013) where they may receive excitatory inputs from local neuronal elements and from the olfactory receptor cell axons. Therefore we suggested that the distal axonal segments of the central motor axons generate motor commands to the flexor and tegumental muscles without the soma

excitation. This suggestion was supported by the present electrophysiological experiments. Electrical stimulation via the olfactory nerve, which innervates the flexor, tegumental and retractor muscles via the tentacle digits, elicited a tension response in each flexor muscle with the same efficiency, indicating that the stimulation excited the common innervation pathway to the tentacle (Hernádi and Teyke 2013).

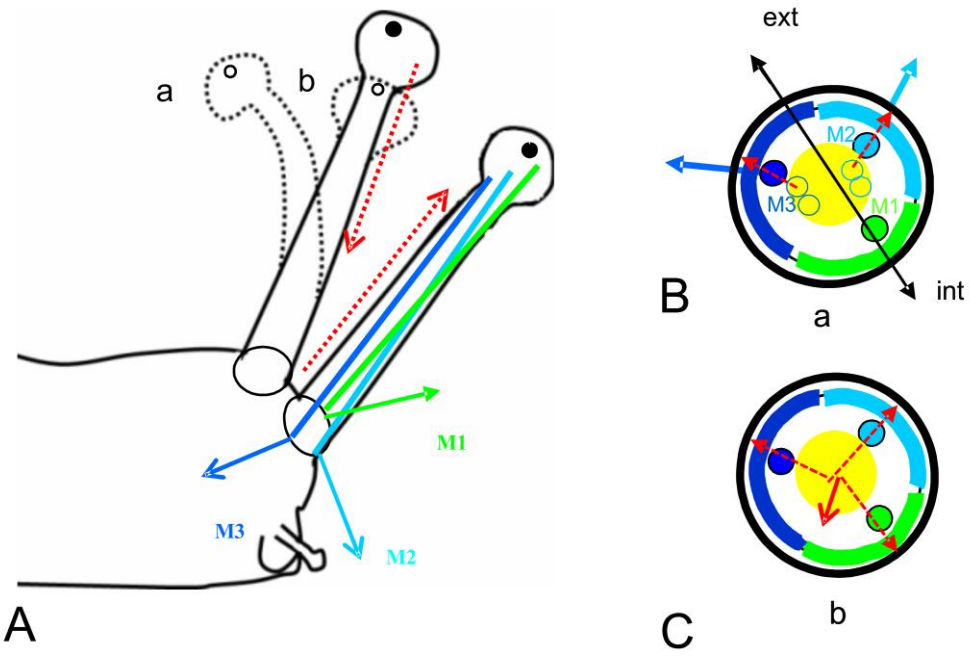

Fig.9A Schematic drawing compares the large scale tentacle movements (right tentacle) as bending around a basal pivot and the local tentacle movements (left tentacle) as the quiver (a) and the contraction (b). Through the central stimulus-response pathway motor commands via the peritentacular nerves initiate contractions in the proximal segment of the flexor and tegumental muscles which spread towards the tip of tentacle. (right tentacle, *red dotted arrow*). Each flexor muscle (*M1, M2, M3*) pulls the tentacle to a given direction (*arrows with different colors*) (for detail see Hernádi and Teyke 2013). Local tentacle movements as quiver (*a*) and brief contraction (*b*) are initiated in the distal segments of these muscles which spreads towards their proximal segments (left tentacle *red dotted arrow*). **B-C** Schematic drawings illustrate the two possible peripheral stimulus-response pathways for local tentacle movements. **B** When motor commands (*dotted red arrows*) to the flexor muscles (*M1 green, M2 light blue and M3 dark blue circle*) and the appropriate bands of tegumental musculature (*colored bands*) are generated by local motor neurons (*dark and light blue open circles*) in the tentacle digits (*solid yellow circle*) to induce quiver. **C** When motor commands (*dotted red arrows*) to these muscles are generated by distal axon segments of central motor axons in the tentacle digits (*yellow circle*) to induce brief contractions or twitch (solid red arrow). *black ring* sensory epithelium, *solid black arrows* external and internal space directions

Electrical stimulation via the different PT nerves was only effective at eliciting tension in the muscle they innervated, supporting the earlier suggestion that each flexor muscle receives a distinct motor innervation via a different PT nerve (Hernádi and Teyke 2013).

The observation that repeated stimulation and increased stimulus frequency via the iPT nerve, which innervates only the M1 muscle, also facilitated tension development in the M3 muscle suggested that when stimulating the distinct pathway via the iPT nerve, the distal axon segments of the common motor axons running in the muscle are also stimulated. Therefore, these stimulated motor axons can send motor commands to other flexor muscles via their side branches, located in other digits (see Fig.8). These features enable the peripheral compartments of the common motor pathway to play a role in the generation of local tentacle movements. This local network may build up another type of peripheral stimulus-response pathway (olfactory stimulus - peripheral compartments of central motor axons - motor response) to local tentacle movements. This type of peripheral stimulus-response pathway could explain the generation of the slow graded contraction of the posterior tentacle following long latency quiver, while the tip of tentacle remains protracted. In conclusion, the two microcircuits described above give a comprehensive neuroanatomical basis facilitating local tentacle movements without the involvement of the CNS.

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