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ULTRASTRUCTURE OF NEUROMUSCULAR CONTACTS IN THE EMBRYONIC POND SNAIL *LYMNAEA STAGNALIS* L.*

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Ultrastructural characteristics of muscle fibers and neuromuscular contacts were investigated during two stages of embryogenesis of the pulmonate snail *Lymnaea stagnalis.* The first muscle cells appear as early as during metamorphosis (50–55% of embryonic development), whereas previously, in the trochophore/veliger stages (25–45%), muscular elements cannot be detected at all. The first muscle fibers contain large amounts of free numbers, a well-developed rER system and only a few irregularly arranged contractile elements. The nucleus is densely packed with heterochromatine material. At 75% adult-like postmetamorphic stage, the frequency of muscle fibers increases significantly, but, bundles of muscle fibers cannot yet be observed. Furthermore the muscle cells are characterized by large numbers of free ribosomes and numerous rER elements. Fine axon bundles and single axon processes, both accompanied by glial elements, can already be found at this time. Axon varicosities with different vesicle and/or granule contents form membrane contacts with muscle fibers, but without revealing membrane specialization on the pre- or postsynaptic side. The late development of the muscle system and neuromuscular contacts during *Lymnaea* embryogenesis correlates well with the maturation of different forms of behavior of adult, free-living life, and also with the peripheral appearance of chemically identified components of the embryonic nervous system of central origin.

Keywords: Neuromuscular contacts – embryogenesis – ultrastructure – snail – *Lymnaea*

INTRODUCTION

One of the major advantages of invertebrates is that their nervous system offers an exceptionally good possibility for study of the organization of the simple reflex arches, responsible for the execution of different forms of behaviors. In these actions, mostly muscle fibers are the target cells of the efferent neurons involved in carrying out the appropriate responses of the animal to the environment. As regards nerve-muscle interactions, numerous data have been published on the formation of neuromuscular interactions under different experimental conditions or in mutants and also during development, although most of our knowledge in this respect originates primarily from arthopods, and mainly insects [7–9, 18, 24]. Neuromuscular contacts

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have also widely been studied at the ultrastructural level in invertebrates [1, 2, 14, 19, 20, 23, 26, 37], including different molluscan species. In pulmonate snails, ultrastructural studies on the genital organs [6], heart [10, 25], osphradium [5], and different somatic and visceral muscles [3, 4, 33] revealed that some of the contacts possess a certain specialization, whereas others show only a close (16–20 nm) apposition of the pre- and postsynaptic membranes.

Novel aspects in studies of the anatomical and ultrastructural characteristics of neuromuscular innervation in gastropods resulted from the application of immunocytochemistry, whereby the neurochemical specifics of the efferentation could be identified by the application of antibodies such as anti-5-HT [12], anti-FMRFamide [15, 16, 22], anti-MIP [13] or anti-buccalin and anti-SCPs [38]. The innervation of different musculatures have been described, demonstrating neuromuscular contacts in the aorta wall [22], heart, intestine, tentacles, buccal mass and connective tissue sheath [12, 13, 15, 16], and in the anterior buccal retractor muscle [38], most of them again without revealing membrane specializations.

In spite of this rather wide range of investigations on adult gastropods, little information is available on the development of peripheral contacts. In the course of a series of light microscopic immunocytochemical investigations on embryos of *Lymnaea* and related species, the aminergic [11, 40, 43] and peptidergic [41, 42] innervation of different peripheral tissues (foot, mantle and buccal mass) has been described. At the ultrastructural level, Marois and Carew [27] have demonstrated the peripheral targets of 5-HT immunoreactive central neurons in larval *Aplysia*, including numerous muscle systems. However, we have no data on the sequence of development of muscle fibers and different musculatures, respectively, or on the ultrastructural characteristics of neuromuscular contacts. Therefore, in this study, we have analyzed the appearance and ultrastructure of muscle fibers and nerve-muscle contacts in two decisive stages of embryogenesis (metamorphosis and the postmetamorphic, adult-like stage) of the pond snail, *Lymnaea stagnalis*, a favorite model animal in both neurobiology and embryology. In this way, we wanted to furnish a basis for future investigations, with data on the maturation of the innervation of different peripheral targets, such as the heart, buccal mass, intestine, salivary gland and certain members of the somatic musculature, and on the neurochemical characterization of the developing nerve-muscle contacts. Such investigations of the nerve-muscle interactions, combined with identification of their central, efferent and intrinsic elements, may bring us closer to an understanding of the cellular background of development of different forms of behavior, necessary for the juvenile and adult foraging life of gastropods.

MATERIALS AND METHODS

Embryos of *Lymnaea stagnalis* from our laboratory colony were used. First, the embryonic development was staged, according to Mescheriakov [29], Morill [30], Marois and Croll [28]: each stage was characterized by a specific set of morphological features. Stages were expressed as a percentage of total embryonic development,

according to Marois and Croll [28], with 0% corresponding to the first cleavage and 100% to hatching. Embryos of the following stages were used for our investigations: 35% veliger, 50–55% metamorphic, and 75% postmetamorphic, adult-like stages. Morphologically, 35% embryos were identified by the appearance of the bilobed foot, 50% embryos by the appearance of the pigmented eyes, 55% embryos by the shell extending over the visceral mass, and 75% embryos by the pigmentation of the head and foot and by the torsion of the shell.

Prior to fixation, the egg capsules were removed, and the embryos were then fixed in a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde diluted in 0.01 M phosphate buffer (PB) for 2 h at 4 °C. Following a 2×5 min washing in PB, postfixation was performed in 1% OsO_4 diluted in 0.01 M sodium cacodylate for 30 min at room temperature. As described earlier [31], the application of buffer solutions of low molarity was the determining factor for achieving good ultrastructural preservation. The embryos were dehydrated in graded ethanol and propylene oxide, and embedded in Araldite (Durcupan, Fluka). Block staining with saturated uranyl acetate was performed in 70% ethanol for 30 min.

From the embryos, 1 μm serial semi-thin sections were cut first for orientation on an LKB Novacut ultramicrotome and stained with toluidine blue. Ultrathin sections were taken from the anterior body (head/foot) region, stained with lead citrate [34] and then investigated in a Tesla BS500 electron microscope. For the investigation of each embryonic stage, a total of 10–12 specimens were used.

RESULTS

50–55% embryonic developmental (metamorphic) stage

Before this stage of embryonic development (35%, veliger stage) no muscle fibers could be found in the embryos.

In the 50–55% stage of embryonic development, during metamorphosis, the first muscle fibers were found in the anterior region of the body. These solitary muscle fibers were distributed irregularly in the rostral body part of the embryos. They were located among other cell components that were either pluripotent embryonic cells or epithelial cells near the ciliated epithelial layer of the foot. The early-developing muscle cells revealed typical ultrastructural characteristics, containing only a few contractile elements and a very high number of free ribosomes, together with rough endoplasmic reticulum (rER) units and mitochondria, whereas the general ultrastructural appearance of the muscle fibers was dominated by free ribosomes (Fig. 1). Ribosomes were also seen along the nucleus membrane (Fig. 1A). The nucleus of the muscle cells contained a rich heterochromatin material. All these observations may be indicative of a very intensive synthesis activity. The few contractile elements that occurred were found both in the vicinity of the nucleus and in more distant sarcoplasmic regions, exhibiting either a parallel or an irregular arrangement (Fig. 1). However, the sarcoplasm was mostly completely free of contractile elements.

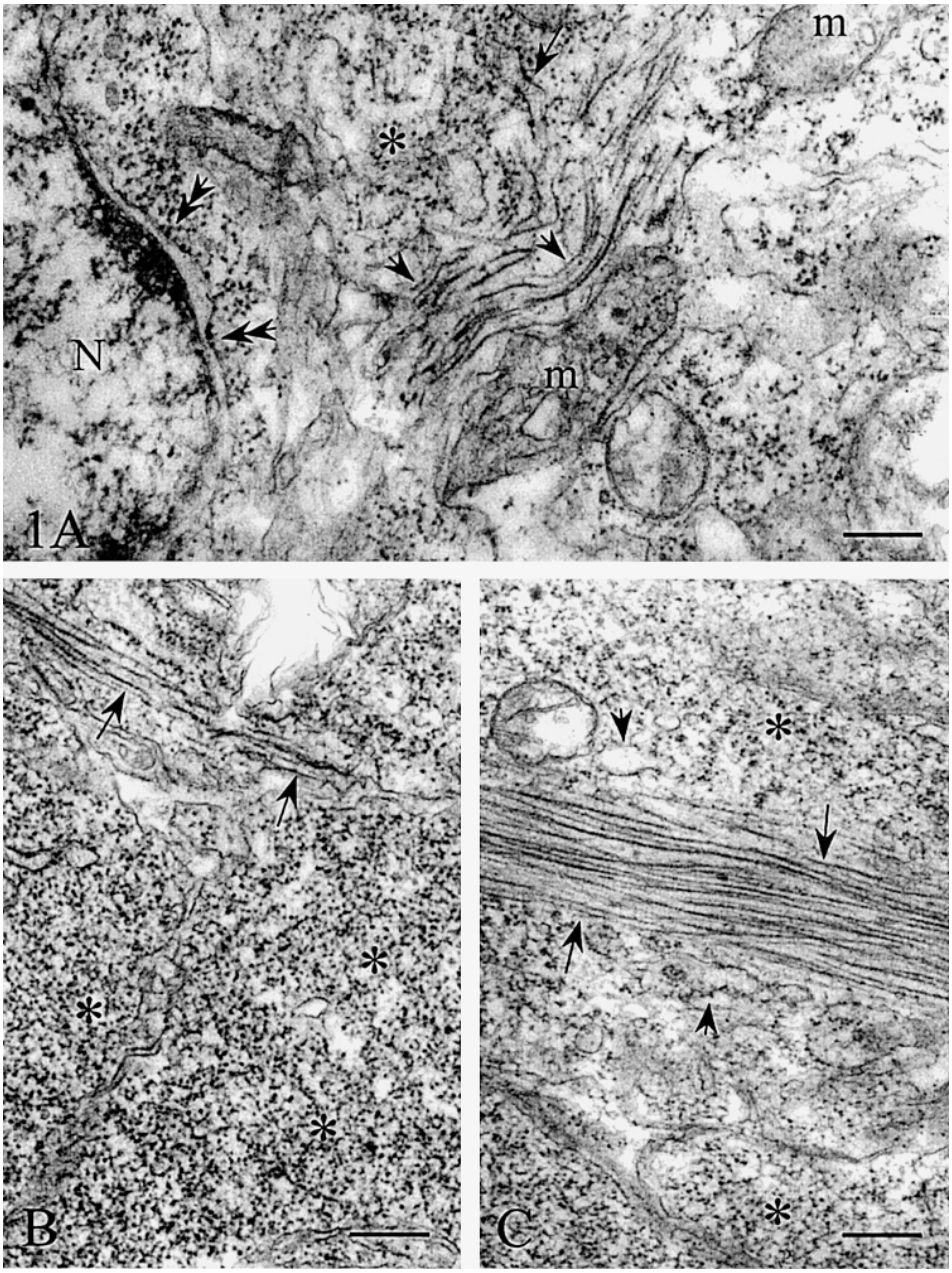


Fig. 1. Ultrastructure of embryonic muscle cells in the 50% embryonic stage of *Lymnaea stagnalis*. A. Perinuclear region showing the nucleus (N), free ribosomes (asterisks), rER elements (arrows), mitochondria (m), and a few irregularly arranged contractile filaments (arrowheads). Note the ribosomes on the nucleolemma (small arrows). B. Detail from a sarcoplasm containing an enormous number of free ribosomes (asterisks). Arrows indicate contractile filaments. C. Contractile elements (arrows) arranged in parallel in the sarcoplasm. Arrowheads – rER elements, asterisks – free ribosomes. Bar for A, B, C: 0.5 μm

75% embryonic developmental (postmetamorphic, adult-like) stage

At this stage of embryonic development, both ultrastructurally well-organized muscle fibers and innervating nervous elements contacting the muscle cells could be observed. Solitary muscle fibers continued to occur rather than in bundle-like arrangements, although many more muscle cells could be observed near each other as compared with the 50% embryos (Fig. 2).

The muscle cells were primarily characterized by containing bundles of contractile elements, which at this stage of development always displayed a parallel arrangement (Figs 2, 3A and 4). At the same time, the numbers of free ribosomes and of rER elements were still significant. In certain muscle cells, the sarcoplasm contained swollen rER elements along the sarcolemma, and the nucleus membrane also exhibited swollen segments, covered by ribosomes (Fig. 3). Golgi units surrounded by numerous budding-off vesicles occurred.

Apart from the numerous muscle fibers and their relatively well-developed ultrastructure, new components of the muscularization of the *Lymnaea* body studied could be seen. These were axon bundles accompanied by glial cells, and neuromuscular contacts. The axon bundles and small nerves were composed of tightly packed axon profiles of different diameters, containing mostly neurotubules, mitochondria and, rarely, granular vesicles and granules (Fig. 4). Axons with very small (less than 0.5 μm) diameters could also be observed occasionally within the bundles, suggesting a mixed, efferent and afferent function.

Along the solitary muscle fibers and among the muscle fibers located near each other axon profiles and varicosities could be discerned (Figs 5, 6). These profiles formed close (16–20 nm) membrane contacts with the muscles cells, but without revealing any membrane specializations (Fig. 6). Some degree of asymmetric vesicle accumulation at the "presynaptic" membrane and also fine intersynaptic cleft material and occasionally subsynaptic cisterns located along the postsynaptic membrane were present (Figs 9–11). The neuromuscular contacts were sometimes formed for a relatively long distance along the opposing membrane segments (Figs 6, 8A). Varicosities of different sizes were found to be deeply embedded in the muscle fiber or surrounded by sarcoplasmic processes (Figs 10, 11). Other varicosities located relatively far from the muscle fibers but with free membrane segments facing towards the muscle cell could also be observed (Fig. 7). In the varicosities contacting the muscle fibers, mostly 50–60 nm agranular and 80–120 nm granular vesicles could be seen (Figs 7–11).

DISCUSSION

The present ultrastructural findings parallel well with the body-pattern and behavioral development of *Lymnaea stagnalis* embryos. In accordance with our earlier observations [43], adult-like locomotion of the embryos (gliding) which is clearly different from the also ciliary-based intracapsular rotation, and adult-like feeding

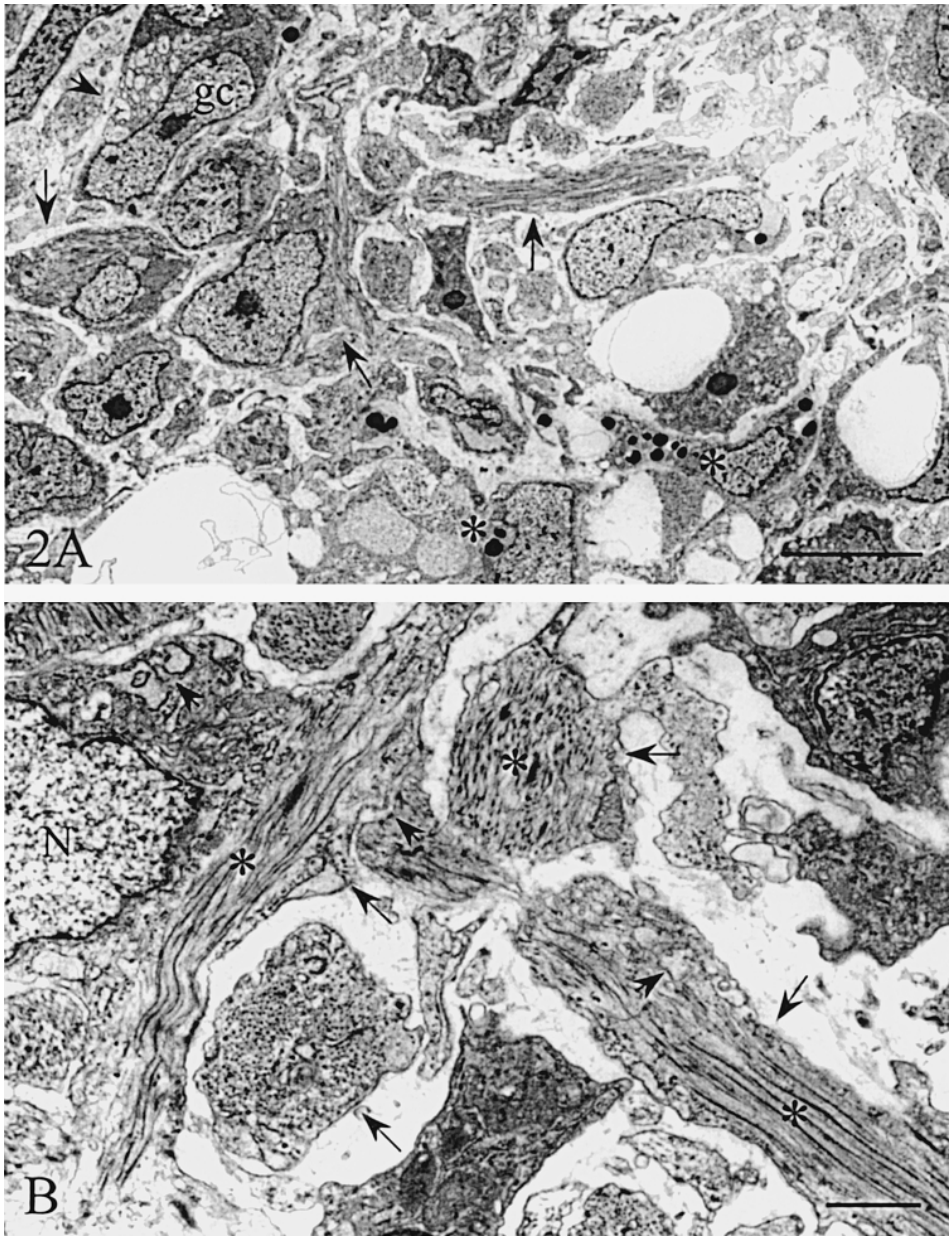


Fig. 2. A. Low-power electron micrograph taken from the rostral body part of a 75% *Lymnaea* embryo. Solitary muscle fibers (arrows), pigmented epithelial cells (asterisks) and an axon bundle (arrowhead) accompanied by a glial cell (gc) are seen. Bar: 2 µm. B. Developing muscle fibers (arrows) in a 75% *Lymnaea* embryo. Note the parallel arrangement of contractile filaments (asterisks) and swollen rER elements (arrowheads). N – nucleus. Bar: 1 µm

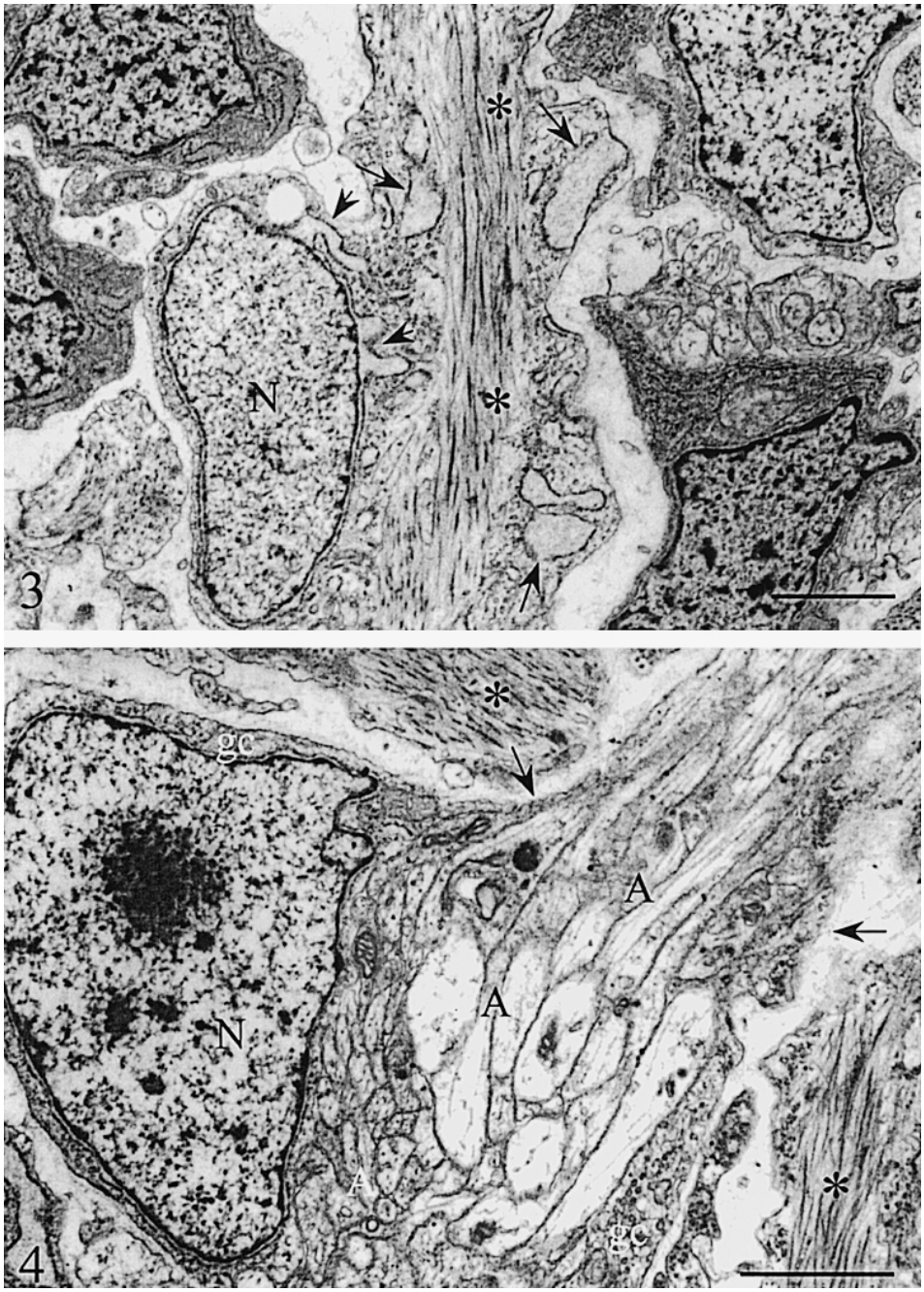


Fig. 3. Developing muscle fiber in a 75% *Lymnaea* embryo, containing numerous large, swollen elements of the rER system (arrows). Note swollen segments of nucleus membrane covered with ribosomes (arrowheads). N – nucleus, asterisks – contractile filaments. Bar: 1 μm. *Fig. 4.* An axon bundle containing axon profiles (A) of different diameters is accompanied and partially covered by a glial cell (gc and arrows). N – nucleus, asterisks – muscle fibers. Bar: 1 μm

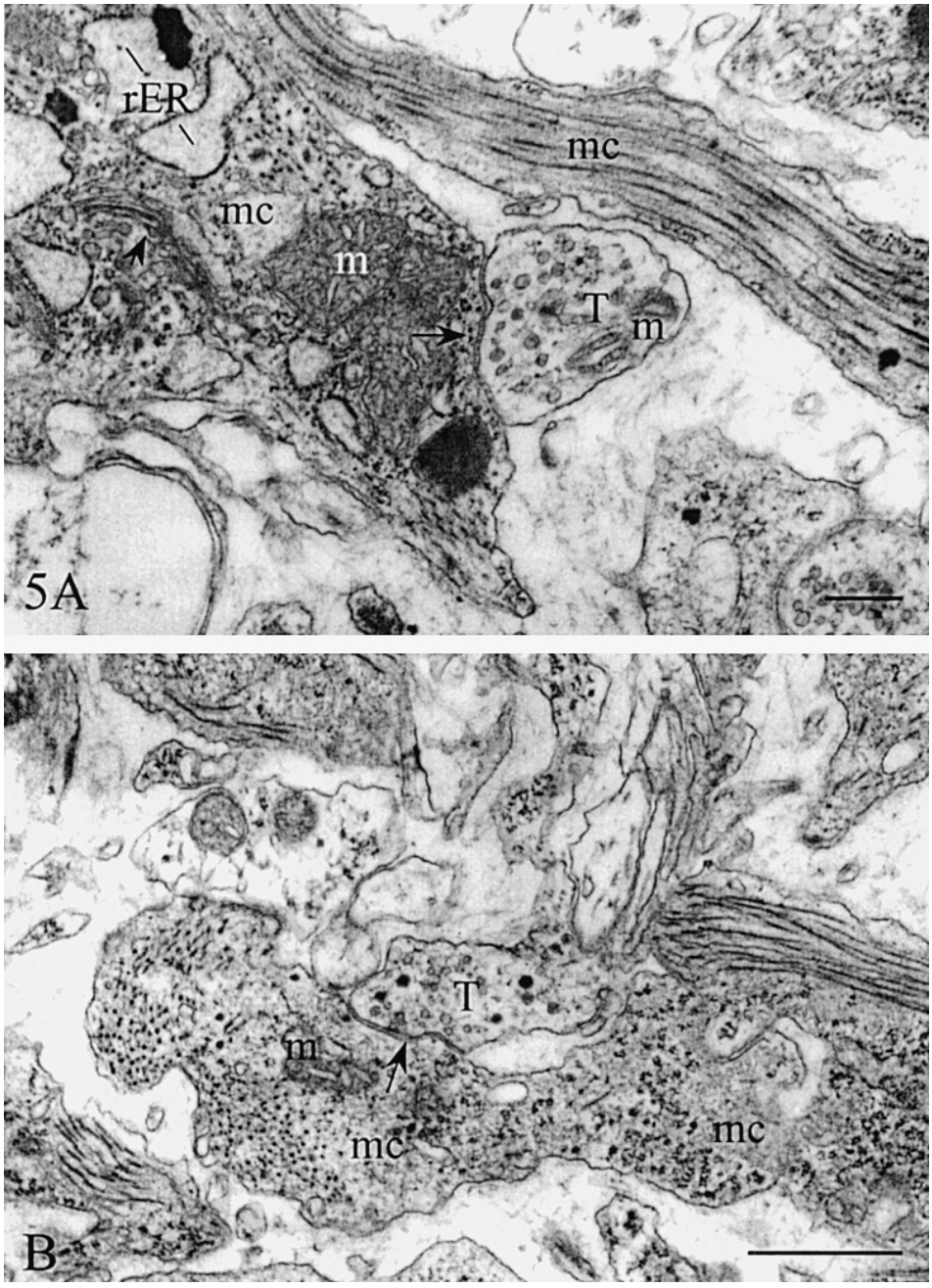


Fig. 5. Varicosities (T) containing both agranular (A) and granular (dense-core) vesicles (B) contact (arrows) muscle fibers (mc) without membrane specializations. m – mitochondria, rER – rER elements, arrowhead – Golgi unit. Bar for A, B: 1 μm

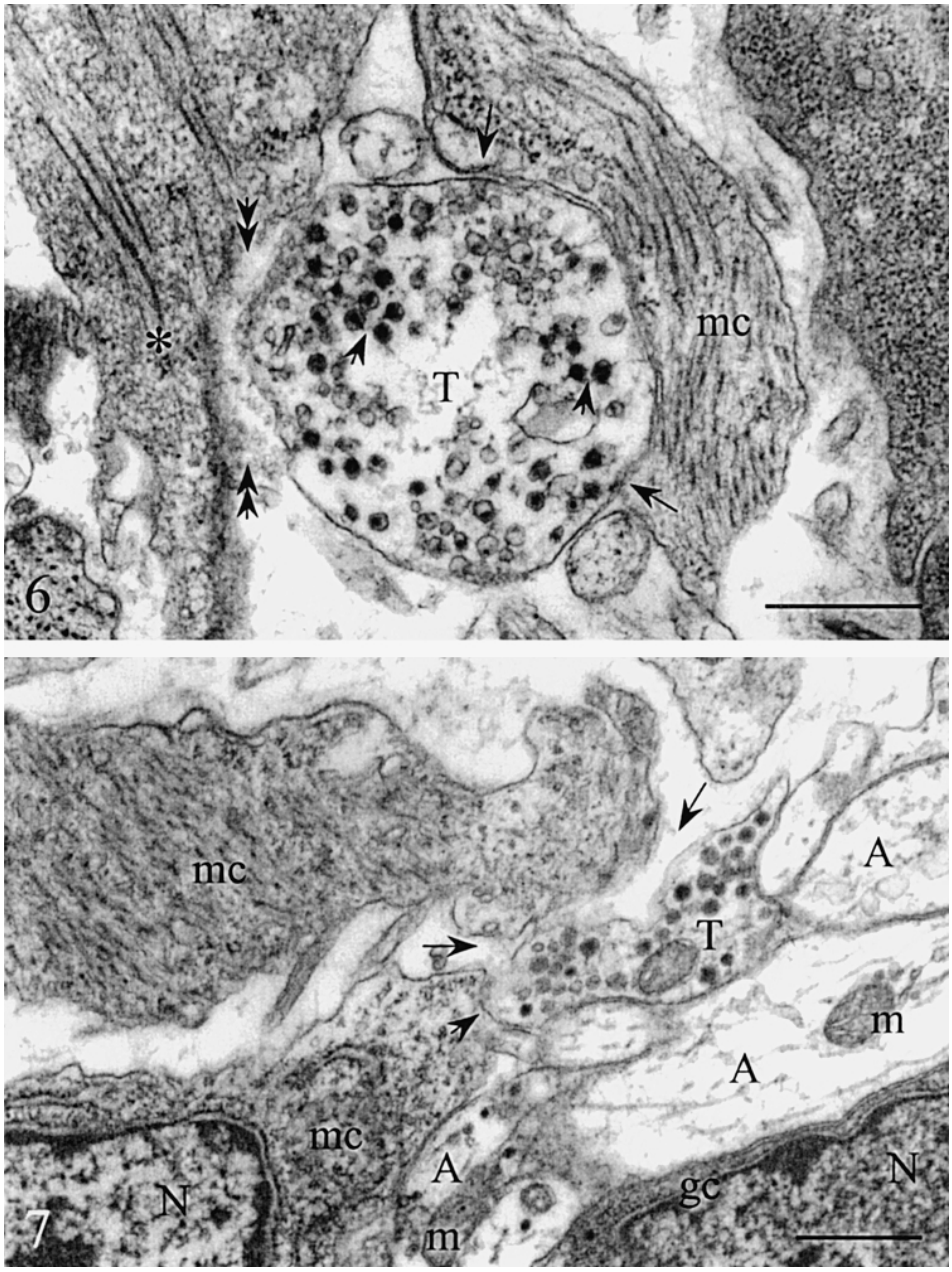
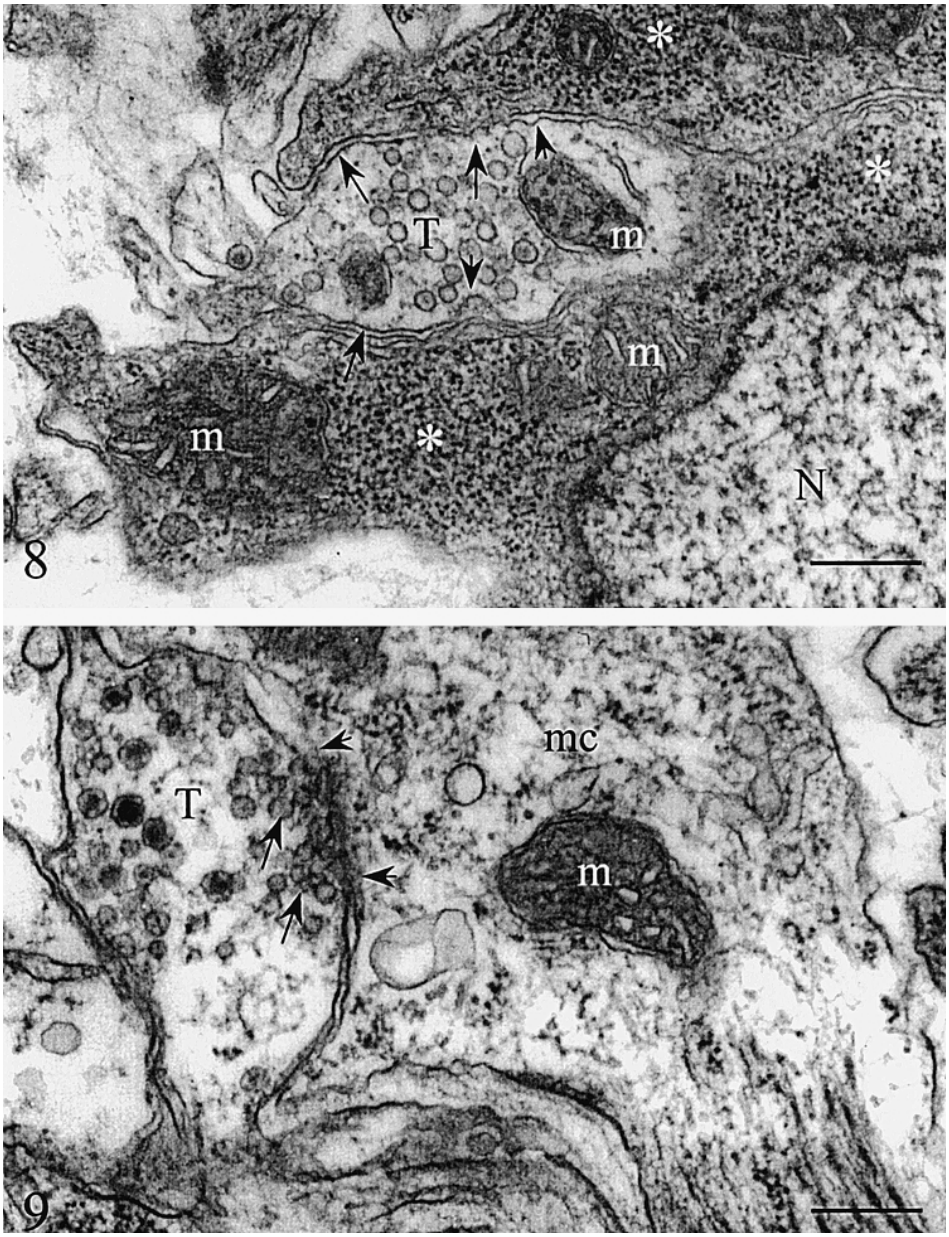
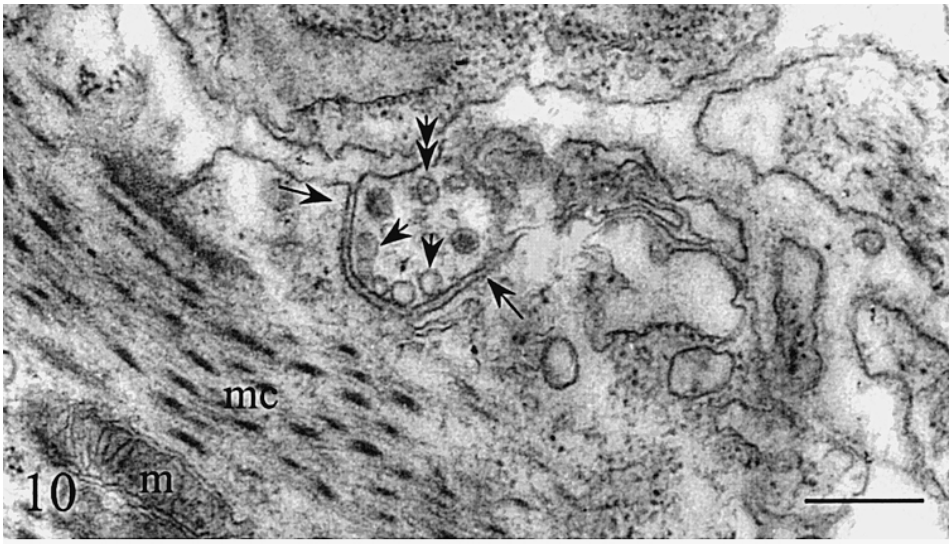
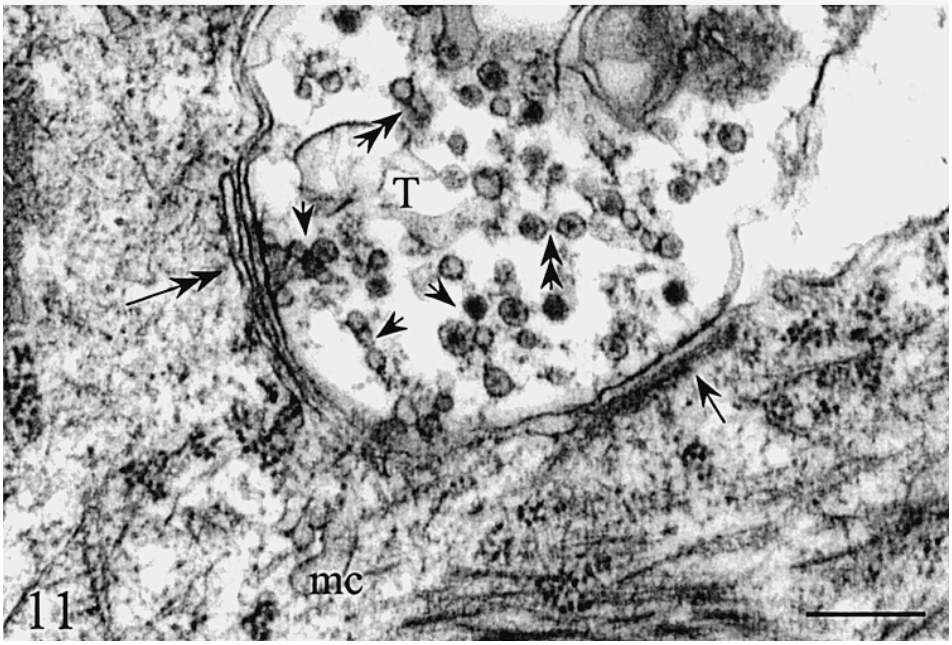


Fig. 6. A large varicosity (T) containing granular (dense-core) vesicles (arrowheads) contacts a muscle cell (mc) with a long, unspecialized membrane segment (between arrows). The varicosity opposes another muscle fiber (asterisk) with a wide intercellular cleft (between double arrowheads). Bar: 1 µm. *Fig. 7.* An axon bundle (A) located between muscle fibers (mc) and a glial cell (gc) contains an axon profile (T) with a free axolemma segment facing towards one of the muscle fibers (between arrows), whereas it forms a close membrane contact with another one (arrowhead). N – nucleus, m – mitochondrium. Bar: 1 µm



Figs 8 and 9. Two types of neuromuscular contacts found in 75% *Lymnaea* embryos. *Fig. 8.* A varicosity (T) contacting sarcoplasmic protrusions with long unspecialized membrane segments (arrows). Note the small membrane indentations and a large coated pit (arrowheads) along the "presynaptic" membrane. The sarcoplasm is almost completely filled with free ribosomes (asterisks). N – nucleus, m – mitochondrium. *Fig. 9.* A varicosity (T) contacting a muscle cell (mc) reveals typical presynaptic clustering of agranular vesicles (arrow) along the active zone of the contact (arrowheads). m – mitochondrium. Bar for A, B: 0.5 μm





Figs 10 and 11. Higher magnification of neuromuscular contacts in 75% *Lymnaea* embryos. *Fig. 10.* A small varicosity (double arrowhead) deeply embedded in the sarcoplasm of a muscle fiber (mc) forms a neuromuscular contact (between arrows). Note the lining-up of agranular synaptic vesicles (arrowheads) along the presynaptic membrane and the presence of intersynaptic cleft material. m – mitochondrium. Bar: 0.25 μm. *Fig. 11.* A varicosity (T) contacts a muscle fiber (mc) with membrane specializations, such as presynaptic vesicle clustering (arrowhead), postsynaptic membrane apposition (arrow), subsynaptic cistern (double arrow), and intersynaptic cleft material. Note the mixed population of agranular and granular (dense-core) vesicles (arrowheads and double arrowheads) in the axon profile. Bar: 0.25 μm

behavior (radula protrusions) start to appear by the end of metamorphosis (E55–65% stage of embryogenesis). In metamorphosing *Lymnaea* embryos, early muscle fibers are found very rarely, containing only "traces" of contractile filaments, but there are enormous numbers of free ribosomes and other elements of the protein synthesis apparatus that are otherwise unusual components of the ultrastructure of adult muscle cells. In postmetamorphic, adult-like snails, however, muscle fibers with relatively well-developed contractile apparatus and neuromuscular contacts can already be found, suggesting a functional need for the involvement of different muscles in the maturation of both somatic (body posturing) and vegetative (feeding) functions.

The ultrastructure of the neuromuscular contacts observed in the adult-like (postmetamorphic) *Lymnaea* embryos in most cases resembles that of the unspecialized close membrane contacts of modulatory character described in adult gastropods, such as *Lymnaea* and *Helix*. In an early detailed ultrastructural analysis of the innervation of the musculature in *Lymnaea stagnalis* [33], different types of nerve endings were found both to form neuromuscular contacts and to be located free in the connective tissue sheath. The neuromuscular contacts visualized were partly characterized by the presence of certain membrane specializations, such as an increased electron density of the pre- and postsynaptic active zones of the opposing membrane segments and intersynaptic cleft material, in addition to presynaptic vesicle/granule clustering. Other contacts lacked these features, but only the opposing membrane segments were separated by a close (16–20 nm) cleft. The developing neuromuscular contacts in *Lymnaea* resemble these latter, suggesting that when the embryos begin adult-like movements and muscle activities, there is less need for a ready-to-go structure (and functions) of the nerve muscle interaction, as a kind of functional "training" for the posthatching juvenile/adult foraging life. Whether these embryonic neuromuscular contacts are functionally immature or already maturing might be decided by receptor and/or ligand localization techniques, whether this be a pharmacological characterization or post-embedding immunogold electron microscopy or with the application of specific immunostainings (for synapsin, connexin, etc.) for the identification of synaptic contacts.

The appearance of the neuromuscular contacts in the *Lymnaea* embryos in the 75% stage disclosed different vesicle and granule types in the nerve endings. The agranular synaptic vesicles, granular (dense-core) vesicles and electron-dense granules, however, revealed less diversity of ultrastructure as compared with that described in the periphery [33] or CNS [35] of adult *Lymnaea*. Especially the lack or relatively low frequency of potentially peptidergic granules is striking. In the course of a preliminary immunogold electron microscopic study using anti-FMRFamide antiserum [32], a very small number of labeled axon profiles containing granular vesicles were demonstrated in *Lymnaea* embryos. The ultrastructure of these granular vesicles was rather variable within the same and different axon profiles, suggesting an immature chemical (peptidergic) character of them. At the neuromuscular contacts found in the periphery of adult *Lymnaea*, altogether nine different axon profiles have been distinguished according to their vesicle and granule contents [33]. Comparison of the embryogenesis of the chemical specificity of different signaling

systems in *Lymnaea* (5-HT [11, 21, 39]; dopamine [43]; octopamine [17]; FMRFamide and related peptides [40–42]; nitrogen monoxide [36] with the relatively late appearance of the neuromuscular contacts suggests a correlation between the time sequences of the two processes, showing that axon profiles start to innervate the muscle cells when the distribution of the immunocytochemically different networks of axon processes becomes apparent in both the target organs and the CNS. It has also been demonstrated that early (30–35%), transient elements of the embryonic 5HTergic [39] and FMRFamidergic [40–42] systems form arborizations in the periphery. Since we failed to find muscle fibers in the embryos before metamorphosis, this observation can be interpreted an indirect evidence of the roles of these early transient cells in general morphogenetic processes and in the regulation of non-muscular, e.g. ciliated epithelial cells.

In the course of a detailed ultrastructural study, we recently demonstrated that the first synaptoid structures in the neuropil of the ganglia of the developing CNS of *Lymnaea* embryos appear by the 75% stage of embryogenesis, whereas true specialized axo-axonic synapses can be observed first at hatching [31]. Until this stage, the unspecialized axo-axonic and axo-somatic contacts dominate. This slow process of synaptogenesis may correspond to the late appearance of neuromuscular contacts in the periphery. Although the ultrastructure of neuromuscular contacts in hatchings remains to be investigated, one may speculate about the existence of a common on-signal, orchestrating the developmental organization of intercellular contacts in both the CNS and the periphery during the embryogenesis of *Lymnaea stagnalis*, in addition to the decisive role of the postsynaptic elements in this process.

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REFERENCES

1. Atwood, H. L., Cooper, R. L. (1995) Functional and structural parallels in crustaceans and *Drosphila* neuromuscular systems. *Am. Zool. 35,* 556–565.
2. Atwood, H. L., Govind, C. K., Wu, C.-F. (1993) Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *J. Neurobiol. 24,* 1008–1024.
3. Barrantes, F. J. (1970) The neuromuscular junctions of a pulmonate mollusc. I. Ultrastructural study. *Z. Zellforsch. 104,* 205–212.
4. Benedeczky, I., Halasy, K. (1988) Visualization of non-synaptic release sites in the myenteric plexus of the snail *Helix pomatia*. *Neuroscience 25,* 163–170.
5. Benjamin, P. R., Peat, A. (1972) Myoneural junctions in the connective tissue sheath of a molluscan ganglion. *Nature 219,* 1371–1372.
6. Bogusch, G. (1972) Zur Innervation des glatten Penisretraktormuskels von *Helix pomatia*: Allgemeine Histologie und Histochemie des monoaminergen Nervenystems. *Z. Zellforsch. 126,* 383–401.
7. Chang, T. N., Keshishian, H. (1996) Laser ablation of *Drosophila* embryonic motoneurons causes ectopic innervation of target muscle fibers. *J. Neurosci. 16,* 5715–5716.

8. Consoulas, C., Kent, K., Levine, R. B. (1996) Remodeling of the peripheral processes and presynaptic terminals of leg motoneurons during metamorphosis of the hawkmoth*, Manduca sexta. J. Comp. Neurol. 372,* 415–434.
9. Consoulas, C., Levine, R. L. (1998) Presynaptic function during muscle remodeling in insect metamorphosis. *J. Neurosci. 18,* 5817–5831.
10. Cottrell, G. A., Osborne, N. (1969) A neurosecretory system terminating in the *Helix* heart. *Comp. Biochem. Physiol. 28,* 1455–1459.
11. Diefenbach, T. J., Sloley, B. D., Goldberg, J. I. (1995) Neurite branch development of an identified serotonergic neuron from embryonic *Helisoma:* Evidence for autoregulation by serotonin. *Dev. Biol. 167,* 282–293.
12. Elekes, K. (1991) Serotonin-immunoreactive varicosities in the cell body region and neural sheath of the snail, *Helix pomatia*: an electron microscopic immunocytochemical study. *Neuroscience 42,* 583–591.
13. Elekes, K. (2000) Ultrastructural aspects of peptidergic modulation in the peripheral nervous system of *Helix pomatia. Micr. Res. Tech. 49,* 534–546.
14. Elekes, K., Hustert, R. (1988) The efferent innervation of the genital chamber muscle by an identified serotoninergic neuron in the female cricket *Acheta domestica. Cell Tiss. Res. 252,* 449–457.
15. Elekes, K., Ude, J. (1993) An immunogold electron microscopic analysis of FMRFamide-like immunoreactive neurons in the CNS of *Helix pomatia*: ultrastructure and synaptic connections. *J. Neurocytology 22,* 1–13.
16. Elekes, K., Ude, J. (1994) Peripheral connections of FMRFamide-immunoreactive neurons in the snail *Helix pomatia*. An immunogold electron microscopic study. *J. Neurocytology 23,* 758–769.
17. Elekes, K., Voronezhskaya, E. E., Hiripi, L., Eckert, M., Rapus, J. (1996) Octopamine in the developing nervous system of the pond snail, *Lymnaea stagnalis* L. *Acta Biol. Hung. 47,* 73–87.
18. Fernandez, J. J., Keshishian, H. (1998) Nerve-muscle interactions during flight muscle development in *Drosophila. Development 125,* 1769–1779.
19. Florey, E., Cahill, M. A. (1982) The innervation pattern of crustaceal skeletal muscle. *Cell Tissue Res. 224,* 527–541.
20. Gerschenfeld, H. M. (1973) Chemical transmission in invertebrate central nervous system and neuromuscular junctions. *Physiol. Rev. 53,* 1–119.
21. Goldberg, J. I., Kater, S. B. (1989) Expression and function of the neurotransmitter serotonin during development of the *Helisoma* nervous system. *Dev. Biol. 131,* 483–495.
22. Griffond, B., Boer, H. H., Wijdenes, J. (1986) Localization and function of an FMRFamide-like substance in the aorta of *Helix aspersa*. *Cell Tissue Res. 246,* 303–307.
23. Hoyle, G. (1983) *Muscles and Their Neural Control.* John Wiley and Sons, New York.
24. Keshishian, H., Broadie, K., Chiba, A., Bate, M. (1996) The *Drosophila* neuromuscular junction: a model system for studying synaptic development and function. *Ann. Rev. Neurosci. 19,* 545–575.
25. Kiss T., Elekes K. (1972) Myo-neural junctions in the ventricle of the snail *Helix pomatia*. *Acta Biol. Hung. 23,* 207–209.
26. Kiss, T., Varanka, I., Benedeczky, I. (1984) Neuromuscular transmission in the visceral muscle of locust oviduct. *Neuroscience 12,* 309–322.
27. Marois, R., Carew, T. J. (1997) Projection patterns and target tissues of the serotonergic cells in larval *Aplysia californica*. *J. Comp. Neurol. 386,* 491–506.
28. Marois, R., Croll, R. P. (1992) Development of serotoninlike immunoreactivity in the embryonic nervous system of the snail *Lymnaea stagnalis*. *J. Comp. Neurol. 322,* 255–265.
29. Mescheriakov, V. N. (1990) The common pond snail *Lymnaea stagnalis* L. In: Detlaff, D. A., Vassetzky, S. G. (eds), *Animal Species for Developmental Studies.* Plenum Press, New York, London, pp. 69–132.
30. Morrill, J. B. (1982) Development of the pulmonate gastropod, *Lymnaea*. In: Harrison, F. W., Cowden, R. R. (eds), *Developmental Biology of the Freshwater Invertebrates.* Alan P. Liss, New York, pp. 399–483.

31. Nagy, T., Elekes, K. (2000) Embryogenesis of the central nervous system of the pond snail, *Lymnaea stagnalis* L. An ultrastructural study. *J. Neurocytology 29,* 43–60.
32. Nagy, T., Ude, J., Voronezhskaya, E. E., Elekes, K. (1998) Ultrastructural and immunogold (FMRFamide) characterisation of the *Lymnaea* CNS in two specific developmental stages. *Neurobiology 6,* 232–233.
33. Plesch, B. (1977) An ultrastructural study of the innervation of the musculature of the pond snail *Lymnaea stagnalis* (L.) with reference to peripheral neurosecretion. *Cell Tissue Res. 183,* 353–369.
34. Reynolds, E. S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol. 17,* 208–212.
35. Roubos, E. W., Moorer-van Delft, C. M. (1979) Synaptology of the central nervous system of the freshwater snail *Lymnaea stagnalis* (L.), with particular reference to neurosecretion. *Cell Tissue Res. 198,* 217–235.
36. Serfőző, Z., Elekes, K., Varga, V. (1998) NADPH-diaphorase activity in the nervous system of the embryonic and juvenile pond snail, *Lymnaea stagnalis*. *Cell Tissue Res. 292,* 579–586.
37. Suzuki, E., Rose, D., Chiba, A. (2000) The ultrastructural interactions of identified pre- and postsynaptic cells during synaptic target recognition in *Drosophila* embryos. *J. Neurobiol. 43,* 448–459.
38. Vilim, F. S., Price, D. A., Lesser, W., Kupfermann, I., Weiss, K. (1996) Costorage and corelease of modulatory peptide cotransmitters with partially antagonistic actions on the accessory radula closer muscle of *Aplysia californica*. *J. Neurosci. 16,* 8092–8104.
39. Voronezhskaya, E. E., Elekes, K. (1993) Distribution of serotonin-like immunoreactive neurones in the embryonic nervous system of lymnaeid and planorbid snails. *Neurobiology 1,* 371–383.
40. Voronezhskaya, E. E., Elekes, K. (1996) Transient and sustained expression of FMRFamide-like immunoreactivity in the developing nervous system *Lymnaea stagnalis* (Mollusca, Pulmonata). *Cell. Molec. Neurobiol. 16,* 661–676.
41. Voronezhskaya E. E., Elekes K. (1997) Expression of FMRFamide gene neuropeptides is partly different in the embryonic nervous system of the pond snail, *Lymnaea stagnalis* L. *Neurobiology 5,* 91–93.
42. Voronezhskaya, E. E., Elekes, K. (2001) Expression of peptides encoded by the FMRFamide gene in the developing nervous system of *Lymnaea stagnalis*. *J. Comp. Neurol.* (submitted, under revision)
43. Voronezhskaya, E. E., Hiripi, L., Elekes, K., Croll, R. P. (1999) Development of catecholaminergic neurons in the pond snail, *Lymnaea stagnalis*: I. Embryonic development of dopamine-containing neurons and dopamine-dependent behaviors. *J. Comp. Neurol. 404,* 285–296.
44. Wojtowicz, J. M., Marin, L., Atwood, H. L. (1994) Activity-induced changes in synaptic release sites at the crayfish neuromuscular junction. *J. Neurosci. 14,* 3688–3703.