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Innervation of Heart and Alary Muscles in *Sphinx ligustri* L. (Lepidoptera)

A Scanning and Transmission Electron Microscopic Study*

Lutz Thilo Wasserthal and Wiltrud Wasserthal**

Lehrstuhl für Spezielle Zoologie, Ruhr-Universität Bochum, Bochum, Federal Republic of Germany

Summary. The origin and orientation of the heart nerves in *Sphinx ligustri* and *Ephestia kuehniella* were investigated by scanning electron microscopy using a special technique which involved pinning the dissected specimens on a stabilizing metal pad. The heart and alary muscles in *Sphinx* particularly their caudal extremity were also examined by transmission electron microscopy. The alary muscles form an incomplete sheath around the heart with a mainly longitudinal fibre orientation, e.i. antagonistically to the fibres of the heart itself. The heart and alary muscles are multiterminally innervated by branches of the transverse segmental nerves. All branches contain a single electron lucent axon; the thickest branches also possess several neurosecretory axons. Swellings of the segmental nerves may indicate the position of nerve cell bodies. There are no lateral heart nerves. Only one type of neuromuscular junction is abundant in the alary muscles but less frequently found in the heart. The terminals originate from the central axon only. They are capped by glial cells, which interdigitate with the muscle cells. They penetrate into the T-system toward the Z-discs and form a complex intercellular space system. Exocytosis of dense-cored vesicles into this “perisynaptic reticulum” seems likely. Sites of neurohaemal release are distributed along the nerve branches and special nerve endings occur at the level of the ostia. The possible nervous influence upon heart activity is discussed.

Key words: Heart innervation – Lepidoptera – Neuromuscular junctions – Perisynaptic reticulum – Neurohaemal secretion.

Send offprint requests to: Dr. Lutz Thilo Wasserthal, Lehrstuhl für Spezielle Zoologie der Ruhr-Universität, Postfach 102148, D-4630 Bochum-Querenburg, Federal Republic of Germany

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Zusammenfassung. Ursprung und Verlauf der Herznerven bei *Sphinx ligustri* und *Ephestia kuehniella* wurden unter Verwendung von Präparationsunterlagen aus Bleiblech rasterelektronenmikroskopisch untersucht. Herz und Alarmuskeln von *Sphinx ligustri* wurden mit besonderer Berücksichtigung des caudalen Herzabschnitts im Transmissionselektronenmikroskop untersucht. Die Alarmuskeln bilden eine unvollständige Hülle um das Herz aus überwiegend längs orientierten Fasern, antagonistisch zum Faserverlauf im Herzmuskel. Herz und Alarmuskeln sind multiterminal über die transversalen Segmentalnerven innerviert, deren größte Zweige ein elektronentransparentes und mehrere neurosekretorische Axone enthalten. Verdickungen der Segmentalnerven enthalten möglicherweise Nervenzellen. Laterale Herznerven wurden nicht gefunden. Synaptische Axonendigungen nur eines Typs sind in den Alarmuskeln häufig, im Herzmuskel seltener. Sie werden nur von dem zentralen Axon aus gebildet. Die neuromuskulären Kontaktstellen sind von Gliazellen bedeckt, die über zahlreiche Ausläufer mit der Muskelzelle verzahnt sind und mit ihr ein „perisynaptisches Retikulum“ bilden. Die Gliazellausläufer dringen in das T-System bis zu den Z-Banden vor. Exozytose von Neurosekret-Vesikeln in das perisynaptische Interzellulärsystem wird angenommen. Neurohämale Abgabeorte finden sich entlang der Nervenäste und in speziellen Nervenendigungen auf der Höhe der Ostien. Der mögliche Einfluß der Innervierung auf die Herzaktivität wird diskutiert.

Introduction

The activity of insect hearts is generally regarded as myogenic with a superimposed regulatory innervation (Wigglesworth, 1972; Richter, 1973; Miller, 1974). In different insect orders the heart innervation has been confirmed ultrastructurally (Johnson, 1966; Miller and Thomson, 1968: Blattaria; Normann, 1972: Diptera). In Lepidoptera, histological and vital stain investigations have led to contradictory results (Ke, 1932; Kuwana, 1932; McIndoo, 1945; Hinks, 1975). In electron microscopic studies in *Hyalophora* no heart innervation was found (Sanger and McCann, 1968a, b). On the other hand, Carlson (1906) reported changes in heartbeat frequency after stimulation of the thoracic ganglia in the *Polyphemus* moth. In electrophysiological investigations no postsynaptic effects could be registered in lepidopterous hearts (McCann, 1970; Richter, 1973; Miller, 1974), although this was the case in the fly *Sarcophaga* (Bruen and Ballard, 1970) and in *Periplaneta* (Miller and Rees, 1973). Temperature experiments were also interpreted as indicating some nervous control of heartbeat frequency (Heinrich, 1970). Elevation of the heartbeat frequency during emergence and wing expansion (Queinnec and Campan, 1972; Moreau and Lavenseau, 1975; Wasserthal, 1975) may also indicate nervous involvement. Recently, it was shown that periodic heartbeat reversal is a regular event, which is interrupted by a prolonged period of forward beating during emergence (Wasserthal, 1975). The periodical changes of heartbeat direction are coordinated with the abdominal respiratory movements (Wasserthal, 1976). The question arises whether the coordination is established directly by mechanical effects on the heart or/and by nervous control.

The present study focuses on the caudal end of the abdominal heart tube where the dominant pacemaker is supposedly located (Tenney, 1953; McCann, 1966).

Material and Methods

Sphinx ligustri larvae were reared under normal daylight conditions in the laboratory and emerged in an air-conditioned room. For transmission electron microscopy hearts of 2 and 6 day-old animals were fixed in situ in 3% glutaraldehyde (0.08 M cacodylate buffer), dissected and then left in the fixative for 14 h at 4° C. After washing in 0.05 M cacodylate buffer containing 4% sucrose, specimens were postfixed in 1% OsO₄ in 0.05 M cacodylate buffer for 1 h at 4° C, washed as before, rinsed in double-distilled water and dehydrated in ethanol. During dehydration the specimens were block-stained in 1% uranyl acetate in 70% ethanol for 60 min. They were then embedded in Epon. Ultrathin sections were cut with glass knives on a Reichert Om U2 microtome, stained in 1% uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined in a Zeiss EM 9 S.

The samples for the SEM were dissected in saline solution (Glaser, 1917). They were fixed as mentioned above, but left in glutaraldehyde at 4° C for 1 to 2 weeks with repeated changes of the fixative. After fixation in OsO₄ the preparations were pinned onto a pad of folded sheet-lead to minimize the danger of damaging the tissue by changes of cuticle tension. Only sheet-lead proved to be sufficiently soft to allow penetration by the steel dissecting needles and to withstand the dehydrating agents (ethanol and acetone) and the drying procedure in a critical point apparatus (Polaron). The gold-coated specimens were examined in a Jeol-U3 (Figs. 1, 2, 6, 7) and Leitz-AMR 1000 (Figs. 3-5).

Results

Mode of Heart Attachment and Suspension by the Alary Muscles

The heart tube of *Sphinx ligustri* is composed of a single layer of muscle cells, the myofibrils of which follow the course of a tightly wound spiral. The inner heart wall is coated only by a basement membrane, the outer wall additionally by scattered connective tissue cells. The heart is suspended by arrays of alary muscles with lateral attachment to the intersegmental cuticular apophyses, as in *Ephestia* (Fig. 1). The heart cells and alary muscle cells in *Sphinx* are connected by myomuscular junctions and by connective tissue strands as in *Hyalophora cecropia* (Sanger and McCann, 1968 a,b). In *Ephestia* a rather irregular network of alary muscle branches is tightly connected to the heart by means of connective tissue (Fig. 3). The alary muscles in *Sphinx* form latticed ramifications around the ventral and lateral heart tube with most branches oriented parallel to its long axis (Fig. 5). Thus the *Sphinx* heart is equipped with an incomplete outer layer of longitudinal muscle fibres (Figs. 8,15). The last abdominal pair of alary muscle arrays extends caudally from the tip of the heart. At the posterior heart segment, tracheae and single alary muscles penetrate into the lumen of the heart (Figs. 6-9). The walls of only this abdominal heart segment receive large tracheae from the inner side, which fill a large part of its lumen (Fig. 8), but there is no conspicuously augmented tracheolar supply of the muscle cells. Another special feature is a meshwork of connective tissue cells on the inner wall of the posterior heart segment (Figs. 7,13).

Origin and Orientation of the Heart Nerves

Segmental nerves can be detected in whole mount preparations of the heart under the dissecting microscope and also in favourable SEM-preparations.

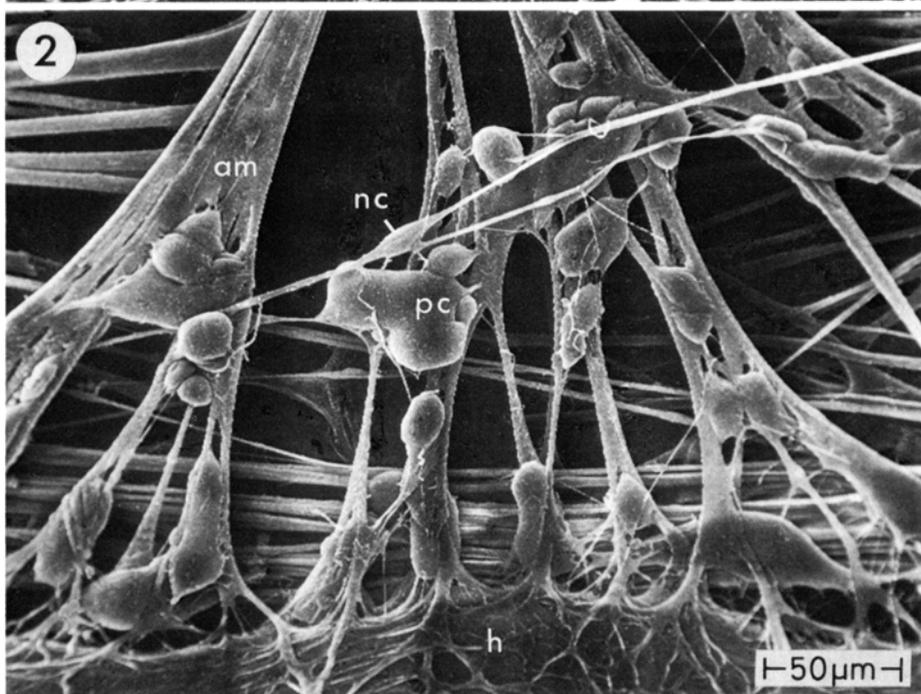
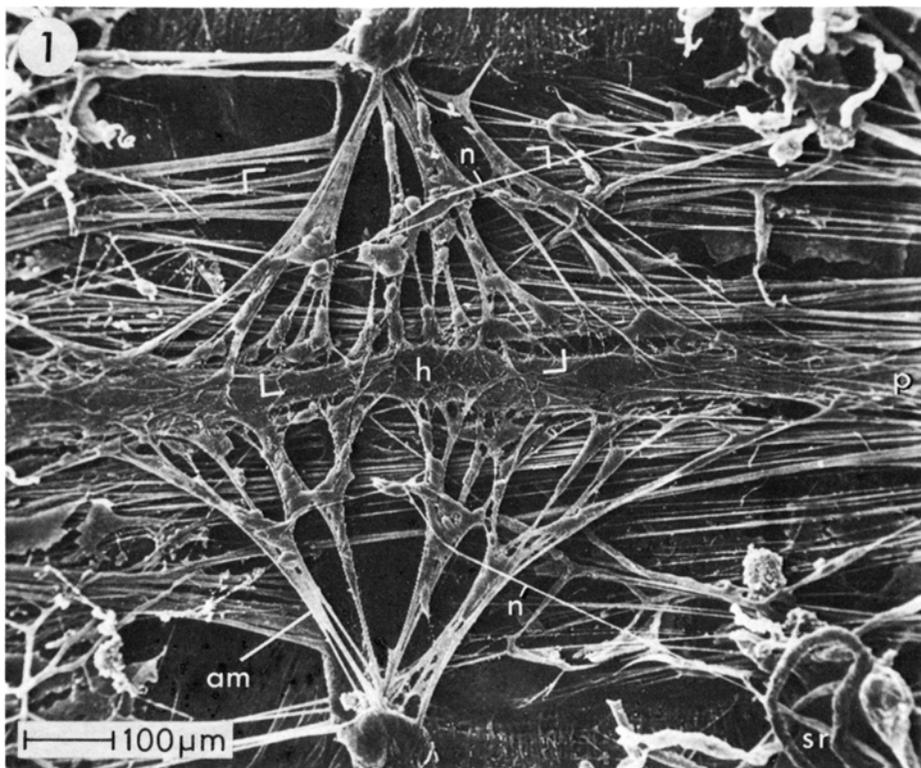


Fig. 1. Ventral view of the transverse segmental nerves (*n*) extending from the spiracular region (*sr*) to the alary muscle arrays (*am*) in *Ephestia*. *h* heart; *p* posterior; SEM

Fig. 2. Ramification of the left nerve with probable nerve cell bodies (*nc*); detail from Figure 1; *am* alary muscle; *h* heart; *pc* pericardial cell; SEM

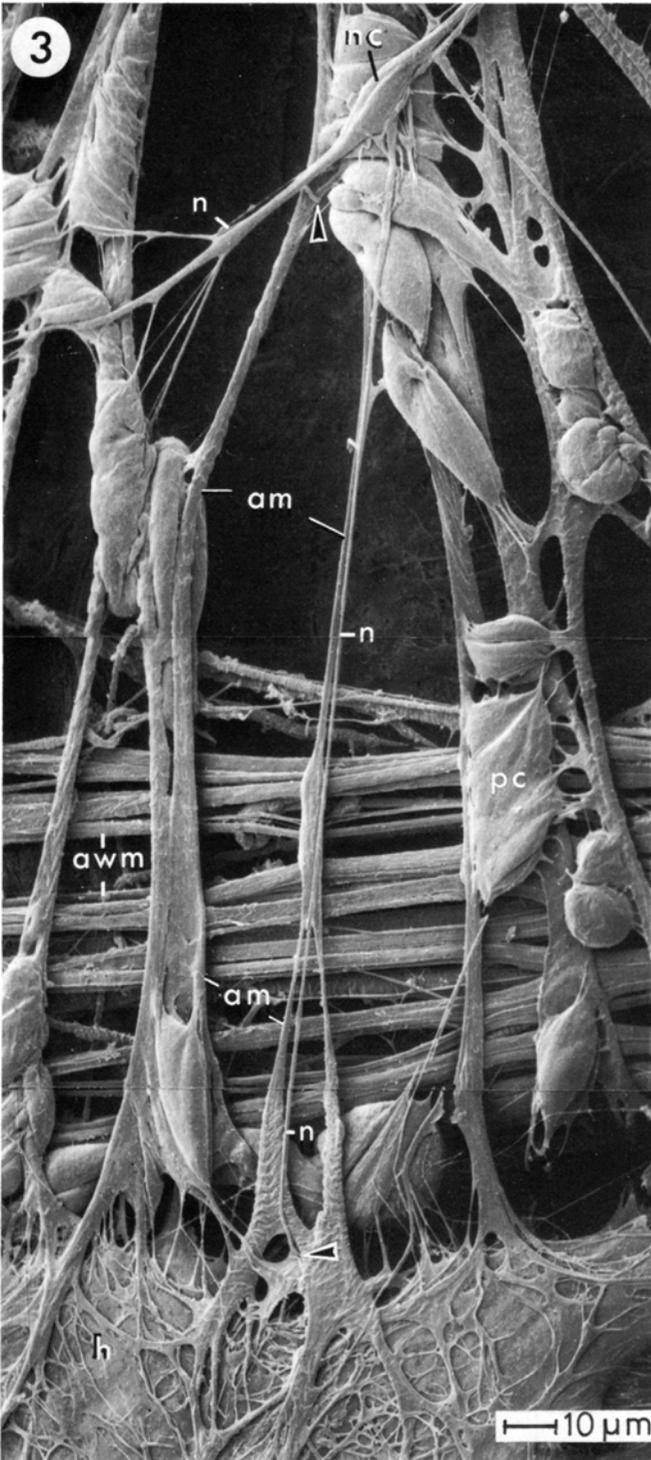


Fig. 3. Segmental nerve supply (arrowheads) of the alary muscles (*am*) and of the sheath of *am*-fibres around the heart tube (*h*) in *Ephesia*; *awm* abdominal wall muscles; *n* nerve; *nc* presumed nerve cell bodies; *pc* pericardial cell; orientation as in Figure 2; SEM

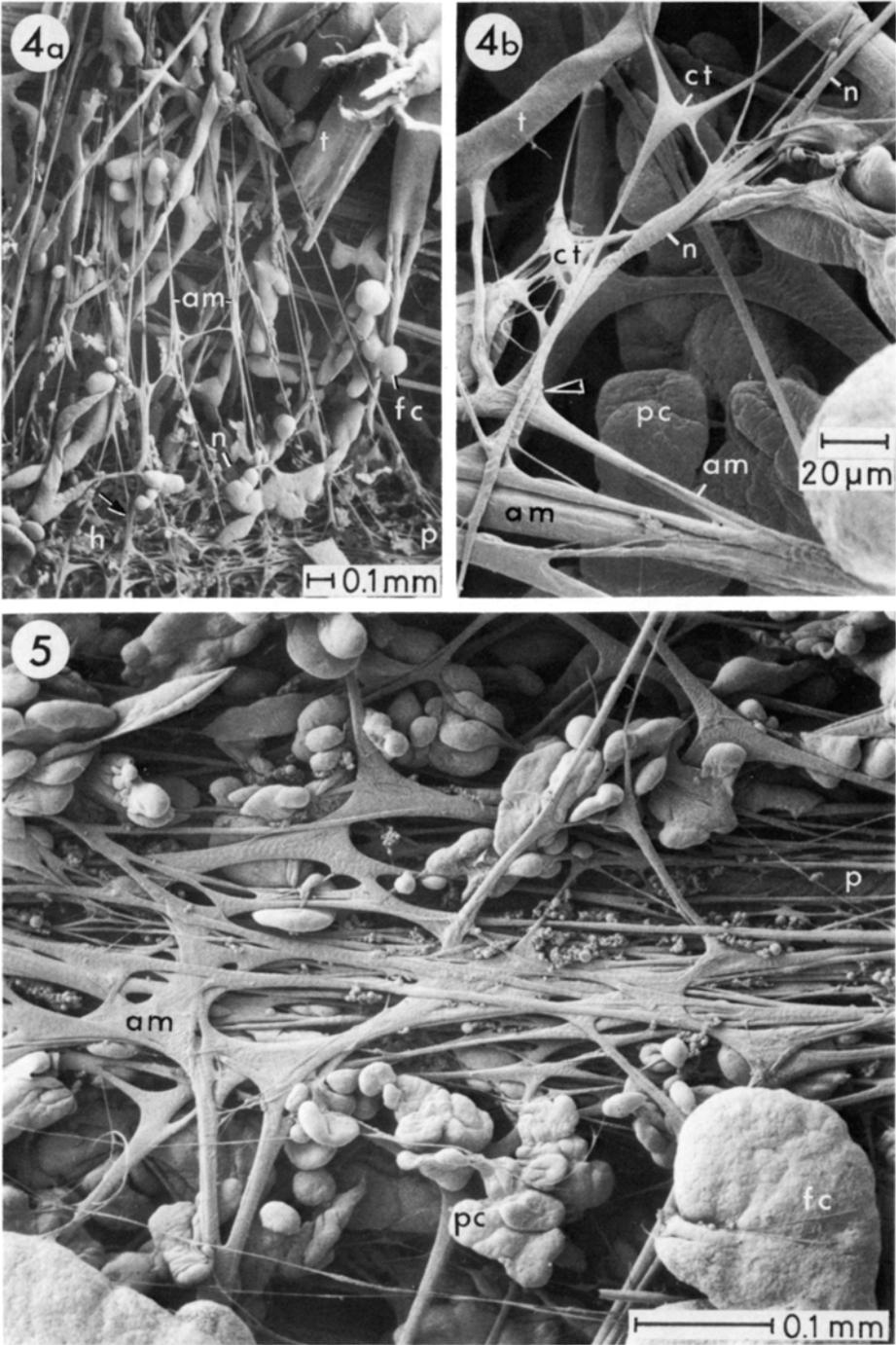


Fig. 4a. Insertion of the alary muscles (*am*) at the *Sphinx* heart (*h*); *fc* fat cell; *n* nerve; *p* posterior; *t* trachea; SEM. **b.** Alary muscles (*am*) and adjoining transverse segmental nerve branch (arrowhead), detail from Figure 4a (at arrow); *ct* connective tissue; *pc* pericardial cell; *n* nerve; *t* trachea; SEM

Fig. 5. Ventral view of the *Sphinx* heart, 6th segment; ramification of the alary muscles (*am*) near the heart tube and longitudinal alary muscle sheath; *fc* fat cell; *n* nerve; *pc* pericardial cell; *p* posterior; SEM

The following characteristics are used to discern different tissue types in the SEM preparations: All strands with an almost round and constant diameter are interpreted as nerves. The tracheae, which can also be round in cross sections, have a more variable diameter and are often compressed. The connective tissue strands and alary muscles are more flattened and diverging, at least at their attachment sites. The muscles can be recognized by their directional arrangement and, when contracted, by the ruffling of the cell membrane (Fig. 3). In contrast to the muscles, the connective tissue strands are very delicate.

From each abdominal spiracular region the distal end of the transverse nerve, which originates from the median nerve of the ventral cord, extends directly toward the dorsal alary muscle array of the preceding intersegment. In *Sphinx* this segmental nerve strand runs parallel to the anterior tracheal trunks of each segmental tuft, which supply the corresponding alary muscle array and heart region (Figs. 4a, b). In a ventral view of the preparation this nerve is almost entirely hidden by these anterior tracheae. The peripheral branching of the segmental nerve can not be demonstrated in *Sphinx* because of the complex arrangement. The arrangement appears to be similar to that in *Ephestia*, where the segmental nerve strands can more easily be traced in SEM preparations, because the tracheal trunks are less developed and the distance between spiracle and alary muscle array is relatively larger (Fig. 1). The nerve ramifies before intimately contacting the alary muscles (Figs. 2, 3). In the branching region a swelling of the segmental nerve can be observed (Figs. 2, 3). Often the main nerve branches accompany the alary muscles before finally reaching the heart (Fig. 3).

In the region of the last lateral array of alary muscles in *Sphinx* the branches of the corresponding segmental nerves are more numerous and conspicuous because they lie closer and more parallel to the heart tube. They could therefore be seen in semithin cross sections of the heart (Figs. 8, 9). A thick nerve strand (maximal diameter 30 μm) approaches the posterior heart dorso-laterally from each side (Fig. 10). This nerve ramifies, one branch extending forward toward the posterior ostia, the other one backward; both continue dorso-lateral and parallel to the heart with further dorsolateral and ventrolateral ramifications. At the heart tip single nerves penetrate the heart tube and enter the heart lumen in the same region as the alary muscle fibres (Fig. 13). No continuous longitudinal nerve accompanying the heart was found that could be interpreted as a lateral nerve.

The nerve branches approaching the posterior heart segment contain a single electron lucent axon, surrounded by glial cells with densely packed microtubules (type II according to Maddrell and Treherne, 1967). An outer sheath of glial cells with fewer microtubules (type I) is covered by a basement membrane. Only normal desmosomes occur between the glial cells. The intercellular space system of the heart nerves appears enlarged after bathing the specimens in saline before fixation (Fig. 11), but not if they are fixed immediately (Fig. 10). At their periphery these nerves contain a number of neurosecretory axons with dense-cored vesicles (maximal size of 1000 \AA). These axons are enveloped by glial cells of type I without complicated folding (Fig. 10). A possible neurohaemal release is indicated by the presence of axon endings covered only by a basement membrane (Fig. 12). Most of the smallest nerve branches lie between the longitudinal ramifications of the alary muscles around the ventral and lateral heart (Fig. 15) and among the caudal alary

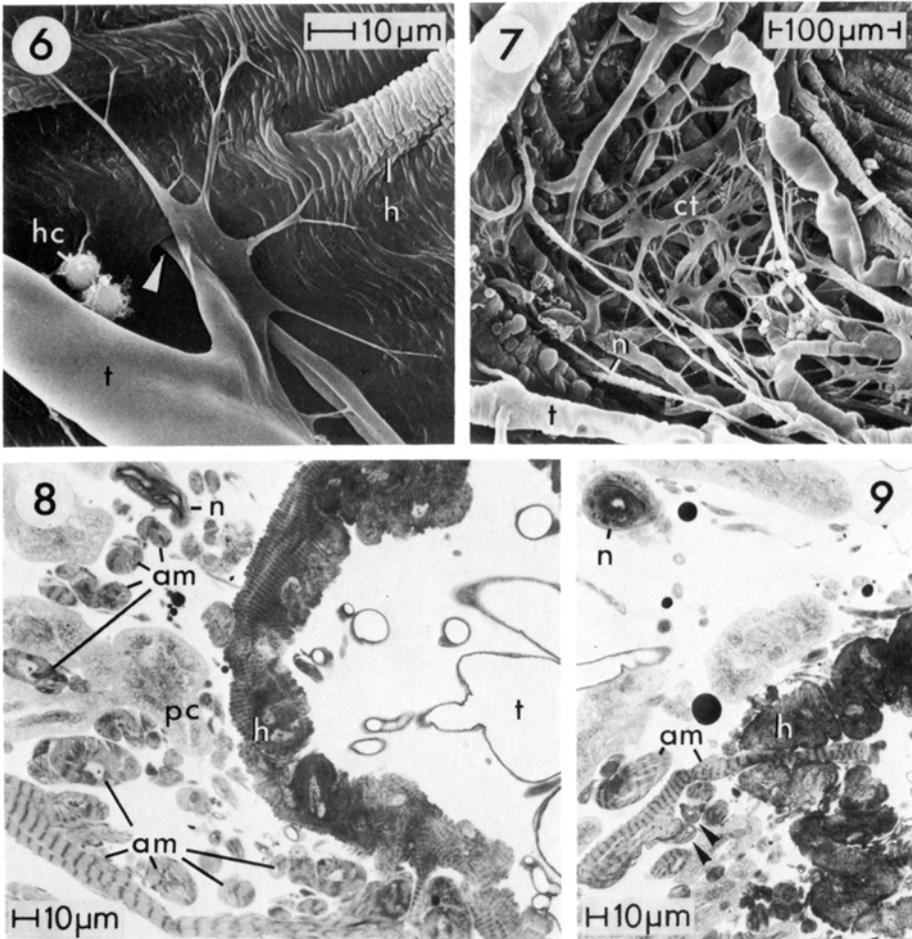


Fig. 6. Tracheal supply (*t*) in the interior of the abdominal heart tip with naked muscle cell surface, anterior to Figure 7; arrowhead: branch of the trachea penetrating the heart muscle (*h*); *hc* haemocyte; *Sphinx*; SEM

Fig. 7. Part of the meshwork of connective tissue cells (*ct*) in the dorsum of the abdominal heart tip; *n* probable nerve branch; *t* trachea; *Sphinx*; SEM

Fig. 8. Cross section of the last heart segment, showing the lateral extension of the longitudinal alary muscle sheath (*am*); *h* heart wall; *n* dorsolateral nerve branch; *pc* pericardial cell; *t* trachea in the heart lumen

Fig. 9. Alary muscle (*am*) penetrating the heart wall (*h*); arrowheads: small ventrolateral nerve branches; *n* dorsolateral nerve branch; cross section

muscles. The fine preterminal nerve processes consist of individual electron lucent axons (Fig. 17). They sometimes contain single dense-cored vesicles, which occur only rarely in the thicker central axons further from the heart (Fig. 14).

Structure of the Synaptic Sites

Synapsing axon terminals are scattered in the wall of the entire posterior heart segment. Only few axon terminals are situated at the inner side of the heart tip. In

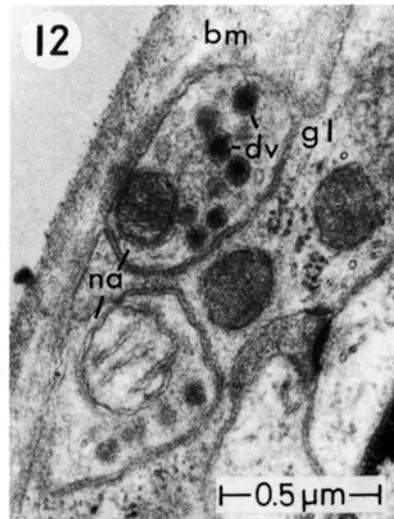
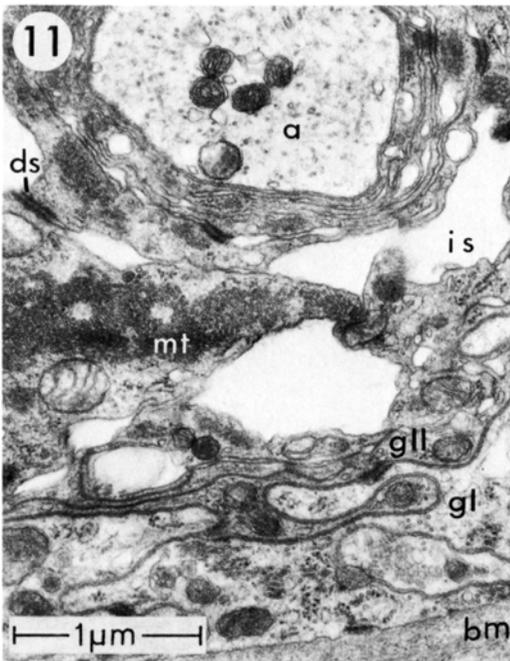
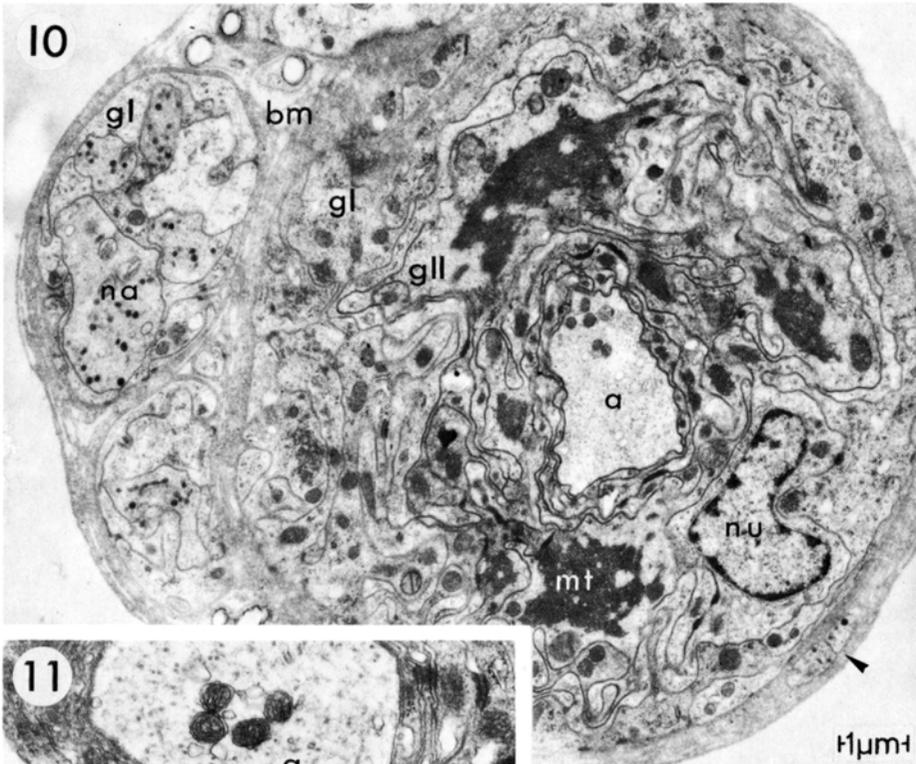


Fig. 10. Dorsolateral nerve branch as in Fig. 9; arrowhead: presumed neurosecretory release site; *a* central axon; *bm* basement membrane; *gl*, *gll* glial cells; *mt* bundles of microtubules; *na* neurosecretory axon; *nu* nucleus

Fig. 11. Dorsolateral nerve branch with artefactually swollen intercellular spaces (*is*) between the inner glial cells, caused by saline treatment; *a* axon; *bm* basement membrane; *gl*, *gll* glial cells; *ds* desmosome; *mt* microtubules

Fig. 12. Neurohaemal release site on a nerve branch; *bm* basement membrane; *dv* dense-cored vesicle; *gl* glial cell; *na* neurosecretory axon

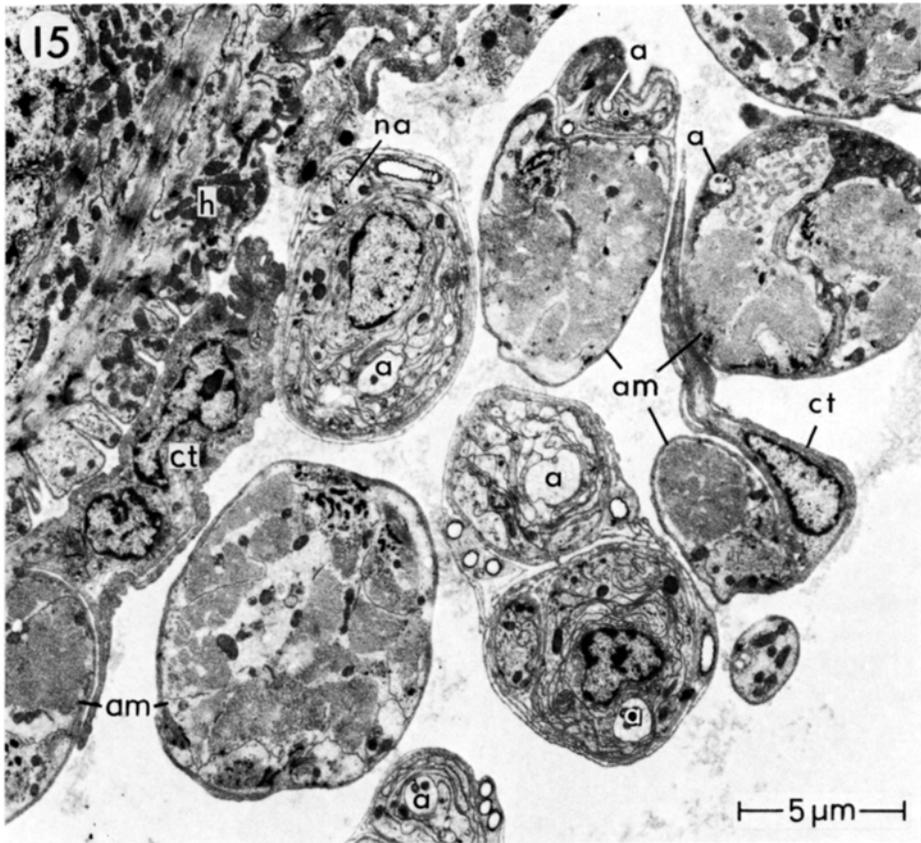
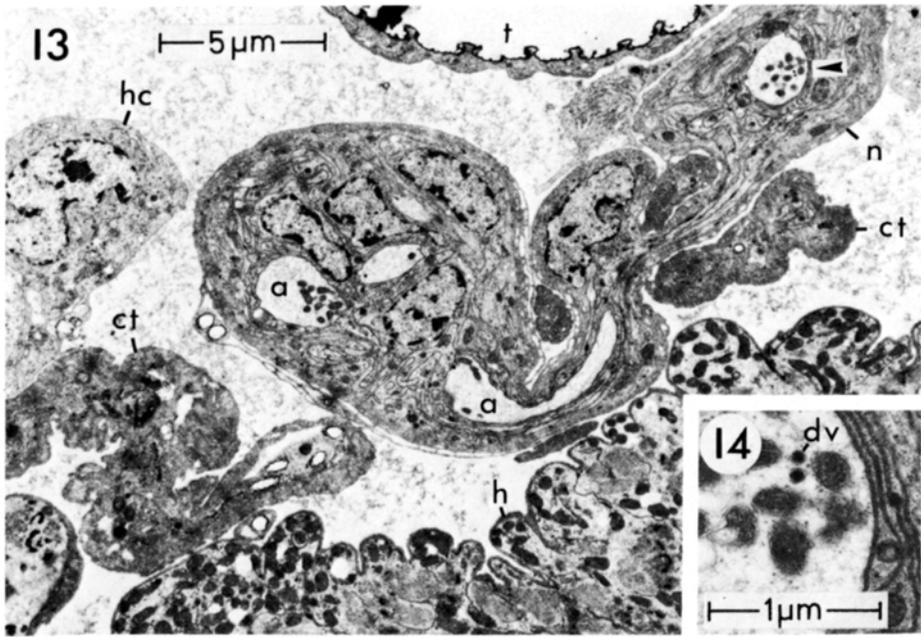


Fig. 13. Interior of the abdominal heart tip, cross section, showing a nerve (*n*), trachea (*t*) and connective tissue (*ct*) in the lumen; *a* axon; *h* wall of the heart tube; *hc* haemocyte

Fig. 14. Central axon with dense-cored vesicles (*dv*) in the heart nerve, detail from Figure 13 (at arrowhead)

Fig. 15. Cross section of the heart wall and alary muscle sheath, showing the rectangular orientation of heart muscle fibres (*h*) and alary muscle fibres (*am*) with nerves and connective tissue cells (*ct*); *a* central

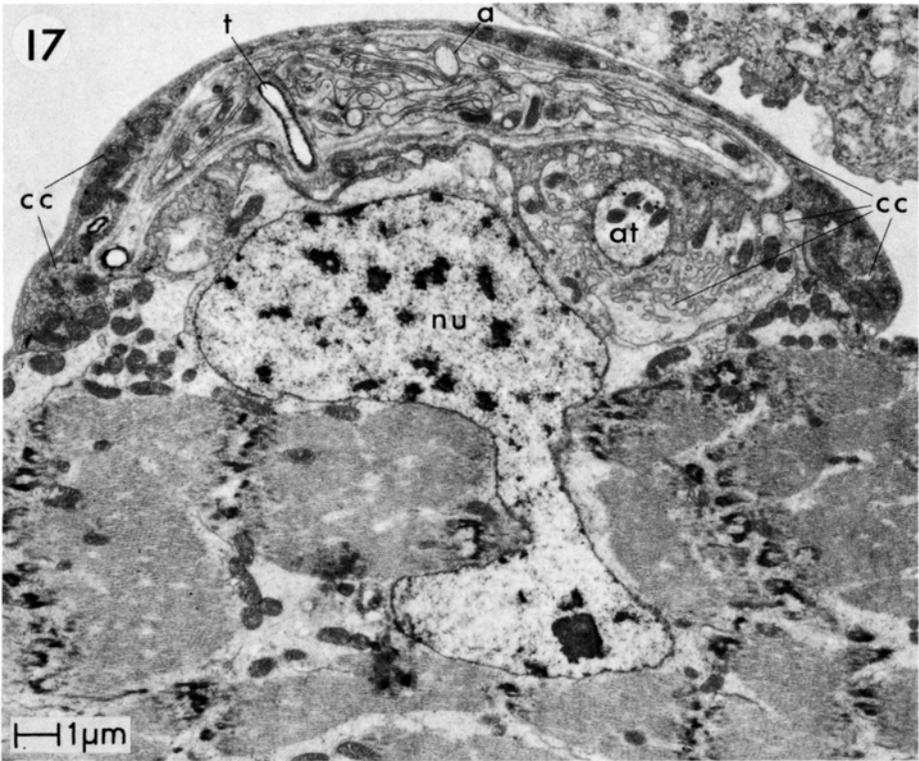
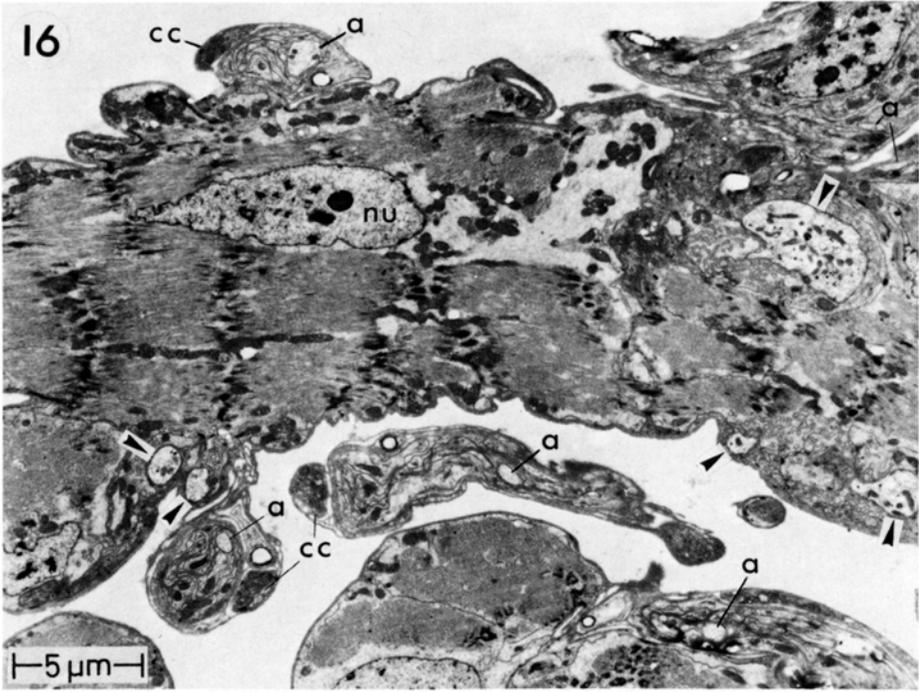


Fig. 16. Nervous supply and distribution of axon terminals (arrowheads) in an alary muscle, longitudinal section; *a* central axon; *cc* glial cap cell; *nu* nucleus

Fig. 17. Glial cell (*cc*) capping the supplying nerve and interdigitating with the alary muscle cell around the axon terminal (*at*); *a* axon; *nu* nucleus; *t* tracheole

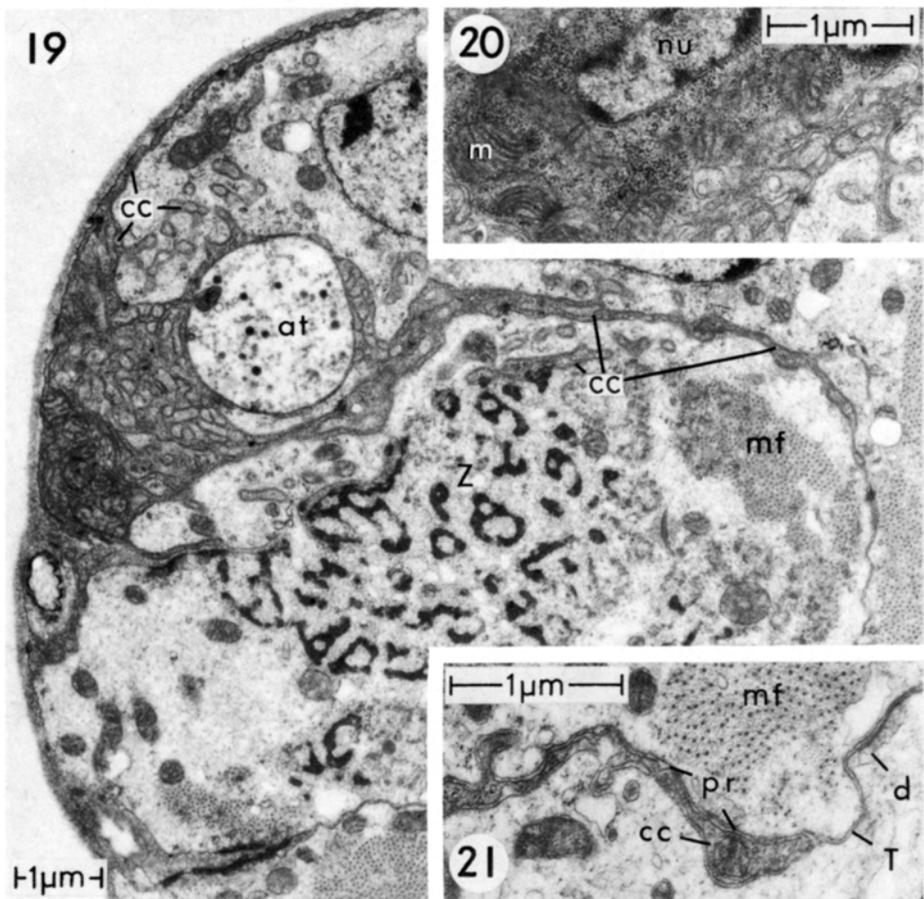
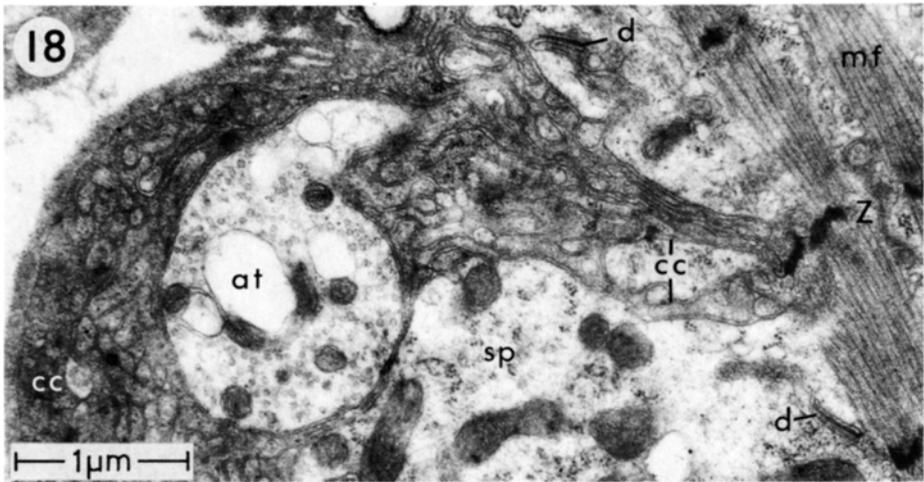


Fig. 18. Neuromuscular junction in a heart muscle cell with interdigitations of the glial cap cell (*cc*) closely contacting the Z-disc (*Z*); *at* axon terminal; *d* dyad; *mf* myofilaments; *sp* sarcoplasm

Fig. 19. Neuromuscular junction in an alary muscle fibre, cross section at the level of a Z-disc (*Z*); *at* axon terminal; *cc* glial cap cell processes; *mf* myofilaments

Fig. 20. Electron-dense cytoplasm with ribosomes and mitochondria (*m*) of the glial cap cell; *nu* nucleus

Fig. 21. Connection between perisynaptic reticulum (*pr*) and T-system (*T*); *cc* glial cap cell process; *d* dyad; *mf* myofilaments of the alary muscle cell

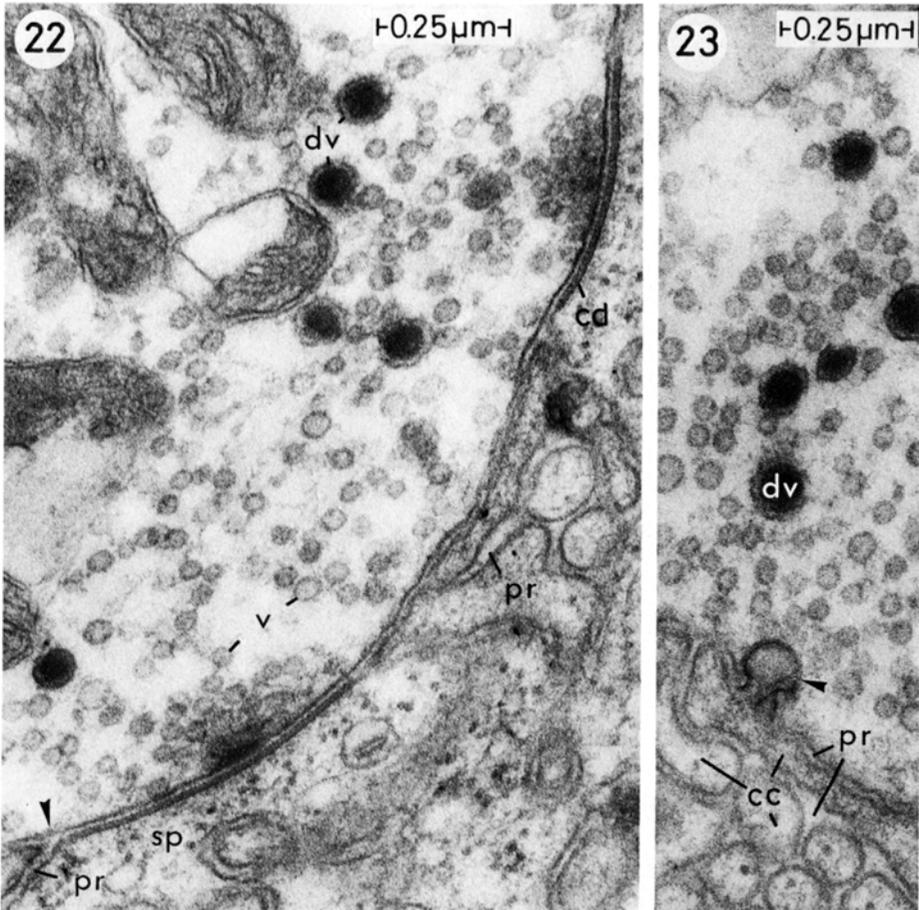


Fig. 22. Synaptic structures in an alary muscle. The synaptic cleft is continuous with the intercellular perisynaptic reticulum (*pr*) (arrowhead); *cd* cleft densities; *dv* dense-cored vesicle; *sp* sarcoplasm; *v* synaptic vesicle

Fig. 23. Omega-shaped profile (arrowhead) in an axon terminal; *cc* glial cap cell process; *dv* dense-cored vesicle; *pr* perisynaptic reticulum

contrast to the heart, the alary muscle cells present numerous axon terminals (Fig. 16). The axon terminals slightly indent the sarcoplasm of the heart and alary muscle cells and are capped by at least one glial cell which also envelopes the corresponding nerve (Fig. 17). The glial cell cytoplasm is rich in ribosomes and mitochondria (Fig. 20). The axon endings are partly surrounded by long processes of the capping glial cell which interdigitate with the muscle cell membrane. The glial cell processes often penetrate into the T-system as far as the Z-discs (Figs. 18, 19) or into intercellular spaces between neighboring muscle cells. Thus a complex intercellular space system between muscle and glial cell membranes is formed, which is continuous with the T-system (Fig. 21). Small normal desmosomes are found between the glial processes and muscle cells.

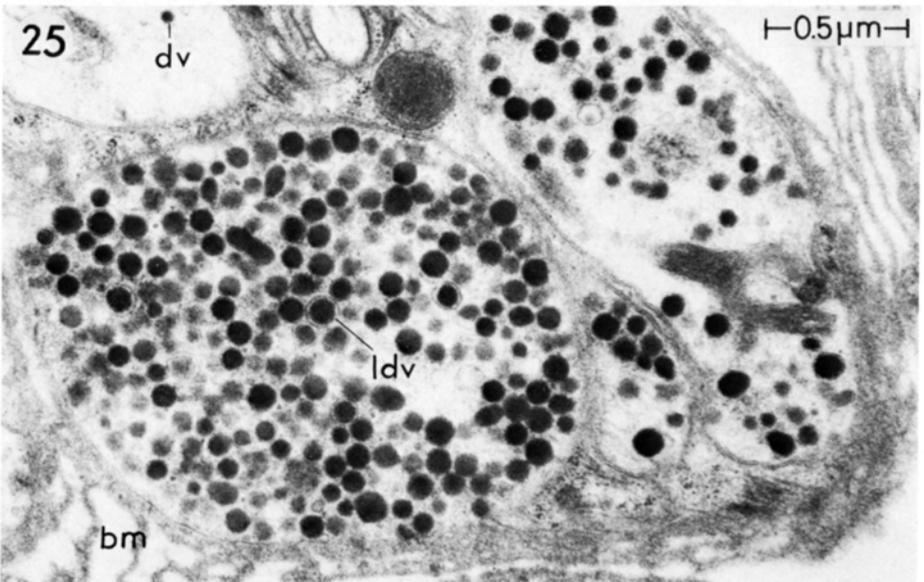
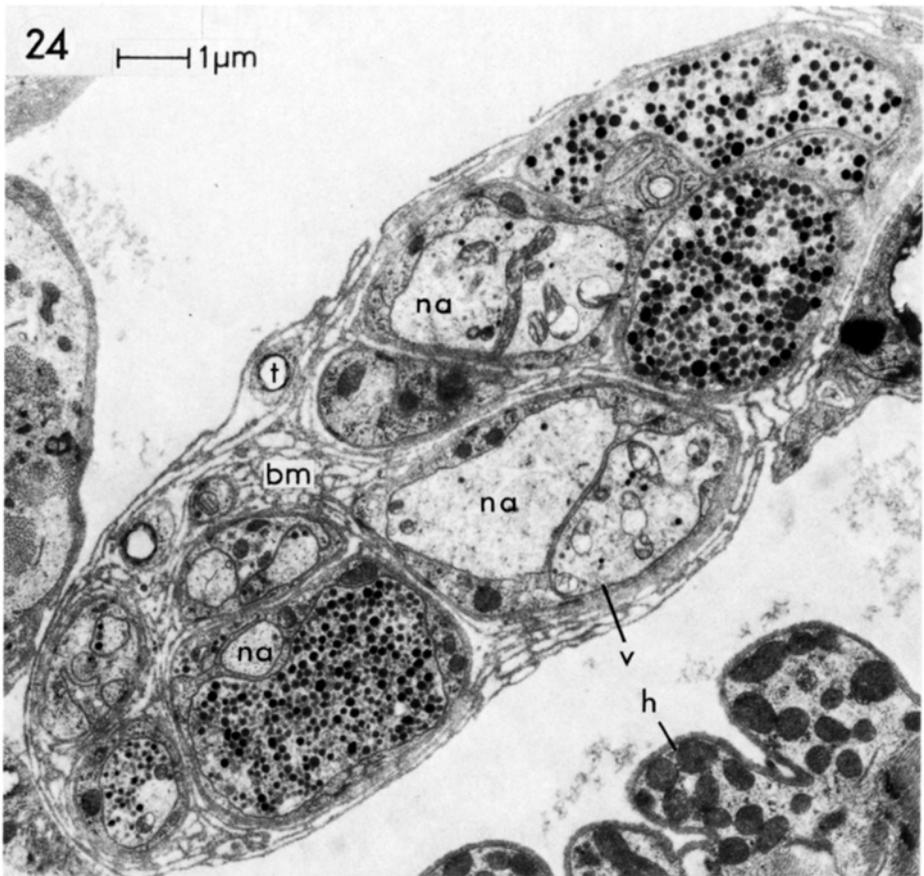


Fig. 24. Neurosecretory nerve ending at the level of the heart ostia; *bm* basement membrane; *h* heart; *na* neurosecretory axon; *v* lucent vesicles
Fig. 25. Neurohaemal release site with large dense-cored vesicles (*ldv*); *bm* basement membrane; *dv* small dense-cored vesicle

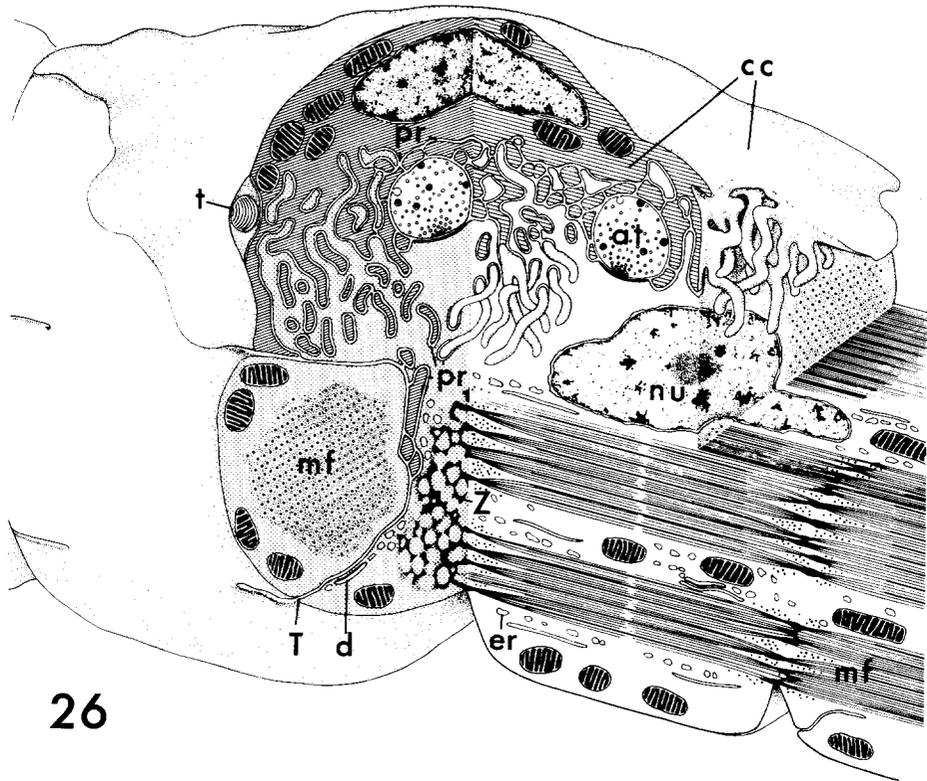


Fig. 26. Semi-schematic view of the capped axon terminals (at) in an alary muscle fibre. The glial cap cell (cc) interdigitates with the muscle cell, forming a perisynaptic reticulum (pr); basement membrane omitted; *d* dyad; *er* endoplasmic reticulum; *mf* myofibrils; *nu* nucleus; *T* T-system; *t* tracheole; *Z* Z-disc

The presynaptic structure contains a single dense projection with an adjacent assembly of synaptic vesicles. The postsynaptic membrane seems to be considerably thicker, due to an outer coating (Fig. 22) ("cleft densities"; Osborne, 1975; Stocker and Nüesch, 1975). The dense cored vesicles are loosely scattered in the axon terminals; their diameter does not exceed 1000 Å. Empty omega-shaped profiles of the same size as the dense-cored vesicles are contiguous to the membrane of the axon terminal (Fig. 23).

Neurohaemal Release Sites

Neurosecretory nerve endings are situated near the outer heart wall in a ventrolateral position at the level of the ostial valves. In this region two successive pairs of alary muscle arrays leave a gap. The neurosecretory endings are packed with large dense-cored vesicles (diameter 1600–1900 Å) (Figs. 24, 25). Additionally, smaller light vesicles (700 Å) are present (Fig. 24). The glial sheath is poorly developed and not plicated. Direct release into the haemolymph is suggested by naked axons which are only covered by a basement membrane. Some axons in the

vicinity of these neurohaemal release sites contain the smaller dense-cored vesicles with a maximal diameter of 1000 Å (Fig. 25).

Discussion

Properties of the Alary Muscles and Heart

The predominant longitudinal orientation of the alary muscle branches parallel to the long axis of the heart may be a specialization peculiar to *Sphinx*. It was not observed in *Ephestia*. In *Hyalophora cecropia* only the ventralmost (outer) of the three alary muscle layers lies parallel to the heart tube (Sanger and McCann, 1968b). The almost rectangular fibre arrangement of the longitudinal alary muscle branches with respect to the spirally organized heart muscle fibrils in *Sphinx* establishes an antagonistic muscle system. If the alary muscles contract alternately with the heart spiral in each segment (de Wilde, 1948) the heart segment may not only expand but also shorten at diastole. The "free play" of the heart tube is facilitated by the meshlike arrangement of this alary muscle sheath.

The heart nerves were found synapsing mainly to the alary muscle branches, which suggests that these muscles may have a mediating function in heart regulation. Relatively few synapses were found on the heart muscle itself.

The exceptional covering of the interior wall at the heart tip with connective tissue cells is probably correlated with the caudad oriented alary muscle suspension and the resulting danger of overstraining by abdominal movements. It is interesting that the dominant pacemaker region is supposed to be located at this site. (Tenney, 1953; McCann, 1966).

Nervous Supply of the Lepidopterous Heart

The abdominal heart and alary muscles in *Sphinx ligustri* are innervated, in contrast to earlier negative reports concerning *Hyalophora cecropia* (Sanger and McCann, 1968a,b). Only segmental heart nerves, but no lateral heart nerves, could be detected. The orientation of the transverse segmental heart nerves in adult *Sphinx ligustri* and *Ephestia kuehniella* corresponds generally to the situation in larval Noctuidae, Arctiidae and Sphingidae when analyzed with the aid of vital staining (Hinks, 1975). The swelling in the branching region of the segmental nerve has the same position as the peripheral neurosecretory cell(s) on the alary muscles (Hinks, 1975). Ke (1932), Kuwana (1932) and McIndoo (1945) describe lateral nerves in addition to the segmental nerves in the silkworm, but McIndoo could not identify the lateral nerves in histological sections, nor did he find them in *Prodenia* larvae (Noctuidae). This and the above mentioned similar observations in larval and adult Lepidoptera suggest the presence of only the segmental heart nerves in both stages. Accordingly, in other adult holometabolous insects only segmental heart nerves are described (*Apis*: Rehm, 1939; *Anopheles*: Iagushinskaia, 1954; *Calliphora*: Normann, 1972). Reliable references confirming the existence of the lateral heart nerves are thus restricted to hemimetabolous insects (Zawarzin, 1911; Alexandrowicz, 1929; Maloeuf, 1935; Opozynska-Sembratowa, 1936; Edwards and Challice, 1960; Johnson, 1966).

Neuromuscular Junctions and Neurosecretory Release

In the posterior heart and alary muscles of *Sphinx* a single type of axon terminal with clear synaptic vesicles and scattered dense-cored vesicles is found. Comparable axons of segmental nerve origin synapse to the heart and alary muscles in *Periplaneta* (Johnson, 1966; Miller and Thomson, 1968; Adams et al., 1973; Miller and Rees, 1973) and on the heart of *Calliphora* (Normann, 1972). Here, however, additional types of neuromuscular junctions have also been described (Normann, 1972; Miller and Rees, 1973).

Two types of neurohaemal release site occur near the *Sphinx* heart. Along the segmental nerve branches the surfaces of some neurosecretory axons containing dense-cored vesicles (900–1000 Å) contact the haemocoel. At the ostial level few free nerve endings contain larger dense-cored vesicles (1600–1900 Å). Neurosecretory granules of the same size are observed at numerous release sites along the lateral heart nerves of *Periplaneta*, which are supposed to function as a neurohaemal organ along their entire length (Johnson, 1966). According to the number of heart nerves and axons as well as the quantity of neurosecretory release sites and diversity of synaptic structures, the nerve supply of the *Sphinx* heart seems to be relatively poorly developed.

The Perisynaptic Reticulum

The organization of the glial cell-capped areas of neuromuscular contacts in insects has not yet been analyzed in detail. As a special feature in the adult *Sphinx* heart and alary muscles, the glial cell involvement in the areas of the neuromuscular junctions is extremely elaborate by mutual interdigitations of the capping glial and muscle cell processes (Fig. 26). A comparable organization of the junction areas was described as a transitory phenomenon in the development of neuromuscular junctions in *Antheraea* flight muscles (Stocker and Nüesch, 1975). Similar membranous foldings near the synapses of other insects were found to be plicated areas of the sarcolemma alone (Smith, 1960; Belton, 1969). The foldings of the sarcolemma and the capping by a glial cell have been dealt with as independent phenomena in the reviews of Osborne (1970; 1975). Edwards et al. (1958), who first described a comparable system of folded membranes, erroneously interpreted it as a part of the smooth endoplasmic reticulum and introduced the term "rete synapticum". Miller and Thomson (1968) employed the English translation "subs synaptic web". This term, however, was used for a different and truly *intracellular* postsynaptic structure (de Robertis, 1967). In order to avoid confusion we propose that the terms "rete synapticum" and "subs synaptic web" in connection with these *intercellular* space systems should both be abandoned. Since the reticulum between glial and muscle cell in *Sphinx* surrounds the axon terminals, we would prefer "perisynaptic reticulum" rather than "subs synaptic reticulum" (Osborne, 1967).

The significance of the glial cell processes and the intercellular reticulum is not known (Stocker and Nüesch, 1975). In *Sphinx* there is evidence of a connection between the perisynaptic reticulum and the T-system and for a tendency of the glial cell processes to invade the sarcoplasm as deep as the Z-discs. This supports the idea of a special amplifying system, which enhances the development or spread of the

postsynaptic potential, as postulated in connection with the subsynaptic reticulum of uncapped axon terminals (Osborne, 1975). The reticulum might also serve for rapid transmitter inactivation analogous to the subneuronal apparatus in vertebrate motor end plates (Couteaux, 1973). The large extension of the intercellular system among the glial processes themselves and between the sarcolemma may additionally suggest some (trophic?) interrelations between the glial and muscle cell. The high content of mitochondria and ribosomes in the capping glial cell indicates some active role in the adult *Sphinx* moth. This is in contrast to adult *Antheraea* flight muscle innervation, where the shrunken glial cell cytoplasm is almost free of organelles (Stocker and Nüesch, 1975). Another difference in *Antheraea* flight muscle axon terminals is the paucity of dense-cored vesicles, which were so common in the adult *Sphinx* heart and alary muscles. Because of similarities in size, the omega-shaped profiles might be interpreted as indicating exocytosis of dense-cored vesicles (Normann, 1974; Jones, 1975).

Possible Nervous Influence upon Heart Activity

Postsynaptic potentials have not yet been recorded in a lepidopterous heart (McCann, 1970). The involvement of the nerves in normal pulse generation is therefore unlikely and the lepidopterous heart is supposed to be myogenic with regulatory nerves (Wigglesworth, 1972; Richter, 1973; Miller, 1974). One possibility of nervous control could be the initiation of directional changes or pauses of heartbeat in resting moths. The regular heartbeat sequences in *Sphinx* consist of a long forward period (26.3 ± 4 min), interrupted by several (7.5 ± 1.6) pauses of about 0.5 min each and followed by a shorter backward period (2.3 ± 0.7 min), ($N=6$ specimens; Wasserthal, in preparation). It can easily be imagined how difficult it must be to record these rather rare events electrophysiologically. Nervous involvement in heartbeat reversal has been repeatedly denied because semi-isolated heart preparations and even single heart segments show reversals (Gerould, 1938; Tenney, 1953; Jones, 1964; McCann, 1970). This, however, can not exclude the possibility of nervous involvement. The arrangement of the nerves in the longitudinal branches of the alary muscles and even in the interior of the heart tip show that it is impossible to deprive the heart of all these nerve branches and to have it functionally isolated.

The heartbeat sequences in *Sphinx ligustri* are coordinated with the abdominal respiratory movements (Wasserthal, in prep.) as also found in *Attacus* and *Argema* (Wasserthal, 1976). Even if the heartbeat reversal should prove to be an autonomous function of the lepidopterous heart muscle, a mechanism must be postulated, which synchronizes the heartbeat sequences with the abdominal movements. This might be effected by the segmental heart nerves.

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