A Multiterminal Stretch Receptor, Chordotonal Organ, and Hair Plate at the Wing-Hinge of *Manduca sexta:* Unravelling the Mystery of the Noctuid Moth Ear B Cell

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ABSTRACT

The present study aims to shed light on the evolutionary origin of the B cell, a sensory element of unknown function in the noctuid moth ear.

Peripheral projections of the metathoracic nerve IIIN1b1, homologue of the noctuid moth tympanic nerve, are described in the atympanate moth *Manduca sexta* on the basis of dissections with the aid of Janus Green B, and intracellular tracer dyes Lucifer yellow and cobalt lysine. A large multiterminal (Type II) neurone, attaching to membranous cuticle ventral to the hind wing axillary cord, was discovered. This cell appears to be homologous to the B cell in the noctuid moth ear. Recordings from the IIIN1b1 nerve in *M. sexta* reveal a continuous train of large, uniform spikes, presumed to originate from the multiterminal cell. This unit increases its rate of firing in response to hind wing elevation, suggesting that it functions as a stretch receptor monitoring wing movements during flight. Also identified in the tympanic nerve homologue, and closely associated with the multiterminal cell, were a chordotonal organ and hair plate. The chordotonal organ consists of a proximal scolopidial region and a distal strand that attaches to the sclerotized epimeron slightly medial to the multiterminal cell. This simple chordotonal organ, having three uniterminal (Type I) sensory cells, is homologous to the auditory cells of the noctuid moth ear.

The significance of these receptors as proprioceptors in *M. sexta,* and as evolutionary precursors to the noctuid moth ear, is discussed. © 1992 Wiley-Liss, Inc.

Key words: proprioceptors, evolution, sensory, neuroanatomy, insect

The B cell of the noctuid moth ear has been regarded as somewhat of an anomaly since it was first described (Treat and Roeder, '59). This multiterminal (Type II; see Finlayson, '68, for definition) sensory neurone attaches to an infolding of the integument, the Bügel, situated within the tympanic cavity, and is in close proximity to the uniterminal (Type I), acoustically sensitive neurones (A cells) of the tympanal organ. Despite extensive examinations of the B cell's physiological characteristics (Treat and Roeder, '59; Lechtenberg, '71), its function remains unknown.

It has been speculated that the noctuid moth B cell and its neighboring tympanal chordotonal organ are derived from proprioceptors monitoring flight-induced movements of the thorax, and that through certain peripheral modifications, such as the development of a tympanal membrane and enlargement of tracheal air sacs, the chordotonal organ became sensitive to airborne vibrations used for the detec-

tion of bat echolocation calls (see Roeder, '67; Yack and Fullard, '90). It is suggested that the B cell remains an unmodified portion of this ancestral proprioceptive system (Treat and Roeder, '59). If so, one might expect the B cell homologue in a primitively atympanate moth such as *Manduca sexta* (Sphingidae) to act as a wing proprioceptor.

Recently a chordotonal organ representing the homologue and evolutionary prototype to the noctuid moth tympanal organ was identified in the atympanate moth *Actias luna* (Saturniidae) (Yack and Fullard, '90; Yack and Roots, '92). However, the homologue to the noctuid B cell was not located, although extracellular recordings from the

IIIN1b1 nerve (the tympanal branch homologue) in *A. luna* indicated the presence of a large spontaneously active cell similar to the B cell. This led me to search for the B cell homologue in the IIIN1b1 branch of another atympanate moth.

This study reports on a previously undescribed complex of sensory receptors located in the wing-hinge of the atympanate hawkmoth *Manduca sexta*. This receptor complex consists of a large multiterminal cell, a simple chordotonal organ, and a hair plate. The functional role of this receptor complex and its homologous relationship to both the noctuid moth tympanal sensilla and the locust wing-hinge receptor complex are discussed.

MATERIALS AND METHODS

Manduca sexta (Linnaeus) were purchased as pupae from North Carolina Biological Supply Company. Adults emerged in an environmental chamber maintained at 26°C on a 12:12 hour light cycle. Adults were used 12–48 hours after eclosion. Noctuid moths, *Feltia herilis* (Grote), used to obtain a representative trace of the IIIN1b1 (tympanal) nerve activity, were provided by the Agricultural Canada Research Branch in Lethbridge, Alberta. Both male and female moths were used, as there were no apparent differences between the two sexes with respect to the characteristics examined.

For both anatomical and neurophysiological examinations, the thoracic nervous system was exposed by a modified dorsal approach (Roeder, '66; Yack and Fullard, '90). Following amputation of the head and legs, the moth was mounted, dorsal side up, on a block of modeling clay into which had been impressed a narrow, shallow groove to accommodate the body. The wings and abdomen were secured into position with wire staples, and the dorsal thoracic scales were brushed off. A shallow cut was made around the perimeter of the mesonotum (scutum and scutellum) followed by two more shallow cuts made on each side just medial to the forewing axillary cord. The mesonotum, along with the attached flight musculature, was then lifted out to expose the thoracic ganglia and their peripheral nerve branches.

Drawings of the peripheral nerve IIIN1 and its associated musculature and skeletal structures were prepared by following the nerve out to its peripheral innervation sites in 18 freshly dissected specimens of *M. sexta*. Following the standard dorsal dissection, the metathorax was removed from the animal, divided in a sagittal plane, and pinned to Sylgard (Dow Corning Corporation, Midland, Michigan) in a petri dish. In the fresh preparation, a few drops of 0.01–0.05% Janus Green B (Miller, '79) were applied to the exposed tissue and replaced after 1 minute with fresh saline (Paul, '74). More stain was applied throughout the dissection as unstained nerves were exposed. The branches of IIIN1b were traced as close as possible to their peripheral destinations and drawn with a Wild M5 dissecting microscope and camera lucida attachment. The chordotonal organ in the Janus Green B-stained IIIN1b1 branch was dissected out of the preparation and mounted on a glass slide with a small amount of saline. The stain increases the differentiation of the scolopale rods and caps (Yack, in preparation), thus facilitating their being counted and photographed.

Nomenclature used for muscles and nerves follows that of Nüesch ('53, '57). Structural details of the metathorax have been introduced only as they relate to the branches of the metathoracic IIIN1 nerve.

Since Janus Green B stains only the nerve branch, neither individual axons nor cell bodies of the neurones could be discerned. Therefore, axons of the first branch of IIIN1b (IIIN1b1) were filled out to their peripheral targets with either 4% Lucifer yellow CH (Sigma Chemical Company) (N = 12) or 10% cobalt lysine (Gallyas et al., '78) (N = 21). After the thoracic nervous system had been exposed by a dorsal approach (see above), the IIIN1b1 branch was severed at the point where it attaches to IIIN1b and its cut end was placed in a boat composed of a small disc of Parafilm surrounded by a rim of silicon grease and filled with distilled water. After a few seconds the distilled water was replaced with one of the two stains. The boat and any exposed tissue were covered with silicon grease in order to prevent dehydration. The preparation was then placed in a partially sealed container (lined with moistened paper towels in order to maintain a high humidity) and kept at 7–10°C for 6–30 hours. Following this period, the silicon grease and boat were removed, and the thoracic cavity was rinsed thoroughly with saline. For Lucifer yellow fills, the chordotonal organ and stretch receptor branches of IIIN1b1 were dissected out of the animal as quickly as possible with the aid of small amounts of Janus Green B to facilitate the dissection. These branches were then transferred to saline on a glass slide and photographed. For cobalt lysine fills, the musculature surrounding the IIIN1b1 branch was partially dissected away in order to expose the peripheral branches. The cobalt was precipitated with ammonium sulfide, and the preparation was fixed (acetic acid:ethanol [1:4]), intensified, dehydrated, and cleared according to Davis ('82). The filled nerve and its peripheral innervation sites were then drawn in situ with a Wild M5 dissecting microscope and camera lucida attachment, or photographed. The chordotonal organ and stretch receptor branches were then dissected out, mounted in Canada balsam on a glass slide, and photographed.

Extracellular recordings were taken of the left IIIN1b1 branch activity in 10 *M. sexta*. The thoracic cavity was exposed by the dorsal approach and recordings were made by hooking the IIIN1b1 branch onto an insulated stainless steel hook electrode. The nerve was drawn up slightly out of the saline and insulated with silicon grease. A tapered stainless steel wire was inserted into the exposed thoracic muscle as a reference electrode. The IIIN1 branch was routinely deefferented by severing it at the ganglion, in order to ensure that recordings were only representing centripetally conducted impulses. A small amount of low melting point wax was applied to the metathoracic scutum in order to eliminate the possibility of interference from the hair plate sensilla, also innervated by IIIN1b1 (described below in the Results). Extracellular axonal impulses were amplified with a WPI preamplifier (model #DAM-5A) and monitored with a storage oscilloscope.

To determine whether the sensory units were responsive to hind wing movements, the left wings were positioned so that their movement was not restricted by the modelling clay and they could be moved freely. Following the recording of spontaneous activity while the wing was held in a horizontal position, the wing was elevated from 0 to 90° (0°

being horizontal) with a glass pipette held in a micromanipulator, and then allowed to fall back to its original horizontal position.

Recordings of the IIIN1b1 nerve in the tympanate moth *F. herilis* (N = 5) were performed in the same manner as described above to obtain a trace (Fig. 7) of the B cell activity while the wing was maintained in a horizontal position.

RESULTS
Principal branches of the IIIN1 nerve

The principal branches of peripheral nerve root IIIN1 were carefully examined in *M. sexta* in order to confirm that the IIIN1b1 branch corresponded to the same (tympanal) branch in the noctuid moth as described in previous studies (e.g., Yack and Fullard, '90).

Figure 1b illustrates the peripheral projections of the main IIIN1 branches with respect to surrounding musculature. The peripheral nerve root (IIIN1) originates from the dorsolateral surface of the pterothoracic ganglion posterior to the junction of the meso- and metathoracic components. It takes a posterolateral course from the ganglion, where IImn runs beside it for a short distance. The first branch that appears along the IIIN1 nerve root innervates the ventrolongitudinal muscles IIvl1&2. The next distal branch (IIIN1a) innervates the intersegmental muscle, IIis. The main nerve trunk (IIIN1c) runs laterally between the meso- and metathorax, where it innervates the base of the metathoracic wing. The third branch (IIIN1b) continues posteriorly, passing across the medial surface of the metathoracic dorsoventral flight muscles (IIIdv), where it bifurcates. The larger of the two branches continues posteriorly and innervates the metathoracic dorsolongitudinal muscles (IIIdl1&2). The smaller of the two branches (IIIN1b1), which is homologous to the noctuid tympanal branch, continues posterolaterally, passing between the dorsoventral and dorsolongitudinal muscles to the moth's periphery. Here IIIN1b1 innervates a group of sensory receptors located near the posterior edge of the metathoracic wing's axillary cord (Fig. 1a,c). The remainder of these results will focus on this previously undescribed wing-hinge receptor complex.

Cobalt lysine and Lucifer yellow fills of IIIN1b1

Of the 21 cobalt lysine preparations, 9 resulted in complete fills (all branches that were previously seen with the Janus Green dissections were filled out to the periphery) and 12 were partial fills. Of 12 Lucifer yellow fills, 5 were complete and 7 were partial fills. Lucifer yellow tended to require shorter filling times than did the cobalt lysine: complete fills could be obtained within 8 hours, while the cobalt preparations required at least 18 hours for complete fills. However, the cobalt lysine proved to be more useful in this particular study, in that the cobalt-precipitated nerves could be easily processed, observed, and drawn in situ, then dissected out to be mounted and preserved indefinitely. Such processing was difficult with the Lucifer yellow fills, since the filled nerves, which were undifferentiated from surrounding musculature under a normal dissecting microscope, could not be easily observed or drawn in situ. Despite differences in their suitability for this study, both stains were equally effective in staining the nerve branches, and produced similar results in terms of the number of units

filled in the IIIN1b1 branch. A description of these results follows.

The IIIN1b1 branch passes directly between the dorsoventral and dorsolongitudinal flight muscles. Approximately midway between the medial and lateral boundaries of these muscles, the IIIN1b1 bifurcates (Figs. 1c, 2). One of the two IIIN1b1 branches follows a short distance between the dorsolongitudinal and dorsoventral musculature, where it divides into the SR (stretch receptor) and CO (chordotonal organ) branches (Figs. 1c, 2).

The SR branch takes a lateral and slightly ventral course, passes through a sheet of tracheal tissue and attaches to the membranous cuticle ventral to the hind wing axillary cord (Figs. 1a,c, 2). The SR branch contains a single axon (~ 3–4 μm in diameter) that terminates in a large cell body (~ 25 × 50 μm) (Figs. 1c, 5, 6b). The soma is ovoid in shape, with the axon at one end and a few tentacular dendrites at the other. The dendrites appeared to be embedded in the membranous tissue ventral to the axillary cord, and when I attempted to separate the cell from its attachment site, the dendrites appeared to contract. Although there did not appear to be muscle or connective tissue associated with the cell, the attachment site should be examined histologically to determine if there is an accessory component associated with the cell. Finally, in 2 of the 23 preparations that were successful in filling the SR nerve, there appeared to be two axons, and in one of these fills, two mirror-image cell bodies appeared to be present.

The CO branch extends slightly dorsad, passes through a sheet of tracheal tissue, and ends in a chordotonal organ (Figs. 1c, 2, 3, 6a). The chordotonal organ consists of a proximal scolopidial region and a distal, nonneural strand. The scolopidial region is ovoid in shape, measuring approximately 250 μm in length and 50 μm in width. Its proximal end is suspended by a ligament that attaches dorsally to the scutum. The CO nerve branch supplies the proximal end of the scolopidial region with three axons, each innervating one of three uniterminal (Type I) sensory cells. Each sensory cell has a proximal cell body (~ 10–15 μm in diameter), and a distal dendrite that is associated with a single scolopale cell. The CO strand (Figs. 1c, 2), which is approximately 1,200 μm long and 50 μm wide, attaches proximally to the distal end of the scolopidial region and distally to the edge of the sclerotized cuticle of the epimeron, where it borders the membranous cuticle ventral to the hind wing axillary cord (Fig. 1a,c).

The other branch of the IIIN1b1 bifurcation continues posterodorsally and turns medially around the periphery of dl2, between this muscle and the metathoracic scutum. Having reached the scutum, this branch innervates a hair plate consisting of at least 15 setae, each supplied by a single neuron (Figs. 1c, 4). The branch continues medially, where it appears to terminate in a small cluster of arborizations directly beneath the scutum. However, the specific peripheral innervation site of this small medial branch is not certain.

Extracellular recordings of IIIN1b1

Extracellular recordings of the deefferented IIIN1b1 branch were performed in 10 *M. sexta*. When the metathoracic wing ipsilateral to the recording electrode was held in a horizontal position, a single unit of large amplitude was spontaneously active, delivering a steady train of impulses during the time the wing was held in position (Fig. 7). (It is assumed here that the multiterminal cell is the source of
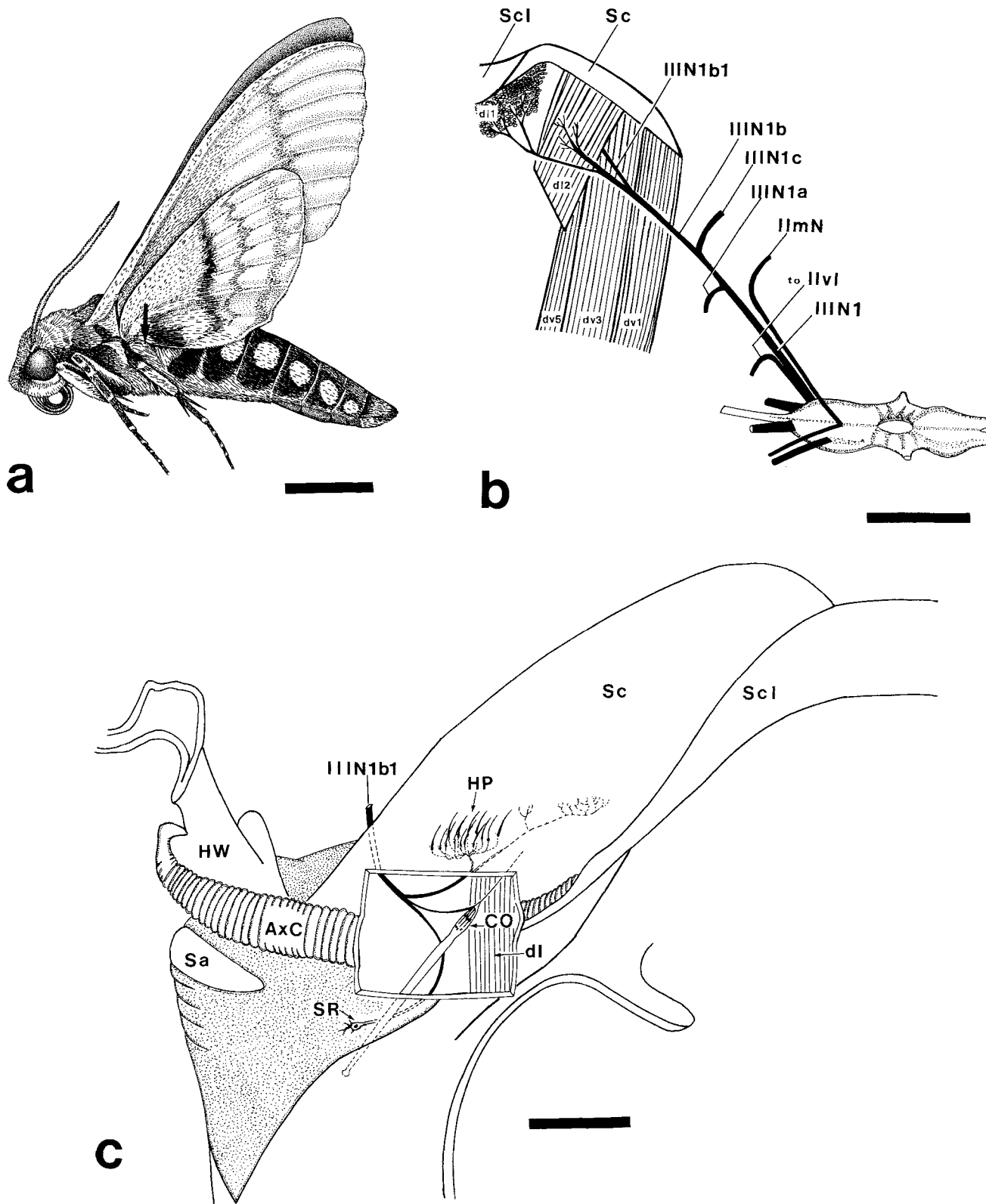
Fig. 1. Diagrams illustrating the location of the metathoracic wing-hinge sensory complex in *Manduca sexta*. **a:** Left lateral view of *M. sexta*. An arrow marks the general location of the wing-hinge receptor complex, as illustrated in c. Scale bar = 1 cm. **b:** Left half of the metathorax viewed from the midline, showing the principal branches of nerve root IIIN1 with surrounding nerves and musculature. The first branch of IIIN1b (IIIN1b1) takes a lateral course between the dorsoventral and dorsolongitudinal musculature, where it innervates the wing-hinge sensory cells. dl, dorsolongitudinal muscle; dv, dorsoventral muscle; Sc, scutum; Scl, scutellum. Scale bar = 2 mm. **c:** Posterior view of the left, descaled metathorax with parts of the cuticle, musculature, and tracheal tissue removed to reveal the wing-hinge receptor complex. The stretch receptor attaches to the membranous region (stippled) ventral to the axillary cord. The strand of the chordotonal organ attaches to the sclerotized cuticle of the epimeron in a slightly ventral position to the stretch receptor. AxC, axillary cord; CO, chordotonal organ; dl, dorsolongitudinal muscle; HW, hind wing; HP, hair plate; Sa, subalar sclerite; Sc, scutum; Scl, scutellum; SR, multiterminal stretch receptor. Scale bar = 500 μm.
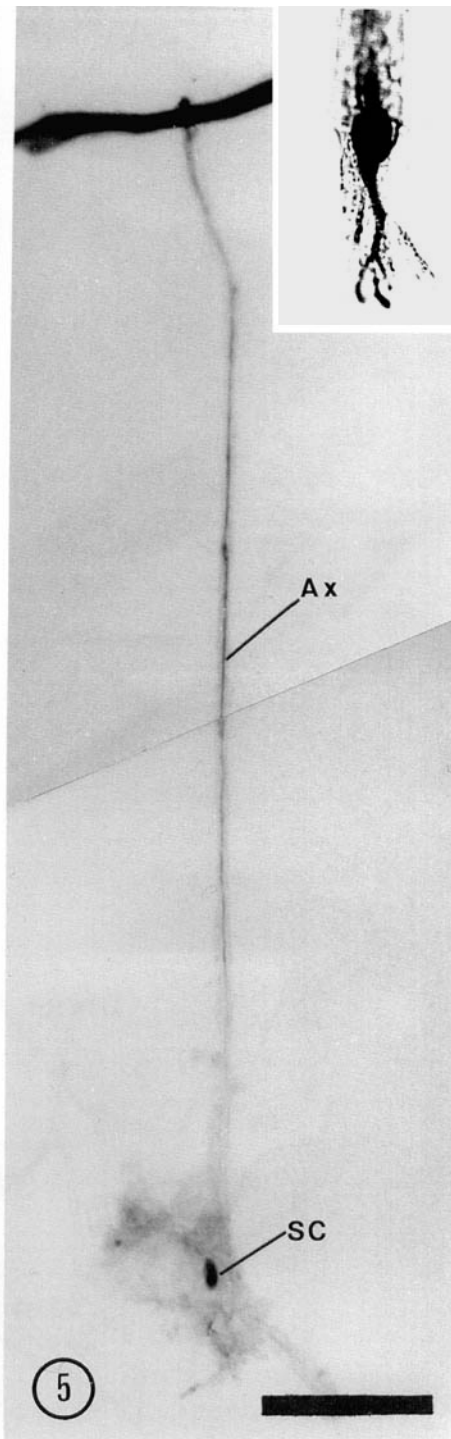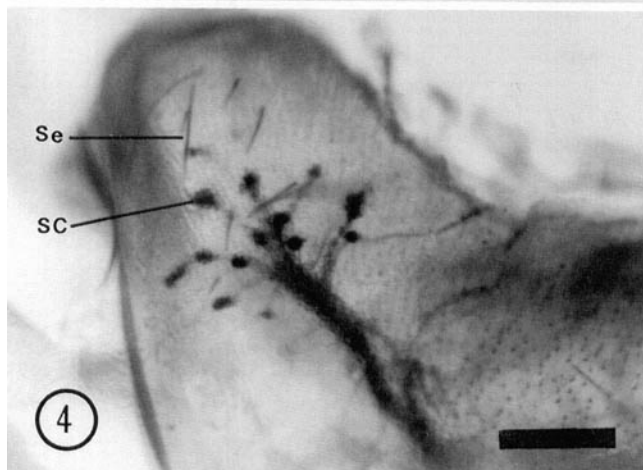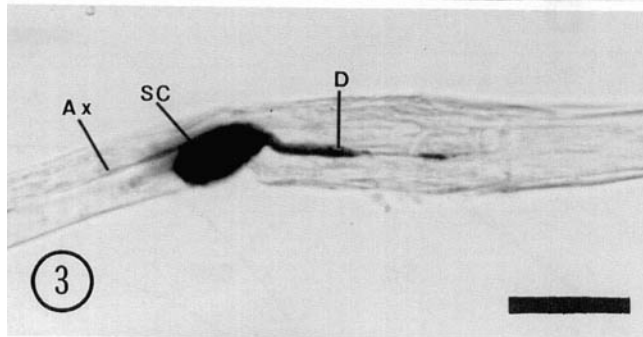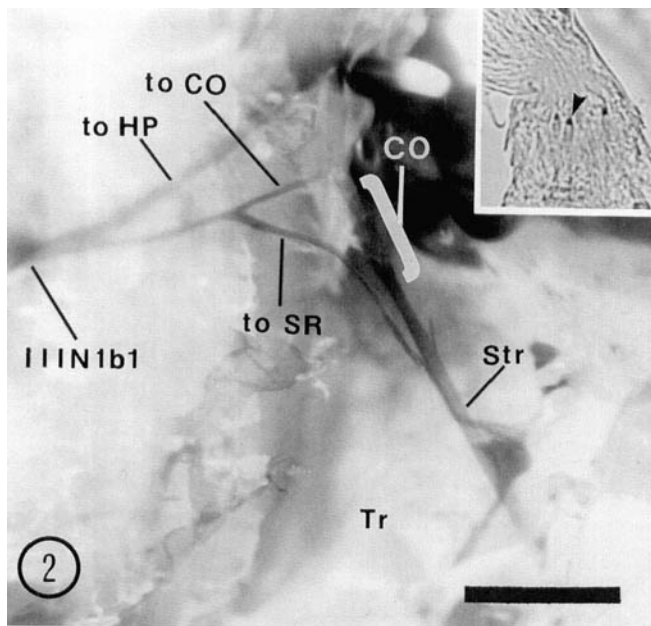
Fig. 2. Janus Green B-stained IIIN1b1 nerve branch viewed from the moth's interior. The dorsoventral musculature has been removed to reveal the branches of IIIN1b1. IIIN1b1 divides into two branches; one of these divides again into the multiterminal stretch receptor (SR) branch and the chordotonal organ (CO) branch. The SR axon and the CO strand (Str) pass through a sheet of tracheal tissue (Tr) before attaching peripherally to the subalar region (not shown). The other of the two IIIN1b1 branches continues in a dorsocaudal direction to the scutum, where it innervates a hair plate (HP). Scale bar = 500 μm. The inset is a Janus Green B-stained wholemount of the distal end of the chordotonal organ's scolopidial region. An arrowhead points to one of the three scolopale caps.

Fig. 3. Wholemount photomicrograph of the chordotonal organ's scolopidial region, showing one of the three uniterminal sensory cells

filled with cobalt. Distal is to the right. Ax, axon; D, dendrite; SC, sensory cell body. Scale bar = 45 μm.

Fig. 4. Cobalt fill of the hair plate on the scutum arising from the IIIN1b1 nerve branch. Each seta (Se) is innervated by a single sensory cell (SC). Some of the setae have been broken in processing the tissue. Scale bar = 250 μm.

Fig. 5. Cobalt fill of the stretch receptor branch, showing the single axon (Ax), which leads to the multiterminal (processes not shown) sensory cell (SC). The inset is also a cobalt fill, showing the cell body and some of the tentacular processes of the SR. Scale bar = 200 μm.
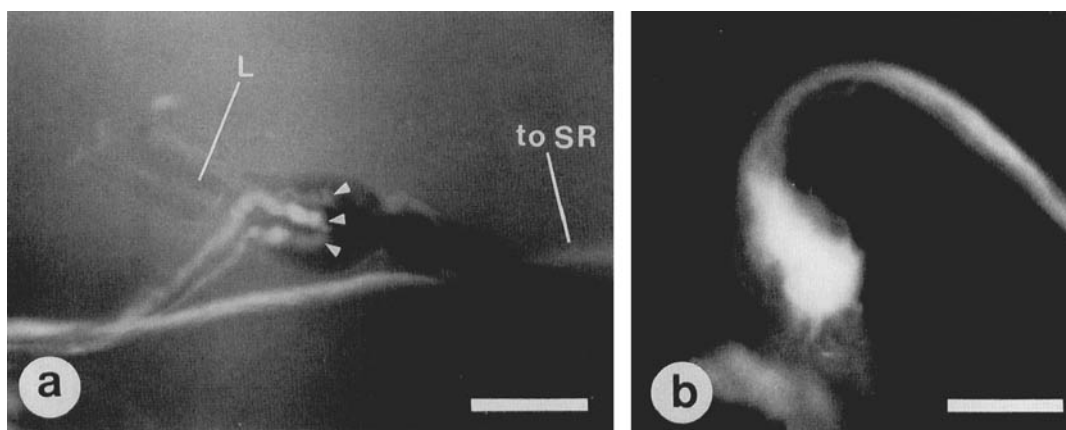
Fig. 6. **a:** Wholemount photomicrograph of the chordotonal organ's scolopidial region stained with Lucifer yellow, showing the three sensory cells (arrowheads). Part of the ligament (L) that suspends the proximal region of the chordotonal organ to the scutum is shown. The single axon that continues on to innervate the stretch receptor (SR) is also shown. Scale bar = 100 μm. **b:** The distal region of the Lucifer yellow-filled SR axon terminates in a single cell body. Scale bar = 35 μm.
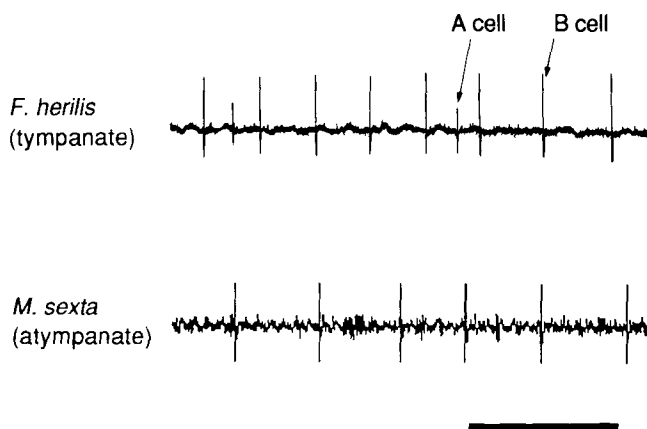

Fig. 7. Extracellular recordings of the deefferented IIIN1b1 nerve branch in a tympanate (*Feltia herilis*) and an atympanate (*Manduca sexta*) moth while the wings are maintained in a horizontal position. A single, spontaneously active cell is prominent in both traces. This unit is the multiterminal B cell in the tympanate moth and presumably, the multiterminal cell in the atympanate moth. A single spike of one acoustic A cell in the tympanate moth is also indicated. Scale bar = 100 milliseconds.

these impulses, since there are only two other types of sensory receptor in the deefferented IIIN1b1 branch: a chordotonal organ, which does not generally exhibit a regular resting discharge, and a hair plate, the setae of which have been waxed. In addition, the spontaneous resting activity of this cell resembles that of other multiterminal stretch receptors [cf. Finlayson, '76; Wright, '76].) While the rate of firing of this unit shows a wide variation between individual moths (from 4 to 25 spikes per second), in any one individual the rate is extremely regular. The rate of discharge in any given individual can be easily altered by raising the wing from the horizontal position. When the wing is raised, the rate of discharge is increased with increasing positional angle of the wing, where it reaches a maximum (from 41 to 79 spikes per second) at the top of the wing stroke. When the wing is allowed to drop passively to

its horizontal resting position, the unit is temporarily silent, and then resumes its original discharge rate (Fig. 8).

The discharge frequency of the tympanic B cell, recorded from the left IIIN1b1 nerve branch in *Feltia herilis* while the ipsilateral wing was maintained in a horizontal position, is presented in Figure 7 for comparison with its atympanate homologue.

DISCUSSION

In *M. sexta*, the peripheral innervation patterns of the principal IIIN1 branches (as shown in Fig. 1b) correspond to those described in the Saturniidae (*Telea polyphemus*, Nüesch, '57; *Actias luna*, Yack and Fullard, '90), the Noctuidae (*Prodenia eridania*, Paul, '73; *Feltia herilis*, Yack and Fullard, '90), and in a previous description of *M. sexta* (Eaton, '74). A more detailed description of the tympanal nerve (IIIN1b1) peripheral projections in the Noctuidae (Treat and Roeder, '59) shows that the IIIN1b1 bifurcates just outside the tympanal cavity. One of the two branches enters the tympanal cavity, where it innervates the two uniterminal sensilla of the tympanal chordotonal organ (the auditory A cells) and a multiterminal sensory cell (the nonauditory B cell). The other branch does not enter the tympanal cavity, but is drawn as a stump (Fig. 1 in Treat and Roeder, '59) headed in a dorsal direction. Paul's illustration ('73, Fig. 1) of the tympanal nerve shows this dorsally routed branch (named "xn") to terminate in the region of the scutum as a cluster of arborizations. In the atympanate moth, Nüesch ('57) briefly describes the whole IIIN1b1 branch as a fine branch that climbs up laterally on the dl2 muscle to the epidermis of the scutum and scutellum, possibly innervating the flat IIIdl3 muscle. Yack and Fullard ('90) also show that IIIN1b1 delivers a branch to the dorsum of the scutum, and further describe a second branch that innervates a chordotonal organ with three uniterminal sensory cells. Neither a hair plate nor a multiterminal cell, however, has been previously reported in the atympanate moth IIIN1b1 branch.

In *M. sexta*, a description of the IIIN1b1 peripheral projections is lacking, except for Eaton's ('74) mentioning

Fig. 8.   Extracellular hook electrode recording of the IIIN1b1 branch in *Manduca sexta*, showing the response of the spontaneously active cell to artificial elevation of the hind wing. Left of the open arrowhead the cell shows resting activity. The open arrowhead indicates the point at which the wing is initially being raised. The rate of firing increases with increasing angle of wing elevation. The closed arrowhead indicates when the wing is dropped from a 90° position. For a short period following release of the wing, the unit is silent, and then it returns to its resting discharge rate. Scale bar = 500 milliseconds.

that it goes to the scutum of the metathorax. Here I further describe the peripheral projections of IIIN1b1 in *M. sexta*, revealing a sensory complex consisting of a multiterminal stretch receptor, a chordotonal organ, and a hair plate. The remainder of this discussion focuses on these structures.

The multiterminal cell of IIIN1b1 described here in *M. sexta* has not been previously described in an atympanate moth, although extracellular recordings from the IIIN1b1 branch in *A. luna* (Yack and Fullard, '90) and numerous other species of atympanate moths (*Automeris io, Anisota virginiensis, Dryocampa rubicunda* [Saturniidae]; *Malacasoma americanum, Phyllodesma americana, Tolype laricis* [Lasiocampidae]; *Prionoxystus robiniae* (Cossidae); *Olceclostera angelica* [Apatelodidae]) (Yack, '87) indicate the presence of a similar cell. On the other hand, the anatomical and physiological characteristics of a multiterminal cell (B cell) in the IIIN1b1 nerve of the Noctuoidea have been well documented (Roeder and Treat, '57; Treat and Roeder, '59; Lechtenberg, '71; Pérez and Coro, '83; Surlykke, '84). Briefly, the B cell of the noctuid moth is a large (~50 × 20 μm) cell situated within the tympanal cavity in close proximity to the acoustic sensilla of the tympanal organ. The B cell in the family Noctuidae attaches to the Bügel (Eggers, '19; von Kennel and Eggers, '33), an apodemal ingrowth of the skeletal frame of the tympanum, which serves for support and attachment of one end of the tympanal organ and its nerve (except in the family Notodontidae, where a Bügel was not observed [Surlykke, '84]) and does not appear to be associated with specific muscles or with other peripheral neurones save those of the acoustic sensilla.

Previous studies show that the resting noctuid B cell delivers a regular train of impulses varying between 4 and 100 per second in different preparations, but remaining the same in any given individual. The firing rate of the cell does not appear to be altered significantly while the moth is in stationary flight, during normal manipulations of the wing, or when subjected to a variety of other experimental manipulations, including exposure to changes in temperature or barometric pressure (Roeder and Treat, '57; Treat and Roeder, '59; Lechtenberg, '71). However, the train of firing can be significantly altered by manually deforming the thorax along its longitudinal axis, or by directly stretching the tracheal sheath associated with the cell by opening the tracheal air sac and pulling it with forceps (Roeder and Treat, '57; Treat and Roeder, '59). Also, the noctuid B cell appears to be entirely unresponsive to acoustic stimulation (e.g., Roeder, '67; Surlykke, '84; Yack and Fullard, '90;

however, see Lechtenburg, '71). To date, the functional significance of the noctuid B cell remains uncertain.

The multiterminal cell in *M. sexta* appears to be homologous to the noctuid B cell. Both are large, multiterminal cells arising from homologous nerve branches, attaching peripherally to the same region ventral to the metathoracic axillary cord, although in the noctuid this region has become modified into a tympanum. In addition to being in an homologous anatomical situation to the noctuid moth B cell, the multiterminal cell of *M. sexta* resembles the B cell physiologically: both deliver a regular train of impulses in the cell's resting state that, although variable in its rate between individuals, remains constant in any one individual. Unlike the B cell, however, the SR does not attach to a Bügel, or any structure that resembles the latter, but rather to the soft, membranous cuticle ventral to the hind wing. In many ways this cell resembles the subepidermal multiterminal stretch receptor whose processes are closely associated with the basement membrane and epidermal cells (Finlayson, '68). Also unlike the noctuid B cell, the SR readily responds to elevation movements of the wing by increasing its frequency. It appears that the SR, but not the B cell, may act as a proprioceptor monitoring wing movements during flight.

In light of these findings, and if the SR represents the evolutionary prototype to the noctuid B cell, it is feasible to propose that the B cell once acted as a proprioceptor monitoring wing movements. Perhaps the B cell, like the acoustic cells of the tympanal organ, once acted as a proprioceptor, but unlike the acoustic cells, remained unmodified. Indeed, detailed anatomical descriptions of the tympanic cavity suggest that the latter was constructed to resist flight-induced deformations of the thorax (Eggers, '19; Treat, '59). The B cell may have become mechanically isolated from the movements of the thorax by the rigid construction of the tympanic cavity and Bügel. It would be interesting to see how the B cell in a notodontid moth (which lacks a Bügel) responds to wing movements. Although the evidence here suggests that the noctuid B cell once acted as a proprioceptor monitoring wing movements, its current function remains uncertain. Its consistent and regular activity suggests that it is still functional and active. Perhaps it detects subtle distortions of the abdomen during flight, or, as proposed by Lechtenberg ('71), responds indirectly to sound via the acoustically active A cells. It is also possible that it functions as a neurosecretory cell influencing the acoustic cells at the central nervous system level.

The chordotonal organ consists of a proximal scolopidial region (comprised of three mononematic, monodynal scolopidia) and a distal strand (see Moulins, '76, for definitions), and closely resembles that which has been recently described in another atympanate moth, *A. luna* (Yack and Fullard, '90; Yack and Roots, '92). Two slight differences were noted. In *M. sexta,* the attachment strand attaches to the sclerotized cuticle adjacent to the membranous cuticle ventral to the hind wing axillary cord, while in *A. luna,* this strand attaches to the membranous cuticle adjacent to the sclerotized epimeron. Also, in *M. sexta* the CO appeared to be more tightly suspended between the scutum and epimeron. The functional significance of this organ in the atympanate moth has not yet been determined, but it is suspected from its topographical position and from preliminary physiological recordings in *A. luna* (Yack and Fullard, '90) that it acts as either a proprioceptor monitoring phasic movements of the hind wing, or as a detector of vibrations, such as those observed during preflight warm-up. Studies are now underway to determine the function of this organ. For an anatomical and physiological comparison between the atympanate and tympanate chordotonal organs, see Yack and Fullard ('90) and Yack and Roots ('92).

The branch of IIIN1b1 that continues to the scutum first innervates a hair plate. The setae of this hair plate are slanted medially and are located in a position where they would appear to receive stimulation from the metathoracic wing in its resting position, folded over the back of the animal. It is possible that this hair plate contributes information about wing position while the animal is at rest. A second group of arborizations was seen just medial to the hair plate, but the details of its innervation are uncertain and require closer examination. Although a similar branch leading to the scutum has been described in both atympanate (Nüesch, '57; Eaton, '74; Yack and Fullard, '90) and tympanate moths (Treat and Roeder, '59; Paul, '73; Yack and Fullard, '90), its peripheral targets are undescribed. Considering the conservative nature of insect nervous systems (e.g., Dethier, '63), this branch might be expected to innervate hair plates in other moths, as it does in *M. sexta,* and should therefore be reexamined.

Finally, it is interesting that a receptor complex similar to and putatively homologous to that described here in *M. sexta* has been described in the metathoracic wing-hinge of the locust. Gettrup ('62) first reported a proprioceptor complex composed of a single multiterminal neuron and a chordotonal organ, both of which attach to the nonsclerotized membrane in the region of the subalar sclerite, and a distal branch that leads to a hair plate. The physiological properties of the multiterminal cell have been studied extensively (e.g., Gettrup, '62; Wilson and Gettrup, '63; Burrows, '75; Altman and Tyrer, '77; Möhl, '85; Wolf and Pearson, '88); it appears to monitor movements of the hind wing, and contributes significantly to the regulation of the flight motor generator. I predict that the multiterminal cell in *M. sexta* described here plays a similar role in flight. The function of the chordotonal organ in the locust is as yet uncertain, although it is suggested that it may monitor vibratory movements of the wing during warm-up (Pearson et al., '89). Similarly, the CO of the atympanate moth has been reported to respond to vibration (Yack and Fullard, '90).

The purpose of this paper was to report on a previously undescribed sensory receptor complex in the metathoracic wing-hinge of *M. sexta.* If, as suggested by the data, the multiterminal cell of this complex acts as a proprioceptor monitoring wing movements, similar to that of the locust wing-hinge sensory receptor, it might be expected to play a dominant role in the regulation of wing beat frequency during flight. Also, as putative homologues and evolutionary prototypes to the noctuid moth tympanal sensilla, the wing-hinge sensory receptors in *M. sexta* could be useful as models for examining changes in the nervous system that accompanied the evolutionary development of the noctuid moth ear.

LITERATURE CITED

Altman, J.S., and N.M. Tyrer (1977) The locust wing hinge stretch receptors. I. Primary sensory neurones with enormous central arborizations. J. Comp. Neurol. *172:*409–430.

Burrows, M. (1975) Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust. J. Exp. Biol. *62:*189–219.

Davis, N.T. (1982) Improved methods for cobalt filling and silver intensification of insect motor neurons. Stain Technol. *57:*239–244.

Dethier, V.G. (1963) The Physiology of Insect Senses. London: Methuen & Co., Ltd.

Eaton, J.L. (1974) Nervous system of the head and thorax of the adult tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae). Int. J. Insect Morphol. Embryol. *3:*47–66.

Eggers, F. (1919) Das thoracale bitympanale Organ einer Gruppe der Lepidoptera Heterocera. Zool. Jahrb. Anat. *41:*273–376.

Finlayson, L.H. (1968) Proprioceptors in the invertebrates. Symp. Zool. Soc. Lond. *23:*217–249.

Finlayson, L.H. (1976) Abdominal and thoracic receptors in insects, centipedes and scorpions. In P.J. Mill (ed): Structure and Function of Proprioceptors in the Invertebrates. London: Chapman & Hall, Ltd.

Gallyas, F., L. Lénárd, and G. Lázár (1978) Improvement of cobalt-transport in axons by complexing agents. Neurosci. Lett. *9:*213–216.

Gettrup, E. (1962) Thoracic proprioceptors in the flight system of locusts. Nature *193:*498–499.

Lechtenberg, R. (1971) Acoustic response of the B cell in noctuid moths. J. Insect Physiol. *17:*2395–2408.

Miller, T.A. (1979) Insect Neurophysiological Techniques. New York: Springer-Verlag, Inc.

Möhl, B. (1985) The role of proprioception in locust flight control. II. Information signalled by forewing stretch receptors during flight. J. Comp. Physiol. A *156:*103–116.

Moulins, M. (1976) Ultrastructure of chordotonal organs. In P.J. Mill (ed): Structure and Function of Proprioceptors in the Invertebrates. London: Chapman and Hall, Ltd.

Nüesch, H. (1953) The morphology of the thorax of *Telea polyphemus* (Lepidoptera) I. Skeleton and muscles. J. Morphol. *93:*589–608.

Nüesch, H. (1957) Die Morphologie des Thorax von *Telea polyphemus* Cr. (Lepid.) II. Nervensystem. Zool. Jahrb. Abt. Anat. *75:*615–642.

Paul, D.H. (1973) Central projections of the tympanic fibres in noctuid moths. J. Insect Physiol. *19:*1785–1792.

Paul, D.H. (1974) Responses to acoustic stimulation of thoracic interneurons in noctuid moths. J. Insect Physiol. *20:*2205–2218.

Pearson, K.G., B. Hedwig, and H. Wolf (1989) Are the hind wing chordotonal organs elements of the locust flight pattern generator? J. Exp. Biol. *144:*235–255.

Pérez, M., and F. Coro (1983) Actividad eléctrica espontánea del órgano timpánico en varias especies de lepidópteros. Cienc. Biol. 9:25–36.

Roeder, K.D. (1966) Acoustic sensitivity of the noctuid tympanic organ and its range for the cries of bats. J. Insect Physiol. 12:843–859.

Roeder, K.D. (1967) Nerve Cells and Insect Behavior. Harvard Books in Biology, 2nd ed. Cambridge, Mass.: Harvard University Press.

Roeder, K.D., and A.E. Treat (1957) Ultrasonic reception by the tympanic organ of noctuid moths. J. Exp. Zool. 134:127–157.

Surlykke, A. (1984) Hearing in notodontid moths: A tympanic organ with a single auditory neurone. J. Exp. Biol. 113:323–335.

Treat, A.E. (1959) The metathoracic musculature of Crymodes devastator (Brace) (Noctuidae) with special reference to the tympanic organ. In: Studies in Invertebrate Morphology. Washington, D.C.: Smithsonian Institution, pp. 365–377.

Treat, A.E., and K.D. Roeder (1959) A nervous element of unknown function in the tympanic organs of moths. J. Insect Physiol. 3:262–270.

von Kennel, J., and F. Eggers (1933) Die abdominalen Tympanalorgane der Lepidopteren. Zool. Jahrb. Anat. 57:1–104.

Wilson, D.M., and E. Gettrup (1963) A stretch reflex controlling wingbeat frequency in grasshoppers. J. Exp. Biol. 40:171–185.

Wolf, H., and K.G. Pearson (1988) Proprioceptive input patterns elevator activity in the locust flight system. J. Neurophysiol. 59:1831–1853.

Wright, B.R. (1976) Limb and wing receptors in insects, chelicerates and myriapods. In P.J. Mill (ed): Structure and Function of Proprioceptors in the Invertebrates. London: Chapman & Hall, Ltd.

Yack, J.E. (1987) The mechanoreceptive origin of insect tympanal organs: A comparative study of homologous nerves in tympanate and atympanate species of Lepidoptera. M.Sc. Thesis, Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

Yack, J.E., and J.H. Fullard (1990) The mechanoreceptive origin of insect tympanal organs: A comparative study of similar nerves in tympanate and atympanate moths. J. Comp. Neurol. 300:523–534.

Yack, J.E., and B.I. Roots (1992) The metathoracic wing-hinge chordotonal organ of an atympanate moth, Actias luna (Lepidoptera, Saturniidae): A light- and electron-microscopic study. Cell Tissue Res. 676:455–471.