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Sound Production in the Cockroach, *Gromphadorhina portentosa*: The Sound-Producing Apparatus

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Summary. 1. The giant Madagascar cockroach, *Gromphadorhina portentosa,* hisses by expelling air from a pair of specialized abdominal spiracles. The anatomy and innervation of serially homologous respiratory and sound-producing spiracles were compared in order to determine the evolutionary steps by which a new behavior has developed.

2. The trachea leading to the sound-producing (fourth) spiracle shows a constriction proximally; distally it is greatly elongated with a conical bore (Fig. 2). These features, which are lacking in other spiracles, are sufficient to account for the character of the sound (Fig. 10).

3. The motoneurons innervating both types of spiracles were located by axonal diffusion of cobalt, and their morphology was determined in whole-mounted ganglia. The number, ganglionic locations, and in some cases branching patterns of motoneurons serving the sound-producing and respiratory spiracles were essentially identical (Figs. 4, 5, 6).

4. Physiological activity was recorded along spiracle nerves and within spiracle muscle fibers; four units were identified for each spiracle, agreeing with the number of cells located anatomically. These included, for each abdominal spiracle, an opener exciter motoneuron, two closer exciter motoneurons, and one closer inhibitor motoneuron (Figs. 7, 8).

5. During normal respiration the output of these 4 units had similar phase relationships in all abdominal spiracles which were examined; lower firing rates in the motoneurons innervating the hissing spiracles rendered these nonfunctional during normal respiration (Fig. 9).

6. The findings are consistent with conservation of motor innervation and of central pattern generators during evolution.

Abbreviations: AI, first abdominal ganglion; *AII,* second abdominal ganglion; *MNHO,* median neurohaemal organ; *N2,* first segmental root (abdominal ganglia); *TI,* first thoracic ganglion; *TIII,* third thoracic ganglion

Introduction

Nervous systems alter in structure and activity, both in evolution and in the lifetime of individuals, and the behavior of species and individuals alters as a result. A number of investigators have traced ontogenetic changes in central and peripheral neural elements and have linked these changes to behavioral ontogeny (Altman and Tyrer, 1974; Bentley, 1973; Bentley and Hoy, 1970; Kammer and Rheuben, 1976; Taylor and Truman, 1974; Truman, 1976; Truman and Reiss, 1976). In these cases it was possible to make direct comparisons between early and late stages of development in a given population. It is not often possible, however, to make equivalent comparisons on an evolutionary time scale. Direct analyses of the physiological and structural modifications which are responsible for behavioral evolution have been rare and have depended on comparisons among species or cross-species hybrids (Bentley, 1971; Hoy et al., 1977; Willows and Dorsett, 1975). The present study deals with a system that provides an unusual opportunity to deduce, by studying a single species, the anatomical and physiological changes underlying the evolution of a unique behavior pattern. This opportunity arises from the fact that *Gromphadorhina portentosa,* the giant Madagascar cockroach, produces sounds (hisses) by expelling air from a pair of specialized spiracles, which are the only modified pair among 6 serially homologous abdominal spiracles. By taking advantage of this segmental repetition

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Table 1. Characteristics of hissing in *Gromphadorhina portentosa*[a]

Type of hiss	Emitted by	Stimulus or context	Acoustical characteristics	Associated behavior
1. Disturbance	Older nymphs and adults of both sexes	Sudden or disruptive stimuli, e.g. substrate vibration, abrupt light onset, rapidly moving shadows, handling	High amplitude, rapid rise to peak amplitude after abrupt onset	"Tilting" response to localized stimuli
2. Courtship (two types)	Adult males	Presence of adult female (olfactory and possibly tactile cues)	Moderate amplitude. Slow onset and abrupt offset (type 1); rapid amplitude modulation ("trilling") (type 2)	Posturing with abdomen extended (type 1); thrusting during copulation attempts (type 2)
3. Copulatory	Adult males	Tactile stimuli from female (?)	Low amplitude, brief duration	
4. Aggressive	Adult males	Intrusion of other males into territory; tactile and olfactory cues (?)	Moderate to high amplitude; slow amplitude modulation; abrupt onset	Aggressive display ("abdomen thrashing")

[a] Data from Nelson and Fraser (in preparation)

of a structural design to compare the morphology of the specialized spiracles with that of their homologues, it is possible to make deductions about how sound production has evolved.

In order to understand how the specialized spiracles attained their present form it is necessary to understand the context in which they are used and the demands which are made on them as the animal behaves. The behavioral aspects of sound production in this species will be discussed in detail in another report (Nelson and Fraser, in prep.) and will only be reviewed briefly here (see Table 1).

The sounds produced by *Gromphadorhina* are hisses with a broad frequency spectrum (2 to 20 kHz), ranging in amplitude from less than 40 to more than 80 dB when measured close to the source. Adult males hiss during courtship, copulation, and territorial defense; nymphs and adults of both sexes hiss when disturbed. The sounds emitted in these different contexts are clearly distinguishable by differences in their duration and absolute amplitude, in the shapes of their amplitude envelopes, and in the temporal patterning of hiss-trains. Hisses function in intraspecific communication and play an essential role in reproductive behavior. Thus since the structural elements responsible for the production of hisses affect reproductive success, they have probably been subject to strong selective pressure.

In this paper I report an analysis of the sound-producing spiracle and its motor apparatus to determine which components (cuticular, tracheal, muscular and neural) are the same as in conventional spiracles, and which have been modified in the course of evolution. I have found that the components of the sound-producing apparatus have become modified to differing degrees. While several non-neural elements are altered, the peripheral motor innervations of sound-producing and respiratory spiracles are indistinguishable. The analysis of these patterns of change may provide clues to the means by which behavior evolves.

Materials and Methods

1. Animals. The cockroaches used in this study were maintained in mass culture at 26 °C and approximately 65% relative humidity. They received a constant supply of water and dry food (Wayne Solo dog food) supplemented by fresh fruits and greens. Most animals had molted 12 to 24 h before their use in this study; such animals have minimal deposits of fat body, and are therefore easier to dissect.

2. Anatomical Methods. Gross morphology of the spiracles and tracheae was revealed through dissections of fresh preparations stained supravitally with methylene blue (1% in saline), and of fixed preparations preserved with 10% formalin in saline. Evans' saline was used throughout (Evans, 1975). Animals of both sexes in the first through the seventh (adult) instar were examined. Scale drawings and measurements of spiracular structures were made from fresh material viewed through a dissecting microscope equipped with an eyepiece grid micrometer. The gross differences between spiracles in tracheal and muscle structure which are described in the next section were already apparent in the first instar; all dimensions are based on adult material. No differences were seen, either in anatomy or in physiology, between males and females, and animals of both sexes are treated together in the Results section.

The location within the ganglia and the patterns of arborization of spiracle motoneurons were determined by axonal backfilling of cells with cobaltous chloride (Pitman et al., 1972; Taylor and Truman, 1974; Strausfeld and Obermayer, 1976). In this procedure the ganglia of origin, the composite spiracle nerve, and the opener and closer muscles of a given spiracle (see Fig. 1) were dissected free in a culture medium (Levi-Montalcini et al., 1973) consisting of 5 parts Schneider's *Drosophila* medium to 4 parts Eagle's basal

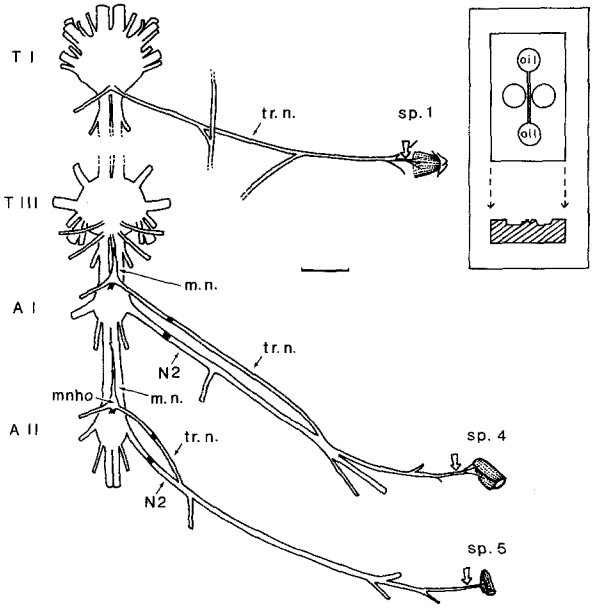

Fig. 1. Dorsal dissection of ventral nerve cord of *G. portentosa*, showing innervation of first (thoracic) and fourth and fifth (abdominal) spiracles. Arrows indicate placement of suction electrodes for extracellular recordings from spiracle nerves. Filled bars along nerves: points at which nerves were cut during recording. TI, first thoracic ganglion; TIII, third thoracic ganglion; AI, first abdominal ganglion; AII, second abdominal ganglion; Tr. n., transverse nerve; m.n., median nerve; mnho, median neurohaemal organ; N2, first segmental nerve root; sp. 1, sp. 4, sp. 5: muscles of spiracles 1, 4, and 5. Scale: 1 mm (lengths of peripheral nerves not drawn to exact scale). *Inset:* Design of incubation chamber used for cobalt diffusion. Two wells connected by a shallow trench contain mineral oil; one of the remaining wells (shown also in cross-section) holds the ganglia immersed in culture medium, while the fourth well holds cut end of spiracle nerve immersed in $CoCl_2$

amino acid mixture (both obtained from GIBCo). The tissues were then transferred to a well in an incubation chamber and the spiracle nerve, with one or both muscles still attached, was draped over a mineral-oil barrier into a second well (see inset, Fig. 1). The second well was filled with distilled water, the muscle was trimmed away, and the cut nerve was subjected to osmotic shock for 8 to 10 min. Cobaltous chloride (400 mM in distilled water, containing 13 mg bovine serum albumen per 100 ml) was exchanged for the distilled water, and the tissue was incubated at 4 °C for 24 to 60 h. It was then rinsed in saline, developed in ammonium sulfide (3 drops in 10 ml saline), treated briefly with protease, 2 mg/ml saline (from *Streptomyces griseus;* Sigma Chemical Co.) to facilitate removal of fat body from the exterior of the ganglia, and fixed in Bouin's or Serra's fixative or 10% formalin in saline. Some preparations were immediately dehydrated in alcohols, cleared in xylene or methyl salicylate, and mounted in Canada balsam. Some preparations were subjected to a wholemount variant of the Timm intensification procedure before dehydration, to enhance the visibility of cobalt-filled structures (Tyrer and Bell, 1974; Bacon and Altman, 1977). A total of 129 preparations were examined, of which 76 were in the 6th or 7th (adult) instar and 63 were in the 2nd through 4th instars. Of these, the 4th spiracle nerve was filled in 76 preparations, the 5th spiracle nerve was filled in 49 preparations, and the 6th spiracle nerve was filled in 4 preparations. Both males and females were used. Unless otherwise specified, all chemicals used in these procedures were obtained from Fisher Scientific Co.

3. Physiological Recordings. To record physiological activity in spiracle nerves and muscles, animals were pinned dorsal side up in a dish coated with Sylgard (Dow-Corning), and the section of cuticle overlying spiracles 4 and 5 was removed along with the superficial tracheae. In some cases the nerve and muscle of spiracle 1 were also exposed by removing the thoracic tergites. The gut was cut far anteriorly and folded back out of the body cavity, and the cavity was flooded with culture medium. A chlorided silver wire in the bath served as indifferent electrode.

Action potentials were recorded *en passant* with saline-filled glass suction electrodes. Muscle potentials were recorded intracellularly with glass microelectrodes (fiber-filled, WPI) containing 0.6 M K_2SO_4 (resistance 30–50 megohms). Ventilatory movements were recorded by running a thread from the anterior margin of the fifth abdominal tergite to a Grass FT.03 force transducer. Oscilloscope traces were photographed and taped for later analysis. Extracellular activity was also monitored with a Grass AM 8 audiomonitor. Since the action potentials travelling in the spiracle nerve were of sufficiently different sizes to produce audibly different sounds, it was possible to identify spiracle-muscle movements, observed directly under the microscope, with the activity of specific units in the spiracle nerve.

In some preparations certain nerves were cut while spontaneous respiratory activity was being recorded, to determine the origin along the ventral nerve cord of particular units. These nerves were the first segmental root (N2, following the terminology of Shankland, 1965), the ipsilateral transverse nerve, the short portion of the median nerve which links the median neurohaemal organ (MNHO) to the ganglion, or the long portion of the median nerve which connects the MNHO to the next anterior ganglion (Fig. 1). The results reported here are based on recordings from 18 preparations all of which maintained stable respiratory activity for several hours.

Results

1. Gross Morphology of the Fourth Abdominal Spiracles

a) The Trachea. *Gromphadorhina's* ability to make sounds is due to a modification of the trachea that joins spiracle 4 to the lateral longitudinal tracheal trunk (Fig. 2). Such a modification was first described, in *G. brunneri,* by Dumortier (1965).

In *G. portentosa* the tracheae leading from the other abdominal spiracles to the lateral tracheal trunk are typically short and broad (diameter ca. 450 μm); they are narrow distally at the spiracle valve and join the trunk proximally without constriction. The trachea leading from spiracle 4 in *G. portentosa* is distinctive in three ways: i) distally, the trachea joins the spiracle valve without constriction; in turn the diameter of the spiracle opening is about 3 times larger than in the other abdominal spiracles. ii) Proximally, the junction between this trachea and the lateral trunk is greatly constricted, forming a narrow, rigid neck (internal diameter ca. 50 μm). ii) Between these two points the trachea is greatly elongated. It

Fig. 2. Dorsal view of tracheae leading to spiracles 4 and 5 in an adult male *G. portentosa*. Animal's abdominal tergites removed; anterior is towards the lower left-hand corner of the plate. The lateral longitudinal tracheal trunk can be seen as a broad tube running from the upper right margin of the Fig. diagonally down to the pointers at the center; it has been removed beyond this point to reveal the trachea, leading to spiracle 4, which it normally covers. Trachea leading from spiracle 4 joins lateral trunk proximally at the narrow constriction indicated by the pointers; it merges distally into the atrium of spiracle 4 (labelled "4") in the region outlined in white. Trachea leading from spiracle 5 is outlined by white in its entirety, from its junction proximally with the lateral trunk to its fusion distally with the atrium of spiracle 5 (labelled "5"). Note the much broader atrial region in spiracle 4 as compared to spiracle 5, the much greater length of trachea 4 as compared to trachea 5, and the much smaller junction of the former trachea with the lateral trunk. Scale: 200 μm

approximates a truncated cone in longitudinal section, typically increasing in external diameter from about 250 μm near the lateral trunk to about 850 μm at the spiracle valve, over a length of 1 cm.

b) The Spiracle Valve and Muscles. Spiracles 4 through 9 all exhibit the same basic design (Fig. 3). Each abdominal spiracle lies in a separate sclerite (peritreme) facing ventrally in the pleural fold between dorsal and ventral sclerites at the anterior border of each abdominal segment. The external opening of the spiracle – the atrium – is at the posterior corner of the spiracular peritreme. From its rigid cuticular opening the atrium extends internally as a tube of flexible cuticle invaginated at one side to form a flap, which normally blocks the opening of the spiracle. Interior to this flap the atrium merges into the trachea. The flap thus forms a valve, which is opened by contraction of an opener muscle that originates in a broad band on the anterior edge of the peritreme and inserts within the cuticular flap (Fig. 3A and B). The flap is held closed or returned to its closed position by contraction of a closer muscle attached to skeletal elements on either side of the atrium. These are a spine running between the fibers of the opener muscle at a slight angle to the long axis of the muscle, and a bar (manubrium) extending medially from the rigid rim of the atrium on the opposite side from the fold (Fig. 3A). The closer muscle originates on this bar and inserts along the spine. Thus when the closer muscle contracts, the spine acts as a piston which pushes the flap against the opposite rim of the spiracle opening. A small group of closer muscle fibers also runs from the closer spine to insert along the anterior edge of the peritreme near the insertion of the opener muscle.

Spiracle 4 retains this basic arrangement of elements. It differs, however, in several details (Fig. 3C). The opener and closer muscles of spiracle 4 are larger in all dimensions than are the corresponding muscles of the more posterior spiracles (Fig. 3C and Table 2). The closer is 2 times greater and the opener is nearly 5 times greater in volume than their homologues in spiracle 5, which is slightly larger in all its dimensions than spiracles 6 through 9. In addition, the opener muscle of spiracle 4 has a second part that is lacking in other abdominal spiracles. This is a massive band of muscle with large fibers running obliquely (Fig. 3C).

2. Anatomy of the Motoneurons

The numbers, locations, and branching patterns of motoneurons serving the abdominal spiracles were determined by cobalt diffusion in the spiracle nerves. When a single spiracle nerve was filled there were never more than two ganglia containing filled cells, nor more than two cells filled per ganglion, although in some preparations fewer than four cells were seen. No difference in the locations or in the maximum number of filled cells was detected across instars or between sexes. The following observations are based primarily on 52 fills of the nerves to spiracles 4 and 5 in adults or last-instar nymphs; a small number of fills of the nerve to spiracle 6 revealed the same pattern. Figure 11 summarizes both anatomical and physiological data on innervation of the abdominal spiracles.

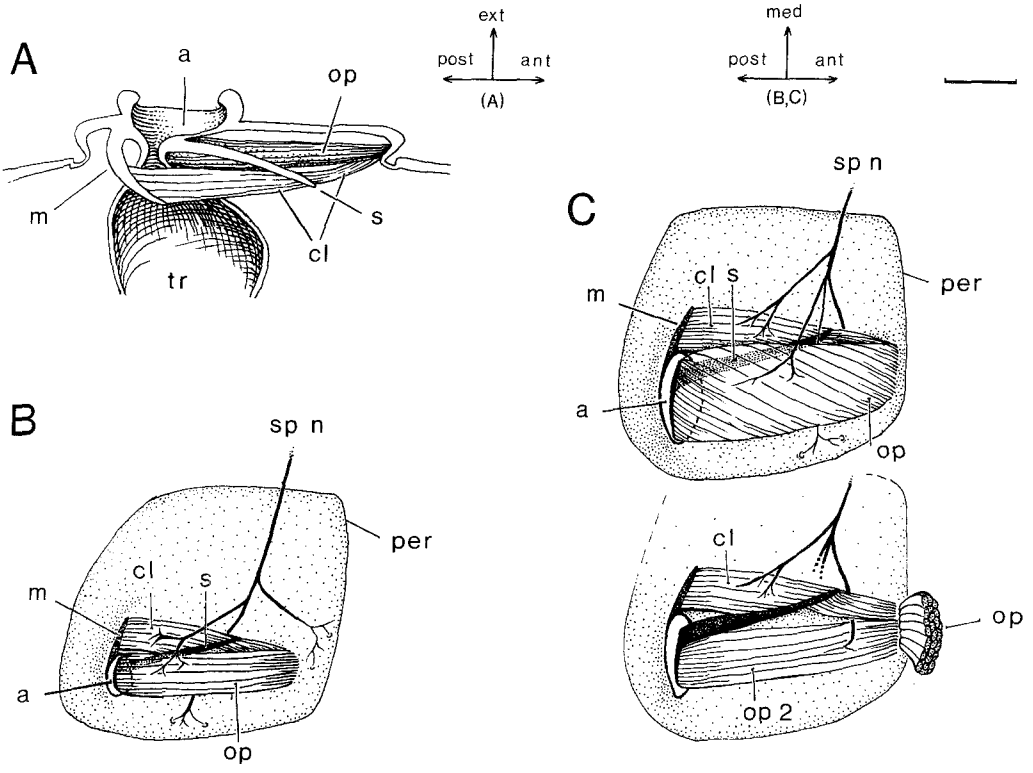

Fig. 3A–C. Anatomy of spiracles 4 and 5 in *G. portentosa*. **A** Side view of an abdominal spiracle. Closer muscle, with its skeletal attachments (spine and manubrium) projects towards the viewer. Spine and opener muscle insert into the atrial flap which closes the spiracle. Atrium and trachea to which it leads shown in section. **B** Dorsal view of spiracle 5. Trachea, which would normally project up out of the page, has been cut away to reveal the opener and closer muscles, with their innervation, lying within the peritreme (spiracular sclerite). In addition to the motor nerves serving the muscles, sensory nerves run to bristles on the surface of the peritreme and spiracle valve. **C** Dorsal view of spiracle 4. Above, with opener muscle intact; below, with dorsal part of opener deflected to reveal the smaller underlying part. *a*, atrium of spiracle; *cl*, closer muscle; *m*, manubrium; *op*, opener muscle; *op 2*, second part of opener muscle; *per*, peritreme; *sp n*, spiracle nerve, *s*, spine; *tr*, trachea. Scale (A, B and C): 500 μm

Table 2. Dimensions of the spiracle valve and its muscles in spiracles 4 and 5. Measurements on adult males weighing between 5 and 6 g (mm and mm^3, $\bar{X} \pm$ s.e.m.; 6 samples

	Spiracle 4		Spiracle 5	
	Opener[a]	Closer	Opener	Closer
Length	1.63 ± 0.07 mm	1.30 ± 0.09	1.50 ± 0.04	1.23 ± 0.06
Width	0.61 ± 0.03	0.35 ± 0.02	0.36 ± 0.02	0.27 ± 0.02
Depth	0.48 ± 0.02	0.26 ± 0.02	0.21 ± 0.02	0.18 ± 0.02
Volume[b]	0.48 ± 0.05 mm^3	0.12 ± 0.02	0.11 ± 0.01	0.06 ± 0.01
Valve diameter	0.60 ± 0.0 mm		0.21 ± 0.01	

[a] Both sections
[b] As a rectangular solid

The pair of muscles (opener and closer) in each spiracle together receive axons from 4 neurons. Two small neurons have their somata (diameter ca. 20–30 μm) on either side of the ventral midline in the ganglion anterior to the segment containing the spiracle (the 3rd thoracic ganglion (TIII) for spiracle 4, the first abdominal ganglion (AI) for spiracle 5, and so forth). Each of these cells sends a single axon out of the ganglion in the median nerve; the axon then bifurcates to send a branch out each transverse nerve, and innervates both spiracles of that segment. This pattern was evident in unilateral fills in which cobalt diffused past the branch point into the contralateral branch as well as the median ascending axonal stem, and in bilateral fills in which cobalt diffused along both axonal branches of each cell, filling only two somata as in unilateral fills. One or both of these cells filled in 13 (25%) of the preparations, and their arborizations could be seen in only a few cases (3 for spiracle 4 and 2 for spiracle 5). In comparing the locations of homologous cells in ganglia TIII and AI it should be noted that TIII is a fused structure which incorporates the embryonic last thoracic and first abdominal ganglia (Shankland, 1965). The pattern of arborization of these cells is shown in Fig. 4, for both spiracle 4 and spiracle 5.

The other two neurons seen in cobalt preparations have their somata in the ganglion of the segment

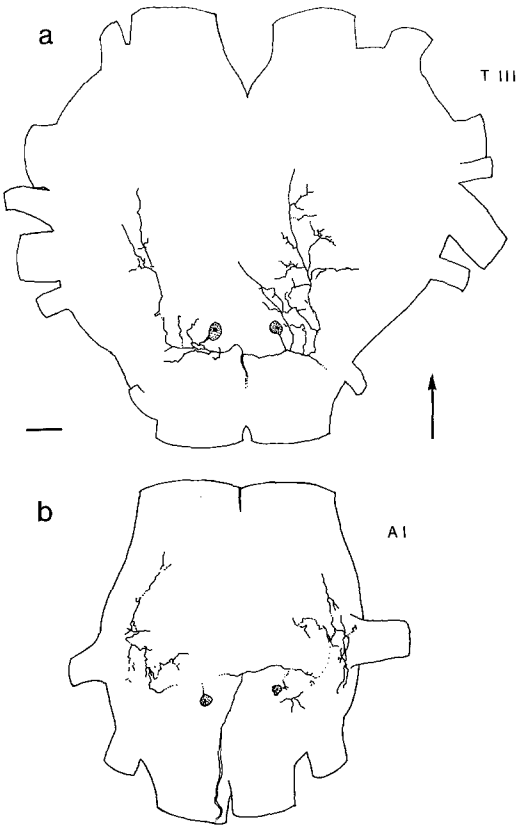

Fig. 4a and b. Camera lucida drawings of motoneurons innervating spiracle 4 (a) and spiracle 5 (b) on right side. These cells send their axons out the median and transverse nerves (see Fig. 1). **a** third thoracic ganglion, dorsal view; intensified preparation. Right spiracle nerve filled. **b** first abdominal ganglion, dorsal view; unintensified preparation. Right spiracle nerve filled. Scale: 100 μm

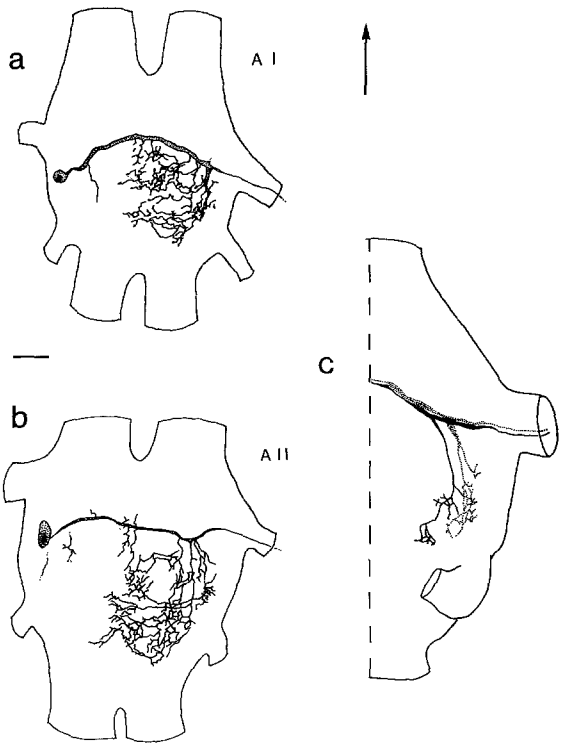

Fig. 5a–c. Camera lucida drawings of motoneurons innervating spiracle 4 (**a**) and spiracle 5 **(b, c)**. These cells send their axons out N2 (see Fig. 1). **a** First abdominal ganglion, dorsal view; intensified preparation. **b** second abdominal ganglion, dorsal view; intensified preparation. **c** second abdominal ganglion, dorsal view; unintensified preparation. In this preparation two cells were visible; note parallel course of main neurites and secondary branches. Scale: a, b: 100 μm; c: 50 μm

containing the spiracle (AI for spiracle 4, the second abdominal ganglion (AII) for spiracle 5, and so forth). Both somata lie contralateral to the spiracle they innervate, and their axons run in N2, the major segmental root leaving the ganglion (Figs. 1 and 11). In 15 out of 27 fills (56%) from spiracle nerve 4 and in 11 out of 25 fills (44%) from spiracle nerve 5, two somata (ca. 40–60 μm) were seen, in close proximity. In the remaining preparations, only one contralateral soma was seen. In a few of the preparations, it was possible to isolate either the closer or the opener branch of the nerve to spiracle 4 and to fill one or the other selectively. In all these cases a single cell was filled, which lay contralaterally in AI.

The arborizations of the contralateral cells could be seen in detail in 16 preparations (9 for spiracle 4 and 7 for spiracle 5). Their arborizations parallel each other at least as far as the secondary branches (Figs. 5c and 6); the major concentration of neurites is ipsilateral to the innervated spiracle, although some branches cross the midline posteriorly and two small branches consistently leave the main neurite at the midline of the ganglion (Figs. 5 and 6). In these samples homologous cells in ganglia AI and AII were indistinguishable with respect to the location and extent of finer branches, and the number of secondary branches (emerging near the base of N2) showed the same degree of variation in both ganglia: in AI there were 1.89 ± 0.26 branches while in AII there were 1.86 ± 0.26 branches ($\bar{X} \pm$ s.e.m.).

3. Physiological Activity in Spiracle Nerves and Muscles

Extracellular recordings from spiracle nerves and intracellular recordings from spiracle muscle fibers during spontaneous respiration reinforced the observations on cobalt-filled cells, and permitted functional identification of some of the cells seen with anatomi-

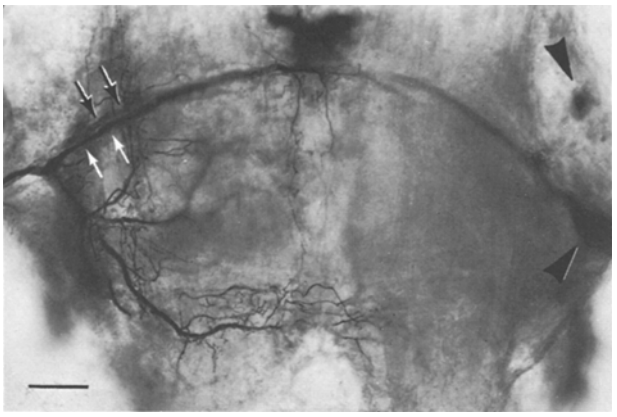

Fig. 6. Dorsal view of a Timm-intensified, whole-mounted first abdominal ganglion in which the motoneurons innervating left spiracle 4 were filled. A portion of the arborization is visible, primarily ipsilateral to the innervated side. Two cells were filled in this ganglion; both cell bodies (arrowheads) are visible out of plane of focus on right-hand side of the figure. One cell filled less densely than the other; its main neurite (double black arrows) is visible, parallel to the darker neurite (double white arrows) of the other cell, in the region where the major descending neurite branches off. Scale: 100 μm

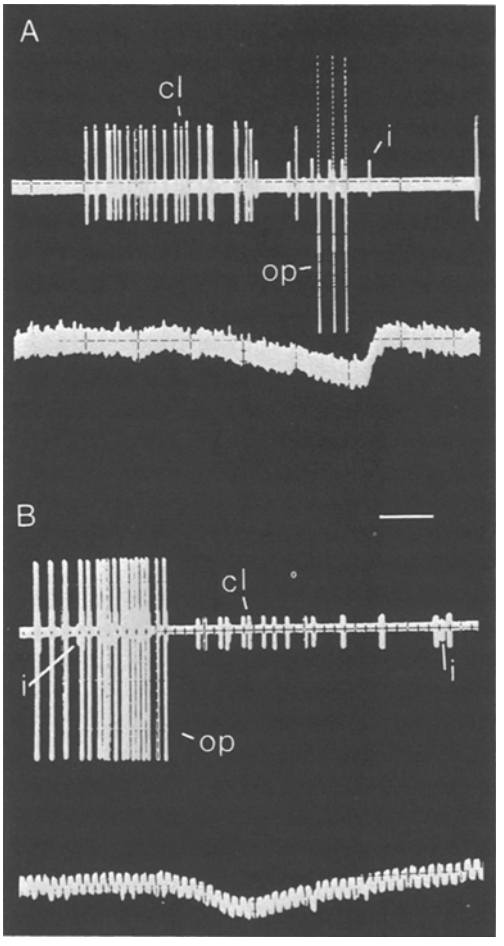

Fig. 7A and B. Action potentials in spiracle nerves during spontaneous respiration. In each case upper trace is an *en passant* recording via a glass suction electrode from spiracle nerve. Note presence of three size-classes of action potentials, labelled op (opener, =class 1, largest), cl (closer, =class 2, intermediate) and i (inhibitor, =class 3, smallest). Lower trace in each case shows ventilatory movement recorded externally via a force transducer attached to an abdominal tergite. **A** Action potentials in nerve to spiracle 4, just before and during expiration; in this preparation expiration registered as a rapid upward deflection following a period of relaxation. Time marker 0.5 s. **B** Action potentials in nerve to spiracle 5, at the time of expiration. In this preparation relaxation phase, upward-going in this case, was less evident; expiration registered as a downward deflection. Time marker 0.1 s

cal techniques. Extracellular recordings from the nerves in the regions indicated by large arrows in Fig. 1 revealed three distinct classes of units which differed in the sizes of their action potentials (Fig. 7). Simultaneous intracellular recordings from muscle fibers indicated that unitary nerve potentials belonging to a given size class were always correlated with the same class of intracellular muscular potentials; thus units could be functionally identified in extracellular recordings on the basis of spike height. The first class of units produced large action potentials which were correlated with excitatory post-synaptic potentials (EPSP's) in fibers of the opener muscle (Fig. 8A), and with contraction of that muscle. In a given nerve, all spikes in this class were essentially the same height, and no summations could be seen, suggesting that a single unit was responsible. The second class contained two units, with action potentials of slightly different sizes in extracellular recordings (Figs. 7, 8C), which correlated with EPSP's of two sizes in closer muscle fibers (Fig. 8B, C) and with contraction of the closer. These units frequently fired in couplets.

The third class consisted of small units whose action potentials correlated with inhibitory (hyperpolarizing) post-synaptic potentials (IPSP's) in closer muscle fibers (Fig. 8D); no IPSP's were seen in opener muscle fibers.

In *Gromphadorhina,* as in other large insects (Miller, 1974), movement of air through the tracheal system is from front to rear. This is achieved by i) relaxing and thus opening the thoracic spiracles during inspiration (the passive phase of respiration) while closing the abdominal spiracles, and then ii) closing the thoracic spiracles and opening the abdominal ones during expiration (the active muscular phase of respiration (Fig. 9a)). Figure 9b compares the phase relationships between expiration and the units described above; the first thoracic spiracle nerve was also monitored in order to obtain a more complete picture of spiracular/ventilatory coupling. Each of the three classes of units fired in a characteristic relationship

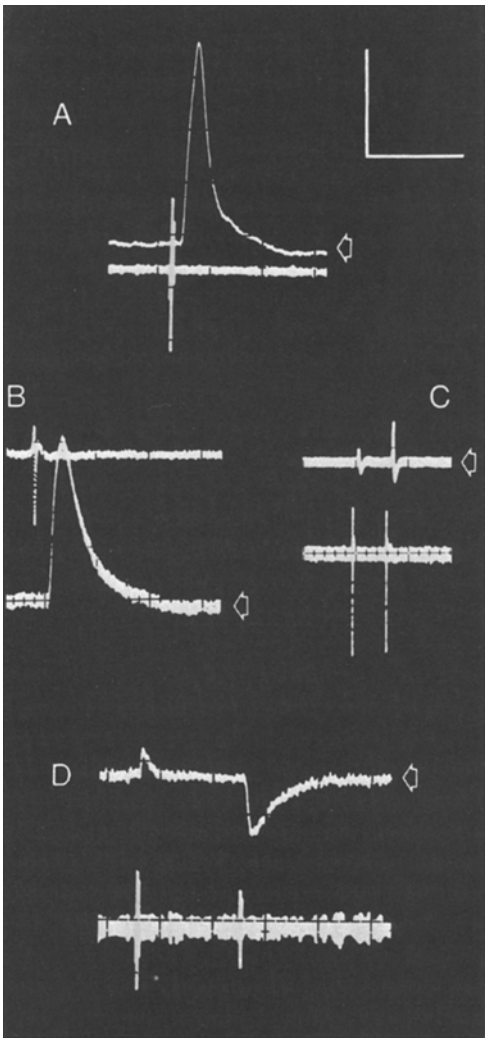

Fig. 8 A–D. Correlation between spike activity in spiracle nerve and intracellular events in spiracle muscle fibers. Arrows indicate recordings from muscle fibers. Recordings from nerve and muscles of spiracle 4. **A** Upper trace: EPSP in an opener muscle fiber. Scale marker: 5 mV, 25 ms. Lower trace: class 1 (largest) action potential. **B** Upper trace: class 2 action potential; lower trace: EPSP in a closer muscle fiber. Scale marker: 4 mV, 20 ms. **C** Upper trace: EPSP's recorded extracellularly from a closer muscle fiber. Scale marker: 0.2 mV, 100 ms. Lower trace: class 2 action potentials. **D** Upper trace: EPSP and IPSP in a closer muscle fiber. Scale marker: 5 mV, 25 ms; lower trace: class 2 and class 3 action potentials which correlate with EPSP and IPSP respectively

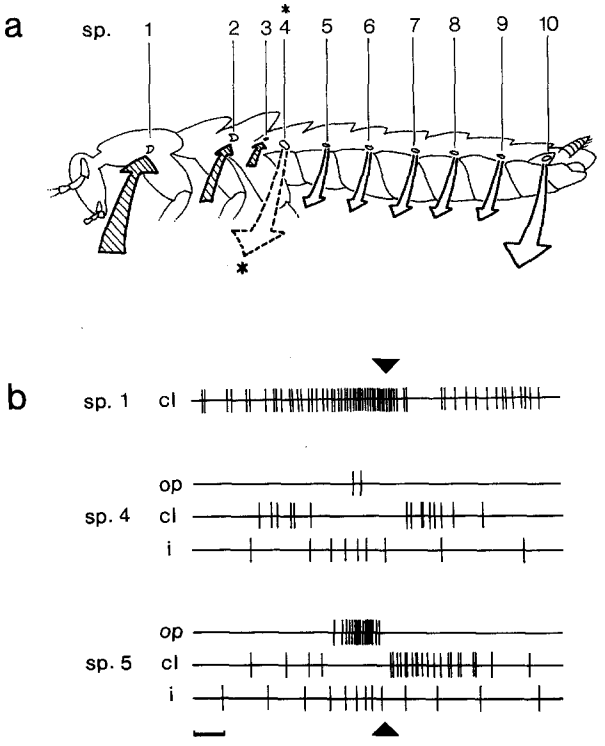

Fig. 9. a Movement of air through tracheal system in *Gromphadorhina* during normal respiration (hatched and clear arrows) and during disturbance hissing (dotted starred arrow). During normal respiration air moves into tracheae through thoracic spiracles and out of tracheae through fifth through tenth spiracles of the abdomen; fourth spiracle remains closed. During disturbance hissing all spiracles except the fourth remain closed and the fourth spiracle opens during expiration. **b** Phase relationship between ventilatory movements and inputs to spiracle muscles during normal respiration (tracings of extracellular records). Arrows: occurrence of an expiratory movement, recorded externally sp. 1, sp. 4, sp. 5: first, fourth and fifth spiracles. *cl*, impulses in closer motoneurons; *op*, impulses in opener motoneuron; *i*, impulses in inhibitory unit. Scale: 0.2 s

to expiratory movement. In the first thoracic spiracle, which has a closer muscle only, activity in the spiracle nerve reaches a peak during expiration, ceases immediately afterwards (=inspiration) and slowly returns to a high firing rate during the interphase. In the respiratory abdominal spiracles (5 *et seq.*) the largest unit (opener exciter) fires in a burst at the onset of expiration, opening the spiracle at each expiratory movement. The two intermediate units (closer exciters) fire briefly before and again towards the end of each expiratory movement. The smallest unit (inhibitor) fires during the opener burst, beginning its activity before and ending it after the opener fires.

A similar pattern applies in spiracle 4, with somewhat different consequences in terms of spiracle valve movements. The opener unit innervating this spiracle fires in phase with other abdominal opener units (compare "op", sp. 4 and sp. 5, in Figs. 7 and 9b), but in each cycle the burst is much briefer and is not sufficient to cause contraction of the muscle, as is evident by direct observation of the muscles during recording. Occasionally (especially at low respiratory rates) the unit is silent throughout the cycle. The closer units and the small inhibitory unit also fire in synchrony with their homologues in other abdominal spiracles. Spiracle movements during disturbance hisses were observed directly in intact animals held under a dissecting microscope. During these hisses

the animal closes all spiracles, thoracic and abdominal, except for the fourth spiracle, which it opens while making an expiratory movement (Fig. 9a).

Transections of the nerve roots supplying axons to the spiracle nerves (see Fig. 1) eliminated specific classes of units from extra- and intracellular recordings. Cutting either the long part of the median nerve or the ipsilateral transverse nerve eliminated the second class of units along with EPSP's in the closer muscle; the largest and smallest units still fired at the appropriate times relative to expiration. Conversely, cutting N2 eliminated large and small units along with EPSP's in the opener muscle and IPSP's in the closer muscle; the two intermediate units still fired in their normal rhythm. Cutting the short part of the median nerve had no effect. When both N2 and the median or transverse nerve from a single ganglion were cut, all activity disappeared from the spiracle nerve and muscles, indicating that all sources of innervation had been removed.

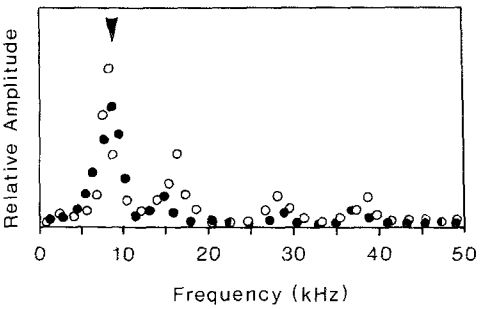

Fig. 10. Relationship between theoretical fundamental frequency of the tracheal "horn" and actual frequency spectra of disturbance hisses. Arrowhead indicates theoretical fundamental frequency for a truncated conical horn 1 cm in length (the average length of the tracheal horn in adult cockroaches). Curves show frequency spectra of disturbance hisses recorded from two adult males (represented by filled and open circles); spectra were obtained by Fourier analysis using a packaged Fast Fourier Transform program (DECUS)

Discussion and Conclusions

The results described above permit some deductions about the evolution of sound production in *Gromphadorhina*. Comparison of spiracle 4 with serially homologous respiratory spiracles in the abdomen reveals a mixture of "conserved" and "innovative" elements.

1. Tracheal Morphology. The production of hisses depends on modifications in the trachea leading from spiracle 4. To hiss, the animal opens spiracle 4, closes all other spiracles (at least in the case of disturbance hisses) and contracts the dorso-ventral expiratory muscles of the abdomen. As a result, much of the air contained in the tracheal system is forced through the narrow, rigid neck of the trachea into the tracheal horn (see Fig. 2). This gives rise to broad-band aerodynamic noise, due to the turbulence set up in the air stream as it passes the constriction (Lighthill, 1952).

The tracheal horn has the conical bore characteristic of a musical horn. An air passage having this shape should act as a resonator (resonating at multiples of that frequency where the length of the horn equals $^1/_4\ \lambda$; Roederer, 1975). For disturbance hisses this is indeed the case (Fig. 10). These hisses represent the simplest situation acoustically, since for a disturbance hiss the animal opens the spiracle valve fully and the mouth of the horn is then essentially unobstructed due to the especially large opening of this spiracle. Tracheal horns from adult males average 1 cm in length; the resonant frequency for a truncated conical horn 1 cm long is 8534 Hz. In the two samples shown in Fig. 10, the major peak agrees well with this figure, with further smaller peaks occurring at multiples of this frequency. Thus it appears that the tracheal modifications contribute to sound production in two ways; the constriction at the proximal end is responsible for the sound *per se*, and the elongated horn leading to the enlarged valve shapes and amplifies this sound.

This modification in tracheal structure is almost certainly general among the members of the genus. I have observed the same device in *G. chopardi*, and Dumortier (1965) described virtually identical structures in *G. coqueleriana* and *G. brunneri*. All of these species are known to produce audible hisses.

Conversion of a trachea to a sound-producing instrument is not without precedent; tracheal-spiracular structures are generally quite malleable in evolutionary terms. They have acquired non-respiratory functions a number of times among the Orthoptera alone. In the Tettigoniids and Gryllids, they have taken on a dual auditory and respiratory function (Lewis, 1974; Nocke, 1975); in the Blaberids and Acridids, they have become adapted independently several times in evolution for the manufacture, storage, and dispersal of defensive secretions (Eisner, 1958; Roth and Stay, 1958; Morse, 1907).

2. Morphology of the Spiracle Muscles. The hisses uttered in different social contexts differ in several ways, but the most consistent indicator of context is the shape of the amplitude envelope (Nelson and Fraser, in prep.). The amplitude of the sound depends on the rate at which air moves past the tracheal constriction (Lighthill, 1952), and several factors which

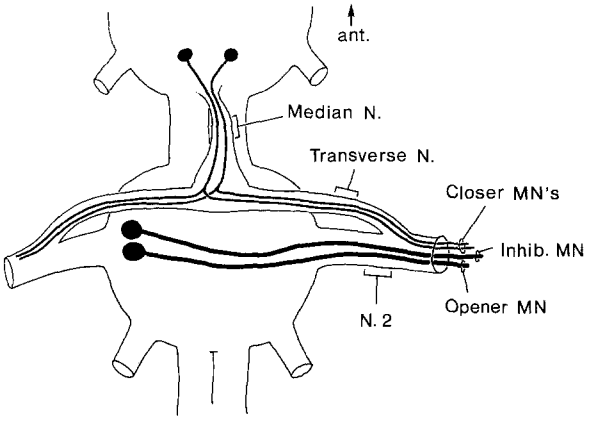

Fig. 11. Innervation of abdominal spiracles in *G. portentosa* showing a typical pair of abdominal ganglia and the locations and identities of spiracle motoneurons within these ganglia. The innervation for one of a pair of spiracles is shown. See text for discussion

affect air flow – the initial tracheal volume, the rate of expiration, and the degree and rate of opening of the spiracle valve – could therefore affect the amplitude of hissing. The movement of the spiracle valve, however, should be most important in generating amplitude modulation, since this occurs within single hisses each of which represents a single expiratory movement. This role presumably imposes special requirements on the muscles controlling the valve. Valve opening requires an inward movement of the cuticular flap, which in some instances, as in forming the abrupt onset of a disturbance or aggressive hiss, must be performed swiftly against considerable internal pressure. Valve closing must be sufficiently rapid to produce the abrupt offset characteristic of courtship hisses; the trills which are also seen in courtship hissing call both opener and closer muscles into play repeatedly. Thus the exceptionally large muscles in the fourth spiracle may find their explanation in the amplitude-modulation of *Gromphadorhina*'s signalling system.

3. Innervation of the Abdominal Spiracles. Cobalt backfilling, physiological recordings, and selective ablations of median, transverse, and segmental nerves revealed little difference between the motoneurons of spiracle 4 and other abdominal spiracles. The anatomical and physiological data agree well and together lead to the picture of the innervation of abdominal spiracles summarized in Fig. 11.

A single opener-exciter motoneuron innervates the opener muscle. Its soma lies contralaterally in the ganglion of the segment containing the spiracle, and its axon exits via N2. Two closer-exciter motoneurons, which correspond to the two intermediate units seen in the spiracle nerve, have their somata in the ganglion of the segment anterior to that containing the spiracle. Their axons reach the closer muscle via the median and transverse nerves. This is consistent with observations on a wide range of insect species, in all of which spiracle closer muscles receive their innervation via the median nerve from cells with bilateral projections (e.g. Case, 1957; Miller, 1974). In *Gromphadorhina* the pair of closer-exciter neurons innervating each closer muscle tends to fire together, but one or the other unit occasionally misses, suggesting that while both units receive the same input they are not directly coupled.

The second contralateral cell whose soma lay near that of the opener motoneuron in about half the cobalt fills was present in all preparations in which only the nerve to the closer was filled. This cell is tentatively identified as the closer-inhibitor unit whose small action potentials correlate with IPSP's in the closer muscle. It appears to be a specific inhibitor serving a single closer muscle in each segment; this interpretation is borne out by its firing pattern, which shows a negative correlation with that of the excitatory motoneurons to the same muscle (Fig. 9). Thus it differs from the common inhibitors seen in several insect species, which do not have this reciprocal relationship with other motoneurons (Pearson and Bergman, 1969).

We might speculate that this pattern of inputs to the spiracle muscles is an innovation which arose in *Gromphadorhina* in association with the evolution of sound production; it is unusual in several respects. Inhibitory innervation of an insect spiracle has been reported only once, for the first thoracic spiracle of another cockroach, *Blaberus discoidalis* (Miller, 1969). While the author suggested no specialized function for the arrangement, it may be significant that *Blaberus,* like *Gromphadorhina,* uses several of its spiracles for nonrespiratory purposes – in this case for expelling the contents of a tracheal gland, probably as a defensive maneuver (Miller, 1973). Given the unity of excitatory input to the two closer muscles of each segment, the presence of paired inhibitory motoneurons that provide separate inputs to the two sides might allow more flexible use of the two spiracles (unilateral sound production does occur in *Gromphadorhina,* primarily in response to localized painful stimulation (personal observation). The presence of inhibitory motoneurons might also be related to the fine control of spiracle-valve movement required for modulation of sounds; the varieties of amplitude modulation which distinguish different types of hisses (Table 1) may depend on specific programs of inhibitory and excitatory inputs to the closer and opener muscles. Both of these speculations about the function of inhibitory innervation in this system must,

however, take into account the fact that inhibition is not restricted to the specialized spiracles. Spiracle innervation has not been exhaustively investigated, and it is possible that inhibitory input is a general feature of the motor control of insect spiracles which has been overlooked heretofore.

The presumptive inhibitory motoneuron is also unusual anatomically in that its branches appear to parallel those of the opener excitor, with which it fires in concert during normal respiration. Such parallel branching of functionally related cells is not unusual where a group of motoneurons all have the same effect on a single muscle (e.g. Simmons, 1977); the phenomenon of cell duplication, which might well give rise to this situation, has been examined experimentally (Goodman, 1977). In the present case, however, the cells in question presumably use different transmitters, and it is difficult to imagine a simple model of this kind for the development of their relationship. An intriguing possibility is that these cells, which act functionally as synergists, have their anatomical layout defined with respect to a common input that is also responsible for their co-activation.

Whatever the basis for the innervation of these spiracles, it is clearly a general pattern for the abdominal spiracles of *G. portentosa*. Moreover, the inputs to spiracle motoneurons during normal respiration are apparently similar in all the abdominal spiracles. During ventilatory movements, spike activity in the spiracle 4 motoneurons is in phase with activity in the other abdominal spiracle motoneurons. In the spiracle 4 opener neuron, however, there are fewer spikes per burst than in other opener neurons, and the muscle does not normally contract during expiration.

These observations suggest that, while the function of spiracle 4 has changed, the "primitive" wiring diagram has persisted. The elimination of spiracle 4 from participation in the normal respiratory cycle may thus have been achieved by a change in the efficacy of the synaptic inputs to the opener motoneuron. During sound production, when spiracle 4 is active, the inputs to its motoneurons must be complex, although the conventional coupling with expiratory movements is maintained. The inputs to other spiracle motoneurons must undergo a 180° phase shift when the transition from respiration to sound production takes place.

The normal function of the respiratory control system is to drive a persistent, stable rhythmic activity. In *Gromphadorhina* this has been modified in the course of evolution so that a non-rhythmic behavior, which conveys information to other members of the species, can be produced. In the periphery it appears that minimal alterations have been sufficient to provide the machinery for sound production. The most drastic innovation has been in the trachea; in cuticular and muscular elements the changes from spiracle to spiracle appear to be primarily a matter of scale. The number and structure of the motoneurons are apparently unchanged; even the pattern of inputs to the motoneurons appears to be similar in all abdominal segments during normal respiration. The findings are consistent with conservation of the peripheral neural elements, along with the creation of a new input to spiracle motoneurons that overrides the respiratory pattern generator when the proper stimuli are present.

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References

Altman, J.S., Tyrer, N.M.: Insect flight as a system for the study of the development of neuronal connections. In: Experimental analysis of insect behaviour. Barton Browne, L. (ed.), pp. 159–179. Berlin, Heidelberg, New York: Springer 1974

Bacon, J.P., Altman, J.S.: A silver intensification method for cobalt-filled neurones in wholemount preparations. Brain Res. **138**, 359–363 (1977)

Bentley, D.R.: Genetic control of an insect neuronal network. Science **174**, 1139–1141 (1971)

Bentley, D.R.: Postembryonic development of insect motor systems. In: Developmental neurobiology of arthropods. Young, D. (ed.), pp. 147–177. Cambridge: Cambridge University Press 1973

Bentley, D.R., Hoy, R.R.: Postembryonic development of adult motor patterns in crickets: a neural analysis. Science **170**, 1409–1411 (1970)

Case, J.F.: The median nerves and cockroach spiracular function. J. Insect Physiol. **1**, 85–94 (1957)

Dumortier, B.: L'émission sonore dans le genre *Gromphadorhina brunner* (Blattodea, Perisphaeriidae), étude morphologique et biologique. Bull. Soc. Zool. France **90**, 89–101 (1965)

Eisner, T.: Spray mechanism of the cockroach *Diploptera punctata*. Science **128**, 148–149 (1958)

Evans, P.D.: The uptake of L-glutamate by the central nervous system of the cockroach, *Periplaneta americana*. J. Exp. Biol. **62**, 55–67 (1975)

Goodman, C.S.: Neuron duplications and deletions in locust clones and clutches. Science **197**, 1384–1386 (1977)

Hoy, R.R., Hahn, J., Paul, R.C.: Hybrid cricket auditory behavior: evidence for genetic coupling in animal communication. Science **195**, 82–84 (1977)

Kammer, A.E., Rheuben, M.B.: Adult motor patterns produced by moth pupae during development. J. Exp. Biol. **65**, 65–84 (1976)

Levi-Montalcini, R., Chen, J.S., Seshan, K.R., Aloe, L.: An in-vitro approach to the insect nervous system. In: Developmental neurobiology of arthropods. Young, D. (ed.), pp. 5–36. Cambridge: Cambridge University Press 1973

Lewis, D.B.: The physiology of the Tettigoniid ear. I. The implication of the anatomy of the ear to its function in sound reception. J. Exp. Biol. **60**, 821–837 (1974)

Lighthill, M.J.: On sound generated aerodynamically. I. General theory. Proc. R. Soc. Lond. A **211**, 564–587 (1952)

Miller, P.L.: Inhibitory nerves to insect spiracles. Nature (Lond.) **221**, 171–173 (1969)

Miller, P.L.: Spatial and temporal changes in the coupling of cockroach spiracles to ventilation. J. Exp. Biol. **59**, 137–148 (1973)

Miller, P.L.: Respiration-aerial gas transport. In: The physiology of insecta, 2nd ed., Vol. VI. Rockstein, M. (ed.), pp. 345–502. New York, London: Academic Press 1974

Morse, A.P.: Further researches on North American Acridiidae. Publ. Carnegie Inst. Wash. **68**, 3–54 (1907)

Nelson, M.C., Fraser, J.: Sound production in the cockroach, *Gromphadorhina portentosa:* Evidence for communication by hissing. (in preparation)

Nocke, H.: Physical and physiological properties of the Tettigoniid ("grasshopper") ear. J. Comp. Physiol. **100**, 25–57 (1975)

Pearson, K.G., Bergman, S.J.: Common inhibitory motoneurones in insects. J. Exp. Biol. **50**, 445–471 (1969)

Pitman, R.M., Tweedle, C.D., Cohen, M.J.: Branching of central neurons: intracellular cobalt injection for light and electron microscopy. Science **176**, 412–414 (1972)

Roederer, J.G.: Introduction to the physics and psychophysics of music (ed. 2). Berlin, Heidelberg, New York: Springer 1975

Roth, L.M., Stay, B.: The occurrence of *para*-quinones in some arthropods, with emphasis on the quinone-secreting tracheal glands of *Diploptera punctata* (Blattaria). J. Insect Physiol. **1**, 305–318 (1958)

Shankland, D.L.: Nerves and muscles of the pregenital abdominal segments of the American cockroach, *Periplaneta americana* (L.). J. Morph. **117**, 353–386 (1965)

Simmons, P.: The neuronal control of dragonfly flight I. Anatomy. J. Exp. Biol. **71**, 123–140 (1977)

Strausfeld, N.J., Obermayer, M.: Resolution of intraneuronal and transynaptic migration of cobalt in the insect visual and central nervous systems. J. Comp. Physiol. **110**, 1–12 (1976)

Taylor, H.M., Truman, J.W.: Metamorphosis of the abdominal ganglia of the tobacco hornworm, *Manduca sexta*. J. Comp. Physiol. **90**, 367–388 (1974)

Truman, J.W.: Development and hormonal release of adult behavior patterns in silkmoths. J. Comp. Physiol. **107**, 39–48 (1976)

Truman, J.W., Reiss, S.E.: Dendritic reorganization of an identified motoneuron during metamorphosis of the tobacco hornworm moth. Science **192**, 477–479 (1976)

Tyrer, N.M., Bell, E.M.: The intensification of cobalt-filled neurone profiles using a modification of Timm's sulphide-silver method. Brain Res. **73**, 151–155 (1974)

Willows, A.O.D., Dorsett, D.A.: Evolution of swimming behavior in *Tritonia* and its neurophysiological correlates. J. Comp. Physiol. **100**, 117–133 (1975)