THE NEUROETHOLOGY OF ACOUSTIC STARTLE AND ESCAPE IN FLYING INSECTS

By RON HOY, TOM NOLEN and PETER BRODFUEHRER

Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

Summary

The acoustic startle/escape response is a phylogenetically widespread behavioral act, provoked by an intense, unexpected sound. At least six orders of insects have evolved tympanate ears that serve acoustic behavior that ranges from sexual communication to predator detection. Insects that fly at night are vulnerable to predation by insectivorous bats that detect and locate their prey by using biosonar signals. Of the six orders of insects that possess tympanate hearing organs, four contain species that fly at night and, in these, hearing is sensitive to a range of ultrasonic frequencies found in the biosonar signals of bats. Laboratory and field studies have shown that these insects (including some orthopterans, lepidopterans, neuropterans and dictyopterans), when engaged in flight behavior, respond to ultrasound by suddenly altering their flight, showing acoustic startle or negative phonotaxis, which serve as bat-avoidance behavior.

A neural analysis of ultrasound-mediated escape behavior was undertaken in the field cricket *Teleogryllus oceanicus*. An identified thoracic interneuron, int-1, was shown to trigger the escape response, but only when the cell was driven (synaptically or electrically) at high spike rates, and only when the insect was performing flight behavior; avoidance steering only occurs in the appropriate behavioral context: flight. Thus, significant constraints operate upon the ability of int-1 to trigger the escape response. The integration of auditory input and flight central pattern generator output occurs in the brain. It is found that neural activity descending from the brain in response to stimulation by ultrasound is increased when the insect is flying compared to when it is not. Although the behavioral act of avoidance steering may appear to be a simple reflex act, further analysis shows it to be anything but simple.

Startle and escape responses as behavioral acts

We all know what it is to be startled by an intense, unexpected stimulus, and it is no surprise that effective responses are ubiquitous in the animal kingdom. A startling stimulus can originate from any sensory modality, but it must generally be of relatively high intensity to be maximally alarming. The response is variable, ranging from bodily escape – by whatever locomotory means are available and appropriate – to subtle movements (such as eyeblinks in primates) and other acts

Key words: crickets, neuroethology, acoustic startle.

that might more aptly be described as 'freezing' rather than escaping. However, the startle response elicited by an alarming stimulus occurs with a characteristically short delay, of the order of tens of milliseconds. It is interesting that the subject of behavioral startle tends to be investigated by physiological psychologists dealing with mammalian subjects, whereas escape behavior is investigated by zoologists who draw heavily upon invertebrate animals (Bullock, 1984; Davis, 1984; Eaton, 1984). Obviously, startle and escape responses are closely linked. In some instances, it is possible to separate startle from the locomotory act of escape, whereas in other cases it is not, as will be seen later. In this review, we will take a zoological, comparative approach to the subject, and will consider startle responses that are brought about by acoustic stimuli. The primary subject will be the acoustic startle response (ASR) of nocturnally active, flying insects.

The acoustic startle response in insects

Insects are among the most successful of life's creatures, because they exploit every possible ecological niche available to them: terrestrial, aquatic and aerial; their activities occur by day, by night or both. However, vertebrate predators have been equally innovative, evolutionarily speaking, and 'track' the habits of their insect prey. Thus, although the 'good news' for nocturnally active, flying insects is that they avoid the predations of sharp-eyed birds that hunt primarily by day, the 'bad news' is that they are vulnerable to the predations of one of the most successful and diverse mammalian groups, the microchiropteran bats, which live a nocturnal, aerial existence and have taken to the air for their foraging activities. Thus, in principle, any insect that flies at night is vulnerable to predation by bats. Microchiropteran bats have evolved remarkable biosonar systems by which they navigate in the dark to detect and localize their insect prey. This remarkable mammalian adaptation is the subject of other papers in this volume, and it is our intention to present this coevolutionary relationship from the point of view of the insects upon which much of bat biosonar activity is focused.

The auditory sense in insects – alarm and social behavior

Ears have evolved at least eight different times in at least six orders of insects and, depending on what one means by an ear, these numbers could be conservative. For example, many insects have vibration-sensitive hairs borne upon specialized appendages (Johnston's organs in dipterans and cerci in many insects) that are capable of detecting airborne vibration signals emitted by conspecifics or predatory animals (Michelsen & Larsen, 1985). Although 'hearing' in this mode is certainly interesting, we will restrict my discussion of hearing to that sensitivity mediated by a particular kind of ear – the highly differentiated tympanate ear, in which specialized receptor cells (scolopales) are associated with tracheal air sacs and some sort of cuticular specialization (tympanal membrane) upon which changes in air pressure play. Tympanate ears tend to be sensitive to higher-

frequency sounds, in the range 1000–100000 Hz, depending upon species. This range includes both the social signals of the most conspicuous (to the human observer) 'singing' insects and the spectral energy of bat biosonar signals. Among these insects are those renowned for their contribution to the din of the night such as bush crickets or katydids and field crickets, whose songs subserve social interactions, as well as other insects such as moths, green lacewings and praying mantises, insects whose activities may be associated with the night, but are not commonly known to produce acoustic signals. All these insects can hear, and they do so by means of scolopophorous hearing organs.

We will present evidence in this article that all the insects just described are potential prey to insectivorous bats that hunt them with the aid of ultrasonic biosonar. Consequently, they have all evolved an acoustic startle response which is part of an escape that is a countermeasure to bat predation. To this end, these insects all possess tympanate ears that permit them to hear the ultrasonic frequencies contained in bat biosonar calls. The available evidence indicates that some of these insects have extended the range of already extant ears to include high ultrasonic frequencies, whereas others may have evolved their ears specifically in response to bat predation.

A brief survey of the acoustic startle response among insects

Moths

That moths have ears has been known since 1950 (Shaller & Timm, 1950), but it was not until Kenneth Roeder and Asher Treat collaborated (1957, 1961) that the linkage between hearing in some orders of moths and predation upon them by bats was firmly established (Roeder, 1967). Roeder's work remains a model for a neuroethological research program: he showed (1) that moth ears are sensitive to ultrasonic frequencies that overlap with the biosonar signals of predacious bats, (2) that the ear responds to natural and electronically synthesized models of bat biosonar signals, (3) that tethered, flying moths respond to playback of bat-like ultrasound by making phonotactic movements in the laboratory, (4) that significant processing of ultrasound occurs in the central nervous system (CNS) of moths, and (5) that observations of free-flying moths under natural conditions show that they respond to attempts by bats to capture them by flying away or making other evasive locomotory acts. Roeder demonstrated the robustness of the moth–bat interaction from nature to the laboratory; from the behavior of unrestrained whole animals to acutely dissected laboratory preparations, and from acoustic signals passed between different organisms to neural signals passing within the nervous system of one animal. Rarely has behavior and neurobiology been so well integrated into a research program. Recent work has extended the earlier findings, including the identification of several central interneurons that may play a role in the phonotactic avoidance behavior (Boyan & Fullard, 1986). However, the basic story has not changed since Roeder. Moths that have tympanate hearing organs (representatives from the noctuids, arctiids, geometrids

and pyralids) show phonotactic avoidance behavior during flapping flight: they fly away from directional soures of ultrasound and often have a second, 'last chance', escape maneuver which can be a nondirectional nose-dive, or a cessation of flight, resulting in a 'drop'. Such nondirectional acts are usually elicited when the ultrasound stimulus is extremely intense (over 90 dB), as would be emitted by a bat closing in so fast and close that a directional steering response would not be sufficient for the moth to escape. As will be seen below, both kinds of escape maneuver are observed in other classes of insects in response to ultrasound.

Green lacewings

Miller (1970, 1971, 1975) originated the work on this group and continues to be its most ardent investigator. Like Roeder, Miller has studied the bat-avoidance behavior of this group both in the field under natural conditions and in the laboratory. The response of green lacewings to ultrasound is nondirectional. Like the 'last chance' maneuvers of moths, green lacewings fold their wings when stimulated by actual or synthetic bat biosonar signals and simply drop to the ground (Miller, 1975). A variation on this response was reported by Miller – some lacewings interpolate a wingflap or two during their descent to the ground – presumably to add an element of unpredictability in the escape behavior. It should be noted that only moths and green lacewings have been investigated in the field to the extent which the data show that the ability to hear affects the insect's chances of surviving bat predation.

Field crickets

Crickets are among the most conspicuous of nocturnal insects, because of their noisy social lives in which males produce loud calling songs to attract females from distances of tens of meters. Less well known is the fact that many species of field crickets disperse widely early in the adult stage, primarily by flight. Flying at night puts any insect at risk from predation by bats, and crickets are no exception. It was Popov & Shuvalov (1977) who first reported that field crickets (*Gryllus bimaculatus*) perform avoidance behavior in the presence of hunting bats, in the field. These authors also demonstrated negative phonotaxis in the laboratory, and from these data the authors concluded that crickets, like moths, actively avoid bats by hearing their biosonar signals and steering away from them. In our study of the flight behavior of *Teleogryllus oceanicus*, we also found that they perform negative phonotaxis in response to bat-like ultrasonic signals, while performing tethered flight behavior, and we, too, interpreted our findings in terms of a bat-avoidance mechanism (Moiseff *et al.* 1978). These steering movements involve directed movements of the antennae, head, wings, legs and abdomen. The elicitation of negative phonotactic behavior in tethered flying crickets is comparable in many respects to that demonstrated earlier in moths and green lacewings: (1) the response latencies are short (40–80 ms); (2) the phonotaxis is always directional: away from the loudspeaker; (3) behavioral thresholds are of the order of

40–70 dB; (4) the adequate stimulus is a brief (tenths of milliseconds) pulse of ultrasound in the range 20–100 kHz; and (5) single pulses, as well as pulse trains, of ultrasound are sufficient to elicit a phonotactic response (Nolen & Hoy, 1986).

The use of single pulses to elicit the escape response (negative phonotaxis) in crickets parallels the experimental paradigm of mammalian psychologists, and permits us to regard negative phonotaxis in crickets as an ASR of the type found in mammals (Nolen & Hoy, 1986; Hoy, 1989). Fig. 1 shows the outcome of three sets of experiments on three different species of field crickets, *Teleogryllus oceanicus*, *Teleogryllus commodus* and *Gryllus bimaculatus*, in which single pulses of ultrasound (30 ms in duration) were used to generate threshold tuning curves for directional turning responses. In their steering behavior, all three species show almost exclusively negative phonotaxis over a broad band of high frequencies. They are most sensitive to acoustic stimuli in the range 15–60 kHz, although considerable variation occurs among the three species (Nolen & Hoy, 1986). It is also clear from these curves that positive phonotaxis, steering towards the sound source, rarely occurs in response to a 4 or 5 kHz single-pulse stimuli, even though these are the frequencies that dominate the species calling songs and normally attract flying crickets (Moiseff *et al.* 1978). On teleological grounds, this is what one would expect – in nature, calling songs consist of trains of sound pulses, never single pulses. However, even a single pulse from a predatory bat might be expected to initiate an ASR or escape response. Thus, it is not surprising that the presentation of a single-pulse stimulus is adequate only in the context of predation, and not of social communication.

It is worth pointing out that recent studies of the cricket ASR refute Davis's (1984) contention that the mammalian ASR differs qualitatively from that of its submammalian counterparts because the mammalian ASR is highly graded in response magnitude, whereas in 'lower species it tends to be an all-or-none response'. Although Davis may not have had crickets in mind when thinking about lower species, we have recently shown that the cricket ASR is also highly graded in response amplitude (May *et al.* 1988). Whereas earlier work on cricket phonotaxis focused on the rudder-like movements of the abdomen in response to stimulation, May focused his work on the effect of ultrasound on the beating of the wings and their effect on flight aerodynamics (active steering). For example, when a cricket makes a turn away from an ultrasound source, it tilts its wings into the turn. Quantitative measurements show that the magnitude of the tilt is linearly related to the ultrasound intensity. Another example of this linear response characteristic can be seen in Fig. 2, which shows the relationship between pitch angle and stimulus intensity when the speaker is placed above (Fig. 2A) and below (Fig. 2B) a tethered, flying cricket that is mounted such that the insect is unrestrained about the pitch axis (May *et al.* 1988). Moreover, when measurements were made of rotations about the two other axes of roll and yaw, the amplitudes of these were also found to be linearly related to the stimulus intensity (May *et al.* 1988). In summary, crickets show a highly developed ASR, similar in many respects to those studied by psychologists in higher mammals.
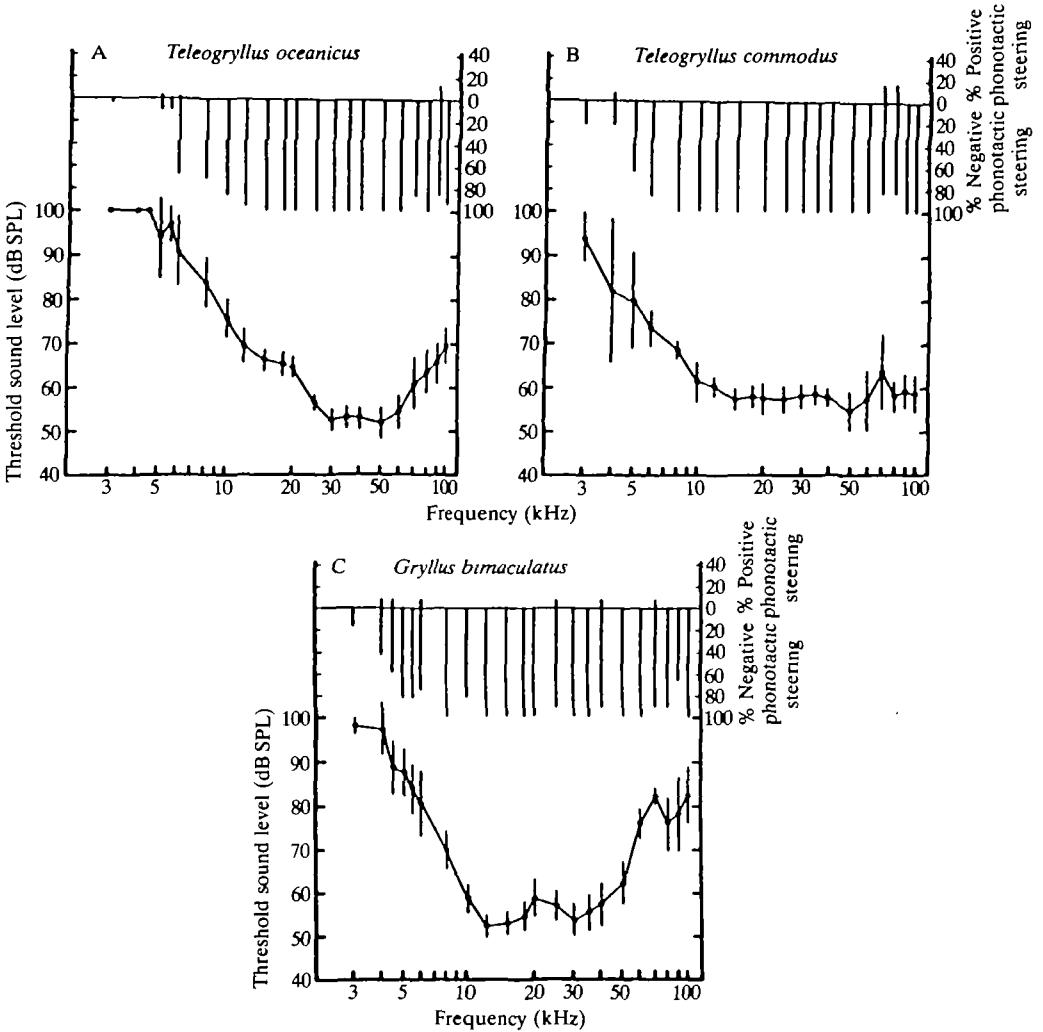
Fig. 1. Behavioral tuning curves for the flight phonotaxis response of three species of field crickets in response to a single 30 ms sound pulse. The bar graph at the top of each figure shows the percentage of animals that performed consistent negative or positive phonotaxis. In the context of this paper, negative phonotaxis, with respect to the direction of the loudspeaker, may be regarded as avoidance steering with respect to a hunting bat. Flying crickets that either did not respond to sound or gave inconsistent responses were not plotted and are reflected in the failure of some bars to add to 100 %. At the bottom of each figure is the threshold tuning curve for avoidance steering. Open circles represent the maximum sound pressure levels (SPLs) available at frequencies where less than 5 % of the animals responded. All other values plotted are mean threshold sound levels. Error bars are 95 % confidence intervals except for cases where less than 30 % responded, where they represent 1 s.D. (A) *Teleogryllus oceanicus*. Thirteen crickets were tested between 3 and 40 kHz, and another 15 were tested between 3 and 100 kHz. (B) *T. commodus*. Eight crickets were tested between 3 and 40 kHz, and another six were tested between 3 and 100 kHz. (C) *Gryllus bimaculatus*. Seven crickets were tested between 3 and 100 kHz. (From Nolen & Hoy, 1986.)
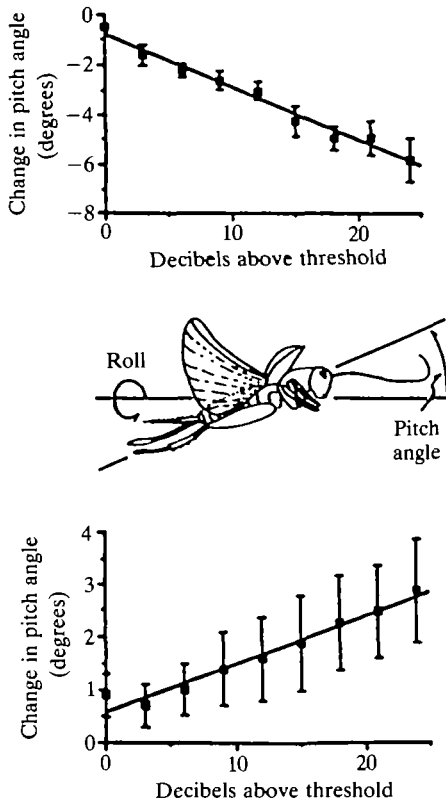
Fig. 2. The effect of speaker position on the pitch angle, in tethered flying crickets. Pitch angle is defined as the angle formed between a line through the cricket's long axis and a horizontal line through the centre of gravity, as illustrated schematically in the drawing. At the top of the figure, the pitch angle is shown to decrease when the loudspeaker is placed above the flying cricket (slope $= -0.21$; $r = 0.99$). At the bottom of the figure, the pitch angle increases when the loudspeaker is placed below the flying cricket (slope $= 0.09$; $r = 0.98$). The lines represent linear regressions, whereas the points represent the mean $\pm$ S.E.M. for 10 crickets. (From M. L. May, P. D. Brodfuehrer & R. R. Hoy, in preparation.)

Praying mantises

Many species of praying mantises are nocturnally, as well as diurnally, active and are capable of flight. Physiological recordings from the CNS of mantises revealed an acoustic sensitivity to ultrasonic frequencies in the same range as that found in moths, lacewings and crickets (Yager & Hoy, 1986). Further investigation revealed that praying mantises have a most unusual hearing organ. A pair of scolopophorous hearing organs, each associated with very closely opposed tympanal membranes lie ventrally in a deep cleft in the *midline* of the metathoracic segment, between the hindlegs (Yager & Hoy, 1986, 1987). This 'cyclopean ear', although anatomically composed of a pair of organs, is functionally a single organ. The role played by such ears in nature is uncertain, but in at least one species,

Creobrotor gemmatus, bat-avoidance is possible (Yager & Hoy, 1985). When *C. gemmatus* was tethered at the thorax, placed in the airstream created by a windtunnel, and stimulated by ultrasound, the mantis reacted, with short latency, by making a pronounced dorsiflexion of its abdomen, a transient wing tilt, and markedly extending its raptorial forelegs. This alteration in flight posture would be likely to cause a change in the insect's flight path. Not all species of mantids can fly, and those that do not (owing to reduced or absent hindwings) are deaf to ultrasound (Yager & Hoy, 1988). Although the full story of these fascinating insects remains to be written, evidence suggests that praying mantis species that fly also have a bat-predation problem and that they have evolved hearing organs sensitive to ultrasound, and take evasive action in flight.

Tettigonids

These insects, also called katydids or long-horned grasshoppers, are related to crickets, and like them, the males sing loud calling songs at night. The auditory system has been particularly well studied in this group, and similarities to that in crickets are obvious (Romer, 1985; Oldfield *et al.* 1986). The mating calls of many of these species are 'noisy' and contain high frequencies. Correspondingly, the auditory system is sensitive to frequencies that extend well into the ultrasonic range (Sales & Pye, 1974). Many species are excellent fliers and, given their nocturnal habits, this leads one to suspect that they are preyed upon by insectivorous bats, and have evolved an ASR. Recent work on *Neoconocephalus ensiger*, from eastern North America, confirms the presence of an ASR (F. Libersat & R. R. Hoy, in preparation). When this insect is tethered, placed in a windstream, and stimulated by ultrasound (at least 80 dB SPL at 30 kHz), the insect abruptly (within 40 ms) ceases to flap its wings. This phonotactic act reminds us of the escape response of green lacewings (a 'drop'), described earlier. In response to single, short-duration pulses of ultrasound, the hesitation in flapping flight may be brief, but to longer trains of pulses of sound, the pause is prolonged, and appears to follow the duration of the stimulus. Another point to be further explored is the observation that when artificial pulse trains contain frequencies in the range 15–20 kHz, an ASR is not elicited, whereas it clearly is at 30 kHz (F. Libersat & R. R. Hoy, in preparation). The relationship between frequency and the insect's auditory world is relevant to this finding: the calling/courtship song of the male contains considerable energy in the 15–20 kHz range, and much less at higher frequencies. Frequency discrimination appears to separate conspecific males from bats in *N. ensiger*. The parallels in phonotactic behavior between *N. ensiger* and the insects described earlier lead us to presume that this tettigoniid, and probably others in this group, have evolved an ASR in response to predation by insectivorous bats.

Locusts

The locust ear has long been a favorite subject of study for bioacousticians and these insects are known to produce sounds during aggression and courtship

(Michelsen & Larsen, 1985; Steven & Bennet-Clark, 1982). Locusts fly by night as well as by day. We have argued that *any* insect that flies at night is, in principle, vulnerable to the predation of bats that use biosonar to detect and locate prey. In fact, D. Robert & C. H. F. Rowell (personal communication) have recently obtained evidence that *L. migratoria* does indeed have an ASR that is elicited by ultrasound. These workers used pulse-train stimuli that mimicked the search calls of insectivorous bats and found that the sensitivity of the behavioral reaction, negative phonotaxis, was best in the range 15–35 kHz. When the locusts were tethered and placed in a windstream, acoustic stimulation caused a steering response – an abdominal 'swing' away from the loudspeaker. Thus, locusts, like crickets, when engaged in the act of flight, react to bat-like ultrasound stimuli in very similar ways, and presumably for the same reason: the ASR is an anti-predator response.

Neural mechanisms underlying the acoustic startle response

Moths

More is known about the peripheral mechanisms of hearing than the central ones. In part, this is due to the difficulty of doing experiments in the moth CNS, rather than in its hearing organ. The sensory physiology of moth hearing has been reviewed elsewhere (Roeder, 1967; Michelsen & Larsen, 1985). Neurally, the ear is extremely simple in these insects, consisting of 1–3 receptor cells, depending on species. Roeder (1969*a,b*) recorded auditory units in the brains of noctuid moths, but his work predated the availability of intracellular dyes, and none of the units was identified. Recently, seven different auditory interneurons have been identified in the thoracic ganglion of *Heliothis virescens*, a noctuid moth (Boyan & Fullard, 1986). When stimulated by bat-like ultrasound, these cells become excited, and various features of the stimulus are adequately encoded among the collective spike patterns of the interneurons. This is an encouraging start, and now work must link sensory input to specific motor acts in the evasion response.

Crickets

The complexity of the cricket ear with its 70 scolopale receptors reflects the increased demands of a more complex auditory world (Michel, 1974; Schwabe, 1906). Crickets must listen to other crickets as well as to bats. The anatomical organization of the scolopophorous receptors of the cricket ear has been shown to be tonotopic, although the precise number of receptors tuned exclusively to ultrasonic frequencies has not yet been determined (Oldfield *et al.* 1986; Zhantiev & Korsunovskaya, 1978).

The receptor axons terminate in the prothoracic ganglion in an anatomically discrete neuropil, and there converge upon a rather limited number of second-order interneurons (Wohlers & Huber, 1982). Since auditory receptor axons

terminate completely and ipsilaterally in the prothoracic ganglion, all neural information carried forward to the brain, or anywhere else in the CNS, must be carried by interneurons. In the Australian field cricket, *Teleogryllus*, there appear to be only two ascending neurons (neurons whose axons project to the brain): one excited at 5 kHz (the carrier frequency of calling songs) and the other excited primarily at higher (15–100 kHz) frequencies, including ultrasound (Moiseff & Hoy, 1983; Hennig, 1988). In this review we will refer to the ultrasound-sensitive, ascending interneuron in *Teleogryllus oceanicus* as int-1, following the designation of Casaday & Hoy (1977). This identified neuron, or its homologue, has been found in virtually every species of field cricket that has been studied by comparative neurobiologists, but it unfortunately bears as many names as laboratories that study it. Nonetheless, whenever its response properties have been examined, it has been found to be sensitive to stimulation by ultrasound, as well as to lower sound frequencies.

In *T. oceanicus*, int-1 was proposed to be a putative 'bat-detector' because its responsiveness to ultrasound closely paralleled the negative phonotactic response of tethered, flying crickets to ultrasound (Moiseff *et al.* 1978; Moiseff & Hoy, 1983). The issue was clarified when Nolen & Hoy (1984) demonstrated that neural activity in int-1 was both necessary and sufficient to initiate a negative phonotactic steering response (Fig. 3). That excitation of int-1 is necessary to initiate negative phonotaxis was shown by depressing the synaptic excitability of int-1 to ultrasound by passing hyperpolarizing current into the neuron at the same time that the acoustic stimulus was presented (Fig. 3B). This depression effectively 'cancelled' the downstream response in the abdominal steering muscles, the dorsal longitudinal muscles. That activity in int-1 is sufficient to initiate negative phonotaxis was shown by stimulating int-1 to discharge vigorously, by current injection (Fig. 3C). About 50 ms after stimulation of int-1, there was a burst of motor activity in the abdominal dorsal longitudinal muscles that brought about an abdominal swing. This steering response in the flying insect occurred in the absence of acoustic stimulation; only high spike-rate activity in int-1 was required and, consequently, int-1 activity is sufficient to initiate steering. Although it is no longer surprising that the activity in single neurons can have potent behavioral effects, especially in invertebrates, this is an especially direct demonstration of a sensory–motor linkage in an acoustic behavior.

It is worth emphasizing three points from this study: (1) the experimental conditions that led to showing necessity and sufficiency of int-1 in initiating the avoidance response also meet the criteria set forth by Kupferman & Weiss (1978) to define a 'command neuron', and we could just as well call int-1 a command neuron as a bat-detector; (2) the behavioral efficacy of int-1 in initiating steering behavior is highly conditional – the cricket must be engaged in flight behavior, otherwise stimulation of int-1, no matter how intense, does not lead to activation of the steering neuromotor system; and (3) there is a minimum spike-rate threshold for int-1 that must be met before its activity initiates avoidance steering (180–200 spikes s^{-1}) – thus afferent activity must be sufficiently intense (as would
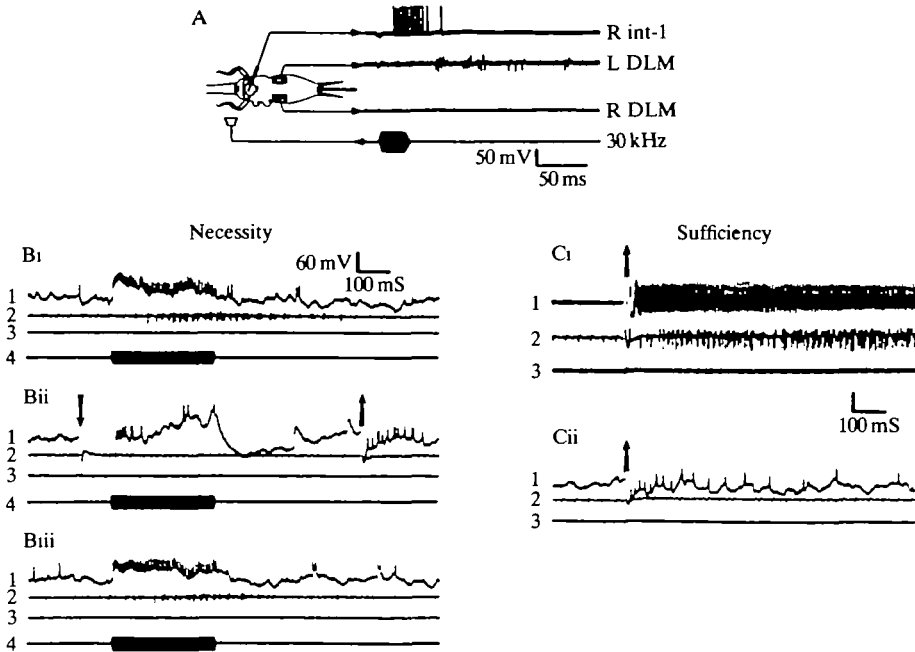
Fig. 3. Phonotactic steering and its physiological correlates. Simultaneous recordings were made from the right (R) int-1 (intracellular record) and both left (L) and right (R) abdominal dorsal longitudinal steering muscles (DLMs) (electromyograph records). A 30 kHz, 85 dB, 30 ms duration sound pulse was played from a loudspeaker at 90° to cricket's right side. The identity of int-1 was established from injection of Lucifer Yellow into the cell. Here, the ultrasound elicited a burst of action potentials (average 400 spikes s^{-1}) in int-1, and this was followed by a burst of muscle action potentials in the contralateral DLMs, 50 ms later. (B) Activity in int-1 is *necessary* to initiate avoidance steering during flight. The recordings are as in A, except that the microelectrode is inserted nearer the integrating segment of int-1 (hence the ability to record EPSPs) instead of nearer the axon (which would register action potentials, as above). Here, the acoustic stimulus parameters are: 30 kHz, 82 dB, 300 ms presented to the right ear. (i) Control: normal int-1 excitation by ipsilateral ultrasound source activates contralateral abdominal steering muscles with a latency of about 50 ms. (ii) Depressing int-1's excitability to ultrasound by injection of −15 nA of hyperpolarizing current at the same time as the sound prevents activation of abdominal steering muscles; the spike rate of int-1 was reduced from 330 spikes s^{-1} in (Bi) to under 170 spikes s^{-1} in (Bii). (iii) Control trial conducted immediately after the hyperpolarization trial in ii. Clearly, when int-1's excitability to ultrasound is restored, so too is the abdominal steering response. (C) Activity in int-1 is *sufficient* to elicit avoidance steering, even in the absence of ultrasound. Recording arrangements as described above. (i) The right int-1 was excited to discharge at a rate exceeding 400 spikes s^{-1} upon anode-break, following prolonged hyperpolarizing current injection. The contralateral (but not ipsilateral) abdominal steering muscle was activated. It was typical that int-1 responded to the termination of hyperpolarizing current injection with a rebound excitation; it was not possible to pass sufficient depolarizing current from our Lucifer Yellow electrodes to excite int-1 at spike rates above 100 spikes s^{-1}. (ii) Lower spike rates (under 170 spike s^{-1}) in int-1 due to smaller anode-break rebound fail to activate steering muscles. (After Nolen & Hoy, 1984; Hoy & Nolen, 1987.)

be elicited by the biosonar pulses of closely approaching bats). In short, although int-1 may be the 'trigger-point' in an escape circuit, connectivity alone does not predict behavioral outcome if int-1 is activated. Its activation must occur in the correct behavioral context (flapping flight) and its activity must exceed a threshold. Once beyond the threshold of activation, however, the magnitude of the steering response is graded, and in fact linear, to stimulus amplitude (Nolen & Hoy, 1984). We have described other manifestations of this linearity between response and stimulus amplitude earlier in this review. The dependence of the steering response on coativation of int-1 and flight behavior can be seen as an example of a neural 'and gate', or neural gating, in which activity of int-1 is gated into the steering circuitry by the central flight pattern generator (CPG).

A provisional 'circuit' for avoidance steering is shown in Fig. 4. It draws heavily on a model advanced earlier by Reichert & Rowell (1985) for flight steering in the locust. The circuit, as drawn, requires connections to and from the brain, in keeping with our knowledge that: (1) decapitated crickets can be made to fly, sometimes even for days after the operation, but they do not perform steering behavior in response to normally suprathreshold acoustic stimuli (Pollack & Hoy, 1981), and (2) int-1's axon ascends within the cervical connective and makes extensively branching terminal fields in the brain (Moiseff & Hoy, 1983; Brodfuehrer et al. 1988). We mention this because, in principle, since the significant appendages for steering – wings and abdomen – lie below the head, and since the auditory input enters and terminates in the prothoracic ganglion, one might not necessarily presume that the brain enters into the neural processing of escape, as it clearly does in the case of *T. oceanicus*. We should point out differences in flight steering movements that are produced by nonphase-locked sensory input in the locust, from which the Reichert–Rowell model was developed, and our version of it. In locusts, the sensory inputs (from ocelli, compound eyes and cephalic sensory hairs) are combined with the flight CPG in the thoracic ganglia, and not in the brain, as we find in crickets, as will be discussed below (P. D. Brodfuehrer & R. R. Hoy, in preparation).

An important question about our hypothetical network is how and where int-1 is actually gated into the steering circuitry by the flight CPG. We have recently recorded descending neural activity in the neck connectives of *T. oceanicus*, in episodes of flight activity as well as in periods of nonflight, during which we activated int-1 with suprathreshold ultrasound stimuli (Brodfuehrer et al. 1988, P. D. Brodfuehrer, M. L. May & R. R. Hoy, in preparation, and Fig. 5). We find that in response to ultrasound stimulation, the level of neural activity descending from the brain, as reflected in total multi-unit activity from extracellular recording, is increased during flight when compared to the nonflight condition (Fig. 6). These data support the hypothesis that output from the flight CPG gates activity from int-1 to drive steering. Moreover, flight activity does not alter the responsiveness of int-1 itself to ultrasound. Although we have not yet identified any auditory interneurons whose ultrasound-evoked activity is enhanced during flight, the extracellular recordings demonstrate their existence.
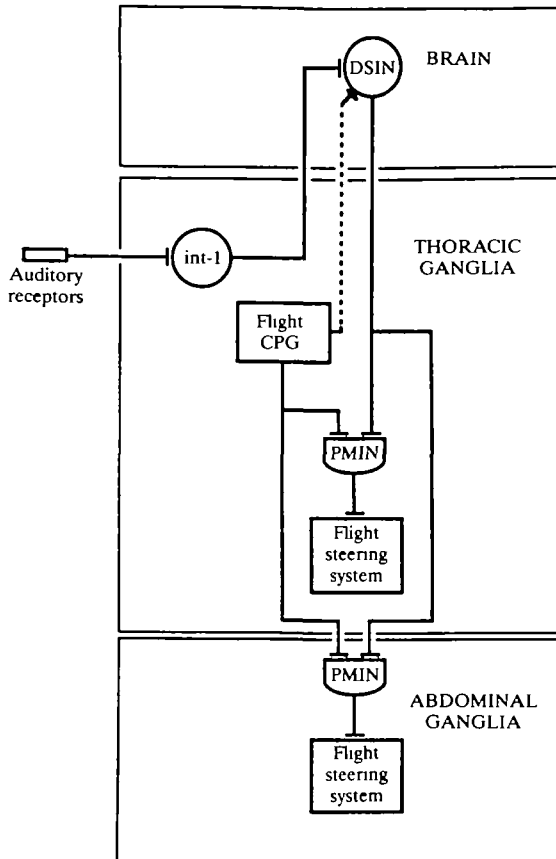
Fig. 4. A hypothetical circuit for negative phonotaxis (bat avoidance) in *Teleogryllus oceanicus*. Ultrasound activates some fraction of the 70 auditory receptors in the tympanal ear, which excite int-1 in the prothoracic ganglion (probably monosynaptic; see Hennig, 1988). Int-1's ascending axon terminates ipsilaterally (with respect to the sound source) in the brain, where it probably activates descending interneurons (DSINs) that are part of the flight steering circuit, analogous to those proposed by Reichert & Rowell (1985) in *Locusta*. Following their reasoning, we suppose that the DSINs activate a group of premotor interneurons (PMINs) in thoracic and abdominal ganglia. Finally, the PMINs are thought to activate the appropriate pools of motor neurons involved in avoidance steering (thoracic flight MNs, and abdominal dorsal longitudinal muscles). Since we know that int-1 initiates steering only in the context of flight behavior, we include the flight central pattern generator (CPG), which we presume to gate descending auditory signals, either in the brain, forming an 'and' gate with int-1, or indirectly, at the level of PMINs at lower levels. (After Hoy & Nolen, 1987.)

Praying mantis

The basis of the hearing in *Mantis religiosa* is mechanotransduction in a scolopophorous hearing organ. There are approximately 30 receptor cells in the tympanal organ, and their axons run within a short tympanal nerve that projects to
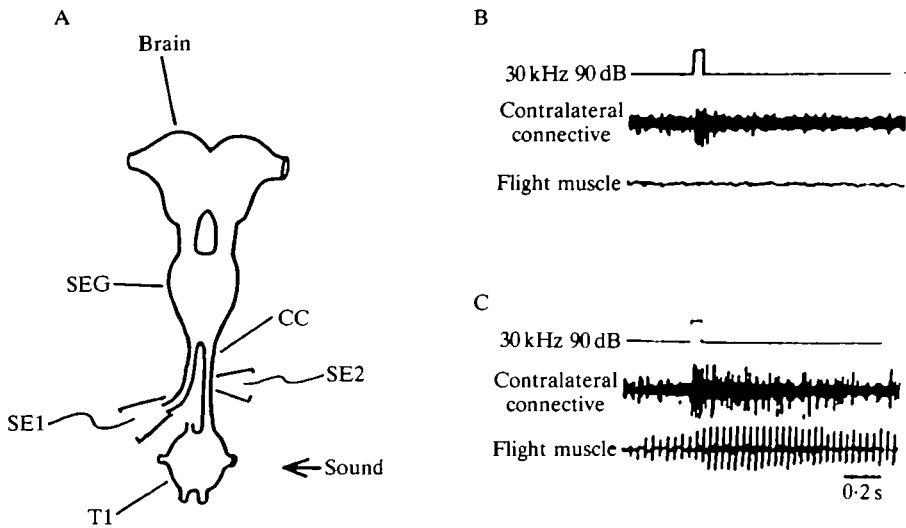
Fig. 5. (A) Method for recording descending activity in cervical connectives in response to ultrasound stimulation. Abbreviations, SEG: subesophageal ganglion; T1: prothoracic ganglion; CC: cervical connective. Suction electrodes (SE1 and SE2) were used to record from either ipsilateral or contralateral neck connectives (ipsi- and contralateral are defined with respect to the sound direction). Multi-unit auditory responses to ultrasound (30 kHz sound pulses at various intensities and durations, as noted) were recorded in this way. Auditory stimulation was administered both when the cricket was flying and when it was not. Flight modulates the auditory response. (B) Nonflying cricket; top trace: onset of ultrasonic stimulus is indicated by rectangular pulse. Middle trace: extracellular recording from the cervical connective in response to the ultrasonic stimulus. Bottom trace: electromyogram recording from a metathoracic wing depressor muscle (muscle 129a) involved in steering. (C) Same animal when it was induced to fly; note enhanced response in neck connective. (From P. D. Brodfuehrer & R. R. Hoy, in preparation.)

the metathoracic ganglion (Yager & Hoy, 1987). It has been possible to record the auditory response in the tympanal nerve, by extracellular recording and, although it is not possible to resolve single-unit activity with much clarity this way, the shape of the tuning curve of the mass response in the nerve matches that of an identified auditory interneuron described earlier (Yager & Hoy, 1986): the range of best sensitivity extends from 25 to 45 kHz, where the threshold is approximately 60 dB SPL (D. D. Yager & R. R. Hoy, 1989).

There are several auditory interneurons of *M. religiosa*, one of which has been identified (Yager & Hoy, 1986). This neuron's response properties match that of the tympanal nerve's, and its sensitivity resides entirely in the ultrasound, extending to 100 kHz, although it is most sensitive in the range 25–45 kHz (Fig. 7). We referred to the mantis ear as cyclopean, in the sense that it functions as a single hearing organ. This is unusual in the animal kingdom, where the sense of hearing is generally mediated by two anatomically separated ears. Given the cyclopean state of hearing in the mantis, it would be expected that the auditory response in
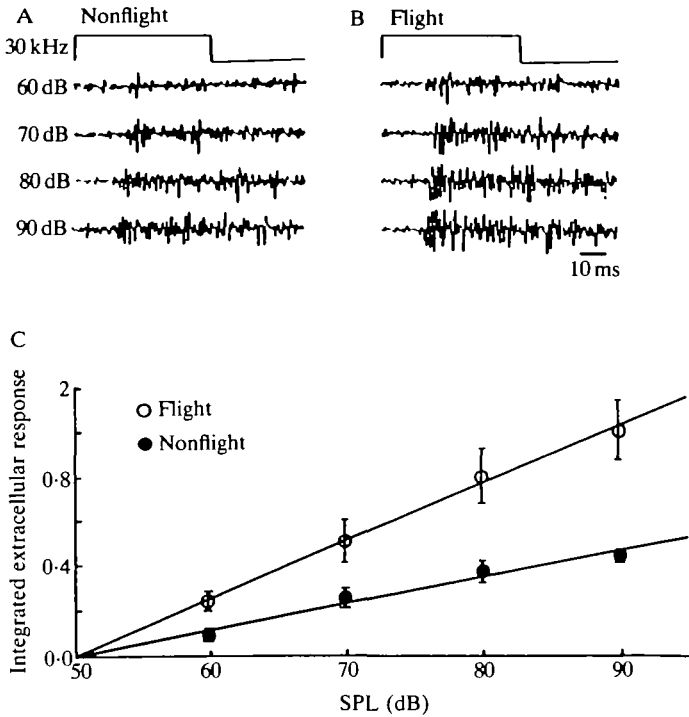
Fig. 6. Flight activity enhances auditory responsiveness of multi-unit descending activity in the cervical connectives. The top trace is a monitor of the ultrasonic stimulus. The lower traces show the multi-unit response in the cervical connectives to the ultrasound stimulus delivered at four different intensities. (A) The response when the cricket is not flying. (B) The response when the same cricket is flying. (C) A graphical presentation of the amount of descending activity elicited by ultrasound (30 kHz) during flight and nonflight, as a function of stimulus intensity (SPL). The multi-unit response was measured as a peak area, from full-wave rectified and integrated extracellular recordings from the cervical connectives. The lines are drawn as linear regressions: for flight $r = 0.997$, for nonflight $r = 0.983$. (From P. D. Brodfuehrer & R. R. Hoy, in preparation.)

this interneuron should be insensitive to stimulus direction, and recently we have found this to be true (Yager & Hoy, 1986, 1989). The morphology of this interneuron is intriguing, in that it shows a remarkable resemblance to the G and B interneurons of the locust *Locusta migratoria* (Rehbein *et al.* 1974), known to be auditory in function. Whether the mantis neuron, MR-501-T3, is truly homologous with the G/B cells of locusts is unknown. However, cell MR-501-T3 occurs as a mirror-image pair of ascending neurons, whose cell body and integrating segment is in the metathoracic ganglion (Fig. 7). Although the crucial tests that would permit the linkage between phonotactic function and neural responsiveness remain to be conducted in the praying mantis, the parallels with the cricket, lacewing and moth systems would lead one to suspect that MR-501-T3 is probably part of a bat-detection system. One is tempted to infer the same of the G/B
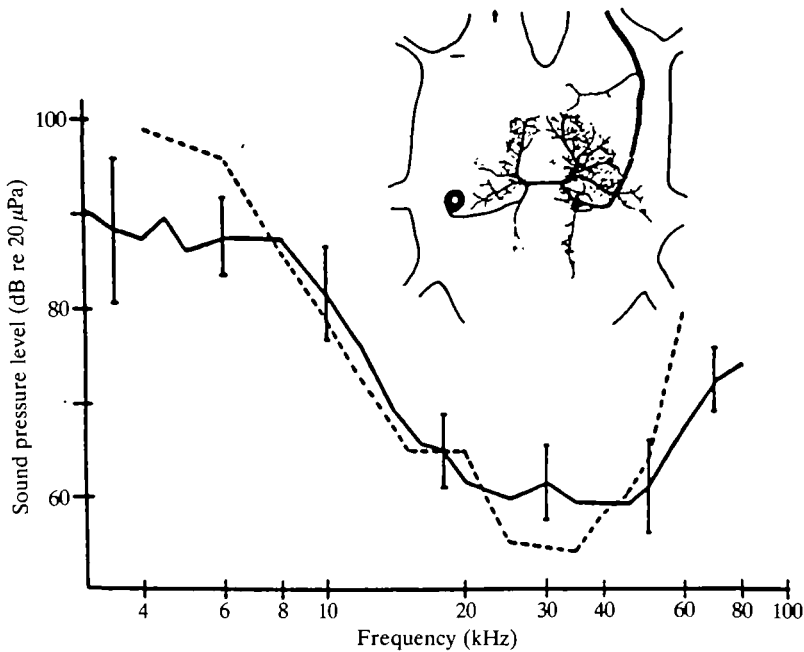
Fig. 7. Frequency threshold curve of the neural auditory response of *Mantis religiosa*, with an inset showing a *camera lucida* drawing of an identified auditory interneuron, MR-501-T3. The solid line is the mean tuning curve based on extracellular recordings from seven males and six females. In this mantis species, no differences occur between the two sexes, although they do in other species (Yager & Hoy, 1988). The dashed line is the tuning curve of the MR-501-T3 (= four animals), which is shown in the inset drawing, in the metathoracic ganglion. (After Yager & Hoy, 1986.)

neurons of *Locusta*, given that this flying insect also exhibits negative phonotaxis in response to ultrasound (D. Robert & C. H. F. Rowell, personal communication).

Conclusions

Tympanate hearing has evolved in at least six different insect orders (Orthoptera, Lepidoptera, Neuroptera, Hemiptera, Homoptera and Dictyoptera). High-frequency hearing is associated with tympanate ears and, in instances where an insect (1) is primarily nocturnally active, (2) engages in winged flight, and (3) has a tympanate ear, it is not unusual to find that auditory sensitivity includes the ultrasound. A reasonable hypothesis for the latter is that it derives from the predation pressure exerted by microchiropteran bats, which use biosonar signals to detect and localize their insect prey. These flying insects detect and escape from hunting bats on the wing, by hearing a bat's biosonar signals and flying away. Thus, in the past few years, the number of insects suspected to have evolved anti-bat escape responses based on ultrasonic hearing has grown. We should not be

surprised to see even more examples. Bat predation appears to be an efficient, relentless force upon nocturnal, flying insects, and the few cases of ASR known at the moment may be but the tip of the iceberg.

Our investigation of what appears to be a 'simple' escape response in a cricket has revealed a complicated set of restrictions about the state of a neural 'circuit' that underlies this escape behavior, and might serve as a caveat for the neural analysis of behavior in other animals. Our initial finding of a strong correlation between the neural activity of a single, identified interneuron (int-1) and avoidance steering encouraged us to investigate its causative role in escape behavior. When we found that activity in just a single auditory interneuron, int-1, could be shown to be both necessary and sufficient to initiate escape behavior, it seemed that the neural substrata might prove to be uncomplicated. However, we also found that activity in int-1 was sufficient to trigger escape behavior only under two restrictive conditions: first, the spike rate of int-1 had to exceed a minimal level $(180\,s^{-1})$, a high rate of activity, and second, even if the spike rate of int-1 exceeded this threshold, the separate multisegmental acts that comprise avoidance steering (involving antennae, head, wings, legs and abdomen) were not triggered unless the cricket was engaged in flight activity – unless the flight central pattern generator was active. The requirement for coactivity between the flight CPG and suprathreshold spike rate in response to an external ultrasound stimulus defines the behavioral context in which avoidance steering occurs. Thus, while ultrasound (in nature, from a bat) may provide the 'instructive' input to the escape system, the response of int-1 to ultrasound (spike-rate threshold) and coactive flight activity serve as 'permissive' conditions for the movements of avoidance steering. Simply, the body movements produced by a flying cricket that allow it to escape from a bat are not relevant in a nonflying insect; the response elicited by ultrasound depends on the animal's behavioral context.

Where in the neural pathways are auditory input and flight-related activity combined to form a behavioral 'and' gate? As described earlier, our evidence points to the cricket's brain, because descending multi-unit activity from the brain that is elicited by ultrasound stimulation is enhanced when the flight CPG is active, compared to when it is inactive. The corollary discharge from the thoracic flight CPG that presumably ascends to the brain remains to be identified, as do desending axons from the brain that drive the motor system for steering. Although these are formidable tasks to accomplish, at least they are now well-defined, and when completed, should provide a much clearer picture of the neural network(s) that underlie this acoustic escape behavior.

We conclude by noting that other investigators of 'simple' invertebrate neural systems view neural networks as multifunctional (Marder & Eisen, 1984; Harris-Warrick, 1988; Getting, 1989). Thus, a single anatomically interconnected set of cells can subserve a host of behavioral functions by having different functional networks 'carved' from the larger interconnected one by means of the differential actions of neuromodulators, or sensory input, and both of these might well be influenced by the animal's behavioral context at the time of performance of the

act. What began for us as an investigation into a simple auditory behavior has taken us into the complex, but fascinating, realm of sensorimotor coordination in which it becomes difficult to parse sensory from motor contributions in a behavioral act.

References

BRODFUEHRER, P., MAY, M. L. & HOY, R. R. (1988). Ultrasonic neurons in the brain of crickets. *Soc. Neurosci. Abstr.* **14**, 311.

BOYAN, G. S. & FULLARD, J. H. (1986). Interneurones responding to sound in the tobacco budworm moth *Heliothis virescens* (Noctuidae): morphological and physiological characteristics. *J. comp. Physiol.* A **158**, 391–404.

BULLOCK, T. H. (1984). Comparative neuroethology of startle, rapid escape, and giant-fiber mediated responses. In *Neural Mechanisms of Startle Behaviour* (ed. R. C. Eaton), pp. 1–11. New York: Plenum Press.

CASADAY, G. C. & HOY, R. R. (1977). Auditory interneurons in the cricket *Teleogryllus oceanicus*: physiological and anatomical properties. *J. comp. Physiol.* **121**, 1–13.

DAVIS, M. (1984). The mammalian startle response. In *Neural Mechanisms of Startle Behaviour* (ed. R. C. Eaton), pp. 287–342. New York: Plenum Press.

EATON, R. C. (ed.) (1984). *Neural Mechanisms of Startle Behavior.* New York: Plenum Press.

GETTING, P. A. (1989). Emerging principles governing the operation of neural networks. *A. Rev. Neurosci.* **12**, 185–204.

HARRIS-WARRICK, R. M. (1988). Chemical modulation of central pattern generators. In *Neural Control of Rhythmic Movements* (ed. A. H. Cohen, S. Rogsignol & S. Grillner), pp. 285–332. New York: John Wiley.

HENNIG, R. M. (1988). Ascending auditory interneurons in the cricket *Teleogryllus commodus* (Walker): comparative physiology and direct connections with afferents. *J. comp. Physiol.* A **163**, 135–143.

HOY, R. R. (1989). Startle, categorical response and attention in acoustic behavior of insects. *A. Rev. Neurosci.* **12**, 355–375.

HOY, R. R. & NOLEN, T. G. (1987). The role of behavioral context in decision making by an identified interneuron in the cricket. In *Higher Brain Functions* (ed. S. P. Wise), pp. 133–155. New York: John Wiley.

KUPFERMAN, I. & WEISS, K. R. (1978). The command neuron concept. *Behav. Brain Sci.* **1**, 3–39.

MARDER, E. & EISEN, J. S. (1984). Electrically coupled pacemaker neurons respond differently to the same physiological inputs and neurotransmitters. *J. Neurophysiol.* **51**, 1362–1374.

MAY, M. L., BRODFUEHRER, P. D. & HOY, R. R. (1988). Kinematic and aerodynamic aspects of ultrasound-induced negative phonotaxis in flying Australian field crickets (*Teleogryllus oceanicus*). *J. comp. Physiol.* A **164**, 243–249.

MICHEL, K. (1974). Das Tympanalorgan von *Gryllus bimaculatus* DeGeer (Saltatoria, Gryllidae). *Z. Morph. Tiere* **77**, 285–315.

MICHELSEN, A. & LARSEN, O. N. (1985). Hearing and Sound. In *Comprehensive Insect Physiology, Biochemistry And Pharmacology* (ed. G. A. Kerkut & L. I. Gilbert), pp. 495–555. New York: Pergamon Press.

MILLER, L. A. (1970). Structure of the green lacewing tympanal organ (*Chrysopa carnea*). *J. Morph.* **131**, 359–382.

MILLER, L. A. (1971). Physiological responses of green lacewings (Chrysopa, Neuroptera) to ultrasound. *J. Insect Physiol.* **17**, 491–506.

MILLER, L. A. (1975). The behavior of flying green lacewings, *Chrysopa carnea*, in the presence of ultrasound. *J. Insect Physiol.* 21, 205–219.

MILLER, L. A. & OLESEN, J. (1979). Avoidance behavior in green lacewings. *J. comp. Physiol.* 131, 113–120.

MOISEFF, A. & HOY, R. R. (1983). Sensitivity to ultrasound in an identified auditory interneuron in the cricket: a possible neural link to phonotactic behavior. *J. comp. Physiol.* 152, 155–167.

MOISEFF, A., POLLACK, G. S. & HOY, R. R. (1978). Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. natn. Acad. Sci. U.S.A.* 75, 4052–4056.

NOLEN, T. G., & HOY, R. R. (1984). Initiation of behavior by single neurons: the role of behavioral context. *Science* 226, 992–994.

NOLEN, T. G. & HOY, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. *J. comp. Physiol.* 159, 423–439.

OLDFIELD, B. P., KLEINDIENST, H. U. & HUBER, F. (1986). Physiology and tonotopic organization of auditory receptors in the cricket *Gryllus bimaculatus* DeGeer. *J. comp. Physiol.* 159, 457–464.

POLLACK, G. S. & HOY, R. R. (1981). Phonotaxis is flying crickets: neural correlates. *J. Insect Physiol.* 27, 41–45.

POPOV, A. V. & SHUVALOV, V. F. (1977). Phonotactic behavior of crickets. *J. comp. Physiol.* 119, 111–126.

REHBEIN, H., KALMRING, K. & ROMER, H. (1974). Structure and function of acoustic neurons in the thoracic nerve cord of *Locusta migratoria* (Acrididae). *J. comp. Physiol.* A 95, 263–280.

REICHERT, J. & ROWELL, C. H. F. (1985). Integration of non-phase locked exteroceptive information in the control of rhythmic flight in the locust. *J. Neurophysiol.* 53, 1201–1218.

ROEDER, K. D. (1967). *Nerve Cells and Insect Behavior*. Cambridge: Harvard University Press.

ROEDER, K. D. (1969a). Acoustic interneurons in the brain of noctuid moths. *J. Insect Physiol.* 15, 825–838.

ROEDER, K. D. (1969b). Brain interneurons in noctuid moths: differential suppression by high sound intensities. *J. Insect Physiol.* 15, 1713–1718.

ROEDER, K. D. & TREAT, A. E. (1957). Ultrasonic reception by the tympanic organs of noctuid moths. *J. exp. Zool.* 134, 127–158.

ROEDER, K. D. & TREAT, A. E. (1961). The detection and evasion of bats by moths. *Am. Sci. June*, 135–148.

ROMER, H. (1985). Anatomical representation of frequency and intensity in the auditory system of Orthoptera. In *Acoustic and Vibrational Communication in Insects* (ed. K. Kalmring & N. Elsner), pp. 25–32. Berlin, Hamburg: Parey.

SALES, G. & PYE, D. (1974). *Ultrasonic Communication By Animals*. London: Chapman & Hall Ltd.

SCHWABE, J. (1906). Beitrage zur Morphologie und Histologie der tympanalen Sinnesapparet der Orthopteren. *Zoologica* 50, 1–154.

SHALLER, F. & TIMM, C. (1950). Das Horvermogen der Nachtschmetterlinge. *Z. vergl. Physiol.* 32, 368–481.

STEVEN, R. O. & BENNET-CLARK, H. C. (1982). The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. *J. exp. Biol.* 99, 279–314.

WOHLERS, D. W. & HUBER, F. (1982). Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket, *Gryllus campestris* L. *J. comp. Physiol.* 146, 161–173.

YAGER, D. D. & HOY, R. R. (1985). Neuroethology of audition in the praying mantis, *Creobroter gemmatus*. *Soc. Neurosci. Abstr.* 11, 202.

YAGER, D. D. & HOY, R. R. (1986). The cyclopean ear: a new sense for the praying mantis. *Science* 231, 727–729.

YAGER, D. D. & HOY, R. R. (1987). The midline metathoracic ear of the praying mantis, *Mantis religiosa*. *Cell Tissue Res.* 250, 531–541.

YAGER, D. D. & HOY, R. R. (1988). Sexual dimorphism in the auditory system of the praying mantis. *Soc. Neurosci. Abstr.* 14, 87.

YAGER, D. D. & HOY, R. R. (1989). Physiology of audition in the praying mantis, *Mantis religiosa. J. comp. Physiol.* (in press).

ZHANTIEV, R. D. & KORSUNOVSKAYA, O. S. (1978). Morphological organization of tympanal organs in *Tettigonia cantans* (Orthoptera, Tettigoniidae). *Zool. J.* **57**, 1012–1016. (in Russian.)