

ACOUSTIC ORIENTATION OF A MOTH IN FLIGHT BY MEANS OF TWO SENSE CELLS

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INTRODUCTION

A most impressive but puzzling aspect of animal behaviour is the extraordinary degree of integration it displays. At a given instant the manifold sensory inputs to an animal's central nervous system, the central mesh of intermediary neural and humoral operations, and the array of effectors are all bent through excitation or inhibition to a oneness of action, be it food seeking, predator evasion, or reproduction. Even in conflicting stimulus situations a normally behaving animal does not seem to be unduly afflicted with coincident conflicting orders and the consequent possibility of becoming stalled 'at dead centre', even though such a situation may produce a succession of contrasting behaviour patterns.

Faced with this oneness or integration that characterizes animal behaviour it is easy for us to succumb to notions of goal and purpose that explain nothing, and to be overawed by the paradox of trying to disentangle the mechanisms of a closely knit entity. As experimental biologists we are presumably undismayed by such depressing thoughts, at least during our working hours. Still, perhaps it is proper that the wider implications of 'integration' as it appears in the title of this Symposium should continue to agitate our collective subconscious.

In recent years we have attempted to probe a situation and a subject which promise some reduction in the complexity surrounding integration in behaviour. Maximum integration has obvious survival value when an animal is evading a predator, for if it is not 'one-minded' while doing so its offspring will never have such an opportunity. For a similar reason evasive behaviour is characterized by unequivocal action and often by speed. These promise economy and simplicity in the neural mechanisms concerned. While these facts make it worth while to analyse the neural mechanisms of evasive behaviour, there is still the nightmarish problem of not activating and perhaps adapting out such mechanisms by the very experimental techniques used to investigate them. To this we have no answer.

THE TYMPANIC NERVE RESPONSE

Adult noctuid and geometrid moths have tympanic organs sensitive to the ultrasonic cries emitted by echolocating bats. These tympanic organs were first described in detail by Eggers (1919), and their properties have been discussed at length (Roeder & Treat, 1957, 1961*a*; Roeder, 1963, 1964; Treat & Roeder, 1959). Each tympanic organ contains two acoustic sense cells (A_1 and A_2) whose axons pass in the tympanic nerve to the pterothoracic ganglion. A_1 is about ten times more sensitive to ultrasound than A_2 . The A_1 receptors of the moth, *Caenurgina erechtea*, show a liminal response to pulsed ultrasound at pressures of -30 ± 5 dB. relative to 1 microbar between 25 and 60 kcyc./sec., falling off in sensitivity by about 20 dB. at 15 and 80 kcyc./sec.

The behavioural responses of moths to high-pitched sounds and to the proximity of flying bats have been noted by many observers. They range from 'freezing' or taking flight in crawling moths to diving, swerves and spirals in airborne individuals. Further, it has been observed that in the field airborne moths will turn and fly directly away from a loudspeaker emitting a train of ultrasonic pulses (Roeder, 1962). This seems to occur only when the source is distant from the moth or the signal relatively faint, the behavioural responses to strong ultrasonic stimulation being non-directional power dives, looping, or dropping passively to the ground.

This turning-away from a faint or distant sound source is of particular interest because (1) it appears to be primarily steered by a differential in the afferent discharges from only two sense cells—one A_1 receptor (the most sensitive of the pair) in each ear, and (2) because it occurs in planes both horizontal and vertical to the flight path, moths turning up or down as well as to right or left in taking a course away from the sound source.

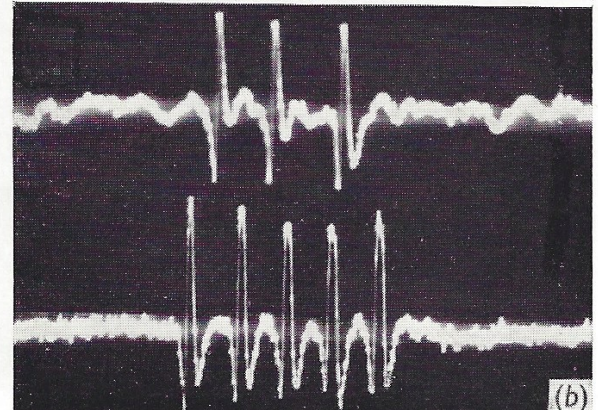
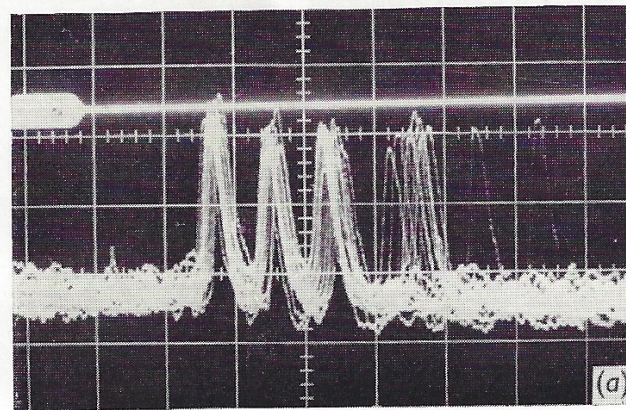
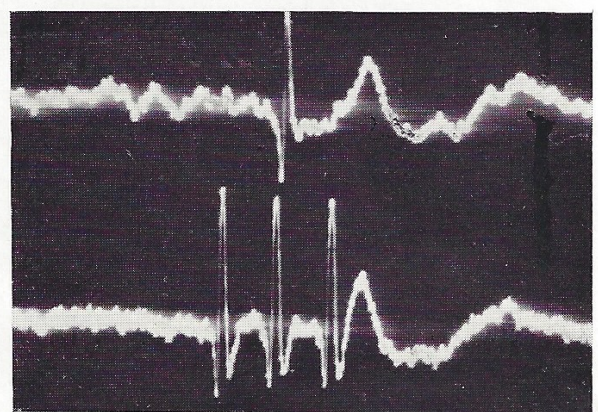
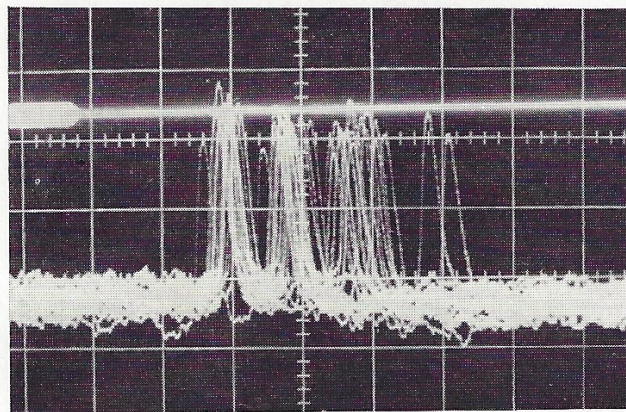
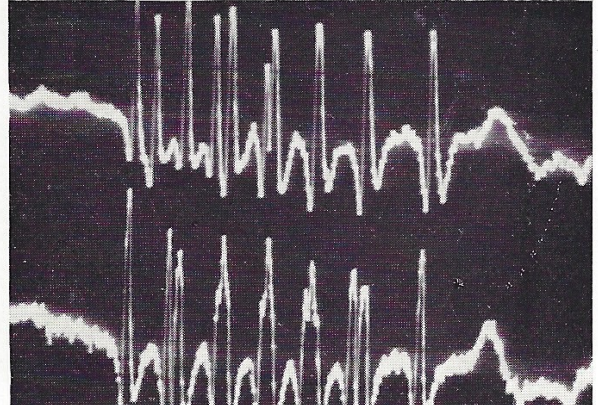
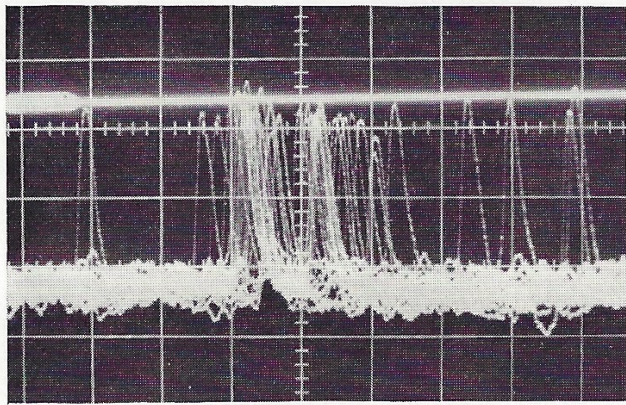
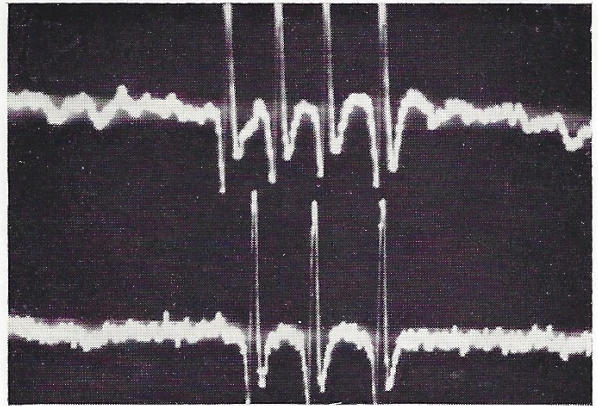
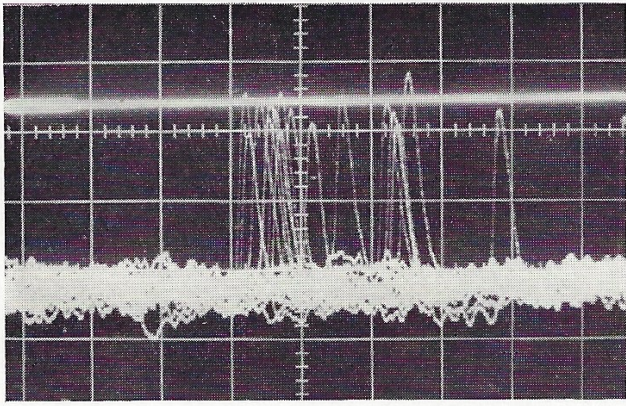
Since the ears of moths are quite directional (Roeder & Treat, 1961*b*; Payne, Roeder & Wallman, 1966) horizontal turning could be steered by a

Plate 1. Tympanic nerve responses to ultrasonic stimulation.

(a) Multisweep records of tympanic responses of *Leucania pseudagyria* male to different sound intensities. Stimulus was a repeated pulse (2.5 msec., 25 kcyc./sec., 15/sec., shown on upper trace). Each frame contains a number of stimulus-synchronized sweeps. Horizontal scale, 2 msec. per major division. Sound intensities relative to that in top frame (in vicinity of threshold) are: 2nd frame +3 dB., 3rd frame +6 dB., 4th frame +9 dB.

(b) Binaural tympanic nerve responses of *Feltia* sp. to the cries of red bats (*Lasiurus*) flying in the field. Individual frames were selected from a tape section of about 5 sec. duration made as a bat flew over the preparation. Responses range from a cry received at low intensity and producing one A_1 spike in one tympanic organ and three in the other (3rd frame) to cry received at high intensity by both tympanic organs and stimulating both A_1 and A_2 receptors (2nd frame). Interaural differentials in latency, spike number, and interspike interval are evidence of asymmetric acoustic stimulation. Compare with *A* above. Marker under last frame, 10 msec.

PLATE I



(Facing p. 252)

difference in the afferent signals delivered by the right and left *A* cells. Such differences have been analysed in some detail (Roeder, 1964). Stimulation with pulses of constant duration and low but increasing intensity causes (1) an increase in the number of spikes delivered by *A*₁, (2) a decrease in the average time interval between consecutive *A*₁ spikes, and (3) a decrease in the latency of the first *A*₁ spike of a train (Pl. 1*a*). These three criteria of sound intensity difference are evident in a binaural recording of tympanic nerve responses made in the field as bats manoeuvred in

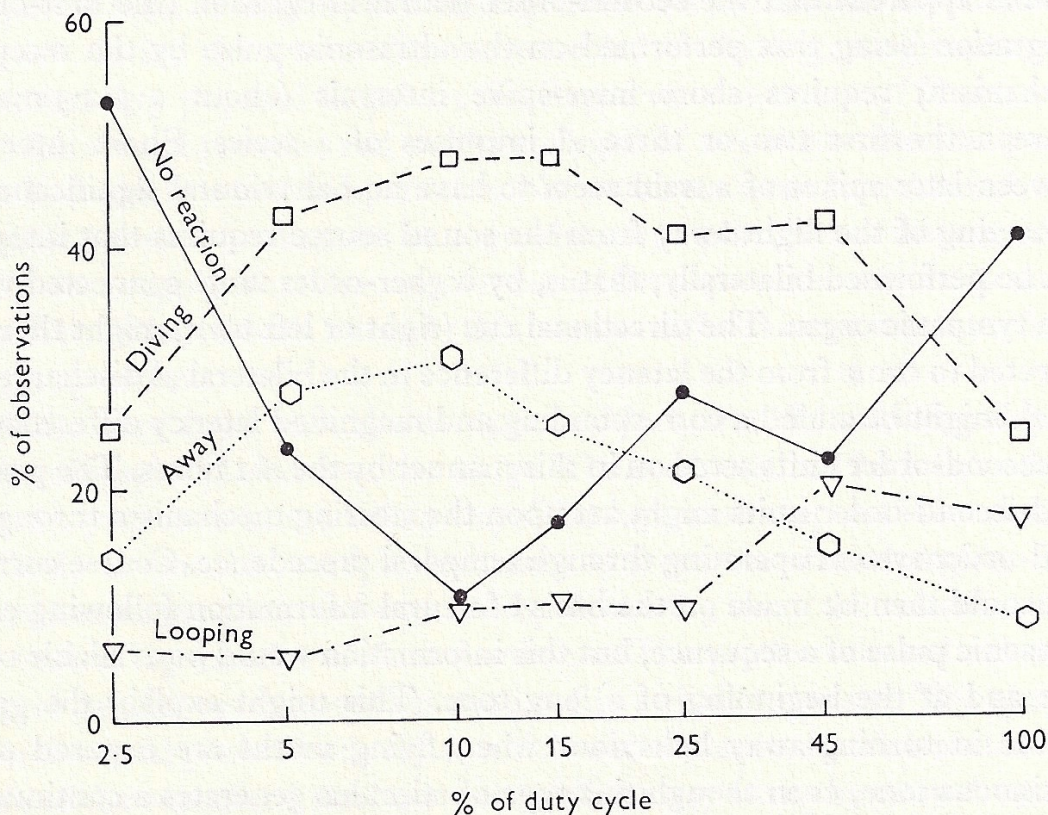


Fig. 1. Summary of behavioural responses shown by assorted and unidentified moths flying in the field when exposed to ultrasonic pulses at various repetition rates and to continuous tones. Based on 1342 separate observations. In making each observation the moth's flight path was observed by incandescent illumination, the ultrasonic source (mounted on a mast) turned on, and a change in behaviour, if any, noted. Responses were arbitrarily categorized as *no reaction*, *turning away* from the ultrasonic source and continuing on a straight course, *diving* to the ground without showing any obvious directional reaction, and *looping* in a series of non-directional turns. On different occasions the ultrasonic stimulus was a series of 5 msec. pulses recurring from 5/sec. (2.5% duty cycle) to 90/sec. (45% duty cycle), and a continuous tone (100% duty cycle). Frequency (50 kcyc./sec.) and intensity were kept constant during all observations. (From Roeder, 1964.)

the area (Pl. 1*b*), and any or some combination of them might be capable of informing the moth whether the sound source is to its right or left. Directional characteristics of the tympanic organs will be considered later.

Indirect evidence as to which of these criteria plays a part in exciting central neurones concerned in 'turning-away' behaviour was obtained from further field experiments. Ultrasonic pulses (50 kcyc./sec., 5 msec. in duration) emitted by a loudspeaker at different repetition rates were compared

with long tones (2 or 3 sec.) of identical frequency and intensity for their effectiveness in causing directional and non-directional types of evasive behaviour in a wild population of naturally flying moths of assorted species (Roeder, 1964). Long continuous tones were found to be markedly less effective than the same frequency presented in pulses in producing all types of reactions, turning-away from the source of ultrasound being practically absent (Fig. 1). When the data on behavioural reactions were compared with tympanic nerve responses to the same types of stimulus pattern it became apparent that the second-order neural integration (the first-order integration being that performed on the ultrasonic pulse by the receptor mechanism) requires short inter-spike intervals (about 1.5–2.5 msec.) between the first two or three *A* impulses of a series. Short intervals between later spikes of a train seem to have no behavioural significance.

Steering of the flight away from the sound source requires that integration be performed bilaterally, that is, by higher-order units connected with each tympanic organ. The directional cue (right or left turn) might then be expected to come from the latency difference in the bilateral *A* discharge, to which might be added a corresponding and magnified latency difference in the second-order units acted on in this manner by the *A*1 fibres. The postulated second-order units might act upon the steering mechanism through a third-order system operating through temporal precedence. Course correction could then be made on the basis of neural information following each ultrasonic pulse of a sequence, but this information would be available only once and at the beginning of a long tone. This might explain the great decline in turning-away behaviour when flying moths are exposed to a continuous tone, even though this type of stimulus generates a continuous train of *A*1 impulses.

An additional finding of these behavioural experiments was that 5 msec. ultrasonic pulses were most effective in causing evasive behaviour when they were presented about 20 times a second (Fig. 1). Higher or lower repetition rates caused a decline in the numbers of moths diving or turning away from the source. These repetition rates correspond to a duty cycle of 10% when 5 msec. pulses are used, which suggests that a relatively long 'off' period between each pulse is required for the resetting of the second-order unit, and they approximate to the repetition rates occurring in the cries of cruising bats.

HIGHER-ORDER NEURONES REACHED BY THE TYMPANIC NERVE FIBRES

Serious attempts to trace central connexions made by the tympanic nerve fibres were begun only during the past year. Several types of distribution

and synaptic connexions have been found in the thoracic nerve cord and suboesophageal ganglion, but most of them have been very incompletely followed and functionally examined, and none has been anatomically identified. However, one type of synaptic connexion made by the tympanic nerve fibres is of particular interest in the present context, so it will be given a preliminary functional description.

Method. Subjects were adults of both sexes of a common grass moth, *Caemurgina erechtea* (Noctuidae). Eggs were obtained from wild females trapped at light in August, and three generations were raised at room temperature during the winter. Larvae were fed on fresh grass grown under glass, and they pupated in 4–6 weeks. Pupae were stored at 5° C., in some cases for several months, and moths emerged in 2–3 weeks after the pupae were brought to room temperature. No problems were encountered with diapause (larvae were kept on an approximately 14 hr. day) or disease, and a continuous supply of moths was maintained throughout the winter. Acoustic sensitivity of individuals raised in this manner differed in no way from field-caught specimens.

Moths were fixed ventral side up on a block of stiff modelling clay by means of staples placed over the wings. The head was held back by embedding the proboscis in modelling clay. The legs were amputated as close to the body as possible, and scales removed from the sternal region. The pterothoracic ganglion was exposed by dissecting away the sternal cuticle and ventral leg muscles of the mesothorax. The prothoracic and suboesophageal ganglia were exposed as required in a similar manner. The ganglia lay in a natural pool of body fluid, which was augmented with insect saline. Some of the superficial tracheae were pulled away from the ventral surface of the pterothoracic ganglion, which nevertheless seemed to remain in good shape (as shown by flight and reflex movements as well as by electrical activity) for some hours.

Extracellular electrical activity was detected within the thoracic ganglia through insulated microelectrodes of electrolytically tapered stainless steel wire. Sometimes silver hooks were placed under the tympanic nerve to obtain a simultaneous record of afferent activity in the first-order acoustic fibres.

Ultrasonic stimuli were delivered by two condenser microphone heads used as loudspeakers and placed opposite each ear on either side of the moth at about 15 cm. This made it possible to stimulate either ear separately provided the sound intensity was not more than 10–20 dB. above the tympanic threshold. For unequivocal monaural stimulation at high intensities one tympanic membrane of the moth was destroyed before mounting the preparation.

Ultrasonic pulses of 50 kcyc./sec., 2–10 msec. in duration, with 0.25 msec. rise and fall time, and repeated 1–20/sec., were routinely used. They were delivered through a calibrated attenuator and driver amplifier (McCue, 1961), and when unattenuated had an intensity of +50 dB. relative to the threshold of the moth. Ramped pulses with sinusoidal rise times up to 25 msec., and single long pulses were also used. Latencies not otherwise defined are expressed in milliseconds relative to the beginning of a pulse at the tympanic membrane.

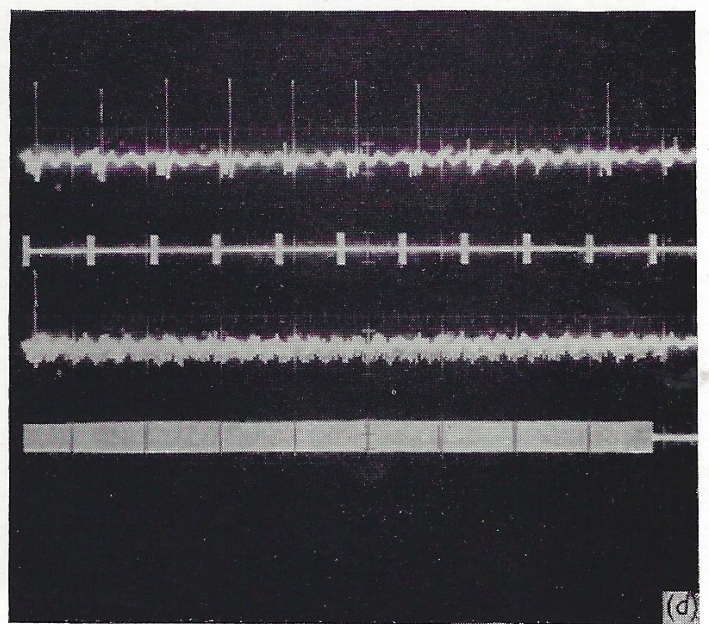
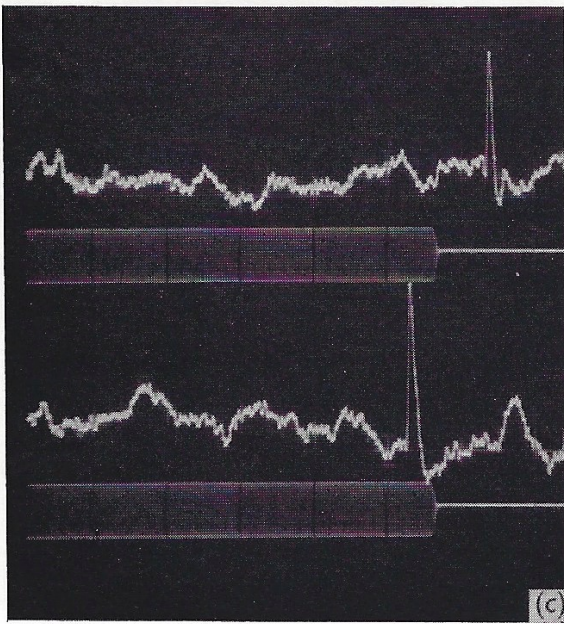
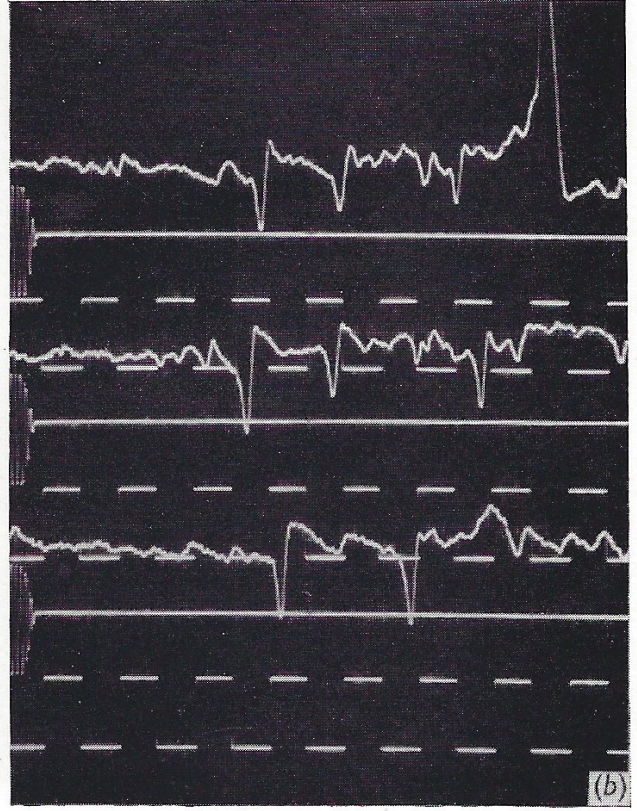
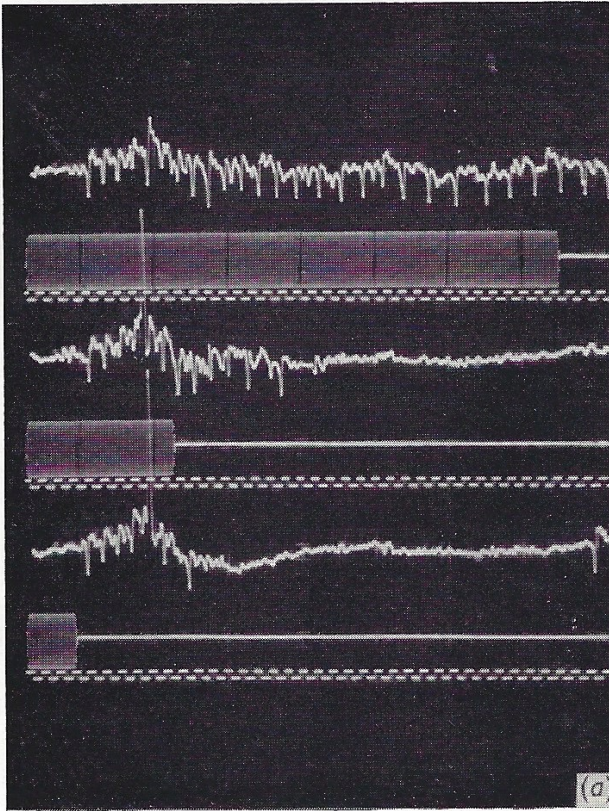
Results. Analysis of the central connexions made by the tympanic nerve fibres is still very incomplete, and emphasis will be placed only on one type of second-order neurone relevant to the present topic. However, it might be noted that apparently undifferentiated tympanic nerve (*A*₁ and possible *A*₂) impulses can be detected at a number of points as they ascend the thoracic nerve cord on the ipsilateral side. These appear in the mesothoracic ganglion with a latency of 3.5–5 msec., the prothoracic ganglion in 5–7.5 msec., and the suboesophageal ganglion in 7–9 msec. after tympanic stimulation. As they pass through the ganglia these fibres appear to synapse with contralateral neurones of a relay type whose course and function have not been followed further.

Electrodes probing the posterior half of the mesothoracic ganglion have frequently encountered spikes generated by another type of second-order neurone activated by the ipsilateral tympanic nerve volley in a significant and quite complex manner (Pl. 2). Repeated probing was often necessary to find this unit, and it was readily lost through a slight movement of the preparation. When found, its externally recorded spike usually registered as an upward (negative) deflexion, and the same electrode sometimes recorded first-order tympanic spikes as low-voltage deflexions of the opposite sign. In one or two cases *A*₁ spikes and the post-synaptic response of this unit were detected separately through two microelectrodes inserted in the mesothoracic ganglion.

This second-order *I* (integrator) unit commonly discharged from 6 to more than 20 msec. after the appearance of the first *A*₁ spike of a train. Sometimes the single spike was closely followed by a second, particularly at higher stimulus intensities. The *I* response was quite sensitive, appearing at sound intensities about +5 dB. relative to the approximate sensory threshold (Roeder, 1964) of the tympanic organ, yet typically it could not be made to fire more than once during a train of *A*₁ impulses elicited by an uninterrupted tone, irrespective of the intensity (+40 dB.) or duration (500 msec.) of the acoustic stimulus. However, in a few cases the *I* unit was seen to fire several times during such a stimulus.

In spite of the insensitivity of the *I* unit to the arrival of long trains of *A*

PLATE 2



(Facing p. 257)

impulses, it could be fired by sound pulses as short as 0.50 msec., and could be driven by these or longer pulses (5.0 msec.) up to 30 times a second. At or above this repetition rate it tended to skip alternate *A* trains (Pl. 2*d*). By using various combinations of pulse duration and repetition rate the unit was found to perform best in response to pulse sequences having less than 10% duty cycle, e.g. 5 msec. pulses repeated 20 times a second. This suggests that reactivation of the *I* unit normally depends on a fairly substantial period of inactivity on the part of the impinging *A* fibres.

The *I* unit responds for long periods in a stable manner if the tympanic organ is exposed to short pulses (2–5 msec.) delivered at low repetition rates (1–3/sec.). If now the stimulus intensity is reduced to the point where the *I* unit fails to respond to an occasional afferent train elicited in this mode, some of the conditions of *I* excitation become evident. Most of the *I* units thus far examined appear to be synaptically excited by the third or fourth *A* spike to arrive (shoulder in Pl. 2*b*) provided the preceding two or three *A* spikes have inter-spike intervals of from 2 to 2.5 msec. or less.

Longer pulses (50 msec.) delivered in this mode, that is, at low repetition rates and with intensities not far from the sensory threshold, elicited single *I* spikes at variable and often long (20 msec. or more) latencies (Pl. 2*c*). From this it is inferred that the somewhat random frequency of impulses evoked in the *A* train under these circumstances did not provide the requirements for *I* excitation—3 or 4 *A* impulses with sufficiently short inter-spike intervals—until fairly late in the *A* impulse train.

One simple explanation for the presence of only one *I* spike per *A* train is that synaptic excitation of the *I* unit depends on straightforward temporal summation. Only one or two *I* spikes might be expected because the *A* receptors adapt rapidly to continuous tones (Roeder & Treat, 1957; Roeder,

Plate 2

Intraganglionic responses to tympanic stimulation in *Caemurgina erechtea*, recorded extracellularly with stainless steel insulated microelectrodes. In *a*, *b* and *d* the electrode tip registered activity in central portions of *A*₁ fibres (small down-going spikes) as well as response of a second-order unit *I* spike (large upward deflexion).

(*a*) Responses to 30, 10, and 3 msec. pulses (50 kcyc./sec., centre trace) delivered at an intensity of about +5 dB. relative to the *A*₁ threshold. The *I* spike occurred during sweeps 2 and 3, but failed to generate in sweep 1 although a generator potential is evident. Square wave, 1000/sec.

(*b*) Three responses to a 0.5 msec. pulse just above threshold intensity. In upper trace the *I* unit is fired by 3 *A*₁ impulses; in middle trace *I* response fails to be generated by 3 slightly more widely spaced *A* impulses; in lower trace *I* response fails to generate on arrival of only 2 *A* impulses. Square wave, 1000/sec.

(*c*) *I* response to stimulation by 10 msec. pulse near threshold (upper trace) and at +20 dB. (upper trace).

(*d*) *A*₁ and second-order *I* responses (large up-going spike) to tympanic stimulation with 5 msec. ultrasonic pulses delivered at a rate of 30/sec. *I* unit drops out during part of the stimulus period. Below, response of the same unit to 300 msec. pulse of the same intensity. A single *I* spike occurs only once at the beginning of response. Lower trace in each sweep indicates stimulus pattern.

1964), and the requisite amount of temporal summation of *A* impulses would be achieved only once and then near the beginning of the *A* train, subsequent inter-spike intervals being rendered too long through sensory adaptation. Two experiments suggest that the excitation mode of the *I* unit is more complex than this, and that an extensive 'off' period of *A* impulses is required to reset the *I* unit so that it can be re-excited by the next *A* train. First, an *I* unit may fire only once during a long *A* train even when the sound intensity is high enough (+40 dB. relative to the *A*₁ threshold) to cause the recurrence of adequately short *A*₁ inter-spike intervals many times during the train. However, it should be noted that a few *I* neurones were encountered which fired more than once during such a train, particularly with stimuli of high intensity. Secondly, in some experiments the sound pulse was ramped so that its intensity built up gradually. Special pains were not taken to adjust the shape and time course of the ramp (a sinusoid of 12.5 or 25 msec. rise time) to the time course of *A* adaptation. Therefore, this stimulus only roughly offset the adaptation of the *A* receptors. Nevertheless, when delivered to the ear at high intensity so that the first *I* spike was triggered near the foot of the ramp, this form of stimulation did not elicit more *I* spikes per *A* train than did stimuli having an abrupt (0.25 msec. or less) onset. It is concluded that, although temporal summation plays a part in excitation of the *I* unit, at least 50 msec. of inactivity on the part of the afferent fibres seems to be a prerequisite. If this inactive period does not follow generation of an *I* spike, then temporal summation of *A* impulses will not initiate a second *I* spike, at least in the majority of preparations.

A great deal remains to be done. The *I* neurone must be identified anatomically and its interaction with its opposite number must be defined. However, it fulfils so neatly some of the conditions of central integration required by the behavioural experiments (Roeder, 1964) that this preliminary report seems justified. Assuming that it is the second step in the chain of nervous integration between sound pulse and evasive behaviour, the mode of the *I* unit would account for the failure of moths to turn away from the sound source when exposed to a continuous tone, and perhaps also for the decline in non-directional diving shown by moths exposed to tones of greater intensity (Fig. 1). The optimum behavioural effectiveness of signals having a 10–20% duty cycle coincides with the properties of the *I* unit as well as with the pattern of cries emitted by cruising bats.

The slight difference in latency (1 msec. or less, Pl. 1) of the first *A*₁ spike of the trains arriving at the central nervous system from right and left tympanic organs has been suggested (Roeder, 1964) as a possible basis for steering the flight mechanism into right or left turns away from an ultrasonic source of pulses. This time differential would be greatest at the low sound

intensities when 'turning-away' is most often observed. In addition to this, it can be seen from Pl. 1 that under conditions of low sound intensity the ear receiving the louder sound also generates shorter inter-spike intervals and more spikes per A_1 train. Behaviour of the I unit suggests that it integrates all of these criteria of intensity, expressing the result in a much greater latency of the single I spike generated on the 'off' side, which ranges from 6 msec. to more than 20 msec. after the arrival of the first A_1 impulse (Pl. 2c). It has not yet been possible to record simultaneously from right and left I units during binaural stimulation, but there seems no reason to doubt the presence of this large latency differential in their spikes following asymmetric stimulation with a faint sound pulse. It is not unreasonable to expect the right and left I units to converge on a third-order system with three 'positions' and operating through temporal precedence. In one of these 'positions'—no I impulses—it would not influence steering; in the other two it would bias the steering either to right or to left depending on whether the right or left I impulse had temporal priority. The pulsed cries made by a distant cruising bat would reset this system of temporal precedence about ten times a second.

ACOUSTIC LOCALIZATION*

The term 'integration' is used in this account to describe the general nature of the processes that combine, smooth, select from, suppress, and otherwise operate on the multitude of sensory inputs to produce that oneness of action immanent in the adaptive behaviour of animals. It is perhaps idle to speculate whether we shall ever have sufficient information about these processes to be able to dis-integrate the behaviour, that is, reconstruct all the inputs from a knowledge of the ultimate action. It is doubtful whether this is even theoretically possible, because if one examines the individual steps of neural integration they seem to involve discarding certain characteristics of the input as well as accentuating others.

For instance, in the first step in the system under consideration—transduction of the ultrasonic pulses into A_1 nerve impulses—the afferent pattern of spikes contains information on pulse duration, intensity, and repetition rate, but the ultrasonic frequency of the stimulus cannot be derived from studying the afferent nerve message. At the second step—the response of the I units information on pulse duration is similarly discarded, although intensity differences are accentuated in differences of the response time of the I unit and pulse repetition rates are presented directly

* The work on acoustic localization, in which Joshua Wallman of Harvard University collaborated, has been reported in greater detail elsewhere (Payne *et al.* 1966).

in the single spikes. A subsequent step, about which we know nothing in fact, may operate through the latency differential of the repeated discharges of the right and left *I* units to steer the moth on a course directly away from a faint source of ultrasonic pulses. Since it is not possible to probe further into the moth's central nervous system at this point we shall return to the afferent train of *A*₁ impulses in order to see how it contains information making possible this directional behaviour.

The existence of a differential in the afferent nerve responses from right and left tympanic organs exposed to an asymmetrically placed source of ultrasound (Pl. 1*b*) implies that the noctuid ear has directional properties. Polar plots of acoustic sensitivity in a plane horizontal to the moth's body (Roeder & Treat, 1961*b*) show the general form of this directional property in one plane. However, field observations (Roeder, 1962) show that moths turn and, after some 'hunting', fly directly away from a source of faint ultrasonic pulses, orienting while doing so in a vertical as well as in a horizontal direction and thereafter maintaining a relatively straight path. While the evidence at hand might be sufficient to account for turns to the right or left in selecting a path away from an ultrasonic source, it gives no hint of the sort of sensory cues that might enable a moth to take a climbing turn away from a sound source below its line of flight, or vice versa. The experiments to be described examine variations in the intensity of *A*₁ cell responses due to variations in sound source placement. Each informative experiment consisted of *c.* 500 measurements made from continuous records with the wings of the subject fixed in some one position approximating to a certain phase of the natural wing stroke. The experiments suggest that the wings act as a rapidly moving acoustic baffle, and that by combining information concerning wing position with that originating from the right and left *A*₁ acoustic receptors a moth could localize sounds coming from above or below as well as from right or left.

Method. Multidirectional measurement of the acoustic sensitivity of a moth with its wings in different attitudes presents two major practical problems: (1) hundreds of measurements from a single individual are necessary to ascertain how intensely the moth's tympanic nerve reports an ultrasonic pulse of constant frequency, duration, distance and intensity from many points on the surface of a sphere with the moth at its centre. Tympanic nerve preparations last but a few hours, since the dissection necessary for implanting an electrode on a moth's acoustic nerve allows the tissues to desiccate. Therefore, one is forced to speed data collection by automation. (2) The ultrasonic signals used (30 and 60 kcyc./sec.) echo readily from small objects near the detector and suffer consequent distortion. This means that the moth cannot be surrounded by electrophysiological equip-

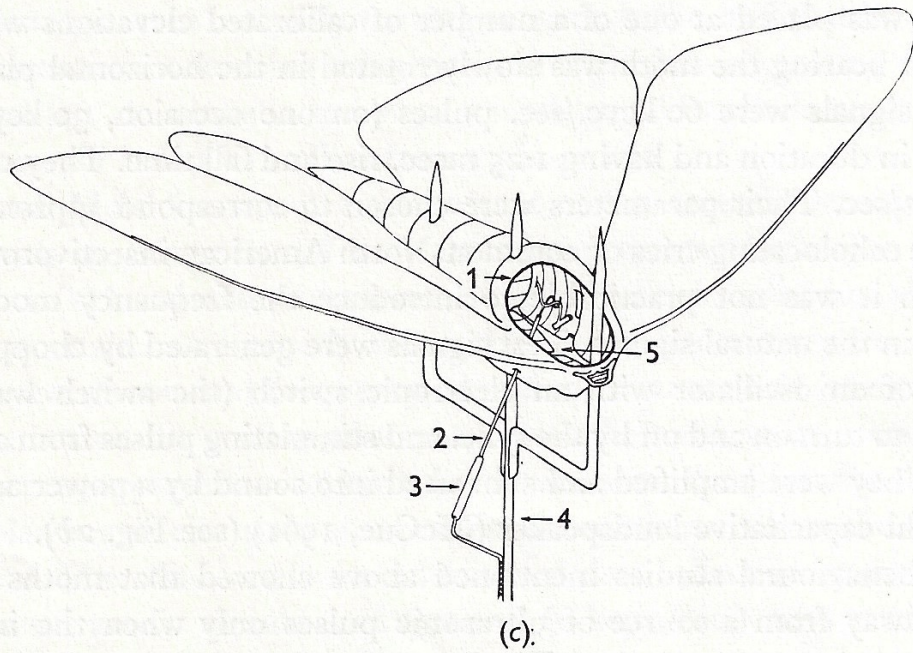
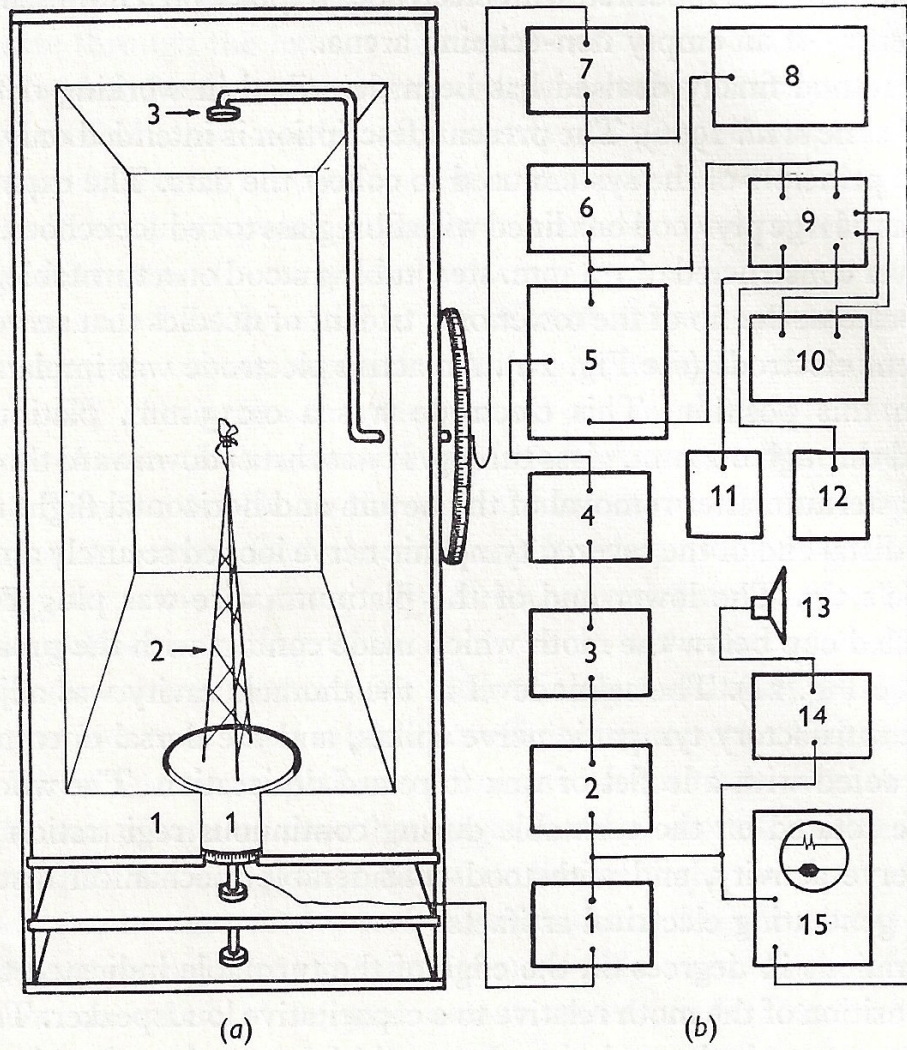
ment, and has to be mounted with electrodes in place on a minimal support in the centre of an empty non-echoing arena.

The method finally devised has been described in working detail elsewhere (Payne *et al.* 1966). The present description is intended only to make clear the principle of the system used to collect the data. The experimental arena was a large plywood box lined with fibreglass to reduce echoes. A 30 in. high tower constructed of 1.2 mm. steel tubing stood on a turntable. A moth was impaled at the tip of the tower on a trident of needles that served as the indifferent electrode (see Fig. 2*a*). An active electrode was implanted in it while in this position. This electrode was a 0.015 mm. platinum wire threaded through 0.2 mm. glass tubing. It was thrust downward through the thoracic sternum after removal of the notum and horizontal flight muscles, and the distal end of the severed tympanic nerve looped securely around the electrode's tip. The lower end of the platinum wire was plugged into a saline-filled cup below the moth which made contact with the preamplifier input (see Fig. 2*c*). The saline level in the thoracic cavity was adjusted to produce satisfactory tympanic nerve spikes, and the dorsal opening in the thorax roofed with a leaflet of wax to retard desiccation. The whole array could be rotated on the turntable during continuous registration of tympanic nerve activity, and withstood considerable mechanical disturbance without generating electrical artifacts.

Calibrations in degrees on the edge of the turntable indicated the horizontal position of the moth relative to a capacitative loudspeaker. The loudspeaker was attached to a boom that could be moved so that the speaker swept through an arc of 180° in the vertical plane. In practice the loudspeaker was placed at one of a number of calibrated elevations while the platform bearing the moth was slowly rotated in the horizontal plane.

Test signals were 60 kcyc./sec. pulses (on one occasion, 30 kcyc./sec.) 6 msec. in duration and having 1.25 msec. rise and fall time. They recurred 20 times/sec. Their parameters were chosen to correspond approximately with the echolocating cries of common North American insectivorous bats, although it was not practicable to introduce the frequency modulation present in the natural signals. Test signals were generated by chopping the output of an oscillator with an electronic switch (the switch was commanded to turn on and off by the sync. and stimulating pulses from a stimulator). They were amplified and converted into sound by a power amplifier and Kuhl capacitative loudspeaker (McCue, 1961) (see Fig. 2*b*).

The behavioural studies mentioned above showed that moths steer a course away from a source of ultrasonic pulses only when the intensity reaching their ears is quite low. Therefore, a near-threshold response in the tympanic nerve of between 2 and 3 *A* 1 spike potentials per ultrasonic pulse



For legend see facing page.

was chosen as a 'criterion response'. This is close to the response level shown in frame 3 of Pl. 1, and is perhaps 5–10 dB. above the ill-defined tympanic threshold. After amplification, spikes were separated from other activity in the tympanic nerve by a pulse-height analyser and converted into standard pulses of the same frequency and number. A frequency meter then transformed this signal into a d.c. potential proportional to the number and frequency of A_1 spikes.

A feedback amplifier adjusted the sound intensity being delivered to the moth to whatever level was necessary to maintain the chosen number of tympanic nerve action potentials per sound pulse. The system thus served to keep the tympanic response at criterion level, increasing the stimulus intensity when the sound was presented from directions to which the tympanic organ was relatively insensitive, and vice versa. A sound-level recorder registered the changing voltage of the stimulus during rotation of the tower bearing the moth.

The final step in preparing a moth for recording was to fix its wings in one of the postures assumed during active flight. This was done by applying minute drops of wax to the wing articulations. In selecting these postures it was not possible to reproduce the subtle changes in camber assumed during the natural wing stroke, and the wings were treated as simple flaps.

With the wings fixed in position the moth was rotated on its platform with the loudspeaker at a certain elevation. This constituted one 'sweep'. A series of sweeps with the loudspeaker fixed at successive elevations 10° apart, from 90° vertical (directly above the moth) to 290° vertical (as far

Fig. 2. (a) Diagrammatic view of the acoustic arena. 1. Fibreglass insulation. 2. Tower with moth at its apex mounted on the turntable. The legs of the tower are seen here emerging from a cylinder of insulation covering the turntable. The periphery of the turntable is calibrated in degrees to show the horizontal plane orientation of the moth with respect to the loudspeaker. 3. Ultrasonic loudspeaker mounted on a boom that can be rotated in the vertical plane. The disc outside the box is used to turn the boom within and is calibrated in degrees to show the vertical plane orientation of the loudspeaker to the moth.

(b) 'Black box' diagram of the electronic apparatus. 1. Preamplifier. 2. Pulse-height analyser. 3. Pulse generator. 4. Frequency meter. 5. Feedback amplifier. 6. Filter. 7. Transducer driver. 8. Graphic level recorder. 9. Electronic switch. 10. Stimulator. 11. Oscillator, 100 cyc./sec. 12. Oscillator, 60,000 cyc./sec. 13. Loudspeaker, for monitoring A spike activity. 14. Power amplifier. 15. Oscilloscope. Explanation of operation as in text with one addition: the use of two oscillators, one at 100 cyc./sec., made it possible to deliver a continuous signal to the graphic level recorder so that it did not 'hunt' between pulses. The 100 cyc./sec. signal was interjected between the 60,000 cyc./sec. pulses and subsequently filtered out.

(c) Diagrammatic view of a headless, legless moth impaled on a trident of needles at the apex of the tower. 1. Periphery of the excavation made into the thorax in order to expose the acoustic nerve. 2. Fine glass tube containing the active electrode wire; the wire may be seen emerging from the tube inside the thoracic cavity. It is there bent into an 'S' and the acoustic nerve draped over it. 3. Saline-filled cup made from a length of no. 20 hypodermic needle soldered to a copper wire that is tied to the tower (but insulated from it). The end of the glass-platinum electrode is inserted into this cup. 4. Tip of the tower, showing the trident of needles (the indifferent electrode) penetrating the moth's body. 5. Pterothoracic ganglion.

below the moth as the tower would allow), produced a 'sphere' of intensity readings. After satisfactory collection of data for a sphere the wings of the moth were fixed at another angle and the process repeated.

Signals at various points in this system were continuously monitored by means of an oscilloscope and loudspeaker. Various steps were necessary in order to control excessive 'hunting' by the frequency meter and sound level recorder. Instability and deterioration of the preparation was checked at various points and compensated for when necessary or possible.

Many moths were tested but only twelve survived long enough to provide one or more complete spheres. Noctuids of the genus *Catocala* (underwing moths) provided all but 5 of the 36 spheres obtained—one individual providing 10 spheres and lasting for 14 hr. Subjects were captured at ultraviolet light traps and used as they became available. Members of the genus, though not particularly common, are known to be subject to capture by bats (Webster, 1962). Their tympanic organs are similar to those of other noctuid moths.

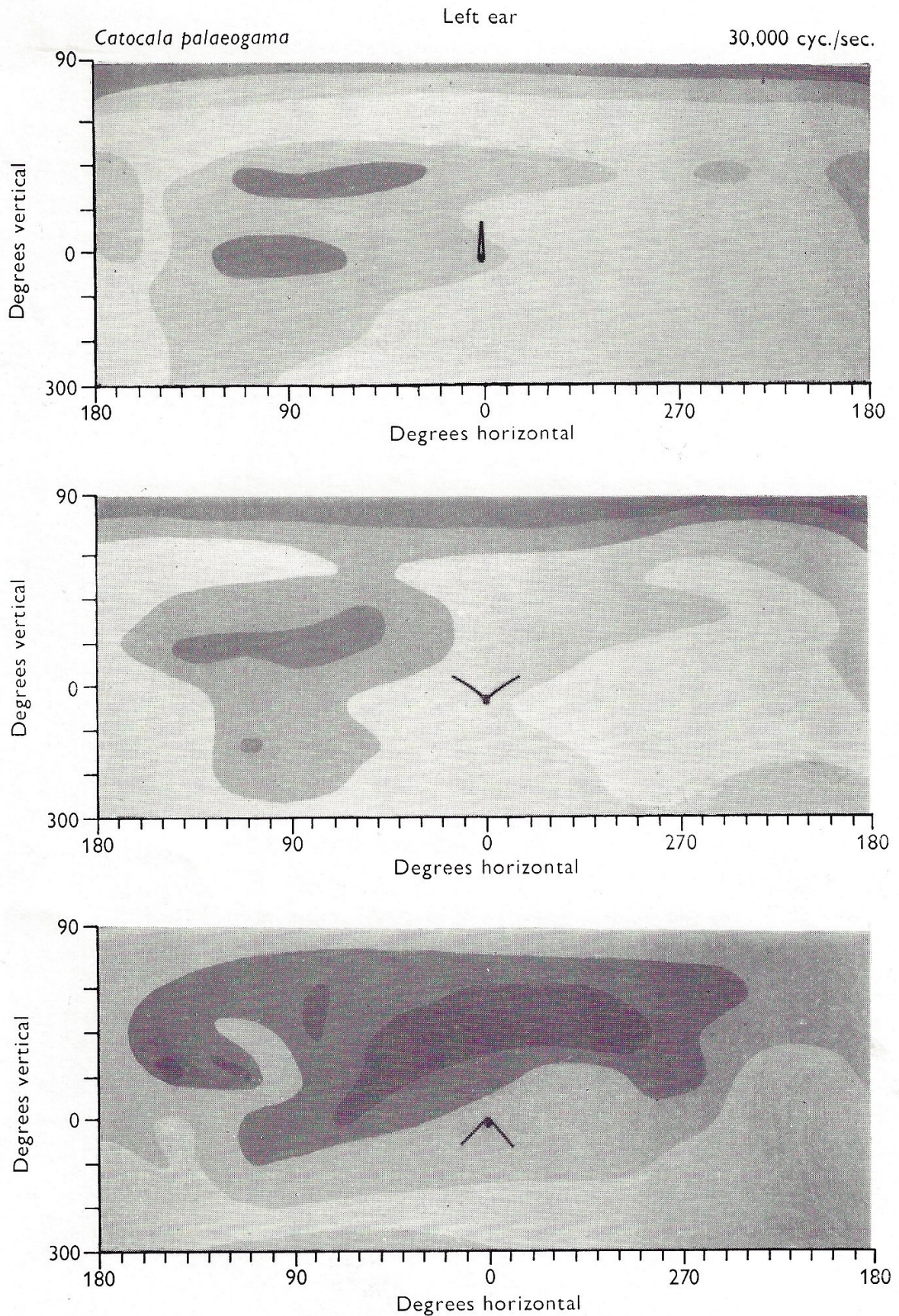
Results. In assessing the data collected by this method it should be borne in mind that the readings made for one sphere of sensitivity, that is, during one run at a given wing position, are internally consistent within ± 2 dB. Intensity discrimination of the tympanic organ is sufficient to expect this accuracy, and replicate sweeps made at several points during each sphere provided sensitivity checks and enabled adjustments to be made where necessary. Therefore, a valid comparison can be made of measurements of sensitivity to sounds coming from any two directions within a given sphere. However, between runs the nerve was usually irrigated with saline and the position of the wings altered. This often changed the overall sensitivity, so that no sensitivity comparisons for any fixed sound source placement can be made between one sphere and the next.

After several attempts to display the data for each sphere of sensitivity readings, Mercator projections were found to be the most satisfactory form for the two-dimensional printed page. When sensitivity readings for all sound source placements at any one wing position had been collected, we had sufficient data to cover a sphere with numbers—one number at each intersection of latitude and longitude lines spaced 10° apart. This is more readily understood by referring to Fig. 3. A moth is pictured at the centre of a globe with its body in the equatorial plane (0° vertical) and headed towards the Greenwich meridian (0° horizontal). Each number at latitude and longitude intersection points (not shown in Fig. 3) indicates how loud (in decibels) a sound source has to be when placed at that intersection to produce the criterion, near threshold, intensity report from the moth's tympanic organ. Clusters of high numbers thus represent regions of poor

PLATES 3, 4, 5

Mercator projections of directional sensitivity data

PLATE 3



Directional sensitivity of the left ear of a single specimen of *Catocala palaeogama*. Sensitivity to the same 30 kcyc./sec. pulse coming from all points on the surface of an imaginary sphere enclosing the moth is shown by using different shades of grey for each 5 dB. change in sensitivity. The darker the shade, the less intense would sounds arriving from those directions (relative to the moth's body axis) be reported by the moth's tympanic organ (from white, 35-40 dB.; to black, 0-5 dB.). The sphere is shown here in Mercator projection. A silhouette of the moth flying out of the plane of the paper towards the reader shows the angle at which the moth's wings were held during collection of data for this sphere. The settings of the loudspeaker in the vertical plane are indicated by dashes along the vertical borders of the projection. Similar dashes along the horizontal borders merely indicate 10° intervals and do not necessarily show all horizontal readings entered on the projection (readings at intermediate points were entered when brief changes occurred that would otherwise be lost).

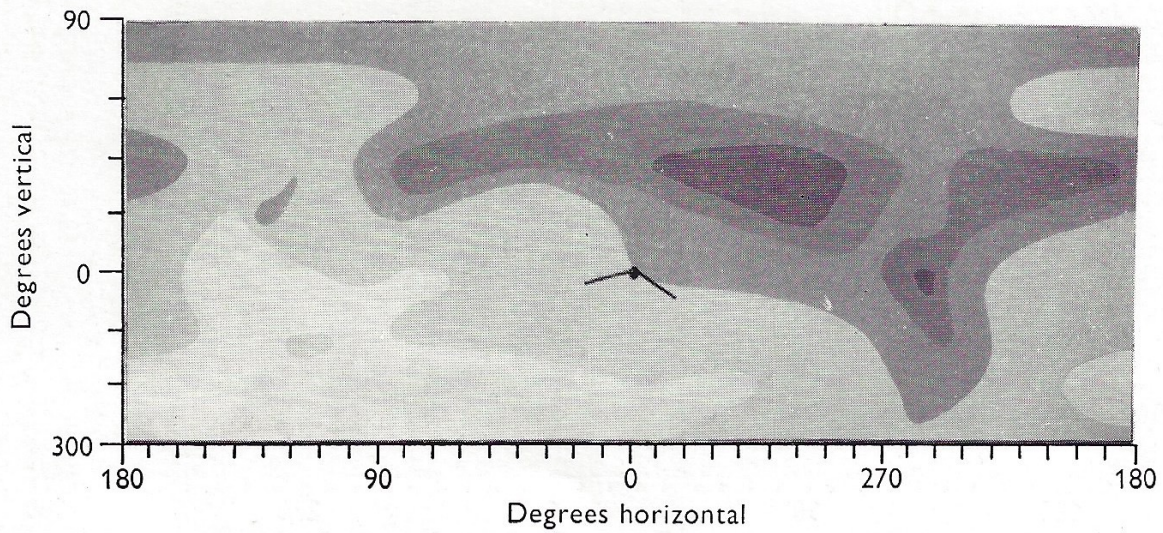
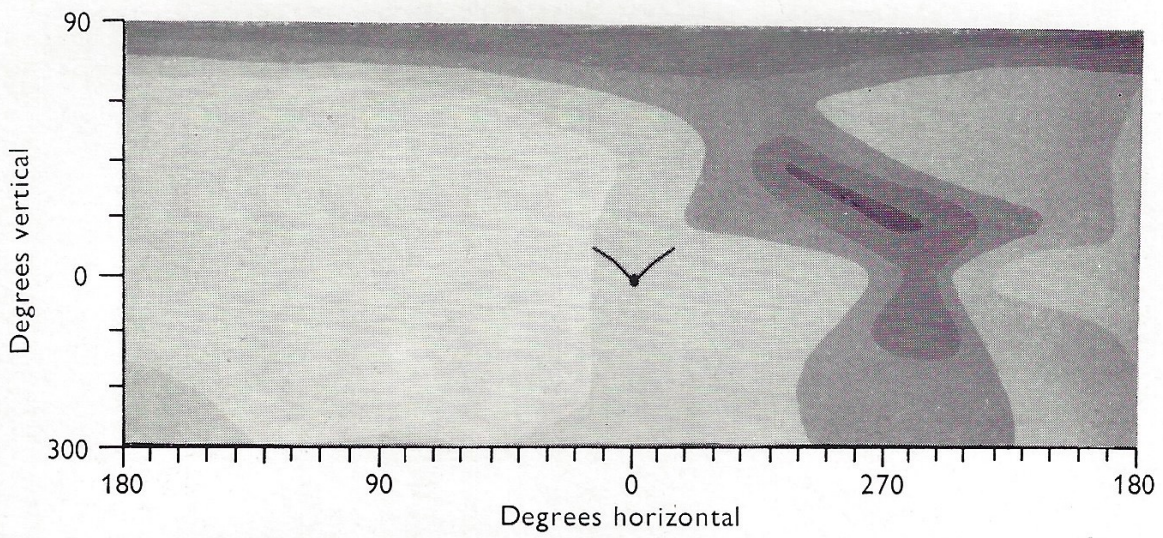
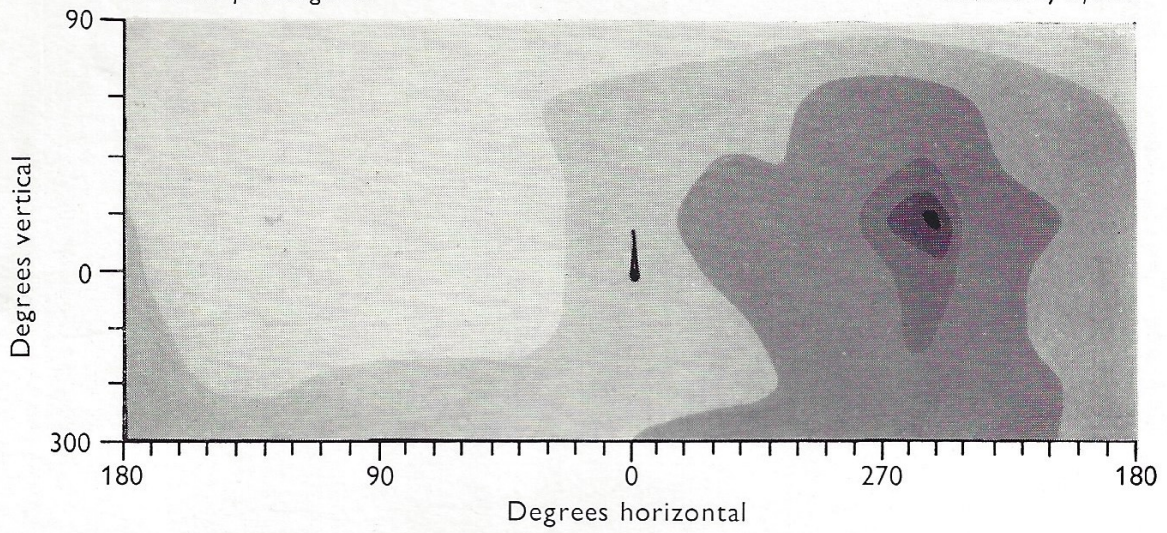
(Following p. 264)

PLATE 4

Right ear

30,000 cyc./sec.

Catocala palaeogama

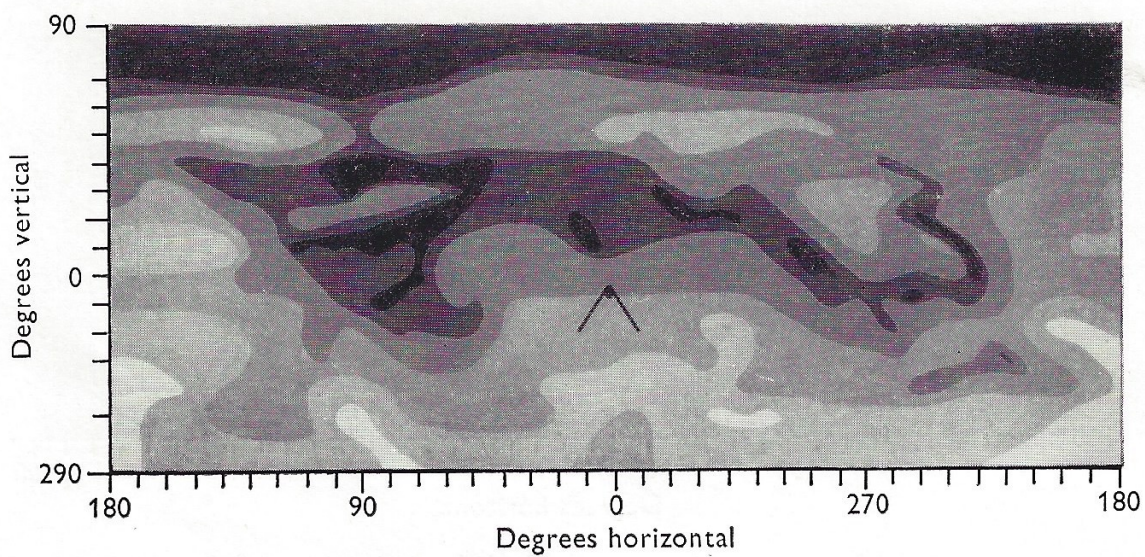
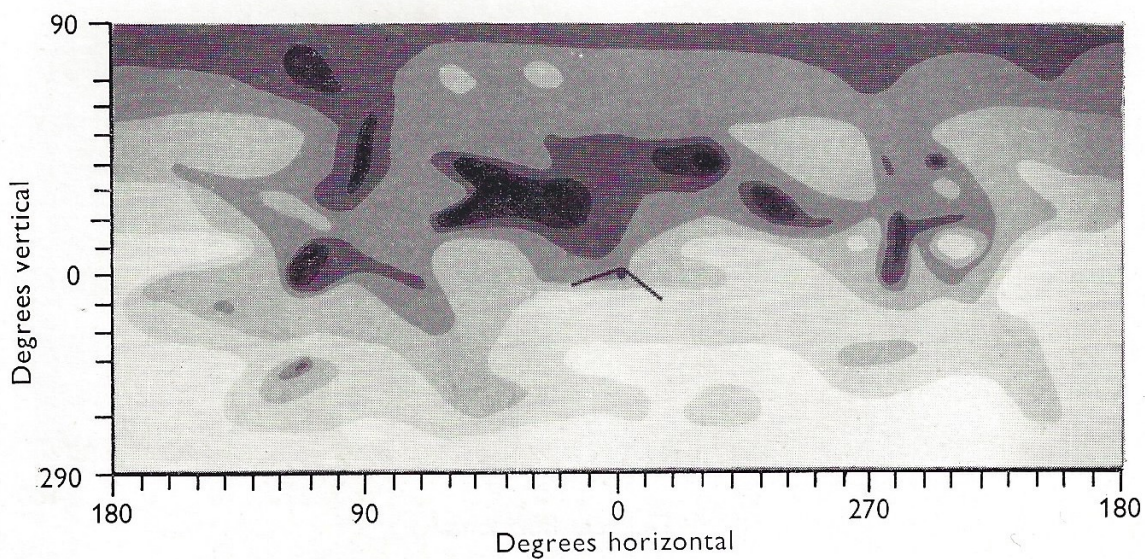
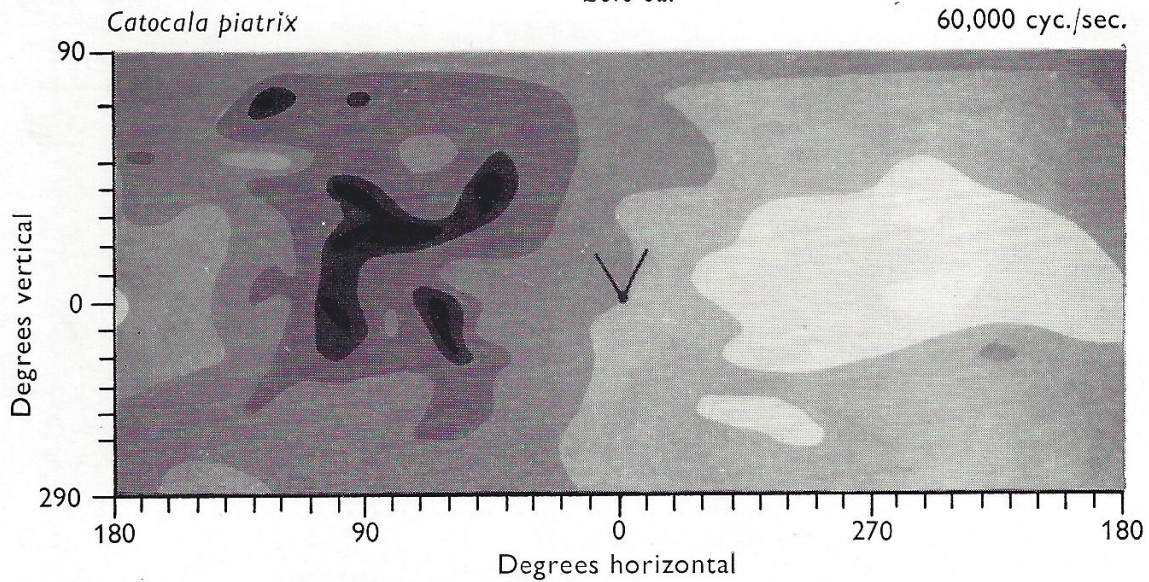


Right ear of the same individual shown in Plate 3.

PLATE 5

Left ear

60,000 cyc./sec.



Same explanation as in Plates 3 and 4 except that frequency in each pulse is 60,000 cyc./sec. and the moth is *Catocala piatrix*.

(Facing p. 265)

sensitivity where the sound has to be loud; clusters of low numbers, regions of better sensitivity where the sound can be softer. By surrounding all numbers of the same value with a line and then surrounding, by another line, all numbers 5 dB. higher (using subsequent lines for numbers 5, 10, 15, 20, etc. dB. higher) one could produce on the globe a pattern of concentric, irregularly shaped rings enclosing iso-intensity regions (i.e. regions within which the loudspeaker can be moved around at will without adjusting its intensity and without the tympanic organ reporting any intensity change).

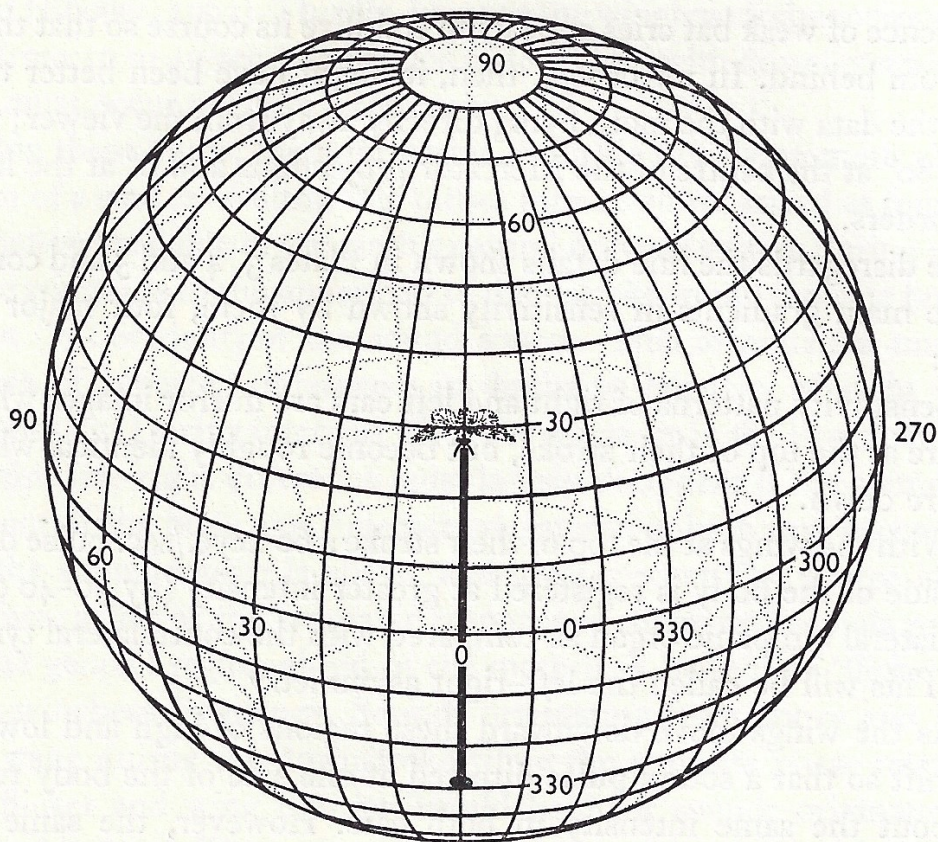


Fig. 3. A 'sphere' of sensitivity readings. A moth, facing the viewer at 0° vertical and 0° horizontal is visualized on a support at the centre of an imaginary sphere. A loudspeaker was placed at the intersection of all latitude and longitude lines 10° apart and sound intensity was adjusted until a constant response from the moth's acoustic nerve was obtained. With data at all points of intersection it was possible to reconstruct a 'sphere' of directional sensitivity readings. The moth's wing position was then changed and process repeated. (Courtesy of *Scientific American*.)

Iso-intensity regions are then tinted, each with a different shade of grey from white to black, the darker shades of grey indicating regions of poorer sensitivity. White regions thus indicate those angular directions in which the tympanic organ is most sensitive; and black, directions of least sensitivity. Intermediate shades indicate directions of intermediate sensitivity in 5 dB. steps.

Plates 3, 4 and 5 show such spheres in Mercator projection. A silhouette of the moth in the centre is shown headed towards the viewer at 0° horizontal

(its wing position for that sphere is shown also). Angular readings in the horizontal plane increase as the sound is moved clockwise round the subject (as viewed from above). Thus, a sound from directly ahead is at 0° vertical and 0° horizontal; from directly behind, 0° vertical and 180° horizontal. A sound coming from directly above the moth is at 90° vertical; one from directly below (this could not actually be measured because of the presence of the tower), at 270° vertical.

Our choice in having the moth flying towards the viewer was, from a behavioural viewpoint, an unfortunate one since a naturally flying moth in the presence of weak bat cries appears to stabilize its course so that the cries come from behind. In retrospect, then, it might have been better to have plotted the data with the moth flying directly away from the viewer; that is, to put 180° at the centre of the Mercator projections and 0° at the left and right borders.

If one disregards the fine details shown in Plates 3, 4 and 5 and considers only the main gradients in sensitivity shown by them, four major points emerge:

(1) Sensitivity patterns of right and left ears are mirror images when the wings are at the top of their stroke, but become roughly identical when the wings are down.

(2) With the wings at the top of their stroke a 60 kcyc./sec. pulse directed at one side of the body is registered at greater intensity (by 30–40 dB.) by the ipsilateral tympanic organ as compared with the contralateral tympanic organ. This will be called the left–right asymmetry.

(3) As the wings move downward these regions of high and low sensitivity shift so that a sound pulse directed at one side of the body registers with about the same intensity in both ears. However, the same sound originating from in front and below the body axis will be reported by the tympanic nerve at higher intensities than it will when it comes from in front and above. This will be called the dorsal–ventral asymmetry.

(4) Throughout a complete wing cycle sound pulses of constant intensity, originating from behind the moth, register on both tympanic organs with little or no fluctuation due to wing position. Sounds coming from any other direction relative to the body axis (except possibly from a narrow zone directly ahead and slightly below the equator) are modulated in intensity by the wing position as described above.

DISCUSSION

Before attempting to extrapolate from the results of these experiments to the 'live' situation—a free-flying moth and a moving source of ultrasonic

pulses—certain facts must be kept in mind. First, the general alternation of left–right with dorsal–ventral acoustic asymmetry seems likely to occur in natural flight even though our experiments (in which wings were treated as simple flaps) are undoubtedly simplifications of the complex wing shape variations observable in normal flight. Secondly, the natural path travelled by a free moth is marked by minor pitching, yawing, rolling and probably by up-and-down vibration of the body at wingbeat frequency. In addition, its course is marked by major turns and loops, although the course finally selected away from a distant source of ultrasonic pulses is often surprisingly straight (Roeder, 1962). Thirdly, because the wingbeat frequencies of moths range from 10 to 40/sec., alternation of left–right with dorsal–ventral asymmetry must occur at these frequencies.

Within these limits, the information available to a flying moth about the location of a source of ultrasonic pulses may be summarized as follows: (1) Information available in the moth's central nervous system during intervals that the wings are in the upper half of their stroke would indicate horizontal position (left or right) of the sound source. This information might take the form of repeated differences in discharge latencies of right and left *I* neurones. (2) During intervals when the wings are in the lower half of their stroke this left–right difference must largely disappear if the sound source is in front of the body axis, intensity registered at both ears becoming less when the sound source is above, and greater when the source is below. Therefore, as far as the tympanic response is concerned sounds presented from the general region ahead of the moth may be said to 'flicker' at the moth's wingbeat frequency. Sounds originating from above and in front would wane during the downstroke while the opposite phase relation between flicker and wing position would hold for sounds originating from below and in front. Thus, assuming that the moth has a signal indicating its own wing position, the direction of a sound source could be located to right or left as well as above or below the body axis by combining the wing position signal with the timing of the impulses generated by the right and left *I* neurones.

With this in mind it is possible to postulate means by which a moth could execute a turn carrying it away from the area in which a distant bat is hunting.

Horizontal localization

If a moth is flying broadside to a bat approaching from its left side and on the same horizontal plane as the moth, the bat's cries would register at higher intensity in the left than the right ear during those intervals when the wings were in the upper half of their stroke. This difference in intensity might be

expected to trigger a horizontal turn by operating through: (1) differences in the response time of the right and left A_1 receptors; (2) differences in their inter-spike intervals; and (3) the amplified response time difference of the I neurones. The information is at hand to make this a right turn, which would place the moth on a course away from the bat. If, however, the turn was random the same bearing relative to the bat could be reached and maintained if the moth's central nervous system contained the following instructions: If the turn produces a relative increase in the intensity reported by the ear on the side to which the turn was made, reverse the turn and stop turning only when both ears give equal intensity reports. If the random turn results in a relative decrease in the intensity report from the ear on the side to which the turn was made, continue turning past the point giving maximum binaural disparity until both ears give equal intensity reports.

It is apparent that the greatest binaural disparity in the tympanic nerve signals (least ambiguity) is achieved when a sound source originates from a source at right-angles to the moth's body axis. It is perhaps less apparent that a moth's theoretical accuracy in locating a bat is greatest when binaural disparity is least (bat approaching from directly ahead or behind). (This apparent paradox has been explained by Mills (1958) in discussing human hearing.) A specific example in each case (with imaginary values) may help to clarify this point. *Case 1*: the minimum requirement for steering a course that will put a sound source anywhere within the moth's sagittal plane is a decision from the central nervous system as to whether there is no delay or simply 'some' delay between first A_1 spikes from each ear. *Case 2*: The minimum requirement for steering a course at some angle to a sound source, say 90° , is a decision as to whether the delay is, say, 0.95 msec. or whether it is 0.05 msec. above or below that value. *Case 1* is a simpler task because it requires no ability to specify a time interval and is limited in accuracy only by how close to a zero delay the moth's central nervous system can judge. *Case 2*, however, involves judgement of an elapsed time interval: the moth needs a timer; since such timers are usually found to be accurate within some set percentage of the full interval, the smaller the interval being measured the greater the accuracy with which it can be measured.

Vertical localization

For sounds arriving from above or below, the appropriate corrective manoeuvre would be a pitch, putting the moth on a course downwards or upwards with respect to its previous flight direction. In order to make such a course correction the following program in the central nervous system might be employed: Pitch at random and if this manoeuvre brings intensities

registered by both ears closer to the same value throughout the wing cycle then continue pitching until there is no acoustic 'flicker' at wingbeat frequency. If, however, the pitch results in increased acoustic flicker, pitch in the opposite direction and continue until the flicker ceases. In point of fact, the initial pitch need not be at random: information is available to the moth to give the pitch the appropriate sign ('up' or 'down'). This is true because pulse intensities from a source above must wax and wane in phase with the top and bottom of the wing stroke, while for sounds originating below similar (though less marked) intensity changes would be 180° out of phase with these extremes in wing position.

Similar reasoning might be applied to rolls. However, rolls will not change a moth's flight direction except when executed as part of a turn, and thus do not concern us further.

If the moth is to be able to follow the sorts of program postulated above it must have a minimum of two types of information. These are: (1) ultrasonic pulse intensity differences encoded in the afferent trains from the right and left *A1* receptors; (2) some marker indicating its own wing position at least once per full wing cycle. The first of these has been discussed at length in the earlier part of this article and in a previous paper (Roeder, 1964). Relatively small differences in the intensity of ultrasonic pulses when not much above the tympanic threshold cause significant changes in the response latency of the *A1* receptor cells, and in the inter-spike interval for the first two or three *A1* nerve spikes. These differences seem to be further amplified by the behaviour of the second-order *I* neurones, which respond to low-intensity ultrasonic pulses with long and variable delays after the arrival of the first *A1* impulses, thus greatly expanding any difference in latency or inter-spike interval of the afferent discharges from the two ears. Since variations in latency are greatest per decibel for low-intensity sounds, they are thus ideally suited for providing directional information about a distant bat. This is supported by the behavioural observation (Roeder, 1964) that 'turning-away' in free-flying moths is reduced or absent at high sound intensities or when a continuous tone is substituted for a pulse sequence. With continuous tones, spike generation time can only be appreciated by the moth's central nervous system for the first spike in the acoustic response train.

The second type of information, that regarding wing position, could come from several different sources. Motor control of the indirect flight muscles in Lepidoptera is of the synchronous type (Roeder, 1951), each efferent volley producing a contraction. The motor neurone discharge could serve as a precisely timed internal marker of wing position. In addition to efferent markers there are a variety of proprioceptors on the wings and thorax that

could report each wing cycle. One of these, the *B* cell, is of particular interest because of its association with the tympanic organ. The *B* cell is a single large proprioceptor whose cell body lies close to the point where the *A* axons leave the scoloparium. Its axon accompanies the *A* axons in the tympanic nerve. It responds without adapting to gradual mechanical deformations of the thorax when these are comparable in magnitude to those occurring during natural flight (Treat & Roeder, 1959). On two occasions during the present study the *B* cell was made to discharge once per wing stroke when the subject's wings were set in motion by bringing them into contact with the wings of a second, intact moth, held next to it in stationary flight.

The foregoing analysis is based on the assumption that moths fly a straight course and make gradual smooth turns. Field observations suggest that this is commonly far from being the case. The cruising flight of most moths is characterized by many erratic changes of direction superimposed on continuous minor yawing, pitching, and rolling. These continuous 'hunting' motions suggest that we may be trying to read too much from the directional sensitivity patterns, and that we have been led to postulate an unnecessarily complex system of directional control. The significant point may be that the only large region of the directional plots that does not change markedly in sensitivity during a wing cycle is the area behind the moth. On this basis, the chief role of the other regions of the sensitivity plots may be to act as an alerting mechanism. Thus, a command by the moth's central nervous system that would carry it away from the hunting ground of a distant bat, regardless of bearing, might be as follows: When the intensity reports of the two ears flicker in synchrony with the wingbeat, initiate a series of random turns until the flickering stops, and continue flying in that direction. Such a command would have the added advantage that when a moth was flying in the general direction of a distant bat the flickering would be most pronounced and thus probably serve as a forceful stimulus. Such a system would also have the advantage of great simplicity—one of the prime attributes of any integrating system that is required to produce that unity of action so important in encounters between predator and prey.

An additional complication has to be considered in attempting to reconstruct the natural acoustic interaction between moths and bats. Recent measurements of the pulses of North American brown bats (probably *Eptesicus fuscus* and *Myotis lucifugus*), tape-recorded as they cruised in the open at dusk, gave pulse durations of 10–20 msec. and repetition rates of 3–8/sec. These measurements excluded the rapid sequence of brief pulses made during the 'buzz', and were limited to the high-intensity search signals made by bats flying at some distance from sources of echoes. They

are the pulses most likely to be encountered by a still distant moth. The wingbeat frequency of moth species commonly ranges from 10 to 40/sec. Assuming a wingbeat frequency of about 30/sec., one of these cruising cries would impinge on a moth for the duration of about half a wingbeat, or about 15 msec. During this interval the wings of the moth could travel from the top to the bottom of their stroke, or from mid-point on the upstroke to mid-point on the downstroke. This might be sufficient to indicate to the moth whether a single cry was modulated by the position of its wings, although it is difficult to say whether the simplicity of the acoustic mechanism outlined above would permit the encoding of this amount of information from a single pulse.

The duty cycle ('on-off' ratio) of the cries described above is roughly 10%. This has been shown to be approximately optimal in causing moths to show evasive manœuvres in response to artificial ultrasonic pulses (Roeder, 1964). However, intervals of up to 300 msec. between the arrival of consecutive pulses must deprive the moth for an appreciable time interval of information enabling it to make the next course correction. Hence, much hunting and overshooting might be expected from a moth while turning away from a distant bat, and the second method of steering suggested above is made more probable.

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