FUNCTIONAL ORGANIZATION OF TWO
TYMPANIC NEURONS IN
NOCTUID MOTHS

Nobuo SUGA*

Department of Physiology, Tokyo Medical and Dental University,
Yushima, Bunkyo-ku, Tokyo

Since SCHALLER and TIMM (1950) suggested ultrasonic reception in the
tympanic organ of noctuid moths, it has been conceived by behavioral studies
that ultrasonic reception may be convenient for the insects to escape from approach
of their predators, bats (WEBB, 1953; TREAT, 1955). ROEDER and TREAT
(1957) further confirmed the ultrasonic reception in this organ by recording
impulses in responses to ultrasonic waves from the tympanic nerve. The tympanic
nerve of a moth consists of only two tympanic neurons and one more neuron
named B by TREAT & ROEDER (1959). The cell bodies of the former are found
immediately inside the tympanic membrane and the latter in the exoskeleton called
Bügel (Eggers, 1919) close to the tympanic membrane. When the tympanic
nerve is hung up in the air by a fine silver wire electrode, impulse discharges
of those three fibres can be observed simultaneously. Both the tympanic neurons
respond to sound, while B neuron does not, with regular discharges. The proper-
ties of the tympanic neurons have been explored pretty well by ROEDER and TREAT
(1957), but still some problem remains there to be studied more. The present
paper will further clarify the difference in the response pattern between two tympanic
neurons.

MATERIAL AND METHOD

Adris tyranus, Lagoptera juno, and Oraesia excavata (Noctuidae) caught at vine-
yards were used as the materials. The moth was pinned on a cork board ventral side
down in a sound-proofed room which was air-conditioned at 25°C. Scales were wiped
away with wet cotton and the ventral exoskeleton of the thorax was removed. Muscles
were cut away so as to see the pterothoracic ganglion and peripheral nerves. The
tympanic nerve was hung up in the air by a silver wire electrode of 100 μ in diameter.
The central part of the hung nerve was cut off in order to record only impulse
discharges of the tympanic neurons and B neuron. An indifferent electrode was put
on the wet cotton in the cut abdomen. The physiological saline solution for insects
was sometimes dropped down on the tympanic nerve, protecting from drying. As

Received for publication July 10, 1961

*署 乃武男
stimulus sounds, tone bursts were used in order to exclude click sounds at the on- and offsets of stimulation. They were delivered through a tweeter and a condenser speaker. If necessary, another speaker delivered a continuous pure tone. The reference level (zero dB.) in this paper corresponded to a sound intensity of approximately 100 dB. above the lowest average human threshold at 2-3 kc./sec., that is, 20 μbar. The recording and stimulating equipments used in the present work were the same as those described in the previous paper (KATSUKI & SUGA, 1960).

RESULTS

I. Difference between the informations sent by two tympanic neurons.

When the tympanic nerve was hung up in the air with a recording electrode, very regular impulse discharges were always observed. Their spike heights ranged between 1 and 2 mV. The rate of impulse discharges increased reversibly by loading on the thorax. Thus it was confirmed that those impulses represented the activity of B neuron. By delivering a sound, impulse discharges of other two neurons were always evoked. Their spike heights were always smaller than that of B neuron, ranging between 0.5 and 1 mV. Both the tympanic neurons discharged at so high rates at the onset of a strong sound stimulus that each impulse of two neurons was hardly discernible. After a while, however, the rate of impulse discharges decreased and each impulse became clearly recognizable one by one. Those two tympanic neurons were distinguished respectively by different spike height, adaptation, and also thresholds for sound stimuli.

In Adris tyranus and Lagoptera juno, the neuron firing with large spikes always adapted slowly to a continuous pure tone while the neuron firing with small ones adapted rapidly. The processes of the adaptations of both the tympanic

![Fig. 1. Adaptation of the tympanic neuron (Adris tyranus).](image-url)

The abscissa and ordinate represent the time after the onset of a stimulus sound (17 kc./sec., -10dB) and the number of impulses per second respectively. 1 and s are the symbols of large and small spikes (See text).
neurons of *Adris tyranus* were shown in Fig. 1. The abscissa represents the time after the onset of −10 dB sound of 17 kc./sec. and the ordinate the number of impulses per second calculated from the number of impulses for 50 msec. The neuron with large spikes discharged with the rate of 310 impulses per sec. at the onset of the sound stimulation. The rate of discharges decreased gradually after that and became constant, 140 in 6 seconds after the onset. On the other hand, the neuron with small spikes discharged at the rate of 250 impulses per sec. at first, but, thereafter the adaptation of this neuron was so rapid that the discharge rate became only 6 in 6 sec. after the onset. The discharge of the former, the slowly adapting neuron lasted long, even if the intensity of a stimulus sound was weak, while that of the latter, the fast one did not, even if the intensity was sufficiently strong. In *Oraesia excavata*, the neuron firing with small spikes always adapted slowly as compared with that firing with large spikes. The discharge curves of *O. excavata* were similar to those of *A. tyranus*.

The weakest intensity of a sound giving rise to impulses on the tympanic neurons, the threshold of the tympanic neuron was measured for various frequencies of tone bursts with the duration of 60 msec. The response areas of two tympanic neurons of *Adris tyranus* were shown in Fig. 2. The abscissa and ordinate represent the frequency and intensity of a stimulus sound. Each curve represents the average of the response areas measured in four specimens. In three species of the noctuid moths, the slowly adapting neuron always had the lower threshold than that of the fast adapting one. The sounds between 10 and 20 kc./sec. activated most effectively the tympanic organ. The frequency range of 0 dB sound by which the neuron with a low threshold was brought into the excitation

![Fig. 2. Response area of the tympanic neuron (*Adris tyranus*). The abscissa and ordinate represent the frequency of the stimulus sound and its intensity. Curves with 1 and s show the response areas of the neurons discharging large and small spikes respectively.](image)
was from 1 to 85 kc./sec. in *Adris tyranus* and *Lagoptera juno* and from 1 to 45 kc./sec. in *Oraesia excavata*. The most effective frequency to the neuron with a high threshold was the same as those to the neuron with a low threshold and the response frequency ranges for 0 dB sounds were from 3 or 4 to 30 kc./sec. in the former two species and from 3 to 25 kc./sec. in the latter. The response areas of the neurons with a high threshold were relatively narrow in comparison with those of the neurons with a low threshold. The difference in the threshold between both the tympanic neurons ranged between 20 and 30 dB for any frequency of sound.

On each neuron, the fast and slowly adapting neurons, the relation between the number of impulses per second and the intensity of a stimulus sound in decibel was explored for different sound frequencies. In Fig. 3, the abscissa and ordinate represent the sound intensity and the number of impulses per second which were calculated from the responses to the tone bursts with the duration of 58 milliseconds. When a stimulus sound became strong or came near to the most effective frequency, impulse discharges were enhanced. The rate of increase of the number of impulses was almost the same without any reference to the difference in the frequency of a stimulus sound. If the frequency of a stimulus

![Fig. 3. Change of the number of impulses with the change of the sound intensity (*Lagoptera juno*).](image)

The abscissa and ordinate represent the intensity of the stimulus sound and the number of impulses per second. The tone bursts used have the duration of 58 msec. and their frequencies are 6, 17, and 45 kc./sec. as shown at the right side of each curve. The open circle shows the change of the number of impulses of the slowly adapting neuron with a low threshold and the closed circle that of the fast adapting neuron with a high threshold. The fast adapting neuron does not respond to the sound of 45 kc./sec.
sound was toward the most effective one on the tympanic neurons, the number of impulses of the neuron with low threshold reached to a maximum value by a strong stimulus and decreased contrarily by a stronger one as shown in the figure (17 kc./sec.). The neuron with a high threshold changed the discharge rate in the same way as that with a low threshold did, but the rate did not decrease even by the strongest sound with the most effective frequency. Besides this, there was no difference in the change of discharge rate between both neurons.

II. Response to two sound stimuli.

Between the responses of two tympanic neurons to a tone burst or a continuous pure tone the difference was only found in the threshold and in the adaptation. However, when a tone burst and a continuous pure tone were delivered at the same time, the reappearance of the response to the tone burst which was masked by the response to a continuous tone was found only on the slowly adapting neuron which had the lower threshold than the other. There were two types in the manner of the reappearance of the response. Those types of the responses were brought about by the two sound stimuli with a certain different relation in frequency and intensity. The response to a tone burst was masked at first by that to a simultaneously delivered continuous pure tone, that is, no difference in the rate of impulse discharges was found between the responses to the continuous pure tone and that to both the tone burst and the continuous pure tone. However, one type of the reappearance of the response to the tone burst showed that in several seconds after the onset of the continuous pure tone, the response to the tone burst became clearly recognizable as the suppression of impulse discharges for the continuous pure tone. The other type showed that the response to the tone burst reappeared with the higher rate of impulse discharges than that to the continuous pure tone. The former and the latter type were shown in Figs. 4 and 5 respectively.

Each photograph in Fig. 4 was picked out from a serial record. The bottom line is a scale representing one second. The largest regular impulses originate from B neuron. The responses of two tympanic neurons were found to a 0 dB tone burst of 17 kc./sec. with a duration of 58 msec. as shown on the left side in A. The tone burst was regularly delivered in a certain constant interval. Such responses never changed even if the same stimulus was repeated so often. When a continuous pure tone of 14 kc./sec. and of −28 dB was delivered as a background one for the tone burst, the response to the latter was masked by that to the former (A). In A, a number of the smallest impulses are found, but the neuron discharging those impulses adapted so rapidly that only a few impulses were found in the subsequent photographs (B-F). In a dozen of seconds after the onset of the continuous pure tone, the response to the tone burst began to appear as the decrease in the rate of impulse discharges to the background tone. Thereafter the response to the tone burst was observed as a perfect pause in a
Fig. 4. Hysteretic response in the inhibitory manner (Adris tyranus).

The frequency of the tone burst with the duration of 58 msec. is 17 kc./sec. and its intensity is 0dB (the middle beam). The intensity of the continuous pure tone of 14 kc./sec. is –28 dB (the bottom beam). Impulse discharges of the tympanic and B neurons are shown on the top beam. The bottom line shows one second. (See text).

train of impulses (B). In other words, the response to the continuous pure tone were temporarily inhibited by the tone burst.

Here it will be expected that a train of impulses to the continuous pure tone is perfectly vanished by the tone burst with such a long duration as the former. The short tone burst was suddenly changed to the long lasting tone burst, a continuous pure tone. Impulse discharges was, however, inhibited only at the on-set. No change was found any more (C). The effect seemed to be the same as that of the tone burst.

However, after the discontinuity of the long tone burst, the silent period of discharges lasted for one hundred milliseconds, that is, the postexcitatory inhibition was observed (D). The rate of impulse discharges gradually increased after that. The tone burst produced the increase of impulses in response to the continuous pure tone at first (E) and then the response to the tone burst was masked by the impulse discharges in response to the continuous pure tone. After a while (in about twenty seconds after the cessation), the silent period of discharges evoked by the tone burst became again observable in a train of impulses.
to the continuous pure tone (F).

Such a curious phenomenon was neither found on the response of the fast adapting neuron with a high threshold nor related to discharges of this neuron. B neuron discharged very regularly during the two sound stimuli.

The other type of the reappearance of the response is shown in Fig. 5. In this case many spontaneous discharges of the slowly adapting neuron with a low threshold were found while those of the other tympanic neuron with a high threshold were very poor, because the adaptation of the latter was so fast. Each photograph was selected from the continuous record of the responses to two sound stimuli at the interval between 1 and 3 seconds. The bottom line represents one second. The tone burst used was 17 kc./sec. and 0 dB. A shows the responses to the tone burst. There the largest regular spikes represent the activity of B neuron. Delivery of the continuous pure tone of 15 kc./sec. and of −7 dB made the number of discharges increase and masked the response to the tone burst (B).

FIG. 5. Hysteretic response in the augmentative manner (Oraesia excavata).

The frequency of the tone burst is 17 kc./sec. and its intensity is 0 dB (the middle beam). The intensity of the continuous pure tone of 15 kc./sec. is −7 dB (the bottom beam). The top beam shows impulse discharges of the tympanic and B neurons. The bottom line shows one second. (See text).
However, the response to the tone burst recovered gradually thereafter (C, D) and returned to the original one in about 10 seconds after the onset of the continuous pure tone (E). By weakening the continuous pure tone, the recovered response became faint, but the original response to the tone burst was found immediately after turning off the continuous pure tone (F). No small impulse was found during the two sound stimuli and the rate of discharges of B neuron also did not change.

The phenomenon that the response to a tone burst masked by that to a continuous pure tone reappeared gradually by suppressing or contrarily augmenting impulse discharges to the latter was not confined to the narrow frequency and intensity relation between such two sounds as described above, but it was also found at fairly different frequency and intensity relation. The tone burst was kept constant at the frequency of 10 kc./sec. and the intensity of 0 dB. The intensity of a continuous pure tone of 10 kc./sec. was changed. When the latter intensity was about -5 dB, the response to the former reappeared in the inhibitory manner at first, but, after a while its pattern changed to the augmentation of impulse discharges. With the weaker intensity (-10 dB), the response reappeared only in the inhibitory manner.

DISCUSSION

Two problems will be discussed here. One of them is about the difference between the informations sent by two tympanic neurons and the other is about the response to two sound stimuli, the hysteretic response.

(I) Difference between the informations sent by two tympanic neurons.

Since the number of impulses sent by a tympanic neuron can be obtained as a function of both the intensity and frequency of a stimulus sound and no characterized pattern of impulse discharges to the frequency of a stimulus sound has been found, a tympanic neuron seems to send the central nervous system no information about the difference among sound frequencies, but rather send something about the degree that the tympanic organ is activated, that is, their intensities. Noctuid moths have, as described above, two tympanic neurons at their tympanic organs. If those two neurons had the different most sensitive frequencies and their impulses elicited were sent to the brain or to some reflex arcs through different neural pathways, the frequency analysis would be possible. However, by the present studies it became clear that two tympanic neurons in three species studied have the same most sensitive frequency in reference to their response areas and showed only the difference in threshold. It may be, therefore, concluded that two neurons can not send the different information about the sound frequency, because the sound frequency can not be uniquely determined only by the number of impulses evoked on the tympanic nerve fibres. In the
previous reports, the present authors have described that even insects which have many tympanic nerve fibres can not discriminate the sound frequency (KATSUKI & SUGA, 1960; SUGA, 1960 and SUGA & KATSUKI, 1961). It is well comprehensible that noctuid moths which are conceived to develop less may therefore not distinguish the sound frequencies.

In two neurons, the number of impulses changed with the change of sound intensity in the same proportion. When the tympanic organ was stimulated weakly, the neuron with a low threshold could well signal the change of sound intensity, and in case of strong sound the other neuron with a high threshold discharged and supplemented the information. Two neurons thus seems to work cooperatively.

On the other hand the large difference in adaptation was further found between two neurons. The neuron with a high threshold adapted more rapidly than the other with a low threshold. Therefore the tympanic organ of a noctuid moth can supply the central nervous system the tonic and phasic informations about the incident sound through two tympanic neurons. Such two types of primary sensory neurons are also found in crayfish stretch receptors (Wiersma, FURSHPAN and Florey, 1953) and it has been said that the slow cell with a low threshold sends a tonic information to the central nervous system as long as stretching lasts, while the fast cell with a high threshold sends a phasic information and can well signal a change in stretching (EYZAGUIRRE & KUFFLER, 1955). Those facts show the validity of the hypothesis that the function of the tympanic sensory cells is originally a proprioceptive one registering the displacement of one part of the exoskeleton with respect to another (HERJWECK, 1931).

Even in the mechanoreceptors of higher animals where the organ is composed of many neurons, two types of the neurons with slow and fast adaptation have been known, e.g. in the lateral-line nerve of an eel (KATSUKI, YOSHINO and CHEN, 1951) and in the stretch receptors of the lung of a rabbit (KNOWLTON and LARRABEE, 1946). When those slow and fast types of the neurons connected with mechanoreceptors as described above are found in the skin, they participate in pressure and touch respectively. Therefore the slow and fast types in the adaptation of neurons may be the fundamental ones in the mechanoreceptors, though each of them has a different function, e.g. proprio- or extero- or teleceptive one. The two different neurons in the tympanic organ of noctuid moths or in the stretch receptor of Crustacea may be the prototype of the highly developed sense organ in the higher animals, the ear or the retina. There are also two different kinds of receptor cells, the inner and outer hair cells or the cone and rod. It is thought that those cells have the recognitory and discriminatory functions respectively. The dual neural system is certainly generalized from the lower animals up to the higher one.
(II) *Hysteretic responses to two sound stimuli.*

The reappearance of the response to a tone burst in the inhibitory or augmentative manner was always observed only on the slowly adapting neuron with a low threshold. A certain physiological state for the reappearance of the response in the inhibitory or augmentative manner may be produced on the neuron during adaptational process to a continuous stimulation and the inhibition or augmentation of impulse discharges by a tone burst stimulation may be evoked by delivering suddenly the tone burst in a little while after the onset of the continuous pure tone. However, the long lasting inhibitory response can not be caused. The inhibitory response is evoked only by the simultaneous stimulation with a continuous pure tone and a tone burst. The peculiarity of the inhibitory response suggests that such a phenomenon may be evoked by the interaction between the slow and fast tympanic neurons. However, the fast neuron with a high threshold adapts to a continuous pure tone stimulation so fast that the response to a tone burst delivered together with a continuous pure tone has not been found. As far as impulses are recorded from the tympanic nerve apart from the endorgan, the fast neuron has no reference to the discharge of the slow one. If there is an interaction between two neurons, it should occur at a more peripheral region than that where the impulses are initiated. The existence of the lateral branch in the endorgan might make the reappearance of the response in the inhibitory or excitatory manner understandable.

However, light- and electron-microscopic studies on the tympanic organ reveal that the sensory cell has peripheralward a cilium and centralward an axon connecting with the thoracic ganglion (Egggers, 1919 and Gray, 1960). There has been no discription about a lateral branch so far. Furthermore, there is no possibility that the sensory cell is controlled by descending impulses from the pterothoracic ganglion, because such a phenomenon was observed even after the proximal part of the tympanic nerve had been separated from the ganglion.

Impulses elicited on the stretch receptor by stretching are blocked by over-stretch (Wiersma, Furshpan & Florey, 1953 and Eyzaguirre & Kuffler, 1955). So it is thought that the inhibitory response might be evoked by over stimulation with two sounds. But, that may not be the case, because the inhibitory response was evoked when the continuous pure tone was weak rather than strong and only by two sounds, a tone burst and a continuous pure tone, stimuli. Conclusive answer to this phenomenon will be given by intracellular recording of the potential change from the sensory cells themselves and by clarifying the characteristics of the membranes where the receptor potential is evoked and where a nerve impulse initiates.

At any rate, the hysteretic response in which the response to a tone burst gradually reappears by inhibiting or augmenting the response to a continuous tone seems to show that the tympanic organ can send the information about
pulsatory sounds to the central nervous system without undergoing masking effect by a background noise. This is indeed an interesting phenomenon not only from the electrophysiological, but also from the ecological point of view. Almost all sounds produced by insects consist of pulsatory sounds. As pointed out in the previous paper (KATSUKI and SUGA, 1960), the tympanic organ is made favorably to receive the sound produced by the insect. The tympanic nerve of a moth sends impulses responding to the wing beat noise produced by other moths flying around. It has so far been unknown whether or not moths produce sounds in some special manner. However it is reasonably supposed that moths produce sounds which involve dominantly the most effective frequency to the tympanic organ.

SUMMARY
The information sent from the tympanic neuron of noctuid moths, *Adris tyrarus*, *Lagoptera junco*, and *Oraesia excavata*, were studied electrophysiologically.
1. The tympanic organ of noctuid moths can send the phasic and tonic information through two tympanic neurons, fast and slowly adapting neurons. The threshold of the former is higher by 20~30 dB than that of the latter.
2. The slowly adapting neuron responds to the sound of 1~85 kc./sec. in *A. tyrarus* and *L. junco* and of 1~45 kc./sec. in *O. excavata*. The fast adapting neuron responds to the sound of 3 or 4~30 kc./sec. in the former two species and of 3~25 kc./sec. in the latter. The most effective frequencies to those neurons are between 10 and 20 kc./sec.
3. The response area of the fast adapting neuron is relatively smaller than that of the slowly adapting one and the most effective frequency is the same in both neurons. The discharge patterns of those neurons bear no reference to the frequency of a sound. Therefore the tympanic neurons can not send the information about the frequency.
4. The number of impulses in two neurons changes with the change of sound intensity at the same proportion. With weak sound the slowly adapting neuron can well signal the change of sound intensity. In case of strong sound, the fast adapting neuron discharges too and supplements the information about the sound intensity.
5. The response of the slowly adapting neuron to a short tone burst regularly delivered is first masked by that to a continuous pure tone, but it becomes recognizable in two hysteretic manners, i.e. in one of them, the response to the tone burst becomes clear gradually by suppressing a train of impulses in the response to the continuous pure tone. In the other case, the response appears by augmenting impulse discharges in the response to the continuous pure tone. Such hysteretic responses are evoked on one and the same neuron by the different relation in intensity and frequency between two sounds.
6. The hysteretic responses are only observed when a tone burst with a short duration is delivered with a continuous pure tone. The mechanism is now unknown, but the hysteretic responses seems to show that the tympanic organ is convenient for detecting pulsatory sounds in a background noise.

7. Two neurons in the tympanic organ are very similar to the fast and slow cells in a crayfish stretch receptor. Those organs with fast and slowly adapting neurons may be a prototype of mechanoreceptors.

The author wishes to express his thanks to Prof. Y. Katsuki for his guidance and correcting the manuscript and to Mr. K. Ogura and Miss H. Miyazaki for their continual assistance. This work was supported by grants from the Rockefeller Foundation (DA. MS. 59115) and also from the Ministry of Education of Japan.

REFERENCES


