STUDIES ON THE ORIGIN AND THE PATTERN OF THE MINIATURE ELECTRICAL OSCILLATION IN THE INSECT MUSCLE

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In the cross-striated muscle of the vertebrate, the spontaneous activity is observed only in special experimental conditions, though it is usually observed in the iterative system such as the smooth muscle. On the other hand it was recently found by Fatt and Katz (3) that miniature spontaneous potentials were generated at the motor end plate.

It has been noticed by Wakabayashi that the tymbal muscle of the cicada shows the miniature electrical oscillation which occurs while it is not active (Wakabayashi and Hagiwara (13)). Recently from the results of investigations of many species of insect muscles, Wakabayashi and Ikeda reported that the occurrence of the oscillation had systematic relation to the stage of phylogenetic development of the insect (14). Similar phenomena are found in the reports by Pringle (7) and Roeder (11) of the observation on the indirect flight muscle of the insect, but without special remarking. In this report the further investigation on the origin of this oscillation and the transition of the pattern of the oscillation is presented.

MATERIALS AND METHODS

Experiments were performed in most cases with the tymbal muscle of Graptopsaltria nigrofuscata. The other species: Terpnosia vacua, Platyleura kaempferi, Cryptolympana japonensis, Tanna japonensis, Oncotympana maculatocollis and Meimna opalifera were also employed with similar results as in the case of G. nigrofuscata.

The morphological aspect of tymbal muscle is outlined as follows: A pair of main sound producing apparatus of a male cicada is stretched in its first abdominal segment as shown in fig. 1. It arises proximally from a Chitinous-V (8) and attached distally to a tymbal with a circular disc and an apodeme in both sides (fig. 1). It is innervated with the nerve No. 9 (tymbal nerve) from mesothoracic ganglion and consists of parallel bundles which contain 5–10 muscle fibres closely surrounded by tracheae (fig. 2 and fig. 3).

In the following experiments, miniature electrical oscillation was led off by means of a pair of fine silver wire electrodes (100 μ in diameter) inserted in a tymbal muscle. Potentials were amplified by a resistance-condensor coupled

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FIG. 1. Tymbal muscle and its accessories.—*Graptopsaltria nigrofuscata.*
Left half: Posterior view. Right half: Anterior view.
1: Tymbal muscle. 2: Chitinous V. 3: Apodeme. 4: Tymbal.
5: Tymbal cover. 6: Operculum. 7: Tensor muscle. 8: Tympanum.
9: Resonant chamber.

FIG. 2. Transverse section of tymbal muscle.—*Graptopsaltria nigrofuscata.*
Though intercellular space is filled with tracheae, they are unnoticed in this section
as tracheae and muscle fibres are mostly cross at vicinity of rect angles each other.
Stained with Haematoxylin-Eosin. ×600. Scale: 20 μ.

FIG. 3. Longitudinal section of tymbal muscle.—*Graptopsaltria nigrofuscata.*
Tracheae reveal their cross sections between two muscle fibres. It can be found
that muscle fibres are surrounded closely with tracheae.
Stained with Haematoxylin-Eosin. ×900. Scale: 20 μ.
amplifier and recorded by an electromagnetic oscillograph and a cathode-ray oscilloscope-camera.

Methods for each experiments were as follows:

A. Tymbal muscle in situ. For the purpose of recording the electrical activity of a tymbal muscle in situ, a small pore was made through the exoskeleton of 2nd, 3rd and 4th abdominal segments in order to insert recording wire electrodes into the tymbal muscle (fig. 4-A).

B. Tymbal muscle preparation with ganglion. For the recording of the electrical activity of tymbal muscle which was innervated by the intact mesothoracic ganglion, abdominal segments caudal from the 2nd one were removed. A pair of tymbal muscles is exposed.

C. Tymbal muscle preparation without ganglion. For recording with a tymbal muscle freed from ganglion, the whole part of body except 1st abdominal segment was removed (fig. 4-C). By this procedure, both tymbals and tymbal muscles were intact, the latter was used as well as in the cases of A and B.

D. Tymbal muscle preparation with destroyed abdominal segment. One half of the preparation of 1st abdominal segment described in fig. 4-C was removed.
as fig. 4-D by vertical cut along its median line leaving another half which still had an apparatus for sound producing. By this procedure, the tension of muscle which depended upon the elasticity of exoskeleton was heavily diminished.

**E. Excised tymbal muscle preparation.** A whole muscle and strips of a muscle whose fibres were reduced to 1/2, 1/4, 1/10 and 1/20 of a whole muscle were used for the experiment.

After the experiment, disappearance of oscillation by applying alcohol to the muscle was observed to make sure that the electrical oscillation was not an artefact.

Experiments were performed in room temperature: 25°-30° C., without applying any saline solution because the muscle, being surrounded closely by well-developed net-work of trachea, could maintain in the air its physiological condition for a long time.

**RESULTS**

The miniature electrical oscillation of tymbal muscle showed usually irregular discharges but sometimes it became regular and showed beat-like waves. In the following paragraph, pictures of irregular electrical oscillations which were generally obtained will be described in Part A, and pictures of regular and beat-like oscillation in Part B.

**Part A: Irregular electrical oscillations**

**A. Tymbal muscle in situ.** The preparation A shown in fig. 4-A might be regarded as not so different from natural state as for sound production, because it sang sometimes spontaneously without any artificial stimulation while recording electrodes were inserted in the muscle. Besides the action potential and the miniature electrical oscillation were recorded at the same time in such a case as shown in fig. 5 (high amplification). At the beginning of the figure normal action potentials in singing went beyond the margin of the photograph and irregular miniature oscillations followed them. The latter was maintained for 40-60 minutes, showing a slight fluctuation of which the pattern displayed some temporal transition. Its amplitude was 800 µV at most while the amplitude of action potential was about 5 mV.

**B. Tymbal muscle preparation with ganglion.** In the experiment with the Preparation B (fig. 4-B), the pattern of miniature electrical oscillation was identical to that of the Preparation A (fig. 4-A) as shown in fig. 6-A.

**C. Tymbal muscle preparation without ganglion.** As shown in fig. 6-B, in a pair of tymbal muscles set free from ganglion (fig. 4-C), no obvious difference was also observed in the general pattern of miniature electrical oscillation as compared with that of the preparation innervated by nerve (Preparation A and B).

**D. Tymbal muscle preparation with destroyed abdominal segment.** In the experiment with the Preparation D (fig. 4-D), after a vertical section of a
Fig. 5. Action potentials in singing and miniature electrical oscillation obtained in Expt. A with the preparation of fig. 4-A. — *Graptoptersia nigrofuscata.* Initial saturated potentials are action potentials in singing. Irregular oscillation following to them is miniature electrical oscillation. Calibration: 500 μV. Time-scale: 50 c/s. Temp.: 26°C. August 30, 1954.

Fig. 6. Miniature electrical oscillations obtained in Expt. B, C and D. — *Graptoptersia nigrofuscata.*
A: Expt. B, with innervated tymbal muscle (Preparation: fig. 4-B).
B: Expt. C, with tymbal muscle freed from mesothoracic ganglion (Preparation: fig. 4-C).
C: Expt. D, with a preparation of fig. 4-D.
Fig. 7. Miniature electrical oscillations obtained in Expt. E.—Graptopsaltria nigrofusca.

E: 1/20 of a muscle. F: Vanishing of oscillation owing to the applying of alcohol.
sound producing apparatus to each half, the miniature electrical oscillation showed small amplitude and low frequency (fig. 6-C).

**E. Excised tymbal muscle preparation.** The results of experiments with a whole muscle or a strip preparation were shown in fig. 7. Fig. 7-A indicates the miniature electrical oscillation of a whole muscle, and B, C, D and E that of strips of reduced fibres to 1/2, 1/4, 1/10 and 1/20 respectively. The disappearance of potentials after applying alcohol to the muscle is shown in F. Any obvious difference of discharge pattern was not recognized among A, B and C. In D, the pattern is somewhat changed, in E (1/20 of a whole muscle) the oscillation became quite regular but small and seemed to vanish periodically.

**Part B: Waxing and waning with beat-like wave**

Though the electrical oscillation of tymbal muscle was generally irregular, its pattern shifted as time went on. A few examples will be shown. In fig. 8, the preparation of fig. 4-C was employed and miniature electrical oscillations (A, B and C) were recorded every other minute. Namely, irregular discharges (A) changed the form gradually by increase in amplitude and took the form of beat (B) for 40-50 seconds and then became irregular (C) again, i.e. waxed and waned. Sometimes discharges showed signs of cessation to diminish ampli-

![Graph](image-url)

**FIG. 8.** Transition of miniature electrical oscillation.—*Graptopsaltria nigrofuscata.* Irregular discharges (A) took form of beating (B) and then became irregularly again (C). Sometimes discharges were diminished as (D). A, B, and C were recorded on every other minute.

Fig. 9. Successive recording of the transition of beating oscillation. — *Graptopsis trigonata nigrofuscata.*

Beating oscillation became regular discharges and then returned to beating again.

tude as fig. 8-D, but the transition used to continue for an hour or so. Another example of successive recording of this transition is shown in fig. 9. Beating discharge changed its pattern to the simple regular one and then returned to beat again.

Though records of fig. 8 and fig. 9 were obtained in different individuals, the frequency of potentials were about 160 c/s. at waning phase, and about 80 c/s. at waxing phase and simple oscillatory phase. It was difficult to measure the frequency in absolutely irregular phase.

The course of transition from irregular to regular, thereafter to waxing oscillation and vise versa was investigated by taking records every five seconds (fig. 10). At the state of waxing, it seemed as the waxing wave consisted of two components slightly different in frequency from each other. To investigate precisely, waxing phases were recorded every five seconds following after the recording of fig. 10 (fig. 11).

Regular, probably synchronized oscillation was recorded with rapid time base in fig. 12 (preparation of fig. 4-C). In this state, the rising phase of a wave was always slightly longer than the falling one, the latter being followed by the next rising phase. This was noticed generally in every species, the fact that is contrary to the action potential.
FIG. 10. Transition of miniature electrical oscillation.—Oncotympana maculaticollis.

Process of transition from irregular discharges to incomplete beating. Recordings were taken on every five seconds from upper one of left column to lower of right column.


FIG. 11. Transition of miniature electrical oscillation.—Oncotympana maculaticollis.

Recordings were taken on every five seconds following after the oscillograms of fig. 10. Complete beatings appear in right column.

DISCUSSION

1) Initiation of the miniature electrical oscillation. It has been already reported (14) that miniature electrical oscillations are in all probability not brought about by the injury caused by the insertion of electrodes. Here in the Experiment A the miniature electrical oscillation was recorded in situ. It is certainly different from the potentials of fibrillation which was described by Case (1) in the case of a denervated insect muscle.

2) Myogenic origin of the miniature electrical oscillation. Miniature electrical oscillations were of the same pattern irrespective of the preparation with or without ganglion (Experiment A, B and C).

By removal of the abdominal segments followed to the second one (Experiment B, Preparation: fig. 4-B), afferent impulses from any abdominal receptor should be dropped out. So it was clearly excluded experimentally that the miniature electrical oscillation might not be brought about by reflex.

To make sure the myogenic origin of the miniature electrical oscillation, similar oscillations as the result of innervated preparation (Experiment B) was recorded with the preparation whose mesothoracic ganglion was deprived of (Experiment C).

3) Kind of muscles which generate the miniature electrical oscillation. As reported by Wakabayashi and Ikeda (14), the appearance of the miniature-
electrical oscillation is correlated to the rapidity and the myogenic determination of contraction rhythm and to the development of the elasticity of thoracic exoskeleton in insect muscle. The tymbal muscle in this report may belong to this kind.

4) Tension and the miniature electrical oscillation. As shown in fig. 6-C, in the Experiment D (Preparation: fig. 4-D) in which one of a pair of tymbal muscles was removed by vertical dissection of the abdominal segment containing it, miniature electrical oscillation showed small amplitude and low frequency compared with that obtained in the Experiment C (Preparation: fig. 4-C). It will be due to the injury issued secondarily from the destruction of Chitinous V (Ventral base of tymbal muscle).

If the generation of the miniature electrical oscillation is considered from the standpoint of Pringle's myogenic rhythm theory (9), the interpretation for the diminution of miniature electrical oscillation after the destruction of Chitinous V will be explained as follows: By the destruction of Chitinous V, the tension of muscle can not develop well enough resulting in the decrease of the activity of muscle. But this interpretation is inadequate in this case because miniature electrical oscillation was also obtained in isolated muscles as shown in the Experiment E.

In the Experiment E (fig. 7), muscle strips of about 1/2, 1/4, 1/10, 1/20 of a whole muscle and a whole muscle were used to compare the results. In fig. 7-E, as numbers of fibres were reduced, the smaller amplitude and lower frequency were recorded, at the same time the patterns were simplified. It is presumed that the diminution of activity occurs because the strip is too fine and may have been injured.

5) Composition and the pattern of the miniature electrical oscillation. As in the Experiment E, the miniature electrical oscillation does not show any noticeable variation in discharge pattern by the procedure of decreasing numbers of fibres which compose the tymbal muscle. In the case of extremely reduced muscle fibres, small irregular, somewhat regularized wave was observed. But it was difficult to know from this the single form of the elementary wave of a single muscle fibre which composed the miniature electrical oscillation. The elementary wave can possibly have no constant wave form but easily change its pattern due to some external influence. By the neurogenic impulse, it may synchronize to evoke the action potential.

As described in Part B, the miniature electrical oscillation shifted its pattern gradually, sometimes regular, waxing and waning, sometimes irregular. In the case of fig. 8-B and fig. 9, numbers of spikes were found to be about 80 per second at the waxing phase, about 160 per second at the waning phase in two different preparation of the tymbal muscle of *Graptopsisaltia nigrofuscata*. It can not be simply explained how the waxing and waning and the multiple relation of frequency as this occurs. On the other hand, it is interesting that the frequency of action potentials has been found usually 80–120 c.p.s. in *Graptopsisaltia nigrofuscata*; that is to say quite comparable frequency of this miniature pattern.
It is possible to assume two or more sinusoidal wave generators in this case. Physically, the waxing and waning can be expected as the result of the beat of two or more sinusoidal waves, frequency of which is similar but different by a few c.p.s.

In this respect some speculation is tried to interpret the phenomena; labile elementary wave which composes the oscillation may variate, fluctuate in frequency and amplitude and interact among each other, as the result it may occur that they constitute, by synchronizing a group of fibres, two or a few groups of oscillation in the whole muscle. This may result in the beating oscillation as physical vibration. But due to the biological fluctuation, the state of this synthetical oscillation may not continue long and soon change into different form. In this way, the waxing and waning and the other transition of patterns may be considered to occur. It was tried to interpret the phenomenon of waxing and waning analogous to the beat of physical oscillation. But the biological oscillation which composes the miniature oscillation has property to change its amplitude and frequency in the course of time, so the circumstances may be quite different and the analogy may conventionally only be applied in a limited time interval. On the other hand the amplitude of the oscillation of one group may decrease periodically as found in the fig. 7-E and consequently the waning of the synthetical oscillation may result.

At first sight, man will be reminded of the pattern of the electrocardiogram of fibrillation in the field of electrocardiography. It was tried to interpret how this was brought about. The multi-forci theory (2, 5), the circus movement theory (4, 6) or the single ectopic focus theory (10, 12) were considered. The resemblance of the pattern of the miniature electrical oscillation of the tymbal muscle to that of the fibrillation of the mammalian heart are interesting, though it may have only superficial similarity. This point will be expected in the future research.

Conclusion. According to the result of the present study, the miniature electrical oscillation was obtained in the tymbal muscle of every species examined. The miniature electrical oscillation is generated in a tymbal muscle whether it may innervated or not.

Though the miniature electrical oscillation presents irregular pattern generally, sometimes it changes the form as time goes on, sometimes takes form of waxing and waning of regular frequency. This is especially indicated in fig. 9, in which the frequency in the waxing shown to be 80 c.p.s. and that in the waning 160 c.p.s. while in the regular phase following thereafter 80 c.p.s. This will be easily understood by assuming the superposition of two waves frequencies of which differ only a few, but vary their frequencies slowly in the course of time.

As the frequency of regular discharges presents approximately the same value as those of the two sources in beating, the regular discharges should be consequent on the synchronization of two sources or the cessation of one of them.

In the tymbal muscle of every species, the frequencies of regular and beat-
ing discharges are comparable in respective species with that of action potentials in normal singing.

SUMMARY

1. The miniature electrical oscillation of the tymbal muscle of the cicada was investigated with the following species: Terpnosia vacua, Platypleura kaempferi, Cryptotympana japonensis, Graptolebia nigrofusca, Tanna japonensis, Oncotympana maculaticollis and Meimna opalifera.

2. The miniature electrical oscillation was observed not only in the silent muscle but also in the shrilling muscle. It was observed either in situ or in isolated preparation.

3. The miniature electrical oscillation was not affected by motor innervation but originated myogenically.

4. The pattern of oscillation was usually irregular but sometimes changed to be regular, waxing and waning.

5. The frequencies of two sources which were supposed to compose the beating oscillation were approximately equal to that which was found when the wave was regular.

6. The frequencies of regular and beating discharges were quite comparable to that of the action potentials of respective species in singing.

7. The cause of the transition of the pattern of oscillation was discussed and its similarity to the electrocardiogram of the fibrillation was noticed.

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