ACTION CURRENTS OF THE SINGLE LATERAL-LINE NERVE FIBER OF FISH

1. ON THE SPONTANEOUS DISCHARGE

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INTRODUCTION

The function of the lateral-line organ of fish has been discussed since the last century by many authors. The results of Hoagland’s research (1-5) on this nerve, executed by means of electro-physiological techniques, clarified some facts so far unknown. Several authors (6-10) followed him and have done more extensive experiments on this problem. But the results can not be said to be perfect, because most of the experiments have been done solely on the nerve trunk. Sand (7,8) accomplished his far-reaching studies on this nerve of several fresh water and marine fishes, especially of the elasmobranchs (raja). He succeeded in recording the action current of a single nerve fiber and studied the function of this sense organ more precisely.

The lateral-line sense organ is known to be a mechanoreceptor belonging, ontogenically and phylogenically to the same series as the auditory and vestibular sense organs. It is innervated by the ramus lateralis of n. vagus (a pure sensory nerve branch), which has a very intimate relation with n. statoacusticus. Recently Zotterman (11) and Löwenstein (12) have proved that a part of the macula statica of the saccule and even of the utricule of fishes are sensible to slow oscillation of water. The recording of the action currents of a single fiber of the lateral-line nerve is so difficult a task that one of the previous authors (Sand (7)), who studied the hyomandibular nerve of the excised preparation of raja, described that he succeeded in recording it only in a few cases, out of 100.

The present author undertook to record the action currents of this nerve in situ, and after having tried several kinds of Japanese fresh water fishes and after long tedious efforts, he finally succeeded to take records of those from Japanese eel (Aniguilla japonica).

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2 吉野隆夫
3 崔 柱

Received for publication April 7, 1950.
TECHNIQUE FOR RECORDING OF THE ACTION CURRENT

The author planned to record the action current of a single fiber after Tasaki's (13) method. The fishes used are relatively large ones, about one foot in length and over 100 g. in weight. The larger the fish, the easier is the technique. First a few cm. of the skin near the operculum must be removed backward along the body, which is fastened to a board. As the lateral-line nerve is buried deep in the muscle, so one must isolate it very carefully, after having exposed about 2 cm. of it. After the nerve has been freed, the fibers are cut off to leave a single fiber cautiously with a needle, using at first a binocular magnifier (×30) and finally a microscope. At the same time the diameter of the isolated single fiber is measured. The electrodes used are relatively large silver plates. The isolated fiber is hung up in the air, while the body is laid on a board or soaked in water. If care is taken to prevent the fiber from drying, then the fiber continues to discharge the impulses for several hours, if it is during cold season.

The artificial solution used is a 0.75% Ringer's solution for frogs, to which a small amount of Mg++ and glucose is added. For recording, a 4 staged resistance-capacity coupled amplifier (120 db.) and an electromagnetic oscillograph were used.

RESULTS OBTAINED

Discharges are obtained from a single fiber, if any kind of stimulus is given to a certain limited skin area (about 7-10 mm.) along the lateral-line, the so-called sensory unit as named by Tower (14).

The spontaneous discharges: Fig. 1 shows the vigorous and complex spontaneous discharges of the lateral-line nerve trunk. Hoagland (1) was the first to describe such spontaneous discharges. The spike heights of the spontaneous discharges are variable. Some are large and some are small as Sand (7) has already found. As one reduces the number of fibers, the difference of the spike height appears more marked. Fig. 2 shows two kinds of such spikes. The spike voltage is over 1 m. volt for the largest and under 100 μ. volts for the smallest.

Naturally a thick fiber elicits a large spike and a thin fiber a small one.
Fig. 2. Spontaneous discharges of two fibers. Diameters of the fibers are 6 μ. and 10 μ. respectively.

Diameters of the fibers range from 4 to 15 μ. Fig. 3 shows the histogram for the fiber diameters, which are measured on fresh living fibers under a microscope. Most fibers are medium sized and the maximum value lies in 10 μ. The number of thinner fibers is larger than that of thicker ones. The fibers are all myelinated, which is verified by staining with osmic acid.

Fig. 3. Histogram of fiber-diameters.

It is very hard to say, which fiber will remain to the last by picking, or whether a large spike or a small spike will be obtained. It is a chance, which of these two will take place. In most cases one of the thicker fibers remained, because all thick fibers continue to discharge for several hours at their best condition. The thin fiber is very weak to the strain from the change in the environmental condition.

The intervals between two successive spikes are not the same, but they are quasi-periodic, as shown in fig. 2 and 4. The average frequency of the smaller

Fig. 4. Spontaneous discharges of a single fiber of the lateral-line nerve. The diameter is 12 μ.
spikes is larger than that of the larger spikes. But if the lateral-line organs are stimulated severely, the attained frequency of a thicker fiber gets much more frequent than that of a thinner fiber.

An example of the histogram of the interval is shown in fig. 5A. All others do not show a normal distribution either, but are asymmetric, in which cases the mode and the mean are not equal. As the frequency increases the histogram approaches the normal distribution. One meets often with such a case of small spikes, because they have always relatively large frequencies.

The author attempted to make a more detailed statistical examination of the spike interval by using the method of correlogram composed of serial correlation coefficients, because the series of impulse discharges are of the stochastical process in the mathematical expression. Fig. 5A is an example of those. As mentioned above, the distribution is not normal there, and the mean value is: \( \bar{x} = 0.134 \text{ sec} \). Fig. 5B is its correlogram. This correlogram appears to have a hidden period (a kind of a cyclic periodicity, which shows the randomness of the amplitude—in this case it means the time-length of the interval—and the phase relation in oscillation).

The sixth interval has the maximal correlation. Therefore, the hidden period is: \( \bar{x} \times 6 = 0.804 \text{ sec} \). Fig. 6 A and B are other examples, representing the histogram and the correlogram of spike intervals on another fiber of the same fish. There is a hidden period in the correlogram, and as the fifth spike interval has the maximal correlation, so the hidden period is: \( \bar{x} \times 5 = 0.790 \text{ sec} \).

Serial correlation coefficient \( r \) is:

\[
r_k = \left( \frac{1}{N-k} \sum_{i=1}^{N-k} x_{i+k} - \bar{x} \right) / \left( \frac{1}{N} \sum_{i=1}^{N} x_i^2 - \bar{x}^2 \right)
\]

where \( x_i \) represents the time length of intervals and \( \bar{x} \) is the serial mean, that is:

\[
\bar{x} = \frac{1}{N} \sum_{i=1}^{N} x_i
\]
These two cases being compared, we know that they have exactly the same hidden period, which shows that the impulse discharges of the two fibers are influenced by a common cause within the fish itself. These two correlograms show other, not so conspicuous, irregular undulations too. Naturally these impulses are set up by various changes of the external environment, but some of the causes can be the changes of the internal environment. The common hidden period above mentioned suggests that the cause of this phenomenon may be a certain constant rhythmical motion inside the body of the fish. Such a motion would be due to respiration or blood circulation. Judging from the time relation of the period, it seems to be most probable that it is due to the heart-beat. For this reason, impulse discharges of a single nerve fiber and ECG of that fish were recorded simultaneously (fig. 7). Fig. 7 A and B are the histogram and the correlogram in such a case. The mean of spike intervals in this case is: $\bar{x} = 0.072$ sec. The correlogram shows that only the 15th spike interval has a relatively significant correlation and all other 14 intervals are inside the confidence limit. These discharges occur at random owing to the various changes of external and internal environment. $\bar{x} \times 15 = 1.080$ sec. is the hidden period. This is nearly equal to the interval of the heart-beat.
In many cases of these investigations there is seen a tendency to show a relatively high value of correlation coefficient, when the discharge frequencies are low, that is, in the cold season, when the hidden period becomes more prominent. This phenomenon appears principally in the case of large spikes. In summer, even when an active single fiber can be isolated, there are generally no spontaneous discharges and, if any, just a very few, and moreover, it is very difficult to take record from such a unit. The simultaneous recording of impulses and ECG shows only a few impulses in one ECG-interval (fig. 8).

In order to investigate where the impulse discharge occurs in one ECG interval, the interval should be divided into ten or more subintervals and the occurrence probability of discharges in each subinterval should be calculated, by a method similar to that for the periodogram. Tab. 1 shows an example of this. If there is one impulse in one subinterval its occurrence probability in that subinterval is 1, and if there is no discharge, then the probability is 0. The average of such probability in each column shows, which subinterval has the highest occurrence probability of discharge (fig. 8A). In this example the highest probability (5/10) lies in the 6th subinterval. The occurrence proba-
Table 1. Occurrence probability of discharges in the subintervals during one heart interval.

<table>
<thead>
<tr>
<th>No. of the subinterval</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population probability</td>
<td>15/80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence interval</td>
<td>0.272 $\leq p \leq 0.127$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8A. Occurrence probability of spike discharges in the subinterval. 

Fig. 9 shows the case with two fibers. The result of a similar calculation of the occurrence probability is shown in tab. 2. The probability of the population is 15/80. If one takes the confidence coefficient as 95%, then the confidence interval $p$ is: $0.272 \leq p \leq 0.127$. As the probability in the 6th subinterval (0.5) is outside of the confidence interval, it is evidently significant in the stochastical sense. Fig. 9 shows the case with two fibers. The result of a similar calculation of the occurrence probability is shown in tab. 2. The

Table 2. Occurrence probability of spike discharges of two fibers during one heart interval.

<table>
<thead>
<tr>
<th>No. of the subinterval</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability</td>
<td>0.08</td>
<td>0.22</td>
<td>0.26</td>
<td>0.22</td>
<td>0.13</td>
<td>0.22</td>
<td>0.04</td>
<td>0.43</td>
<td>0.08</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Population probability = 44/230 = 0.191
Confidence interval $0.245 \leq p \leq 0.151$
occurrence probability of the population is $44/230 = 0.191$ and its confidence interval $p$ is: $0.245 \leq p \leq 0.151$, where the confidence coefficient is taken as 95%.

The probabilities in the 3rd and the 8th subintervals are both outside the confidence interval.

The author thinks that the position of the sensory unit which is innervated by the fiber used in this experiment, that is, the distance from the heart to this receptor, will determine, in which subinterval the highest occurrence probability will be seen.

In the case of several fibers taken altogether, there appear many more impulses in one pulse interval, and a different calculation-modus must be used. Each compartment contains the number of impulses (table 3). The average number of impulses in each column will be unequal and the significance of the difference between the maximum and minimum values should be tested in the stochastical procedure. If there is a difference, it can naturally verify the fact that impulses from different fibers are not elicited to the same extent at the same time. Each fiber may have a subinterval of its own of the highest occurrence probability. The number of peaks on the curve in fig. 10 coincides nearly with the number of fibers which can be deduced from the difference in the spike-size.

<table>
<thead>
<tr>
<th>Occurrence frequency</th>
<th>19/VIII, 28° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>mean f.</td>
<td>0.69</td>
</tr>
</tbody>
</table>

(Several fibres)

Fig. 10. The mean occurrence frequency of spike discharges in the subintervals during one heart interval.

The mathematical management above mentioned has proved that the spontaneous discharge of a single fiber of the lateral-line nerve has an intimate relationship with the heart-beat. Of course there may be many discharges due to any other stimuli caused by the change of external and internal conditions. Nothing is known about these, but the small and irregular undulations appeared in the correlogram, suggest many of those.

DISCUSSION

1. Hoagland (2) and others (8) have already described that the spontaneous
The discharge of the lateral-line nerve has a close relationship with the temperature. The author's experimental results on the discharge of a single fiber show a similar phenomenon. The data obtained from experiments through a whole year show marked seasonal differences according to the temperature of the external milieu. In winter, the discharges are steady but not so frequent. As the temperature rises step by step, the impulse frequency increases gradually to get most frequent at 15-16°C and to decrease suddenly at over 20°C. At this time of the year the isolation of active fibers becomes very difficult as above mentioned. These phenomena are very interesting if we look at the fish from an ecological standpoint of view, because it is well known that poikilothermic animals are generally influenced markedly by the environmental temperature. As to the relationship between the sensation of temperature of fishes and the function of the lateral-line organ, which will be clarified by the reaction of this nerve to the acute change of the water temperature, the author et al. will be able to report in the near future.

2. The nervous impulses, which seem to be elicited under the influence of the heart-action, have been already found in both efferent or afferent nerve fibers and in many parts of the body. There are many examples on the side of the autonomic nerve (15,16,17), especially in the afferent nerves. Matthews (18) saw such phenomena in the nerve fiber from the muscle spindle of cat. In this case he used the nerve preparation in situ, and the phenomenon above mentioned was found very easily because of the lack of spontaneous discharges.

The most conspicuous examples are found in the cases of stretch afferents for the blood pressure, that is, sinus nerves, aortic nerves and the splanchnic nerve which comes from Vater's corpuscles in the mesentery of cats. Those are their essential functions. These receptors are found in the wall of blood vessels or just near them, even in the heart muscle and elicit impulses of two kinds which differ in their discharge pattern from one another (Bronk and Stella (19), Tasaki and Sato (20), Gammon and Bronk (21), Jarisch and Zotterman (22)).

3. Furthermore histological studies made by many authors showed the presence of two different kinds of nerve endings, innervating each single receptor (de Castro, 1926-28. Sunder-Plassman, 1930-33. Seto, 1935-49. etc).

With the cooperation of J. Chen the histological structure of the lateral-line organ was clarified. Fig. 11 is the lateral-line organ of a Japanese eel. The receptor is composed of many sensory cells and supporting cells. In many literatures there are described the sensory cells as having sensory hairs, but we could not find any. This will need further investigation. We always found blood capillaries just near the receptors, which is clearly shown in fig. 12, representing a lateral-line organ of the sea eel (Rhyncocymba). Even in the fine structures the organ is wonderfully similar to the Corti's organ in the cochlea of higher mammals, which belongs to the same phylogenical category. At the Corti's organ there is a spiral vessel buried in the basal membrane just under the hair cells. A similar construction can be seen also in the macula of the vestibular organ. One may easily comprehend that the most important and sensitive sensory cells would need a large blood supply.

4. The lateral-line organ and the Corti's organ are essentially the receptors
for the change of mechanical forces directed to them. The former is stimulated directly by the pressure change of water and the latter by that of the endolymph, to which the pressure change of air is transmitted through the complex mechanism of the middle ear.

The already discarded hypothesis suggested by Bunge and Macallum, that the body fluid might have originated from the sea-water, would make the above description easy to understand. The author thinks that this hypothesis can also explain very clearly the developmental relationship among the lateral-line organ, the vestibular organ and the auditory organ. The lateral-line organ can be stimulated essentially by the external forces as well as by the change of intrinsic conditions within the body. Galambos and Davis (23) found in their splendid works on the auditory nerve fiber vigorous spontaneous discharges, and they suggest that those discharges should be due to respiration, heart beat or blood flow through the cochlear structure. The author's experiment can be said to have proven the validity of their suggestion. Such spontaneous discharges will also be the cause of ear-tinnitus in an accoustic dead room or in calm midnight, and further more of a pathological vascular ear-tinnitus.

5. Histological studies on the lateral-line nerve show also that there are two kinds of fibers of different sizes (fig. 13). In the figure there are a few thicker fibers and much more thinner fibers. There are no ganglion cells on
the way to the region (24), where the action currents are led off from. Naturally large spikes come from the former and small spikes from the latter. The thinner fiber has a low threshold and the thicker a high one. But there are some exceptions to this, that is, a very few thicker fibers, which show the phasic adaptation, have relatively low thresholds, while other thicker fibers of tonic nature have high thresholds.

The author and his coworkers have also found that these two kinds of fibers innervate the sensory cells in different locations.

Some authors (9, 10) said that the meaning of the small spikes from the lateral-line nerve or from the vestibular nerve were obscure. Some others (23, 25) who used the needle electrode for recording the action currents of a single unit have not examined the small spikes in detail. Only Granit (26) has discussed the existence of different kinds of fibers in retina. It may be difficult to discuss about the small spikes or about the special kind of fibers as their origins, because the recording methods are different in different workers. But the author is of the opinion that the thinner fibers are for the perception of sensory stimuli.
and the thicker fibers for the discrimination.

The next report on the action currents of the lateral-line nerve fiber artificially stimulated will prove this in detail experimentally.

SUMMARY

1. The lateral-line nerve of Japanese eel yields vigorous spontaneous discharges as many other fishes do.
2. These spontaneous discharges are much influenced by temperature of the external milieu.
3. Each fiber of this nerve refers to a certain limited skin area of 7-10 mm. in length along the lateral-line. This is a sensory unit.
4. The spike discharges of a single fiber of this nerve vary in size. Some are large and the others are small.
5. These spontaneous discharges occur quasi-periodically and the average frequency of small spikes is higher than that of large spikes. In stimulation of the receptor, however, the frequency of the large spikes become much higher than that of small spikes. Naturally large spikes are elicited from thick fibers and small spikes from thin fibers.
6. The statistical treatment of the spike-intervals shows that most of these spike discharges occur at random, owing to the changes of the external as well as internal environments.
7. Some of these discharges have a relatively high correlation with the heart-beat.
8. Histologically the lateral-line organ of the fish shows, even in its fine structure, a marked similarity to the Corti’s organ of the higher mammal.
9. The intimate relationship between the spontaneous discharges of the fibers of this nerve and the heart-beat is suggestive of the mechanism of the human ear-tinnitus.
10. A few thicker fibers and many thinner fibers innervate together a receptor, each innervating different groups of sensory cells respectively.
11. It seems probable that the thinner fibers are related to the threshold, that is, to the perception of stimuli, and thicker fibers to the discrimination of sensation.

We wish to express our appreciation of the advices kindly given to us by Prof. T. Ogawa on the histological study and by Dr. I. Tasaki on the experimental method.

The cost of this research was defrayed by a grant from Prof. Y. Suehiro.

REFERENCES

LATERAL-LINE NERVE FIBER