ACTION CURRENT OF THE SINGLE LATERAL-LINE NERVE FIBER OF FISH.

II. ON THE DISCHARGE DUE TO STIMULATION.

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In a previous paper (1) a precise analysis was made of the spontaneous discharges of the lateral-line nerve of fish. In this paper are described the responses of the same nerve, especially of the single fibers, to natural and artificial stimulation such as pressure, touch, water-flow and mechanical vibration.

The fibers which compose this nerve are different in size (from 4 μ to 15 μ in diameter). They are also different in many aspects of impulse discharge, i.e., in the spike height, the rhythm, the conduction velocity, etc. In the present work, therefore, experiments were executed on as many as over 200 single nerve fibers. The technique and the procedure were the same as before and most of the materials employed were Japanese eels (Anguilla japonica).

RESULTS

1) Stimulation with water-flow. The body of a fish, except the prepared part of the nerve from where the action current of a single fiber was led off, was submerged in water contained in a long wooden box. The side wall of the box was provided with a relatively large aperture, through which any required amount of water could be withdrawn at any time. The water flowing out thereby stimulated the receptors. As described before, vigorous spontaneous discharges were seen in every fiber of the nerve and these impulse discharges were generally divided into two groups according to their spike heights, that is, one of relatively large and one of relatively small size, which were due to the diameter of the fibers.

Now, the number of discharges increased as soon as the water began to flow. When the flow was very slow, the alteration was found only in the smaller discharges, the larger spikes remaining almost the same. Fig. 1 represents an example of these. As the rate of flow increased gradually, the larger fibers and...
consequently larger spikes came into play one by one, together with a gradual increase in the number of small spikes (a recruitment phenomenon).

These results tell us that the weak stimuli excite the thin fibers, and the stronger ones the thicker fibers one by one. We see, thus, that the thin fibers have lower thresholds in comparison to the thick fibers, as far as the iterative discharge is concerned. It may be related to the accommodative resistance of nerve fibers (2). Adrian (3) described such phenomenon on the afferent vagus fiber, too. It is to be noted here that, regarding the threshold for a single stimulus, it is generally accepted that the thicker fibers have lower thresholds than the thinner.

As mentioned in the previous paper, the spontaneous discharge of a single thin fiber is much more frequent than that of a thicker one. Their responses to stimulation showed, however, just the contrary. As the discharge gets more frequent, and consequently the spike interval becomes shorter, then the discharges appear more rhythmic and the distribution curve approaches that of the normal symmetric curve, in contrast to the asymmetric one in the case of spontaneous slow discharges (fig. 2).

In order to investigate the mode of discharge of each fiber more precisely, a better method was devised, that is, to apply a water jet through a small glass tube on the so-called sensory unit of Tower or Hartline’s “receptive field” (4).
of the referred fiber. The water jet was provided from a large water reservoir, the pressure of which is controlled by means of another large glass vessel connected to it and provided with an air bellow (fig. 3). In this way, the rate of

![Diagram of apparatus supplying a constant water flow.](image)


flow could be regulated simply at one's wish. The direction of the water flow relative to the skin surface was made to vary; tangential, perpendicular, oblique, headward or tailward.

All fibers reacted to all of these, of which the horizontal (tangential) flow was found to be least effective, and the perpendicular one most effective, while all oblique ones were intermediate respectively (fig. 4). This means perhaps

![Record of discharges due to tangential and perpendicular flow.](image)

that it is the water pressure that excites the recepter. There were, however, also a few fibers which responded to very calm horizontal flows. They were those of the thickest type with phasic adaptation.

![Graph showing relation between average number of spike discharges and flow rate.](image)

Fig. 5. Relation between the average number (during the first 0.1") of spike discharges and the flow rate (in a logarithmic scale).
The relationship between the flow rate and the average frequencies of spike discharges (during the first 0.1") is illustrated in fig. 5, where we see a linear relationship between the average frequency of impulse and the logarithm of the stimulus intensity (the flow rate here), as in the cases of the optic nerve (Hartline (5)), the stretch afferents from muscles (Matthews (6)) the carotid sinus nerve (Bronk and Stella (7)) and the auditory nerve (Galambos and Davis (8)).

This linear relationship exists, however, only in a limited part of the middle range of the whole curve. With extremely weak or strong stimuli, this linear relationship gradually disappears. This is more easily seen in the case of electrical stimuli, which will be described later. The rate of increase of the number of discharge is, on the other hand, different from fiber to fiber. The striking point is that, the number of impulse discharges can be altered by the flow rate, particularly by its perpendicular component to the skin surface (fig. 6), and that there was no inhibitory effect exerted by any direction of the flow, whether tailward or headward.

2) Stimulations by pressure and touch. As already noted by many authors, a light touch or stroke on the skin evoked manifest discharges. Each fiber then showed respective frequencies and adaptations. For the most sensitive fibers even the smallest shock to the building, for example, due to gentle walking in the room, was effective. Some thick fibers (over 12 μ) showed low thresholds and phasic discharges, while some others grouped discharges.

In these fibers there could be seen an initial burst associated with a post excitatory inhibition (the silent period) which lasted for the whole period of a relatively long stimulation, and then conspicuous discharges (the post inhibitory excitation) soon after the cessation of the stimulus (fig. 7). These phenomena are very similar to Granit’s observations (9) on the optic nerve.

All thin fibers were slow in adaptation, impulses being discharged without cessation during the stimulation. These discharges were absent for a little while...
after the stimulation, then began to appear again spontaneously.

In order to investigate the effect of stimulus strength upon the impulse frequency quantitatively, various weights were applied to, or taken off from, the skin area in question, whereby the "off" and "make" mechanism in an electromagnetic coil circuit served the purpose. It was thus found that massive weights evoked more frequent discharges with shorter latent periods. The relationship between the weight \( W \) and the average spike frequency \( N \) during the first 0.1" is approximately expressed by \( N = k \log W \), \( k \) being a constant (fig. 8).

![Fig. 8. Relation between the average number (during the first 0.1") of spike discharges and weights in a logarithmic scale.](image)

![Fig. 9. Discharges of the single fiber evoked by slow mechanical vibrations (upper). The current of stimulating vibration (middle). Time 1/50".](image)
3) **Stimulation by mechanical vibration.** The stimulator adopted was a small short glass rod attached to the vibrating membrane of a magnetic speaker, which was activated by an oscillator. The oscillator circuit contained, besides a thyatron, a limiter, to make the shape of the current rectangular and so to make the vibration of the glass rod forceful.

The experimental results were nearly the same, whether they were obtained from fishes laid in the air or from those in water, and therefore most experiments were made in air. In this experiment, too, the results obtained were different from fiber to fiber according to their sizes. However, the tendency of the spike discharges to increase in number with increase of the stimulus frequency was common in all cases. In most of the fibers we recognized that the impulse is discharged while the skin is being pressed or while it is being released from the pressure, or in both cases, provided that the stimulus frequency is very low. In each cycle the onset of spikes had a nearly constant time relation to the stimuli (fig. 9).

When the stimulus frequency was increased, the number of spikes in each cycle decreased step by step, finally to just one (the authors call this one-for-one appearance). In the case of long continued stimulation such one-for-one appearance was seen for a little while at the beginning, after which the spike fell out often, owing perhaps to the adaptation (fig. 10).

![Fig. 10. Discharges of the single fiber evoked by mechanical vibration (upper). The current of stimulating vibration (middle). Time 1/50".](image-url)
In most cases this one-for-one appearance appeared when the skin was stimulated with a frequency less than 50 cps. and with a moderate intensity. With increase of the stimulus frequency, the falling out of the spike came in earlier and more often, and we had finally only one spike for many cycles. However, the one-for-one appearance seems somewhat to be related to the thickness of the fiber, for in some thick fibers it also appeared in frequencies much higher than 50 cps., whereas in the thinnest fiber only in frequencies less than 10 cps., although the upper limit could not be determined exactly in the present instrumentation. In the case of low frequency (a few cps.) stimulation, the number of spikes in each cycle was dependent on the intensity, i.e., it was just a few to begin with and increases one by one with increasing intensity. When the frequency became higher, and if the stimulus was weak, the spikes fell out often but irregularly, and if the stimulus was strong enough, 2 or more spikes were observable for each cycle, provided that the frequency was not too high (fig. 10).

The results mentioned above led us to the conclusion that with the thin fibers the spikes appeared only in the case of low frequency stimulation, while with thick fibers (except phasic ones), they appeared in higher frequencies as well, despite their high thresholds.

These results explained to us the observations of Parker and Hoagland (10) that the lateral-line organs of fishes appear to respond to vibratory stimuli from tuning forks at frequencies ranging from 20 to 70 cps. and not to those with frequencies of 100 cps., 200 cps. or over.
4) **The electrical stimulation.** From the experimental results mentioned above one can see how the lateral-line nerve fibers respond to various types of adequate mechanical stimuli. In order to know more precisely and quantitatively the nature of these responses, electrical stimulation was tried.

Of the electrodes used, one was a cotton thread soaked in Ringer’s solution and placed on the referred sensory unit, and the other was a sharp steel needle, totally insulated except at the tip, which was pierced into the subcutaneous tissue just beneath the thread electrode. In such an arrangement, the true point stimulated cannot exactly be determined, that is, whether it is the receptor or the nerve fiber. It was, however, the receptor itself, because iterative discharges could be evoked in the nerve very easily in the following ways:

a) **D.C. stimulation.**

The stimulation of receptor organs by D.C. has often been executed by many authors. (On the muscle spindle by Matthews (11), on the tongue by Pfaffman (12), on the retina by Granit (13), etc.).

Weak currents provoked a few irregular impulses. The increase of the current intensity resulted in provoking frequent and rhythmical discharges (fig. 11). The number of impulses decreases, not continuously with time (fig. 12).

![Fig. 12. The adaptation of nerve fiber to D.C. stimulation.](image)

There were some fibers which showed the grouped discharge when stimulated by strong current, despite the slow adaptation, which is a common nature of most of the fibers.

Generally, the ingoing current (the electrode on the skin being positive) elicited in most of the fibers marked and continuous discharges during the current
flow, which were inhibited after the stimulus cessation. The outgoing current, on the contrary, inhibited the spikes during the flow, evoking only one or two spikes at the "make," and a few after the "off." But some fibers showed a reversed reaction in relation to the direction of the current.

Some differences were found in the mode of discharge, which are attributable to the fiber diameter, as in the cases of mechanical stimulation mentioned above.

The maximum discharge frequency attained by thin fibers with low thresholds was much less than that of thick fibers with high thresholds. The relation between the current strength and the number of spikes was illustrated much clearer than in the cases described above (fig. 13).

![Fig. 13. Relation between the average number of spike discharges and the strength of D.C. (in a logarithmic scale).](image)

b) A.C. stimulation.

The oscillator circuit and the recording method are as illustrated in fig. 14. As the time constant (RC) of the amplifier was made small, the stimulating current of low frequency could not be recorded, so, for recording, it was led off directly from the oscillator circuit, which was of course unnecessary in the case of high frequency.

![Fig. 14. A diagram of a low frequency oscillator circuit.](image)
A weak current of a very low frequency elicited a few discharges in every cycle. With gradual increase of stimulus frequency, the number of spikes in each cycle decreased one by one. The ratio was, for example, one to three, one to two and then one to one. Such one-for-one appearance continued for fairly a long time until the falling out of the spike began to appear in relatively high stimulus frequency. The falling out was rare and irregular at first, then it increased gradually. In frequencies higher than a certain limit, the spikes appeared very often in the positive phase of the current, probably due to the temporal relation between the basic excitatory and recovery processes (fig. 14).

With the circuits system employed, it was unable to determine the upper limit of frequency for the one-for-one appearance. Further precise investigations are needed for that.

Various current strengths also yielded different results. Generally in the low stimulus frequency the increase of current strength brought an increase of the impulse number in each negative phase (fig. 16A).

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Fig. 15. Discharges of the single fiber due to A.C. stimulation of the end organ. The negative phase is upper. Time 1/50°.
Fig. 16A. Different aspects of spike discharges due to different strength of stimulating current. The frequency of stimulating current is 12.5 cps. Time 1/50". (The current increasing from up to down.)

Fig. 16B. Different aspects of discharges of the nerve fiber due to different strength of stimulating current. $f$ of the stimulating current 3.8 cps. (The current increasing from up to down.) Time 1/50".
In the case of very low frequency one could see also another feature of discharges as showed in fig. 16B. When the strength was weak (a), there could be seen some spikes in the negative phase; with a stronger current (b) more spikes in one phase; with a much stronger current (c) there appeared a few spikes even in the positive phase; in (d) many spikes appeared in both negative and positive phases. They were perhaps cathodal and anodal excitations. The author did not encounter with impulses being discharged only in the positive phase of low frequency current.

The relatively constant time relation of the occurrence of spike in each cycle is a phenomenon similar to that in Galambos' observation (8) on the auditory nerve fiber, in which the spikes occurred in the relatively constant phase of the microphonic wave. These responses to D.C. and A.C. stimuli are very suggestive of the electrotonic or generator potential of receptors described by Granit et al. (14).

ADDENDUM. CHEMICAL STIMULI

The application of various chemicals on the skin surface showed no marked changes in discharges. The chemicals used were various salts (Na, Ca, K) and acids. Even the acetic acid solution, which certainly stimulates our skin, was not effective. Naturally, the spontaneous discharges disappeared soon after the application of a cocaine solution. Sod. citrate solution showed no marked stimulative effect when applied to the skin surface, but if applied directly to the nerve, could, as is well known, excite it.

We conclude that the lateral-line organ is a pure mechanoreceptor and not a chemoreceptor.

DISCUSSION

The function of the lateral-line organ of fishes has already been subjected to electrophysiological studies by some authors. The results obtained, however, do not wholly conform with ours mentioned above.

Sand (15) reported that in the lateral-line nerve of rays, there are 2 kinds of fibers, which give small and large spikes respectively. They were shown to be different in the flow direction, to which they respond, namely, the fibers with large spikes were excited by a tailward flow and inhibited by the headward flow, while the fibers with small spikes responded in a contrary way. But the present author never observed such dual phenomena in the nerve fiber of Japanese eel. It is true that there are fibers with small and large spikes, but as previously described there are not only two but many groups of fibers, which are different in size and spike height as well. They also differ from one another as to the threshold and adaptation associated, seemingly, with their diameters (4 μ-15 μ).

Moreover, they show different maximum occurrence frequencies of spikes when stimulated by various mechanical or electrical stimuli, such as continuous, slowly changing, vibratory or oscillatory.

The author did not recognize the peripheral inhibition of nerve fibers observed by Sand, but he observed the dual responses of fibers to D.C. stimulation. A plausible explanation for this phenomenon might be that there are two different
kinds of fibers which are activated by currents of opposite directions respectively. Some examples of this kind are actually found in the “on” and “off” fibers of optic (16, 17) and auditory nerves (18), and also in the motor nerve fibers supplying flexor and extensor muscles (19).

However, the present author has a different view. In the case of electrical stimulation in the present experiments, the exact position of the stimulating electrode in relation to the receptor organ is obscure, because the organ, with its surroundings, is of a very complicated structure.

It was not that the author had never seen the phenomenon which might be regarded as that due to inhibition. But it was always on fibers of phasic nature, which were activated by a strong stimulus. This is perhaps the phenomenon which Granit explains as due to the cathodal depression (20).

The phenomenon of adaptation of the sensory nerve fiber is generally believed to be an important characteristic attributable to the end organ itself (21). We saw, however, in certain definite fibers the adaptation changing from phasic to slow with time, but never from slow to phasic. Granit (22) described the change of on-off ratio in the case of optic nerve fibers, but it was concerned with second or third order neurons and he attributed the phenomenon to the change of dark and light adaptation before the experiment.

The author thinks, that the change observed may possibly be analogous with the “hysteresis” in the physical senes, and may be due to the very complicated conditional changes of the internal and external environments. In other words, we believe that the difference between the tonic and phasic fibers are probably not absolute.

When the fish moved actively, the impulse discharges appeared vigorously as observed by Hoagland (10) and Sand (15). Hoagland believed this to be of a proprioceptive nature, but Sand did not. His opinion, to which the present author agrees, that it might be due to the motion of the fluid in the canal, accompanying the body motion, and not to the proprioceptive impulses from the muscles. The proprioceptive mechanism of fish muscles is believed to be not so developed as in the higher animals (Fessard (23)), and the impulses from the lateral-line organ may partly be playing a similar role.

Adrian’s (21) original conception of the adaptation of cutaneous sensations is well known. In skin tissues many receptors are distributed diffusely and react independently of one another. In other types of receptors, composed of many sensory cells, there are tonic and phasic fibers supplying them, for example, in stretch afferents of muscles, in the sinus (7), aortic and splanchnic nerves, and also in the vagus nerve (24, 25, 26). The one responds to the pressure itself, the other to its change, that is, to the first derivative in mathematical expression.

There is Adrian’s (21) another discovery concerning the relation of the intensity of stimuli to the number of impulses and active fibers. The results of the author’s experiments on the lateral-line nerve fibers show that weak stimuli excite the thin fibers with low thresholds and stronger stimuli the thicker fibers one by one (recruitment) accompanying the increase in discharge frequencies in each fiber. Besides, the thickest fibers respond to rapidly changing stimuli, too.

The lateral-line organs belong to those of a relatively higher order in phylo-
genetical sense. Perhaps it represents the fundamental mechanism of sensory organs of higher orders, even those of vision and audition of higher animals.

Adrian et al. (27) examined the responses of skin nerve fibers supplying the tactile receptor of frogs to intermittent stimuli and showed that the responses follow the stimuli of frequencies of 200-300 cps.

Pfaffman (28) described also the responses of the nerve supplying the teeth of cats to vibratory stimuli. He confirmed, as the present author did, that the stimulus frequency, which the impulse could follow, was different from fiber to fiber, the maximum frequencies ranging from 80 to 900 cps., beyond which the spikes began to fall out more and more. The maximum frequency obtained is akin to that of the auditory nerve fiber of cats (Galambos and Davis (8)).

These data make us conclude that higher animals have higher sensory organs in the sense that they can respond to vibrations of higher pitches.

SUMMARY

The function of the lateral-line organ of Japanese eel has been examined precisely by means of recording the impulse discharges from a single nerve fiber supplying the organ, which was activated by various kinds of natural and artificial stimulations. The results obtained are summarised as follows.

1) Examinations by water flow.

With a calm water flow only thin nerve fibers could be excited. Somewhat higher rates of flow first excite the thicker fibers. The aspect of discharges was not changed by the direction of the water flow. The number of discharges from a single nerve fiber increased with increase of the flow rate. The maximum discharge frequency attained was different in fibers with different diameters. The relation between the average number of spikes and the logarithm of the flow rate was nearly linear in every single fiber examined.

2) Examinations by pressure and touch.

Applying pressures of various grades on the skin surface along the lateral-line, results were obtained, which were similar to those above mentioned qualitatively and quantitatively. However, some very thick fibers of phasic nature were very sensitive to the impulse shock and had a relatively low thresholds. They showed sometimes inhibitory phenomena during stimulation, and facilitatory effect after the cessation of the stimulation. The lateral-line organ of fish has, too, a dual innervation of slow and phasic fibers, as many other sensory organs have.

3) Examination by mechanical vibrations.

In all fibers examined, the discharges were seen to appear in every cycle of mechanical vibrations, provided that the vibration frequency was relatively low. With very low frequencies we had generally a few spikes in each cycle, which changed into one-for-one relation in the middle range of frequency (20-50 cps.). When the stimulus frequency was further increased, there appeared the discharge deficit oftener, the more frequent the stimulus. The largest frequency producing one-for-one appearance of the spikes was different in different fibers, owing to the different diameters. The thicker the fiber the higher it was. The thinnest fiber could follow the vibrations of under 10 cps. only. On the contrary most of
the thicker fibers could follow those of 50 cps., and the thickest one even to
those near 100 cps.

4) The D.C. stimulation of the receptor easily evokes the iterative firing
of the nerve fiber. The average number of spikes had a linear relationship with
the logarithm of the current strength. The threshold for repetitive discharges
could not be determined precisely, but it was certain that weak currents made
the thinner fibers discharge more easily than the thicker fibers. Many fibers
were excited by the ingoing current and a few by the outgoing one.

5) The A.C. stimulation of the receptor evoked, also, discharges in every
stimulus cycle. For the low stimulus frequency there were a few spikes in the
negative phase only. With the increase of the stimulus frequency the number
of spikes in one stimulus cycle decreased gradually, to become the one-for-one
appearance, to random falling out of the spikes, and finally to a stage where
there resulted only one impulse in many stimulus cycles. The maximum fre-
cquency for one-for-one appearance of the spikes was related to the fiber diameter,
the thicker fiber being able to follow a higher frequency. The upper limit could
not be determined owing to the disturbances brought by the stimulus current
itself.

The strength of the stimulating current caused changes in the aspect of the
discharges. Namely, an increase in strength brought more spikes to appear in
the negative phase. With very low frequency it resulted in some spikes appear-
ing in the positive phase, too. If the stimulus frequency is high, and if the
current is sufficiently strong, then the one-for-one appearance of the spikes con-
tinued to follow much higher frequency, than when it is weak.

6) Some chemicals were not effective on the lateral-line organ.

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REFERENCES