THE SLOW POTENTIAL OBSERVED
IN DORSAL COLUMN-ROOT PREPARATIONS

PART 1. ON THE ORIGIN OF THE SLOW
POTENTIAL IN THE SPINAL CORD

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Much research has been performed since the first records of electrical activity in the spinal cord made by Gotch and Horsley (5). Gasser and Graham (4) put the recording electrodes directly on the dorsum of the spinal cord and named the recorded potential “cord potential.” Putting the electrodes on a dorsal root, Barron and Matthews (1) recorded the potential originated from the spinal cord, and called it “dorsal root potential.” In both experiments, it was observed that the potentials had very long durations compared with potential changes in the peripheral nerve.

Much discussion has centred on the origin of such slow potentials. Graham and Gasser attributed them to the activity of the internuncial neurons, while Barron and Matthews thought that they were originated from the terminations of dorsal root fibers.

Recently Lloyd (9) classified the dorsal root potential into five components. He identified the component V with the slow potential observed by Barron and Matthews and attributed its origin to the result from polarization of primary afferent fibers by current flow about secondary neurons. He thought that the activity of the somata rather than axons of secondary neurons generated the polarizing current.

In our department, employing the dorsal column-root preparations of toads, Sakamoto, Mashima and the author (11) have carried out experiments concerning slow potentials. In this paper the experiments of which the author has taken charge will be stated and the origin of the slow potential which he has observed will be discussed.

METHOD

a) A dorsal column-root preparation. The preparation consisting of the dorsal column, the dorsal root and sometimes the peripheral nerve branches i.e. the dorsal column-root preparation has been devised by Sakamoto and used first by Tsumuraya (11) for research. The method to make this preparation is as follows:

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Japanese toads (*Bufo vulgaris japonica*) were employed as materials. The spine is opened from the dorsum and the spinal cord is safely taken out together with dorsal roots and if necessary sciatic nerves as well. Ventral roots are cut from sciatic nerves. When the spinal cord is cut longitudinally along the central line, the white matter can be clearly discriminated from the grey matter on the section. The border lines between both are distinct to our naked eyes. Lifting up the ends of the dorsal roots or sciatic nerves and cutting exactly along the line between the dorsal white matter and the grey matter, the dorsal column is separated from just below the entrance point where dorsal root enters the cord up to the medulla with sharp scissors.

b) The 9th and 8th dorsal roots, or either of them, were usually used, because these two are much bigger than the others.

c) Using Helmholtz’s pendulum, a brief shock was given to a dorsal root or a sciatic nerve and the potentials were led off from the same root or an adjacent root with silver electrodes.

d) The amplifier used was a 4 staged R-C coupled one, each stage having a 4 μF condenser and 1 meg ohm leak, thus having 1 second time constant as a whole. A test unit function introduced showed only a slight height-decrease (just negligible) after 100 msec. However this amplifier is not sufficient for the accurate research in the meaning that the slow potential has sometimes a duration of over several hundred msec.

RESULTS

1. Potential changes along the dorsal root and dorsal column. The preparation is sketched in fig. 1. The dorsal column is drawn much bigger than what it is for the advantage of discrimination. In fact it is made nearly as big as the dorsal root itself. To make the analysis easier and simpler, the grid pole of the recording electrodes (G) was put on the upper end of the dorsal column and the earth pole (E) was moved along the root and column. Thus changes were observed in the shapes of potentials (fig. 2).

   The spike potential is followed by the slow potential. As long as the earth pole is put on the dorsal root, the spike potential is big, but it gradually becomes smaller and disappears at last when the pole is moved upwards along the column (fig. 2: 1-7). In the dorsal column the slow potential becomes biggest at the entrance point of the dorsal root and gradually decreases when the earth pole is put higher on the dorsal column. It has a duration of several hundred msec. The magnitude of the slow potential is various according to some unknown reasons. It changes between 10 μV and over 10 mV.

   2. Summation of the slow potentials. The magnitude of the slow potential increases in proportion to the strength of stimulus as long as it is submaximal. But after it becomes maximal, the magnitude of the potential no longer increases.

   When two dorsal roots are simultaneously stimulated by a weak enough shock, the potential produced is equal to the sum of the two potentials which are produced by the stimulation of each dorsal root in isolation. Thus the phenomenon of spatial summation is exhibited. However when the stimulus
Fig. 1. A dorsal column-root preparation.

Fig. 2 (right). Change of potential shapes when recorded on the various points of the preparation. (Traced from the original pictures.) S: Stimulating electrode. E: The earth pole of the recording electrodes. G: The grid pole of the recording electrodes. Time: 50 cycle.

Fig. 3 (left). Temporal summation and occlusion. Top: Control. Others show the grade of summation and occlusion when stimulated by two successive stimuli with various intervals. (Traced from original pictures.)

approaches maximum, the potential produced becomes smaller than the sum. Thus the phenomenon of occlusion is observed.

If a dorsal root is stimulated by two weak enough successive shocks, temporal summation is observed. However when the stimuli become big enough, the phenomenon of occlusion is likewise observed.

3. The slow potential as a generator potential. After the preparation is kept in cold Ringer's solution covering the range of 2° to 9°C., spikes appear superposing on the slow potential. In the arrangement of electrodes shown in fig. 2, they are observed to travel antidromically towards the stimulating electrode (fig. 4a) and orthodromically towards the medulla as well (fig. 4b). This fact indicates that the slow potential can act as a generator potential and perhaps the "isolated conduction" may sometimes be broken.
4. Antagonistic effects between potassium and calcium ions on the slow potential. The recording electrodes were put as seen in fig. 2: 3. when one or two drops of Ringer solution containing 0.082 per cent potassium chloride were dripped on the dorsal column just above the earth pole, the slow potential became much smaller, while the spike potential slightly decreased or remained unchanged (fig. 5). On the contrary one or two drops of Ringer solution with 0.088 per cent calcium chloride increased the magnitude of the slow potential while it decreased the spike potential (fig. 6).

Fig. 6 (left). Calcium effect. Top: Control. Middle: 2.5 min. later. Bottom: 6 min. later (retouched).

Fig. 7 (middle). T.E.A.B. effect. Top: Control. Middle: 1.5 min. later. Bottom: 4.5 min. later (retouched).

Fig. 8 (right). Nicotine effect. Top: Control. Middle: 6 min. later. Bottom: 16 min. later. Notice that slow potential still remains 16 min. after administration. The slight decrease can be seen, but such a grade of decrease frequently occurs without administration of any agent, when the preparation is laid in the air. Time: 50 cycle (retouched).
5. Veratrine effect. The preparation was kept for some time in Ringer solution containing 0.0001 to 0.00002 per cent veratrine. Using the same arrangement as above, slow potential was observed to become strikingly larger and longer. It showed a duration of over 3 seconds even with the R-C amplifier. The complete restoration of the slow potential was only observed 1 to 5 minutes after the preceding stimulation.

6. T.E.A.B. effect. 1 per cent tetraethyl ammonium bromide dissolved in Ringer solution was dropped on the preparation in the same manner. The slow potential, though not so remarkable as in veratrine case, became elongated and augmented as well, while the spike potential remained unchanged or became slightly smaller. It seems that this agent elongates the slow potential rather than augments it (fig. 7).

7. Nicotine effect. 0.1 per cent nicotine dissolved in Ringer solution did not decrease the slow potential, while it did the spike potential. Though nicotine worked as a spinal cord depressant in Barron and Matthews' case, it did not inhibit the slow potential in this experiment (fig. 8).

8. Histological studies of the dorsal column-root preparation. The preparations used were histologically observed after the end of experiments. Branching of dorsal root fibers was confirmed. They suddenly branched into many fibers and became much smaller in size as soon as they entered the dorsal column. Although there were numerous glia cells, no internuncial neuron somata could be discovered in most preparations.

DISCUSSION

I. The potential observed in the cut-ends of nerve fibers.

One who makes the above stated dorsal column-root preparation, may suspect that the numerous cut-ends of nerve fibers in the dorsal column are the origin of the slow potential. In order to ascertain this doubt, the following experiments have been done.

A sciatic nerve is taken out of a Japanese toad. In nearly the same way as when a single nerve fiber preparation is made, all nerve fibers surrounded by the perineurium are cut at a little distance from the nerve end, leaving a part of perineurium intact. Then a nerve preparation having cut-ends of nerve fibers in it is completed. The earth pole of the recording electrodes (E) is put on the cut-end, the other pole (G) on the part of nerve beyond the cut-end (fig. 9).

Fig. 9. The sciatic nerve preparation used to observe the action potential on the border of the cut-end. S: Stimulating electrode. E: The earth pole of the recording electrodes. G: The grid pole of the recording electrodes. Distance between the stimulating electrode and the “E” of the recording electrodes is within 1 cm.
Results:— (1) Potentials having a duration of 20 to 100 msec. are recorded (fig. 10). (2) The magnitude of the slow potentials have the order of 10 μV. (3) Successive stimulation causes summation (fig. 11). (4) Antagonistic effects between potassium and calcium ions on the potential are observed. The height-changes are parallel with those of the slow potential on the dorsal column-root preparation (fig. 12). (5) Acetylcholine (× 1,000-10,000) dissolved in Ringer solution makes the potential bigger (fig. 13a). Eserine (× 100-500) makes it smaller (fig. 13b). This relation is likewise observed in the slow potential on the dorsal column-root preparation. (To be published in a next paper.) (6) Veratrine (× 1,000-10,000 in Ringer solution) causes its latter part (negative after-potential?) much longer and bigger. The duration is over several hundred msec (fig. 14). (7) Successive stimuli cause summation phenomena on the veratrinized nerve preparation (fig. 14).

Conclusion:— When these results are compared with those on the dorsal column-root preparation, the differences are as follows: (1) The magnitude of the duration of the two kinds of potentials is different by one place. Namely, the change of the action potential in the cut-ends of nerve fibers may not produce a slow potential of long duration. (2) Concerning the veratrine effect, the summation phenomenon could not be obtained on the dorsal column-root preparation.
Fig. 12. Antagonistic effects between KCl and CaCl₂.

a) Left top: Control. Bottom: 9 min. after administration of CaCl₂. Time: 50 cycle.

b) Right top: Control. Bottom: 3.5 min. (up) and 6.5 min. (down) after administration of
KCl. Time: 1,000 and 50 cycle (up) and 1,000 cycle (down).

Fig. 13 (left). Antagonistic effects between acetylcholine and eserine.


b) Eserine. Top: Control. Bottom: 7.5 min. later. Time: 1,000 cycle.

Fig. 14 (right). Veratrine effect.
Cut-end was made after soaking in 0.1% veratrine for 30 min. Top: Potential immediately
after cut-end was made. Normal form. Time: 1,000 cycle. Middle up: 3 min. later.

Middle down: Summation by 3 shocks. Bottom: 7 min. later. Time: 50 cycle.
preparation. Considering these two differences, the potential occurred on the cut-ends of nerve fibers may compose a small part of the slow potential in the dorsal column-root preparation, but never be its cardinal component.

II. Similarities between the slow potential and the negative after-potential in the peripheral nerves.

<table>
<thead>
<tr>
<th>No.</th>
<th>Slow potential</th>
<th>Negative after-potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CaCl₂</td>
<td>becomes larger, while spike decreases.</td>
</tr>
<tr>
<td>2</td>
<td>KCl</td>
<td>becomes smaller.</td>
</tr>
<tr>
<td>3</td>
<td>T.E.A.B.</td>
<td>is augmented and prolonged.</td>
</tr>
<tr>
<td>4</td>
<td>Veratrine</td>
<td>is strikingly augmented and prolonged.</td>
</tr>
<tr>
<td>5</td>
<td>Relation to spike</td>
<td>always follows spike.</td>
</tr>
</tbody>
</table>

The above data on the negative after-potential were obtained by different investigators, i.e. No. 1 and 2 by Graham (7), No. 3 by the author, No. 4 by Graham and Gasser (6), Fromherz (3), and No. 5 by Erlanger, Gasser and others.

Thus some qualitative similarities are observed between both potentials. Though they are quantitatively different, it may probably be originated from the differences in the structure.

The author often observed the transition of potential form the negative after-potential-like form to the slow potential (fig. 15). This phenomenon was usually observed immediately after the preparation was made. This may be a very important fact in the study of the nature and origin of the slow potential.

Fig. 15. Change of potential shape on the same preparation.
Top: Negative after-potential-like shape. Bottom: Typical slow potential. Middle: potential shape observed between both shapes. This transition of shape from top to bottom rarely occurs immediately after the preparation is made. Time: 50 cycle (retouched).
The negative after-potential in the peripheral nerve is conceived to be the restorative process after excitation. From the similarities between these two, it is suspected that the slow potential belongs to the process of the sort, as well. But it would be better to refrain from speculation and wait for future experiments.

III. On the D.R.V. named by Lloyd

Lloyd (9) studied the dorsal root potential, dividing it into five components. He identified his component V with the slow potential observed by Barron and Matthews (1). This component resembles the author's slow potential very well, too. But there is an important difference between these two potentials. While the component V is said to be most sensitive to asphyxia, the author's slow potential remained constant for over seven hours, even when oxygen was supplied neither to the preparations nor to the Ringer solution containing them. They always were simply kept in Ringer solution exposed to air at room temperature ranging from 5° to 25°C.

When the reflex activity of spinal cord isolated from the animal body was studied for hours, oxygen was said usually to be supplied to the preparation. Otherwise, reflex activity disappeared within an hour or so. The author has likewise confirmed that the spinal reflex activity disappears in a few hours when oxygen is not specially supplied, which indicates that the internuncial neurons are especially sensitive to asphyxia.

Moreover nicotine failed to abolish the slow potential in this case, though it did the dorsal root potential by Barron and Matthews. These two slow potentials may therefore be principally of different origins.

IV. On the observation of Dun and Feng (2).

They reported that the dorsal root potential has two components. They were frequently observed on this preparation as well (fig. 5 and 8). They attributed the first one to the activity of the internuncial neurons in the thoracic region of the spinal cord, because they discovered that it disappeared when the dorsal column was cut in the midway between the thoracic and lumbar regions. However in this preparation where no internuncial neurons were found, the first component was frequently observed as well. This is all the author can say now, because he has not studied specially this component yet.

HYPOTHESIS ON THE ORIGIN OF THE SLOW POTENTIAL

Gasser and Graham (4) performed their experiments on cats. Leads were made by putting recording electrodes upon the dorsum of the cord. When the dorsal root was electrically stimulated, the so-called "cord potentials" were obtained following spike potential. They were divided into negative and positive intermediary potentials. The former had a duration of about 10 msec., and the latter about 80 to 100 msec.

Concerning the origin of the intermediary potentials, comparative studies were performed between this and the negative after-potential in the peripheral nerve. Qualitatively there were many resemblances but quantitatively considerable
differences were discovered. Simultaneous stimulation of adjacent homolateral roots resulted in a negative wave much smaller than the sum of the two component waves. Convergence was indicated to take place.

According to their opinions, if the convergence occurs in the terminations of the primary neurons, it would be necessary to postulate some form of mutual antagonism between simultaneously active terminations on the motor cells in order to explain the failure of the two roots to produce an effect equal to the sum of the effects of each one singly. Such a postulate has the weakness of not having any basis in the known properties of nervous tissue. Therefore the terminations of the primary neurons are probably not the origin. Moreover it was also examined if the potentials were originated from the motor cells, and proved not to come from these cells. In accordance with the three data, the most probable origin of the intermediary potentials was thought to be in the internuncial neurons.

Hughes and Gasser (8) studied the cord potential more precisely. From the fact that convergence occurred, they judged that the intermediary potentials were generated beyond the point at which the pathways from two roots overlap.

Barron and Matthews (1) performed an elaborate and precise research on cats, frogs and monkeys. Recording electrodes were put near the spinal cord on dorsal roots, and the potentials were led from the excited dorsal root or from an adjacent one. The so-called “dorsal root potentials” had a duration of about 1/10 sec. in the cat and 1/4 sec. in the frog. The phenomena of spatial and temporal summation and occlusion were also exhibited by these potentials.

Concerning the origin of the potentials, they indicated that the conclusion stated by the above authors lacked any conclusive evidences and expressed an hypothesis that the slow potential might be originated from the terminations of the dorsal root fibers. The mechanism of this origination was thought to be the similar one as that of the negative after-potential in a peripheral nerve. They explained the possibility that it could really exist, and moreover the convergence observed in the intermediary potential could occur in the primary neurons. (See the original p. 310-314.)

Lloyd did not add much on the origin of D.R.V. deflection. However, in the light of his proposition considered in connection with his nerve model, he discussed the fact that the slow negative wave appeared in an active root and a neighboring root alike with the same electrical sign, and concluded that secondary neurons are responsible for the polarization that produces this deflection.

In this preparation no internuncial neuron was discovered. Moreover the slow potential could not be abolished by nicotine, and it survived for several hours without supply of oxygen. This indicates that the slow potential must be originated from other sources than those stated above. When a dorsal root enters the spinal cord, it branches suddenly into many twigs and collaterals. Consequently the total surface of nerve fibers in the dorsal column is thought to become extremely broader at the region of the entrance of the dorsal root, as we have already noticed (10). Furthermore, it is microscopically observed that numerous fibers run closely together in the dorsal column. The space between fibers is very narrow and fibers run in winding ways.
These two facts suggest: When a dorsal root is stimulated, the fiber branches at the entrance point to the dorsal column are excited nearly in synchronism. This produces a potential change in the interstitium and it will take a considerable time to restore the initial state.

When the mechanism of excitation is studied, discussions have centered solely on nerve fibers. However, especially in cases like the spinal cord where numerous nerve fibers are surrounded by interstitium, the movement of ions outside nerve fibers cannot be neglected.

Sakamoto supposes that such a potential change (and its restoration) in the dorsal column is an important origin of the slow potential (10). There are some electro-chemical facts, for instance ζ-potential, which justify his idea. However, he does not at all intend to deny the hypotheses stated by the forerunners, but wishes to add another hypothesis from physico-chemical point of view. This will be communicated by him or his collaborator sometime in future.

In addition the author wishes to mention the fact that there are numerous glia-cells in the dorsal column, unknown at present what kind of electricity they produce when stimulated. This must be studied in future.

For the research of the origin of the slow potential, the progress in the knowledge of metabolism and enzymology in nerve is thought important, as well.

ON THE ORIGIN OF THE POSITIVE SLOW POTENTIAL

Gasser and Graham (4), Hughes and Gasser (8) and Lloyd have discussed on the origin of the positive slow potential. In the present experiments, the earth pole of the recording electrodes being put on the dorsal root, the positive potential was observed. Successive stimuli produced the summation of positive potentials.

For the analysis of the mechanism, the following data are important. In the case of fig. 16 the dorsal column was crushed at a little distance from the

Fig. 16. Potential change due to the arrangement of recording electrodes-I. Diagram shows the position of the electrodes and the crushed point in the dorsal column. (See fig. 2.)
upper end and the potential was led in the same arrangement as fig. 2: 1. Positive potential was observed. However, when the earth pole was moved to the point where the dorsal root entered the dorsal column, it disappeared and only the negative slow potential having the same duration as that of the positive potential appeared.

This fact may be explained as follows: When an impulse reaches the point where the dorsal root enters the dorsal column, the negative slow potential is produced. The impulse goes upwards (and downwards as well) producing the negative slow potential behind it. Branching of nerve fibers being most abundant at the entrance point the slow potential is maximum at this point as above discussed. Some of the fiber branches ascend, sending collaterals and end gradually at segments on the way. Consequently the number of branches becomes progressively less from down above and the slow potential becomes smaller at upper segments (fig. 2). The slow potential travels electrotonically along the dorsal column, even if the conduction of an impulse is interrupted on the way.

Now with the above hypothesis in mind, the negative potential following the spike potential in the upper one of fig. 16 is thought to be produced by the electrotonic and extrinsic conduction to the earth pole of the slow potential at the entrance point, while the following positive potential is caused by the electrotonic conduction to the grid pole of the slow potential produced before the crushed point. The form of potential results from the sum of both potentials of opposite signs.

In the lower one of fig. 16, the slow potential produced at the entrance point is thought to be overwhelming in size compared with the conducted potential at the grid pole. In this case, the slow potential was often found to be very small in size but long lasting. This is explained as follows: As the electrotonic and extrinsic conduction of negativity is sometimes remarkable due to

Fig. 17. Potential change due to the arrangement of recording electrodes-II. Diagram shows the position of recording electrodes. Positive slow potential appears when the grid pole (G) is put on a little distance from the upper end of the dorsal column. However when the pole is moved to the upper end, it is no longer observed. In both cases, the earth pole (E) remains unchanged. Time: 50 cycle.
SLOW POTENTIAL IN SPINAL CORD

some unknown conditions, the negative potential may be electrotonically conducted all over the dorsal column and the difference of negativity between both poles becomes small.

The hypothesis stated above is assured by moving the grid pole, while the earth pole is not changed, as shown in fig. 17. In the upper one, the positive slow potential appears. The slow potential is produced earlier at the entrance point than at the point where the grid pole is located. The slow potential disappears in the same order, and the positive slow potential appears.

In the lower one, the grid pole is located at the upper end. Therefore the negativity at the upper end is thought to be overwhelmed by the bigger one at the entrance point and could not be observed.

SUMMARY

1. The slow potential which occurs in the dorsal column-root preparation has been recorded on Japanese toads (*Bufo vulgaris japonica*).
2. The shapes of the slow potentials depend upon the position of the recording electrodes and change in a definite way.
3. The phenomena of spatial and temporal summation and occlusion are exhibited by the slow potential.
4. The slow potential acts as a generator potential when the preparation is kept in cold Ringer solution for some time.
5. Antagonism is observed between potassium- and calcium-effects on the slow potential.
6. The slow potential is strikingly augmented and prolonged by veratrine. Similar but much less effects are observed by T.E.A.B. (Tetraethyl ammonium bromide), as well. Nicotine can not abolish the slow potential.
7. Similarities are observed between the slow potential and the negative after-potential in the peripheral nerve. This indicates that the slow potential has some connections with the restorative process.
8. Some dorsal column-root preparations have been microscopically examined after experiments. No internuncial neurons have been discovered in them. The slow potential of the spinal cord can be produced without internuncial neurons.
9. The slow potential is shown not to be cardinally originated from the cut-ends of nerve fibers in the dorsal column, though some minor part of it may be so.
10. The origin of the slow potential is explained to be due to the potential change in the interstitium of the dorsal column after an impulse is conducted.
11. The origin of the positive potential is stated in the same way.

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REFERENCES