EXTRACELLULAR POTENTIALS FROM SINGLE SPINAL MOTONEURONS

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ABSTRACT

Extracellular action potentials found close to the surface of motoneurons are related to the intracellular spikes. Evidence is cited to support the assumption that the extracellular spikes have the same time course as the membrane current at the site of recording. Simultaneously recorded intracellular and extracellular spikes are compared. Intracellular spikes are transformed, by means of a circuit which is equivalent to the extracellular recording situation, into transients that are like those appearing extracellularly. Evidence is given that the recordings are from the cell bodies of motoneurons. The results show that the membrane at the extracellular recording site does not produce a spike since the time course of the extracellular potentials is determined by the passive properties of the membrane.

INTRODUCTION

This is an analysis of the extracellular potential differences that are found between the tip of a micropipette, which is very close to the surface of a motoneuron, and a remote electrode. The assumption is made that the time course of these potential differences is proportional to the time course of the membrane current at the site of recording. In a study of the principal cells of the lateral geniculate nucleus of the cat, Freygang (3) employed this assumption and was led to suggest that these cell bodies, and possibly their dendrites, did not become electrically active; they were not like a peripheral nerve during a nerve impulse because the electrical constants of the membrane did not change.

Dr. K. S. Cole suggested that the assumption of proportionality between extracellular potential and membrane current might be tested most easily by building a resistance-capacity-network model of an axially symmetrical motoneuron in a volume conductor. Therefore, Mr. A. J. McAlister built such a model and his results (6) support the assumption very well. Also, they support a suggestion made by Dr. W. Rall, namely, that with reference to a remote electrode, one may assume, for practical purposes, that all points

within the cell body are at the same potential at all times. Rall's suggestion is based upon a consideration of the low impedance presented by the intracellular space in relation to the high impedance of the membrane. When only a small portion of the somatic membrane is active, the intracellular recording has the same time course as it would have if the whole soma fired. The same consideration applies when an active area of membrane is coupled to the inside of the cell body by a small resistance; the potential difference across the passive somatic membrane is nearly the same as that across the active locus.

In view of the results which were derived from the network model of the motoneuron in a volume conductor, the situation of the extracellular micropipette relative to the motoneuron can be represented by the equivalent

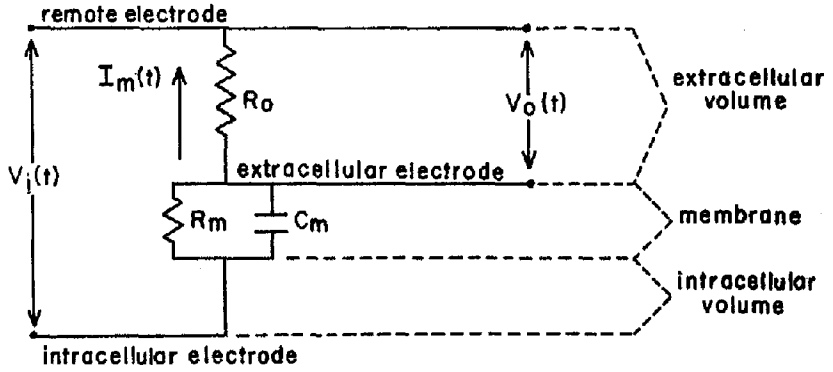

FIG. 1. Equivalent circuit of the recording situation.

circuit shown in Fig. 1. The time courses of the intracellular and extracellular recordings are signified by $V_i(t)$ and $V_o(t)$, respectively. Both are measured with respect to the remote electrode. The membrane resistance and capacity are represented by R_m and C_m, respectively, and their product is the time constant of the membrane, T_m. There is a resistance, R_o, between the outside surface of the membrane and the remote electrode. If one assumes radial symmetry for the cell body, the resistance across each unit of radial distance declines as the inverse square of the distance away from the membrane. It follows that most of the resistance, R_o, between the outer surface of the membrane and the remote electrode lies close to the membrane. Therefore, the potential difference across R_o is produced primarily by the flow of membrane current at the site of the extracellular electrode, with little contribution by the spread of current from other portions of the cell. It is likely that pressure from the extracellular micropipette increases R_o by dimpling of the membrane. If R_o is small compared to R_m, it can be seen by inspection of Fig. 1

that the membrane current, $I_m(t)$, and the extracellular recording, $V_o(t)$, will both have a time course that can be approximated by the expressions

$$\frac{V_i(t)}{R_m} + \frac{C_m\, dV_i(t)}{dt}, \quad \text{or} \quad V_i(t) + \frac{T_m\, dV_i(t)}{dt} \tag{1},$$

which describe the sum of the conductive and capacitative components of the membrane current. These expressions were employed by Freygang (3). In this study, the equivalent circuit in Fig. 1 was used to compute $V_o(t)$ from $V_i(t)$.

Methods

This research was performed on cats that were either decerebrated and immobilized with curare or tri(diethylaminoethoxy)benzene triethyliodide (flaxedil), or on cats anesthetized with pentobarbital sodium (nembutal). Reference is made to previous reports by Frank and Fuortes (2) and Freygang (3) for details of the methods used for preparing the animals and for recording unitary neuronal activity from intracellular and extracellular locations.

Two concentrically oriented glass micropipettes similar to those of Tomita (8) were employed in this study. A diagram of the model used most successfully is shown in Fig. 2. The inner pipette was filled with 3 M KCl and the outer was filled with either saline or Tyrode's solution. The position of the inner pipette relative to the outer was adjusted by means of the holder shown in Fig. 3. The outer pipette was rigidly attached to the holder while the inner pipette was waxed to an assembly mounted in rubber tubing. A spring, deformed by rotation of a screw, determined the position of the assembly holding the inner pipette.

In addition to the usual problem of limited frequency response produced by the resistance at the tip of a micropipette and the capacity from the inside of the pipette to ground, there is the additional problem introduced by the inter-electrode capacity. The effect of this capacity is to allow cross-talk between the two recording channels. Fig. 4 is a diagram illustrating the technique employed to compensate for these effects. The resistances at the tips of the inner and outer electrodes are represented by R_e and R_e', respectively, and the capacities between their grid leads and ground are represented by C_e and C_e', respectively. Loss of frequency response produced by R_e and C_e and by R_e' and C_e' is minimized by positive feed back from amplifiers A_1 and A'_1 to capacities C_1 and C'_1, respectively. Amplifiers A_1 and A'_1 were of the type described by Bak (1). The outputs from amplifiers A_1 and A'_1 were amplified with phase inversion by amplifiers A_2 and A'_2, respectively. Outputs from amplifiers A_2 and A'_2 were capacitatively coupled to the inputs of A'_1 and A_1, respectively, in order to oppose the signals that crossed the inter-electrode capacity C. As pointed out by Tomita (8), the capacity C is then equivalent to an extra capacity from each of the input leads to ground. When the input capacity is large, it becomes difficult to obtain a reasonably good frequency response. Since the capacities can be reduced as the gain of the ampli-

fiers is raised, the gains of amplifiers A_1 and A'_1 were variable and had maximum values of 8 for D.C. signals. The gains of amplifiers A_2 and A'_2 were also variable and had

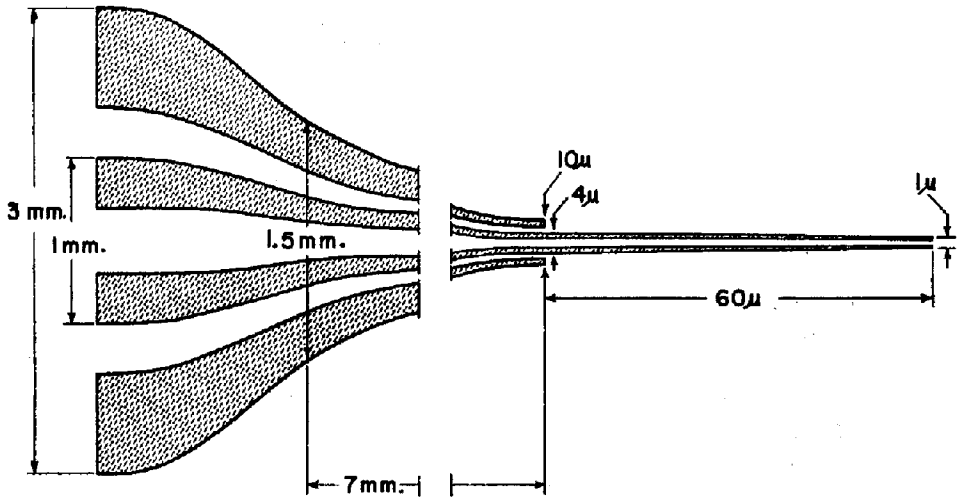

FIG. 2. Diagram of the concentric micropipettes.

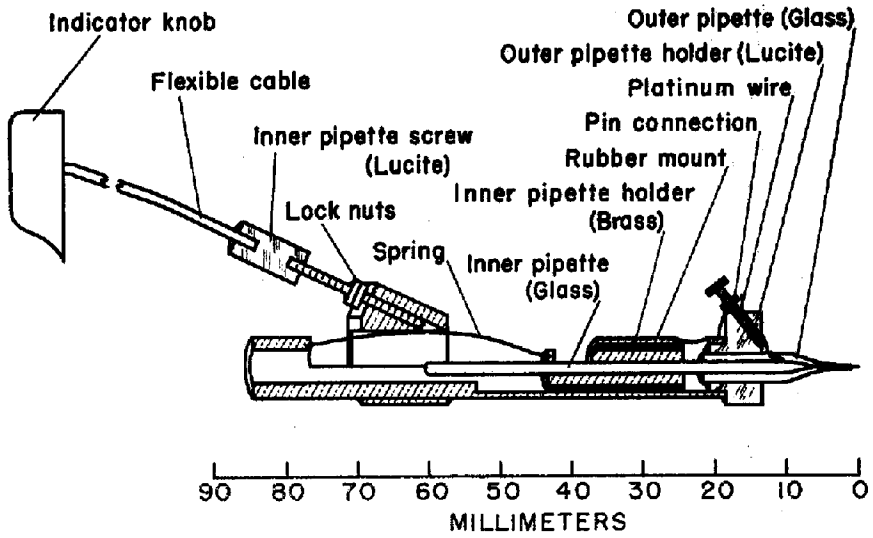

FIG. 3. Diagram of the concentric micropipette holder.

maximum values of 10 for D.C. signals. The values of C_1 and C'_1 were 5 pf. and C_2 and C'_2 were 1.5 pf. Two additional capacities, C_3 and C'_3 of 1.5 pf. each, were attached to the inputs of amplifiers A_1 and A'_1. When a sawtooth of voltage, $V_3(t)$ or $V'_3(t)$, was applied to one of these capacities, a pulse of constant current flowed through a pipette

and produced the signal used to monitor electrode resistance and frequency response, as is described in reference 3. The voltage pulse that is developed across the resistance at the tip of the micropipette is the same as that which would appear if a square pulse of voltage could be applied to the tip of the micropipette *in situ*. By using these voltage pulses as test signals, the gains of the amplifiers were adjusted so that the effects of

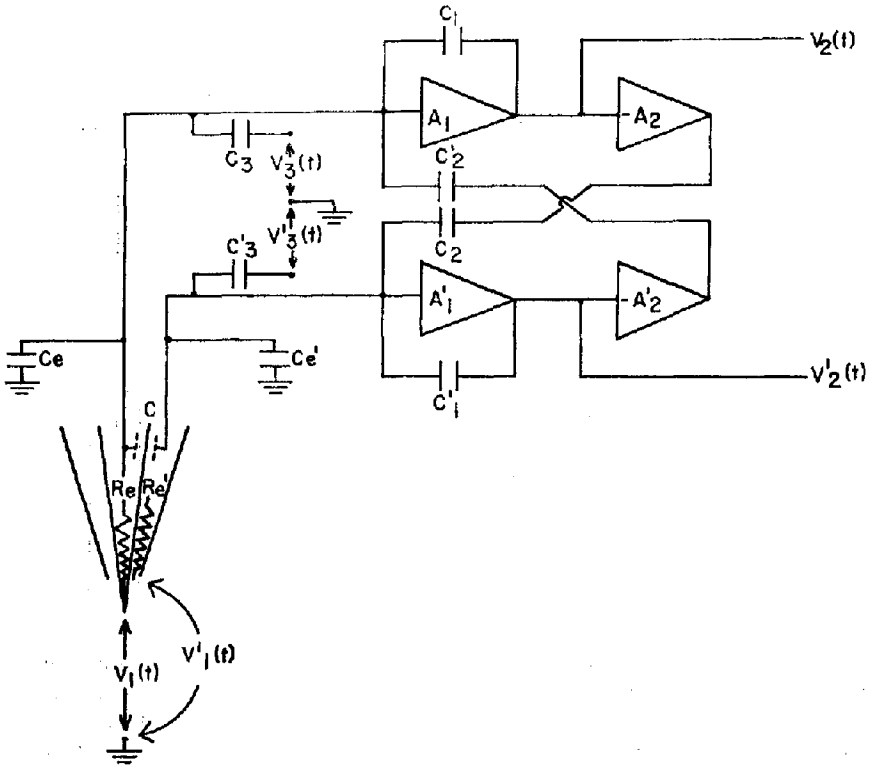

FIG. 4. Diagram illustrating the method used to minimize capacitative loss and cross-talk, as described in text.

C_e, C_e', and C were negligible. The output signals $V_2(t)$ and $V'_2(t)$ were led to a dual beam oscilloscope. *Positivity is indicated by an upward deflection in all figures.*

RESULTS

Approximation of Equivalent Circuit.—From Fig. 1 it can be shown that, if $V_i(t) = V_{i(\max)}e^{j\omega t}$,

$$\frac{V_o(t)}{V_i(t)} = \frac{j\omega + \frac{1}{T_m}}{j\omega + \frac{1}{\alpha T_m}} \qquad (2),$$

in which T_m is the time constant of the membrane, $\alpha = \frac{R_o}{R_m + R_o}$, $j = \sqrt{-1}$, $\omega = 2\pi f$, and f is the frequency. Therefore, $V_o(t)$ is related to $V_i(t)$ by a transfer function that depends on the constants T_m and α. A circuit can be made that is equivalent to the conditions of recording *in situ* if the constants T_m and α are known. Rall (7) has proposed a value of 4 milliseconds for T_m. This value was adopted in constructing the equivalent circuit since it is derived from experimental data and a consideration of the effects of the dendrites on the flow of membrane current. The value of α necessary to produce "giant" extracellular spikes, such as those of Matthews, Phillips, and Rushworth

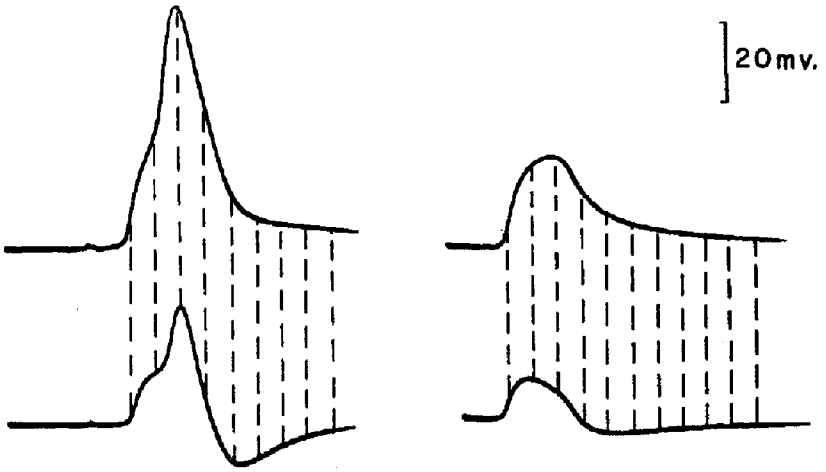

FIG. 5. Transformation of intracellular spikes into extracellular wave forms. Upper traces: Intracellularly recorded AB spike on left, A spike on right. A spike elicited during refractory period of B area. Lower traces: Transformation of intracellular spikes by means of equivalent circuit. The dashed lines are 0.2 millisecond apart.

(5), was estimated from a comparison of intra- and extracellularly recorded data and a value of 0.06 was chosen. In the equivalent circuit employed, therefore,

$$R_m = 40K\Omega, \quad C_m = 0.1\mu f, \quad \text{and} \quad R_0 = 2.5K\Omega.$$

Intracellular recordings from an antidromically activated motoneuron are shown in the upper traces of Fig. 5. In accord with Fuortes, Frank, and Becker (4), the smaller of these spikes is called the "A spike" and the portion that is added to it to produce the larger spike is called the "B spike." Their sum is called the "AB spike." It is convenient to call the portion of the motoneuron that generates the smaller spike "the A area," and the portion that generates the later phase of the total spike "the B area." By means of the equivalent circuit, intracellular recordings were converted into the

form of the extracellular transients. The result is shown in the lower traces of Fig. 5. They were obtained immediately after the upper traces. The shape

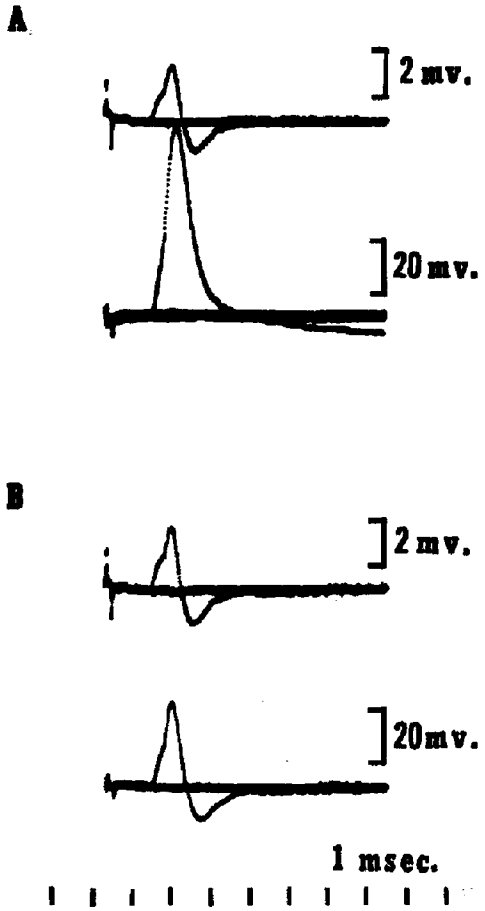

FIG. 6. Comparison of intracellular and extracellular spikes obtained simultaneously by concentric micropipettes. Upper traces are extracellular spikes recorded by the outer pipette. Lower trace in part A is the intracellular spike recorded with the upper trace. Lower trace in part B is an intracellular spike recorded with the upper trace and transformed by the equivalent circuit. Antidromic excitation, multiple sweeps superimposed, threshold stimulus at start of each sweep, cell fired once.

of the intracellular recordings was unchanged after the lower traces were photographed. It was concluded from the data in Fig. 5 that a value of $\alpha = 0.06$ was large enough to produce "giant" extracellular spikes; *i.e.*, 40 mv. peak to peak.

With respect to Equation 2, it can be noted that as R_o increases, α

approaches its maximum value of 1 and the form of the extracellular recordings approaches that of the intracellular recordings. On the other hand, when R_o and α are small, Equation 1 becomes a better approximation than it was when α was larger. It may be that an increasing value of α occurs as the extracellular micropipette approaches the external surface, perhaps by increasing R_o by putting a dimple in the membrane. This may be responsible for the changing wave form in the extracellular recording that is frequently observed.

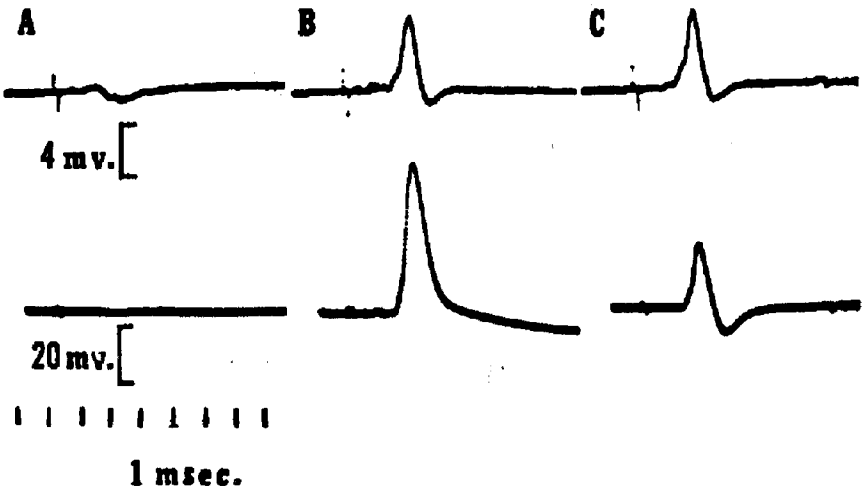

FIG. 7. Comparison of intracellular and extracellular spikes obtained simultaneously by concentric micropipettes. Upper traces are extracellular recordings from the outer pipette, lower traces are intracellular recordings from the inner pipette. Threshold stimulus did not excite in part A, but did in parts B and C. Lower trace in part C is an intracellular spike that has been transformed by the equivalent circuit.

Data from Concentric Double Micropipettes.—A threshold stimulus was applied at the start of each sweep photographed in Fig. 6. A number of sweeps are superimposed in this figure. The motoneuron was excited antidromically during one of the sweeps. The upper traces in parts A and B were recorded simultaneously with the lower traces. Part B was obtained immediately after part A was photographed. The outer pipette was extracellular and recorded the upper traces while the inner pipette recorded a resting potential and provided the lower traces. The lower trace of part B, however, was obtained from an intracellular recording that had been transformed by the equivalent circuit. The similarity to the extracellular recording (upper trace) should be noted. Probably the value of α in the equivalent circuit was higher than the true value when part B was photographed because the amplitude of the response in the lower trace is greater than that of the upper.

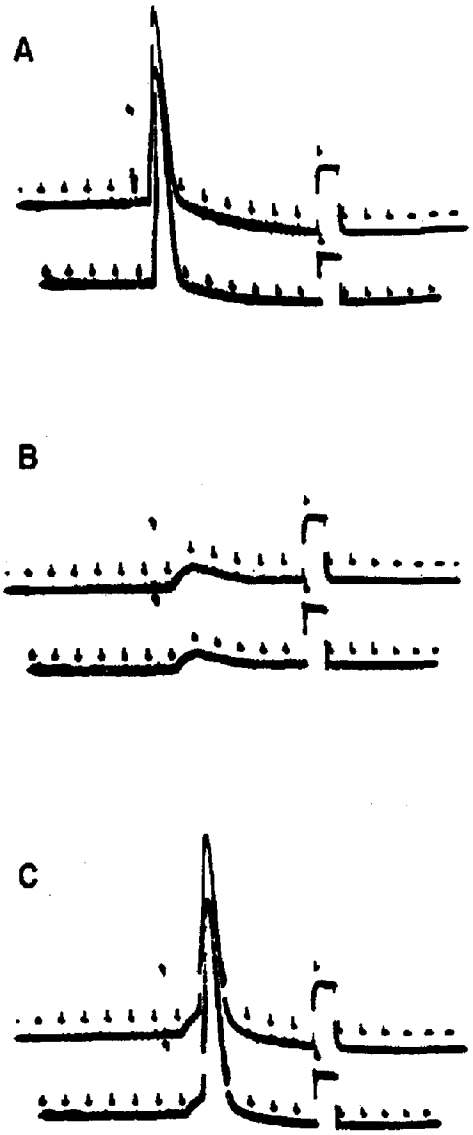

FIG. 8. Intracellular recordings from both tips of a concentric micropipette. About 20 μ between tips. Part A: antidromic excitation; part B: subthreshold synaptic excitation; part C: threshold synaptic excitation. One millisecond time marks. Twenty millivolt pulse at end of sweeps.

Fig. 7 presents the same kind of result shown in Fig. 6, but it was obtained from another motoneuron. Again the upper traces are extracellular recordings from the outer pipette and the lower traces were obtained from the inner, or intracellular, pipette. The antidromic stimulus was below threshold for

this motoneuron when part A was photographed. Part B was photographed when the cell was excited. The equivalent circuit was utilized to transform the intracellular recording in the lower trace of part C. The similarity between the upper and lower traces in part C should be noted.

It seems reasonable to suppose that these recordings were obtained from cell bodies, rather than some other part of the motoneuron, because recordings like those in Fig. 8 could be obtained frequently. The traces in this figure were photographed when both the inner and outer pipettes were intracellular and the distance between the tips was about 20 μ. This suggests that the impaled structure was too large not to have been a cell body.

DISCUSSION

It is well known that the electrical changes occurring across the membrane of an excited nerve fiber are associated with an inwardly directed membrane current. The simultaneous recordings in part A of Fig. 6 and part B of Fig. 7 show that the membrane current during the rising phase of the spike is directed outward; *i.e.*, the extracellular recording has a positive deflection. If the membrane in proximity to the extracellular electrode became active, it would not be possible to approximate the extracellular recordings with the equivalent circuit, as was done in preparing part B of Fig. 6 and part C of Fig. 7. It is possible only when the electrical constants of the membrane are invariant.

Not all cell bodies produce extracellular spikes that are consistent with the view that the membrane is not excited electrically. Dr. M. V. L. Bennett has allowed us to present one of his figures, which he obtained from a supramedullary neuron of the puffer (blowfish), *Spheroides maculatus*. It is shown in Fig. 9. The upper trace is the extracellular recording and it was recorded simultaneously with the intracellular recording in the lower trace. It should be noted that at the start of activity of the B area, the membrane current reverses and becomes inward. This suggests that the membrane at the site of the extracellular recording became excited at that time. The membrane was not excited during the part of the activity that produces a transient like that of the A spike.

Although the evidence strongly suggests that the membrane in proximity to the extracellular micropipette has not been excited, the possibility remains that it has been made inexcitable by the presence of the extracellular electrode. Matthews *et al.* (5) also found that the KCl in the pipette does not seem to be responsible for the kind of extracellular spikes described in this paper. Furthermore, they were able to maintain an extracellular position for 4 hours without a decline in the amplitude of the extracellular spike. The recordings of Bennett that are reproduced in Fig. 9 are probably obtained from excited membrane and such evidence indicates that pressure from an

extracellular electrode need not deprive the membrane of its excitability. Despite these arguments in favor of the view that the somatic membrane has not lost its excitability because of some effect of the extracellular electrode, the possibility cannot be eliminated completely.

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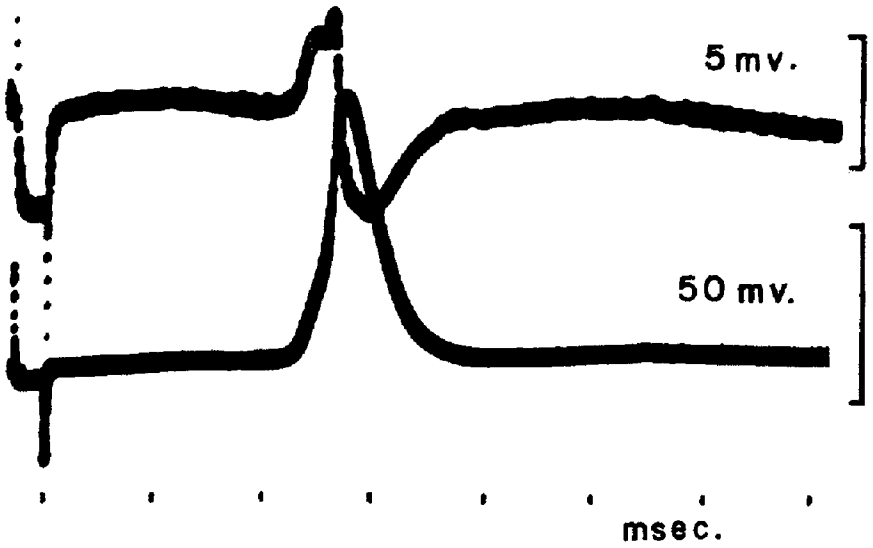

FIG. 9. Simultaneous extracellular (upper trace) and intracellular (lower trace) recordings from a supramedullary neuron of the puffer (courtesy of Dr. M. V. L. Bennett).

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