Voltage Clamp of Motoneuron Soma

Abstract. Concentric microelectrodes were used to voltage-clamp spinal motoneurons. With depolarizing voltage steps, current transients often appear, with some latency and in all-or-none fashion. Voltage-current relations indicate a two- to threefold reduction in resistance between inside and outside when the cell fires. These results suggest that activity does not involve the whole soma-dendritic membrane.

Spikes recorded from spinal motoneurons with intracellular electrodes present two components which have been called A and B (*1*). It has been suggested (*1–3*) that the first component results from activity of the initial segment of the axon, and the second component, from activity of the soma (and perhaps of the dendrites). Alternatively (*4*), the first component has been ascribed to activity of the soma, and the second, to activity of the dendrites. More recently, evidence has been produced suggesting that the cell soma is not fully invaded by activity even during the second component (B) of the spike (*5, 6*). Voltage-clamp techniques have been applied to the study of motoneuron firing in the hope that some features of their impulse production could be clarified by the results obtained with this method.

Concentric microelectrodes, similar to those previously used by Tomita (*7*), were employed for impaling motoneurons. The smaller microelectrode was used to record the potential difference between cytoplasm and indifferent electrode while the currents required to clamp at any desired voltage were passed through the larger microelectrode. Both microelectrodes were connected to a unity-gain "negative capacitance" cathode follower (*8*), and circuits were available for neutralizing the capacity between the two microelectrodes. The feedback amplifier used to deliver currents through the external microelectrode had a gain of 5000 for direct current, and the current was measured as the potential drop occurring across a 0.5-megohm resistor. Measurement of the resistance of each electrode, and of the fraction of these resistances which was common to both electrodes, was made possible by accessory circuits not shown in the block diagram of Fig. 1*A*. The potential records led off by the internal microelectrode (Fig. 1*C*) revealed the degree of clamp of the region surrounding the electrode tip, but it should be noted that these records can give no indication of the spatial extent of the voltage clamp.

Tasaki and Spyropoulos (*9*) have found that the squid giant axon behaves in a nonuniform manner under the voltage-clamp conditions which they employed. With depolarizing voltages near threshold, some parts of the membrane became fully active while other parts remained at rest. If this same phenomenon occurred in motoneurons, interpretation of the results would become ambiguous. However, nonuniformity has not been demonstrated in motoneurons, and it appears reasonable, as a first approach, to consider that the cell soma is isopotential, as has already been assumed by previous workers (*1, 3, 5, 10*) on theoretical grounds. The results of our experiments will be examined in light of this assumption, but the assumption may have to be abandoned if contrary evidence is found.

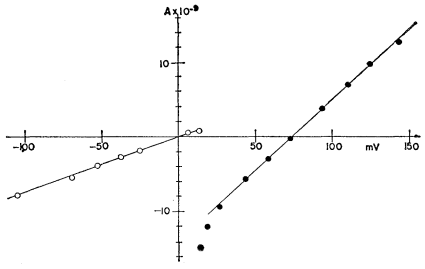

Fig. 2. Plot of data from experiment illustrated in Fig. 1*B*. (Abscissa) Potential recorded from inner pipette during clamping pulse measured from resting value; (ordinate) intensity of current through external microelectrode. Outward current is positive.

Typical results obtained with this method are illustrated in Fig. 1*B*. Hyperpolarizing voltage steps produce inward currents which start at high value and decay as shown in the top record. With weak depolarization (not illustrated), the current is the mirror image of that produced by weak hyperpolarizing voltages, but with stronger depolarization (13 mv in Fig. 1*B*) there is a delayed transient reversal of the current, which follows a course similar to that of a conventional spike. As the depolarization is increased (23 to 93 mv in Fig. 1*B*), the transient inward current becomes less delayed and decreases in amplitude until finally the current is always outward-directed.

Latency of the transient inward current can be explained if it is assumed that the low threshold A area is separated from the clamped area by a series resistance through which the membrane capacity must be charged. Since the depolarization required for firing the B area (arrow in Fig. 1*C*) is much higher than threshold for the A area (*1, 11*), the B area must be at least partly unclamped because a B current transient frequently occurs as a consequence of A activity when the clamp is just threshold for A (13 mv, Fig. 1*B*).

Figure 2 is a plot of the data illustrated in Fig. 1*B*. The open circles in this figure measure the final values of the currents required to clamp the potential of the cytoplasm at different hyperpolarizing or weak depolarizing values. The slope of the line through these points measures conductance between cytoplasm and outside fluids at rest, and the values obtained are in agreement with those determined by different methods

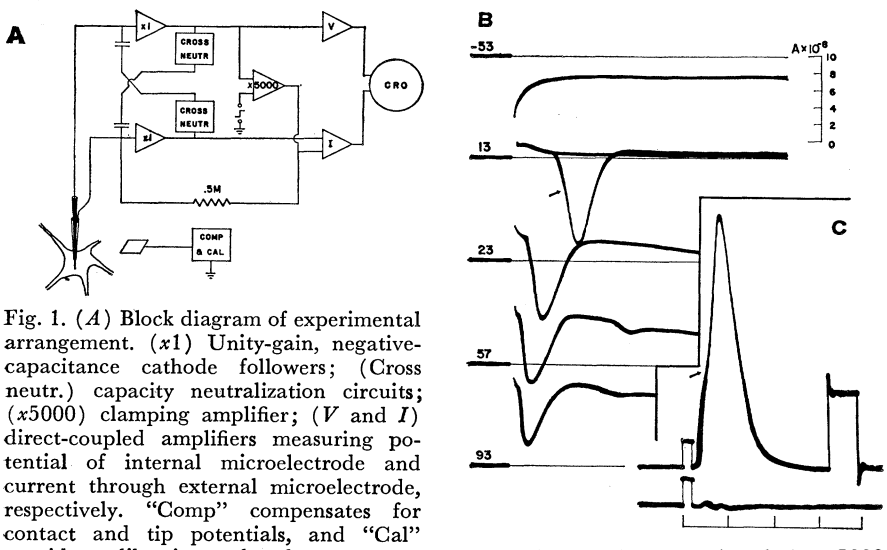

Fig. 1. (*A*) Block diagram of experimental arrangement. (*x*1) Unity-gain, negative-capacitance cathode followers; (Cross neutr.) capacity neutralization circuits; (*x*5000) clamping amplifier; (*V* and *I*) direct-coupled amplifiers measuring potential of internal microelectrode and current through external microelectrode, respectively. "Comp" compensates for contact and tip potentials, and "Cal" provides calibrating pulses between preparation and ground. The gain of the *x*5000 amplifier must be reduced at higher frequencies to prevent oscillation. (*B*) Currents through external microelectrode during clamp at different voltages. Clamping voltages are indicated (in millivolts) by numbers at left. At 57 and 93 mv there is evidence of repetitive firing. (*C*) Potentials recorded by internal microelectrode following antidromic stimulation. The upper record was made in the absence of clamp; the lower record was made during voltage clamp. The square wave is a 20-mv calibration. Arrows indicate A-B inflection; 1-msec time marks apply to records B and C.

(*3, 12, 13*). The solid circles measure the values of the current at the peak of the transient phase evoked by depolarization. The line through them intercepts the abscissa at 70 mv, a value similar to the height of the spike recorded in the absence of clamp (Fig. 1*C*). It can be argued that the slope of the line through the solid circles should give an approximate value of the conductance between cytoplasm and external fluids at the peak of activity. If this is correct, the results indicate that the resistance measured between inside and outside with the method described decreases by a factor of only 2 to 3 during activity. Two principal sources of error should be considered: On the one hand, capacity of the unclamped regions of the motoneuron tends to decrease the value of the resistance measured during activity, since this measurement is made a short time after application of the clamping pulse; on the other hand, any resistance (other than membrane resistance) common to both electrodes will not only decrease the effectiveness of the clamp on the membrane but will also decrease the percentage change in measured resistance between rest and activity by adding a constant quantity to each.

When the clamped region separates one part of the neuron from another, voltage changes in one part cannot affect the other. Thus, the clamped area cannot lie between A and B regions, since A activity elicits B firing even in the presence of the clamp. The foregoing results are consistent with the original hypothesis (*1–3*) that the A spike originates in the axon but suggest that the B spike does not involve more than a part of the soma-dendritic membrane.

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Cortical Correlates of Auditory Localization

Abstract. Responses were recorded simultaneously from a number of electrodes on the auditory cortex of one hemisphere of cats. Response amplitudes at different electrodes reached maxima and minima at different real or apparent locations of a click stimulus.

Although we have fairly extensive knowledge of the stimulus correlates of auditory localization, the physiological correlates still appear to be undetermined. The experimental evidence presently available indicates two major possibilities. One is that spatial location of the auditory stimulus is translated into locus of maximal activity within one side of the auditory system. This type of theory, generally referred to as a place theory, has been seen often, not only in audition (*1*) but in the "local sign" theories of other sensory modalities as well. The other possibility is that auditory localization is related to the ratio of the response magnitudes of the two sides of the auditory system (*2*). This may be referred to as a bilateral ratio theory. The research reported here was designed to test the validity of a place principle in auditory localization.

A six-channel electroencephalograph was used in conjunction with a bank of six oscilloscopes to photograph and measure simultaneous responses to click stimuli at six locations on the auditory cortex (AI and AII) of one hemisphere. Changes in the magnitudes of the responses recorded from each of the electrode locations were explored as a function of changes in the real and apparent location of the click stimuli (*3*). The subjects were 15 cats anesthetized with Nembutal.

Moving an actual (click) source in space gives the type of results illustrated in Fig. 1, which shows response amplitudes at different electrode locations reaching maxima or minima, or both, at different source locations. These data suggest (i) that there may be in the auditory cortex of one hemisphere elements that are differentially sensitive to the spatial location of a stimulus source (*4*) and (ii) that these elements are not distributed homogeneously through the auditory area.

When apparent location of the click stimuli is manipulated by changing the binaural time interval while holding the binaural intensity ratio constant, results of the type illustrated in Fig. 2*A* are obtained. These data were obtained with click stimuli of equal loudness in both ears. It can be seen that response amplitudes at different electrode locations reach peaks or troughs at different binaural time intervals (or apparent locations). When the loudness of the first click is made greater than the loudness of the second click, the relationship among average or general response magnitudes at the various electrodes changes, as is illustrated by comparing Figs. 2*A* and 2*B*. In Fig. 2*A* electrodes 2, 3, 4, and 5 show similar average response magnitudes, while in Fig. 2*B* the response magnitudes are clearly different, the average response magnitude at electrode 2 being enhanced, that at electrode 4 being relatively unaffected, and those at electrodes 3 and 5 being depressed. The curves in Fig. 2*B* also are more nearly parallel than those in Fig. 2*A*. The extent to which these changes take place as the result of manipulation of the binaural intensity ratio is related, as might be expected, to the amount by

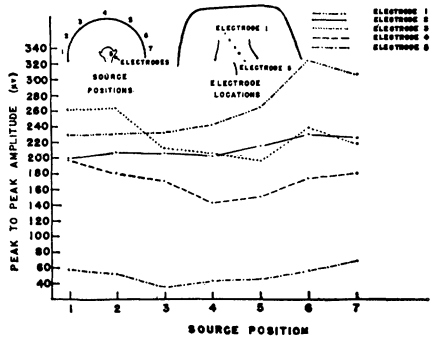

Fig. 1. Amplitude of cortical response to clicks (28 db SL at electrode 1) as a function of source position and electrode location.

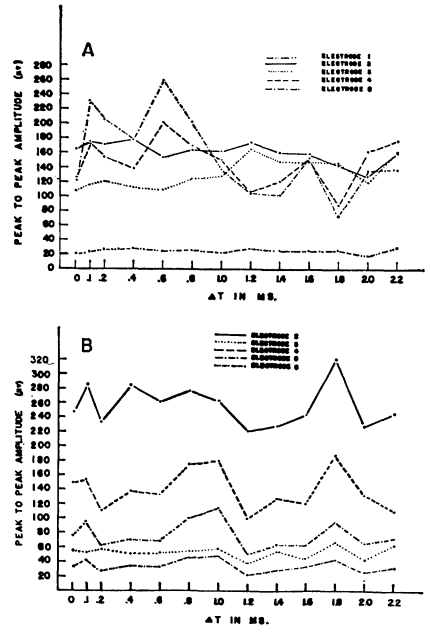

Fig. 2. Amplitude of cortical response to binaural click stimuli as a function of binaural time delay (Δt). First click, ipsilateral. *A*, First click, 20 db SL; second click, 20 db SL. *B*, First click, 30 db SL; second click, 10 db SL.