CHARACTERISTICS OF THE RESPONSES OF VARIOUS SPINAL NEURONS TO DIRECT STIMULATION

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Modern microelectrode technique permits us not only to record the potentials that arise in single neurons while the latter are participating in various sorts of reflex activity, but also to study the functional characteristics of these cells by direct stimulation. The recording of potentials of single neurons during reflex activity clearly shows that the three main types of elements in the spinal cord—the nerve fibers, and the cell bodies of internuncial and motor neurons—are quite different in their functional properties. But the method of direct stimulation has so far been employed only on motor neurons. We have utilized this method to determine the differences in activity among all three types of elements in the spinal cord.

METHODS

Direct stimulation of the cell with an intracellular microelectrode is possible with single-barreled microelectrodes [2, 6], and also with double-barreled microelectrodes [4]. In the first case, the microelectrode is incorporated in one arm of a bridge network, and the input of an amplifier system is used as a null instrument. But this method requires very complex regulation in picking up potentials, and is therefore not very convenient. We have employed double-barreled microelectrodes, prepared by drawing two Pyrex glass pipettes, fused along their length, in an automatic puller. The microelectrodes were filled with 3M KCl solution. One of the barrels was used for recording potentials, and was connected to the input of a cathode follower; the other was used for polarization of the cell. The polarizing current took the form of rectangular pulses produced by a rectangular-pulse generator and fed to the microelectrode through an output stage with rf coupling [1]. The polarizing circuit contained resistance equal to 80 meg; the polarizing current was 10^{-8} amp. Stimulation was produced by a current passing out of the cell (microelectrode positive, external electrode negative).

The main drawback of the double-barreled microelectrodes is the presence of a common resistance in the two electrodes and capacitative coupling between them. The resistance of the electrodes is usually especially high at the very tip; the passage of current often produces a much greater potential drop in this resistance than in the resistance of the cell membrane. For this reason determination of the true magnitude of the change in the resting potential of the cell is impossible, and polarization can be measured only by the strength of the polarizing current. Double-barreled microelectrodes are ordinarily selected from a large number of electrodes, and those are used in which the common resistance is not greater than 300 kohm, on account of certain special features of the breaking of the tip [4]. But such electrodes usually have a tip of rather large diameter (up to 1μ); they can be used for recording motoneuron potentials, but they do not penetrate internuncial neurons and nerve fibers. We have therefore been forced to use finer double-barreled microelectrodes, although they had considerable common resistance. Accordingly, the rectangular pulse in the oscilloscope pictures presented below reflects not only the change in the resting potential of the cell but also, to a considerable degree, the potential drop in the vicinity of the microelectrode tip.

The effect of capacitative coupling between the electrodes can be compensated to some extent by special circuitry [3]; in the present study, we have not used this type of compensation.

The experiments were performed on cats under pentobarbital anesthesia.

RESULTS

In view of their large diameters, motoneurons are the most suitable object for the insertion of double-barreled microelectrodes. When movements of the cord are dependably eliminated, the microelectrode may remain in the cell for a long time, and the effect of direct stimulation can be compared in detail with the activity that arises under the influence of synaptic or antidromic impulses.

Spike potentials (SP) generated in motoneurons in all three types of stimulation are identical, as has been shown previously [5]. The duration of SP (including weak trace depolarization) is 2–3 msec; there then develops a rather intense and prolonged hyperpolarization of the cell surface. The amplitude of the SP reaches 80 mv or more. The rising phase of the spike is distinctly divided into two parts with different slopes. The slower potential rise is replaced by a steeper one when the depolarization reaches about 30-40 mv. In some instances, particularly in polarization of near-threshold intensity and in recording from partially damaged cells (with a considerably reduced resting potential), only the first component of the SP develops; but it still obeys the "all or none" law, as a maximal spike does. The duration of this component is shorter (about 1 msec), and no perceptible hyperpolarization develops afterward.

Figure 1 shows examples of SP obtained from the same motor cell during direct stimulation, synaptic excitation, and antidromic excitation (by stimulation of the ventral root). As can be seen, the only difference in these oscillograms upon synaptic excitation was a slow variation in the resting potential of the cell (postsynaptic potential). We also note some diminution in the amplitude of SP upon direct stimulation—the usual phenomenon in a region of catelectrotonus.

Along with the motoneurons, which are easily differentiated on the basis of antidromic excitation, are found cellular elements with very different response characteristics from those described above. These cellular elements are subdivided into two groups, distinguished primarily by the shorter duration of their SP (1 msec or less).

In one group of cellular elements, a slightly suprathreshold depolarization evokes rhythmic discharge of SP, which does not happen in motoneurons. As the polarizing current increases, the number of impulses in the discharge increases. With sufficient depolarization, the rhythmic discharge is generated as long as the current is flowing. The duration of the SP in these cells is about 1 msec, and the discharge frequency is as high as 400-500 impulses/sec. Therefore, the refractory period in these neurons does not last more than 1-2 msec. The amplitude of the SP in these neurons is perceptibly lower than in the motoneurons, being around 20-30 mv.

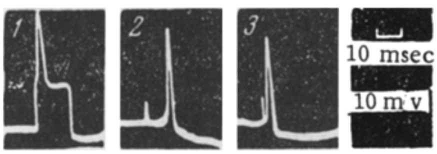

Fig. 1. Recording from motoneuron. 1) Direct stimulation; 2) response to stimulation of cutaneous nerve; 3) response to antidromic impulse.

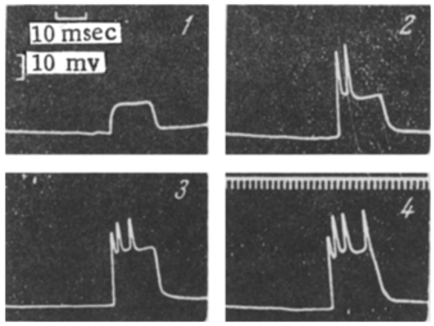

Fig. 2. Recording from internuncial neuron showing effects of direct stimulation at various current strengths.

In natural reflex activity these neurons ordinarily display high-frequency discharges in response to a single afferent impulse; the duration and amplitude of the SP is the same in both cases. Figure 2 illustrates the response of a neuron of this type to depolarization of gradually increasing intensity.

The second group of cellular elements responds to a depolarizing pulse with only one SP, even if the intensity of the depolarization is increased. In this group the SP is of even shorter duration (about 0.75 msec). The amplitude of the spikes is low in these elements, too, amounting to 20-30 mv as a rule. Trace hyperpolarization is not seen. An example is shown in Fig. 3.

There is every reason to classify the cellular elements of this last type as neuronal axons. Elements with these characteristics are the only type found in recording from the region of the white matter. The SP observed are identical in their characteristics with the SP recorded from various cellular elements in the white and gray matter upon stimulation of peripheral nerve fibers. These elements never display convergence, and the latent period of excitation of these elements is very short. Of course, in this case we may be dealing only with recording from those axons having the largest diameters.

The elements that react with a rhythmic discharge to direct stimulation must be regarded as the cell bodies of intermediate neurons. In this case we lack such definite criteria for cell differentiation as antidromic excitation in the case of the motoneurons and the short latent period and the impossibility of demonstrating

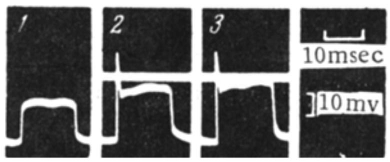

Fig. 3. Recording from axon showing effects of direct stimulation at various current strengths.

convergence in the case of the afferent fibers. The identification of elements with responses of this type as internuncial neurons is justified by a comparison of their responses on direct stimulation with the characteristics of their electrical activity during reflex excitation. Elements with this type of activity almost always show broad convergence of influences from various afferent sources, and a long latent period pointing to one or even several preliminary synaptic delays. Thus, the data that we have obtained about the main types of cellular elements in the spinal cord, as a result of direct stimulation, correspond completely to the classification established by recording of intracellular potentials during reflex excitation [7]. Consequently, the type of activity a cell displays is a quite firmly fixed property of the cell, which is not dependent on the source—an external electric current or synaptic activity—of the depolarization that gives rise to this activity.

The types of activity the various elements display are precisely adapted to the tasks they perform. Neuronal axons, by responding in a 1:1 fashion, can transmit information with the least distortion. The internuncial neurons, on the other hand, are particularly suited to the generation of sustained discharges in response to any incoming signal; in this way, excellent possibilities are created for temporal summation of the postsynaptic excitatory and inhibitory processes evoked by them in motoneurons, and for generation of prolonged inhibitory and excitatory states—the bases of central coordination—in these neurons. Finally, since the motoneurons have a mechanism for intensive trace hyperpolarization (almost absent in the internuncial neurons), they generate, on the basis of these prolonged depolarization processes, discharges of propagated impulses with a very low frequency. This frequency is adequate to maintain a fused tetanic muscular contraction; at the same time, it is maximally economical, and eliminates the possibility of peripheral blocking resulting from the development of Vvedenskii inhibition.

SUMMARY

Responses of various units in the cat's spinal cord to direct stimulation were studied by means of double-barreled intracellular microelectrodes. Three main types of units were found, corresponding to the three types of single-unit responses during reflex stimulation.

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