PREPARATION OF A SINGLE MAMMALIAN NERVE FIBER

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O. B. Il'inskii

Laboratory of General Physiology (Head, Academician V. N. Chernigovskii)
I. P. Pavlov Institute of Physiology AN SSSR, Leningrad
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At the present time in their study of nerve fibers most physiologists restrict themselves to myelinated fibers of the frog and to the giant unmyelinated axons of certain invertebrates. However, a study of the activity of mammalian nerve fibers is made comparatively seldom. The reason apparently is that there is no convenient animal on which this study may be made.

Here, we wish to draw attention to a wide range of experimental preparations of single mammalian nerve fibers, discovered long ago, but not well known, We refer to fibers connected with single mechano receptors, the Pacinian corpuscles, lying in the mesentery of the small and large intestine.

Morphologists have long ago shown that the Pacinian corpuscles are the endings of single nerve fibers from which run quite thick myelinated nerve fibers [5-9]. The fiber diameter varies between 4 and 10μ [2, 3, 7, 8], and their conduction time varies between 13 and 56 m sec [1]. An interesting feature of the Pacinian corpuscles is the presence in them of a connective tissue sheath which is thick, particularly near the corpuscle to the point at which it enters the nerve trunk, and which makes it possible to separate it from the surrounding tissue without damage. Usually the fiber is exposed for a length of 1.5 - 2.5 cm, and sometimes for 3 cm or more. If it is remembered that the stimulating electrodes are placed as a rule on the nerve trunk, and only the lead-off electrodes are placed upon the single fiber, it can be seen that the distance between the 2 pairs of electrodes may be 4-6 cm or more.

In making the preparation of the nerve fiber and in leading off potentials from it there are certain points which we will consider in some detail. To prepare the fiber it is best to use eye scissors having fine long curved blades, needle forceps, 2 straight fine preparation needles, one needle bent into the shape of a hook, a small scalpel, and a hydraulic device consisting of a syringe, rubber tube, and glass capillary.

The dissection was made under a lowpower binocular microscope (MBS-2; ocular 12.5, objective 0.6). The animal was anaesthetized and a portion of mesentery was selected which contained several separate Pacinian corpuscles, whose axons run separately for a considerable distance without adjoining other nerve fibers. It is advisable to select an area where there are a few fat cells. As a rule the best place is the mesentery of the large intestine.

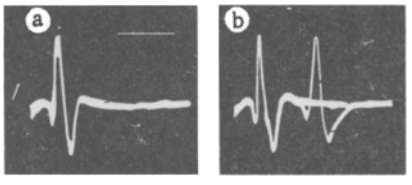

Fig. 1. Response of a myelinated nerve fiber to stimulation (a) single, and (b) with paired pulses. Superimposed traces. Time base frequency 1 cycle. Time marker 2 m sec.

The mesentery may be held in place by various methods according to the purpose of the experiment. If the work is on a nerve fiber which retains a blood supply (experiments in vivo), the mesentery is fixed in the same way as for a study of the properties of intact Pacinian corpuscles [1]. If the experiment is to be made in vitro the selected portion of mesentary is stretched on a round ebonite plate, fixed with a flexible metal ring lying close up against the plate, and is the dissected free from the surrounding tissue. Next the ebonite plate with the mesentery stretched upon the surface is transferred to a thermostatically controlled water both at 37°, and

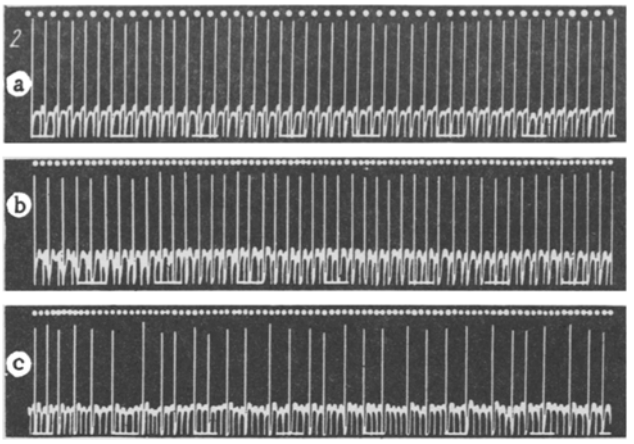

Fig. 2. Response of a myelinated nerve fiber to rhythmical stimulation (a) at 320 cycles; (b) 630 cycles 10 sec after the start of stimulation; (c) at 630 cycles 120 sec after the onset of stimulation. Time marker 20 msec. Top line—stimulus marker.

fixed in it. Into the bath is poured oxygenated Hank's solution (industrial), or Krebs-Henselwhite solution (composition of the solution in mM: NaCl - 115, KCl - 4, $CaCl_2$ - 2.46, $NaHCO_3$ - 24.1, KH_2PO_4 - 1.15, $MgSO_4$ - 1.15, glucose - 10). The pH of the solutions was first brought to 7.4. In the first case this was done by the addition of bicarbonate, and in the second case by bubbling through a small amount of CO_2.

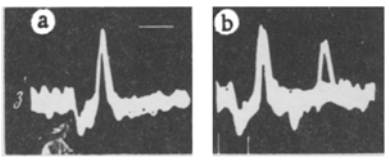

Fig. 3. Responses of an unmyelinated nerve fiber during stimulation (a) with single, and (b) double stimulae. Superimposed traces. Sweep frequency 1 cycle. Time marker 2 msec. Lines at base—stimulus marker.

Next by means of the hook, forceps, scalpel, and the scissors the superior fold of mesentery was cut, and all tissue which stood in the way was removed (fat cells, connective tissue cords, etc.).*

Further preparation is carried out by means of 2 sharp needles and a hydraulic micromanipulator while applying physiological saline, until a preparation of the nerve fiber has been achieved. During the work the fiber is slightly displaced by taking up the Pacinian corpuscle with a needle. The whole work was carried out at a magnification of 12.5 - 25 times.

To lead off the potentials, metal electrodes, or fluid and a bridge may be used. The latter arrangement is used for leading off from solitary nodes of Ranvier [4, 10]. The platinum electrodes proved more convenient than liquid electrodes. In order for the fiber not to be damaged at the electrodes, and also to improve the contact between the fiber and the electrodes, the latter were bound round with small pieces of mesentery. Whether metal or fluid electrodes are used, a covering of vaseline is used.

The stimulating electrodes, which are always metallic, are placed as a rule on a thick nerve trunk which the fiber under study enters. The fiber is grounded between the stimulating and pick-up electrodes.

Under these conditions normal action potentials from the nerve fiber could be recorded for many hours.

Figure 1 is a record of the activity of a nerve fiber in response to rhythmical stimulation. At moderate frequencies of 300 cycles a spike occurred at each stimulus (Fig. 2a), at higher frequencies (600 cycles) and prolonged stimulation the spike rate was stepped down (Fig. 2b, c).

To conclude we must note that by this method of preparation and leading off the potentials the properties of a single unmyelinated nerve fiber may be studied. In this case a single active nerve fiber was separated from a single nerve branch by the method of trans-section. Fig. 3 shows the response of such a fiber. The velocity of conduction in it was 2.5 meters per sec.

* In work in vivo, to maintain the normal blood supply it is important to protect the surrounding tissue and to dissect out the fiber only for a small distance in the neighborhood of the Pacinian corpuscle.

SUMMARY

A method is described for preparaing single mammalian nerve fibers connected with Pacinian corpuscles lying in the feline mesentery.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
