OSCILLOGRAPHIC METHOD FOR MEASUREMENT OF FUNCTIONAL LABILITY AND REFRACTORY PHASE OF NERVES

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The measurement of the refractory phase of nerves or muscles is most often done by applying a single stimulation consisting of two impulses with a known time interval between them. The refractory phase can, however, also be measured under conditions of continous stimulation [3, 4, 7].

Advances in electronic technique have provided the possibility of producing two-stimulus stimulators, which are used for measurement of the refractory phase. Single- or double-beam oscillographs are generally used for this purpose [7]. We have devised a method for measurement of the refractory phase using a four-beam cathode oscillograph, with continuous or single stimulation.

Measurement of the refractory phase and lability of a nerve, applying continuous stimulation

By making use of automatic synchronization of processes on the screen of a cathode ray oscillograph, with periodic stimulation of the nerve by one of the stimuli, we obtain a stationary representation of the process on the screen of the oscillograph, which may be observed visually, or photographed [1]. If under these conditions of synchronization we apply the second stimulus to the nerve, at a given interval after the first one, then a second action potential may result, and the stationary image on the screen will represent two stimuli and two action potentials simultaneously. After the first action current, in response to the first stimulus, the nerve will be in a state of modified excitability, expressed as the absolute and relative refractory phases. The incidence, the extent, and the duration of these phases may be determined by applying the second stimulus at definite intervals of time. If the second stimulus is applied to the nerve at about 0.5-1 millisecond after the first, i.e., within the limits of the absolute refractory phase, the second action potential wave will not appear. It can be made to appear by gradually lengthening the interval between stimuli, and to pass through the various stages of the relative refractory phase, thus permitting the recording of the whole of the curve expressing restoration of excitability of the nerve.

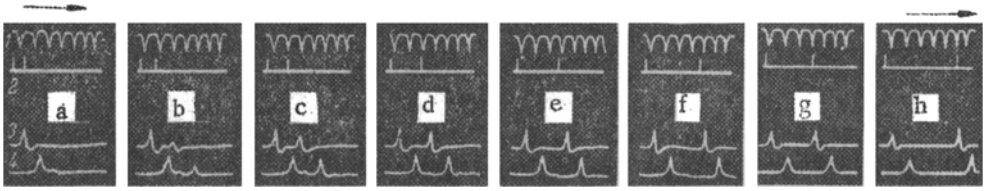

Fig. 1. Recording of the refractory phase of a nerve on the screen of a cathode ray oscillograph. a) to f): Oscillograms showing gradual development of the second action potential; g) oscillogram recorded with an adequate time interval between two series of stimulations. 1) Time marker, 500 cps; 2) exciting stimuli; 3) and 4) action potentials of the nerve.

For the measurement of the refractory phase by this method we used a 3-channel electronic stimulator of our own design, which allowed us to apply two stimuli to the nerve, the interval between which could be varied by turning a knob on the instrument. The stimulator generates three stimuli, the intervals between which can be varied within the limits 0-100 milliseconds. The amplitude, duration, and polarity of each stimulus can be varied independently of each other. The stimuli are generated either in the form of square waves or of that of a condenser discharge. The amplitude can be varied from 0 to 50 v, and the duration from 0.1 to 4 milliseconds. The

yield of the stimulator is symmetrical in relation to earth. In the experiments described below two stimuli were applied to the nerve from one and the same stimulating electrode, but our design of a 3-point stimulator would have permitted the application of the stimuli independently of each other, at different points along the nerve.

Figure 1 shows the whole process of origination of action potentials at different stages of the relative refractory phase, recorded on a 4-channel cathode ray oscillograph. Channel 1 shows time, in the form of periodic spikes, registered from a tuning-fork generator of frequency 500 cps; the interval between two spikes is therefore 2 milliseconds. Channel 2 registers the amplitude, form, and duration of the stimuli, and the interval between them. Channel 3 registers the diphasic action potential curve of the nerve, led-off from one pair of electrodes located at uninjured sections of the nerve. Channel 4 registers a monophasic action potential taken from a second pair of electrodes, of which one was placed in contact with a part of the nerve which had been killed by thermocauterization. It will be seen from the Figure that the action potentials of Channel 4 are displaced, with reference to those of Channel 3, by the time interval needed for propagation of the nerve impulse from one set of registering electrodes to the other. Knowing the distance between these electrodes, and the time interval between the stimuli, we can calculate the velocity of propagation of the nerve impulse.

Figure 1 shows successive stages of origination of a second action potential at different times of the relative refractory phase, applying square wave stimuli to the nerve. It is evident from oscillograms a to f that the second action potential appears and increases in amplitude as the time interval between the stimuli is lengthened.

The reason why we applied continuous stimulation was because two stimuli were applied to the nerve periodically (in the given case at a rate of $62\frac{1}{2}$ per second) for a previously determined period of time.

Figure 2 represents diagrammatically the entire process of measurement, recorded on a length of film moving at uniform speed. As is evident from Figure 2, not only the interval a, but also the intervals b and c, are of significiance. In investigating the refractory phase under conditions of continuous stimulation, it is essential that the time interval between the first and the second stimulus should be adequate for restoration of full excitability of the nerve, before the next pair of stimuli is applied. Unless this condition is satisfied, and the first of the succeeding pair of impulses falls within the relative refractory period of the second impulse of the previous pair, the amplitude of the response to the second stimulus of the pair will be greater than to the first (Figure 1, g and h). In such a case, it is necessary to increase the time interval, i.e., to lower the frequency of repetition of pairs of stimuli, for example, from 62.5 to 50. The length of the interval will then be 20 milliseconds, and this would permit the study of the entire excitability cycle of normal nerves.

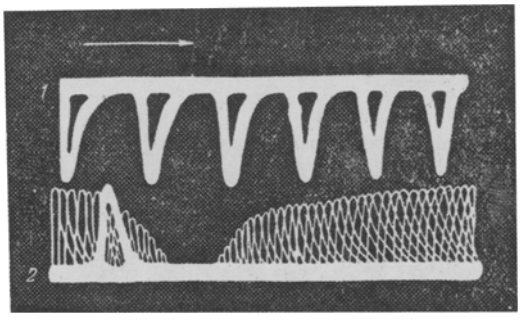

Fig. 2. Measurement of the refractory phases, recorded on a continuous moving strip of film. 1) Time marker; 2) stimulating impulses; 3) action potentials of the nerve; a), b), and c) intervals between stimulating impulses.

The procedure described above allows us to measure not only refractoriness, but also to assess the functional lability of the nerve. According to N. E. Vvedensky, lability is derived from the maximum frequency of stimulation which the nerve can reproduce without transformation, at a strength of current sufficient for the stimulation of all the fibers of the nerve. We also measured the lability of nerves under conditions of continuous stimulation, but instead of applying the maximum frequency we took that of the absolute refractory phase. From this value,

measured under conditions of tetanic contraction, we could calculate lability, according to N. E. Vvedensky, from the formula:

$$L = \frac{1000}{ar}$$

where L is lability, a is a constant, and r is the length of the absolute refractory phase, in milliseconds.

The procedure for deriving the lability coefficient from the length of the absolute refractory phase was first applied by A. A. Ukhtomsky [5, 6], and achieved in practice by P. O. Makarov [2]. The procedure devised by this author differs considerably from that of N. E. Vvedensky, since P. O. Makarov measured the length of the absolute refractory phase using single stimuli, whereas according to N. E. Vvedensky the lability coefficient is determined in the same way as in our method. Our method of calculation differs from that of A. A. Ukhtomsky in our introduction of the constant a, not equal to unity. The necessity of introducing this constant is illustrated by the following example. The length of the absolute refractory phase of the unfatigued nerve is usally about 1 millisecond. Hence if the value of the constant a were to be equal to 1, that of the lability coefficient would be 1000; according, however, to N. E. Vvedensky's measurements it is about 500. It follows that in the given case the constant a is equal to 2. The value of a should be determined by means of special experiments.

Measurement of refractory phase applying single stimuations

It is possible, taking a single time cycle of the cathode ray oscillograph, to measure the refractory phase, applying not continuous, but single stimulations of the nerve, as is done with the metronome ordinarily applied for the purpose. In such a case, the nerve is excited by two stimuli only, the second following the first at a known interval. The single stimulation method permits the recording on a film of the same effects as are recorded by the continuous stimulation method (shown in Figure 1), but is not adapted to continuous observation.

The advantages of the oscillographic over the metronome procedures reside in its greater objectivity and in its permitting of direct recording and prolonged visual observation of all the magnitudes concerned which cannot be achieved using a metronome equipment.

Automatic recording of the whole cycle of restoration of nerve excitability

The oscillographic method permits the observation and measurement of refractoriness at different points of the curve representing restoration of excitability, but does not, as usually applied, show the curve as a whole, in the form that the excitability curve is usually represented in textbooks. This can, however, be achieved in the following way. One stimulus is applied at close to zero time, while a series of second stimuli moves continuously along the screen, i.e., their incidence falls at different moments of the refractory phase. In consequence, the whole of the curve of recovery of excitability will be visible on the screen, as a line joining the summits of the action potential waves. An oscillogram of this type is shown in Figure 3. Channel 1 is used for the time marker

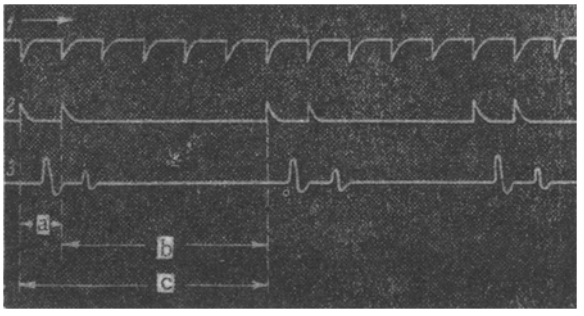

Fig. 3. Automatic recording on a cathode ray oscillograph screen of the whole of the curve expressing recovery of excitability. 1) Time marker; 2) action potentials of a nerve, arising at different stages of the relative refractory period.

(frequency 500cps). Channel 2 records the action potentials arising in response to stimulation. As is evident from the Figure, continuous stimulation of the nerve gives a picture on the screen in which the whole of the curve of recovery of excitability is visible, in the form of a line enclosing the action potential waves.

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PROCEDURE FOR LEADING OFF ELECTRICAL POTENTIALS FROM AND FOR STIMULATING THE BASAL REGIONS OF, THE BRAIN OF DOGS, UNDER CONDITIONS OF SERIES EXPERIMENTATION

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A number of procedures are used in modern physiology for permanent leading off biopotentials from and for applying stimuli to, various parts of the brain. Of these procedures, the most useful has been that devised by A. B. Kogan [1], depending on the insertion of indwelling electrodes. By this procedure, action potentials can be led off from, and electrical stimulation can be applied to, the basal areas of the brain of dogs, using indwelling electrodes. This is a drawback of the method, since the electrode introduced into the brain injures conducting pathways and nuclear formations when being inserted into the desired locality, and may interfere with the normal physiological relations existing between different parts of the central nervous system. In this connection, we have devised a procedure for the direct leading off of bioelectric potentials from, and for the stimulation of, the basal areas of the brain under conditions of series experimentation in dogs, involving the designing of a Plexiglass screw-in electrode 10 mm long, with a diameter of the thread of 4 mm, and with 7 turns of the screw-thread.

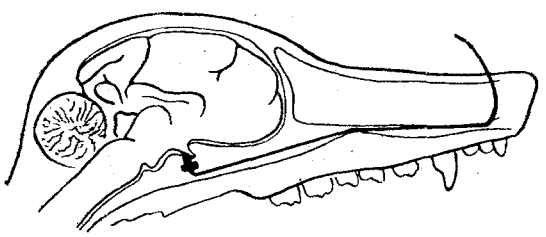

Fig. 1. Diagrammatic representation of the location of a basal screwed-in electrode, in the skull of a dog.

* In Russian.