SYNAPTIC POTENTIAL IN THE MOTOR GIANT AXON OF THE CRAYFISH

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ABSTRACT

Some electrical properties of the synapses between central giant axons (presynaptic) and the motor giant axon (postsynaptic) of the crayfish abdominal nerve cord have been investigated. Postsynaptic potential change in response to presynaptic volleys contains two components: a spike potential and a synaptic potential of very long time course. Amplitude of the synaptic potential is graded according to the number of active presynaptic axons. Conductance increase in the synaptic membrane endures over most of the period of potential change, and it is this rather than the "electrical time constant" of the membrane that in large measure determines the form of the synaptic potential. Temporal summation of synaptic potential occurs during repetitive presynaptic stimulation, and after such stimulation the rate of decay of synaptic potential is greatly slowed.

The third root of each abdominal segment of the crayfish contains a giant fiber (Johnson, 1924) that receives synaptic connection from the ipsilateral lateral giant fiber and from both median giant fibers of the central nerve cord. Because of its size, and because the exact loci of synapsis can be seen by microscope without staining, the giant axon preparation is excellent material for the study of phenomena occurring at the synaptic region, with which question the present account is concerned.

The experiments were performed on the central nerve cord of the crayfish (*Procambarus clarkii*) isolated together with its abdominal segmental roots and suspended, by means of two forceps, one at the thoracal part of the cord, the other at the fourth abdominal segment, in crayfish saline[1] under paraffin oil. The root to be used for recording, usually of the first or second abdominal segment, was brought into the oil so that the cord-root junction was at the oil-saline interface. Records were obtained

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[1] The saline used had the following composition after Van Harreveld (1936): NaCl, 12 gm.; KCl, 0.4 gm.; $CaCl_2$, 1.5 gm.; $MgCl_2$, 0.25 gm.; H_2O, 1000 ml. (pH adjusted to 7.5 with NaOH).

by electrodes one at the distal end of the root in oil, the other in the saline. For internal recording micropipettes of tip diameter less than 0.5 μ and filled with 3 M KCl were used. A D.C. amplifier with cathode follower input and adjustable negative capacitance was employed to achieve a low grid current and to compensate for distortion.

The cord was stimulated at its caudal end by means of square wave pulses of 0.1 msec. duration. Usually the lateral giant axons displayed the lowest threshold, with increase in stimulus strength recruiting in succession first one medial giant axon, then the other, and finally the non-giant axons.

RESULTS

Responses Recorded by Means of External Recording Electrodes.—External recording with the electrodes placed as described reveals the potential differences developed between the root-cord junction and the distal end of the third root of the first abdominal segment. In this lead the passage of impulses in the central giant axons appears as a polyphasic deflection, the second phase of which indicates arrival at the region of the presynaptic impulses. This deflection also serves to indicate the number of presynaptic giant axons activated by a given stimulus.

To obtain the records A, B, and C of Fig. 1 the presynaptic stimulus was increased progressively. In Fig. 1 A, the stimulus being subliminal, there was no appreciable potential change. When one central giant axon was activated (Fig. 1 B), its impulse was recorded as the small diphasic deflection following the stimulus artifact. This deflection is followed after somewhat less than 1 msec. by a large diphasic spike potential indicating postsynaptic response in third root axons. There follows in turn a slow potential change of low amplitude and of negative sign at the cord-root junction. Further increase in the stimulus brought other central giant axons into action (Fig. 1 C). This is indicated by an increase in the initial deflection. There is no change in the postsynaptic spike potential of the third root, but the slow potential is markedly increased and prolonged as a consequence of the increased central giant fiber activity.

Records D, E, and F of Fig. 1 were obtained at higher sweep speed from another preparation. Stimulation was subliminal for the recording 1 D. At strength sufficient to stimulate a single central giant axon (Fig. 1 E) the simple initial deflection signalling its activity again is seen followed this time, however, only by the slow potential, there being no postsynaptic spike potential in the third root. Upon further increase in stimulus strength (Fig. 1 F) the initial deflection becomes complex, indicating response of other central giant axons, and a postsynaptic spike potential, of which only the onset is seen, appears in the third root.

Intracellular Recording of Potential Change in the Motor Giant Axon.—There follows an analysis of these responses made with the aid of intracellular recording from the motor giant fiber in the root.

Fig. 2 presents intracellular recordings from the motor giant axon at the

point of its exit from the cord, the experiment otherwise being similar to those illustrated in Fig. 1. The left and right columns are similar responses recorded at different sweep speeds. Below a critical stimulus intensity no response occurs in the motor giant axon (Fig. 2, A and D). Upon increasing the stimulus there appears (Fig. 2, B and E) a spike potential after which there is a redevelop-

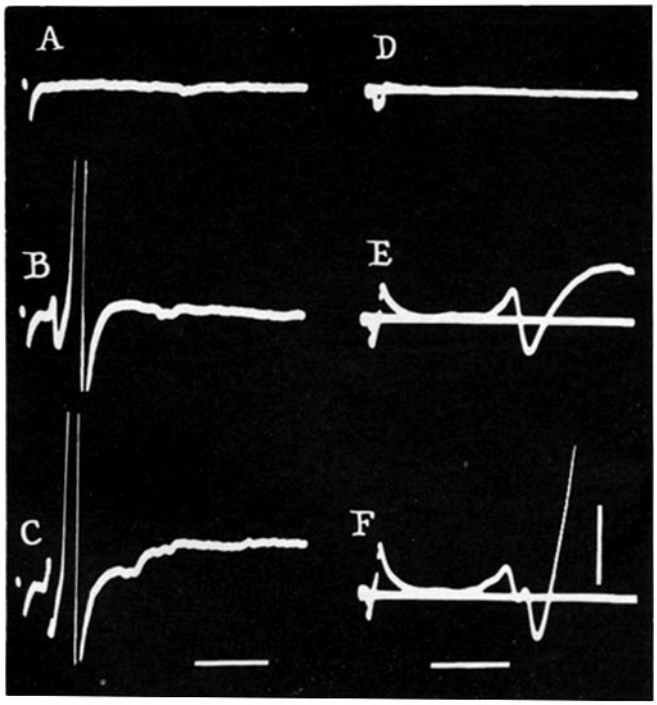

FIG. 1. Potential changes in the third root of the second abdominal segment recorded by external electrodes following stimulations of the caudal abdominal cord. A, B, and C: responses in a single preparation following stimuli of increasing strength. Time: 5 msec. D, E, and F: similar responses from another preparation. Time: 1 msec. Calibration: 0.5 mv. for all records.

ment of membrane depolarization that starts from the base line, reaches maximum a few msec. after the spike potential, and decays over a very short time course. Further increase in stimulus intensity did not in any way alter the spike potential response, but there is a step-like increase and great prolongation of the slow depolarization (Fig. 2, C and F). Still further increase in stimulus caused no further change in the recorded potential.

The step-like changes seen in Fig. 2 clearly are due to the successive recruitment of central giant axons. Although one might suppose the slow depolariza-

tion in records B and E of Fig. 2, appearing as it does in a single step with the spike potential, to be an after-potential, the increment that occurs with further increase of stimulus cannot be so designated. It is rather a synaptic potential

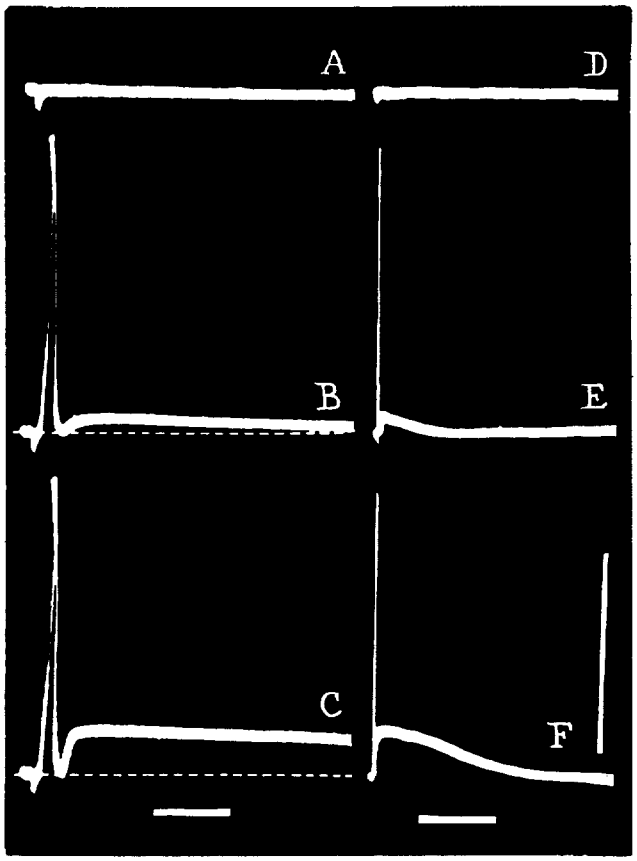

FIG. 2. Potential changes during reflex response recorded internally from the synaptic region of the motor giant axon. A, B, and C: responses to incrementing stimuli. Time: 10 msec. D, E, and F: the same slower sweep. Time: 100 msec. Calibration: 50 mv. for all records.

of the motor giant fiber evoked by presynaptic action. Parenthetically, the question of after-potential is considered further in connection with Fig. 3. Gradation of the slow depolarization demonstrates summation of the synaptic potentials produced at the several contacts between the central giant axons and the postsynaptic motor giant axon.

If the slow depolarization indeed is a synaptic potential, it should be elicitable in the absence of spike potential production. This is shown in Fig. 3. Rec-

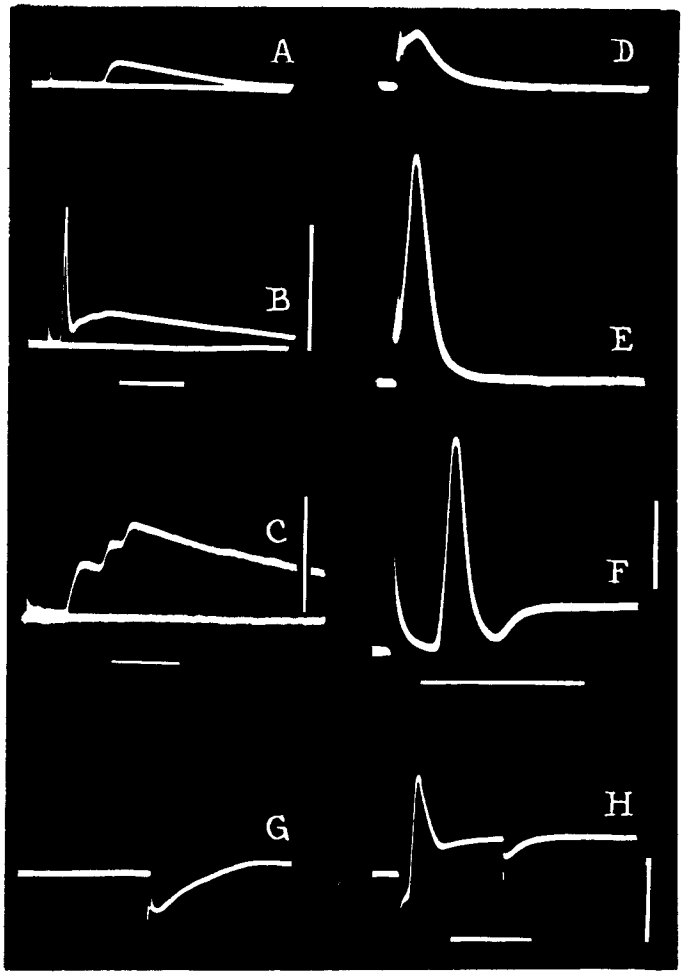

FIG. 3. Internally recorded potential changes of motor giant axon. A, B, and C: responses to presynaptic stimulation. A single central axon causes response A. All central giant axons active for responses B and C. C: preparation deteriorated. Calibration: A and B 50 mv.; C 5 mv. Time: 10 msec. D, E, and F: comparison between directly elicited responses (D and E) and synaptically elicited response (F). Calibration: 25 mv. Time: 5 msec. G: effect of brief inward current pulse on resting membrane potential. H: effect of similar pulse during plateau of synaptic potential. Calibration: 25 mv. Time: 5 msec.

ords 3 A and 3 B were obtained in the same manner as were those of Fig. 2. However, in this instance the smallest effective stimulus caused a slow depolarization in the absence of a spike response (3 A). Upon increasing the stimulus other presynaptic axons were brought into action, resulting (3 B) in the dis-

charge of a postsynaptic impulse and increased amplitude of the slow depolarization. The rising phase of this increased slow depolarization displays a succession of peaks indicating that summation has occurred between synaptic potentials produced by asynchronously arriving presynaptic impulses. It is notable that the lowest threshold central axon in the circumstance of experiment acted upon the motor axon after the longest latency, the synaptic potential in 3 A corresponding to the last peak of synaptic potential in 3 B.

During progressive deterioration of the preparation yielding recordings 3, A, B, and C, the slow depolarization decreased in amplitude, the spike potential at first remaining essentially unaltered. At a critical stage the motor axon failed to generate an impulse; the spike potential disappeared leaving the three-step synaptic potential, of decreased amplitude, illustrated in Fig. 3 C.

A Comparison of Direct and Synaptic Excitation of the Motor Giant Axon.—To stimulate the motor axons directly a current of brief duration was applied to the third root between an electrode placed at the distal severed end of the root and one immersed in the saline bath. The third root being suspended in oil, with the oil-saline interface at the root-cord junction, where the internal recording electrode was placed, stimulus current density across the motor giant axon membrane should be maximal at the region of the internal recording electrode. Hence the potential change recorded should be that of the stimulated region. Synaptic stimulation was secured in the manner earlier described.

A directly applied stimulus current of subthreshold intensity gave rise to the brief depolarization illustrated in Fig. 3 D. This response in amplitude was graded according to intensity of the stimulating current until, at a certain value, the stimulus gave rise to a spike potential such as that depicted in Fig. 3 E. The spike potential directly elicited, as seen in 3 E, is not associated with a slow depolarization whereas the orthodromically elicited spike potential, seen in record F of Fig. 3, is followed by a slow depolarization. Records 3 E and 3 F were recorded through the same microelectrode.

Membrane Resistance.—When a current of given intensity is caused to flow from the electrode at the distal end of the third root to the saline bath, amplitude of the potential change recorded through the microelectrode should vary with the resistance of the axonal membrane at the region of the microelectrode. Record G of Fig. 3 displays the potential change obtained, in this manner, the axon membrane being in the resting state. By way of contrast Fig. 3 H shows the potential change obtained when a current pulse, of equal intensity and duration, is applied during the plateau of slow depolarization following an orthodromically elicited response. The potential change produced by the current in the latter instance is greatly reduced, a result that obtained even when the current pulse was applied during the declining phase of the slow depolarization. Otherwise put, there is associated with the slow depolarization, well into its declining phase, a decrease in membrane resistance.

Potential Changes during Repetitive Presynaptic Activity.—Fig. 4, A, B, and C illustrate potential changes caused by the action of two successive presynaptic stimuli applied at divers intervals to the cord. In each recording the response to a single stimulus and to two stimuli are superimposed by double exposure. The experiment shows the slow potential capable of temporal summation (by successive impulses in presynaptic giant fibers) as it was seen, in connection

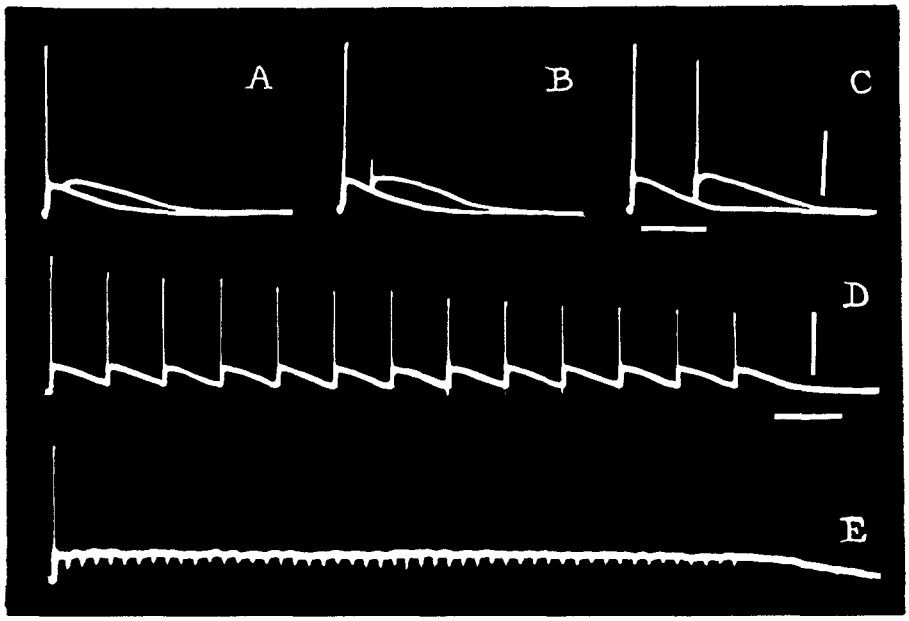

FIG. 4. Internally recorded responses produced by repetitive presynaptic stimulation. A, B, and C: responses to two shocks at various intervals superimposed on single responses. D: 12 per sec. E: 30 per sec. Calibrations: 25 mv. Time: 100 msec.

with Figs. 2 and 3, to be capable of spatial summation (by single impulses in several presynaptic giant fibers). The smaller spike potential appears to be due in part to conduction block at some point in the motor giant axon proximal to the recording locus and in part to reduction in amplitude of the electrotonically conducted blocked spike by virtue of decreased membrane resistance during the slow depolarization. Such conduction failure was not encountered in the very fresh preparation for which reason it may be considered a consequence of deterioration.

Fig. 4, D and E depict events as recorded during and following repetitive presynaptic stimulation. At a frequency of 12 per second (4 D) there is only partial summation of the slow depolarization and that which follows the last stimulus has essentially the duration of slow depolarizations caused by single

stimulations. At approximately 30 per second, however (4 E), summation is virtually complete and the membrane potential is, in effect, clamped at a level close to that of the peak of synaptic potential caused by a single presynaptic stimulus. Moreover, the potential remains at the "clamped" level for a long time after the close of stimulation and then returns to the original level over a time course that is much slower than that of return following a single stimulation.

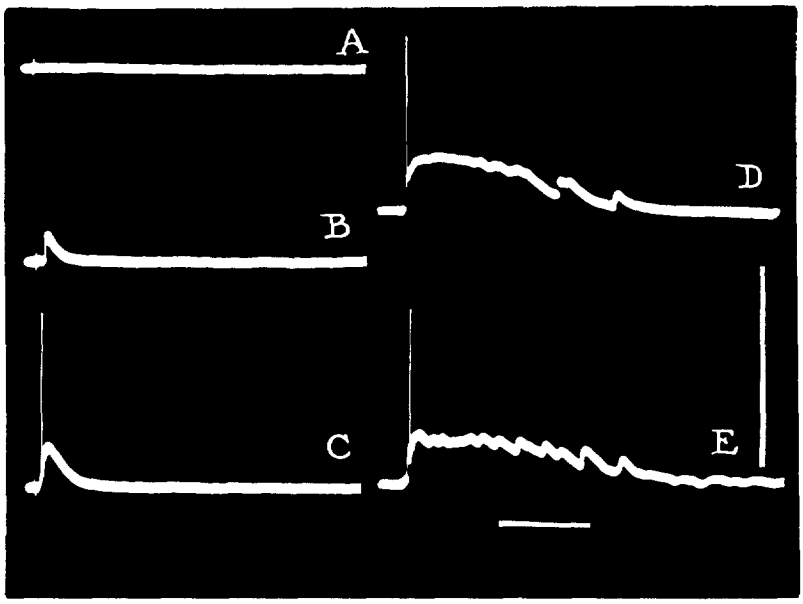

FIG. 5. Internally recorded potential changes of motor giant axon produced by a single presynaptic stimulation. A, B, C, and D (E): responses to incrementing stimuli. The stimulus intensity in D and E are the same but the time course of potential change is different in D and E. Time: 100 msec. Calibration: 50 mv. for all records.

At some invertebrate synapses, such as that between the first and second order giant axons in the squid, amplitude of the synaptic potential declines during the course of repetitive stimulation, even when the frequency of stimulation is too low for summation to occur. This phenomenon of fatigue, as described by Bullock (1948), although not encountered at the synapses under present discussion, is seen to occur in the crayfish synapses between central giant axons and the medium-sized axon of the third root.

Prolonged Synaptic Potentials Caused by Single Presynaptic Stimuli.—On some occasions a strong stimulus applied to the cord will give rise to prolonged synaptic potentials such as those exemplified in Fig. 5. The successive recordings

in Fig. 5 represent responses elicited by single stimuli of incrementing intensity. For record 5 A the stimulus was subthreshold for the central giant fibers. The weakest effective stimulus secured the synaptic potential presented in record 5 B. Increase in stimulus then produced a spike response of the motor giant axon and a synaptic potential increased in amplitude and to a small degree in duration (record 5 C). To this point the recorded potential changes are those customarily encountered. However, upon further increase in stimulus intensity, the enormously prolonged and complicated synaptic potentials represented in Fig. 5, D and E were recorded. The numerous small peaks seen on the plateau and declining phase of the synaptic potential bespeak the arrival at the motor giant axon of a succession of presynaptic impulses, from which fact it would appear that prolongation of the synaptic potential results from temporal summation. Since the strength of stimulation requisite for causing synaptic potentials such as those depicted in 5 D and 5 E usually is quite in excess of that sufficient to excite the central giant axons, it is reasonable to suppose that non-giant axons in the caudal part of the cord contribute to generation of the train of presynaptic impulses. Whether the non-giant axons act directly upon the motor giant axon or whether, in accord with the observations of Kao and Grundfest (1956), the non-giant axons secure this result by causing repetitive response of the central lateral giant axon which in turn acts upon the motor giant axon cannot be decided definitively, although the latter alternative appears the more probable.

DISCUSSION

It is the usual custom to regard synaptic potentials, or in a more general designation junctional potentials, as being caused by a transient conductance increment of the postsynaptic membrane produced by the action of presynaptic impulses (Fatt, 1954). At the neuromuscular junction the conductance increment endures only for the rising phase of the synaptic potential, the decay of the potential representing merely the discharge of charged membrane capacity through the resting membrane resistance (Fatt and Katz, 1951). The concept of brief junctional action and passive decay of potential has been justified for the neuromuscular junction by the finding of Fatt and Katz (1951) that analyses of end-plate potential and of electrotonus yield similar values for membrane resistance and capacity. A similar mechanism has been assumed for synaptic potentials of vertebrate motoneurons (Coombs, Eccles, and Fatt, 1955) without the same experimental justification. At the crustacean synapses under present consideration however, the conductance increase at the synaptic membrane does not appear to be "instantaneous," but rather to be enduring, indeed up to a fairly late stage of the synaptic potential.

True, a synchronous arrival of presynaptic impulses at different synaptic contacts might contribute, by virtue of temporal summation, to prolongation

of conductance change. However, the observations: (1) that rate of decay of the synaptic potential is substantially slower than the simple decay of charged membrane capacity through the resting membrane resistance; (2) that the synaptic potential redevelops after impulse discharge; and (3) that a decrease of membrane resistance is measurable at a late stage of the synaptic potential all can be made with respect to synaptic potential elicited by single action in a single presynaptic giant axon. Hence, one must conclude that time course of the synaptic potential in question, unlike that of the end-plate potential, is determined largely by the conductance change of the membrane, rather than by the "electrical time constant" of the membrane.

Inasmuch as the two aforementioned factors, at least, may be concerned in determining the course of a synaptic potential, and in varying importance, it is clearly a fallacy to argue that the decay of synaptic potential measures the time constant of the membrane and from this, that the "active phase" has a certain duration. For instance, it appears now that a distinction must be drawn between synaptic potentials of mammalian spinal motoneurons and end-plate potentials, for Frank and Fuortes (1956) have shown by direct test that the time constant of the motoneuron membrane is on the average 1.18 msec., a much lower value than the approximately 4.0 msec. value obtained by assumption as to the nature of the synaptic potential. One can only agree with Frank and Fuortes (1956) "that the long duration of the potential change evoked in the motoneurone soma membrane is not a consequence of the long time constant of this membrane itself, but rather of a similarly long-lasting change occurring elsewhere."

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