INTRACELLULAR RECORDING FROM THE GIANT SYNAPSE OF THE SQUID*

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(Received for publication, September 10, 1956)

Several authors have described intracellular recording from the post-junctional cell in synaptic regions, for example, in the neuromuscular junction (Fatt and Katz, 1951, 1953), in the brain and spinal cord (Brock, Coombs, and Eccles, 1952; Woodbury and Patton, 1952; Tasaki, Polley, and Orrego, 1954; Tasaki, Hagiwara, and Watanabe, 1954; Araki and Otani, 1955; Coombs, Eccles, and Fatt, 1955; Frank and Fuortes, 1955, 1956), in the sympathetic ganglion cell (R. Eccles, 1955), the electroplaque of electric fish (Albe-Fessard and Buser, 1952; Albe-Fessard and Chagas, 1954; Altamirano, Coates, and Grundfest, 1955), and in the large monopolar ganglion cells of certain invertebrates (Tauc, 1955; Arvanitaki and Chalazonitis, 1956; Hagiwara and Watanabe, 1956). Only in the nerve-muscle junction of the frog and the electroplaque of the eel, however, have the records been obtained from a region of the postjunctional cell known to be near the prefiber ending and only in the former is the junction supplied with but one input fiber. The giant synapse in the stellate ganglion of the squid between the giant fibers of the second and the third order offers a particularly favorable object for such recordings. Especially valuable is the opportunity to place microelectrodes internally in the presynaptic fiber close to the ending as well as in the postsynaptic unit, close to the junction. This preparation was studied some years ago with macroelectrodes on the surface of the ganglion (Bullock, 1948). A preliminary account of the present observations has appeared (Bullock and Hagiwara, 1955).

Materials and Methods

The stellate ganglion was isolated together with several centimeters of preganglionic nerve and postganglionic (last stellar) nerve from the squid *Loligo pealii*. The dissection was carried out in running and aerated, chilled sea water at about 14°C. After testing for the presence of normal transmission, the preparation was mounted on a glass slide inclined at a slight angle to facilitate insertion of the micropipette nearly parallel to the long axis of the nerve. The ganglion rested in sea water while the ante-

* Aided by grants from the National Institute for Neurological Diseases and Blindness (B-21) and the University of California.

rior end of the prenerve and the posterior end of the postnerve were lifted up into air for stimulation and external recording respectively. The whole vessel was surrounded by ice water, keeping the temperature between 15 and 20°C.

When the preparation is properly oriented and in a favorable light it is possible to see not only the tributary processes of the third order giant fiber but also the terminal digitations of the presynaptic second order giant (Fig. 1). The glass micropipettes filled with 3 M KCl were inserted, after a minimum of dissection, into the pre- or postsynaptic fiber approximately as shown in Fig. 1 and pushed longitudinally from 1 to several millimeters towards the region of the synapse. Long shank electrodes of 10 to 30 megohms resistance were used leading to cathode follower input units of 10 micromicrofarad capacity or less. Following the input stage, amplification was provided to a final sensitivity of 1 mv. per inch.

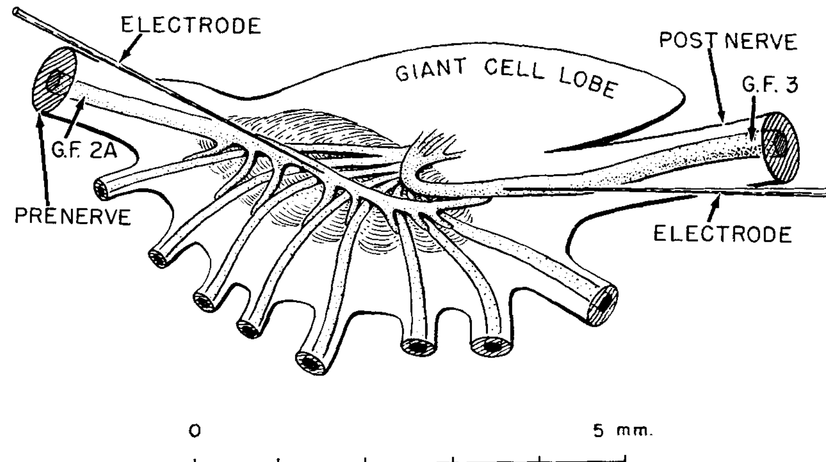

FIG. 1. Slightly diagrammatic representation of the preparation used, drawn to scale.

The preparations lasted up to several hours and those which failed early seemed to be associated with excessive effort to clean the preganglionic nerve of small fibers in order to prevent small fiber spikes from entering the ganglion. This precaution proved to be unessential since intracellular records showed little or no sign of deflection attributable to small fibers.

RESULTS

Form of the Postsynaptic Response to a Presynaptic Impulse.—The fresh synapse transmits in a one-to-one manner, and we therefore record a full spike. This looks very much like the ordinary spikes in the axon, but appears to be somewhat longer in duration. Measured from the first inflection to the beginning of the undershoot, values of 1.5 to 2 msec. are common at 18–20°C. We have always seen an undershoot in this position of recording, but it varies greatly in amplitude from about 2 to 5 or more millivolts.

The synaptic potential has been studied by fatiguing the spike until it drops out in an all-or-none manner. The potential that remains, which we have regarded as the synaptic potential, is not higher than 10 mv. and may be as small as 1 mv. as recorded at this distance. Since the process of fatigue has decreased the rate of rise and the amplitude of the synaptic potential, the fully normal unfatigued synaptic potential is not available to us for observation (*cf.* Kao and Grundfest, 1955 on squid axon local response).

The form of the synaptic potential recorded 2 mm. from the junction, that is much less than 1 space constant away is seen in Fig. 2. Its rise requires about 1 to 1.5 msec. and its fall about 2.5 to 3 msec. at a temperature near 21°C. The falling phase does not fit a simple exponential form. There is often a small undershoot which also would not be expected of a purely passive potential in

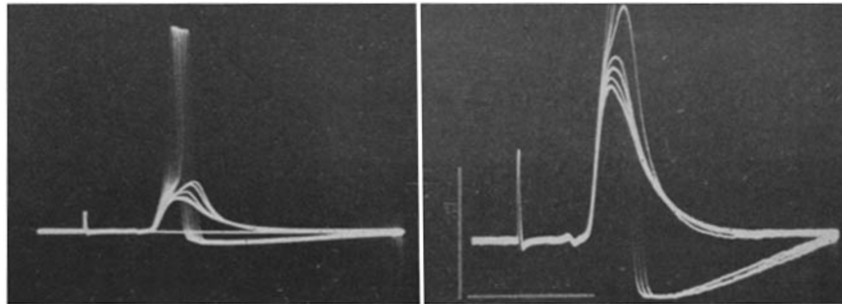

FIG. 2. The orthodromically transmitted response at the moment of greatest alteration with fatigue under high frequency stimulation, recorded inside the postfiber, at lower (left) and higher (right) amplification. Calibration, 10 mv. and 5 ms.

a simple leaky condenser cable. Further stimulation, bringing on further fatigue, seems to involve not only a decrease in the rate of rise of the synaptic potential, but a shortening of the duration of the rising phase, and a slowing of the falling phase. Every one of the features mentioned is quite variable from preparation to preparation. Thus no noticeable undershoot is recorded in some cases. There may be a considerable decline in amplitude with fatigue without any noticeable change in the duration of the falling phase. The crest time may not shift considerably to the left in some cases.

A few experiments were carried out in which an intracellular electrode in the prefiber close to the synapse was used as stimulating electrode. An indication that the amplitude of the prefiber action can determine the postfiber response was seen. It was found that we had control of the postsynaptic response amplitude. Over a small range of stimulus intensity transmission occurred but without setting up a postspike, only a postsynaptic potential of variable size. A slightly stronger shock elicited a postspike.

With the exceptions of the special experiments described in the last para-

graph, all effects of preganglionic nerve stimulation upon the third order giant fiber were all-or-none. Young (1939) described two giant fibers in the preganglionic nerve which end in relation to the third order giant fiber in the stellate ganglion at different levels, one closer and one farther from the cell bodies. As in the earlier experiments (Bullock, 1948) no physiological sign could be found of two separate effects. We have no direct evidence of which junction we are

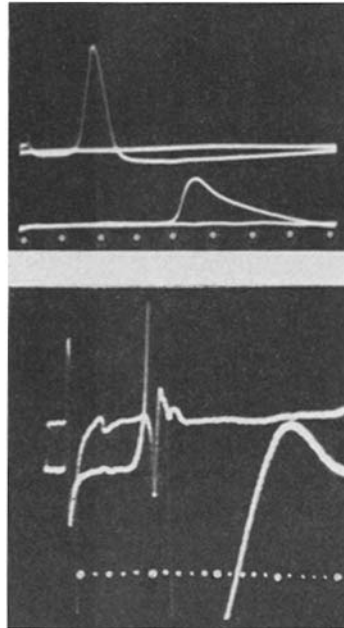

FIG. 3. Pre- and postsynaptic potentials recorded simultaneously. Upper, both recorded intracellularly at the positions shown in Fig. 1, the prespike at low amplification is a full spike, the postresponse, at higher amplification is a synaptic potential after the spike has been eliminated by fatigue. Lower, the postresponse, here a full spike going off scale, is recorded intracellularly on the lower beam; the upper beam is a steel microelectrode extracellularly recording both pre- and postspikes. Time marks, 1000 per sec.

studying but believe it more reasonable to attribute the observed synaptic potentials to the distal one, as shown in Fig. 1.

Synaptic Delay.—The suggestion has frequently arisen in the literature that in at least some preparations the true delay after correcting for prefiber conduction time would prove to be vanishingly small. Accordingly special effort was made in the present material to test this point. The evidence obtained is contrary to this suggestion. Simultaneous recording with microelectrodes inside the prefiber and inside the postfiber (Fig. 3) shows a delay between the

prespike and the postsynaptic potential of some 1 to 2 msec. The tips of these two electrodes are about 2 mm. apart and both are in fibers of large diameter. In the case of the postfiber recording, we can reasonably assume that at high gain the first deflection we see represents activity in the very region of initiation of postunit response, even if it is at some distance proximal to the electrode. The time of activity in the terminals of the presynaptic fiber is not so easy to establish, as distinct from preterminal activity. The internal electrode in the presynaptic fiber gives an approximation. We have measured from the crest of the prespike (Fig. 3) but there would be a considerable delay even if we measured from the beginning of the undershoot.

We have probed around the ganglion with an extracellular microelectrode of steel sharpened to 3 to 10 micra, hence visualizing on the same electrode both pre- and postspikes. In some cases the glass micropipette electrode has been inserted into the postunit while the steel electrode was in place. The delay as recorded is not sensitive to the position of the extracellular recording electrode. The latter may be in any part of the ganglion. At 15–20°C. the postsynaptic deflection starts 1 to 2 msec. after the prespike crosses the base line. The results agree with those obtained by intracellular recording. They also agree, within the range of variation, with the data reported in 1948 which were obtained by surface recording with macroelectrodes on preparations in air and hence assigned to the points of emergence of the pre- and postnerves from the ganglion. In the present measurements with extracellular steel microelectrodes there is no reason to assign the records to the same points because the preparations were immersed in sea water. Furthermore, in simultaneous records with intracellular electrodes in the postfiber and extracellular electrodes in the ganglion, as in Fig. 3, it can be seen that the discrepancy in time of first deflection of the postsynaptic response is only a small fraction of the whole delay—and can largely be attributed to the difference in amplification. If the same is true of the prespike, then there is a negligible correction for conduction in the extracellular microelectrode records. We did not succeed in simultaneously recording with this electrode and one inside the prefiber.

The exact value of synaptic delay is not the same from preparation to preparation but there is always a distinct delay.

Fatigue cannot be the main explanation of the variation. We find that fatigue produced by repetitive stimulation alters the rate of rise of the postsynaptic potential and hence the spike time but not the delay measured to the beginning of the postsynaptic deflection, in agreement with the 1948 report.

Amplitude of Prespike Seen across Postsynaptic Membrane.—A still better way to observe the delay would be to record the electrotonic sign of the prespike across the postsynaptic membrane. As long as it was possible to believe that there may be no real delay, it could not be expected with assurance that this prespike would be distinguishable from the postsynaptic potential. But now

we believe it must be separately discernible and the main question whose answer cannot be predicted is how large it will be. In most of our records, when the amplification permits recognition of signals as small as 1 mv., there is no sign of a prespike inside the postfiber (Fig. 4). In some of the high amplification records we see a deflection which must represent the prespike (Fig. 2, right, and 3, lower) but it is only 100 to 300 μv. which is similar to the amplitude

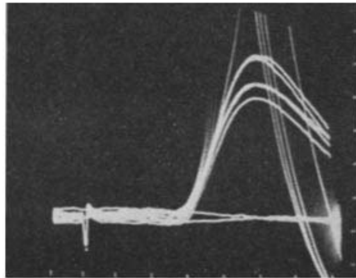

FIG. 4

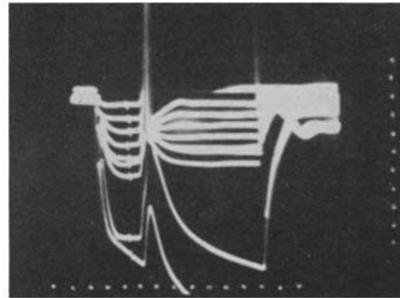

FIG. 5

FIG. 4. Intracellular record in the postunit at high amplification showing the usual finding of no detectable deflection attributable to the field potential of the arriving presynaptic spike. Calibration in millivolts and milliseconds.

FIG. 5. The effect of hyperpolarization of the postsynaptic membrane by applying a long square pulse through one penetrating electrode, recorded inside the postunit by a second electrode. The first deflection is the hyperpolarizing potential. Several sweeps are superimposed at different levels of membrane polarization from 3 mv. (above) to about 50 mv. (below, subtracting an artifactual component). The second deflection is the stimulus artifact of the shock to the prefiber. The third is the postsynaptic response—a spike in this unfatigued case, until high polarization is reached. At the off of the polarizing pulse another spike occurs as a consequence of the direct stimulus to the postmembrane. Calibration, 10 mv. and 2 ms.

of prespike recorded with an extracellular steel electrode. Smaller spikes could not be reliably discerned due to the noise level.

The reference electrode was not just outside the postunit membrane but far away, so that the true field voltage across the membrane is probably much smaller than that observed. After allowing for the decrement due to the 1 to 2 mm. between synapse and intracellular recording electrode, we still can estimate that the prespike is less than 1 mv. and often is not visible at all.

Polarization of the Synaptic Membranes.—At the junction of nerve and muscle Fatt and Katz (1951) in the frog and Hagiwara and Watanabe (1954) in the insect, found the endplate potential to be greatly enhanced by hyperpolariza-

tion of the postsynaptic membrane. In the motoneuron of the spinal cord Coombs, Eccles, and Fatt (1955) found no enhancement of e.p.s.p. amplitude.

Fig. 5 shows one record from a typical experiment on the squid synapse. We sometimes saw a small enhancement of the synaptic potential and in other preparations saw some depression, especially of the rate of rise, when hyperpolarizing up to 90 mv. above resting membrane potential. In these experiments the polarizing electrode lies close to the recording electrode, inside the postfiber and between 1 and 2 mm. from the synapse. Since the space constant is longer than this, a significant fraction of the imposed potential must be felt at the synapse. Unless we can assume that the fraction of the synaptic potential felt at the recording electrode has decremented more steeply under polarization than before, the near constancy of size of the observed potential indicates a real difference from the other junctions mentioned.

Hyperpolarization during transmission of the unfatigued spike first replaces the undershoot with a slowly increasing inside negativity, then at some critical level blocks the spike. Up to 80 mv. could be imposed, adding that much to the resting membrane potential, before blocking the spike, in some cases. The *absolute* level of membrane potential at which the synaptic potential flared up into a spike became higher than normal, even higher than the initial resting potential; *e.g.*, the height of the *potential change* from the 40 mv. hyperpolarized base line to flare up of spike was about 30 mv. in Fig. 6. This is probably somewhat above the unpolarized value although in the same experiment the preparation was too fresh to show a step before polarization. The spike exceeded 200 mv. in peak amplitude, in some cases.

Imposed potentials in the opposite polarity, decreasing the membrane potential, slightly decreased the synaptic potential if the base line had been shifted more than 10 mv. Reversal of the sign of the synaptic potential was not seen but greater depolarizations were not studied.

Interaction of Synaptic Potential and Antidromic Spike.—If an antidromic spike is allowed to enter and occupy the synaptic region just ahead of the beginning of a synaptic response to an orthodromic impulse arriving in the presynaptic fiber, the contribution of the latter depends on the phase of the former during which it occurs. We find no addition or subtraction of any appreciable potential to the peak or early falling phase of the antidromic spike. Only if the synaptic potential is elicited late enough that it can rise out of the later falling phase or the phase of undershoot is an addition to the potential seen (Fig. 6). It begins minimally and contributes more potential as it occurs later, but may not be fully normal in amplitude for more than a millisecond after the peak of the antidromic spike. The synaptic delay is considerably increased and progressively more so as the synaptic potential arises earlier in the recovery phase of the antidromic spike.

The question has been raised whether synaptic potentials can occur during

the absolute refractory period to direct electrical stimulation (Altamirano *et al.*, 1955; Grundfest, 1956). We cannot answer this by direct test in this preparation but only by analogy with the axon. We cannot say that a direct stimulus applied through our internal electrode or through external electrodes in the vicinity of the junction really tests the excitability of the synaptic membrane

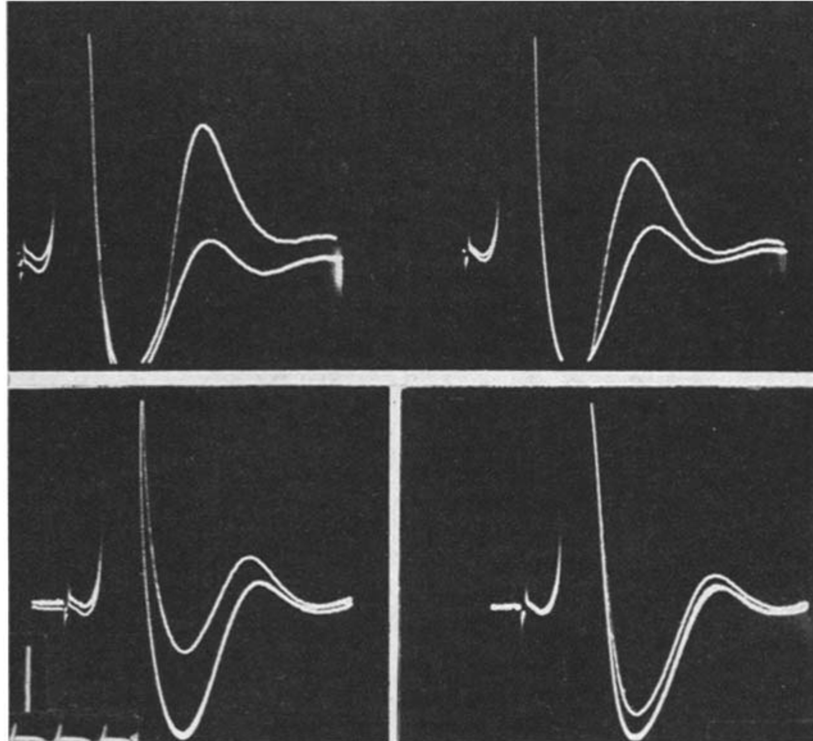

FIG. 6. Interaction of orthodromically transmitted synaptic potential and antidromic spike. The latter arrives first in each case and the former at an earlier phase in each successive picture. A large synaptic potential can rise out of the undershoot and a small one from the later part of the falling phase. Calibration, 1 mv. and 3 ms.

rather than an adjacent region of axonal membrane. By analogy with the axon the membrane would be refractory even to strong stimuli during the falling phase of a spike and we can therefore regard the synaptic potential as occurring during a time when that membrane is probably refractory to electrical stimuli.

Absence of "Synaptic Noise" and "Miniature Potentials."—In this preparation we have not seen any sign of "synaptic noise" or of "miniature potentials" at high amplification, in the absence of intentional prenerve stimulation.

DISCUSSION

One of the most interesting points discovered is the control of the postsynaptic response by the amplitude of the presynaptic action. By directly stimulating through the intracellular electrode in the prefiber close to the synapse, it was possible to vary the size of the postsynaptic potential over some range. At a critical intensity a postspike appeared. The stimuli used could not have excited the postfiber directly and must have elicited graded prefiber action. From the present evidence we cannot say whether the prefiber response was subthreshold or all-or-none. It is of the most general neurological interest to inquire whether specific neuronal interaction can occur without impulses; that is, whether a presynaptic neuron can excite at least a postsynaptic potential although itself not propagating a full all-or-none spike but only undergoing a graded, local response. It has been proposed (Bullock, 1953) that such interaction may be an important part of normal excitation by synaptic pathways, particularly in gray matter among short axon neurons and in invertebrate neuropiles in which afferent and efferent processes of a given neuron may be close together. If the present case proves to involve subthreshold prefiber activity, it would represent such a transmission without impulse, although, in all likelihood this is not a normal event for this particular junction.

With respect to synaptic delay, the evidence obtained forces us to the conclusion that there is a real delay between the arrival of the presynaptic impulse at the junction and the initiation of the postsynaptic potential, which cannot be attributed to conduction time in the presynaptic terminals. We can add nothing from these results concerning the suggestion (Bullock, 1952) that a considerable part of the synaptic delay is consumed inside the postsynaptic unit after transmission time has been completed and before visible response begins, but we can confirm the earlier report that there is no necessary increase in the synaptic delay with fatigue induced by repetitive stimulation.

Since the last conclusion removes the possibility that the presynaptic impulse would be lost in the beginning of postsynaptic response as seen inside the postsynaptic unit, it becomes possible to look for signs of the field potential of the presynaptic impulse across the postsynaptic membrane. The evidence reported indicates that this must be smaller than the noise level of our system in most cases, in a few however, reaching an amplitude of 100 to 300 microvolts. Since this is an order of magnitude or more smaller than the potential across the membrane necessary to stimulate the fiber with an abrupt electric shock, we must conclude that the available transmembrane current provided by the presynaptic impulse is too small for any known means of exciting the postsynaptic unit electrically. Terzuolo and Bullock (1956) have shown the physiological effectiveness of even such small potential gradients as these—of the order of tens of microvolts, in other preparations, but the essential condition for such

high sensitivity to potential fields seems to be that the cell is already active and this weak field modulates the frequency of firing. They report that the activation of a silent cell, even one which has been poised fairly close to firing, requires currents of at least 20 times greater intensity.

Del Castillo and Katz (1954) and Brock, Coombs, and Eccles (1952) also show small or indetectable presynaptic spikes in their records from intracellular electrodes in postunits, motor endplates in muscle, and ventral horn cells, respectively. It is impossible in these cases to establish the true delay, independent of conduction time in the terminals. The small prespike sometimes seen may be recorded at a discontinuity in the extracellular resistance some distance from the junction; *e.g.*, where the prenerve enters the main mass of tissue. The true prespike at the junction could therefore be obscured by the rising phase of the endplate or postsynaptic potential. These authors therefore, correctly, did not make a special point of the evidence against electrical transmission from the absence of a noticeable prespike across the postsynaptic membrane in those preparations.

Del Castillo and Katz (1954) placed weight instead upon the absence of detectable sign in the endplate of an electrotonic potential imposed on the nerve terminal adequate in amplitude to alter the frequency of miniature endplate potentials. While we now agree with their conclusion, it may be said that the argument suffers from the finding (Terzuolo and Bullock, 1956) that extremely small voltage gradients are adequate to modulate frequency of already active cells. Even the external field across the unit directly influenced (presumed to be the prefiber terminals) is probably less than 100 microvolts, the potential across any given membrane in the field still smaller. Thus the absence of visible potential change upon imposing the polarizing current is expected and does not represent a special attenuation across the postmembrane.

An attenuation of the normal prespike does occur, according to the present evidence, such that its field is weaker than 300 microvolts across the nearby postsynaptic membrane and this would appear to be a crucial argument against electrical transmission.

Hyperpolarization of the postsynaptic membrane has produced surprisingly large spikes (200 mv.) before blocking. There is some decrease in the overshoot, but it is not in proportion to the increase in membrane potential. The potential level, where the spike flares up out of the synaptic potential, shifts with hyperpolarization towards higher membrane potential levels. The postsynaptic potential is either slightly altered or not altered in amplitude—in agreement with the results of Coombs, Eccles, and Fatt (1955) but in contrast to the condition reported by Fatt and Katz (1951), and Hagiwara and Watanabe (1954). If this finding is correct it suggests that in the present preparation, the synaptic potential does not represent a short circuit across the membrane or have an equilibrium potential at which its amplitude becomes zero.

Also, in contrast to the neuromuscular junction and to the results with

the electroplaque of the electric eel, in the present synapse—a presynaptic impulse cannot cause any additional potential change on the crest or early in the falling phase of an antidromic spike in the postunit but begins to appear only late in the falling phase or during the undershoot. However, it does appear that the earliest part of this phase of possible addition of synaptic potential to falling phase of spike coincides with the period of complete refractoriness of postsynaptic axon. This rests upon analogy with the axon for we cannot directly test the excitability of the synaptic region to electric shocks at various phases of the recovery after an antidromic spike with any assurance that we are not exciting regions of the axon adjacent to the synapse. This finding is in agreement with the report of Fatt and Katz (1951) and of Grundfest (1956), although it is not as marked in the squid.

SUMMARY

1. Recording with glass micropipette electrodes inserted close to the synaptic region, in the presynaptic and in the postsynaptic fibers of the giant synapse in the stellate ganglion of the squid, has been accomplished.

2. The forms of the spike and of the synaptic potential are very much like those reported earlier (Bullock, 1948) from macroelectrodes. The crest time and the rate of fall are labile and depend on the state of fatigue, though the time of initiation of the postsynaptic potential does not.

3. It is concluded after examination of both intra- and extracellular recordings that there is a real synaptic delay of the order of 1 or 2 milliseconds at 15–20°C.

4. There is sometimes a very small and sometimes no visible deflection in the intracellular postsynaptic record attributable to the presynaptic spike. It is concluded that transmission cannot be electrical.

5. The amplitude of the postsynaptic potential can be controlled over some range by the amplitude of the presynaptic potential.

6. Hyperpolarization of the postsynaptic membrane results in increase in amplitude of spikes up to 200 millivolts, in increase in the membrane potential level at which the spike flares up, but in no considerable change in the amplitude in postsynaptic potential.

7. The postsynaptic potential can add to the late falling phase and the undershoot of an antidromic spike in the postfiber but cannot add to the crest or early part of the falling phase. The earliest part of the antidromic spike during which the postsynaptic potential can add is probably a period of refractoriness to electrical shock by analogy with the properties of the axon.

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