

# PROPERTIES OF A SINGLE SYNAPSE IN THE STELLATE GANGLION OF SQUID

THEODORE HOLMES BULLOCK\*

Department of Anatomy, University of Missouri School of Medicine, Columbia, and Department of Zoology, University of California, Los Angeles

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RECENT ADVANCES in understanding of the synapse have been made with preparations which approach the simplicity of the unit junction (2, 3, 16, 25, 26, 27). Study of the junctions on a single post-synaptic neuron has been achieved in the crayfish abdominal ganglion by Prosser (29) and in the vertebrate autonomic ganglion by Larrabee and Bronk (24). Kuffler (21) has succeeded in dissecting and recording from single neuro-myel junctions in the frog. The only certain neuro-neural junction between single units which has been studied appears to be the ephapse or artificial synapse created by two single giant peripheral fibers arranged in particular geometric relation (1). Closely approximating this condition are the preparations of Pumphrey and Rawdon-Smith in the cockroach (30, 33), and of Wiersma between giant fibers and motor neurons in the crayfish (36, 37). These have been discussed in recent reviews (8, 16).

The monograph of Young (39) on the giant system of the squid elegantly describes two sets of synapses, between second and third order giant nerve fibers in the stellate ganglion. The junction is remarkable for its size, relative simplicity and accessibility. Young (38) stimulated the preganglionic nerve and observed contraction of the mantle representing transmission through this synapse; he noted its ability to transmit the response to a single shock, to become fatigued reversibly and to conduct in the absence of cell bodies. In 1946 experiments were initiated (7) which showed this junction to be amenable to preparation in a manner permitting recording at the ganglion, as well as ahead of it and beyond it, while transmission continued apparently normally for some hours. The present paper describes the properties revealed by such a preparation.

## MATERIALS AND METHODS

The Woods Hole experiments were done upon *Loligo pealii*, those at Pacific Grove upon *L. opalescens*. No differences reasonably attributable to the species were noted. The lower temperature of the sea water and the greater abundance of squid in good condition may have been factors conducing to longer survival of the preparations at Pacific Grove. But this varied so greatly that the factors responsible were not successfully isolated. The longest-lived preparation transmitted impulses across the isolated ganglion for

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13 hours. More usual times were two to six hours and short-lived transmission lasted 15 minutes or less after the dissection was completed. The amount of stimulation required to precipitate fatigue also varied greatly but was never more than a minute or two at a high frequency (100 or more per sec.), proportionately much longer at low frequencies.

The usual preparation was made in the following manner. The squid was decapitated in front of the posterior wall of the brain case and the mantle opened with a midventral longitudinal incision. The mantle was pinned out in a special tray arranged with running sea water to a depth of about 2 cm. and with a window illuminated from below to provide transmitted light through the center of the animal. The viscera were removed. The special compartment of the body cavity accommodating the digestive gland was opened and the latter removed from behind forwards. Care had to be exercised as the forward and dorsolateral limits of this space were approached. The pallial nerves which include mantle connectives were seen running from the cartilaginous brain case, near the midline and dorsal wall of the compartment, backwards and laterally to perforate the side walls 2 or 3 cm. back. Keeping instruments to the midline and pulling the digestive gland, esophagus and salivary glands out piece by piece, there was little danger of touching the nerve (hereafter called the prenerve). When cleanly exposed, each was ligated as far forwards as possible, cut in front of the knot, and was then ready to follow through the cartilaginous lateral wall of the compartment, from which it emerged and very shortly joined the stellate ganglion. This was rather simple due to the generous size of the foramen which will admit the point of fine scissors without pressing on the nerve. If the lateral wall was first trimmed down from the ventral exposure to within a few millimeters of the foramen, it could then be opened by insertion of the scissors. Very little freeing of nerve from connective tissue remained to bring it to the ganglion. The last stellar nerve (one of 8-10 postnerves, each containing one giant postfiber) was dissected in a manner similar to the standard preparation of the so-called giant axon of the squid (actually one of many giant fibers in the squid). The pen was removed, starting from the posterior end. The last stellar nerve was picked up in a portion of its course, about 2 cm. behind the ganglion, where it is least bound to the mantle by connective tissue and carefully followed backwards as far as desired for the proper length of postnerve, where it was ligated and cut. Very little dissection was required to follow it forward to the ganglion. An important step, if the prespike was to be recorded, consisted in removing the fin nerve component of the pallial nerve because small fiber spikes otherwise interfere with or prevent recognition of the giant. This was best begun at the ganglion where fine forceps could readily be inserted between the fin nerve, here an independent nerve, and the ganglion. Holding the fin nerve with the forceps, the fork between it and the prenerve could be carefully forced proximally with the point of the scissors. When sufficient length of prenerve thus freed of fin nerve was obtained (usually all the way to the proximal ligature), the latter was cut and then removed distal to the ganglion. The ganglion was then readily separated from the mantle to which it is only lightly bound with connective tissue, the anterior stellar nerves cut near their origins and the isolated preparation was complete.

In earlier experiments each of the stellar nerves was tied off individually, but this proved to have no advantage and in most experiments the ones from which recordings were not to be made were simply cut off close to the ganglion. In either case, tied or cut close to their origins, the effect was an early cessation of excitability in the corresponding giant fiber apparently extending proximally effectively to the origin of the fiber in the ganglion. No local response or other sign of activity of these units was recorded.

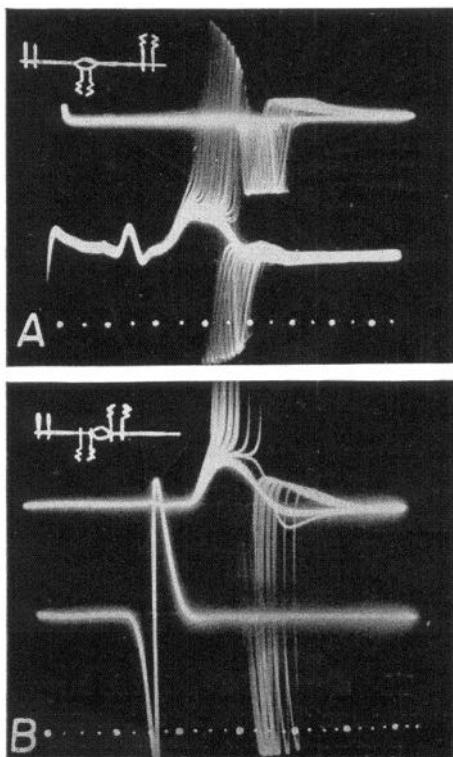
A number of experiments were done with the ganglion *in situ*, the mantle connective being freed and lifted on electrodes and one or more stellar nerves either lifted or simply approximated with electrodes while lying undisturbed on (last stellar only) or in (other stellar) the mantle musculature. Giant spikes were easily picked up through the mantle, even at some distance. The preparations were studied, except as otherwise noted, not at the temperature of the sea from which the squid came, but close to the air temperature of the laboratory, 22°-25°C. Sea water was used as the saline throughout. Electrodes were of fine silver or platinum wire. Stimulation was accomplished by means of rectangular or condenser discharge spikes of duration generally shorter than 0.1 msec. These were delivered to the tissue through low impedance, shielded, coupling transformers. They were generated by a circuit which provides two output pulses at separate posts with a variable interval between them, each separately controlled as to form and intensity. The first stimulus triggered the sweep of the cathode ray oscilloscope. Recording was accomplished

by two channels of identical, differential input, condenser-coupled amplifiers of standard design deflecting the two beams of a dual-gun cathode-ray tube. Controls showed that the frequency limits employed did not appreciably affect any of the dimensions described herein and that interaction between stimulators and amplifiers was negligibly small.

## RESULTS

*Character and identification of the response.* Figure 1A shows the character of the response in a fresh preparation undergoing progressive fatigue when both pickup electrodes are on the ganglion and a single, brief shock is delivered to the prenerve every 30 milliseconds (msec. hereafter). The first deflection is an all-or-none spike occurring at a time corresponding to a

FIG. 1. The prespike and postspike during development of fatigue. A: Single shock delivered at start (left) of each sweep of cathode ray beams recurring 33 times per sec. Inset diagrams show electrode arrangement in this and following figures: anterior (prenerve) always to the left. Simple vertical pairs of lines represent stimulating electrodes, pairs with terminal zig-zag represent leading-off electrodes, those directed upwards recording on upper beam, downwards on lower beam. Time in 0.5 msec. Note that prespike recorded at ganglion shows no fatigue, postspike does by arising later out of local potential and by falling amplitude, finally failing abruptly leaving only local response at ganglion and base line at distal electrodes. B: Same, prespike recorded just ahead of ganglion. Note abortive spike. Time in 0.25 msec.



conduction speed in the prenerve of 5.8–16 m./sec. Its amplitude is larger than any other all-or-none component of the prenerve response. It can be followed, by moving the pickup electrodes, into and up the prenerve, but not into the postnerve. These properties, together with its sequelae in the ganglion, lead to the suggestion that it is the spike of the larger of the two presynaptic giant fibers of the second order described by Young (39).

These two giant fibers can be seen in the prenerve in both *Loligo pealii* and *L. opalescens* in the living state, under favorable illumination, the larger being from 1.1 to 1.7 times the diameter of the smaller. According to Young

the larger has its origin in a single cell in the visceral ganglion of the brain and there receives, in a giant synapse, endings of the first order giant fiber coming back from the lobus magnocellularis as well as other synapses. The smaller or "accessory giant fiber" has an entirely different origin in the brain, without obvious connection with the first order giant, and has a different relation to the third order giant (our postfiber). Its synapse with the latter is proximal to that of the larger prefiber, on the processes of the cells of origin of the postfiber before they have fused into a single giant fiber. I have not succeeded in recognizing its spike or effects on the postfiber.

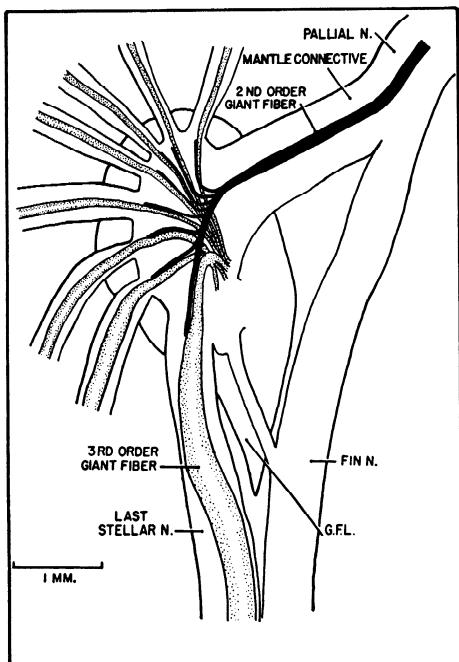


FIG. 2. Diagram of stellate ganglion in *Loligo* to show synapses believed to be responsible for the transmission under study. Redrawn from Young (39) and confirmed in detail by original observations on living and on fixed and cleared ganglia. Finer anatomy of synapse, for which see Young, was also confirmed in celloidin sections by J. Rabin. G. F. L., lobe of stellate ganglion occupied by cells of origin of third order giant fibers.

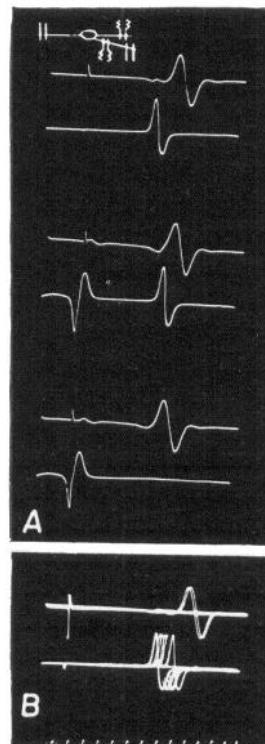
The second deflection is larger, unless electrodes are closer to the pre-nerve than the middle of the ganglion, is all-or-none, and can be traced into the postnerve but not into the prenerve. Its speed of conduction in the postnerve, its amplitude, and its exact correspondence with the lowest threshold, "giant" spike response to direct stimulation of the stellar nerve indicate that it is the action current of the third order giant fiber (our postfiber), the "giant axon of the squid" of many previous authors. Under pre-nerve stimulation it never occurs without the first described deflection preceding it, and in a fresh preparation it always occurs if the first deflection does.

If the above identifications of the spikes are correct, we must be recording transmission through the remarkable giant synapse described by Young (39). The general anatomy of this synapse is shown in Figure 2. The follow-

ing facts bear on the question whether the recorded spikes represent activity at one or at several of the unit junctions between the same prefiber and different postfibers.

Ordinarily all the postfibers are activated together since the whole mantle contracts every time a giant spike is known to be initiated either artificially or naturally in the prenerve of an intact preparation or one not artificially fatigued. Giant spikes can be recorded from any of the stellar nerves under these conditions by electrodes resting on the mantle (inside surface) over the nerve. But this synchronous activation is not due to an organic connection between postfibers—for example, at their origin in the giant fiber lobe of the stellate ganglion—since antidromic excitation of one postfiber does not activate others (38). Thus each postfiber must be separately excited through its own synapse with the prefiber. Furthermore, each can be independently fatigued without affecting others (Fig. 3). When any stellar nerve is cut within a few millimeters of the ganglion, transmission through its synapse soon fails and no

FIG. 3. Separate fatigue and lack of antidromic interaction in the Y-preparation (two postnerves used). A: A single shock is delivered to the prenerve (note artifact near left end of sweep) and results in transmitted spikes in both postnerves (initial phase up). On next sweep an antidromic impulse is initiated in lower postnerve by stimulus at start of sweep (spike with initial phase down), delaying transmitted spike in this nerve but not affecting other nerve. Third sweep same but transmitted spike in lower nerve failed to appear. B: Prenerve stimulated only, transmitted spikes in the upper postnerve fail early, those in lower nerve later although they show more increase in latency with fatigue. Time in 0.5 msec. applies to both A and B.



postspike is seen on recording from the proximal stump. Since the usual preparation in this study consists of a ganglion with all postnerves cut short but one, it is to be expected that all the synapses except that one will have failed after the first few minutes. That this is true can be shown by the character of the failure of the recorded postspike when the last remaining stellar nerve is damaged or under other conditions such as progressive fatigue. The failure is always all-or-none, whereas in the presence of one or more additional functional postunits failure does not usually occur at the same time for the separate units and the postspike recorded at the ganglion fails in steps. It may be concluded that in the usual preparation the responses recorded at the ganglion represent the behavior of a single synaptic junction.

*Delay.* The apparent synaptic delay, as seen in Figure 1A, is 0.8 msec. (24°C.), measured (34) from the first reversal point of the triphasic prespike (0.6–1.2 in other cases). It seems probable that, because of the conditions of leading off, the prespike recorded is essentially that of the point of entrance of the prenerve whereas the first deflection of the postsynaptic response is synchronous with the actual beginning of that response in the ganglion. The former conclusion is supported by the fact that if one electrode is placed on the prenerve and the other on the ganglion within a millimeter of the prenerve a good prespike will be picked up but no postspike or a very small one; if one electrode is placed on the postnerve and the other on the ganglion a millimeter from the origin of the postnerve, a large postspike but no prespike is led off. The second conclusion is supported by the fact that the moment of first deflection is not altered if the leading-off electrodes are moved distally so that the nearest is a millimeter or more from the ganglion (Fig. 5A). This is to be expected from the known electrotonic conduction of the local potential (see below). There is therefore intraganglionic conduction time included in the above figures, amounting to at least 2 mm. at something less than the conduction rate of the extraganglionic prefiber. The fiber is giant right to the synapse but is considerably smaller intraganglionically. Allowing 6 m./sec., which is more than half the maximum extraganglionic speed in this specimen, 0.3 msec. would be required as a minimum. It would seem that delays of 0.5 msec. and less must be attributed to this synapse until microelectrodes or other refinements permit recording more locally. Modifications of the delay are discussed below under fatigue and temperature effects but these act only to increase it.

*Polarization.* Transmission through this synapse is strictly polarized. Antidromic impulses in two postfibers simultaneously reaching the ganglion are as ineffective as a single one in producing an impulse in the prefiber. Attempts to detect even a small local potential of the prefiber in response to antidromic stimulation of the postfiber have been uniformly unsuccessful. Such stimulation may be continued for a long time without fatiguing the junction sufficiently to prevent normal transmission. It is more difficult to test whether any effect at all is left by antidromic stimulation; a small effect cannot be excluded but none was demonstrated in our experiments.

*Relation of pre- to postactivity.* Transmission through this synapse is always 1:1, that is, a single postspike follows every prespike in the unfatigued preparation. There is never an afterdischarge. Temporal summation occurs only in the partially fatigued state (see below). The frequency to which transmission will follow is at least 475 per second for short periods (this figure obtained in a preparation 13 hours old!). It is limited not by the properties of the junction but by the refractory period of the prefiber.

*Fatigue* readily supervenes with continued stimulation at high or even moderate frequencies in this preparation. It takes place only at the junction, pre- and postfibers being virtually impossible to fatigue in a reasonable length of time. As shown in Figure 1, the fatigue does not involve any change

in the recorded prespike close to the synapse. The same is true as far posteriorly as the prespike may be traced. In so far as the spike is a sign of the nerve impulse in the ganglion, fatigue does not occur in the presynaptic unit at all but entirely in the postunit. As recorded in or within several millimeters of the ganglion, failure in the postsynaptic unit is shown in Figure 1. The spike starts later and later, without changing its form or time relations, finally failing altogether in an abrupt, all-or-nearly-nothing manner. The disappearance of the spike does not represent the loss of all sign of response in the postunit. There is left a smaller deflection which, from its similarity to them, may be identified with the local potential of Katz (19) and of Hodgkin (18), the synaptic potential of Eccles (11) and the end-plate potential of Kuffler (21).

*The synaptic potential.* This potential is not all-or-none but graded according to the state of fatigue; it is not conducted any considerable distance but occurs with a decrement for a few millimeters beyond the ganglion and is then undetectable. It can be elicited by direct stimulation applied to the ganglion, even though subthreshold for a spike, and is then graded according to the strength of the stimulus. The progressively later origin of the spike and its failure while the synaptic potential is still present strongly suggest that the spike is normally initiated by a local response of a certain threshold amplitude and that fatigue is primarily a reduction in the amplitude of the synaptic potential—as has already been clearly indicated in the single fiber and the multisynapse and the isolated neuromuscular junction preparations of the authors previously cited. The local process is clearly detectable in the presence of the spike after some fatigue has taken place, presumably depressing its amplitude and delaying the spike origin sufficiently to notch the rising phase. It is not noticeable in the fresh state, presumably because its amplitude is sufficiently high that the threshold for spike initiation is reached while the ascent is still steep. In certain cases, when the recording has been confined to the ganglion and the ratio local response/spike is highest, the amplitude of the spike appears to fall with fatigue (Fig. 1A). This may represent the declining contribution of the synaptic potential to the whole response in the ganglion.

*The shape and time course of the local response* at the junction depend partly on the site of recording electrodes. As expected from earlier work, the crest occurs later the farther the electrodes are from the locus of maximum response, though the time of first deflection may not be later—conduction is at least partly electrotonic. As recorded from the ganglion in the region of the junction, the response is typically a monophasic negativity, rising to its peak in 0.5 msec. (extremes 0.3 and 1.5 at 23°C) and falling to 37 per cent of its peak height in 0.5 msec. (extremes 0.45 and 1.8 msec.). In some experiments a well-marked biphasicity, with a positive peak up to a third of the amplitude of the negative, was observed under conditions of recording excluding the possibility of a conducted response reaching the distal electrode (thus, one electrode on the ganglion or at the origin of the stellar nerve, the

other distally on the stellar nerve at a point separately shown to be entirely inactive during these responses). With the progress of fatigue the time of initiation of the synaptic potential does not change, *i.e.*, the synaptic delay measured to the first deflection is not increased though it is strikingly so measured to the start of the conducted spike. Similarly, there is no or very little change in the temporal course of the local response until fatigue has gone so far that the potentials are less than about one-tenth the just subthreshold height (for spike initiation).

An expression of the amplitude of the local response at the junction has little meaning if given in absolute units (volts); the most significant measure is in proportion of the spike height at the same place and only the maximum such proportion can have much meaning. Even this may be vitiated if the activity recorded at a certain electrode is not actually that of the same

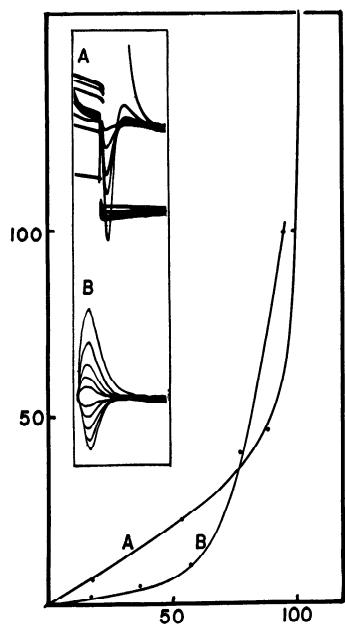


FIG. 4. Stimulus-response relation of subthreshold local potential at ganglion. Stimulating ganglion directly and leading off from distal pole of ganglion. Two experiments: A—Cathodal stimuli of amplitude shown by upward deflected square tops give local potentials of amplitude shown by downward deflections plus one spike which goes off scale and is seen as descending phase above base line. Plotted as per cent of threshold on abscissa, per cent of maximum recorded local potential on ordinate (spike off scale). B—Cathodal stimuli give responses above base line, anodal stimuli of same intensity below (stimuli not shown). Plotted as above but ordinates are cathodal response minus anodal to show development of nonlinear or nonelectrotonic, active, local potential.

part of the neuron in the case of the synaptic potential (which must be maximal in a certain junctional region and is conducted to nearby regions only with decrement) when measured alone and in the case of the spike (which because of increase in diameter may be much larger a short distance from the junction). The error will be in the direction of too small an estimate of the maximal subthreshold local response. It is considered to be a relatively small error on the basis of the large size of the synapse and the rather localized leading off, but microelectrode evidence on this point is lacking. The maximum fraction of a spike reached by a local response, without setting up a propagated spike, was 20 per cent. This is not very different from the figures of Hodgkin (18) and Katz (20) on single crab fibers (20 per

cent) and vertebrate myelinated nerve (10 per cent) respectively and those of Kuffler (21) and Eccles (11) on junctional potentials from the single myoneural junction (33 per cent) and multi-unit sympathetic ganglion (12 per cent) respectively.

The relation between amplitude of local response and intensity of stimulus directly applied to the ganglion is shown in Figure 4. Over most of the range of intensities it is a nonlinear relation. Weak shocks produce little or no active response and anodal and cathodal stimuli result in nearly symmetrical recorded potentials; stronger shocks produce an active response in the ganglion if they are cathodal and this grows out of proportion to the increase in intensity with an accelerating rate as the spike threshold is approached. This confirms the findings on peripheral nerve and the ephapse (1, 18, 19, 20).

*The problem of how local is the local response* has some interest and the present preparation lends itself to studies of the consistency of the area occupied and effects of grading the response since short term and reversible fatigue is so readily induced without the use of drugs. The most convenient measure is the half length or space constant, the distance within which the peak voltage falls to a half or other specified fraction of the height at the proximal of two leading-off electrodes, each referred to an indifferent distant electrode. The proximal electrode was placed for the following measurements at the locus of maximum amplitude of synaptic potential, *i.e.*, at the posterior pole of the ganglion where the last stellar nerve takes origin. This measure of area is not without shortcomings. If the two active leading-off electrodes are kept at a constant distance, different ratios between proximal and distal amplitude will be recorded in different preparations and these cannot safely be computed to a common space constant since this involves the assumption of purely electrotonic conduction, *i.e.*, the fall in voltage may not be a simple or passive function of the distance. If the distal active electrode is made movable and the point of 50 per cent amplitude is found by exploration, different distances will be found in different preparations and these cannot safely be compared since neither a common nor a uniform diameter of the stellar nerve may be assumed. This would also operate in the first case and any active, decremental propagation would complicate the second if it occurred to a variable extent in a fiber of nonuniform diameter. This is to say that the evidence indicates that the curve of voltage against distance has different shapes in different preparations. Even the curve from a given specimen has a doubtful validity, especially at the greater distances, under the conditions of recording used: the potential recorded at successive points along the nerve starts later and reaches a peak later, often becomes diphasic with initial positivity at the greater distances (above 8 mm.) and may finally be monophasic positive.

The amplitude at various points on the ganglion, referred to a far distal point on the stellar nerve, was usually not more than 5 per cent different; the probable actual point of leading off is the origin of the stellar nerve. The

observed point of 50 per cent amplitude ordinarily lay about 3 mm. from this origin. Extreme figures were 1.5 and 5.5 mm. At 3 mm. the amplitude in various specimens was from 30 per cent to 70 per cent of that at the ganglion.

Of particular interest is the fact that the area occupied by the synaptic potential may vary in the same preparation from moment to moment. Thus in different specimens the ratio of amplitudes at two fixed points was observed to be consistent within 2 per cent or less, to vary slightly in the region of questionable reliability of measurement and to vary by considerable and reliable amounts. In each case determinations were from photographs taken within a few seconds or minutes under relatively steady conditions such that the amplitude of the local response at the ganglion was constant or very nearly so. Typical figures from cases in which the variation is considered significant are: distal response fluctuates between 50 and 60 per cent of proximal at 1.5 mm. from ganglion, between 28 and 37 per cent at 9 mm. in another experiment, between 35 and 44 per cent at 3 mm. in another. The extreme figures were 26 and 46 per cent at 3 mm. The estimated limit of reliability varied from 2 to 5 per cent depending on the absolute size of the measured potentials.

A special study was made of the possibility of a systematic variation in area with fatigue and the diminishing size of the synaptic potential which is its sign. The same technic was used as above, *i.e.*, recording at two fixed points simultaneously to permit measurement of the ratio of proximal to distal amplitude while stimulating the prenerve at a frequency which produces a conveniently progressing fatigue. Rest periods of a few seconds then allow recovery of the local response amplitude and the cycle can be repeated many times (Fig. 5B and C). No reliable, systematic variation in area was found though local responses falling to a third and less were measured.

*Abortive and growing spikes.* The most conspicuous case of a variation in area of a local response is the abortive spike. These have been described by Eccles and O'Connor (14) and Kuffler (22) from the motor end-plate. They have often been encountered in the squid synapse preparation at a certain stage of fatigue and are easily recognized as distinct from the pure synaptic potential because they rise out of a typical synaptic potential in the same manner as a spike, with a marked notch and change of slope, only to fall again after achieving some fraction of the amplitude of a spike and without being propagated more than a few millimeters. The rate of rise of potential of the abortive spike is typically less than that in a normal spike. An example is seen in Figure 1B. The expression "all-or-none spike" retains its validity only because the abortive spike is very uncommon under ordinary conditions.

Closely akin to abortive spikes are slow-growing spikes. These begin like an abortive spike but gradually grow up to full size and propagate (Fig. 6). Related in form to these are the spikes of normal character which arise from the falling phase of the local response (last spikes of Fig. 1A).

Hodgkin (18) described and discussed such cases, showing that the spike actually arises some distance from the point of leading off, where the peak of the local response occurs later.

*Excitability cycle: refractoriness, subnormality, supernormality.* Any refractory period of the local response at the ganglion in the fresh preparation is

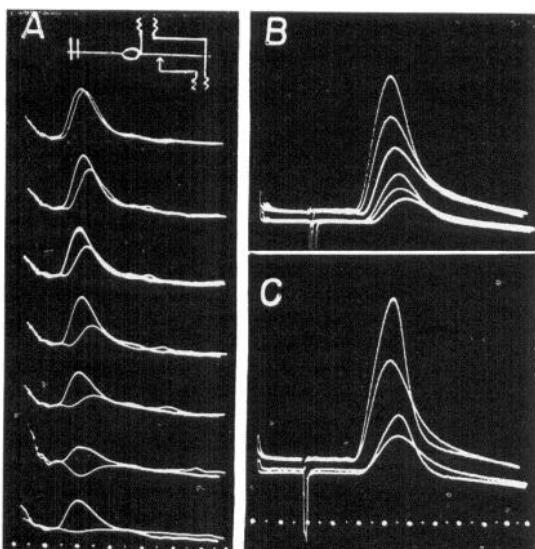


FIG. 5. A: Decrement of synaptic potential with distance. Roving electrode at (top to bottom) 0, 2, 3, 8, 11, 12, and 14 mm. from the posterior pole of the ganglion. Time in 1.0 msec. Fatigue has abolished the postspike. B: Area of local potential under fatigue. Three successive stimuli at a suitable frequency produce progressive reduction in height of synaptic potential (already fatigued till spike has failed). Electrodes as in A, roving stationary at 3 mm. Successive sweeps occur to the right of preceding ones—note stimulus artifacts. Allowing for this, form, time relations and area are unchanged by fatigue. C: Same as B but a significant change in form of descending phase. Amplitude of largest response = 2.2 mv. Time in 0.5 msec.

phase, grows up to a full spike by the time it passes the distal electrode. The local responses show various degrees of propagation.

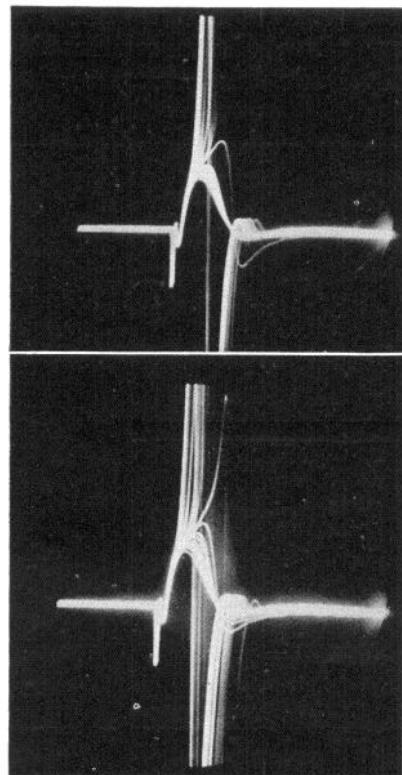


FIG. 6. Abortive and growing spikes. A: Repeated stimuli produce fatigue, spike failing intermittently, leaving local synaptic potential and once an intermediate, briefly propagated but dying "abortive spike." B: Same but the intermediate response, though short of total on the initial

shorter than that of the prefiber so that it can be tested only by direct stimulation of the ganglion. Under these conditions we cannot be certain that the portion of the system under test is the same as the actual synaptic region tested by indirect stimulation. In some experiments an absolutely refractory period of about 1 msec. was found, followed by a long period during

which the maximal response was less than the conditioning response—at first barely detectable and growing with longer intervals. Although the amplitude had returned to within 20 per cent by the end of 2 msec., some effect was detectable more than 5 msec. after the first shock (see Fig. 7).

In other experiments no absolute refractory period was found. Furthermore, the response at short intervals (0–0.3 msec.) is not a small one but actually greater than after long (1–2 msec.) intervals, and summates with any phase of the conditioning response (supernormality or facilitation, Fig. 8). In this manner two local responses, each subthreshold for a spike,

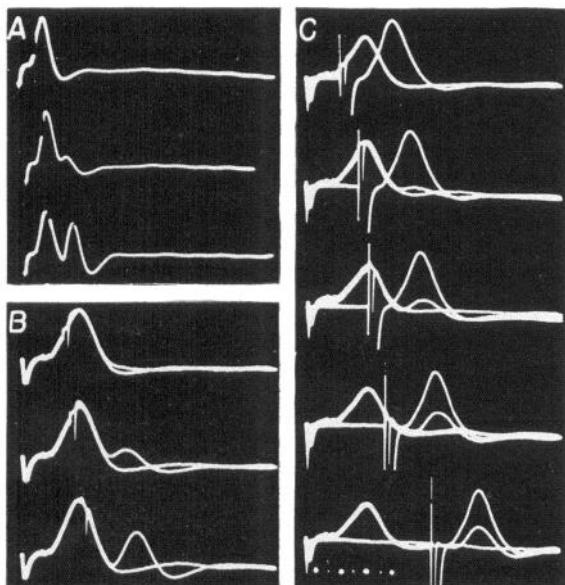


FIG. 7. Refractory period of local response at ganglion with progressive fatigue. Example of experiments in which a clear refractory period occurred. Conditioning shock at start of sweep delivered to ganglion directly (local response only—spike has failed). Test shock, shown by its artifact, delivered to same place; leading off from posterior pole of ganglion. A, B, and C are successive experiments on the same preparation. In B double exposures give conditioning shock alone and with test shock. In C triple exposures give these plus test shock alone (which is not identical in time-constant or intensity with first stimulus; both are near but not quite maximal). Time in 0.5 msec. applies to B and C. Comparison of responses at similar intervals between shocks in A, B and C shows progressive increase in refractoriness.

could be made to summate to a height adequate to initiate a spike. The conditioning response may have a depressive effect on the amplitude of the second response beginning at about 0.5 msec. and detectable for 5 msec. or more. Even when the effect was so small as to be uncertain by inspection of photographs, it could be demonstrated by making the testing shock just threshold for a spike by itself, whereupon a conditioning shock, even if it is strong enough to produce only a local response, prevents the spike after the testing shock (Fig. 9). In one experiment the effect of a conditioning local response lasted 1.8 msec. and that of a conditioning spike, 44 msec.! This depression by an antecedent response increases markedly with time and stimulation and is one of the primary aspects of fatigue. It early progresses to the point where the refractory period of the prefiber is no longer the limiting factor in frequency of transmission and the last experiment can be performed just as well by indirect stimulation.

It would seem that such fatigue could account for the experiments in

which an absolute refractory period was found. Therefore the results indicating no absolute refractory period are considered more significant of the state of a fresh preparation. That these results are not unconnected with the actual synapse is suggested by the following variation of a typical refractory

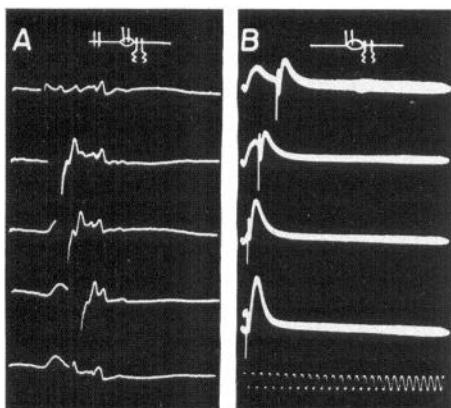


FIG. 8. Summation of local response. Examples of experiments in which an absolute refractory was lacking. A: First shock delivered to prenerve in fatigued preparation, spike having just failed, produces local response (first deflection, bottom record). Second shock is delivered to ganglion at times before (top two), during (next two) and after (bottom) local response. Itself a subthreshold shock which does not even produce noticeable local responses, it sums with the synaptic potential to give transmitted spikes in middle three records, blocks the transmission of even the synaptic potential in the top. Fin nerve not well removed, small fiber activity pronounced. B: Both shocks delivered to ganglion, resulting in directly stimulated local responses (the two shocks are of nonidentical time-constant and intensity and are both submaximal, hence the difference between the two responses). Summated response at short intervals is greater than the sum of the two responses at longer interval. Time in 0.5 msec.

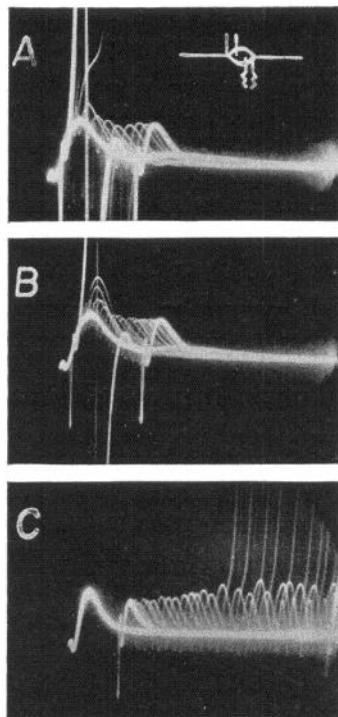


FIG. 9. The excitability cycle, summation, facilitation, depression and recovery. Pairs of shocks delivered directly to ganglion at various intervals, both shocks subthreshold and of nonidentical form and intensity. A and B: Summation at short intervals producing spikes (no refractory period), local responses depressed at middle intervals, facilitated at shortest. C: Recovery from depression—at longer intervals second shock, which has been increased to just threshold intensity by itself, can increasingly often produce a spike.

period determination. The conditioning shock is applied to the prenerve of a preparation fatigued just sufficiently to give only synaptic potentials and no postspikes. At various intervals after the arrival of the prespike a testing shock is applied to the ganglion directly. Responses led off from the origin of the last stellar nerve show that a practically undiminished response to the testing shock can occur on top of the response to the prespike, starting

simultaneously with it and running the same time course. It is not likely that the testing response comes from a portion of the postfiber significantly different from the conditioning synaptic potential, considering the conditions of leading off, the correspondence in crest times and the ready summation to postspike threshold (Fig. 8A) starting with proper-sized local responses. Pointing to the same conclusion is the experiment in which the second shock at the ganglion is reversed in polarity, *i.e.*, made anodal. The response to the first shock, whether it was a spike or a local response, is abruptly terminated. All the findings are in close agreement with the measurements on the peripheral giant by Pumphrey, Schmitt and Young (31).

*Antidromic stimulation* has revealed the following properties. Not only does no spike cross the synapse into the prefiber but no local response has been detected attributable to it. There is no evidence of a special cell body potential at the ganglion. The antidromic spike at the ganglion is all-or-none. If a prespike is sent into the ganglion at various intervals before and after the arrival of an antidromic impulse, a clash occurs just as though it were a peripheral nerve (Fig. 10). If the antidromic impulse arrives just after the local response to a prespike has started, it will cancel the latter and come through but with reduced amplitude. If the local response has risen nearly to its peak, the antidromic spike is canceled, *i.e.*, the clash occurs just distal to the ganglion. If the antidromic spike arrives first, no effect of the prespike is seen until its arrival coincides with the falling phase of the former when a local response can be seen superimposed. A fully normal transmitted spike can occur 1.25 msec. after the arrival of an antidromic spike. The transmission time in this case is longer than normal. An antidromic spike in one stellar nerve does not interfere with the transmission of a normal spike in another adjacent stellar nerve, nor does it itself produce a spike in any other stellar nerve. Prolonged stimulation of one stellar nerve, sending antidromic impulses into the ganglion, does not fatigue transmission in adjacent nerves.

*The effect of cold* was observed in a few experiments by substituting chilled oil at about 9°C. for oil at room temperature, about 24°C., as the fluid bathing the ganglion (Fig. 11). The effect on transmission, as distinct from the effects on the propagation of the spike which will not be dealt with here, can be divided into at least two separate actions, a lengthening of the delay measured to the first deflection of the local process and a depression of the slope and peak amplitude of the local process. The second results in a delay in the origin of the spike. Average figures from a considerable series of measurements on the same preparation are: interval between arrival of prespike at ganglion and start of local process increases from 1.14 msec. to 1.62 (maximum 4.30 in another preparation), interval between start of local process and start of spike increases from 0.33 to 0.86 (maximum, 2.53, another preparation, or infinity, *i.e.*, no spike initiated). In all preparations the effect is greater on the latter (delay in origin of spike) than on the former (apparent minimum synaptic delay) but the unknown intraganglionic conduction time may represent a large fraction of the apparent minimum

delay so that neither this relation nor  $Q_{10}$  values can be reliably stated on the present evidence. The change in duration of the synaptic potential, measured on a preparation fatigued sufficiently to initiate no postspikes but still with a large local potential, is not great—about 33 per cent with respect both to crest time and to decay time (to 1/e of crest).

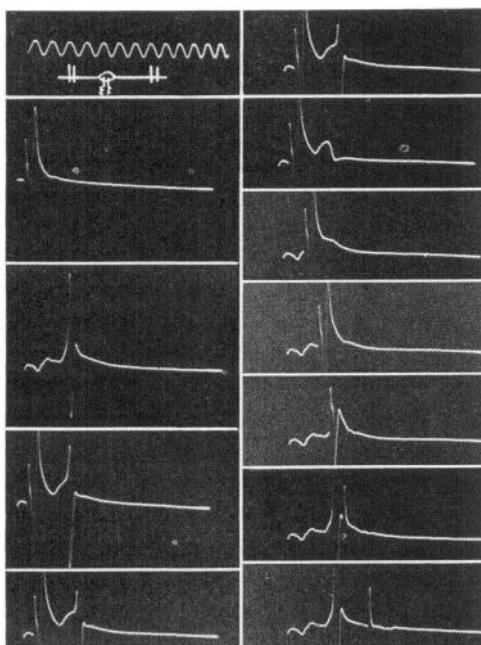


FIG. 10. Interaction of orthodromic and antidromic impulses. First record shows antidromic impulse alone, second normally transmitted impulse, preceded by small prespike. Successive records with antidromic impulse arriving first, then second. When it arrives after local response of orthodromic impulse has just started (third from last), its initial phase is depressed and second phase virtually lost, but it does enter the ganglion and finds the region of local response not absolutely refractory, preventing the development of the synaptic potential into a spike. Fourth record shows depression of synaptic potential and of initial phase of orthodromic spike, but the latter propagates into increasingly recovered regions and grows to normal in its second phase. Time in 1.0 msec.

(only a short length of prenerve cooled), large effect on origin of synaptic potential, its rate of rise, time of origin of spike and on second phase of spike. Time in 0.5 msec.

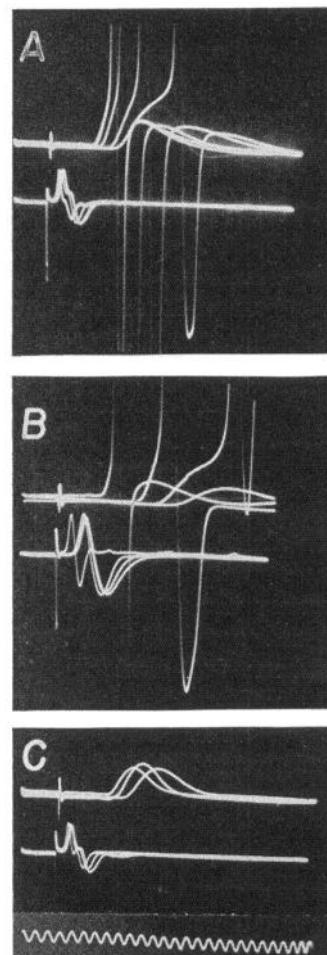


FIG. 11. Effect of cold on synaptic transmission. Earliest response in each case in oil at approximately 24°C., then preparation immersed in oil at approximately 9°C. and succeeding sweeps occur while it cools. Electrodes as in Fig. 1B, prespike on lower beam, postspike on upper. Note small effect on prespike

*Anoxia* has a similar action on both time of origin and slope of local potential. It has not been studied quantitatively.

An anticholinesterase, DFP (di-isopropylfluorophosphate), was tried in a few experiments to see how the present junction compared with those already tested under such agents (33). It was found that the junction is not significantly, if at all, more sensitive than peripheral giant fibers of *Loligo pealii* (10), that recovery may take place as easily as in the peripheral fiber and that no hypersensitive state or afterdischarges developed. Thus, a solution of 0.33 mg. per ml. of sea water required 14 minutes to block transmission, recovery took place in 9 minutes in sea water (and had not taken place at 6 min.). A solution of 1 mg./ml. required 7 minutes to block and recovery took place in 15 seconds in air. Re-exposure of the same preparation to DFP resulted in block in 2 minutes, recovery in 40 seconds, block in 30 seconds, recovery in 60 (in air), block in 30 seconds again and recovery in 60 seconds in air again. In each case recovery was not sufficient to permit a spike before the time stated. In each case the ability of the postfiber to conduct an antidromic spike was tested to make certain the effect was not taking place beyond the synapse, but the possibility that the effect was taking place in the prefiber ahead of the synapse could not be eliminated due to interference of small fibers since the giant fiber threshold was raised.

#### DISCUSSION

The single junction preparation has permitted evidence to be obtained on questions not readily answered when many units are in parallel. Thus, concerning the nature of the local potential, when a variety of shapes of rising and especially falling phase are recorded, even in the same preparation without moving electrodes, some significance may be attached to the observation. Often the decline of the potential, including the later portions, is far from exponential and may even be diphasic. Such facts suggest that the synaptic potential, while local and graded, is often propagated for a short distance while suffering decrement. The wide scatter of individual measurements of half length or space constant (from less than 1.5 to 5.5 mm.) in different preparations bears this out. There is still a clear distinction between ordinary synaptic potentials and abortive spikes (Figs. 1B, 6A), but if this suggestion is correct, they have a basic similarity in being propagated briefly. On this view, the variance noted in the ratio of peak heights at given distances as between successive local responses is perhaps more surprising for its small magnitude than for the inconsistency of the rate of decrement. Indeed, in many preparations no inconsistency in the area of the response could be reliably measured. And it is interesting to note that during fatigue, while the size of the potential drops to a small fraction, there is no appreciable trend in variations of area occupied.

On this view, also, the differences in distance to half amplitude of local potentials in other preparations reported in the literature take on a somewhat different aspect. Rather wide variations have been noted in the few

cases measured and it has been implied (13) that medullated nerve may differ systematically from unmedullated in the greater half length of the former. Crab nerve (18) shows a decay to half height in about 0.5 mm., frog medullated nerve (20) in about 3 mm., squid peripheral giant axon (31) in about 5 mm. (inferred from excitability tests). The squid falls into the class of the crab nerve with respect to thinness of myelin sheath but in the property under discussion it equals or exceeds frog nerve. Kuffler (21) was unable to give accurate figures for the single neuromuscular junction but it is apparent that there is quite a sharp fall normally. A great difference would seem to exist between this, the only other case in which the potential has been recorded at a single natural junction, and the squid synapse. Possibly this may be interpreted to mean that the squid synapse lacks a specialized end-plate region at the edge of which the decrement in the junctional potential could be expected to be steep. The area of contact between pre- and post-fibers is extensive—a matter of 800 micra—but this alone would not explain the difference if a sharply bounded specialized junctional region were present to localize the response. In the neuromuscular junction Kuffler was able to show that the end-plate potential right at the end-plate is as large as a spike. This may be true in the squid synapse but until the spike has been recorded in the short segment of postsynaptic fiber before full diameter is attained, it cannot be assumed.

With respect to the polarized transmission at the giant synapse in the squid stellate ganglion, it may be asked why this transmission should not be expected to be two-way, in view of the relatively simple, symmetrical gross anatomy of the junction and recent suggestions that polarized transmission is largely the result of asymmetrical geometry of contact (4, 5, 9, 28). "Explanations" of the observed one-way transmission can only be suggested at present, in the absence of additional information. They may be based on references to the lack of actual symmetry. Fiber diameter may be significantly different, the intimate contact is known to be asymmetric. Young (39) shows that contact is by numerous small perforations in an otherwise continuous sheath, with complex, short projecting processes of the postfiber abutting on a plane or slightly indented surface of the prefiber. It is possible that the effect described by Arvanitaki (1) in ephapses plays a role: artificial synapses arranged such that the impulse in the prefiber comes to the junction and ends there may transmit (their effect on the postfiber is terminated in cathodal excitation), whereas those arranged such that the pre-impulse can conduct past the junction do not transmit (presumably because their action is terminated in anodal depression of the postunit). An antidromic impulse in the natural synapse in the stellate ganglion presumably can continue past the junction, up the processes of origin of the giant fiber, into the lobe occupied by the cell bodies and the diameter of the critical region of the third order giant (which is still large for several hundred micra proximal to the junction) may be adequate to exert the necessary anodal effect. That this portion of the fiber, proximal to the synapse, is functional is

strongly indicated by the presence of the synapses with the accessory giant fibers (not yet physiologically recognized) on the tributaries of the third order fiber before they have all joined to form a single giant.

The magnitude of the synaptic delay takes on interest when compared with certain other values reported. Although precise figures could not be given with the methods here employed, it is apparent that the delay may be at least as short as 0.5 msec. ( $24^{\circ}\text{C}.$ ), probably shorter. This is of the same order as the shortest delays known at normal junctions—in the spinal cord of warm-blooded, higher vertebrates. Uncertainties still exist in these figures, too, and from essentially the same cause, but the recent values of Brooks and Eccles (2) are the shortest yet reported—0.3 msec. at  $37^{\circ}\text{C}$ . The value of 0.6 msec. at  $25^{\circ}\text{C}.$ , reported by Roeder *et al.* (33) in the cockroach, likewise indicates that evolution of the synapse in higher vertebrates, on present evidence, appears not to have achieved significantly faster transmission than that in some of the higher invertebrates.

The achievement of the squid, in evolving a giant synapse of special anatomy, is well shown by comparison with figures given in Fröhlich (15) for delay at the stellate ganglion of octopods. These animals lack the giant system but the response tested is the comparable one—a contraction of the mantle musculature. An average of 10 msec. ( $15^{\circ}\text{C}.$ ) is lost in the ganglion in unfatigued preparations. The squid has gained quite materially therefore and if a comparable gain may be assumed at the other synapse between (first and second order) giant units in the motor pathway from the brain, we may refine Pumphrey and Young's (32) estimate of the saving in reaction time due to development of a giant system. They estimate that *Loligo* should get under way after a visual stimulus in half the time (55 msec.) it would require if lacking giant fibers. This figure does not take into account the saving in time at synapses. Comparison with octopods would seem to justify a considerably greater estimate of time saved, both because of junctional delays and because of conduction rates in nerve fibers. Pumphrey and Young cautiously compared the squid figures (22 m/s) with an assumed 5 m/s in an animal lacking giants. The maximum rates reported by Fröhlich in the octopus, *Eledone*, are less than 1.2 m/s. Aside from these indications of the direction it would take, actual recalculation of expected total latencies would not be profitable in the absence of further quantitative information.

It has been shown that transmission in this synapse is 1:1, follows up to high frequencies and, in the absence of fatigue, shows no summation or facilitation. These properties, almost identical with those of the familiar vertebrate nerve-muscle junction (but not with those of the small-fiber system recently described by Kuffler and Gerard (23) in frog muscles), are probably less integrative than any other known neuro-neural synapse. The question may then be raised what functional significance in the life of the animal has such a junction. Unless in its normal function it suffers what we have called fatigue in isolated preparations, the presence of the synapse can hardly make any difference in the number and spacing of impulses reaching

the effectors from the brain. Even the loss of time in the ganglion is probably insignificant in the reaction time of the animal. One might be led to believe that this is an anatomical junction, whose meaning is developmental or trophic and that it has little if any functional importance in life—in these respects reminiscent of the present state of understanding of the junctions at segmental intervals in the central giant fibers of annelids and crayfish (5, 6, 8, 9, 35, 36). Before such a conclusion can be accepted it will be necessary to learn how the giant system is used in the intact animal and what effect is exerted upon the third order giant by the accessory giant fiber, an anatomically known mechanism which provides the possibility of some kind of integration before the final common path.

The study has revealed a similarity between the behavior of this synapse and that of peripheral nerve fibers (18), of the ephapse (1) and of the neuromyal junction (21). The significance of this lies in the further strengthening of the concept of fundamental similarity of the mechanism of propagation and synaptic transmission already enunciated by many authors. The potent and quantitatively precise arguments for an essentially electrical mechanism of propagation in the fiber which have been presented by a large number of investigators (see, for example, 17, 18, 19, 31) have been shown to a very considerable degree and in some detail to be applicable to transmission at the squid synapse. Finally it may be pointed out that the description of properties given here corresponds exactly to expectations from the electrical theory elaborated by Eccles (12).

#### SUMMARY

1. It is shown that a preparation consisting of the mantle connective, stellate ganglion and one or more of the postganglionic stellar nerves isolated from the body of *Loligo* can transmit impulses for periods of hours and is amenable to recording giant impulses in single units from preganglionic and postganglionic nerves and from the ganglion.

2. The observed spikes are identified with the giant system described by Young (39) which has a giant synapse in this ganglion for each of the efferent giant fibers, one to each stellar nerve. The preparation can be arranged to permit recording from several of the synapses or from only one.

3. The physiologic behavior of the system confirms the described anatomy with respect to isolation of the postfibers. The several synapses fatigue independently and antidromic impulses in one do not affect others.

4. No physiologic sign of the accessory giant fiber in the preganglionic nerve, which has a separate synapse on the same postfibers, has been recognized.

5. The properties of the junction are described. Transmission is polarized. Synaptic delay may be less than 0.5 msec. at 24°C., a value of the same order as the shortest known in mammals and 10–20 times less than that in close relatives lacking giant synapses.

6. Transmission is always 1:1. An afterdischarge has never been ob-

served. Temporal summation occurs only when the junction is partly fatigued. Under the same conditions facilitation is present but not marked. The synapse can transmit incoming impulses faithfully up to frequencies at least as high as 475 per second.

7. Fatigue does not change the action current of the prefiber as far into the ganglion as it can be traced. The postspike is exclusively affected, becoming later, then in some cases losing amplitude (probably due to reduction in the contribution made by the synaptic potential), and finally dropping out abruptly. After the cessation of the propagated spike there may still be recorded in or near the ganglion a graded, local potential which is identified with the local process, end-plate potential, and synaptic potential of Katz, Hodgkin, Eccles, Kuffler and others. This potential can be recognized before the spike drops out as an inflection in the rising phase of the spike but cannot be separated from the spike in the freshest preparations.

8. The synaptic potential of the unit junction rises to a peak in approximately 0.5 msec., falls to 37 per cent amplitude in about the same time, may reach a height of 20 per cent of the spike and decrements to half amplitude at about 3 mm. from the ganglion.

9. The synaptic potential becomes smaller with fatigue but, unlike the spike, it does not arise later. The synaptic delay measured to the first deflection at the ganglion does not increase though the sign of transmission in the postfiber, the propagated spike, is significantly postponed. The time course and the rate of decrement of the synaptic potential are not altered.

10. Significant variations in the area occupied by the local potential from moment to moment in the same preparation were observed. Reasons are given for the tentative conclusion that the synaptic potential is propagated for a short distance, decrementally, rather than being conducted entirely electrotonically from its site of initiation. Distinct abortive and growing spikes are recorded.

11. The local potential can be elicited by direct stimulation of the ganglion. The relation of its amplitude to the intensity of stimulation is given. The refractory period may be absent altogether in good preparations or it may be apparent and of various durations depending on the state of fatigue. The time course of excitability after a local response passes through a supernormal, a subnormal and then another supernormal phase in suitable preparations, but these relations are not fixed and wide differences are seen.

12. The consequences of antidromic impulses meeting dromically transmitted ones in the ganglion are described.

13. Cold can greatly prolong the synaptic delay and separately depress the local potential. The latter results in an even greater delay of the spike which may not be initiated for 6 msec. after the presynaptic impulse arrives in the ganglion. The change in time course of the local process is not great and is about the same for rise time and decay.

14. Anoxia has a similar action on time of origin and slope of local potential.

15. DFP blocks transmission, reversibly, in a manner similar to its action on the peripheral giant fiber as described elsewhere, and in about the same concentration. No hyperexcitable phase occurs.

16. The great similarity between the behavior of this preparation, the ephapse of Arvanitaki and the vertebrate nerve-muscle junction for twitch response, isolated by Kuffler, is pointed out. Comparison with the latter—the only other natural junction which has been locally studied—leads to the suggestion that the squid junction lacks a sharply bounded specialized junc-tional region comparable to the end-plate in its properties.

17. Discussion of the functional significance for the animal of such a simple, relay-type, 1:1 synapse without apparent integrative action, leads to the conclusion that any real meaning of the junction physiologically must depend on (a) the accessory giant fiber having a separate action on the third order giant from that exerted by the prefiber studied here or on (b) the junction in normal use suffering such long-continued, high-frequency incoming barrages that it is reduced to the stage called fatigue in the isolated preparations, when integrative mechanisms can come into play.

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