Chemically Mediated Transmission at a Giant Fiber Synapse in the Central Nervous System of a Vertebrate

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ABSTRACT The hatchetfish, Gasteropelecus, possesses large pectoral fin adductor muscles whose simultaneous contraction enables the fish to dart upwards at the approach of a predator. These muscles can be excited by either Mauthner fiber. In the medulla, each Mauthner fiber forms axo-axonic synapses on four “giant fibers,” two on each side of the midline. Each pair of giant fibers innervates ipsilateral motoneurons controlling the pectoral fin adductor muscles. Mauthner fibers and giant fibers can be penetrated simultaneously by microelectrodes close to the synapses between them. Electrophysiological evidence indicates that transmission from Mauthner to giant fiber is chemically mediated. Under some conditions miniature postsynaptic potentials (PSP’s) are observed, suggesting quantal release of transmitter. However, relatively high frequency stimulation reduces PSP amplitude below that of the miniature potentials, but causes no complete failures of PSP’s. Thus quantum size is reduced or postsynaptic membrane is desensitized. Ramp currents in Mauthner fibers that rise too slowly to initiate spikes can evoke responses in giant fibers that appear to be asynchronous PSP’s. Probably both spikes and ramp currents act on the same secretory mechanism. A single Mauthner fiber spike is followed by prolonged depression of transmission; also PSP amplitude is little affected by current pulses that markedly alter presynaptic spike height. These findings suggest that even a small spike releases most of an immediately available store of transmitter. If so, the probability of release by a single spike is high for any quantum of transmitter within this store.

INTRODUCTION

An important problem in the study of synaptic transmission is the relation between pre- and postsynaptic potentials. Although this relation has been determined in a number of instances of electrical transmission (5, 7, 8, 15,
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37), it is little known at most synapses where transmission is chemically mediated, because intracellular electrodes cannot be placed in both pre- and postsynaptic structures simultaneously. The only previously known exception is the giant synapse of the squid (17, 23, 27, 28, 33) but considerable data have also been obtained from the neuromuscular junction (21, 22), the chick ciliary ganglion (29, 30), and certain electroreceptors (cf. reference 6).

This paper describes a chemically transmitting synapse between large nerve fibers in the brain of the hatchetfish, Gasteropelecus. Although the fibers cannot be visualized in vivo, they can be penetrated by independently controlled microelectrodes and identified using electrophysiological criteria. Both pre- and postsynaptic structures can be recorded from simultaneously, and the input-output relation resembles in most respects that demonstrated or inferred for other chemically transmitting synapses. There are, however, a number of important differences, most of which can be explained by assuming that a single presynaptic impulse releases a large fraction of an immediately available store of transmitter.

The hatchetfish, Gasteropelecus, is a common aquarium fish imported from South America, and is so named because of its characteristic shape. Its enlarged, fan-shaped coracoid bones are fused and protrude to form attachment sites for the powerful adductor muscles of the pectoral fins (Fig. 1 A, Fig. 2). The fish is a surface feeder and its pectoral fins enable it to jump appreciable distances into the air, in what is apparently a fast escape reaction. It is also reported to use its pectoral fins to taxi along the surface with only the ventral portion of the body submerged. It has even been said to fly by flapping its fins rather than gliding as do other forms of flying fish (11, 38).

The chemically transmitting synapse described in this paper is involved in the control of the pectoral fin adductor muscles. The paper following this one describes the next lower synapse in the control system and the over-all reflex activity. This second synapse is electrotonically transmitting and is the first to be discovered in a vertebrate where the junctional membrane rectifies.

Preliminary communications of some of this work have appeared (1, 2).

METHODS
Animals about 1½ inches in over-all length (probably G. sternicla) were employed for most experiments. Curare (8–10 mg/kg) was given to prevent movement. The medulla and upper spinal cord were exposed from the dorsal side. Respiration was maintained by perfusion through the mouth with physiological saline for Electrophorus (25). The composition of this saline is 169 mM NaCl, 5 mM KCl, 3 mM CaCl₂, 1.5 mM MgCl₂, 1.2 mM Na₂HPO₄, and 0.3 mM NaH₂PO₄. Saline solution instead of aquarium water was used for respiration because of difficulty in keeping the perfusate out of the exposed region.

Conventional microelectrode techniques were employed. Intracellular stimu-
tion was ordinarily carried out by means of a bridge circuit and all illustrations of intracellular stimulation except Fig. 3 F, Fig. 4 C–E, and Fig. 6 A, A' were obtained using this technique. When the bridge was used, the membrane potential during the applied current could be determined indirectly by measuring the change in amplitude of a spike evoked during the current pulse (14). The relation between spike height and current gives a measure of input resistance on the assumption that the effective resistance at the peak of the spike is low compared to that at rest.

In most experiments, two independent microelectrodes were used. Usually a grounded shield was inserted between them to reduce cross talk, and any remaining contribution of cross talk due to spikes could be evaluated by grounding one or the other electrode. When the two electrodes penetrated the same fiber, direct measurements of potential during applied currents could be obtained. Electrotonic spread was also measured by applying current through one electrode in a bridge circuit while recording potential with a second electrode. In these experiments a spike was evoked by spinal stimulation during the current pulse. As a function of the current, $I_o$, three voltages were measured: the change in spike amplitude at the polarizing electrode, $\Delta V_{eo}$; the change in spike amplitude at the second electrode, $\Delta V_{e2}$; and the change in membrane potential at the second electrode, $V_s$, the second electrode being at a distance, $x$, from the first electrode. As seen below all three of these relations are linear over a sizeable range, and their slopes define resistances which can be denoted as $R_{eo}$, $R_{e2}$, and $R_s$, where for single measurements $R_{eo} = \Delta V_{eo}/I_o$, $R_{e2} = \Delta V_{e2}/I_o$, and $R_s = V_s/I_o$. $R_{eo}$ and $R_{e2}$ are effective resistances and $R_s$ is a transfer resistance (that is, the ratio of potential change at one point to inducing current applied at a different point). The “true” input resistance, $R_o$, is the membrane potential change recorded at the first electrode, $V_o$, divided by the current ($R_o = V_o/I_o$). $R_o$ and $V_o$ approach $R_s$ and $V_s$ as $x$ becomes small (provided radial voltage drops in the cytoplasm can be neglected), but where $x$ is significant, $R_o$ and $V_o$ can only be calculated from the directly measured resistances.

On the assumption that the change in spike amplitude is the same proportion of the change in membrane potential at each electrode, one can write $V_o$ as

$$V_o = \frac{\Delta V_{eo} V_s}{\Delta V_{e2}}$$

(1)

The same relation holds for the corresponding resistances as shown by dividing each voltage of this equation by the value of polarizing current.

$$R_o = \frac{R_{eo} R_s}{R_{e2}}$$

(2)

This value of input resistance is corrected for the effective resistance at the peak of the spike and for decrement due to separation of the two electrodes. The same data allow calculation of a space constant, $\lambda$, from the equation for electrotonic spread of potential along a uniform core conductor:

$$V_s = V_o e^{-x/\lambda}$$
or

$$\lambda = x / \ln \left( \frac{V_o}{V_x} \right)$$

(3)

Instead of using single pairs of voltage values, greater accuracy can probably be obtained by using the resistances $R_{so}$ and $R_{sz}$. From equation (1),

$$\frac{V_o}{V_x} = \frac{R_{so}}{R_{sz}}$$

thus,

$$\lambda = x / \ln \left( \frac{R_{so}}{R_{sz}} \right)$$

(4)

In some experiments, three independent microelectrodes were used. The caudal spinal cord and right muscle nerve were stimulated using pairs of fine silver wire electrodes 125 $\mu$ in diameter and insulated except at the tips. The electrodes were placed close to the structure to be stimulated after making a small incision in the skin.

RESULTS

Morphology

Hatchetfish possess two large Mauthner fibers which run the length of the spinal cord as in many other species. At the level of the fourth ventricle, these fibers are 40–60 $\mu$ in diameter including the thick myelin sheath (Fig. 1 B–D). On each side of the midline at this level there are also two other exceptionally large fibers which we have termed giant fibers. The cell bodies of origin are located somewhat rostrally, but since the axons taper markedly in this direction, the cell bodies have not yet been identified. Each giant fiber forms several axo-axonic synapses with the ipsilateral Mauthner fiber (Fig. 1 B–D) usually by short (10–20 $\mu$) myelinated processes from the Mauthner fiber, but there may also be a similar process from the giant fiber. Each giant fiber has a large (about 30 $\mu$ diameter) myelinated branch that crosses the midline and passes dorsal to the contralateral Mauthner fiber and then terminates ventrolaterally in the neuropil (Fig. 1 B). Each transversely running branch makes a single synaptic contact with a short process from the Mauthner fiber. The several ipsilateral synapses lie over an anterior-posterior distance of less than 0.6 mm. The cross-branches run transversely about 0.2–0.3 mm before synapsing on the contralateral Mauthner fibers. Electrophysiological evidence indicates that the space constants of both Mauthner and giant fibers are quite long. The morphological data are in agreement in that no naked axonal membrane has been seen other than that at the synapses, and the heavy myelin sheaths must provide substantial surface insulation for the fibers.
Fig. 1. Mauthner and giant fibers in the hatchetfish, Gasteropelecus. A, front and side views of the fish. B, section through the medulla showing a Mauthner fiber and two giant fibers on each side of the midline. The floor of the fourth ventricle lies in the upper part of the figure. The transversely running branch of one giant fiber crosses the midline from right to left. It synapses with the contralateral Mauthner fiber about 30 μ caudally. The Mauthner fiber on the left synapses with an ipsilateral giant fiber. (Toluidine blue stain of osmic acid-fixed material embedded in Epon and sectioned at 2 μ.) C, higher magnification of the synapse in B. D, a section about 10 μ rostral to B. The Mauthner fiber on the right of the midline synapses with each of the ipsilateral giant fibers.
From the synaptic region, the giant fibers send processes ventroposteriorly to the region of the large motoneurons that innervate the pectoral fin adductor muscles. Direct contacts have not been seen between giant fibers and motoneurons but electrophysiological data establish a synaptic relationship (3). The motoneurons innervating the adductor muscle lie primarily in the first spinal segment, although there are also a few in the second segment. The axons of the motoneurons are about 20 µ in diameter which is larger

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**Figure 2.** Diagram of the fish and reconstruction of the relations between Mauthner fibers, giant fibers, and pectoral fin adductor motoneurons. The diagram of the fish shows the central nervous system, the pectoral fin adductor muscle (m), and its innervation. The anterior of the muscle is supplied by a nerve running from the first spinal segment (n₁). Some caudal muscle fibers are innervated by a nerve from the second segment (n₂). The coracoid bones underlie the entire muscle. In the medulla, each Mauthner fiber (mf) makes several synapses with each ipsilateral giant fiber (gf) and a single synapse with each contralateral giant fiber. The cross-branches of the giant fibers are paired; the pairs are about 100 µ apart. Processes of each giant fiber synapse with each ipsilateral adductor motoneuron (mn) in the first spinal segment. There are about 40 motoneurons on each side of the midline, but for clarity only 3 motoneurons on one side are shown. Transmission from Mauthner fiber to giant fiber is mediated by chemically transmitting synapses (cs). Electrically transmitting synapses (es) couple the giant fibers and ipsilateral motoneurons.
than the other axons in the peripheral nerve, and they are readily traced in their course to the muscle (Fig. 2). There are 40–50 motoneurons on each side as determined by counts of fibers in the ventral root. The Mauthner fibers, giant fibers, and several motoneurons on one side are diagrammed in Fig. 2.

Identification and Properties of the Fibers

The Mauthner fibers were penetrated in the medulla and first spinal segment, generally somewhat caudal to the region of the cross-branches of the giant fibers. At this level, they were usually found about 50–100 μm on either side of the midline at a depth of 400–500 μm from the surface. The resting potential in the Mauthner fibers was usually about 70 mv inside negative, and the spike was about 80–90 mv in amplitude. The fibers were identified by the short latency (about 0.3 msec) of their response to stimulation of the caudal spinal cord (Fig. 3 A). This delay corresponded to a conduction velocity of about 80 m/sec, and simultaneous external recordings indicated that these fibers were the most rapidly conducting and lowest threshold elements in the spinal cord. In response to paired stimuli or brief tetani, they could conduct impulses separated by as little as 1.2 msec (Fig. 3 B). The rising phase of the spike was slightly faster than the falling phase, and neither phase showed an inflection or “shoulder” in uninjured axons. The duration at the base of the spike was about 0.5 msec. The spike was followed by a brief hyperpolarizing afterpotential that often was separated from it by a distinct inflection (arrow, Fig. 3 A). In two experiments, a Mauthner fiber identified by these characteristics was marked by intracellular iontophoretic injection of methyl blue (34). Dissection following formalin fixation confirmed the electrophysiological identification.

The giant fibers were also penetrated in the medulla and first spinal segment, usually at a depth somewhat greater than the Mauthner fibers. The resting potential was about 90 mv and the spike amplitude was often as large as 120 mv. Spikes evoked by stimulation of the caudal spinal cord had a latency of about 0.7 msec (Fig. 3 C). In response to graded spinal stimuli, a characteristic all-or-none component could be observed on the falling phase of the response (arrow, Fig. 3 D). As shown below, this component was the PSP due to activity of the higher threshold Mauthner fiber (the PSP produced by the lower threshold Mauthner fiber having evoked a spike at a lower stimulus strength). When a pair of spinal stimuli was given separated by an interval of about 5–100 msec the second spike in the giant fiber failed, revealing the underlying PSP from the Mauthner fiber (Fig. 3 E). This PSP could also be demonstrated by moderate hyperpolarization (Fig. 3 F) or by repetitive stimulation at frequencies that often could be less than 10/sec (Fig. 3 G). Another property was that graded depolarizations
could be evoked in a giant fiber by stimulation of the ipsilateral peripheral nerves (Fig. 3 H). These depolarizations were about 0.4 msec in latency, and often became large enough to excite the giant fiber. They were due to electrotonic spread from antidromically activated motoneurons, as will be shown in the following paper (3). The foregoing characteristics were used to identify a giant fiber, and in two experiments, iontophoretic injection of methyl blue and subsequent dissection confirmed the identification.

Figure 3. Characteristic responses of Mauthner and giant fibers. A, the spike in a Mauthner fiber evoked by spinal stimulation at the level of the dorsal fin. The arrow indicates a characteristic inflection preceding the afterhyperpolarization. B, spikes in a Mauthner fiber evoked by a pair of spinal stimuli separated by 1.2 msec. C, a spike in a giant fiber evoked by spinal stimulation. D, superimposed sweeps showing an all-or-none component (arrow) on the falling phase of the spike in a giant fiber as the strength of spinal stimulation was varied. This component was the PSP due to excitation of the higher threshold Mauthner fiber. E, the responses in a giant fiber to paired spinal stimuli that excited both Mauthner fibers. Failure of the second spike occurred at an interval between stimuli of 10 msec. The middle portion of the sweep is omitted. F, upper trace, hyperpolarizing current applied in a giant fiber. Lower trace, potential recorded by a second microelectrode in the same fiber. The spike was evoked by spinal stimulation during the current pulses. Superimposed sweeps show the spike and the underlying PSP, which was revealed when the hyperpolarizing current was increased sufficiently to block the spike. G, superimposed sweeps showing failure of the spike in a giant fiber when the spinal cord was stimulated at a rate of about 10/sec. H, graded potentials in a giant fiber produced by graded antidromic stimulation of the nerve innervating the ipsilateral pectoral fin muscles. All time calibrations, 1 msec. Voltage calibrations in A–G, 50 mv. In this and subsequent figures mf and gf signify voltages recorded from Mauthner fiber and giant fibers, respectively.
Input resistances of Mauthner and giant fibers were measured for hyperpolarizing current applied through a single electrode in a bridge circuit (see Methods). In six experiments, the input resistance of the Mauthner fibers had a mean value of 0.74 megohm and ranged from 0.63 to 0.98 megohm. In five experiments the mean input resistance of the giant fibers was 0.51 megohm, with a range from 0.35 to 0.73 megohm. These input resistances as measured would be somewhat lower than the actual values because of the finite resistance at the peak of the spike.

A more accurate method using two intracellular electrodes permitted simultaneous measurement of both input resistance and space constant (see Methods). In Fig. 4 B and 4 C are shown records from an experiment on a Mauthner fiber. The middle trace is the recording from one of the electrodes in a bridge circuit, the upper trace is the potential recorded by the second electrode 0.6 mm away, and the lower trace is the polarizing current. A spike was evoked during the current pulse by spinal stimulation. The graph of Fig. 4 A is from the same experiment and shows the change in spike amplitude recorded by the polarizing electrode, $\Delta V_{sp}$, the change in spike amplitude at the second electrode, $\Delta V_{ss}$, and the change in membrane potential at the second electrode, $V_{ss}$, all plotted against polarizing current, $I_o$. These three relations are linear. The calculated relation between the change in membrane potential at the polarizing electrode, $V_o$, and polarizing current (equation 2) is the line in Fig. 4 A with the greatest slope; the slope of this line is the corrected input resistance as discussed above. In this experiment the calculated input resistance was 0.86 megohm. The space constant calculated from the slopes of the change in spike amplitude at the two electrodes was 2.7 mm. In two similar experiments the input resistances were 0.76 and 1.02 megohms and the space constants were 3.6 and 2.9 mm. The means of the three values of input resistance and space constant were 0.88 megohm and 3.1 mm, respectively.

In three similar experiments on giant fibers, the input resistances were 0.38, 0.50, and 0.67 megohm giving a mean value of 0.52 megohm. In the same experiments, calculated values of the space constant were 3.2, 2.8, and 2.9 mm, respectively, giving a mean value of 3.0 mm. The values of input resistance of the giant fiber are for hyperpolarizations less than about 30 mv in which range the current voltage relations are linear. As shown in the following paper, large hyperpolarizations cause a 20-30% increase in input resistance of the giant fiber ($V_o/I_o$ where $x$ is small) and the current-voltage relations become somewhat nonlinear.

Fig. 4 D and 4 E show characteristic records obtained from the Mauthner fiber when linearly increasing or ramp currents were applied through one electrode while recording voltage through a second electrode. This type of current application was used, as described below, to produce large depolariza-
Figure 4. Input resistance and space constant of the Mauthner fiber. A–C, application of rectangular current pulses. Sample records in B and C, voltage-current relations in A. Two electrodes separated by about 0.6 mm simultaneously penetrated the Mauthner fiber. Rectangular hyperpolarizing current pulses were passed through one electrode in a bridge circuit (current on lower traces, voltage on middle traces) while recording directly with the second electrode (upper traces). Mauthner fiber spikes were evoked during the current pulses (C) and when no current was passed (B). Changes in spike amplitude as a function of applied current, $I_a$, were determined from recordings by the second electrode ($\Delta V_x$) and by the electrode in the bridge circuit ($\Delta V_o$) and plotted in A. Changes in membrane potential at the second electrode ($V_x$) were directly measured and also plotted as a function of $I_a$. The lines drawn through these three sets of points were fitted by eye. Changes in membrane potential at the electrode in the bridge circuit were recorded along with an unknown amount of potential due to bridge imbalance. The actual change in membrane potential, $V_o$, shown in A, was calculated as described in the methods (equation 1). (The values of $V_o$ indicate that the bridge was fairly well-balanced in C.) The calculated input resistance (equation 2) was $R_o = 0.86$ MΩ. The calculated space constant (equation 4) was 2.7 mm. D–E, effects of linearly increasing ramp currents. Separate experiments in D and E. Two electrodes separated by about 0.5 mm simultaneously penetrated the Mauthner fiber. Ramp currents (lower traces) were applied through one electrode while recording voltage with the other electrode (upper traces). D, two superimposed sweeps of equal amplitude hyperpolarizing and depolarizing currents. The slopes of the hyper- and depolarizing potentials were equal up until ±30 mV, corresponding to an input resistance of about 0.8 MΩ. At larger hyperpolarizing potentials, the hyperpolarizing resistance $V_x/I_a$ remained constant. At larger depolarizing potentials, the depolarizing resistance $V_x/I_a$ gradually decreased until at about 60 mV it was down by about 30%. E, independence of depolarizing resistance of the rate of current increase. Six superimposed sweeps. The voltage produced by a given value of current was little affected when the rate of rise was changed over the fourfold range illustrated. Calibrations the same for B and C.
tions in the Mauthner fiber without exciting a spike. The input resistances for currents of both polarities were approximately equal over the first 30 mv of potential change (Fig. 4 D). When hyperpolarizing currents were further increased, the resistance remained constant; i.e., the voltage trace remained linear with the same slope. When depolarizing currents were further increased, the resistance began to decrease; i.e., the slope of the voltage trace decreased. The decrease in depolarizing resistance \( (V_e/I_o) \) was presumably due to delayed rectification, and averaged about 20–30% when the potential was about 60–100 mv above the resting potential (Fig. 4 D and E). The rate of change of polarizing current had little effect on the shape of the voltage trace (Fig. 4 E) except when ramps were rising sufficiently rapidly to initiate spikes.

**Relation between Mauthner and Giant Fibers**

The synaptic relation between Mauthner and giant fibers was established in experiments in which the fibers were simultaneously penetrated. The Mauthner fiber was presynaptic, i.e. a directly evoked spike in the Mauthner fiber produced a PSP in the giant fiber (Fig. 5 A), whereas a directly evoked spike in a giant fiber did not lead to a PSP in the Mauthner fiber (Fig. 6 B). The PSP usually initiated a spike (Fig. 5 A and B), but hyperpolarizing the giant fiber (Fig. 5 E) or repetitively stimulating the Mauthner fiber (Fig. 7) could cause failure of impulse initiation and demonstrate the underlying PSP. The latency of the PSP was 0.3–0.4 msec measured from onset of the directly evoked spike in the Mauthner fiber to onset of the PSP in the giant fiber (arrows, Fig. 5 A).

As indicated in Fig. 2, a Mauthner fiber activates both ipsilateral and contralateral giant fibers. This relation was shown in many experiments using two electrodes where it could be clearly seen on which side of the midline the penetrated Mauthner and giant fibers lay. A directly evoked spike in a Mauthner fiber was always followed by a PSP in a giant fiber whether the fibers were ipsi- or contralateral. This relation between fibers was further demonstrated using three electrodes. In two experiments, a Mauthner fiber and one ipsilateral and one contralateral giant fiber were recorded from simultaneously. Direct stimulation of the Mauthner fiber excited both giant fibers (Fig. 5 C). In two additional experiments, both Mauthner fibers were penetrated while simultaneously recording in a giant fiber. Direct stimulation of each Mauthner fiber produced a PSP in the giant fiber which was of necessity ipsilateral to one Mauthner fiber and contralateral to the other (Fig. 5 F).

Stimuli were also applied to the spinal cord while simultaneously recording from Mauthner and giant fibers (Fig. 5 B, D, G, and H). As the stimulus strength was increased, excitation of a Mauthner fiber was always followed by a corresponding PSP component in the giant fiber. When the PSP from the
FIGURE 5. Synaptic relation between Mauthner and giant fibers. A, a directly evoked spike in a Mauthner fiber (upper trace, current on the lower trace) was followed by a spike in a giant fiber (middle trace); i.e., the Mauthner fiber was presynaptic. B, same fibers and display as in A. Spinal stimulation evoked spikes in the Mauthner and giant fibers that were separated by the same interval as when the Mauthner fiber was directly excited. C, a directly evoked spike in a Mauthner fiber (first trace, current on the fourth trace) evoked spikes in both an ipsilateral and a contralateral giant fiber (second and third traces). D, same fibers and display as in C, but spikes were evoked by spinal stimulation. Activation of the giant fibers by both Mauthner fibers is indicated by the occurrence of an additional component on the falling phase of each giant fiber spike. E, a directly excited spike in a Mauthner fiber (upper trace) produced a PSP in a giant fiber (middle trace) that initiated the giant fiber spike. The PSP was demonstrated by hyperpolarizing the giant fiber (current on lower trace, two superimposed sweeps in one of which the giant fiber spike was blocked). F, PSP's produced in a giant fiber by direct excitation of each Mauthner fiber. The two upper traces show directly evoked spikes in each Mauthner fiber; the third trace shows the PSP's in the giant fiber, and the bottom trace shows the intracellular current applied to one Mauthner fiber. G, upper and lower traces, recording from each Mauthner fiber; middle trace, recording from a giant fiber. Spinal stimulation. The threshold of the Mauthner fiber recorded on the lower trace was somewhat lower than that of the other Mauthner fiber. At threshold for this fiber, the giant fiber was excited only when the Mauthner fiber was excited (superimposed sweeps showing the Mauthner fiber excited and not excited). The inflection on the falling phase of the Mauthner fiber spike is an artifact caused by capacitative coupling between the microelectrodes which had not been adequately shielded. H, same fibers and display as in G, but stimulation at threshold for the other Mauthner fiber. When the second Mauthner fiber was excited, an additional component appeared on the falling phase of the giant fiber spike. All time calibrations 1 msec. All voltage calibrations 50 mv unless otherwise indicated.
lower threshold Mauthner fiber initiated a spike (Fig. 5 G), the second Mauthner fiber produced an additional component on the falling phase (arrow, Fig. 5 H; cf. Fig. 3 D, Fig. 5 C and D).

The latency of PSP's following spinal stimulation was identical to that for direct stimulation of the Mauthner fibers, provided a correction was made for the different time course in reaching the threshold of the Mauthner fiber spike (arrows, Fig. 5 A and B). Although there are several ipsilateral synapses and only one contralateral synapse, the PSP components from the two Mauthner fibers were usually of about the same size. Occasionally, the amplitudes could differ by a factor of up to four, but it was not determined which Mauthner fiber produced the smaller PSP's.

Mode of Transmission

A number of observations indicate that transmission at the Mauthner fiber, giant fiber synapse is chemically mediated. The most important evidence can be summarized as follows: (a) The PSP could be inverted by sufficiently large outward (depolarizing) currents. (b) No electrotonic coupling could be measured between the Mauthner and giant fibers. (c) There was a delay of about 0.4 msec between the presynaptic spike in the Mauthner fiber and the PSP in the giant fiber. (d) At low to moderate frequencies of stimulation, the presynaptic spike remained constant in amplitude, but the PSP could vary randomly. On the other hand, controlled variation of the amplitude of the presynaptic spike produced little or no change in the average amplitude of the PSP. (e) In certain circumstances, there were indications of transmitter release in discrete packets or quanta, as has been observed at a number of chemically transmitting synapses (18, 29). These observations are discussed more fully below.

Inversion of the PSP by outward currents is illustrated in Fig. 6 A. For these experiments it was necessary to pass currents too large to allow the use of the bridge circuit. Therefore, separate recording and current-passing electrodes were placed in the giant fiber and the PSP was evoked by spinal stimulation. The inversion indicates that there is a conductance increase associated with generation of the PSP that is more or less independent of the potential across the membrane (4, 16). An electrically mediated PSP cannot show this inversion, and the inference is that transmission must be chemically mediated. Measurement of the reversal potential of the PSP was complicated by the increased conductance due to the polarizing current. In Fig. 6 A, no PSP was observed when the potential was about 90 mv positive to the resting potential. In two other experiments, the measured reversal potential was about 100 mv above the resting potential. From these experiments and the estimated resting potentials, the reversal potential was probably close to zero membrane potential.
Figure 6. Reversal of PSP's in the giant fiber and absence of electrotonic coupling between Mauthner and giant fibers. A, inversion of the PSP by depolarizing current (five traces superimposed photographically). A spike was evoked in the giant fiber by spinal stimulation. Directly excited spikes can be distinguished at the beginning of the two weakest depolarizing pulses and the amplitude of the subsequent spikes diminished in these records. The PSP was not detectable about 90 mv above the resting potential which presumably was close to the reversal potential. Inverted PSP's are seen on the two upper traces. A', augmentation of the PSP by hyperpolarizing current (six superimposed traces, one without polarizing current). The orthodromic spike was blocked by the smallest current applied, and increasing hyperpolarization increased the amplitude of the PSP. B–C, the absence of electrotonic coupling between Mauthner and giant fibers. B, a spike in a giant fiber (middle trace) was directly evoked by depolarizing current (lower trace), but produced no measurable potential in the Mauthner fiber (upper trace). C, approximately 0.1 μamp of hyperpolarizing current in the Mauthner fiber (upper trace) blocked propagation of a Mauthner fiber spike evoked by spinal stimulation (bottom trace). Based on resistance measurements from other fibers this current would have produced about 60-100 mv of hyperpolarization. No measurable hyperpolarization was recorded in the giant fiber (middle trace). The spike in the giant fiber was unaffected. Presumably, the other Mauthner fiber produced the PSP that initiated the giant fiber response since with the usual sites of electrode placement, and if only a single Mauthner fiber were active, block of a spinaly evoked spike in the Mauthner fiber blocked the PSP in a giant fiber (Fig. 10 D). D, approximately 0.1 μamp of hyperpolarizing current (upper trace) blocked the giant fiber spike evoked by spinal stimulation (bottom trace). This current would have produced from 40-80 mv hyperpolarization. There was no measurable hyperpolarization in the Mauthner fiber (middle trace) and the Mauthner fiber spike was unaffected. Calibrations the same in C and D.
Hyperpolarization augmented the PSP (Fig. 6 A'), as is observed at many chemically transmitting synapses, but as discussed in the following paper (3), this property can also be exhibited at an electrotonic synapse. An estimate of the PSP reversal potential can be obtained from extrapolation of the change in PSP amplitude as a function of hyperpolarization. In Fig. 6 A' this value is only a few millivolts positive to the resting potential and in other experiments, the values ranged between 30 and 50 mv positive to the resting potential. Two factors probably contributed to the discrepancy between these estimated values and the directly measured ones. First, the measured values may be somewhat high because delayed rectification decreased the space constant, and the electrodes were at some distance from the synapses. Second, there was some increase in input resistance of the giant fiber as it was hyperpolarized (3). This change would have increased the degree of augmentation of the PSP produced by hyperpolarization, and caused the extrapolated reversal potential to be too low.

The degree of electrotonic coupling may be described in terms of the coupling coefficients (the ratio of voltage in the second cell to voltage in the first cell when current is applied in the first cell). The coupling coefficients for hyperpolarization were always less than the measurable limit of about 0.005 whether current was applied in Mauthner or giant fibers (Fig. 6 C and D). Depolarization that evoked spikes in a giant fiber caused no depolarization in a Mauthner fiber (Fig. 6 B), but, of course, a spike in a Mauthner fiber was followed by a PSP in a giant fiber.

To be valid, the measurement of synaptic delay and the demonstration of absence of coupling require that the electrodes be close to the synaptic region. In these experiments, the electrodes were always less than 0.5 mm apart and close to the synapses as judged by both their proximity to the fourth ventricle and the presence of a large PSP in the giant fiber. As noted above, the calculated space constants in both Mauthner and giant fibers are about 3 mm and the conduction velocity in the Mauthner fiber is very high. The processes forming the actual synapses are smaller in diameter than the main parts of the fibers, but calculations given in the discussion indicate that they are too short to allow for significant decrement or conduction time.

The presence of synaptic delay is characteristic of chemically mediated transmission although comparable delays can occur in electrotonic transmission (5). The delay measured at chemically transmitting synapses in cold-blooded forms is usually 0.5 msec or greater at 20–25°C (cf. reference 8). At the frog neuromuscular junction there is a minimum delay at 20°C of 0.4 msec measured from the peak negativity of the externally recorded presynaptic spike to the onset of the PSP (19, 20). The value of the synaptic delay in hatchetfish would be slightly shorter if measured in the same way.
Effects of Repetitive Stimulation

As noted above, transmission at the Mauthner fiber, giant fiber synapse was fatigued by repetitive stimulation at rather low frequencies (Fig. 3 G). This reduction in the PSP (postactivation depression) was studied by giving pairs of stimuli with periods of 1–5 sec between pairs. PSP's due to the second of a pair of directly evoked Mauthner fiber spikes failed to excite a giant fiber spike at intervals between stimuli as large as 100–500 msec. When the second Mauthner fiber spike followed the first at successively shorter intervals, the

**Figure 7.** Effects of repetitive stimulation on PSP amplitude. A, postactivation depression following a single directly evoked spike in a Mauthner fiber. Upper trace, depolarizing current in the Mauthner fiber; middle trace, recording in the Mauthner fiber; lower trace, recording in a giant fiber. Pairs of stimuli separated by varying intervals were given at about one pair per sec. The two stimuli and the evoked spikes in the Mauthner fiber remained of constant amplitude in all records. A1, the PSP due to the first spike in the Mauthner fiber initiated a spike in the giant fiber and this part of the sweep is omitted in subsequent records. The PSP due to the second stimulus 10 msec later was only about 4 mv in amplitude. A2, when the interval between stimuli was about 50 msec, the second PSP recovered to about 6 mv. A3, when the interval between stimuli was about 100 msec, the second PSP became threshold for a giant fiber spike (two superimposed sweeps with and without a spike). Recovery was incomplete because the PSP initiating the spike in A1 rose faster than the PSP in A2. Calibrations in A2. B, PSP amplitude in a giant fiber during a train of 51 directly excited Mauthner fiber spikes separated by intervals of 160 msec. The PSP remained below threshold for the giant fiber (about 15 mv) after the initial response. The three PSP's in response to the second through fourth stimuli successively increased in amplitude. The next 10 responses varied widely in amplitude, perhaps periodically. The remaining responses appear to have varied randomly except for a small downward trend.
PSP decreased in amplitude (Fig. 7 A). If pairs of spinal stimuli were used that were strong enough to excite both Mauthner fibers, the reduction of the second PSP to the same amplitudes as observed with direct stimulation of a single Mauthner fiber required shorter intervals between stimuli, since PSP's from the two Mauthner fibers summated. When a pair of spinal stimuli was separated by the shortest interval that still permitted excitation of both Mauthner fibers, the second PSP was only about 2–3 mv in amplitude. Except for the first few milliseconds of the period of postactivation depression, there was no alteration in the Mauthner fiber spike. The reduction in the PSP cannot be attributed to increased conductance of the postsynaptic cell, since excitability of the giant fiber measured by direct stimulation was unchanged following the PSP (other than for a brief period of refractoriness if the first PSP initiated a spike). Furthermore, stimulation of one Mauthner fiber had no effect on the PSP produced by stimulation of the other provided a spike was not evoked in the giant fiber. A possible explanation of the depression of transmission seen with paired stimulation is that the transmitter immediately available for secretion is depleted by the first stimulus. Recovery of PSP amplitude following such depletion would then be a consequence of replenishment of this transmitter.

The effects of previous activity on transmission were also studied by giving trains of stimuli at various frequencies separated by periods of rest. At moder-
ate frequencies of stimulation, the second PSP was greatly reduced (as in Fig. 7 A) but the amplitude of subsequent PSP's recovered to some extent (Fig. 7 B). In the next 10 or so responses, the PSP amplitude varied quite widely. These variations may have had a periodic component with a frequency of 2-4/sec. After several seconds, the variability decreased but in the steady state there continued to be considerable variation. Although further study is required, the amplitude histogram is probably unimodal and symmetrical about the mean. It does not appear to fit a Poisson distribution, because the dispersion of amplitudes is much smaller than would be expected for this distribution.

The mean amplitude of the PSP during the steady state varied inversely with frequency (Fig. 8). After several minutes of stimulation at frequencies of 10-20/sec, the PSP often broadened (Fig. 9) revealing many small components resembling “quanta” seen at a number of chemically transmitting synapses (9, 18, 26, 29, 31). After a short period of low frequency stimulation or rest, the shape of the PSP returned to normal. The changes observed suggest a desynchronization in the release of transmitter. When resolvable, the components were about 0.3–0.5 mv in amplitude. As described below, similar components could be evoked by depolarization of the Mauthner fiber. Under normal conditions, the rate of spontaneous occurrence of small potentials...
resembling the PSP components in Fig. 9 was no more than a few per second. During a period of desynchronized release, these small potentials occurred much more frequently, and one can be seen at the start of the sweep in Fig. 9 D. Probably these potentials were due to spontaneous release of transmitter, but the possibility that they were PSP’s produced by impulse activity in other neurons was not excluded.

When the frequency of spinal or direct stimulation of the Mauthner fiber was increased to 20–40/sec, PSP’s in the giant fiber further diminished in amplitude (Fig. 8 A–F). The PSP’s exhibited a continuous range of amplitudes and after some seconds of stimulation approached the amplifier noise level, which was usually about 50–100 μV. No “failures” of transmission were observed; that is, there was always at least a small PSP. The coefficient of variation (standard deviation divided by the mean) did not increase, as it would have been expected to do if the reduction in PSP size were due to reduction in the number of quanta released. Assuming that the 0.3–0.5 mv components observed correspond to normal sized quanta of transmitter, either the size of a quantum or its postsynaptic action must be considerably reduced at higher frequencies of stimulation.

**Relationship between Presynaptic Potential and PSP Amplitude**

The PSP in a giant fiber was unaffected by current pulses that altered the amplitude of a Mauthner fiber spike over a wide range. The amplitude of a propagated spike in a Mauthner fiber could be changed ±25% by current pulses applied close to the synaptic region, but these changes had no effect on the amplitude or time course of the PSP’s. The amplitude variations normally observed (Fig. 8) were still present, but their mean and distribution were changed little, if at all, as may be seen from the superimposed sweeps in Fig. 10 A–C. In this same experiment, 50 measurements of PSP amplitude were also made for each case, i.e. with no current applied in the Mauthner fiber, as in Fig. 10 A; with depolarizing current, as in Fig. 10 B; and with hyperpolarizing current, as in Fig. 10 C. The means and amplitude distributions in each case were essentially identical. Fig. 10 E and 10 F from another experiment show the absence of an effect in successive sweeps with and without polarization. These records are representative of many additional trials. In these experiments, propagation in one Mauthner fiber was blocked by injuring it with a coarse microelectrode, and then the other Mauthner fiber was penetrated. The spinal cord was stimulated at a rate adequate to cause failure of impulse initiation in the giant fiber. The recorded PSP resulted solely from activity of the penetrated Mauthner fiber, because if propagation in it was blocked by hyperpolarization, the PSP failed completely (Fig. 10 D). The range over which spike height could be varied was limited to the changes produced by subthreshold depolarization and by hyperpolarization insuffi-
Figure 10. The lack of dependence of PSP amplitude on presynaptic spike height. A single Mauthner fiber was activated by spinal stimulation (see text) at a rate sufficiently fast to reduce the PSP below threshold for excitation of the giant fiber. Stimulation was maintained at this rate long enough for mean PSP amplitude to reach a steady-state value. The amplitude of the spike in the Mauthner fiber was augmented by hyperpolarizing currents (C and E), or diminished by depolarizing currents (B and F). Polarizing currents are shown on the upper trace in each case. The PSP’s in the giant fibers were recorded on the middle traces in A–D but during the pulses the lower traces recording the Mauthner fiber potentials crossed over the middle traces. The middle and lower traces in E and F are recordings from the Mauthner and giant fibers, respectively. There are superimposed sweeps in A–C. A, the PSP due to the normal Mauthner fiber spike showed random variations. B, depolarizing current decreased the presynaptic spike height by about 25%. The mean amplitude and variability of the PSP were apparently unchanged. C, hyperpolarizing current increased the presynaptic spike amplitude by almost 30%. The mean amplitude and variability of the PSP showed little change. D, demonstration that the PSP was due solely to the spike in the one Mauthner fiber. A spinal stimulus adequate to excite both Mauthner fibers was given. A strong hyperpolarization caused a large component of the recorded spike to fail indicating that propagation along the fiber was blocked. Correspondingly, the PSP in the giant fiber failed completely. E and F, successive sweeps superimposed with and without polarizing currents. E, hyperpolarizing current slightly delayed the presynaptic spike and increased its amplitude about 20%. The PSP in the giant fiber was slightly delayed, but it was unchanged in amplitude. F, depolarizing current diminished the amplitude of the presynaptic spike by about 20%. The PSP was unchanged. Calibrations identical within each series.
cient to block propagation. The amplitude variation of the spike at the synapse must have been approximately equal to that recorded. As already noted in respect to the absence of electrotonic coupling, there would have been little decrement of the hyperpolarizing potentials in reaching the terminals. The depolarizations in these experiments would also have shown little decrement, because they must have been too small to cause appreciable delayed rectification (cf. Fig. 4 D and E).

The relative independence of PSP amplitude from presynaptic spike height contrasts markedly with results of similar experiments on the squid synapse in which the PSP was greatly affected (33). The results in the hatchetfish suggest that maximal secretion of transmitter is evoked by even a small spike; i.e., the secretory processes are easily saturated. One mechanism that would explain the data is that even a small spike causes sufficient secretion to deplete the immediately available transmitter store and that mobilization of additional transmitter is relatively slow. This explanation is also consistent with the observation that a single stimulus produces a pronounced depression of transmission.

The relation between pre- and postsynaptic potentials was also studied by using linearly rising (ramp) depolarizing currents which increased at a rate too slow to initiate spikes. This procedure allowed controlled depolarization of the Mauthner fiber in the range between a subthreshold pulse and a minimum sized spike. These experiments were carried out using one electrode in the Mauthner fiber and one in the giant fiber. The currents required changed the electrical properties of the polarizing electrode in the Mauthner fiber and during the ramps prevented accurate potential recording from this electrode by means of the bridge circuit. Nonetheless, when a too rapidly rising ramp initiated a spike, the response could be seen on the bridge recording and was also signalled by the occurrence of a large PSP in the giant fiber. As shown in Fig. 4 D and E a ramp current produced a more or less proportional ramp potential change.

When sufficiently large ramp depolarizing currents were applied in a Mauthner fiber, these stimuli evoked depolarizations in a giant fiber that presumably resulted from the release of transmitter (Fig. 11). The responses appeared to consist of many small components (Fig. 11 F) like those seen following prolonged repetitive stimulation (Fig. 9). The responses were graded, and larger stimuli evoked larger responses (Fig. 11 A1-A4) until a maximum was reached (Fig. 11 A4 and C). The responses began at approximately the same current value when the slope of the ramp was changed (Fig. 11 B1-B4). However, the more rapidly rising ramps evoked more synchronous responses, which is consistent with the graded increase in response amplitude as the ramp current was increased. By comparison with experiments in which two electrodes in a Mauthner fiber were used to determine the potentials
produced by ramp currents, the voltage threshold for secretion was about 25–30 mv above the resting potential (Fig. 11 A2). This value is close to that observed at the squid synapse (23, 27). When a ramp current continued to rise beyond the threshold, the responses continued for a period of 15–30 msec

![Figure 11](image_url)

**Figure 11.** The effects of ramp currents applied in the presynaptic fiber. The stimuli (lower traces) were applied in the Mauthner fiber at a rate of repetition of 0.20/sec. The slope was kept below that required to initiate spikes. Responses in the giant fiber (upper traces) appeared to consist of bursts of small potentials which summed and reached a maximum amplitude of 4–5 mv. All data in this figure were taken from the same fibers. A1-A4, ramp currents of increasing amplitude and constant slope. Small responses were evoked when the current reached a value of about 30 namp (A2). Larger and more synchronous responses were evoked by larger currents (A3 and A4). B1-B4, ramp currents of decreasing slope and constant peak amplitude. The slope was decreased by a factor of about 5 from B1 to B4. The responses were more dispersed in time when the ramp was more slowly rising. However, the responses began at approximately the same current level in each case. C, D, the responses became very small after about the same period whether the ramp current continued to rise (C) or whether it was held at a plateau value adequate to evoke maximal responses (D). E, when the current was terminated during the response, the response greatly diminished after very little delay. A few miniature potentials continued for 20–40 msec after the stimulus. F, two examples of expanded sweeps to show the shape of the responses in detail. The response to the more slowly rising ramp shows seven individual components clearly. Individual components are not distinguishable in most of the larger response. Calibrations for A-E are identical.

and then decreased greatly, although usually there continued to be a few miniature potentials as long as the ramp current was maintained (Fig. 11 C). The same decrease in responses was observed if the current was held at a constant level slightly above the threshold (Fig. 11 D). If the current was terminated, the responses stopped almost completely after a latency too short to be measured at the slow sweep speed used (Fig. 11 E).
The rapid decrease in the response during ramp currents is consistent with the data showing marked depression of transmission following a single spike. If the depression in each case were due to virtually complete depletion of transmitter, it would be predicted that the maximum amount of transmitter released by a ramp would be more or less equal to that produced by a single spike. In this situation, the response amplitude integrated over time should be approximately the same in the two cases. The data appear to be consistent with this hypothesis, although the comparison is difficult to make accurately because of electrode noise and the "noisy" nature of the responses due to the ramps. The time integrals of the largest responses due to the ramps in Fig. 11 are about 20 mv msec which is not far from that for a maximal PSP (Fig. 5 E). Furthermore, when a suprathreshold ramp current was followed at varying intervals by a directly evoked spike in the Mauthner fiber, the PSP due to the spike was depressed initially and recovered over a time course similar to that observed using a pair of directly evoked spikes. The degree of depression has yet to be correlated with the amount of secretion produced by the ramp, although the depression tended to be greater with larger ramps. The similarity in the time integrals of response amplitude and in the depressions evoked by ramp currents and spikes suggests that the two forms of depolarization cause secretion by the same mechanism. However, large ramp currents clearly had effects in addition to depletion of transmitter. The ramps had to be given at much lower frequencies than spikes, if the responses were not to become successively more depressed.

DISCUSSION

The Mauthner fiber, giant fiber synapse is unique among synapses in vertebrate brain in that both pre- and postsynaptic fibers can be penetrated by separate microelectrodes. Several of the transmissional properties are different from those of other known synapses. The PSP is unaffected by current pulses that change presynaptic spike height; depression produced by a single stimulus is very pronounced; and maintained depolarization does not cause maintained transmitter release. All three of these features could be explained by one property, that the immediately available supply of transmitter is easily exhausted. If even a small spike releases all the available transmitter, a large spike can release no more, and PSP's evoked by subsequent spikes will cause little release of transmitter until the immediately available store is replenished. Depletion of transmitter is, of course, not the only explanation of these results. For example, the mechanism of transmitter release could become refractory, or less likely, the postsynaptic membrane could become desensitized.

Another unusual property is that during high frequencies of stimulation, PSP amplitude is reduced to well below the size of the miniature PSP's seen.
under other conditions and there are no complete failures of transmission (Fig. 8 F). This finding indicates that either the amount of transmitter per quantum is reduced or the sensitivity of the postsynaptic membrane is decreased; i.e., there is desensitization (24). Reduction in quantum size was observed at the neuromuscular junction under conditions where acetylcholine resynthesis was blocked (12), but not when the nerve was repetitively stimulated (10, 13). Repetitive nerve activity does not appear to cause desensitization at the neuromuscular junction (10, 13, 32), but probably does do so at a synapse in *Aplysia* (36). These comparative data do not lead to a preference for one of the alternative explanations of the effects in the hatchetfish.

A critical point in the interpretation of these experiments is the degree to which the potentials recorded by electrodes in the axonal cores represent the potentials in the terminals. The experiments using ramp currents prove that currents applied in the Mauthner fiber do reach the terminals. Thus applied currents should affect spike height in the terminals even if the spike amplitude differs somewhat from that recorded in the main trunk of the axon. The experiments using ramp currents also validate the experiments showing the existence of a synaptic delay and the absence of electrical coupling.

Calculations indicate that the decrement in electrotonic spread from axonal core to terminals and from terminals to axonal core is small in both the Mauthner and giant fibers. First, in respect to spread from axonal core to terminals in the Mauthner fiber, the entire input resistance of about 1 MΩ may be ascribed to eight contralateral and two ipsilateral terminals, each of which then has an input resistance of 10 MΩ. This value is likely to be much too low because the space constant is so long (Fig. 4) that the resistance measurements must involve spread to nodes distant from the synaptic region. If each terminal has a 20 μ long, 5 μ diameter myelinated portion whose surface resistivity is infinite and whose axoplasmic resistivity is 100 MΩ cm, the intracellular access resistance to the terminal’s unmyelinated portion is about 1 MΩ. As the total input resistance of each terminal exceeds 10 MΩ, only a small voltage drop could occur in the myelinated part of the terminal. If the unmyelinated portion of each terminal has a total surface area of 200 μ², and if an input resistance of 9 MΩ is ascribed to this membrane, the calculated membrane resistivity is 18 Ω cm². Again, this value is likely to be a marked underestimation because of the long space constants. If one assumes a fiber 5 μ in diameter with 18 Ω cm² membrane resistivity and axoplasmic resistivity of 100 Ω cm, the space constant would be greater than 45 μ. Since the unmyelinated portion of the terminals is considerably shorter than 45 μ (Fig. 1 C and D) and since it constitutes a core conductor with a closed end, there can be little decrement within the unmyelinated part of the terminals. It can be concluded that a large part of the potential recorded in the axonal core due to applied current is developed across the membranes of the terminals. The degree of nonisopotentiality could be greater during a spike because of membrane capacity and increased conductance during activity. However, spikes would not be expected to decrement significantly in inactive terminals. The time constant of the terminal
membrane would be very short compared to spike duration if its resistance were as low as 10 Ω cm² and its capacity were 1 μF/cm². If its resistivity were higher, there would be even less decrement in the terminal although the membrane time constant would be greater. Assuming that the terminals generate spikes, a 10-fold decrease in resistance would lead to about a 3-fold decrease in space constant, and the spike at the tip of a terminal might differ somewhat from that in the axonal core. However, it would be difficult to explain the postactivation depression by failure of impulses to propagate into the terminals, because there is no change in the Mauthner fiber spike over most of the period of depression. Furthermore, the PSP’s are little affected by marked changes in spike height.

In respect to current spread from terminal to axonal core in the Mauthner fiber, there are two limiting cases. If all the input resistance is ascribed to the terminals, there can be no decrement in the myelinated portion of the terminals, and, as before, negligible decrement in the unmyelinated portion. If on the other hand, the terminal membrane is assumed to be of infinite resistance, the total access resistance to the core provided by four myelinated terminals would be 0.2 MΩ, a small fraction of the fiber’s input resistance. One can conclude that any electrotonic spread from giant fiber to ipsilateral Mauthner fiber would be little affected by loss in the Mauthner fiber terminals. Electrotonic spread from giant fiber to contralateral Mauthner fiber would show some decrement along the single Mauthner fiber terminal connecting them, but should nevertheless be detectable if the PSP’s were electrically transmitted.

In respect to the giant fibers, the input resistance is about 0.5 MΩ but the resistance ascribable to processes synapsing with the Mauthner fiber is greater than 1 MΩ because of spread into the motoneurons (3). Furthermore, the giant fiber processes are considerably shorter and thicker than the Mauthner fibers terminals (Fig. 1 C, D). Thus, there is likely to be even less decrement than is indicated for the Mauthner fiber. In any case, attenuation of PSP’s in spreading from terminal into axon would not affect the observation of reduction of PSP amplitude during high frequency stimulation to below that of the normal miniature PSP’s.

If one accepts the hypothesis that the immediately available transmitter is largely exhausted by a single spike, the probability of release by a spike is high for each quantum in this store. At a number of synapses, the amplitudes of the PSP’s are described by a Poisson distribution (29). This type of distribution is usually ascribed to a process in which a small probability of release of each quantum operates on a large number of quanta. At the hatchetfish synapse, the steady-state amplitude variations at moderate frequencies of stimulation do not appear to fit a Poisson distribution because the coefficient of variation is too small. Attenuation of PSP’s in spreading from terminals to axonal core could not explain the deviation from a Poisson distribution. There would be no effect on the measured distribution if the attenuation were the same at each terminal; if the attenuation differed at different terminals, there would be an increase in the coefficient of variation. Although further study is required, the variance of the PSP amplitude distribution differs from that of a Poisson
distribution in the direction expected for a binomial distribution of amplitudes where the probability of quantal release is high. The magnitude of the coefficient of variation appears consistent with the prediction from a binomial distribution in which the probability of quantal release is that which was measured by the degree of depression using paired stimulation (Fig. 7 A).

It should be noted that a large probability of quantal release does not require that the amplitudes of the PSP's have a non-Poisson distribution, since the output can also reflect the statistics describing the immediately available store. Thus, if a process having a large (or small) probability of release operates on an immediately available store whose size varies according to a Poisson distribution, the output of transmitter will be distributed according to Poisson statistics (35). This fact is obvious for the limiting case of release probability equal to one. To give a physical picture, let the immediately available store be the number of vesicles occupying release sites at a given instant. This number would have a Poisson distribution if vesicles containing quanta were freely diffusing near many release sites, each of which had only a low probability of being occupied by a vesicle. In these circumstances, output would be Poisson-distributed whether probability of quantal release were low or high. If the probability that a release site were occupied and the probability of quantal release were both large, the amplitude distribution would become binomial (35). Restricted diffusion of vesicles within the terminal and replenishment of the immediately available store might also lead to a non-Poisson distribution of PSP amplitudes. In the hatchetfish the latter factor could be important in the brief period of greater variability observed shortly after the onset of a stimulus train (Fig. 7 B).

If release probability were in fact high, the size of a PSP would be a measure of the size of the immediately available store of transmitter, which could be little more than the total mobilized since the previous stimulus. A figure for the number of quanta in the immediately available store can be estimated from quantal size, about 0.4 mv, and PSP amplitude, 20-40 mv. The value of 50-100 quanta is somewhat smaller than those given for neuromuscular junctions, but comparable to those for the sympathetic and ciliary ganglia (9, 29, 31). The decrease in PSP size produced by a short period of high frequency stimulation suggests that the total amount of mobilizable transmitter may also be small. Study of the time course of amplitude changes during and after tetani should make it possible to estimate the size of the mobilizable store as well as the rate at which it is refilled, perhaps by resynthesis, and the rate at which it is emptied by movement of transmitter into the immediately available store. Analysis of variance of PSP amplitudes may also be useful in defining the changes that occur as a result of tetanic stimulation.

Further study of this synapse should prove valuable in elucidating the mechanism of chemically mediated transmission and the relation between
presynaptic potential and release of transmitter. Although this synapse is different in several respects from others studied to date, it may well be representative of many synapses in the central nervous system.

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REFERENCES


