tivity of the earlier assay (*6*). The amount of plasma 1,25-$(OH)_2D_3$ that is recovered during extraction and purification is one-third more than the amount recovered in previous methods, and the concentration of 1,25-$(OH)_2D_3$ in 5-ml samples of plasma can be estimated in triplicate. Because both 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ can be measured, a potential source of error is removed in estimations of 1,25-$(OH)_2D$ in the plasmas of persons on diets rich in either form of vitamin D. The concentrations of 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_2$ that we found in the plasma of adults are somewhat lower than those found by Haussler and his coworkers (*6*), but this may reflect the age differences of the subjects. This assay is being used in investigations of several metabolic bone diseases.

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Modulation of Synaptic Transmitter Release by Repetitive Postsynaptic Action Potentials

Abstract. *The effect of repetitive action potentials in the postsynaptic axon on the release of synaptic transmitter from the presynaptic terminal was investigated at the squid giant synapse. Repetitive antidromic stimulation of the postsynaptic axon resulted in a reduction in the excitatory postsynaptic potential (EPSP). The reduction in transmitter release was accompanied by a decrease in the presynaptic spike afterhyperpolarization (AH). Increasing the concentration of extracellular potassium ions also reduced the EPSP and decreased the amplitude of the presynaptic spike AH. The reduction in transmitter release resulting from repetitive postsynaptic impulses is attributed to the accumulation of extracellular potassium ions. It is proposed that the accumulation of extracellular potassium ions resulting from repetitive postsynaptic activity may modulate synaptic transmission and function as an integrative mechanism in the nervous system.*

The modulation of synaptic transmitter release plays an important role in information processing in the nervous system and may be involved in the cellular mechanisms of learning and behavior (*1*). The mechanisms which have been reported to modulate synaptic transmission (*2*) include repetitive activity in the presynaptic terminals which can produce facilitation (*3*, *4*), depression (*4*, *5*), or posttetanic potentiation (*6*) of transmitter release. Other modulating effects

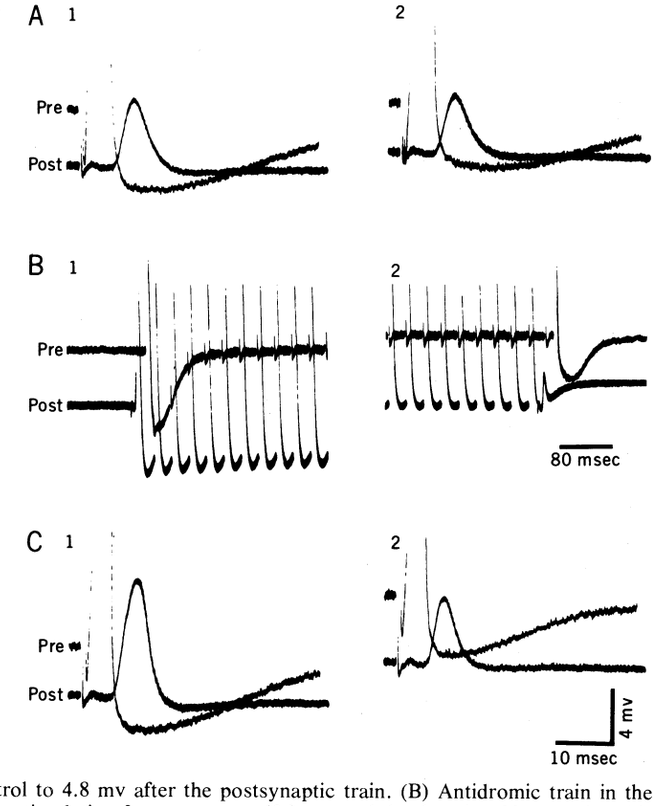

Fig. 1. Effect of postsynaptic stimulation and increased $[K]_o$ on synaptic transmission at the squid giant synapse. (A) The top trace is the record from an intracellular electrode in the presynpatic terminal showing the presynaptic spike AH. The bottom trace is the record from an intracellular electrode in the postsynaptic axon showing an EPSP. (A1) Control, recorded 10 seconds before the antidromic train in the postsynaptic axon shown in (B). (A2) Recorded 10 seconds after the antidromic postsynaptic train shown in (B). Note the reduction in the EPSP from 5.2 mv in the control (A1) to 4.2 mv after the postsynaptic train (A2). Note also the reduction in the presynaptic spike AH from 6 mv in the control to 4.8 mv after the postsynaptic train. (B) Antidromic train in the postsynaptic axon. The stimulation frequency was 40 hertz and the duration 20 seconds. This was recorded with standing spots on the oscilloscope and moving film; the spots were out of line such that the presynaptic record (top) is shifted to the right relative to the postsynaptic record (bottom). (B1) Beginning of an antidromic train in the postsynaptic axon (bottom trace). The top trace shows the AH of a presynaptic spike at the beginning of the postsynaptic train. The presynaptic axon was stimulated orthodromically every 10 seconds before, during, and after the postsynaptic train. (B2) End of the antidromic train of impulses in the postsynaptic axon. The top trace shows a presynaptic spike AH at the end of the postsynaptic train. Note the reduction in the postsynaptic spike AH from 5.2 mv at the beginning (B1) to 1.9 mv at the end (B2). Note also the reduction in the presynaptic spike AH from 6 mv at the beginning (B1) to 3.2 mv at the end (B2) of the postsynaptic train of impulses. (C) Effect of changing $[K]_o$. (C1) Control EPSP and presynaptic spike AH, as in (A1). (C2) Effect of increasing $[K]_o$ to 12 m*M*. Note the decrease in the EPSP from 8.6 mv (C1) to 4.9 mv (C2), and the decrease in the presynaptic spike AH from 6.4 mv (C1) to 4.6 mv (C2). All recordings (A, B, and C) were from the same synapse. The temperature was 7.0°C in (A) and (B) and 9.2°C in (C).

involve the presynaptic action of other synapses resulting in presynaptic inhibition (*7*) or heterosynaptic facilitation (*8*). There is, however, no evidence for a direct postsynaptic role in the modulation of transmitter release. This fact, together with evidence that increasing the extracellular potassium concentration, $[K]_o$, depressed transmitter release (*9*), prompted us to investigate whether the accumulation of extracellular K resulting from a train of postsynaptic impulses might modulate transmitter release at the squid giant synapse. We now report that repetitive action potentials in the postsynaptic axon can modulate the release of synaptic transmitter and suggest that this effect is due to the accumulation of extracellular K ions.

The stellate ganglion was dissected from the squid *Loligo pealii* and placed in a small Lucite chamber perfused by oxygenated artificial seawater of the following composition (m*M*): NaCl, 424; KCl, 9; $CaCl_2$, 9; $MgCl_2$, 23; $MgSO_4$, 25; and $NaHCO_3$, 2. The bath temperature was lowered until the excitatory postsynaptic potential (EPSP) no longer initiated spike generation (*10*). Experiments were then performed at a constant temperature (± 0.1°C) between 6° and 12°C. The pre- and postsynaptic axons were stimulated by suction electrodes. In the experiments reported here, the presynaptic axon was stimulated every 10 seconds before, during, and after repetitive antidromic stimulation of the postsynaptic axon. Standard electrophysiological techniques were used to record from electrodes placed intracellularly in the midregion of the presynaptic terminal and in the postsynaptic axon near the synapse (*11*).

Repetitive antidromic action potentials in the postsynaptic axon produced a depression in the release of synaptic transmitter from the presynaptic terminal; this is illustrated in Fig. 1. Figure 1A1 shows the EPSP and the presynaptic spike after-hyperpolarization (AH) (*12–14*) recorded 10 seconds before a train of repetitive postsynaptic action potentials.

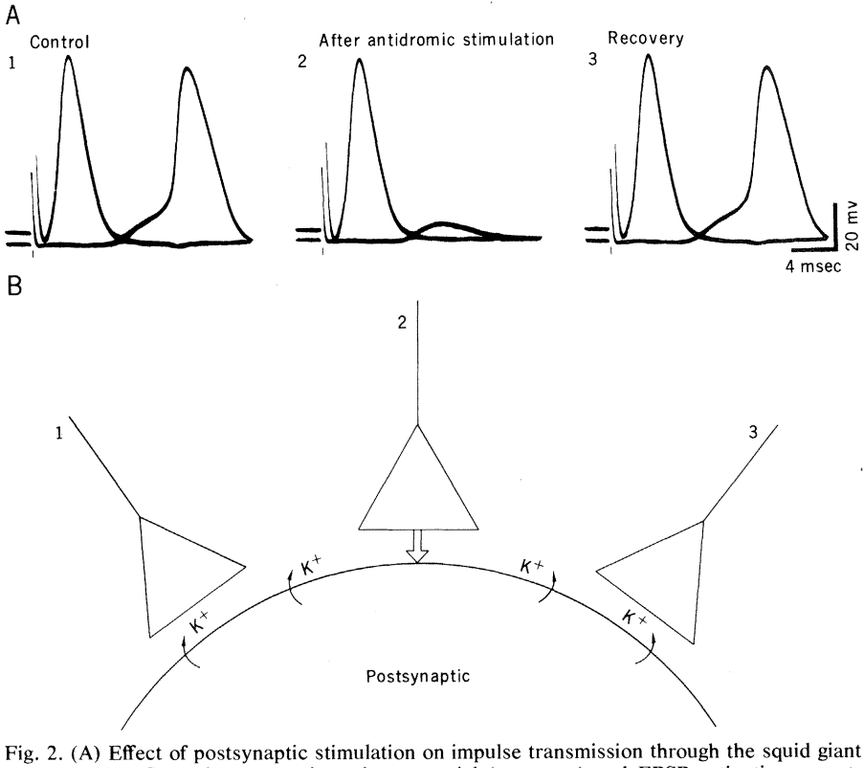

Fig. 2. (A) Effect of postsynaptic stimulation on impulse transmission through the squid giant synapse. (A1) Control presynaptic action potential (top trace) and EPSP activating a postsynaptic action potential (bottom trace), recorded 10 seconds before an antidromic train in the postsynaptic axon. (A2) Recorded 10 seconds after an antidromic train in the postsynaptic axon; the frequency of the train was 25 hertz and the duration 20 seconds. Note that because of the reduction in EPSP amplitude, resulting from repetitive postsynaptic impulses, the EPSP no longer initiates an action potential in the postsynaptic axon. (A3) Recovery of EPSP initiation of action potential generation in the postsynaptic axon, 3 minutes after the end of the antidromic postsynaptic train. (B) Schematic diagram of hypothetical neuron (postsynaptic) with three separate covergent synaptic pathways (1, 2, and 3). Repetitive firing of one excitatory pathway (2) would cause repetitive firing of the postsynaptic neuron, which would result in accumulation of extracellular K, which could in turn modulate transmission in the other convergent synaptic pathways (1 and 3). See text for details.

The records in Fig. 1A2 were taken 10 seconds after the train of postsynaptic action potentials; the frequency of the train was 40 hertz and the duration 20 seconds. It can be seen that the EPSP amplitude was reduced after the postsynaptic action potentials. The average percentage reduction in EPSP amplitude was 27.6 ± 7.9 (standard deviation; $N = 9$) for postsynaptic stimulation at a frequency of 25 hertz for 20 seconds. Figure 1B shows specimen records from the beginning (B1) and the end (B2) of the antidromic train. The lower trace in Fig. 1B was recorded from the postsynaptic axon and shows the AH's of the antidromic spikes during the train; note the reduction in AH amplitude produced by the repetitive impulses. Frankenhaeuser and Hodgkin (*13*) have shown that the reduction in spike AH during repetitive action potentials is the result of the accumulation of extracellular K ions.

The repetitive impulses in the postsynaptic axon also reduced the amplitude of the presynaptic spike AH. This is shown in the top trace of Fig. 1B, which was recorded from the presynaptic terminal. The reduction in the presynaptic spike AH can be seen by comparing it at the beginning of the postsynaptic train (B1) and at the end of the train (B2). Since the reduction in amplitude of the AH can be attributed to the accumulation of extracellular K ions (*13, 15*), the reduction in the presynaptic spike AH suggests that K ions accumulating as the result of repetitive action potentials in the postsynaptic axon can diffuse across the synaptic cleft and affect the presynaptic terminal.

The effect of increasing $[K]_o$ on transmitter release at the squid giant synapse is illustrated in Fig. 1C. Increasing $[K]_o$ to 12 m*M* produced a reduction in the amplitude of both the EPSP (*16*) and the presynaptic spike AH. By comparison, the amplitudes of both the EPSP and the presynaptic spike AH were also reduced by repetitive antidromic stimulation of the postsynaptic axon (Fig. 1A). This suggests that the reduction in EPSP amplitude resulting from repetitive postsynaptic action potentials is due to the accumulation of extracellular K ions around the presynaptic terminal (*17*).

The functional consequences of reduction in amplitude of the EPSP produced by repetitive postsynaptic action potentials can be significant. Figure 2A illustrates the effect of repetitive postsynaptic action potentials on synaptic transmission. Figure 2A1 shows the EPSP initiating an action potential in the postsynaptic axon, resulting in impulse

transmission through the synapse. The record in Fig. 2A2 was taken 10 seconds after an antidromic train of impulses in the postsynaptic axon and shows that the reduction in transmitter release produced by the postsynaptic train was sufficient to prevent activation of spike generation in the postsynaptic axon. Figure 2A3 shows recovery of synaptic transmission and impulse generation 3 minutes after the end of the postsynaptic train.

The modulation of transmitter release by repetitive postsynaptic action potentials can function as a type of negative feedback. Figure 2A shows that this feedback is effective in modulating transmission through the synapse. Previous investigations on the mammalian central nervous system (CNS) have shown different types of negative feedback. For example, recurrent inhibition can be mediated by pathways through axon collaterals and interneurons, as in the case of the motoneuron (*18*) and hippocampal pyramidal cell (*19*), or through dendrodendritic interactions with interneurons, as in the case of the olfactory mitral cell (*20*). The main feature of these negative feedback systems is that they feed back synaptic inhibition on the output neuron. However, a direct feedback inhibition of transmitter release from nerve terminals impinging on a neuron has not, to our knowledge, been previously reported. This investigation provides evidence for a direct inhibitory feedback from the postsynaptic structure that modulates transmitter release from the presynaptic terminal.

Figure 2B illustrates schematically how such a feedback inhibition might function in the CNS. Repetitive action potentials in one excitatory synaptic pathway (2 in Fig. 2B) would result in repetitive firing of the neuron. The accumulation of extracellular K resulting from the repetitive neuronal activity could, in turn, result in feedback inhibition of synaptic transmitter release from other synaptic pathways (1 and 3 in Fig. 2B) impinging on the neuron (*21*). In this way, the accumulation of extracellular K may modulate synaptic transmission and function as an integrating mechanism in the nervous system.

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16. The small depolarization of the postsynaptic membrane resulting from the increase in $[K]_o$ would reduce the EPSP by only 0.2 to 0.6 mv per millivolt change in membrane potential [S. Hagiwara and I. Tasaki, *J. Physiol. (London)* **143**, 114 (1958); R. Miledi, *Nature (London)* **223**, 1284 (1969); R. Llinas, R. W. Joyner, C. Nicholson, *J. Gen. Physiol.* **64**, 519 (1974)]. On the other hand, if the EPSP is due to an increased Na and K conductance, increasing $[K]_o$ would decrease the K equilibrium potential, which would be expected to increase the amplitude of the EPSP. Separate analysis (*11*) indicates that the reduction in EPSP amplitude is associated with and can be accounted for by a decrease in presynaptic spike height.
17. The possibility that synaptic transmitter release might be reduced by a synaptic pathway impinging on the presynaptic terminal seems most unlikely in this experiment for several reasons. First, there is no known anatomical pathway from the postsynaptic nerve with synapses on the giant termi → [J. Z. Young, *Philos. Trans. R. Soc. London Ser. B* **229**, 465 (1939); K. Hama, *Z. Zellforsch. Mikrosk. Anat.* **56**, 437 (1962); O. J. Castejon and G. M. Villegas, *J. Ultrastruct. Res.* **10**, 585 (1964); J. Z. Young, *Brain Res.* **57**, 457 (1973)]. Second, continuous recording in the presynaptic terminal (Fig. 1B) does not reveal synaptic potentials in the presynaptic terminal upon antidromic stimulation of the giant axon. Third, the reduction in the presynaptic spike AH indicates an accumulation of extracellular K (*13, 15*).
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22. We thank G. R. Siggins and B. J. Hoffer for critical review of the manuscript. This investigation was conducted at the Marine Biological Laboratory, Woods Hole, Mass., and was supported in part by PHS grant NS 12211.

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Monoamine Changes in the Brain of Cats During Slow-Wave Sleep

Abstract. *We have found that the metabolism of 5-hydroxytryptamine increases in the hippocampus and that the metabolism of dopamine decreases in the striatum and thalamus during slow-wave sleep, and we suggest that these changes are related to this stage of sleep. We have also found that the concentration of dopamine increases in the hippocampus during slow-wave sleep, and suggest that this may be related to the subsequent appearance of paradoxical sleep. These data raise new questions on the hippocampal role in the sleep-wakefulness cycle.*

The hypothesis that monoamines participate in the regulation of sleep has been supported by the findings that lesion of the raphe neurons containing 5-hydroxytryptamine (5-HT) (*1*) leads to a state of permanent desynchronization with behavioral arousal (*2*) and that bilateral destruction of the nucleus locus coeruleus, rich in catecholamines (*1*), is followed by a total disappearance of paradoxical sleep (*3*). While behavioral, electroencephalographic (EEG), and pharmacologic evidence also substantiates the role of monoamines in sleep, chemical correlates have been lacking because of the difficulty of obtaining brain tissue for chemical analysis during sleep. By using a brain biopsy technique, we have found that the metabolism of 5-HT and the concentration of dopamine (DA) increases in the hippocampus, while the metabolism of DA decreases in the striatum and thalamus during slow-wave sleep (SWS). We suggest that the increased 5-HT metabolism in the hippocampus and decreased DA metabolism in the striatum and thalamus is related to SWS, and that the increased concentration of DA in the hippocampus is related to the subsequent appearance of paradoxical sleep.

Adult cats were used as the experimental subjects. Under pentobarbital anesthesia, the left parieto-temporal bone of the skull and underlying dura mater were removed (*4*). After screw electrodes for EEG recording were implanted into the frontal cortex, electrode wires were sol-