Synaptic Excitation May Activate a Calcium-Dependent Potassium Conductance in Hippocampal Pyramidal Cells

Abstract. *In hippocampal CA1 pyramidal cells, orthodromic synaptic excitation is followed by an early hyperpolarization mediated by γ-aminobutyric acid (GABA) and a late non-GABA-mediated hyperpolarization that has properties consistent with an increase in potassium conductance. Depolarizations produced by iontophoretically applied glutamate are followed by hyperpolarizations that have features in accordance with an increase in potassium conductance. The hyperpolarizations are independent of chloride and resistant to tetrodotoxin but are blocked by a low-calcium, high-cobalt medium. Voltage clamping the glutamate depolarization does not reduce the subsequent hyperpolarization, indicating that the hyperpolarization results from a direct increase in calcium conductance produced by glutamate, rather than from activation of voltage-sensitive calcium channels. A single transmitter, possibly acting on one type of receptor and channel, may initiate both excitation and inhibition in the same postsynaptic cell.*

At both vertebrate and invertebrate neuromuscular junctions, a postsynaptic influx of calcium ions accompanies excitatory synaptic transmitter action (*1, 2*). There is evidence that excitatory amino acids can activate a calcium conductance in the vertebrate central nervous system (*3*). Sodium is responsible for the major depolarizing effects of the excitatory transmitter in these cases, but the role of calcium ions is not known. In many neurons (*4, 5*), including hippocampal pyramidal cells (*6, 7*), activation of voltage-sensitive calcium channels is followed by a long-lasting conductance increase to potassium. We investigated the possibility that a similar calcium-dependent potassium conductance might be activated by excitatory transmitters in the hippocampus.

The rat hippocampal slice preparation that we used has been described (*8*). Stimulating electrodes were placed on stratum radiatum for orthodromic stimulation of CA1 pyramidal cells and on the alveus for antidromic stimulation.

In more than 50 cells we observed that hyperpolarization after orthodromic stimulation in normal media has a considerably longer duration than hyperpolarization after antidromic stimulation and usually has two discrete components, the early and late hyperpolarizing potentials (Fig. 1A1), with different properties. Neither is dependent on an action potential in the recorded cell. Both are associated with a conductance increase, but the early component always has a much larger increase that can be seen as a reduction in the size of constant current hyperpolarizing and depolarizing pulses during the response (Fig. 1A2). In addition, the early component (Fig. 1B1) reverses polarity with moderate hyperpolarizing currents applied through the recording electrode, as does the antidromic response (*9*). The less sensitive late component is usually abolished at membrane potentials of approximately − 85 mV (Fig. 1B).

Gamma-aminobutyric acid (GABA) is considered the major transmitter for inhibitory postsynaptic potentials (IPSP's) in the mammalian central nervous system, including the hippocampus (*10*). When the GABA antagonist bicuculline methiodide is added to the superfusate or applied iontophoretically to the dendrites, the early component is reduced while the late hyperpolarization still follows the excitatory postsynaptic potential (EPSP) (Fig. 1C) (*11*). Varying the strength of the orthodromic stimulus in the presence of a GABA antagonist demonstrates that the late hyperpolarization increases in size together with increases in the size of the preceding EPSP. Finally, when cells (N = 15) are loaded with chloride from a KCl-filled recording electrode, the early component becomes strongly depolarizing, while the late phase remains hyperpolarizing. The sensitivity of the late phase to membrane hyperpolarization is similar with electrodes filled with either KCl or $KMeSO_4$. To exclude the possibility that insensitivity of the late phase to intracellular chloride infusion is due to incomplete reversal of the chloride concentration gradient throughout remote regions of the cell, we lowered the chloride concentration in the bathing medium (N = 8). In this case the early phase becomes depolarizing, as expected for a chloride-dependent potential, and the late phase remains hyperpolarizing (Fig. 1D), indicating that it is chloride-independent. These observations suggest that orthodromic stimulation produces an EPSP, a fast GABA-mediated IPSP, and a slower non-GABA-mediated late hyperpolarization, which has properties consistent with an increase in potassium conductance (*12*).

To determine if this hyperpolarization might arise as a consequence of excitatory transmitter action, we examined the effects of glutamate, the major putative excitatory transmitter in this system (*13*). Glutamate was applied iontophoretically to the proximal dendrites within 100 μm of the cell body layer. We recorded glutamate effects using 3*M* KCl-filled microelectrodes in the presence of tetrodotoxin ($10^{-6}M$) and the GABA antagonist picrotoxin ($10^{-4}M$) in order to avoid possible confusion of indirect GABA-dependent chloride hyperpolarizations with true glutamate responses. Intracellular chloride diffusion will invert the normal chloride gradient, and tetrodotoxin and picrotoxin will block voltage-dependent sodium potentials and GABA receptors, respectively. Under these conditions glutamate iontophoresis

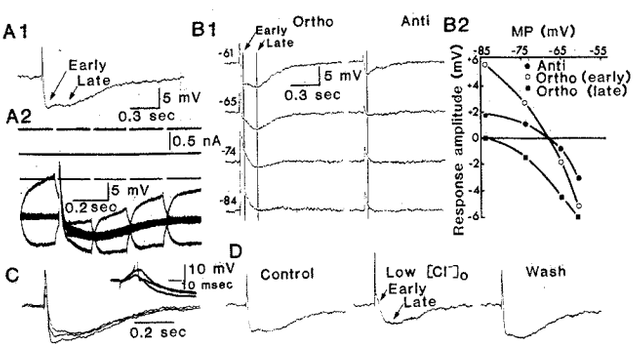

Fig. 1. Synaptic responses in CA1 pyramidal cells. (A1) Pen record of response to orthodromic stimulation showing the early and late components of the hyperpolarization. (A2) Superimposed film records from the same cell in which constant-current hyperpolarizing and depolarizing pulses of varying delays were injected through the recording electrode. The current monitoring trace is shown above the voltage trace. (B1) Records of orthodromic and antidromic responses obtained at different membrane potentials. Membrane potential was shifted by passing direct current through the recording electrode. (B2) The sizes of the responses in (B1), measured at the vertical lines, are plotted against the membrane potential (*MP*). (C) Superimposed tracings of responses to orthodromic stimulation before and during the onset of effects from bicuculline methiodide ($10^{-5}M$). The gain is the same as in (A1). The inset shows fast superimposed film records of the same responses. (D) Switching for 6 minutes to a medium containing low chloride reverses the early hyperpolarizing response. The recovery (*Wash*) was obtained 15 minutes after returning to the control solution. Calibration is the same as in (A1).

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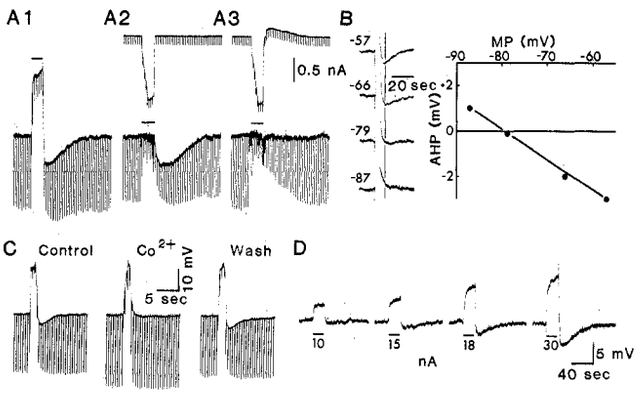

Fig. 2. Responses of CA1 pyramidal cells to iontophoretically applied glutamate. (A1) Constant-current hyperpolarizing pulses applied every 3 seconds reveal a decrease in membrane resistance during the glutamate-induced depolarization and hyperpolarization. (A2) Hyperpolarizing current was applied through the recording electrode to counteract the glutamate depolarization. (A3) The entire response was manually voltage-clamped (*18*). The current record is shown above the voltage record. The calibration is the same as in (D). (B) Glutamate responses were elicited at different membrane potentials (*MP*) and the amplitude of the afterhyperpolarization (*AHP*), measured at the vertical line, is plotted against the membrane potential. The gain in (D) also applies to the responses in (B). (C) A solution containing low calcium and high cobalt, applied for 5 minutes, selectively blocked the hyperpolarization. There was a modest increase in the resistance of the resting membrane. The recovery (*Wash*) was obtained 7 minutes after returning to the control solution. (D) Increasing iontophoretic currents expressed in nanoamperes produce increasingly larger hyperpolarizations.

causes a depolarization followed by a hyperpolarization associated with a conductance increase (Fig. 2A1; $N = 17$). Hyperpolarizing responses have not been observed in these conditions unless preceded by a depolarization. We found that the hyperpolarization is reduced in size as the membrane is hyperpolarized with direct current and reverses polarity at approximately -80 mV (Fig. 2B; $N = 4$) (*14*). Saline solutions containing low calcium concentrations (0.25 m*M*) and the calcium antagonists magnesium (10 m*M*; $N = 2$), cadmium (2 m*M*; $N = 2$), or cobalt (5 m*M*; $N = 4$), markedly reduce the glutamate hyperpolarization (Fig. 2C) (*15*). These observations suggest the glutamate hyperpolarization is a calcium-dependent potassium potential (*16*).

Glutamate hyperpolarizations are graded in size by the amount of glutamate applied and can be seen with doses of glutamate producing depolarizations as small as 4 mV. This level of depolarization is considerably below that normally required to activate voltage-sensitive calcium conductances in these cells (approximately 20 to 30 mV) (*17*). We therefore wanted to distinguish between the glutamate hyperpolarization due to calcium entry that is (i) voltage-dependent or (ii) follows directly from a glutamate-increased calcium conductance. Because the responses are slow, we were able to use a manual voltage clamp and prevent voltage-dependent calcium entry (*18*). When the glutamate depolarization is prevented by clamping, the hyperpolarization is not only not blocked, it is actually increased (Fig. 2A2). This increase in the response is seen in most of the cells that we clamped and probably results from the negative clamp current driving more calcium into the cell during the glutamate action than enters normally. In Fig. 2A3, the entire response was manually voltage-clamped and the conductance increase was clearly seen after glutamate application.

These results suggest that after the action of glutamate, a hyperpolarization results from an increase in potassium permeability (*19*). The observation that the response is blocked by solutions low in calcium and high in cobalt indicates that it is dependent on calcium entry into the cell. Since the response can be generated by glutamate depolarizations that are considerably less than those required to activate voltage-sensitive calcium channels, and since preventing the depolarization by voltage clamping does not reduce the hyperpolarization, we conclude that the hyperpolarization results from a conductance increase to calcium coupled to a glutamate receptor. The resulting increase in intracellular calcium activates a potassium conductance (*4–7*).

Although we have not established a linkage, we suggest that the late hyperpolarization following orthodromic stimulation in normal conditions occurs in part as a consequence of the preceding EPSP and has the same ionic mechanism as the glutamate-induced hyperpolarizations, which it closely resembles. Voltage-dependent calcium entry, which is known to occur in these cells (*17*), would also contribute to a synaptically activated calcium-dependent potassium potential. Eccles and his collaborators (*20, 21*) reported that the monosynaptic EPSP, uncontaminated by any polysynaptic IPSP's, is followed by a small prolonged hyperpolarization, and they concluded that the hyperpolarization "may thus be viewed as due to the partial activation of a process that is activated much more intensely by the spike" (*20*). The spike after hyperpolarization is now known to be largely a calcium-dependent potassium conductance (*5*).

The finding that calcium influx during excitatory synaptic transmitter action can activate a delayed conductance increase to potassium ions, indicates that a single transmitter, possibly acting on one type of receptor and channel (*2*), can initiate both excitation and inhibition in the same postsynaptic neuron. While such an excitatory-inhibitory sequence would appear to be coupled, variation in the calcium gradient or in the intracellular sequestration of calcium might alter this coupling.

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8. R. A. Nicoll and B. E. Alger, *J. Neurosci. Methods*, in press. The normal superfusion medium consisted of 116.4 m*M* NaCl, 5.4 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.3 m*M* $MgSO_4$, 26.2 m*M* $NaHCO_3$, 0.92 m*M* Na_2PO_4, and 11 m*M* glucose. When $CoCl_2$ or $CdCl_2$ was present, $MgCl_2$ replaced $MgSO_4$ and Na_2PO_4 was omitted to avoid precipitating insoluble salts. This modified medium did not alter responsiveness. Low-chloride medium was made by replacing 70 percent of the NaCl with sodium isethionate. Iontophoretic pipettes were filled with 1*M* sodium glutamate (*p*H = 8) or with 10 m*M* bicuculline methiodide in 0.2*M* NaCl. Glutamate was ejected with negative current and bicuculline with positive current.
9. In the majority of cells, antidromic responses contained a small EPSP contamination, which could usually be reduced by adjusting the position of the antidromic stimulating electrode.
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11. Continued application of GABA antagonist results in burst responses to orthodromic activation with a further reduction in the early component and an increase in the amplitude and duration of the late component. This increase in size of the late component at a constant stimulus strength is considered to result in large part from the activation of voltage-sensitive calcium channels (*7*).
12. The late orthodromic hyperpolarization has

been interpreted by Y. Fugita [*Brain Res.* **175**, 59 (1979)] as a remote IPSP. Our results indicate that this potential is neither primarily chloride-dependent nor mediated by GABA, although it may be remote. R. H. Thalmann and G. F. Ayala [*Neurosci. Abstr.* **6**, 300 (1980)] arrived at the same conclusion regarding the late hyperpolarization after orthodromic stimulation in CA1 cells and observed similar potentials in CA3 pyramidal cells and in granule cells of the dentate gyrus. We would agree with Fugita that the response is in the olfactory cortex, a late hyperpolarization with many features similar to the one we report has been described [K. Mori, M. Satoh, S. F. Takagi, *Proc. Jpn. Acad. Ser. B.* **54**, 484 (1978); A. Constanti, J. D. Connor, M. Galvin, A. Nistri, *Brain Res.* **195**, 403 (1980)].
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14. The hyperpolarization following the glutamate depolarization usually had a reversal potential approximately 5 to 10 mV more positive than the afterhyperpolarization following direct depolarizing current pulses. This difference is probably due to a residual glutamate depolarization during the early part of the hyperpolarizing response.
15. We have not determined if the reduction in the hyperpolarization is due to lack of calcium or to the presence of calcium antagonists. Calcium antagonists are known to pass through excitatory transmitter channels at peripheral synapses and thus, if the antagonists are exerting an effect, it may be occurring at an intracellular site (*2*).
16. P. Ascher, A. Marty, and T. O. Neild [*J. Physiol. (London)* **278**, 177 (1978)] noted that acetylcholine activates a calcium-dependent potassium current in *Aplysia* neurons. Glutamate-induced afterhyperpolarizations associated with a conductance increase have also been reported in dissociated spinal neuron cultures (J. M. Wojtowicz, M. Gysen, J. F. MacDonald, *Brain Res.*, in press) and in cells of the olfactory cortical slice [A. Constanti, J. D. Connor, M. Galvin, A. Nistri, *ibid.* **195**, 403 (1980)].
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19. The coupling of the hyperpolarization with the preceding depolarization suggests that the hyperpolarization occurs as a consequence of the depolarization. It might be proposed that the glutamate depolarization of the pyramidal cell activates a reciprocal synaptic inhibition [C. E. Jahr and R. A. Nicoll, *Science* **207**, 1473 (1980)] mediated by a transmitter that feeds back onto the pyramidal cell and increases potassium conductance. However, the fact that voltage clamping the glutamate depolarization does not reduce the response indicates that such a pathway cannot account for the hyperpolarization.
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Rapid Forgetting of a Spatial Habit in Rats with Hippocampal Lesions

Abstract. *Rats with lesions of the hippocampus or mammillary bodies were impaired in learning reversal problems in a T-maze. Test trials given after learning each reversal disclosed little forgetting in the mammillary body group but rapid forgetting in the hippocampal group. These findings resemble those recently reported in patients with amnestic syndromes.*

It has been known for a number of years that humans suffering damage to either the medial temporal region (including the hippocampus) or the diencephalon (particularly the mammillary bodies, medial thalamus, or both) exhibit a dramatic disturbance in memory, commonly called the "amnestic syndrome" (*1*). Patients with these lesions may show virtually normal memory of remote events preceding the onset of the amnestic syndrome, but are apt to display a profound disturbance in learning new material. Recently, Huppert and Piercy (*2*) have presented evidence suggesting that there may be at least two classes of the amnestic syndrome. In one, the formation of new memories is impaired, but retention over time of the newly formed memories is unimpaired. The second is characterized by an impairment (or no impairment) in the formation of new memories, but retention over time of the newly formed memories is impaired. Huppert and Piercy propose that the former type of amnesia is associated with diencephalic lesions, whereas the latter type is associated with hippocampal lesions.

The maze performance of adult male Wistar albino rats with either hippocampal or diencephalic lesions seems to extend Huppert and Piercy's dichotomization of the amnestic syndrome to lower mammalian forms (at least with respect to memory of spatial tasks) and provides further evidence for a dissociation between learning defects and retention defects in brain-damaged subjects. Control (normal and sham-operated) rats and rats prepared with dorsal hippocampal or medial mammillary body lesions (Fig. 1) were trained on a single-unit T-maze under the motive of escape-avoidance of footshock. On the day after preliminary training, each rat was required to choose the nonpreferred arm of the T (first reversal). On the next 3 days, each rat was trained to choose the arm of the T that had been incorrect on the immediately preceding day (reversals 2, 3, and 4). The correct arm led to an end box that could be entered by displacing an unlocked gray card. The incorrect arm, on the other hand, led to a locked gray card, which prevented the animal from entering the end box on that side. If the incorrect arm was chosen, the animal had to return to the choice point and choose the other arm of the T. An error (approach to within 7.5 cm of the locked gray card) was automatically punished by mild footshock. Training to the correct arm was continued until the animal reached the criterion of five consecutive errorless trials, the intertrial interval being held constant at 75 seconds. Each day the animal was given a test trial 5 minutes, 60 minutes, and 240 minutes after the criterion was met. During these three daily test trials of retention, the card on the incorrect side for that particular day was locked and punishment was given for errors.

A test trial of retention 24 hours after learning consisted of trial 1 on reversals 2, 3 and 4. Because the normal and sham-operated rats were virtually indistinguishable from each other in learning and retention scores, they were combined into one major control group for statistical purposes.

Both brain-damaged groups showed a learning defect; they were significantly inferior to the controls in mastering the four reversal problems (Table 1). While the animals with hippocampal lesions

Table 1. Mean learning errors per reversal, mean total errors on retention test trials, and fraction of subjects making at least one error on a given test trial.

Group	*N*	Learning errors	Retention errors	Fraction of subjects making errors			
				5 minutes	60 minutes	240 minutes	24 hours
Control	13	1.4	0	0	0	0	0
Hippocampus	8	6.9*	2.1*	1/8	4/8†	6/8†	2/8
Mammillary body	8	4.5*	0.5	1/8	1/8	0	2/8

*Significantly different from control group, $P < .05$ (Mann-Whitney U test). †Significantly different from control group, $P < .05$ (Fisher exact probability test).

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