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## Hyperpolarizing responses to application of glutamate in hippocampal CA<sub>1</sub> pyramidal neurons

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Micropressure injection of glutamate onto the apical dendrites of hippocampal CA<sub>1</sub> pyramidal cells usually produces a fast rising, brief depolarization. However, hyperpolarizing responses with longer durations (300–500 ms) can be produced over a range of drug electrode locations. These hyperpolarizations can be reversed with intracellular injection of hyperpolarizing current. Localized application of glutamate in the stratum radiatum produces a depolarizing response in intracellularly recorded CA<sub>1</sub> interneurons. Previous studies have shown that the dendrites of GABA-ergic basket cell interneurons extend into the stratum radiatum and are involved in mediating feedforward inhibition of pyramidal neurons. The glutamate-induced hyperpolarizations observed in pyramidal neurons are probably due to direct excitation of dendrites of interneurons, which in turn produce a synaptic inhibition in pyramidal cells.

Glutamate, or a glutamate-like substance, is thought to be the neurotransmitter at the Schaffer collateral/pyramidal neuron synapse in CA<sub>1</sub> hippocampus (see refs. 5 and 15 for reviews). Previous studies have shown that localized application of glutamate to the apical dendritic region of CA<sub>1</sub> pyramidal neurons produces fast-rising, brief depolarizations [7, 12]. We report here that, in addition to depolarizing glutamate responses, application of small amounts of glutamate in the stratum radiatum can produce hyperpolarizations in CA<sub>1</sub> pyramidal neurons.

Transverse slices, 400  $\mu$ m thick, were prepared from adult guinea pig hippocampus and maintained *in vitro* at 35°C. The slices were kept at a liquid/gas interface. Their undersurfaces were perfused with an oxygenated artificial CSF solution (pH 7.4) containing (in mM): NaCl 124, KCl 5, NaH<sub>2</sub>PO<sub>4</sub> 1.25, MgSO<sub>4</sub> 2, NaHCO<sub>3</sub> 26, glucose 10, CaCl<sub>2</sub> 2, and their upper surfaces exposed to a warmed, moistened 95% O<sub>2</sub>–5% CO<sub>2</sub> atmosphere.

Somatic intracellular recordings were obtained with glass micropipettes (tip resistance approximately 80 M $\Omega$  filled with 4 M K<sup>+</sup> acetate and 0.01 M KCl, from CA<sub>1</sub>

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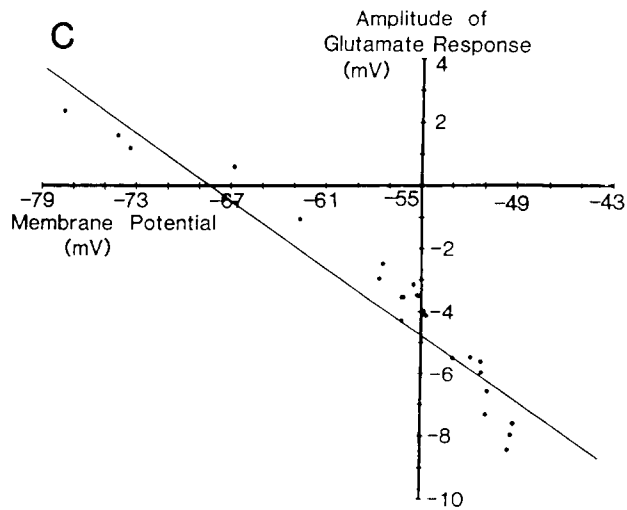
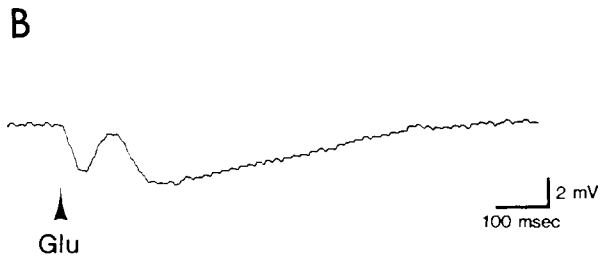
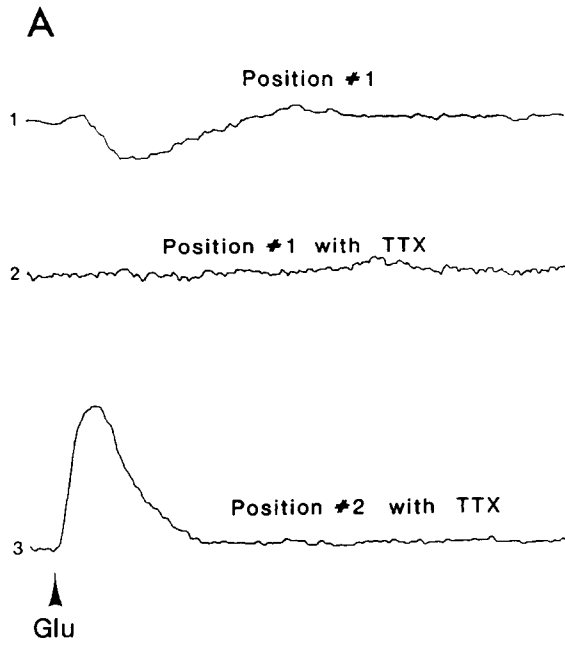
pyramidal cells or from CA<sub>1</sub> interneurons located along the pyramidale/oriens border. A high input resistance intracellular amplifier with internal bridge circuit was used for intracellular recording and for intracellular stimulation via constant current pulse injection. Bridge balance was continuously monitored and adjusted when needed. All acceptable pyramidal cell recordings showed resting membrane potentials greater than 50 mV, input resistance greater than 20 M $\Omega$ , and fired a train of 4–8 spikes in response to a 100 ms injection of 0.5 nA depolarizing current [11]. Amplified voltage signals were displayed on an oscilloscope and stored on tape. Quantitative off-line data analysis was performed later using a Norland 3001 processing digital oscilloscope system.

One mM sodium glutamate (Sigma) was dissolved in saline solution and micro-pressure ejected (via a glass micropipette, tip resistance of 15–30 M $\Omega$ ; 30 psi applied to the pipette) in CA<sub>1</sub> stratum radiatum, onto the apical dendrites of pyramidal cells and interneurons. Interneurons were easily identified and distinguished from pyramidal cells based on their electrophysiological characteristics [14]: (1) interneurons fired a rapid train of 10–25 impulses in response to a 100 ms, 0.5 nA depolarizing current pulse; (2) spike duration was considerably shorter than that of pyramidal cells; (3) input resistance was usually greater than 40 M $\Omega$ ; (4) there was a rapid and abrupt undershoot of the membrane potential following an action potential (whether spontaneous or evoked); (5) spontaneous baseline activity, presumed to be reflective of synaptic activity, was high; and (6) the stimulus threshold for evoking a synaptically driven action potential was always much lower than threshold in nearby pyramidal cells.

Localized application of glutamate in the stratum radiatum usually produced a brief (50–100 ms) depolarization in intra-somatic recordings from CA<sub>1</sub> pyramidal cells. However, hyperpolarizations were often seen that had longer durations (Fig. 1A<sub>1</sub>, B). When a hyperpolarization was recorded in a pyramidal cell in response to dendrite glutamate application, the hyperpolarization could be produced over a wide range of drug electrode locations. As the drug electrode was moved through the tis-

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Fig. 1. A: hyperpolarizing and depolarizing glutamate responses recorded in the same CA<sub>1</sub> pyramidal neuron. Two different intracellular responses were recorded in response to glutamate ejection from the same drug pipette as it moved in a track through the str. radiatum. One mM glutamate was micro-pressure ejected (30 psi) for a duration of 8.5 ms (arrow at onset). A hyperpolarizing response to glutamate was observed at drug electrode position no. 1 (trace 1); 30–50  $\mu$ m deeper into the tissue, at drug electrode position no. 2 (trace 3), a depolarizing glutamate response was observed. The hyperpolarizing glutamate response was observed when drug was ejected at points separated by tens of micrometers, above and below the site at which the depolarizing glutamate response was observed and was blocked by TTX application (trace 2). The depolarizing glutamate response was not blocked by TTX, and was very localized; i.e. small movements of the drug electrode caused a large reduction in the peak amplitude of the response. B: an intracellular recording from a different CA<sub>1</sub> pyramidal cell showing a biphasic response to 1 mM glutamate pressure ejected into the str. radiatum; the depolarizing response appears embedded in a much longer-lasting hyperpolarizing response. C: graph showing the voltage-dependency of the hyperpolarizing glutamate response. The membrane potential was varied by passing hyperpolarizing or depolarizing current through the recording electrode. Values for the peak amplitude of the glutamate response versus membrane potential of the neuron are shown along with the best-fit line for these points ( $r=0.956$ ). In this neuron the reversal potential was  $-68$  mV.



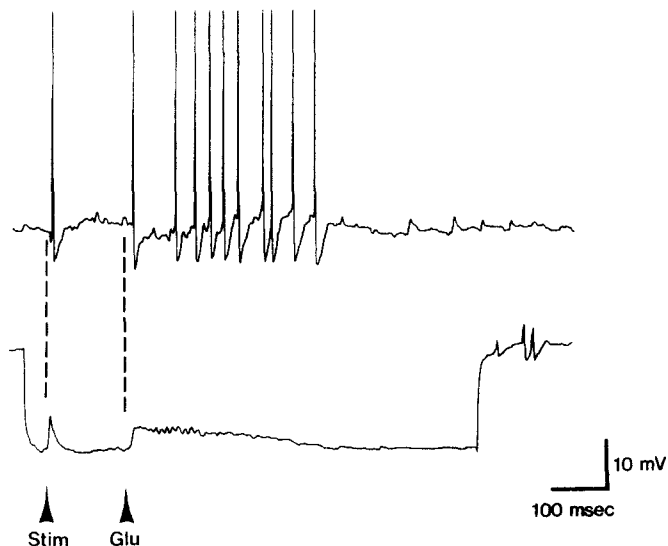


Fig. 2. The excitatory effects of glutamate (Glu), recorded intracellularly from a CA<sub>1</sub> interneuron. One mM glutamate was micropressure ejected (at arrow; 30 psi, 8.0 ms pulse duration) into the str. radiatum. The initial discharge shows the cell's synaptic response to stimulation of the str. radiatum (Stim). Action potentials in the top trace have been clipped. Lower trace shows the same interneuron, with the responses embedded in a 0.5 nA, 750 ms hyperpolarizing current pulse to block action potentials (average of 5 responses). Note the prolonged depolarization, responsible for the burst of action potential discharge, produced by glutamate.

sue, however, a depolarizing response could usually be found at a more precisely restricted location (Fig. 1A<sub>3</sub>). During the transition from pure hyper- to pure depolarizing response, a depolarizing response was occasionally found embedded in the longer lasting hyperpolarization (Fig. 1B). The glutamate-induced hyperpolarization was sensitive to membrane potential, and could be reversed with an intracellular injection of hyperpolarizing current. The mean reversal potential was  $-65.9 \pm 4.48$  mV ( $n = 13$ ) (Fig. 1C).

In 3 experiments where hyperpolarizations were seen, a micro-drop of  $10^{-4}$  M tetrodotoxin (TTX) was applied to the surface of the slice in the stratum radiatum in order to block synaptic transmission supported by action potential generation. When stratum radiatum-evoked EPSPs and action potential generation in response to intracellular current injection were abolished, pyramidal cell responses to glutamate were tested again. In all cells monitored after TTX, the glutamate-induced hyperpolarization previously observed was lost (Fig. 1A<sub>2</sub>). With movement of the glutamate electrode, depolarizing glutamate responses could be found (Fig. 1A<sub>3</sub>), but hyperpolarizations were never seen.

Intracellular recordings were obtained from two interneurons in the stratum pyramidale, and their responses tested to micropulses of 1 mM glutamate delivered in the stratum radiatum. In both cells, glutamate application produced a burst of 5–8 action potentials (Fig. 2, top trace); when glutamate was applied during intracellular pas-

sage of a hyperpolarizing current pulse (to block action potential discharge), a long-duration (0.5 s) depolarizing response was seen (Fig. 2, bottom trace). This depolarizing response was sensitive to small movements of the drug electrode.

We have shown in this study that hyperpolarizing responses to glutamate application may be evoked in CA<sub>1</sub> pyramidal cells. Our results suggest that the hyperpolarization is due to direct excitation of the dendrites of inhibitory interneurons; the interneurons, in turn, make inhibitory synaptic contact onto pyramidal cells. Several findings are consistent with this hypothesis. In our studies, intrasomatic recordings from interneurons located in the stratum pyramidale showed that these cells could be excited (i.e. depolarized) when glutamate was micro-pressure ejected into the stratum radiatum, at locations which could induce pyramidal cell hyperpolarizations. Inhibitory interneurons located in this region are thought to mediate inhibition via the release of  $\gamma$ -aminobutyric acid (GABA) onto somata of CA<sub>1</sub> pyramidal cells [1, 3, 8, 15]. Our results show a pyramidal cell reversal potential for the hyperpolarizing response which is near the GABA reversal potential reported by others using exogenously applied GABA to pyramidal cells [2, 4, 9]. The reversal potential of the glutamate-induced hyperpolarization was also similar to that of the early component of the IPSP evoked by orthodromic stimulation [2, 4, 6, 9]. In our experiments, TTX abolished the glutamate-induced hyperpolarizations, but had no effect on the usual depolarizing responses. This result suggests that spike discharge (and subsequent synaptic transmission) was necessary for generation of the glutamate-induced hyperpolarizations. Finally, the glutamate-induced hyperpolarizing response was observed over a wide area of drug application, whereas depolarizing responses required a more precise localization of the drug electrode.

These data, and the fact that glutamate-induced depolarization of interneurons required precise drug-electrode positioning, suggest that glutamate ejections activate a number of interneurons (over a wide area) which converge on the impaled pyramidal cell. This convergence of CA<sub>1</sub> interneurons onto pyramidal cells was hypothesized in previous examination of interneuron/pyramidal cell connections based on simultaneous recording of synaptically connected pyramidal cell/interneuron pairs [8].

Our results do not distinguish among the types of interneurons that might be excited by glutamate applications (e.g. basket cells as opposed to interneurons with somata located in the stratum radiatum). However, the GABAergic basket cells located in the stratum pyramidale and at the pyramidale/oriens border are known to send their dendritic arbors into the stratum radiatum [13]. Afferent fibers in the stratum radiatum can directly activate inhibitory interneurons in a feedforward manner [1].

Given these morphological and physiological features of CA<sub>1</sub> organization, it is probable that the hyperpolarizing glutamate response observed in our studies can be attributed to activation of the GABAergic inhibitory interneurons along the pyramidale/oriens border. Parallel to our observations of hyperpolarizing glutamate response in hippocampal pyramidal cells, a recent study of cerebellar Purkinje cells reported hyperpolarizing responses to localized applications of excitatory amino

acids; the authors attributed these hyperpolarizations to activation of GABAergic inhibitory interneurons [10].

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