observed in other lesioned groups (Fig. 1). Thus, ethanol abolished two parameters of lesion-induced axonal sprouting in the dentate gyrus. This could reflect inhibition of sprouting in the CA or septal cholinergic neuron systems to various degrees. Alternatively, sprouting of CA fibers may have occurred without being observable because the AChE-positive fibers did not retract from the CA zone. Indeed, the outer (AChE-positive) zone on the side of the lesion was larger in the ethanol-fed lesioned rats than in the pair-fed lesioned and lesioned control groups. However, the failure of the AChE-positive fibers to withdraw from the CA zone would be inconsistent with the critical afferent theory (*16*) of sprouting axons. Further experiments are required to determine which of these systems is most sensitive to the inhibitory effects of ethanol on axonal sprouting.

There is little doubt that prolonged heavy consumption of alcohol interferes with the structural and functional integrity of the brain. In rats, long-term exposure to ethanol results in loss of dendritic spines from neurons in the hippocampus and dentate gyrus (*3*). Moreover, ethanol exacerbates the destructive effects of cerebrospinal trauma in cats (*20*). Increased cerebral atrophy in alcoholic patients has been identified by computerized axial tomography (*4, 5, 21*), pneumoencephalography (*6*), measurement of brainstem-evoked potentials (*22*), and autopsy (*7*). We have now shown significant inhibition of axonal sprouting with daily exposure of rats to ethanol for 2 weeks before and 9 days after an entorhinal lesion. High levels of ethanol may exert a toxic effect on sprouting axons.

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18. Rats in the ethanol-fed lesioned group were given unlimited access to ethanol in a liquid diet (Bio-Serv). This diet, which was the sole source of food and water, was imbibed through calibrated drinking tubes. The individually housed rats were fed the ethanol-containing diet for 2 weeks. After receiving unilateral electrolytic lesions (*14*), the rats were allowed to recover while receiving the liquid diet. Body weight dropped slightly in the ethanol-fed and pair-fed lesioned groups when the liquid diet was started, but was not significantly different on the day of the lesion. The rats were killed 9 days after surgery and their brains were processed for AChE histochemistry by a modification of the method of F. A. Geneser-Jensen and T. W. Blackstad [*Z. Zellforsch.* **114**, 460 (1971)]. For each animal the section used for analysis was taken at the point along the septotemporal axis of the dorsal hippocampal formation where the dorsal and ventral limbs of the dentate gyrus first become continuous. Each section was projected and a MOP-AMO-3 digitizer (Zeiss) was used to measure the widths of each zone in the molecular layer at 15 randomly selected points along the dorsal limb of the dentate gyrus. The measurements for each group were pooled and the mean width of each layer was computed (Table 1). The minimum number of samples required per section (*15*) was determined by the technique of progressive means (M. A. Williams, *Quantitative Methods in Biology* (Elsevier/North-Holland, Amsterdam, 1977).
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Synchronous Neural Afterdischarges in Rat Hippocampal Slices Without Active Chemical Synapses

Abstract. *Extracellular field potential and intracellular recordings from neurons in rat hippocampus show that, even with synaptic transmission blocked, antidromic electrical stimuli can trigger afterdischarges of up to 9 seconds duration; during these discharges action potentials of a single neuron were synchronized with extracellularly recorded population spikes. Apparently mechanisms other than recurrent chemical synapses can synchronize and recruit epileptiform events. Measurements of transmembrane potential indicate that transient extracellular electrical fields (ephaptic interactions) contribute to the observed synchrony; electrotonic coupling and changes in the concentration of extracellular ions may also contribute.*

During seizures, bursts of action potentials and membrane depolarizations are abnormally synchronized across large populations of cortical neurons. The electrical properties of individual neurons that appear epileptic have been investigated (*1, 2*), but the mechanisms that synchronize epileptiform discharges and cause their local spread are not known. Seizures can spread from one area of the brain to a remote region through projection pathways involving chemical synapses (*3*). Some investigators (*1, 2, 4–6*) have suggested that chemical synapses also locally synchronize and propagate epileptiform activity, especially by recurrent excitation. However, other local synchronizing mechanisms may include electrotonic synapses (*5, 7–9*), electrical field effects (ephaptic interactions) (*10*), and changes in concentration of extracellular K^+ and Ca^{2+} (*1, 11, 12*). We investigated the possibility that synchronized epileptiform events can occur when chemical synaptic transmission is inoperative.

Extracellular and intracellular recordings of CA1 pyramidal cells were made by conventional techniques (*13*). Transverse slices (450 μm thick) of freshly

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dissected rat hippocampus (Fig. 1A) were bathed in an oxygenated physiological solution (*14*) containing Mn^{2+} (2.3 m*M*) and lowered concentration of Ca^{2+} (0.5 m*M*), which blocked evoked chemical postsynaptic potentials (Fig. 1B). With prolonged incubation (> 1 to 2 hours), however, the excitability of neurons gradually increased, and in each of 14 experiments antidromic extracellular stimulation evoked afterdischarges of 0.2 to 9 seconds. The cause of such hyperexcitability is not known (*15*). During the afterdischarges, repetitive population spikes (*16*) of large amplitude (Figs. 1C and 2A) revealed that action potentials were synchronized across a large portion of the CA1 population. Intracellularly recorded action potentials coincided with large population spikes more frequently than with small ones. In two cases additional bursts of population spikes followed the evoked burst after a delay of 1 or 10 seconds.

Several observations indicated synchronizing effects other than the electrical stimulus. (i) Intracellularly recorded neurons that became silent during an afterdischarge always resumed firing synchronously with population spikes (Fig. 1C). (ii) Instead of progressive desynchronization, large population spikes were still seen at the end of an afterdischarge, indicating maintained synchrony (Fig. 1C). (iii) In one experiment synchronous firing occurred without any electrical stimuli (Fig. 2, A and B). (iv) When early action potentials of the afterdischarge were blocked with hyperpolarizing current in the impaled neuron, subsequent action potentials were synchronous with the population spikes. We conclude that one or more mechanisms other than chemical synapses or the applied electrical shocks synchronized firing in these experiments. The synchronizing mechanisms must have involved interactions among neurons.

Depolarizations due to electrically remote action potentials (*17*) or electrotonic postsynaptic potentials (*7*) initiate action potentials in hippocampal neurons. In five of nine intracellular recordings during afterdischarges, depolarizations were observed that were synchronous with population spikes. The two depolarizations shown in Fig. 2B were of similar amplitude, but in another recording the depolarizations ranged from 2 to 15 mV. Relatively weak hyperpolarization (~ 10 mV) blocked these potentials, which suggests that they were active responses generated electrically near the recording site. These depolarizations could be electrotonic postsynaptic potentials or partially blocked spike activity in the impaled cell; our data do not distinguish between these possibilities.

In the experiments described above, intracellular membrane potential was measured in a conventional manner (that is, with reference to a distant bath electrode). However, since large extracellular population spikes would distort conventional intracellular recordings, a different method was also used: the voltage from a nearby extracellular micropipette was subtracted from the intracellular voltage, thus producing a differential voltage recording across the cell membrane (Fig. 2, C and D) (*18*). These experiments revealed small rapid subthreshold depolarizations that had been previously masked by population spikes and were closely related to them in time, wave form, and amplitude (Fig. 2C, bottom trace). If these subthreshold depolarizations were electrotonic postsynaptic potentials, one would expect the time course of the depolarizations to reflect the slow decay of the depolarizing afterpotentials observed intracellularly in all CA1 pyramidal cells incubated in Mn^{2+} medium (*9*). Hyperpolarizing current injection had no observable effect on the depolarizations revealed with differential recording. Accordingly, we suggest that these masked depolarizations represent a voltage drop of the extracellular field

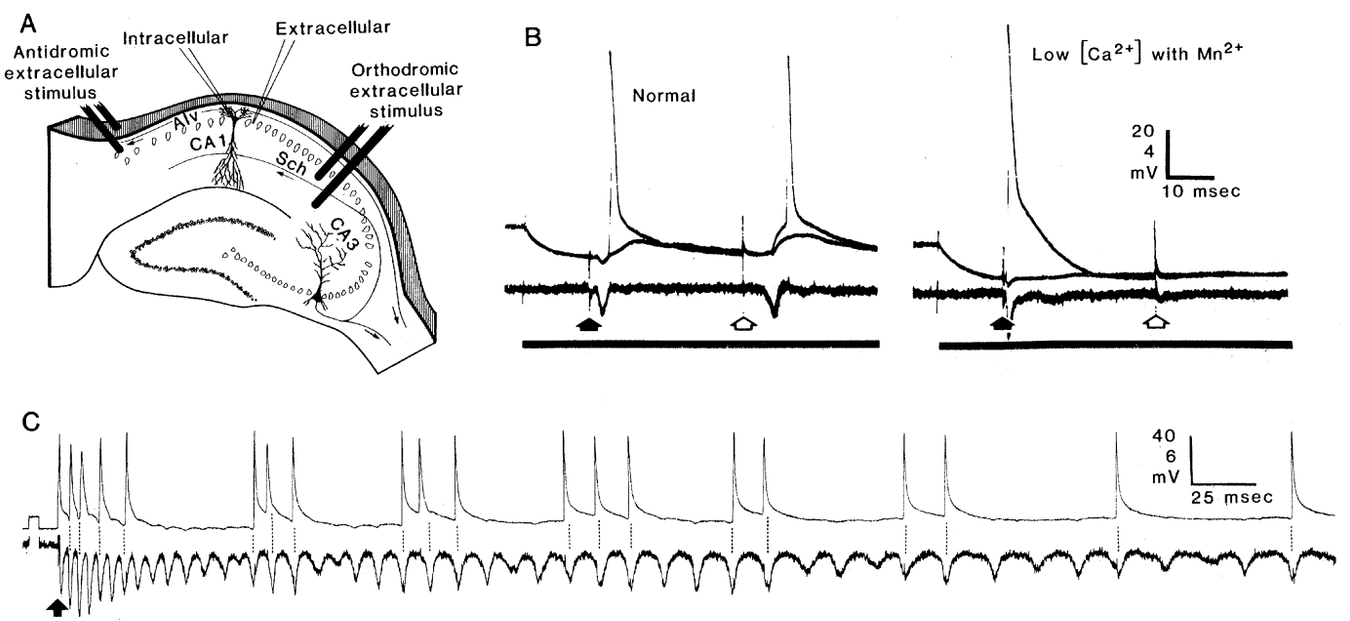

Fig. 1. (A) Diagram of hippocampal slice and electrode placement. Antidromic electrical stimuli were applied to the alveus (*Alv*). Stimulation of CA3 Schaffer axon collaterals (*Sch*) caused an orthodromic excitatory postsynaptic potential (EPSP) in CA1. (B) Blockade of chemical synapses. In both superimposed intracellular recordings (top traces), 1 nA of hyperpolarizing current (dark bar) accentuated EPSP's, probably reversed intracellular PSP's and delayed action potentials of the impaled cell. In normal physiological solution, an alvear stimulus (black arrow) caused an action potential (top traces) and a negative population spike recorded extracellularly (bottom traces). Orthodromic stimulation (open arrow) caused an EPSP that could fire the cell and also evoked a population spike. In a solution with Mn^{2+} and lowered Ca^{2+} concentration (*14*), EPSP's of a nearby cell were blocked even at five times the original stimulus intensity (right panel). Chemical synapses on remote dendrites, which would normally cause smaller EPSP's at the somatic impalement site, were also presumably blocked. (C) Afterdischarge produced by antidromic stimulus (arrow) after 3 hours of incubation in Mn^{2+} medium. Note synchrony of individual action potentials (upper trace) with population spikes (broken lines). All but two intracellularly recorded action potentials occurred within 2 msec of the negative peaks of individual population spikes (S.D. = 1.14 msec, N = 20 intracellular action potentials). Deflection at the beginning of the record is a 10-mV, 5-msec calibration pulse.

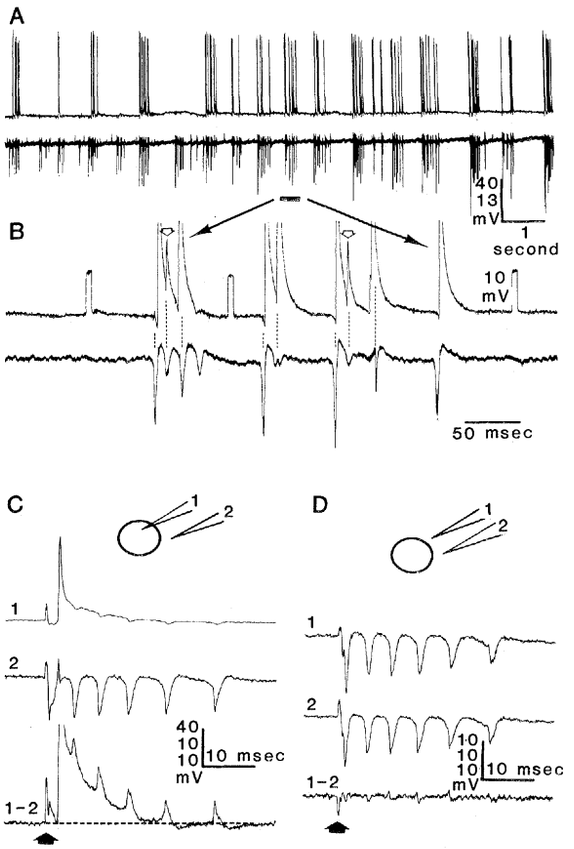

Fig. 2. (A) Spontaneous synchronous bursting recorded from hippocampal slice that was bathed in solution containing Mn^{2+} (2.3 m*M*) and Ca^{2+} (0.5 m*M*) for 4 hours. Intracellularly recorded action potentials (top trace) occurred synchronously with many of the spontaneously recurring bursts of population spikes. A portion of this record (dark bar) is expanded in (B). Note depolarizations (open arrows). (C) Extracellular electrode was moved close to the intracellular recording site (inset). Differential voltage recording indicates true membrane voltage of CA1 pyramidal cell (bottom trace) and reveals small subthreshold depolarizations which mirror the extracellularly recorded field potential. Dashed line indicates neuron resting potential. (D) Proximity of the two electrodes used in (C) was confirmed by withdrawing the intracellular electrode 5 μm. Differential recording reveals only a small error potential (bottom trace) and indicates that the error in the differential recording of (C) was probably insignificant.

potential across pyramidal cell membranes and thus are purely passive events—that is, electrical field effects. The subthreshold depolarizations could sum with depolarization from current injected through the recording electrode to elicit somatic action potentials (*19*). Therefore, the mechanism that caused these depolarizations contributed to synchronization.

Our data indicate that synchronization of hippocampal pyramidal cells can occur without active chemical synapses, probably through some combination of mechanisms including fluctuations in extracellular ions, electrotonic coupling, and electrical field effects. Although slow changes in the concentrations of extracellular ions such as K^+ probably occurred during the afterdischarges, it seems unlikely that the concentrations could oscillate rapidly enough to synchronize individual spikes (*20*). Other evidence has indicated electrotonic coupling between some CA1 pyramidal cells (*7, 9, 21*), but whether it is sufficient to synchronize neuronal firing is not known. Weak or strong electrotonic junctions might be undetectable in our recordings. The rapid depolarizations revealed with differential recordings of transmembrane potential from hippocampal pyramidal cells, which are tightly packed and arranged in parallel (*22*), indicate that transient electrical fields (population spikes) contribute to the predisposition of the hippocampus to epilepsy.

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15. The threshold for spike initiation by intracellular current injection declined after prolonged incubation in a static bath containing 2.3 m*M* Mn^{2+} and 0.5 m*M* Ca^{2+}. The passive component of the depolarizing afterpotential [R. K. S. Wong and D. A. Prince, *J. Neurophysiol.* **45**, 86 (1981)] after intracellularly recorded action potentials increased in amplitude and duration, and neurons tended to fire in doublets or short bursts. Increased excitability and afterdischarges occurred in all slices which appeared viable, as judged by large population spikes (10 to 25 mV) and transparency of the cell body layer [T. J. Teyler, *Brain Res. Bull.* **5**, 391 (1980)]. The ionic composition of the bathing solution may have gradually changed because replacement with fresh medium sometimes prevented afterdischarges. Similar afterdischarges sometimes occurred in normal medium; other workers have evoked afterdischarges electrically in vivo [E. R. Kandel and W. A. Spencer, *Exp. Neurol.* **4**, 162 (1961); D. P. Purpura, J. G. McMurtry, C. F. Leonard, A. Malliani, *J. Neurophysiol.* **29**, 954 (1966)]. However, in solution with Mn^{2+} and low Ca^{2+}, blockade of recurrent inhibition and depression of Ca^{2+}-activated K^+ conductance may have increased the tendency for afterdischarge.
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