Activity-Dependent Disinhibition. I. Repetitive Stimulation Reduces IPSP Driving Force and Conductance in the Hippocampus In Vitro

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SUMMARY AND CONCLUSIONS

1. Intracellular recording techniques were used to investigate the mechanisms underlying the activity-dependent lability of inhibitory synaptic potentials indirectly evoked in CA3 pyramidal neurons by stimulation of the mossy fiber afferent pathway in organotypic slice cultures of hippocampus.

2. Repetitive stimulation (3–10 Hz, 30–60 s) was found to reduce the amplitude of the inhibitory postsynaptic potential (IPSP) and occasionally lead to repetitive, epileptiform discharge.

3. Under single-electrode voltage-clamp, the current underlying the inhibitory postsynaptic potential (IPSC) was found to have the same reversal potential (E_{IPSC}) as the response to iontophoretically applied γ-aminobutyric acid (E_{GABA}), and both were blocked by bicuculline. Reducing the extracellular Cl^- concentration from 153 to 89 mM shifted E_{GABA} in the depolarizing direction by 9 mV from -64.7 to -55.6 mV, an amount close to that predicted by the Nernst equation. We therefore presume that the IPSC is mediated by GABA and that the reversal potentials of both are equal to E_{Cl^-}.

4. Under single-electrode voltage-clamp, repetitive stimulation (3–10 Hz, 30–60 s) was found to cause a mean decrease in the conductance underlying the IPSC (g_{IPSC}) of 22%. This decrease was independent of the membrane potential at which stimuli were delivered.

5. Under single-electrode voltage-clamp, repetitive stimulation (3–10 Hz, 30–60 s) was found to cause a 2–8 mV depolarizing shift in E_{IPSC} when the membrane potential was held constant 5–15 mV depolarized from E_{IPSC}. The mean decrease in IPSC driving force was 49%. If membrane potential was held 10–20 mV hyperpolarized from E_{IPSC}, there was no change in driving force.

6. Currents activated by iontophoretically applied GABA were decreased in amplitude following repetitive stimulation at depolarized, but not hyperpolarized, holding potentials.

7. The decrease in IPSC driving force following repetitive stimulation at depolarized holding potentials was less after decreasing the extracellular K^+ concentration from 5.8 to 1 mM.

8. We conclude that the decrease in driving force following repetitive stimulation results from an increase in the intracellular Cl^- concentration, and that the activity-dependent decrease in g_{IPSC} results from a decrease in presynaptic release rather than from postsynaptic receptor desensitization.

INTRODUCTION

Several experimental models of human epilepsy are in use that allow investigation of different aspects of the disease process. Application of convulsant drugs to isolated and intact CNS structures has provided significant insight into the cellular mechanisms underlying the focal interictal event (for reviews see Refs. 16, 32, 44). The finding that a wide range of convulsants exert their effect by interfering with the efficacy of inhibitory synaptic transmission (32) has suggested a critical role for the inhibitory postsynaptic potential (IPSP) in limiting the excitability of the cell population. Under normal conditions, activation of afferent pathways to hippocampal pyramidal cells produces a short-latency excitatory postsynaptic potential (EPSP), which may be large enough to evoke a single action potential, followed by a pronounced hyperpolarizing IPSP. This IPSP results from the activation of inhibitory interneurons that release the neurotransmitter γ-aminobutyric acid (GABA) (e.g., Ref. 18). Substances that block hippocampal GABAergic synapses directly (e.g., penicillin) or indirectly (e.g., opiates) increase excitability by unmasking recurrent excitatory interactions, causing cells to discharge synchronously and drive follower populations (31, 39, 61). Manipulations that increase excitation (e.g., removal of extracellular Mg^{2+}) can also produce epileptiform discharge (e.g., Ref. 59). While providing significant insights into the nature of epileptiform discharge, this class of model has the limitation of producing epilepsy by seemingly nonphysiological manipulations of the ionic milieu or pharmacologic intervention.

The kindling model of epilepsy has provided complementary information regarding the origin of epilepsy (for review see Ref. 36). Long-lasting changes in cell excitability are observed following repeated application of high-frequency, high-intensity, kindling-like stimulation to many parts of the brain, including the hippocampus (e.g., Refs. 34, 50). This model shares many features with human epilepsy: *1)* seizures occur spontaneously after kindling (43); *2)* spontaneous interictal spikes are observed in between seizures (43); *3)* similar, specific patterns of neuronal damage are seen (49); and *4)* epileptiform discharge can be projected from sites of focal seizures to target populations, leading to the development of secondary foci (58). Although the changes responsible for kindling are as yet unclear, kindling has been shown to produce a long-term depression of GABAergic inhibition (49, 51).

Repetitive stimulation at relatively low frequencies has also been shown to result in a gradual increase in the excitability of hippocampal pyramidal cells in situ and in vitro, as shown by the transition of the neuronal firing pattern from single spikes to multiple-spike bursts, synchronized throughout the population (e.g., Ref. 4, 48, 62). Coincident

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with the development of burst discharge there is a large reduction in the amplitude of the IPSP (7, 27, 35, 62). The similarity of these bursts to epileptiform discharge, and the ability to evoke them with physiologically relevant stimulation frequencies and without pharmacologic intervention, suggests that the depression of the IPSP that follows repetitive stimulation, or activity-dependent disinhibition, may represent a significant endogenous process underlying some aspects of epilepsy. Importantly, repeated applications of low-frequency, low-intensity stimulation to the hippocampus can produce kindling similar to that seen following high-frequency stimulation (11).

The exact mechanisms underlying activity-dependent disinhibition have not yet been fully described. Although initially attributed to potentiation of partially overlapping excitatory postsynaptic potentials (4), or depolarizing GABA-mediated responses (62), it has now been established that IPSPs are themselves depressed following a train of stimuli (7, 35). How the IPSP is depressed is not known, although two aspects of the IPSP have been implicated: the driving force for inhibition (the difference between membrane potential, V_m, and the IPSP reversal potential, E_{IPSC}) and the magnitude of the underlying evoked conductance (g_{IPSC}). Decreases in driving force are believed to result from accumulation of Cl^- intracellularly (e.g., Ref. 29), as the extracellular concentration of Cl^- is effectively constant (17). Decreases in g_{IPSC} could result from either presynaptic processes, such as a decrease in evoked transmitter release (e.g., Ref. 15), or postsynaptic processes, such as desensitization (e.g., Refs. 31, 42).

Previous observations on the effects of repetitive stimulation have been largely made with current-clamp techniques, making it difficult to separate clearly these potential mechanisms. We have therefore reexamined the effects of repetitive stimulation on the processes that produce the IPSP using the single-electrode voltage-clamp technique to directly measure IPSP conductance and driving force. The cultured hippocampal slice is particularly well suited for these experiments due to the excellent visibility of, and access to, the neurons, as well as because of the maintenance of the normal synaptic arrangements (20, 21, 64). We have chosen to investigate the IPSP evoked indirectly in CA3 pyramidal cells following orthodromic activation of the mossy fiber system because of the apparent importance of this population as a pacemaker for seizure discharge (e.g., Ref. 61).

Portions of the data in this series of papers have been presented in abstract form (53, 54).

METHODS

Organotypic hippocampal slice cultures were prepared and maintained as described previously (20, 21). In brief, 4- to 6-day-old neonatal rats were decapitated and the hippocampus was dissected free. Transverse slices of 400 μm nominal thickness were cut with a McIlwain tissue chopper. Most slices were X-irradiated (1,000–1,500 roentgen over 5–10 min) to reduce glial proliferation and thus improve the ability to visualize and to impale individual neurons. Cells in irradiated slices were physiologically indistinguishable from those in nonirradiated slices. Slices were then attached to cleaned glass coverslips in a film of chicken plasma clotted with thrombin. Slices were placed in sealed test tubes containing 0.5–1.0 ml medium and maintained at 36.5°C

on a roller drum (10 revolutions/h) in an incubator. Incubation media consisted of 50% basal medium (Eagle), 25% balanced salt solution, and 25% heat-inactivated horse serum (GIBCO). The concentration of HCO_3^- in the incubation medium was varied with use of either Hanks' or Earle's salt solutions to buffer metabolic lactic acid production. Cultures were fed once per week.

After 2 wk in culture the slices had flattened out to a thickness of only 1 or 2 cell diameters in S. pyramidale, yet had retained much of the cellular and local synaptic organization found in the hippocampus in situ (see Refs. 20, 21, 64). Cultures were then transferred to a recording chamber mounted on the stage of an inverted microscope (Zeiss, IM 35) equipped with phase-contrast optics, where they were continuously superfused and maintained at 35°C. The volume of the chamber was <1 ml allowing for rapid exchange of contents in <2 min. Control saline (modified Hanks' balanced salt solution, GIBCO) consisted of the following (in mM): Cl^- 153, Na^+ 141, K^+ 5.8, glucose 5.6, HCO_3^- 4.1, Ca^{2+} 3.3, Mg^{2+} 2.4, SO_4^{2-} 0.4, $H_2PO_4^-$ 0.4, and HPO_4^{2-} 0.3. The pH in room air was 7.4, as monitored with the presence of phenol red (10 mg/l) in all solutions. Alteration of the concentration of ions was always done as equimolar substitution of the appropriate salt for NaCl.

After 11–33 days in vitro, intracellular recordings were made from visually identified pyramidal neurons located in area CA3 using thin-walled micropipettes (1 mm OD, 0.75 mm ID), pulled on a Brown-Flaming puller (Sutter Inst.). Unless otherwise noted, electrodes were filled with 2 M potassium acetate (pH = 7) and had tip resistances of 40–80 MΩ. Tip potentials were compensated for by adding the bath potential, measured upon withdrawing from the cell, to the command voltage. Synaptic currents were measured using a sample-and-hold switching voltage-clamp amplifier (Axoclamp 2, Axon Inst.) (for review of the technique see Ref. 19). The head stage output was continuously monitored and capacitance compensation appropriately adjusted to insure that the microelectrode voltage transient resulting from the injected current pulse had fully settled before the membrane potential was sampled. Experiments were stopped if this became impossible. Due to the relatively high resistance of acetate-filled electrodes, switching frequency was limited to 2.0–2.6 kHz; however, clamp gain was typically 5–6 nA/mV. Voltage commands were delivered to the amplifier and the sampled membrane potential and membrane currents were digitized and stored on-line by a computer-controlled (IBM-AT; pClamp software, Axon Inst.) analog/digital converter (Labmaster, Tecmar, Inc.). The digitization rate was 2–5 kHz.

Synaptic potentials were evoked with 100 μs anodal current pulses from 5 MΩ, 2 M NaCl-filled micropipettes positioned in S. radiatum at the mouth of the dentate hilus so as to predominantly activate the mossy fiber pathway (for anatomy of mossy fibers in hippocampal slice cultures see Ref. 64). Current intensity (5–50 μA) was adjusted to yield the maximal postsynaptic response. In some experiments drugs were applied iontophoretically. GABA, baclofen, or muscimol were prepared as 0.25–1.0 M solutions in H_2O and titrated to pH = 3.5–4.5 with NaOH or HCl. They were applied from micropipettes (1–3 μm tip diam.) with cathodal current. Anodal retaining currents were used between applications. The electrophoretic pipette was visually positioned directly adjacent to the soma of the impaled cell. All drugs were obtained from commercial sources except baclofen, which was a gift of Dr. H.-R. Olpe, Ciba-Geigy.

As shown previously (e.g., Ref: 21–23), CA3 cells in slice cultures have active and passive membrane properties that are indistinguishable from those observed in situ and in acutely prepared hippocampal slices. Cells were considered acceptable for study if they had resting membrane potentials greater than −55 mV, resting membrane conductances of <50 nS, and overshooting action potentials. A total of 175 neurons were studied for this and the

subsequent two papers. The average resting membrane potential of 25 randomly selected cells was -63.6 ± 4.0 (SD) mV, and the average membrane conductance at rest was 20.9 ± 8.2 (SD) nS.

RESULTS

Measurement and interpretation of IPSP and GABA driving force and conductance

Interpretation of the results of this and the subsequent two papers relies on the ability to measure accurately both the reversal potential and slope conductance of the IPSP under voltage-clamp. Despite the limitations inherent in the single-electrode technique, as well as the difficulties in obtaining an adequate space-clamp in a cell with such a complicated geometry, we believe that the impact of these potential problems on the conclusions drawn are minimal for the following reasons: *1)* GABAergic synapses are located primarily at or near the soma of cultured pyramidal cells (unpublished observations) as they are in situ (46) allowing for minimal distortion of the response due to the cable properties of the cells, *2)* hippocampal cells are electrically compact (e.g., 9), and *3)* we have limited our measurements to the range of membrane potential (-50 to -90 mV) in which the resting current/voltage relationship is relatively linear (see Fig. 2*B*) thus eliminating activation of potentially spurious voltage-dependent currents (see also Ref. 10).

The current/voltage relationship of the IPSP was measured by eliciting synaptic currents 100–200 ms after the start of voltage command pulses of varying amplitude and polarity (Fig. 1*B*). In this way any current through resting and voltage-dependent conductances has reached a steady-state before stimulation. The difference between this steady-state current and the peak synaptic current, measured 20–30 ms after the stimulus, at which time the IPSP reaches maximum amplitude (Fig. 1*A*), is thus due solely to current flow through the synaptic conductance. This measured inhibitory postsynaptic current (IPSC) is then plotted as a function of the corrected command voltage (Fig. 1*C*). The magnitude of the synaptic conductance evoked (g_{IPSC}) is calculated as the slope of the best fit line (using linear regression analysis) and the reversal potential (E_{IPSC}) is, by definition, the voltage at which the regression line crosses zero current. Regression lines were typically determined from 14 measurements within the range of membrane potentials between -90 and -50 mV and had correlation (r^2) values > 0.95. The standard error of the estimated reversal potential was typically <1 mV. In describing the results, holding potential (V_{hold}) will be used to refer to the voltage maintained during an experimental maneuver, such as changing a solution or delivering repetitive stimulation, whereas the voltage during a given command pulse at which an individual IPSC was evoked will be refered to as the membrane potential (V_m).

The mean E_{IPSC} was -65.7 ± 4.7 mV ($n = 57$) and the mean g_{IPSC} was 129.9 ± 60.5 nS ($n = 57$) in control saline with acetate-filled electrodes. These values agree quite closely with those obtained under current- and voltage-clamp in the hippocampus in situ and in acute slices (e.g.,
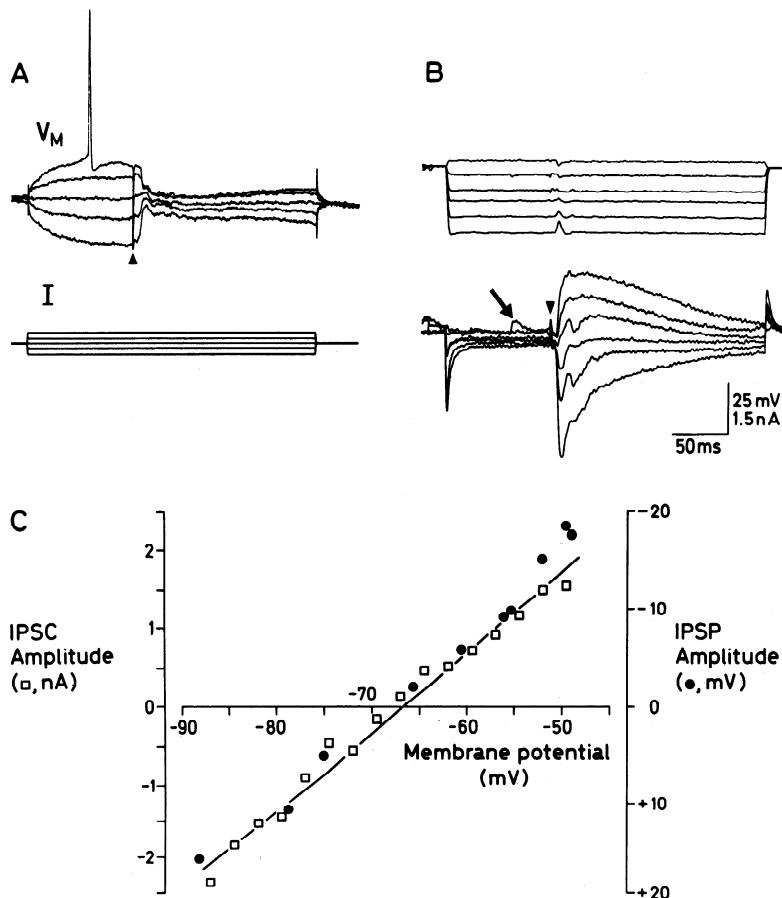

FIG. 1. Calculation of IPSP reversal potential and conductance. *A*: intracellular recording of IPSPs evoked with brief (100 μs) monopolar stimulation of the mossy fiber afferent pathway (at time indicated with *solid arrowhead* in this and subsequent figures) while the membrane potential (V_m) was varied with intracellular current pulses (I) of varying amplitude and polarity. *B*: same cell and stimulation as in *A*, but under single-electrode voltage-clamp. Step voltage commands were delivered (sampled V_m shown in *upper traces*) and the resulting membrane currents are shown. At depolarized V_m the synaptic current is predominantly outward, whereas at hyperpolarized V_m synaptic currents are predominantly inward. In this and all subsequent figures inward currents are downward. Although the initial portion of the synaptic response is poorly clamped (note escape of V_m in upper traces), V_m is well clamped at the time of the peak of the IPSP (25–35 ms after stimulation). Currents resulting from the presence of spontaneous IPSPs are sometimes apparent in this and subsequent records (*arrow*). *C*: amplitude of the IPSP (under current-clamp, *solid circles*) and the IPSC (under voltage-clamp, *open squares*) are plotted as a function of the membrane potential at which they were evoked. g_{IPSC} is given by the slope of the regression line calculated from the voltage-clamp data (98 nS) and E_{IPSC} is the voltage at which no synaptic current flows (-67 mV, not different from the IPSP reversal potential). IPSP and IPSC amplitudes measured 35 ms after stimulation.
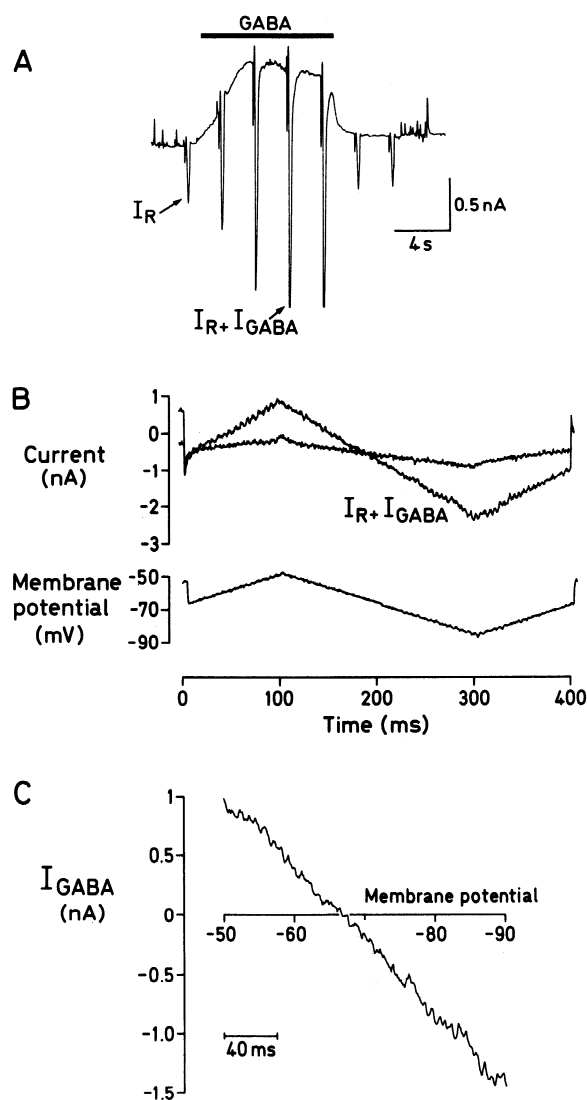
several holding potentials, and the resulting responses plotted as a function of the holding potential. This process is rather slow, however. Alternatively, ramp voltage commands were used to measure simultaneously both the reversal potential (E_{GABA}) and conductance (g_{GABA}) of the response to GABA. This was done by first measuring the current flowing through the resting conductance with a ramp voltage-command (−45 mV to −85 mV over 200 ms) (Fig. 2A, B: I_R). Then, the same command protocol was repeated at the peak of a GABA response; the resulting current being the sum of the currents flowing through both the resting and the GABA-activated conductances (Fig. 2A, B: $I_R + I_{GABA}$). The computer then digitally subtracted the resting current, measured before GABA application, from the currents during GABA application to isolate the portion of the current that flowed through the GABA-activated conductance (I_{GABA}). When this current is plotted as a function of the command voltage the magnitude of g_{GABA} is given by the slope of the line and E_{GABA} is the voltage at which there is no current flow (Fig. 2C). Identical results were obtained with both methods. The mean E_{GABA} under control conditions was −66.4 ± 4.2 mV ($n = 11$) and was not significantly different from E_{IPSC} when measured in the same cell. g_{GABA} was a function of the magnitude of the iontophoretic current (range of g_{GABA} used = 30–250 nS). Rectification of the GABA-activated conductance (e.g., 6) was not significant within the range of V_ms examined (Fig. 2C). In the presence of bicuculline (10^{-5} M) these focal iontophoretic applications of GABA were incapable of significantly activating the GABA$_B$-mediated K$^+$ conductance (22). It is therefore reasonable to assume that the response to iontophoretic GABA is mediated solely by GABA$_A$ receptors.

Multiphasic GABA responses, including a prominent depolarizing component, have been observed in neurons in acutely prepared hippocampal slices, and are most prominent when applications are localized to neuronal dendrites in S. radiatum (2, 3, 62). It has been suggested that during repetitive stimulation, presynaptically released GABA may "spill over" onto receptors at these "extrasynaptic" loca-

FIG. 2. Calculation of E_{GABA} and g_{GABA}. A: current activated by an 8-s, 10-nA iontophoretic application of GABA. Vertical deflections result from ramp voltage commands used to assess E_{GABA} and g_{GABA}. Ramps before application of GABA activate currents (I_R) that flow through only resting conductances. Ramps at the peak of the GABA response activate currents that flow across both resting and GABA-activated conductances ($I_R + I_{GABA}$). B: membrane current before (I_R) and at the peak of the GABA response ($I_R + I_{GABA}$, as indicated in A), and sampled V_m during ramp voltage command. Note large increase in conductance during GABA application as shown by the larger current needed to effect the same voltage ramp. C: I_{GABA} is isolated by digitally subtracting (I_R) from ($I_R + I_{GABA}$) and plotting the result as a function of V_m during the ramp command. Note that the polarity of the abscissa is reversed from convention in this and subsequent figures using this technique. g_{GABA} is given by the slope of this plot, and E_{GABA} is the zero current intercept. E_{GABA} is thus −67 mV, not different than E_{IPSC} measured in the same cell (Fig. 1). g_{GABA} is 63 nS, electrode resistance = 63 MΩ.

7, 10, 35, 37). The IPSC could be blocked with (±) bicuculline methochloride (10^{-5} M, $n = 3$), confirming that it is GABA-mediated; and E_{IPSC} was shifted in the depolarizing direction when recorded with KCl-filled electrodes ($n = 3$), confirming that it is carried by Cl$^-$ ions (18).

The reversal potential and conductance of responses activated with iontophoresis of GABA could be obtained in two ways. First, brief pulses of GABA can be applied at
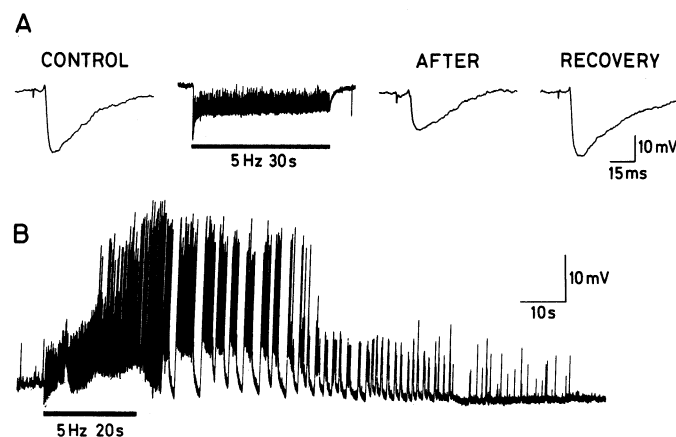

FIG. 3. Depression of IPSPs following repetitive stimulation. A: IPSPs under current clamp recording at $V_m = -55$ mV before, during a train of stimuli at 5 Hz for 30 s (slower time base), immediately after the stimulus train, and after 2–3 min recovery. IPSP amplitude is decreased 45% after stimulation. B: development of spontaneous, rhythmic afterdischarge in another cell following a train of stimuli at 5 Hz for 20 s, $V_m = -59$ mV.
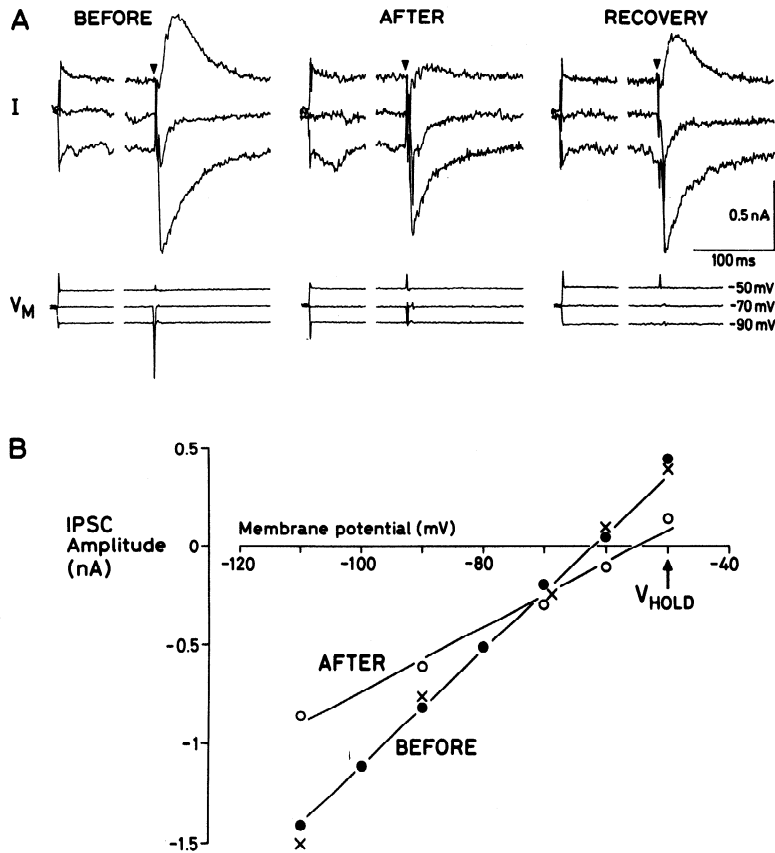
FIG. 4. Effects of repetitive stimulation at depolarized V_{hold} on E_{IPSC} and g_{IPSC}. A: sampled V_m and membrane currents (I) in response to step voltage commands and synaptic stimulation before, after repetitive stimulation at 2 Hz for 60 s at V_{hold} = -50, and after 3 min recovery. Note that the synaptic current at V_m = -50 mV is more depressed than the synaptic current at V_m = -90 mV. B: calculation of E_{IPSC} and g_{IPSC} before (●), after repetitive stimulation (○), and after recovery (×) shows that both g_{IPSC} and driving force are reversibly decreased (45 and 56%, respectively) following repetitive stimulation.

tions, thus causing an apparent depolarizing shift in E_{IPSC} (62). We therefore examined responses to iontophoretic applications of GABA in S. radiatum. Neither multiphasic nor depolarizing GABA responses were ever observed in dendritic (n = 4 cells) or somatic applications. We may therefore rule out the involvement of such a process in activity-dependent disinhibition in our preparation.

If I_{GABA} is a purely chloride-mediated current, then its equilibrium potential should be predicted by the Nernst equation for Cl^-. That is, E_{GABA} should be dependent on the Cl^- gradient such that a 10-fold decrease in the extracellular Cl^- concentration ($[Cl^-]_o$) should produce a 61-mV depolarizing shift in E_{GABA}. $[Cl^-]_o$ was reduced from control (153 mM) to 89 mM by equimolar replacement of NaCl with sodium isethionate. E_{GABA} after 5–10

min in the low $[Cl^-]_o$ solution was found to be significantly less negative than control (-55.6 ± 1.8 mV, n = 7; $P <$ 0.001, Mann-Whitney U test), close to the value of -52.1 mV predicted by the Nernst equation. It was not possible to determine the effects of reducing $[Cl^-]_o$ on E_{IPSC} because of the development of epileptiform discharge in this low $[Cl^-]_o$ saline (63).

Taken together, these data thus strongly support the assumption that the IPSC is GABA mediated, and that the reversal potential of both responses may be considered to be equal to the Cl^- equilibrium potential (E_{Cl^-}).

Effects of repetitive stimulation on the IPSP

The lability of the IPSP during repetitive stimulation was examined in CA3 neurons in hippocampal cultures. As in

TABLE 1. *Effects of repetitive stimulation at depolarized V_m on inhibition*

	Control	After	Recovery	Mean Decrease/Cell	n
E_{IPSC}, mV	-62.5 ± 6.5	-57.8 ± 7.6*	-61.5 ± 6.8	4.7 (2–8)	15
Driving force, mV	10.6 ± 4.3	5.9 ± 4.2*	9.7 ± 4.3	49† (17–90)	15
g_{IPSC}, nS	124 ± 61	95 ± 47*	115 ± 60	22† (0–44)	14
I_{GABA}, nA	0.73 ± 0.17	0.25 ± 0.11*	0.65 ± 0.1	63† (40–90)	4
Resting conductance, nS	23 ± 6	24 ± 7	24 ± 7		14

Values are means $\pm$ SD; numbers in parentheses are ranges. Stimulation at 3–10 Hz for 30–60 s delivered with V_{hold} 5–15 mV depolarized with respect to E_{IPSC}. V_m, membrane potential; E_{IPSC}, mean IPSC reverse potential; g_{IPSC}, conductance underlying the IPSC; GABA, γ-aminobutyric acid; I_{GABA}, GABA-activated current; V_{hold}, holding potential; GABA iontophoretic currents, 4–5 s and 5–20 nA. *Statistically different from control ($P < 0.01$) as assessed with paired t test. †Values expressed as percent.
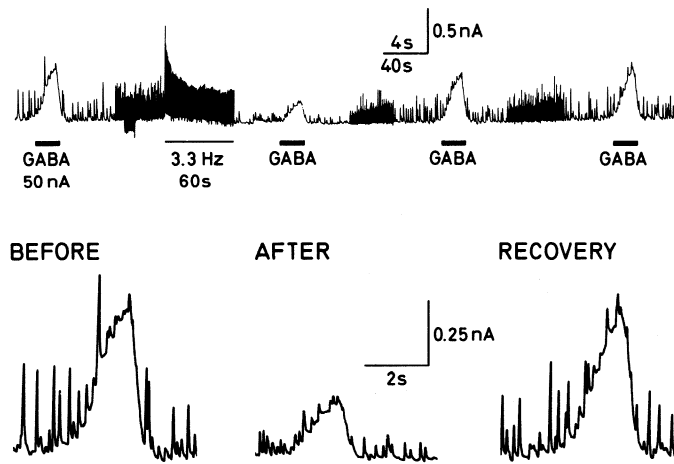
FIG. 5. Effects of repetitive stimulation at depolarized V_{hold} on GABA responses. *Upper trace*: continuous chart recording of membrane current. Responses to iontophoretic application of GABA were obtained before and at varying times after a train of repetitive synaptic stimuli (3.3 Hz for 60 s) at $V_{hold} = -54$ mV. In between the GABA responses the chart speed was reduced as indicated. Vertical deflections are spontaneous IPSCs. Note that they are also significantly reduced in amplitude, although their frequency remains roughly constant, after repetitive stimulation. GABA responses are shown again at higher gain below.

TABLE 2. *Effects of repetitive stimulation at hyperpolarized V_m on inhibition*

	Control	After	Recovery	Mean Change/Cell	n
E_{IPSC}, mV	-68.9 ± 7.0	-68.8 ± 6.8	-68.5 ± 7.8	-0.1 $(1.5--1)$	4
g_{IPSC}, nS	101 ± 76	$69 \pm 45^*$	104 ± 80	$-29\dagger$ $(5-41)$	4
I_{GABA}, nA	-0.57 ± 0.12	-0.56 ± 0.13	-0.59 ± 0.14		7

Values are means ± SD; numbers in parentheses are ranges. Stimulation at 3–10 Hz for 30–60 s with V_{hold} 20–30 mV hyperpolarized with respect to E_{IPSC}. GABA iontophoretic currents, 4–5 s and 5–20 nA [Note that at hyperpolarized V_{hold} there was no significant shift in E_{IPSC} although g_{IPSP} decreased an amount similar to that observed with depolarized V_{hold}s (cf. Table 1)] other abbreviations, see Table 1. *Not significantly different (Mann-Whitney U test) than effects of repetitive stimulation at depolarized V_{hold}. E_{IPSC} and I_{GABA} not significantly different after stimulation (paired t test). †Values expressed as percent.

previous observations in situ and in vitro, stimulation at frequencies >3 Hz was found to result in a depression of IPSP amplitude (Fig. 3A) and, given sufficient stimulation intensities, the generation of synchronous epileptiform afterdischarges (Fig. 3B). Previous experiments using current-clamp recording techniques have left it unclear whether this activity-dependent depression of the IPSP results from a decrease of the evoked conductance underlying the IPSP, or from a decrease in the driving force for the IPSP as a result of an accumulation of intracellular Cl⁻, or from both. We therefore examined this process under voltage-clamp so that both aspects could be independently assessed.

The control E_{IPSC} and g_{IPSC} were determined and tetanic stimulation was then delivered to mossy fiber afferents at 3–10 Hz for 30–60 s under voltage-clamp. The resulting effect on the IPSC was assessed immediately following the
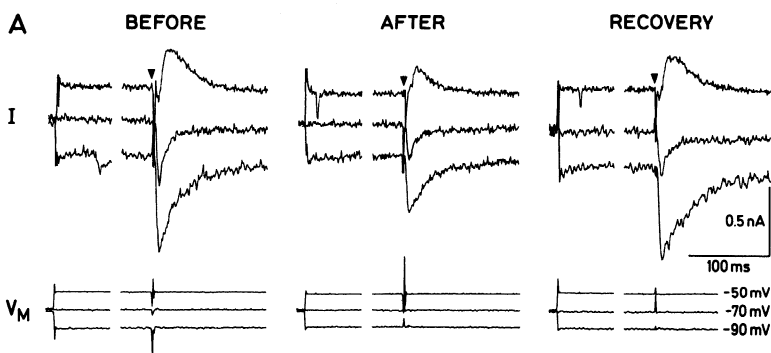

FIG. 6. Effects of repetitive stimulation at hyperpolarized V_{hold} on E_{IPSC} and g_{IPSC}. A: sampled V_m and membrane currents (I) in response to step voltage commands and synaptic stimulation before, after repetitive stimulation at 2 Hz for 60 s at $V_{hold} = -90$, and after 3 min recovery. Note that the synaptic current at $V_m = -50$ mV is depressed approximately the same amount as the synaptic current at $V_m = -90$ mV. B: calculation of E_{IPSC} and g_{IPSC} before (●), after repetitive stimulation (○), and after recovery (×) shows that g_{IPSC} is reversibly decreased by 45% following repetitive stimulation, but driving force is unchanged unlike the effects of repetitive stimulation at depolarized V_{hold} (cf., same cell Fig. 4).
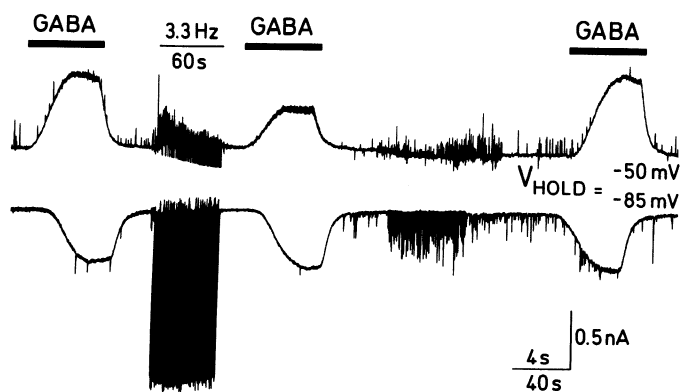
FIG. 7. Effects of V_{hold} on the ability of repetitive stimulation to depress GABA responses. Traces show continuous chart recordings of membrane current. Responses to iontophoretic application of GABA (5 s, 20 nA) were obtained before, and at varying times after a train of repetitive synaptic stimuli (3.3 Hz for 60 s) at either V_{hold} = −50 or −85 mV. In between the GABA responses the chart speed was reduced as indicated. Vertical deflections are spontaneous IPSCs. Note that GABA responses are depressed following repetitive stimulation only when V_{hold} is depolarized with respect to E_{IPSC} and E_{GABA}, because E_{Cl^-} is unaltered by repetitive stimulation at hyperpolarized V_ms (see Fig. 6).

tetanic train. As shown in Fig. 4, when the tetanus was delivered while holding the membrane potential 5–15 mV less negative than E_{IPSC}, i.e., as the cell would be under normal conditions in situ, two effects were consistently observed. First, there was a 22% reduction in g_{IPSC}. Second, there was a 2–7 mV shift in E_{IPSC} towards the holding potential. These data are summarized in Table 1. Given that the holding potential was 5–15 mV depolarized from E_{IPSC}, this depolarizing shift in E_{IPSC} results in a 17–90% reduction in the driving force for the IPSP (mean = 49%). Decreases of a similar magnitude have been indirectly inferred from current-clamp data in vitro and in situ (7, 35). No change in the resting conductance of the cells was observed following repetitive stimulation (Table 1).

The observation of a depolarizing shift in E_{IPSC} might be explained in several ways. First, as the IPSC results from an increase in Cl^- conductance, the decrease may reflect a depolarizing shift in E_{Cl^-} due to an increase in the intracellular chloride concentration ($[Cl^-]_i$). Alternatively, the apparent decrease in E_{IPSC} could arise from an increased contribution in some overlapping conductance that has a reversal potential less negative than E_{IPSC}. Such an effect could occur, for example, as a result of potentiation of the EPSP. Likewise, a decreased contribution of a component of the synaptic response with a more negative reversal potential could also produce an apparent depolarizing shift in E_{IPSC}. We therefore performed the following experiment to distinguish between these possible explanations.

If the observed decrease in E_{IPSC} represents a true decrease in the driving force for inhibition and not an artifact, then responses to iontophoretically applied GABA should also be depressed following repetitive stimulation. Again, trains of tetanic stimuli were given at 3 Hz for 60 s while holding the membrane potential 5–15 mV depolarized to E_{GABA}. The amplitude of the GABA-activated current was found to be reduced an average of 63% (n = 4) (Fig. 5 and Table 1). This evidence thus supports the conclusion that the decline in E_{IPSC} observed following repetitive stimula-

tion reflects a true decrease in the efficacy of GABAergic inhibition and not potentiation of the EPSP.

Is the activity-dependent decrease in E_{IPSC} due to Cl^- accumulation?

GABA-mediated increases in Cl^- conductance generate large inward movements of Cl^- ions (outward currents) when the membrane potential is less negative than E_{Cl^-} (Fig. 1). Huguenard and Alger (29) have presented evidence which suggests that repetitive activation of GABAergic synapses can lead to the accumulation of Cl^- within hippocampal cells. If the shift in E_{IPSC} that we have observed following repetitive stimulation is due to such an accumulation of Cl^-, it should be possible to reduce or reverse this shift by holding the membrane potential more negative than E_{Cl^-} and thus reverse the flow of Cl^- ions to outward (i.e., an inward current). If, on the other hand, the shift in E_{IPSC} following repetitive stimulation is due to some other mechanism, then E_{IPSC} should still shift in the depolarizing direction following repetitive stimulation at hyperpolarized holding potentials.

Repetitive stimulation at 3–10 Hz for 30–60 s, while holding the membrane potential 10–20 mV more negative than E_{IPSC}, was found to result in decreases in g_{IPSC} not significantly different from those seen with depolarizing holding potentials (29 ± 14%, n = 4; Table 2). In contrast, however, E_{IPSC} was unaffected by repetitive stimulation delivered at hyperpolarized holding potentials. The mean change was <1 mV (not statistically significant, n = 4) (Fig.
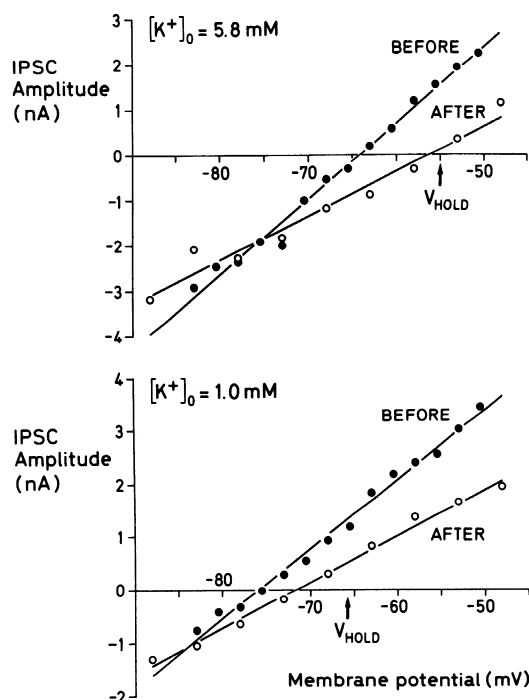

FIG. 8. Influence of $[K^+]_o$ on activity-dependent disinhibition. Calculation of E_{IPSC} and g_{IPSC} before (●) and after (○) repetitive stimulation at 3.3 Hz for 45 s (○) for a single cell in control saline ($[K^+]_o$ = 5.8 mM) and after reducing $[K^+]_o$ to 1 mM. V_{hold} was adjusted to yield approximately the same initial driving force in both salines. Although the decrease in g_{IPSC} was similar in both salines (35–40%), driving force decreased 70% in control saline but only 40% in the low $[K^+]_o$ saline.

TABLE 3. *Effects on reduced $[K^+]_o$ on activity-dependent disinhibition*

[K$^+$]$_o$	Control		After		Recovery		Decrease in DF/Cell, %	Decrease in g_{IPSC}/Cell, %	n
	DF, mV	g_{IPSC}, nS	DF, mV	g_{IPSC}, nS	DF, mV	g_{IPSC}, nS			
5.8 mM	8.6 ± 1.9	121 ± 41	2.4 ± 1.7	75 ± 19	6.9 ± 1.4	102 ± 30	75 ± 17	35 ± 13	4
1.0 mM	10.0 ± 1.5	127 ± 50	8.2 ± 1.9	86 ± 32	11.2 ± 1.8	105 ± 30	18 ± 14*	32 ± 10	5†

Values are means ± SD. Repetitive stimulation at 3.3 Hz for 45 s with V_{hold} 5–15 mV depolarized with respect to E_{IPSC} in control saline ($[K^+]_o$ = 5.8 mM) and in saline containing $[K^+]_o$ = 1 mM. Single cells were held through both salines in either order of presentation. DF, driving force; $[K^+]_o$, extracellular potassium; other abbreviations, see Table 1. *Significantly different than in control saline ($P < 0.05$) as assessed with paired t test. †Inclusion of 1 cell that was not also tested in $[K^+]_o$ = 5.8 mM.

6, Table 2). In addition, when iontophoretic GABA applications were compared following repetitive stimulation at hyperpolarized holding potentials, no decrease in amplitude was seen ($n = 7$) (Fig. 7, Table 2). These data therefore support the hypothesis that E_{IPSC} has shifted in the depolarizing direction following repetitive stimulation as a result of accumulation of intracellular Cl$^-$.

Is the decrease in g_{IPSC} pre- or postsynaptic?

Decreases in g_{IPSC} following repetitive stimulation could arise from either pre- or postsynaptic mechanisms. For example, desensitization of postsynaptic GABA receptors would decrease g_{IPSC} by reducing the conductance activated by a constant amount of synaptically-released GABA. Alternatively, postsynaptic receptors could be unchanged, but if less GABA is released from presynaptic elements following repetitive stimulation then g_{IPSC} would decrease. Responses to exogenous GABA may be used to distinguish between these possibilities. Because E_{Cl^-}, as indicated by E_{IPSC}, was not changed by repetitive stimulation at hyperpolarized holding potentials, only postsynaptic desensitization could cause a decrease in GABA responses. However, GABA currents were *not* depressed following repetitive stimulation at hyperpolarized holding potentials (Fig. 7, Table 2), thus postsynaptic desensitization did not occur under these conditions. Desensitization is therefore unlikely to have contributed significantly to either the decrease in GABA currents or the decrease in g_{IPSC} observed following repetitive stimulation at depolarized holding potentials. Rather presynaptic GABA release is apparently decreased following repetitive stimulation.

Are increases in $[K^+]_o$ involved in effects of repetitive stimulation?

Repetitive stimulation produces a well-characterized, transient elevation of the extracellular K$^+$ concentration ($[K^+]_o$) to as high as 12 mM (e.g., 8, 26, 33, 35). Previous studies have suggested that this increase in $[K^+]_o$ may underly part of the reduction in IPSP driving force following repetitive stimulation (35, 62). We therefore tested this hypothesis by reducing $[K^+]_o$ in the bathing medium from control (5.8 mM) to 1 mM by equimolar substitution with NaCl. Although the amount of K$^+$ released is presumably unchanged under these conditions, the lower base-line $[K^+]_o$ will reduce the maximum, absolute concentration of $[K^+]_o$ achieved during stimulation (26).

Repetitive stimulation at 3.33 Hz for 45 s, at holding potentials 5–15 mV less negative than E_{IPSC}, in saline in which $[K^+]_o$ had been reduced to 1 mM was found to be as effective at decreasing g_{IPSC}, but less effective at decreasing the driving force, than similar trains of stimuli in control saline ($[K^+]_o$ = 5.8 mM). The mean decrease in driving force was reduced from 75 ± 17% in 5.8 mM $[K^+]_o$ to 18 ± 14% in 1 mM $[K^+]_o$ ($n = 5$), whereas the mean change in g_{IPSC} was 35 ± 13% and 32 ± 10%, respectively (Fig. 8, Table 3). These data therefore suggest that activity-dependent increases in $[K^+]_o$ may exert a strong influence on the ability of repetitive stimulation to cause a depolarizing shift of E_{IPSC}. Moreover, it appears that the absolute concentration of $[K^+]_o$ attained is more significant than the amount of K$^+$ released, since this should not be significantly changed in the low $[K^+]_o$ solution (26).

DISCUSSION

The fragility of GABAergic inhibition was first suggested in the observation that the ability of exogenous GABA to inhibit spontaneous discharge in the spinal cord gradually decreased during prolonged iontophoretic applications (13). Since this time, the IPSP has also been shown to be remarkably labile both in situ and in vitro.

Mechanisms of activity-dependent disinhibition

Experiments in hippocampus have shown that IPSPs become decreased in amplitude up to 90% following trains of stimuli at low frequencies (3–5 Hz) (4, 7, 27, 35, 62). In this paper we have examined several mechanisms that together account for this lability. The amplitude of the IPSP is determined by many factors: the amount of GABA released in response to an impulse in the presynaptic element, the magnitude of the conductance activated by a given GABA release, the amount of current that flows across a given conductance, and the input resistance of the postsynaptic cell. Indeed, evidence has previously been presented that all of these may be altered after repetitive stimulation. Using single-electrode voltage-clamp recording techniques we have been able to examine the role and relative contribution of each of these factors. When trains of stimuli were delivered while holding the membrane potential of the cell constantly depolarized with respect to E_{IPSC}, i.e., so that Cl$^-$ ions flowed into the cell during the IPSC as in situ, then two effects were consistently observed. First, there was a shift in E_{IPSC} in the depolarizing direction toward the holding potential of, on average, 4.7 mV. Given a holding po-

tential 5–15 mV from E_{IPSC}, this shift effectively reduces the driving force for Cl$^-$ ion flow, on average 49% (Fig. 4, Table 1). Second, the size of the conductance activated with the same intensity of stimulation was significantly decreased relative to control following a train of repetitive stimuli. On average, g_{IPSC} was decreased 22% (Fig. 4, Table 1). Taken together these two effects could thus reduce the amplitude of an IPSP recorded in current clamp by as much as 60%. No effect of repetitive stimulation on the resting membrane conductance was observed (Table 1). Previous indirect measurements have estimated decreases in g_{IPSC} of 60–75% and depolarizing shifts in E_{IPSC} of 5–15 mV (7, 35), somewhat larger than we report here. As the effects of repetitive stimulation on g_{IPSC} and E_{IPSC} can be attributed to an accumulation in the tissue of substances (K$^+$ and GABA) released during the tetanus (see below), the smaller changes seen in slice cultures may result from the relative lack of diffusional barriers between the cells and the bathing saline in this preparation which therefore allows these substances to diffuse away more quickly.

Mechanisms of activity-dependent decreases in IPSC driving force

Activity-dependent decreases in IPSC driving force could result from several causes. Since several conductances are activated in CA3 cells following stimulation of the mossy fiber afferent pathway, including an EPSP and a late postsynaptic potential (1, 24), either a change in the reversal potential or a change in the relative contribution of one of these responses to the composite synaptic response could give the appearance of a change in E_{IPSC}. For example, potentiation of the EPSP following repetitive stimulation would shift the apparent reversal potential in the depolarizing direction by increasing its contribution to the synaptic response, and this has been suggested to underlie the activity-dependent shift in E_{IPSC} (4). Likewise a recruitment of the so-called depolarizing GABA response would shift the apparent E_{IPSC} in the depolarizing direction (62). Our data allow us to rule out these possibilities. First, depolarizing GABA responses were not observed in our preparation. Second, repetitive stimulation at depolarized holding potentials also depressed the response of the cell to iontophoretic application of exogenous GABA (Fig. 5). Third, both the shift in E_{IPSC}, and the depression of GABA responses, following repetitive stimulation were eliminated if the direction of Cl$^-$ flow was reversed from inward to outward by holding the membrane potential hyperpolarized with respect to E_{IPSC} (Figs. 6 and 7; Table 2). Rather, our data demonstrate that the activity-dependent decrease in IPSC driving force reflects a true change in E_{Cl^-} due to shifts in the transmembrane distribution of Cl$^-$ ions. Since [Cl$^-$]$_o$ has been shown to be insignificantly altered following tetanic stimulation (17), this shift in E_{Cl^-} must result from accumulation of Cl$^-$ inside the cell.

Accumulation of intracellular Cl$^-$ has been shown to occur during prolonged applications of GABA due to the inward flux of Cl$^-$ ions carrying the GABA-activated current (e.g., 29). Similarly, our data suggest that during repetitive stimulation the influx of Cl$^-$ during the overlapping IPSCs transiently elevates [Cl$^-$]$_i$. This increase in [Cl$^-$]$_i$

accounts for the decrease in the amplitude of responses to exogenous GABA as well. Consistent with this mechanism, no increase in [Cl$^-$]$_i$ is observed when repetitive stimulation is delivered at hyperpolarized holding potentials so that here is an outward movement of Cl$^-$ during the IPSC. One may wonder why, given the outward driving force for Cl$^-$ flux in this experiment, no hyperpolarizing shift of E_{IPSC} was observed. One explanation is that the effect of this outward Cl$^-$ flux was obscured by a some additional mechanism which promotes Cl$^-$ accumulation, so that the net effect was no apparent change in E_{Cl^-}. For example, we observed that the activity-dependent decrease in driving force was greater when stimulation was delivered in saline containing a higher [K$^+$]$_o$. The correlation between [K$^+$]$_o$ and Cl$^-$ accumulation has been noted previously (35), and may reflect an inhibition of active, outwardly directed Cl$^-$ transport by elevated [K$^+$]$_o$ (14, 52). This process will tend to increase [Cl$^-$]$_i$. Homeostasis of [Cl$^-$]$_i$, and the influence of [K$^+$]$_o$, are described in detail in the following paper (55).

Mechanisms of activity-dependent decrease in g_{IPSC}

Activity-dependent decreases in g_{IPSC} could arise either from a presynaptic decrease in evoked GABA release or from a decreased sensitivity of postsynaptic receptors to released GABA, such as might occur after desensitization. Desensitization of the GABA receptor complex has been suggested to underlie the decrease in g_{IPSC} following a train of stimuli (7, 62). However, our experiments in which the effect of repetitive stimulation on responses to exogenous GABA was tested (Figs. 5 and 7) indicate that significant desensitization did not occur after the tetanus. Decreases in the amplitude of GABA responses occurred only when there were concomitant decreases in E_{Cl^-}, such as after repetitive stimulation at depolarized holding potentials, and not when E_{Cl^-} was unchanged, such as after repetitive stimulation at hyperpolarized holding potentials. The postsynaptic receptors activated by the exogenous GABA were therefore unaffected by the stimulus train in the later case. Of course, we can not be certain that the population of receptors activated by the exogenous GABA are the same population as those activated by the IPSC. Thus it remains possible that the receptors responsible for the IPSC have become desensitized, whereas the independent population responsible for the GABA response were unchanged. This seems unlikely however given our ability to localize the GABA application visually to the cell soma where the GABAergic presynaptic terminals are most dense. Since desensitization is apparently not responsible for the activity-dependent reduction in g_{IPSC}, we conclude that presynaptic GABA release has decreased following the train of repetitive stimuli. One possible mechanism, described in detail in the last paper of this series (56), is that prolonged GABA efflux during the tetanus has effected a negative feedback of its own release mediated by presynaptic GABA$_B$ receptors, as suggested by Deisz and Prince (15) for the neocortex.

Relationship of activity-dependent disinhibition to epilepsy

The defining feature of activity-dependent disinhibition is a short-term reduction in the efficacy of GABAergic,

Cl -mediated synaptic inhibition following a train of repetitive stimuli. Synchronized, epileptiform discharge resulting from application of GABA antagonists such as bicuculline, penicillin, and picrotoxin due to the large blockade of inhibition is well described (e.g., Ref. 32). Previous studies have suggested that this activity arises from a release of excitatory interactions in the CA3 region, that then serves as a pacemaker in driving synchronous discharge in follower populations such as CA1 (61). Could the partial, short-lasting disinhibition described here also result in epilepsy? Models of neuronal networks in the hippocampus have suggested that synchronized discharge can occur in hippocampal slices following only partial disinhibition, provided that excitatory interactions between CA3 cells are sufficiently strong (57). Such capabilities are also shown by our preparation, such as the epileptiform discharge resulting from repetitive stimulation illustrated in Fig. 3B, where disinhibition is presumably incomplete. Disinhibition may actually increase excitatory interactions through several mechanisms, including reducing the voltage-dependent block of N-methyl-D-aspartate (NMDA) receptor-activated ion channels by Mg^{2+} ions (28) and increasing the probability that an EPSP will generate an action potential in the postsynaptic cell (60). Indeed, Miles and Wong (38, 39) have shown that following suppression of inhibition with either picrotoxin or tetanic stimulation, latent excitatory interactions between CA3 pyramidal cells become unmasked.

Repeated application of stimuli that evoke transient, activity-dependent disinhibition could lead to chronic increases in excitability as well. For example, both a strengthening of excitation (41), perhaps via an NMDA-receptor-mediated process such as long-term potentiation (e.g., Ref. 25), and a long-lasting decrease in inhibition (51) have been described following multiple applications of repetitive stimulation. Such processes might be involved in the development of secondary epileptic foci in regions where the initial propagated event is inhibitory in nature, but gradually becomes transformed into synaptic excitation (47). In addition, such mechanisms could facilitate the transition from interictal to ictal discharge by causing a progressive decrease in the strength of surround inhibition (45).

Activity-dependent disinhibition thus results from several naturally occurring mechanisms which are capable of producing transient or chronic increases in hippocampal excitability. As such, these mechanisms may represent an important endogenous factor in the genesis of epilepsy.

REFERENCES

1. ALGER, B. E. Characteristics of a slow hyperpolarizing synaptic potential in rat hippocampal pyramidal cells in vitro. J. Neurophysiol. 52: 892–910, 1984.
2. ALGER, B. E. AND NICOLL, R. A. Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied in vitro. J. Physiol. Lond. 328:125–141, 1982.
3. ANDERSEN, P., DINGLEDINE, R., GJERSTAD, L., LANGMOEN, I. A., AND MOSFELDT LAURSEN, A. Two different responses of hippocampal pyramidal cells to application of gamma-amino butyric acid. J. Physiol. Lond. 305:279–296, 1980.
4. ANDERSEN, P. AND LOMO, T. Counteraction of powerful recurrent inhibition in hippocampal pyramidal cells by frequency potentiation of excitatory synapses. In: Structure and Function of Inhibitory Neuronal Mechanisms, edited by C. von Euler, S. Skoglund, and U. Soderberg. Oxford, UK: Pergamon, 1968, p. 335–342.
6. ASHWOOD, T. J., COLLINGRIDGE, G. L., HERRON, C. E., AND WHEAL, H. V. Voltage-clamp analysis of somatic gamma-aminobutyric acid responses in adult rat hippocampal CA1 neurones in vitro. J. Physiol. Lond. 384: 27–37, 1987.
7. BEN-ARI, Y., KRNJEVIC, K., AND REINHARDT, W. Hippocampal seizures and failure of inhibition. Can. J. Physiol. Pharmacol. 57: 1462–1466, 1979.
8. BENNINGER, C., KADIS, J., AND PRINCE, D. A. Extracellular calcium and potassium changes in hippocampal slices. Brain Res. 187: 165–182, 1980.
9. BROWN, T. H., FRICKE, R. A., AND PERKEL, D. H. Passive electrical constants in three classes of hippocampal neurons. J. Neurophysiol. 46: 812–827, 1981.
10. BROWN, T. H. AND JOHNSTON, D. Voltage-clamp analysis of mossy fiber synaptic input to hippocampal neurons. J. Neurophysiol. 50: 487–507, 1983.
11. CAIN, D. P. AND CORCORAN, M. E. Kindling with low-frequency stimulation: generality, transfer, and recruiting effects. Exp. Neurol. 73: 219–232, 1981.
12. COLLINGRIDGE, G. L., GAGE, P. W., AND ROBERTSON, B. Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. J. Physiol. Lond. 356: 551–564, 1984.
13. CURTIS, D. R., PHILLIS, J. W., AND WATKINS, J. C. The depression of spinal neurones by gamma-amino-n-butyric acid and B-alanine. J. Physiol. Lond. 146: 185–203, 1959.
14. DEISZ, R. A. AND LUX, H. D. The role of intracellular chloride in hyperpolarizing post-synaptic inhibition of crayfish stretch receptor neurones. J. Physiol. Lond. 326: 619–633, 1982.
15. DEISZ, R. A. AND PRINCE, D. A. Frequency-dependent depression of inhibition in the guinea-pig neocortex in vitro by $GABA_B$ receptor feedback on GABA release. J. Physiol. Lond. In press.
16. DICHTER, M. A. AND AYALA, G. F. Cellular mechanisms of epilepsy: a status report. Science Wash. DC 237: 157–164, 1987.
17. DIETZEL, I., HEINEMANN, U., HOFMEIER, G., AND LUX, H. D. Stimulus-induced changes in extracellular Na+ and Cl− concentration in relation to changes in the size of the extracellular space. Exp. Brain Res. 46: 73–84, 1982.
18. ECCLES, J. C., NICOLL, R. A., OSHIMA, T., AND RUBIA, F. J. The anionic permeability of the inhibitory postsynaptic membrane of hippocampal pyramidal cells. Proc. R. Soc. Lond. B Biol. Sci. 198: 345–361, 1977.
19. FINKEL, A. S. AND REDMAN, S. Theory and operation of a single microelectrode voltage clamp. J. Neurosci. Methods 11:101–127, 1984.
20. GÄHWILER, B. H. Organotypic monolayer cultures of nervous tissue. J. Neurosci. Methods 4: 329–342, 1981.
21. GÄHWILER, B. H. Development of the hippocampus in vitro: cell types, synapses, and receptors. Neuroscience 11: 751–760, 1984.
22. GÄHWILER, B. H., AND BROWN, D. A. $GABA_B$-receptor-activated K^+ current in voltage-clamped CA_3 pyramidal cells in hippocampal cultures. Proc. Natl. Acad. Sci. USA 82: 1558–1562, 1985.
23. GÄHWILER, B. H. AND BROWN, D. A. Effects of dihydropyridines on calcium currents in CA3 pyramidal cells in slice cultures of rat hippocampus. Neuroscience 20: 731–738, 1987.
24. HABLITZ, J. J. AND THALMAN, R. H. Conductance change underlying

a late synaptic hyperpolarization in hippocampal CA3 neurons. *J. Neurophysiol.* 58: 160–179, 1987.

25. HARRIS, E. W. AND COTMAN, C. W. Removal of magnesium induces NMDA receptor mediated LTP by triggering epileptiform activity. *Soc. Neurosci. Abstr.* 11: 845, 1985.

26. HEINEMANN, U. AND LUX, H. D. Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of the cat. *Brain Res.* 120:231–249, 1977.

27. HERRLING, P. L. AND GÄHWILER, B. H. High-frequency stimulation of hippocampal pyramidal cells in situ and in vitro. *Experientia Basel* 37: 610, 1981.

28. HERRON, C. E., WILLIAMSON, R., AND COLLINGRIDGE, G. L. A selective N-methyl-D-aspartate antagonist depresses epileptiform activity in rat hippocampal slices. *Neurosci. Lett.* 61: 255–260, 1985.

29. HUGUENARD, J. R., AND ALGER, B. E. Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. *J. Neurophysiol.* 56: 1–18, 1986.

30. JOHNSTON, D. AND BROWN, T. H. Giant synaptic potential hypothesis for epileptiform activity. *Science Wash. DC* 211: 294–297, 1981.

31. KRNJEVIC, K. Desensitization of GABA receptors. *Adv. Biochem. Psychopharmacol.* 26: 111–120, 1981.

32. KRNJEVIC, K. GABA-mediated inhibitory mechanisms in relation to epileptic discharges. In: *Basic mechanisms of Neuronal Hyperexcitability,* edited by H. Jasper and N. van Gelder. New York: Liss, 1983, p. 249–280.

33. LEWIS, D. V. AND SCHUETTE, W. H. NADH fluorescence and $[K^+]_o$ changes during hippocampal electrical stimulation. *J. Neurophysiol.* 38:405–417, 1975.

34. LOTHMAN, E. W., HATLELID, J. M., ZORUMSKI, C. F., CONRY, J. A., MOON, P. F., AND PERLIN, J. B. Kindling with rapidly recurring hippocampal seizures. *Brain Res.* 360: 83–91, 1985.

35. MCCARREN, M. AND ALGER, B. E. Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. *J. Neurophysiol.* 53: 557–571, 1985.

36. MCNAMARA, J. O., BONHAUS, D. W., SHIN, C., CRAIN, B. J., GELLMAN, R. L., AND GIACCHINO, J. L. The kindling model of epilepsy: a critical review. *CRC Crit. Rev. Clin. Neurobiol.* 1: 341–391, 1984.

37. MILES, R. AND WONG, R. K. S. Unitary inhibitory synaptic potentials in the guinea-pig hippocampus in vitro. *J. Physiol. Lond.* 356: 97–113, 1984.

38. MILES, R. AND WONG, R. K. S. Latent synaptic pathways revealed after tetanic stimulation in the hippocampus. *Nature Lond.* 329: 724–726, 1987.

39. MILES, R. AND WONG, R. K. S. Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. *J. Physiol. Lond.* 388: 611–629, 1987.

40. MISGELD, U., DEISZ, R. A., DODT, H. U., AND LUX, H. D. The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science Wash. DC* 232: 1413–1415, 1986.

41. MODY, I. AND HEINEMANN, U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature Lond.* 326: 701–704, 1987.

42. NUMANN, R. E. AND WONG, R. K. S. Voltage-clamp study on GABA response desensitization in single pyramidal cells dissociated from the hippocampus of adult guinea pigs. *Neurosci. Lett.* 47: 289–294, 1984.

43. PINEL, J. P. J. AND ROVNER, L. I. Electrode placement and kindling-induced experimental epilepsy. *Exp. Neurol.* 58: 335–346, 1978.

44. PRINCE, D. A. Neurophysiology of epilepsy. *Annu. Rev. Neurosci.* 1: 395–415, 1978.

45. PRINCE, D. A. AND WILDER, B. J. Control mechanisms in cortical epileptogenic foci: surround inhibition. *Arch. Neurol.* 16: 194–202, 1967.

46. RIBAK, C. E., VAUGHN, J. E., AND SAITO, K. Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Res.* 140: 315–332, 1978.

47. SAWA, M., NAKAMURA, K., AND NAITO, H. Intracellular phenomena and spread of epileptic seizure discharges. *Electroencephalogr. Clin. Neurophysiol.* 24: 146–154, 1968.

48. SCHWARTZKROIN, P. A. AND PRINCE, D. A. Cellular and field potential properties of epileptogenic hippocampal slices. *Brain Res.* 147: 117–130, 1978.

49. SLOVITER, R. S. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science Wash. DC* 235: 73–76, 1987.

50. STASHEFF, S. F., BRAGDON, A. C., AND WILSON, W. A. Induction of epileptiform activity in hippocampal slices by trains of electrical stimuli. *Brain Res.* 344: 296–302, 1985.

51. STELZER, A., SLATER, N. T., AND TEN BRUGGENCATE, G. Activation of NMDA receptors blocks GABAergic inhibition in an in vitro model of epilepsy. *Nature Lond.* 326: 698–701, 1987.

52. THOMPSON, S. M., DEISZ, R. A., AND PRINCE, D. A. Outward chloride/cation cotransport in mammalian cortical neurons. *Neurosci. Lett.* 89: 49–54, 1988.

53. THOMPSON, S. M. AND GÄHWILER, B. H. Repetitive synaptic stimulation reduces both IPSP driving force and conductance in the hippocampus in vitro. *Neuroscience* 22(S): S690, 1987.

54. THOMPSON, S. M. AND GÄHWILER, B. H. Extracellular K^+ and membrane potential affect hippocampal IPSPs in vitro. *Experientia Basel* 43: 720, 1987.

55. THOMPSON, S. M. AND GÄHWILER, B. H. Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on E_{Cl^-} in hippocampal CA3 neurons. *J. Neurophysiol.* 61: 512–523, 1989.

56. THOMPSON, S. M. AND GÄHWILER, B. H. Activity-dependent disinhibition. III. Desensitization and $GABA_B$ receptor-mediated presynaptic inhibition in the hippocampus in vitro. *J. Neurophysiol.* 61: 524–533, 1989.

57. TRAUB, R. D., MILES, R., AND WONG, R. K. S. Models of synchronized hippocampal bursts in the presence of inhibition. I. Single population events. *J. Neurophysiol.* 58: 739–751, 1987.

58. WADA, J. A. AND SATO, M. Generalized convulsive seizures induced by daily electrical stimulation of the amygdala in cats: correlative electrographic and behavioral features. *Neurology* 24: 565–574, 1974.

59. WALTHER, H., LAMBERT, J. D. C., JONES, R. S. G., HEINEMANN, U., AND HAMON, B. Epileptiform activity in combined slices of the hippocampus, subiculum, and entorhinal cortex during perfusion with low magnesium medium. *Neurosci. Lett.* 69: 156–161, 1986.

60. WIGSTRÖM, H. AND GUSTAFSSON, B. Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. *Nature Lond.* 301: 603–604, 1983.

61. WONG, R. K. S. AND TRAUB, R. D. Synchronized burst discharge in disinhibited hippocampal slice. I. Initiation in CA2-CA3 region. *J. Neurophysiol.* 49: 442–458, 1983.

62. WONG, R. K. S. AND WATKINS, D. J. Cellular factors influencing GABA response in hippocampal pyramidal cells. *J. Neurophysiol.* 48: 938–951, 1982.

63. YAMAMOTO, C., AND KAWAI, N. Seizure discharge evoked in vitro in thin sections from guinea-pig hippocampus. *Science Wash. DC* 155: 341–342, 1967.

64. ZIMMER, J. AND GÄHWILER, B. H. Cellular and connective organization of slice cultures of the rat hippocampus and fascia dentata. *J. Comp. Neurol.* 228: 432–446, 1984.