ate-induced currents with marked inward rectification (*17*). These GluRs probably lack a GluR-B subunit. The properties of heteromeric GluRs (having at least GluR-B) are comparable to those of native non-NMDA GluRs, for example, in hippocampal neurons. The *I-V* curve for the peak current is less curved than that for the steady-state current in GluRs native to rat hippocampal CA3 pyramidal neurons, and the desensitization rate of these hippocampal GluRs is similar to that of heteromeric GluR-A/B (*18*). Excitatory postsynaptic currents in pyramidal neurons of the CA1 (*19*) and CA3 (*20*) region also show linear *I-V* relations. It seems likely that the voltage-dependent properties of excitatory postsynaptic currents mediated by non-NMDA GluRs are determined by the GluR-B subunit.

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9. The shape of agonist-induced *I-V* relations was quantified by fitting of the data points to a polynomial equation. Fourth order polynomials were sufficient to fit the simple outwardly rectifying *I-V* curves, but tenth-order equations were often necessary for description of the more complex doubly rectifying curves. These polynomials were used for calculation of the reversal potential and chord conductances at particular membrane potentials.
10. Ramp *I-V* curves were constructed by application of a ramp voltage command that went from 0 to ±120 mV in 2 s. Data obtained in the absence of agonist were subtracted from those acquired during the steady-state component of the agonist response to produce agonist-activated *I-V* curves. Digitized data points were averaged over 4 mV, and the average values were used for fitting to polynomials.
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13. Voltage jump experiments were used to estimate the voltage dependence of the single-channel conductance in whole-cell recording because the single-channel conductance of these GluRs is low (<3 pS), presenting difficulties in direct measurement of channel amplitudes. From a holding potential of 0 mV the voltage was stepped to various test voltages for 50 ms. Currents occurring in response to voltage steps applied in the absence of agonist were digitally subtracted from those recorded during the steady-state component of the agonist response. Four to six such subtracted current records were averaged for display and measurement. Currents during the voltage pulse were fit to the equation $I = I_o e^{-t/\tau} + B$, where t = time, τ = apparent time constant of decay, and I_o = extrapolated current amplitude at the start of the voltage pulse (instantaneous current). B was set to the current amplitude reached at the end of the pulse. I_o was taken to represent the instantaneous current, and B was used for the late current amplitude in the *I-V* curves in Fig. 4. If the current during a step showed no obvious relaxation, it was fit to a straight line. The line was extrapolated to the beginning and end of the pulse, and these values were used for the instantaneous and late currents, respectively. Currents were filtered at 4 kHz (−3 dB) before being digitized at 10 kHz. Leak and capacitive currents prevented accurate measurement of agonist currents within 1 ms of a step voltage change.
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Facilitation of the Induction of Long-Term Potentiation by $GABA_B$ Receptors

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Long-term potentiation (LTP), an in vitro model of learning, was induced in hippocampal slices by 5-hertz stimulation. During induction, γ-aminobutyric acid A ($GABA_A$) inhibition decreased, causing the *N*-methyl-D-aspartate receptor–mediated excitation to increase. 2-OH Saclofen, a $GABA_B$ receptor antagonist, prevented the reduction of inhibition, the increase of excitation, and the induction of LTP. Therefore, disinhibition caused by $GABA_B$ receptors is required for induction of LTP by 5-hertz stimulation. $GABA_B$ receptor modulation of synaptic plasticity occurs at frequencies in the range of the endogenous hippocampal theta rhythm, which has been shown to modulate LTP in vivo.

LTP, A MODEL OF LEARNING IN THE central nervous system, often requires *N*-methyl-D-aspartate (NMDA) receptor activation (*1*). However, blockade of NMDA channels by Mg^{2+} is normally relieved only by depolarization (*2*). Therefore, LTP is usually induced with stimulus trains of sufficiently high frequency to depolarize the postsynaptic cell.

Inhibition limits postsynaptic depolarization during a stimulus train. When inhibition is blocked with picrotoxin, a $GABA_A$ receptor antagonist, depolarization during a train is enhanced, facilitating LTP induction (*3*). Although blockade of inhibition facilitates LTP, it is unclear if disinhibition is normally required for LTP induction. Inhibition can be reduced by "priming" stimulation (*4*). Priming is so effective that LTP can be produced with as few as two to three stimuli delivered about 200 ms after a single priming stimulus (*4, 5*). In addition, the interstimulus interval for the production of LTP with priming stimulation approximates the periodicity of the hippocampal theta rhythm, which has been linked with LTP in vivo (*5, 6*). We report that $GABA_B$ receptor–mediated disinhibition is required for LTP induction with stimulation in the frequency range of the theta rhythm.

We performed experiments using standard procedures (*7, 8*) in the dentate gyrus of rat hippocampal slices maintained in a submersion chamber perfused with artificial cerebrospinal fluid (ACSF). Responses of granule cells to electrical stimulation of the medial perforant path in the inner two-thirds of the molecular layer were recorded intracellularly.

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Table 1. Effect of 2-OH saclofen on the development of the burst response and the underlying reduction of inhibition when single stimuli were delivered 200 ms apart. All values are mean ± SEM and n is the number of cells for each observation. The asterisks indicate that there was a significant change in the second response as compared to the first (*$P < 0.01$; **$P < 0.001$; paired t test).

Parameter (n)	Second response (% first response)		
	Control	2-OH Saclofen	Wash
EPSP width (5)	174 ± 11*	101 ± 7	164 ± 13*
IPSP area (7)	43 ± 7**	103 ± 11	38 ± 6**

We examined excitatory postsynaptic potentials (EPSPs) elicited by paired pulses with varying interstimulus intervals (n = 10). At intervals of less than 20 ms, the second EPSP was briefer than the first and below threshold. With interstimulus intervals between 150 and 400 ms, the second EPSP was facilitated, or primed, with the peak effect occurring at 200 ms. The facilitated EPSPs resembled burst responses, with the prolonged EPSP triggering two to four action potentials (Fig. 1A, top). Finally, with interstimulus intervals greater than 2 s, the second EPSP was unaffected.

NMDA receptor–mediated currents can prolong EPSPs; therefore, we applied D-2-amino-5-phosphonovaleric acid (D-APV), an NMDA receptor antagonist (*9*). Burst responses were reversibly blocked by D-APV (50 μM) (Fig. 1A, middle). In seven cells the EPSP duration measured at its half width increased by 102 ± 26% ($P < 0.01$) in control but only by 12 ± 7% ($P > 0.05$) in D-APV (*10*). These results and previous reports (*4*) indicate that widening of the EPSP is due to enhancement of an NMDA current (*11*).

Because $GABA_B$ receptors can reduce inhibition and produce burst responses (*7*), we determined whether the increase in the NMDA current required $GABA_B$ receptor activation. Addition of 400 μM [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]sulfonic acid (2-OH saclofen), a $GABA_B$ receptor antagonist (*12*), had no effect on the first EPSP but blocked the burst response 200 ms later (Fig. 1A, bottom, and Table 1). Application of 2-OH saclofen concentrations between 10 and 400 μM demonstrated that the elongation of the EPSP was reduced by approximately 50% at 40 μM (n = 5). Addition of 50 μM D-APV to the 2-OH saclofen containing ACSF caused no further reduction of the second EPSP (n = 3), suggesting that both drugs were reducing the same component of the EPSP.

These results suggested that GABA, released by the first stimulus, activated $GABA_B$ receptors and indirectly increased the NMDA component of the second EPSP by reducing inhibition. We tested this hypothesis by measuring the inhibitory postsynaptic potentials (IPSPs) evoked by the paired stimuli. IPSPs were isolated by application of 50 μM D-APV and 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX), an antagonist at non-NMDA glutamate receptors (*13*). Stimulation in this preparation elicited no EPSP but did evoke a biphasic IPSP with an early component that was blocked by 50 μM picrotoxin (reversal potential, −73 ± 2 mV; n = 9) and a late component blocked by 2-OH saclofen (reversal potential, −91 ± 3 mV; n = 9). Application of both picrotoxin and 2-OH saclofen eliminated all evoked responses (n = 3).

Stimuli delivered 200 ms apart, with the cell hyperpolarized to the reversal potential of the late IPSP, evoked depolarizing $GABA_A$ IPSPs. The area of the second IPSP was significantly less than that of the first (*14*). This reduction was reversibly blocked by 400 μM 2-OH saclofen (Fig. 1B and Table 1) (*15*). The time course for the reduction of the $GABA_A$ IPSPs resembled that of the bursts. The second IPSP was not reduced and temporally summed with the first at short (<20 ms) interstimulus intervals; it was reduced between 150 and 400 ms, with a maximum reduction at about 200 ms, and it recovered to the same area after about 2 s.

We tested the possibility that 2-OH saclofen was blocking the bursts by directly antagonizing NMDA receptors. The NMDA component of the EPSP was isolated with 10 μM DNQX and 50 μM picrotoxin. Addition of 400 μM 2-OH saclofen did not reduce the NMDA receptor–mediated EPSP, whereas application of 50 μM D-APV blocked the EPSP entirely (Fig. 2) (n = 3). Thus, 2-OH saclofen was not blocking bursts through a direct effect on the NMDA receptor.

Table 2. Effect of stimulation frequency during a ten-pulse train on the induction of LTP measured 30 min later. All values are mean ± SEM and n is the number of slices in each group.

Parameter (n)	Response after train (% response before train)		
	100 Hz	0.5 Hz	5 Hz
Population EPSP slope (6)	102 ± 2	86 ± 5*	134 ± 10*
Population spike amplitude (6)	106 ± 5	82 ± 5*	194 ± 24*

*$P < 0.05$, significant change in response after the train (paired t test).

Because enhancement of the NMDA component of the EPSP critically depended on the interstimulus interval, we tested the ability of trains of different frequencies to induce LTP. We delivered ten stimulus pulses at a constant intensity and altered only the stimulation frequency, first to 0.5 Hz, then to 100 Hz. Neither frequency induced LTP in measurements carried out to 30 min after the trains (*16*). In these same slices, lasting LTP was produced when ten pulses were delivered at 5 Hz, the frequency at which $GABA_B$ receptor–mediated disinhibition and, consequently, facilitation of the NMDA component were maximal (Table 2).

During the 5-Hz trains, but not the 0.5-Hz or 100-Hz trains, the population responses progressively increased in width and in number of population spikes until reaching a plateau by the fourth or fifth response.

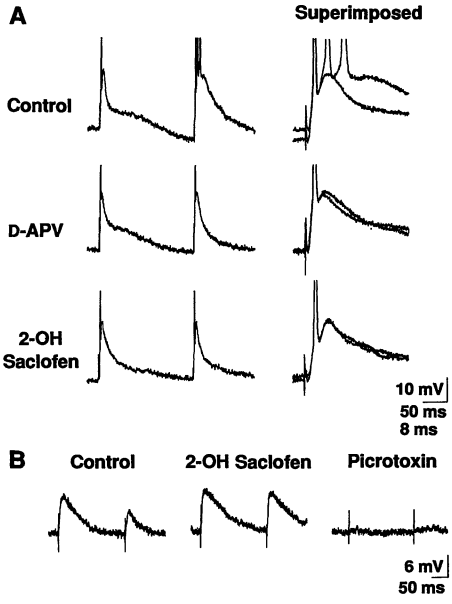

Fig. 1. Effect of 2-OH saclofen on bursts and IPSP reduction. (**A**) Intracellular records of granule cell responses evoked 200 ms apart by paired stimuli. Action potentials are clipped. (**B**) $GABA_A$ receptor–mediated IPSPs recorded from a single granule cell in response to stimuli delivered 200 ms apart. IPSPs were pharmacologically isolated by application of D-APV (50 μM) and DNQX (20 μM). All records were recorded from the same cell hyperpolarized to −91 mV, the reversal potential of the late IPSP.

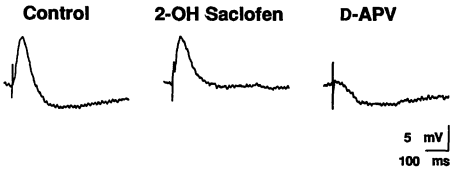

Fig. 2. NMDA receptor–mediated EPSPs recorded in the presence of DNQX (10 μM) and picrotoxin (50 μM). All responses are from a single cell with a resting membrane potential of −76 mV.

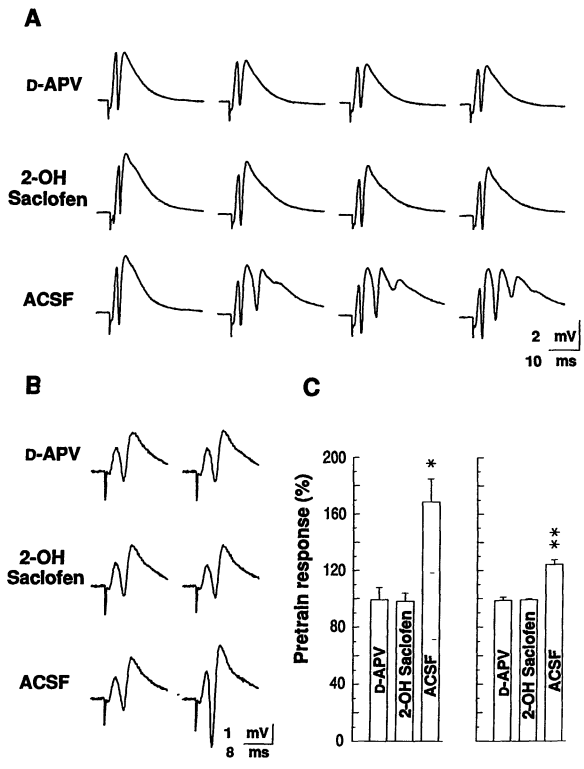

Fig. 3. Blockade of LTP induction by 2-OH saclofen. (**A**) Extracellular, granule-cell layer responses to a 5-Hz, ten-pulse train. Only the first four responses are shown because subsequent responses appeared similar to the fourth response. The stimulus intensity was set to evoke the maximal population spike. All records are from a single slice. (**B**) Granule cell layer responses recorded before (left) and 30 min after (right) a 5-Hz, ten-pulse train. Test stimulus was set at 25% of the intensity eliciting maximal population spike. Application of D-APV (50 μM) or 2-OH saclofen (400 μM) was terminated immediately after the train. All responses are from a single slice. (**C**) Averaged data showing the effect of D-APV (50 μM) and 2-OH saclofen (400 μM) on the induction of LTP of both the population spike amplitude (left) and the population EPSP slope (right). Bars and error bars represent mean ± SEM (n = 7). The asterisks indicate that the response 30 min after the train was significantly different from the response before the train (*$P < 0.04$; **$P < 0.001$; paired t test).

This facilitation was blocked by application of either 50 μM D-APV or 400 μM 2-OH saclofen (Fig. 3A). Intracellular recordings confirmed that $GABA_A$ IPSPs were reduced throughout the trains, and this reduction was blocked by 400 μM 2-OH saclofen.

To determine if the LTP was dependent on NMDA receptor activation, we delivered a 5-Hz, ten-pulse train in the presence of 50 μM D-APV. The drug was washed off immediately after the train, and the response amplitude was monitored for 30 min. No LTP developed. Next, to determine whether $GABA_B$ receptor–mediated disinhibition was required for development of the LTP, we applied 400 μM 2-OH saclofen and delivered a 5-Hz, ten-pulse train. 2-OH saclofen was washed off immediately after the train and, again, no LTP was seen 30 min after the train. Finally, in these same slices, a 5-Hz, ten-pulse train delivered in ACSF produced stable LTP (Fig. 3, B and C).

These data indicate that LTP produced by 5-Hz stimulation is dependent not only on NMDA receptor activation but also on the reduction of inhibition caused by $GABA_B$ receptor activation. This disinhibition enhances the NMDA currents elicited by the train, thereby triggering LTP. These results agree with the disinhibitory effects of $GABA_B$ receptors in several regions of the brain (*7, 17–19*) and with the enhancement of LTP by the $GABA_B$ agonist baclofen (*20*). Disinhibition may be produced by reduction of GABA release via $GABA_B$ autoreceptors on GABAergic terminals (*21*), perhaps in concert with inhibition of firing of GABAergic interneurons by $GABA_B$ receptors on these cells (*22*).

The frequency dependence of LTP induction reflected the time course of the underlying $GABA_B$ receptor–mediated disinhibition, with the peak effect occurring at a frequency in the range of the hippocampal theta rhythm (*6*). Our findings raise the possibility that $GABA_B$-mediated disinhibition might occur during normal theta activity in vivo, thereby facilitating LTP.

$GABA_B$ receptor effects on LTP induction may vary with the frequency of the stimuli used to induce LTP. Olpe and Karlsson (*23*) found LTP induced by high-frequency stimulation was enhanced by $GABA_B$ receptor antagonists. Enhancement of LTP by $GABA_B$ antagonists might be related to the reported suppression of NMDA currents by hyperpolarizing $GABA_B$ IPSPs (*24*).

A recent study in area CA1 of the hippocampus has confirmed our findings in the dentate gyrus that $GABA_B$ receptors facilitate LTP induction (*25*). This evidence, together with the disinhibitory effects of baclofen in the cerebral cortex (*17*), suggests that $GABA_B$ receptors may be important regulators of synaptic plasticity in many brain regions.

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