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# Inhibition of the production and maintenance of long-term potentiation in rat hippocampal slices by a monoclonal antibody

(synaptic plasticity/dentate gyrus/pyramidal CA1 hippocampal neurons/cell surface protein)

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ABSTRACT **Monoclonal antibodies generated to 5-day-old postnatal rat dentate gyri were tested for their effects on long-term potentiation (LTP) in rat hippocampal slices. One antibody, B6E11, was found to block the production of LTP and to suppress established LTP in both area CA1 of the hippocampus and the dentate gyrus. In CA1, B6E11 was effective only when applied to the apical dendrites synapsing with the potentiating input. The production of LTP could not be blocked if B6E11 was applied to the cell bodies or to the basal dendrites of CA1. In field CA1, B6E11 had no effect on the production of short-term potentiation. Another monoclonal antibody from the same panel as B6E11, of the same immunoglobulin class, which also binds to hippocampal neurons similarly to B6E11, had no effect on LTP production. These results supply evidence that B6E11 modulates LTP by an interaction with a specific cell-surface protein associated with the dendrites of neurons in both dentate gyrus and CA1 regions.**

Brief, high-frequency electrical stimulation to either extrinsic or intrinsic hippocampal fiber tracts can produce long-term potentiation (LTP) of synaptic transmission (1–3). The long duration of LTP (3–6) and the implication of the hippocampus in human memory (7) have led to the suggestion that LTP may be a useful model of memory mechanisms (8).

There is growing evidence that synthesis and modification of proteins is involved in the production of LTP. In hippocampal slice preparations, increased incorporation of [$^{32}$P]phosphate into proteins after LTP production has been reported (9–11). Marked increases in the secretion of newly synthesized proteins after production of LTP (12), and the blockade of LTP production by protein synthesis inhibitors, has also been observed (13). A molecular model of LTP formation involving the regulation of the expression of glutamate receptors has been proposed (14). Moreover, a number of cell-surface macromolecules (e.g., ion channels and neurotransmitter receptors) have already been established to be functionally involved in neural transmission. These results suggest that a number of membrane-associated proteins may be necessary for the production of LTP.

Our objective was to generate immunological probes that could be useful in identifying molecules involved in regulating synaptic plasticity and thus perhaps play a role in learning. Because monoclonal antibodies (mAbs) can display a high degree of binding specificity and can potentially perturb the function of the molecules they bind to, experiments were undertaken to screen a panel of mAbs generated against the developing rat dentate gyrus (15–17) for their ability to affect evoked potentials and LTP in hippocampal slices. One antibody of this panel, designated B6E11, was able both to block the production of LTP and to suppress already established LTP in hippocampal slice preparations. In contrast, another antibody from this panel, designated G6E3, with immunohistochemical properties similar to B6E11, had no effect on LTP formation. Neither B6E11 nor G6E3 appeared to have any effect on normal synaptic transmission as measured by population spike amplitude.

## MATERIALS AND METHODS

**Animals.** Male Sprague–Dawley rats (150–250 g) were obtained from Hilltop Lab Animals (Scottsdale, PA).

**mAbs.** mAbs were made by conventional techniques using BALB/c mice and NS-1 myeloma cells (18, 19). Freshly dissected [by the micropunch method of Palkovits and Brownstein (20)] five-day-old postnatal rat dentate gyri served as immunogen. Approximately 50 μg of tissue, homogenized in Dulbecco's phosphate-buffered saline (GIBCO, pH 7.2), was injected.

**mAb Screening.** The mAbs chosen for these studies were required to fulfill four criteria before being tested for their ability to affect hippocampal electrophysiological properties. They were required to have the following characteristics: (*i*) to bind to hippocampal neurons in unfixed, frozen sections of adult hippocampus, (*ii*) to be IgGs, (*iii*) to bind to the cell surface of hippocampal neurons in culture, and (*iv*) to recognize antigens bound to nitrocellulose sheets by immunoblot analysis. The details of the methods and results of this screening protocol can be found elsewhere (15–17).

**Electrophysiology.** Preparation of slices and electrophysiological recording were as described (13, 21–24). Two extracellular recording sites in the cell body layer (200–400 μm apart) were employed in each slice, with one of the two sites randomly selected for mAb application. The other site served as the control evoked response. Ascites fluid containing antibody was diluted 1:20 in 0.9% NaCl and applied by pressure ejection (Picospritzer, General Valve, Fairfield, NJ) from a pipette placed at the same depth as the mAb-recording site electrode, in either the cell body or dendritic layer (see Figs. 1*A*, 3*A*, and 4*A*).

Antibody pipette ejection pressures and times were selected to supply 100- to 200-μm-diameter droplets in air (12 psi/1 sec; 1 psi = 6.895 kPa). Two or 3 ejections were performed, spaced 1 min apart. Under these conditions ≈0.1–1.0 μg of antibody (≈1 pmol of antibody assuming $M_r$ 150,000 as the average molecular weight of an IgG1 molecule) were delivered per ejection. Repetitive stimulation (100 Hz for 2 sec) was applied 15 min after B6E11 ejection to insure binding of the antibody.

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Abbreviations: LTP, long-term potentiation; mAb, monoclonal antibody; STP, short-term potentiation.

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**Data Analysis.** Extracellular responses were sampled on line, digitized, and stored as the average of eight responses. Spike amplitude was defined as the average of the amplitude from the peak early positivity to the peak negativity, and the amplitude from the peak negativity to the peak late positivity (3). LTP was defined as a spike amplitude increase of greater than 2 SDs over prestimulated baselines measured 30 min after a train of repetitive stimuli. Forty-eight slices were examined, and only those that exhibited LTP at the control site 30 min after repetitive stimulation (37 slices) were used. In no case did the mAb site exhibit any potentiation that the control site in the same slice failed to exhibit.

## RESULTS

**Blockade of LTP in Field CA1 Produced by Apical Dendritic, but Not Somatic, Application of B6E11.** Fig. 1 illustrates the blockade of LTP production produced by pretreatment with B6E11 in the apical dendritic field in stratum radiatum (400–500 $\mu$m from the cell body layer). Fig. 1*A* depicts the recording paradigm. In Fig. 1*B*, LTP is shown in a control site not exposed to B6E11 (30-min control). Fig. 1*C* shows the evoked response at another site, in the same slice, treated with B6E11 (30-min mAb). At this site, LTP was almost completely blocked. In those experiments where B6E11 was applied to the pyramidal cell apical dendritic field in stratum radiatum 15 min before repetitive stimulation, LTP was markedly reduced compared to the paired control LTP (Fig. 1*D*, $P < 0.05$, paired $t$ test compared to control untreated sites). However, the decrease in spike latency associated with LTP was not blocked by B6E11 pretreatment (control site, $-7.63 \pm 4.2\%$; mAb site, $-7.72 \pm 2.9\%$). B6E11 did not produce long-lasting alterations in spike amplitude or waveform before high-frequency stimulation (Fig. 1*D*).

When B6E11 was applied to pyramidal cell somata or basal dendrites 15 min before repetitive stimulation, neither normal synaptic transmission nor LTP was impaired (Fig. 2). The experimental paradigm was identical to the apical dendritic experiments, except that the B6E11 pipette was placed 20–50 $\mu$m from one recording electrode in the pyramidal cell body layer in stratum pyramidale (Fig. 2*A*). LTP at the control site is shown in Fig. 2*B* (30-min control). However, Fig. 2*C* illustrates the evoked response at the other site, in the same slice, treated with B6E11 (30-min mAb). LTP was unaffected by somatic B6E11 application. Fig. 2*D* summarizes these experiments.

Experiments summarized in Fig. 3 further characterize the ability of B6E11 to impair LTP in field CA1. Only when B6E11 was applied to pyramidal cell apical dendrites (Fig. 3, mAb apical dendrites) in stratum radiatum 15 min before repetitive stimulation was LTP markedly reduced compared to the paired control LTP ($P < 0.05$, paired $t$ test). Application of B6E11 to pyramidal cell basal dendrites (Fig. 3, mAb basal dendrites) did not impair subsequent production of LTP.

**Blockade of Established LTP in CA1 by Apical Dendritic B6E11 Application.** LTP was not significantly different in the two recording sites prior to B6E11 injection (control site, $138.5 \pm 13.5\%$; mAb site, $164.7 \pm 25.6\%$; $n = 3$). However, after B6E11 application, LTP was completely abolished, in all slices, only at the site receiving mAb (Fig. 3, mAb apicals after 30-min LTP). When evoked population spike amplitudes were measured in these slices 15 min after B6E11 was administered, LTP was still absent from the mAb site compared to control LTP ($P < 0.05$, paired $t$ test). This elimination of LTP is irreversible over a period of many hours (data not shown).

**Blockade of LTP in the Dentate Gyrus Granule Cell Body Layer by B6E11.** In Fig. 4*B*, LTP is shown in a control site that was not exposed to B6E11 (30-min control). Fig. 4*C* shows the evoked response at another site, in the same slice,

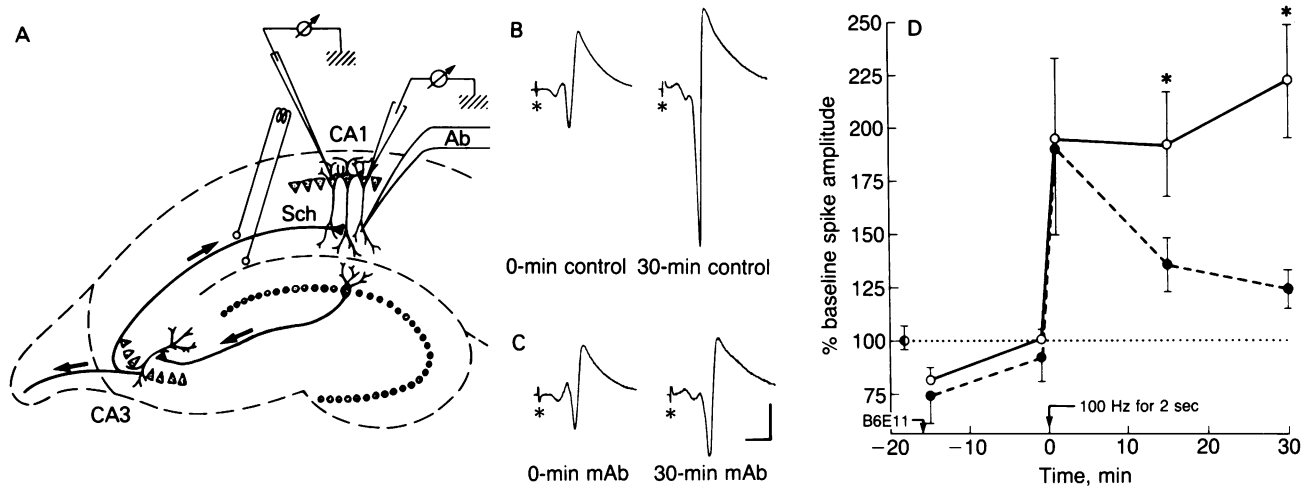


FIG. 1. Blockade of LTP by apical dendritic application of B6E11. (*A*) Experimental arrangement for application of antibody to field CA1 apical dendrites. The field CA1 apical dendritic experimental arrangement; two recording microelectrodes (2 M NaCl) were positioned 200–400 $\mu$m apart in the CA1 pyramidal cell layer. A bipolar stimulating electrode was placed in stratum radiatum. One recording site was then randomly selected for the B6E11-containing pipette, which was placed in the apical dendrites in stratum radiatum 400–500 $\mu$m away from, and at the same depth as, the recording electrode. The other recording site served as the control evoked response. (*B*) LTP at a control site in field CA1. The population spike was recorded just before (0-min control) and 30 min (30-min control) after repetitive stimulation in stratum radiatum. The asterisk denotes the stimulus artifact. (*C*) Blockade of LTP at the antibody site in the same slice as in *B*. This site received three injections of B6E11 in the apical dendrites 15 min prior to repetitive stimulation of the stratum radiatum. At this site, LTP was completely blocked, and the population spike was unchanged 30 min after repetitive stimulation (30-min mAb). The calibration bars for *B* and *C* are 2 mV and 5 msec. Simultaneous recordings were performed to demonstrate that the focal application of B6E11 to pyramidal cell apical dendrites can prevent the full expression of LTP that would have otherwise occurred, as evidenced by LTP at the control site of the same slice. (*D*) Blockade of LTP by B6E11 in slices ($n = 6$) where B6E11 was applied to pyramidal cell apical dendrites. The B6E11 sites (closed circles) received two or three injections. Control sites (open circles) received no antibody. The baseline spike amplitude at each site prior to B6E11 ejections was defined as 100% of control, and all amplitudes are expressed as mean ± SEM. These results show the marked reduction in LTP produced by dendritic application of B6E11, and they also indicate that B6E11 had no measureable effect on normal synaptic transmission prior to high-frequency stimulation.

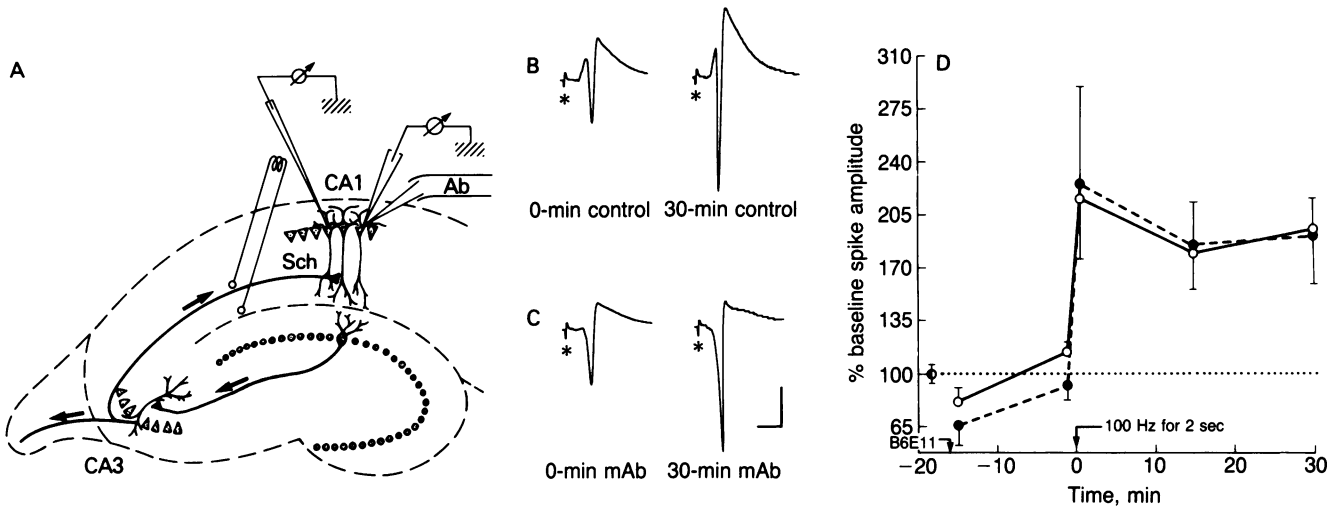


FIG. 2. Lack of blockade of LTP by somatic application of B6E11. (*A*) Experimental arrangement for antibody applied to field CA1 soma. Two recording electrodes were positioned, as in Fig. 1*A*, 200–400 μm apart in the CA1 pyramidal cell layer. The stimulating electrode was placed in stratum radiatum. One recording site was randomly selected for the antibody-containing pipette, which was placed within 20–50 μm of, and at the same depth as, the recording electrode. (*B*) LTP at a control site in field CA1. The population spike was recorded just before (0-min control) and 30 min (30-min control) after repetitive stimulation in stratum radiatum. The asterisk denotes the stimulus artifact. (*C*) LTP at the antibody site in the same slice as in *B*. This site received three somatic injections of B6E11 15 min prior to repetitive stimulation in stratum radiatum. In contrast to apical dendritic B6E11 application, somatic application of B6E11 did not block LTP measured 30 min after repetitive stimulation (30-min mAb). The calibration bars for *B* and *C* are 5 mV and 5 msec. (*D*) Lack of blockade of LTP by B6E11 in slices ($n = 4$) where B6E11 was applied to the pyramidal cell body layer. The B6E11 sites (closed circles) received either two or three injections 15 min before high-frequency stimulation in stratum radiatum. Control sites (open circles) received no antibody. The baseline spike amplitude at each site prior to B6E11 injections was defined as 100% of control, and all amplitudes are expressed as mean ± SEM. In contrast to the results of dendritic B6E11 application, somatic application of B6E11 exhibited no ability to impair production of LTP in field CA1.

treated with B6E11 (30-min mAb). At this site LTP was completely blocked.

When B6E11 was applied to the granule cell body layer 15 min before repetitive stimulation, LTP was markedly reduced compared to the paired control LTP ($P < 0.05$, paired $t$ test) (Figs. 4*D* and 5, soma mAb application). In addition, the decrease in spike latency associated with LTP at the control site (3.36 ± 0.81%; $P < 0.05$, paired $t$ test) was blocked by B6E11 pretreatment (−0.33 ± 0.77%). B6E11 did not produce long-lasting alterations in spike amplitude or waveform before repetitive stimulation (Fig. 4*D*). This was true for a number of stimulus strengths tested in each experiment.

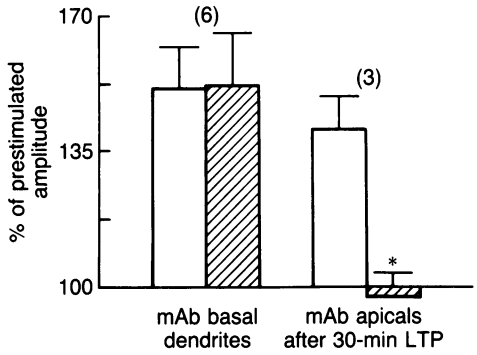


FIG. 3. B6E11 is effective in blocking LTP only when applied to the dendritic synaptic field of the potentiating input. The mean increase in CA1 population spike amplitude in slices that showed LTP for at least 30 min after repetitive stimulation of the Schaffer collateral/commissural axons in stratum radiatum. Amplitude (mean ± SEM) in control sites (open bars) is paired with the amplitude at sites from the same slice that received B6E11 ejections (hatched bars). B6E11 was injected 15 min before repetitive stimulation into basal dendrites (mAb basal dendrites). mAb apicals after 30-min LTP refers to experiments in which B6E11 was injected into the apical dendritic layer 30 min after repetitive stimulation, when LTP was already established, and the amplitudes shown were measured 15 min after B6E11 application. Only in those experiments where B6E11 was applied to the same dendritic synaptic field that received the potentiating input, the apical dendrites, did B6E11 markedly reduce LTP in comparison with the untreated sites in the same slices. The number in parentheses is the number of slices examined. *, $P < 0.05$, paired $t$ test.

**Blockade of LTP in the Granule Cell Dendritic Layer by B6E11.** B6E11 was also applied focally to the granule cell dendritic layer (100–200 μm from the granule cell body layer) to test its ability to impair population spike LTP when applied dendritically. The results of these experiments (Fig. 5, dendritic mAb application) were similar to those in which B6E11 was applied to the granule cell bodies. LTP was significantly reduced ($P < 0.05$, paired $t$ test) when B6E11 was injected in the dendritic layer 15 min before repetitive stimulation.

**Reduction of Established LTP in the Dentate Gyrus by B6E11.** In the next series of experiments, also shown in Fig. 5 (soma mAb after 30-min LTP), B6E11 was applied to the granule cell body layer 30 min after the production of LTP. The mean LTP was not significantly different in the two recording sites prior to B6E11 injection (control site, 234.7 ± 34.1%; mAb site, 233.7 ± 26.6%; $n = 5$). But after B6E11 application, LTP was depressed or completely abolished only in the site receiving mAb. When evoked potential amplitudes were measured in these slices 30 min after B6E11 was administered, the mAb site was still significantly reduced compared to control LTP ($P < 0.05$, paired $t$ test). In one slice, the reduction in LTP by B6E11 was monitored for 4 hr. No reversal of the LTP blockade was observed (data not shown).

**G6E3 Is a mAb that Does Not Impair LTP.** The control experiments in these studies demonstrated that B6E11 did not block LTP nonspecifically. Another mAb in the original panel from which B6E11 was derived was tested for its effects on LTP. Fig. 6 shows that this antibody, G6E3, was unable to impair LTP in the dentate gyrus. Furthermore, G6E3 was also unable to impair LTP when applied to apical dendrites in

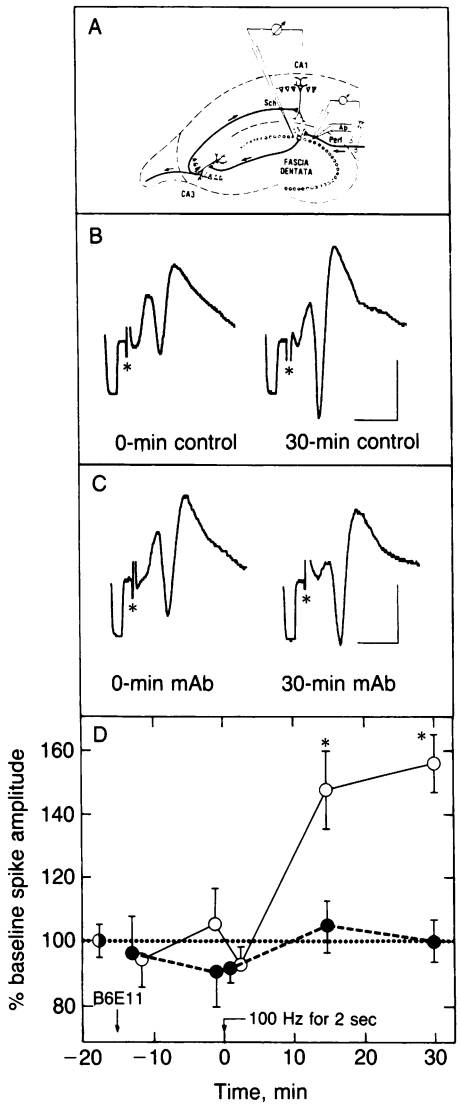


FIG. 4. The blockade of LTP in the dentate gyrus by the mAb B6E11. (*A*) Experimental arrangement; two recording microelectrodes (2 M NaCl) were positioned 200–400 μm apart in the dentate gyrus granule cell layer. A bipolar stimulating electrode was placed on the perforant path axons. One recording site was then randomly selected for the antibody-containing pipette, which was placed within 20–50 μm of, and at the same depth as, the recording electrode. (*B*) LTP at a control site in the dentate gyrus. The population spike was recorded just before (0-min control) and 30 min (30-min control) after repetitive stimulation of the perforant path. The asterisk denotes the stimulus artifact, which is preceded by an internal calibration pulse of 1 mV amplitude, 1 msec duration. (*C*) Blockade of LTP at the antibody site in the same slice as in *B*. This site received three injections of B6E11 15 min prior to repetitive stimulation of the perforant path. At this site, LTP was completely blocked, and the population spike was unchanged 30 min after repetitive stimulation (30-min mAb). The calibration bars for *B* and *C* are 1 mV and 5 msec. Simultaneous recordings were performed to demonstrate that the focal application of B6E11 to the dentate gyrus granule cell body layer can prevent the full expression of LTP that would have otherwise occurred, as evidenced by the LTP in the control site of the same slice. (*D*) Blockade of LTP by B6E11 in slices ($n = 7$) where B6E11 was applied to the dentate granule cell body layer. The B6E11 injection sites (closed circles) received either two or three injections 15 min before high-frequency stimulation of the perforant path. Control sites (open circles; *, $P < 0.05$, paired $t$ test, compared to prestimulated baseline amplitude) received no antibody. The baseline spike amplitude at each site prior to B6E11 injection was defined as 100% of control, and all amplitudes are expressed as mean ± SEM.

field CA1 (data not shown). G6E3 is an IgG1 as is B6E11. It binds to thin sections of adult rat hippocampus and neuronal

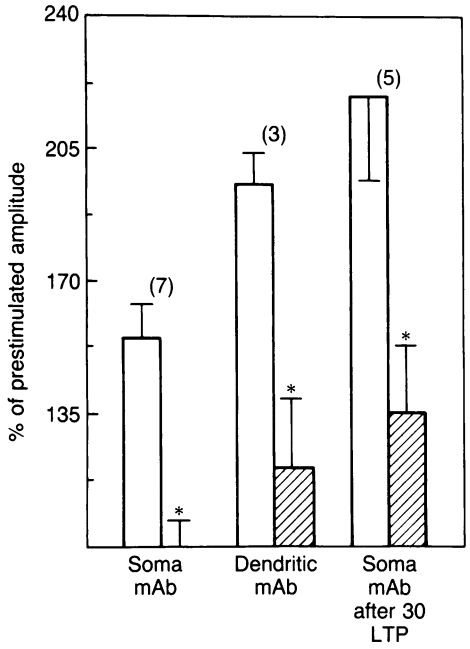


FIG. 5. The mean increase in dentate gyrus evoked population spike amplitude in slices that showed LTP for at least 30 min after repetitive stimulation of the perforant path. Amplitude (mean ± SEM) in control sites (open bars) is paired with the amplitude at sites from the same slice that received B6E11 injections (hatched bars). B6E11 was injected into the granule cell body layer 15 min before repetitive stimulation (soma mAb) or injected into the granule cell dendritic field 15 min before repetitive stimulation (dendritic mAb). Soma mAb after 30-min LTP refers to experiments in which B6E11 was injected into the granule cell body layer 30 min after repetitive stimulation, when LTP was already established, and the amplitudes shown were measured 30 min after B6E11 application. The numbers in parentheses are the number of slices examined. *, $P < 0.05$, paired $t$ test).

cell surfaces of live hippocampal primary cultures indistinguishably from B6E11, based on immunohistochemical analysis. Immunoblot analysis of adult rat hippocampal proteins

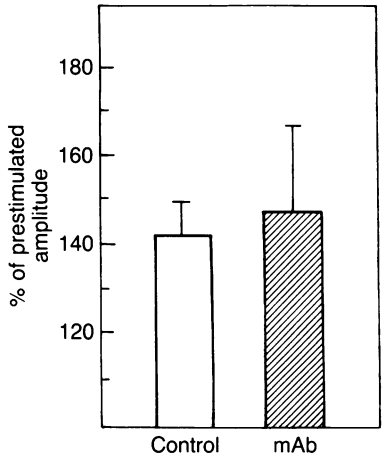


FIG. 6. The mAb G6E3 was unable to block LTP. G6E3 and B6E11 are from the same panel of antibodies, belong to the same immunoglobulin class, and show identical immunohistochemical staining in the dentate gyrus. The antibody was applied to one of a pair of recording sites 15 min before repetitive stimulation (hatched bar; population spike amplitude; mean ± SEM), while the other site was used as the control (open bar; $n = 3$). The amplitudes of these paired responses 30 min after repetitive stimulation were, for the three experiments (control site and G6E3 site): 150 and 133, 150 and 184, 126 and 124% prestimulated amplitude, respectively.

revealed that B6E11 and G6E3 each recognized a single protein of approximately the same molecular weight ($M_r$, 42,000).

## DISCUSSION

The mAb B6E11 blocks the production of LTP and inhibits established LTP in both the dentate gyrus and field CA1. The specificity of these effects is indicated by our demonstration that (*i*) in both dentate gyrus and CA1, B6E11 affected LTP only at the recording site where it was applied, but not at the paired recording site; (*ii*) in CA1, only application of B6E11 to the apical dendrites, where the stimulated synapses are located, blocked LTP, whereas application of B6E11 to the soma or basal dendrites did not affect LTP; and (*iii*) G6E3, a mAb from the same panel and of the same immunoglobulin class as B6E11, which also binds to hippocampal neurons, did not block LTP in either dentate gyrus or CA1. Furthermore, injection of comparable volumes of γ-aminobutyric acid-containing solutions before or after repetitive stimulation has no effect on LTP; only if γ-aminobutyric acid blocks action potential firing during the repetitive train is LTP blocked (22, 23). Thus, we are not inhibiting LTP by a volume effect. It appears that inhibition of LTP occurs through binding of B6E11 to a specific cell surface protein that, when properly expressed, plays a key role in the ability of the dentate gyrus and hippocampus to produce and maintain LTP.

Since B6E11 and G6E3 have been found to induce capping and endocytosis of antibody–antigen complexes *in vitro* (17), B6E11 could affect LTP by the removal of a key protein from the cell surface. B6E11 may bind to and thereby prevent the normal function of a neurotransmitter receptor, ion channel, or regulatory protein. It is intriguing that B6E11 not only prevents but also reverses established LTP. Thus, it seems unlikely that B6E11 acts as an antagonist of the *N*-methyl-D-aspartate receptor, whose blockade prevents but does not reverse LTP (25).

The results of our experiments suggest a dendritic locus for the control of LTP. B6E11 is able to block the production of LTP and to suppress LTP at already potentiated sites only when it is applied to synapses from the potentiating input on CA1 pyramidal cell apical dendrites. It is likely that the potentiation of the excitatory postsynaptic potential was suppressed because spike potentiation was completely blocked. Since it appears that B6E11 does not bind to neurons that synapse with dentate gyrus neurons (15–17), it may be argued that B6E11 exerts its effects postsynaptically.

B6E11 blocked the production of LTP when applied to the dentate granule cell layer but not the CA1 pyramidal cell layer. These results may be explained by the relatively short distance (100–200 μm compared to 400–500 μm for CA1 apical dendrites) between the granule cell bodies and the stimulated excitatory synapses on their dendritic arbors. B6E11 may have diffused into the granule cell dendritic area when applied to the cell body region. Alternatively, the functional localization of protein $A_{b6}$ may be different in the dentate versus CA1.

Another interesting dichotomy between the dentate gyrus and CA1 is the ability of B6E11 to block the decrease in spike latency produced by repetitive stimulation in the dentate but not in field CA1. In CA1 pyramidal cells, application of B6E11 at a site more remote from the soma than in dentate granule cells may permit membrane changes that affect latency to occur at the soma. This observation suggests that potentiation of population spike amplitude may be dissociable from spike latency decreases during LTP.

B6E11, while impairing LTP production, did not significantly alter evoked population responses prior to high-frequency stimulation. The transient depression of the population spike immediately after B6E11 or G6E3 injection was probably due to the volume of liquid injected. Furthermore, although B6E11 blocked LTP, it did not significantly affect amplitude or waveform of the evoked potentials measured after high-frequency stimulation relative to control baseline evoked potentials. Consequently, B6E11 does not seem to be nonspecifically impairing neural transmission. It appears that $A_{b6}$ is not required for normal synaptic transmission, but is specifically required for expression of LTP.

These data also indicate that there are separate mechanisms underlying different phases of hippocampal plasticity. In slices where LTP was impaired by B6E11 applied to the apical dendrites of area CA1, the short-term potentiation (STP) seen 1 min after repetitive stimulation was unaffected (Fig. 1*D*). These results further support the conclusion that LTP and STP are mediated by differing mechanisms. This had been suggested by Stanton and Sarvey (13), who found that inhibitors of protein synthesis block LTP production but do not impair STP, Abraham *et al.* (26), who found that heterosynaptic changes accompany LTP but not STP, and Scharfman and Sarvey (22), who found that blockade of spike firing during high-frequency stimulation blocked LTP but not STP.

The results presented here indicate that a specific mAb, B6E11, probably via its interaction with a specific cell-surface protein, can block both the production and maintenance of hippocampal LTP. Furthermore, the data from field CA1 supply direct evidence that this protein functions primarily in the pyramidal cell dendrites synaptically activated by the stimulated input.

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