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# Hyperexcitability in Combined Entorhinal/Hippocampal Slices of Adult Rat After Exposure to Brain-Derived Neurotrophic Factor

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**Scharfman, Helen E.** Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. *J. Neurophysiol.* 78: 1082–1095, 1997. Effects of brain-derived neurotrophic factor (BDNF) in area CA3, the dentate gyrus, and medial entorhinal cortex were examined electrophysiologically by bath application of BDNF in slices containing the hippocampus and entorhinal cortex. Bath application of 25–100 ng/ml BDNF for 30–90 min increased responses to single afferent stimuli in selective pathways in the majority of slices. In area CA3, responses to mossy fiber stimulation increased in 73% of slices and entorhinal cortex responses to white matter stimulation increased in 64% of slices. After exposure to BDNF, these areas also demonstrated evidence of hyperexcitability, because responses to repetitive stimulation (1-Hz paired pulses for several s) produced multiple population spikes in response to mossy fiber stimulation in CA3 or multiple field potentials in response to white matter stimulation in the entorhinal cortex. Repetitive field potentials persisted after repetitive stimulation ended and usually were followed by spreading depression. Enhancement of responses to single stimuli and hyperexcitability were never evoked in untreated slices or after bath application of boiled BDNF or cytochrome C. The tyrosine kinase antagonist K252a (2 $\mu$M) blocked the effects of BDNF. In area CA3, both the potentiation of responses to single stimuli and hyperexcitability showed afferent specificity, because responses to mossy fiber stimulation were affected but responses to fimbria or Schaffer collateral stimulation were not. In addition, regional specificity was demonstrated in that the dentate gyrus was much less affected. The effects of BDNF in area CA3 were similar to those produced by bath application of low doses of kainic acid, which is thought to modulate glutamate release from mossy fiber terminals by a presynaptic action. These results suggest that BDNF has acute effects on excitability in different areas of the hippocampal-entorhinal circuit. These effects appear to be greatest in areas that are highly immunoreactive for BDNF, such as the mossy fibers and the entorhinal cortex. Although the present experiments do not elucidate mechanism(s) definitively, the afferent specificity, similarity to the effects of kainic acid, and block by K252a are consistent with previous hypotheses that BDNF affects acute excitability by a presynaptic action on trkB receptors that modulate excitatory amino acid transmission. However, we cannot rule out actions on inhibitory synapses or postsynaptic processes.

## INTRODUCTION

In addition to its well-documented effects on neuronal growth and survival of peripheral (Acheson et al. 1995; Hofer and Barde 1988; Yan et al. 1992) and central neurons (Alderson et al. 1990; Cheng and Mattson 1994; Ghosh et al. 1994; Knüsel et al. 1991; Kokaia et al. 1994; Lindholm et al. 1993; Lowenstein and Arsenault 1996; Morse et al. 1993), it has become clear that brain-derived neurotrophic factor (BDNF) has acute effects on synaptic transmission (Berninger and Poo 1996; for review see Lo 1995). One of the first studies to show that BDNF had effects on synaptic transmission demonstrated that the frequency of miniature and evoked excitatory postsynaptic currents at neuromuscular synapses increased after BDNF application (Lohof et al. 1993). Subsequently it was shown in cultured hippocampal neurons that excitatory postsynaptic currents are enhanced by BDNF (Leßmann et al. 1994; Levine et al. 1995, 1996). In addition, it has been shown in adult rat hippocampal slices that BDNF produces a long-lasting potentiation of extracellularly recorded excitatory postsynaptic potentials (EPSPs) (Kang and Schuman 1995). Figurov et al. (1996) showed that BDNF facilitated induction of long-term potentiation (LTP) in area CA1, and Korte et al. (1995) demonstrated deficits in area CA1 LTP in BDNF knockout mice. Patterson et al. (1996) showed that BDNF knockouts had a deficit in basal synaptic transmission, as well as LTP, which could be reversed by exogenous application of BDNF.

Despite the rapid progress in awareness of the acute synaptic effects of BDNF in peripheral neurons, cultured preparations, or area CA1 of slices, effects have not been reported in adjacent areas of the hippocampus such as area CA3 and the dentate gyrus or adjacent areas of the limbic system such as the entorhinal cortex. Nevertheless, BDNF message has been found throughout the brain (Hofer et al. 1990; Phillips et al. 1990; Wetmore et al. 1990). Both message and protein have been found in the dentate gyrus and area CA3 (Dugich-Djordjevic et al. 1995; Kawamoto et al. 1996; Schmidt-Kastner et al. 1996a,b). Staining in the granule cell layer and pyramidal cell layer for BDNF message and protein is prominent, indicating that these cells are likely to produce BDNF. BDNF mRNA expression has also been demonstrated in entorhinal cortex (Castrén et al. 1993), and, as is the case for other areas of neocortex (Schmidt-Kastner et al. 1996a,b), it appears to be located diffusely, throughout layers II–VI. Messenger RNA encoding trkB, the high-affinity receptor for BDNF, has also been found in the granule cells, area CA3 pyramidal cells, and neocortex (Altar et al. 1994; Anderson et al. 1995; Cabelli et al. 1996; Wetmore et al. 1994). trkB receptors are located on the somata and dendrites of granule cells, pyramidal cells of hippocampus,

and pyramidal cells of neocortex (Cabelli et al. 1996; Fryer et al. 1996; Zhou et al. 1993). Recent anatomic data illustrate that BDNF may be preferentially localized in the dentate gyrus granule cells (Conner et al. 1997; Yan et al. 1997) and the superficial layers of the entorhinal cortex (Conner et al. 1997). Therefore we examined whether BDNF has acute effects in these areas.

## METHODS

### *Preparation and maintenance of slices*

Animal care and use was in accordance with the guidelines set by the National Institutes of Health and the New York State Department of Health. Sprague-Dawley rats were anesthetized with ether and decapitated. The age range was 28–42 days old, mostly 30–35 days (1 rat was <30 days and 2 rats were >35 days). The brain was removed and placed in 4°C buffer (composition, in mM: 126 sucrose, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, 1.25 $NaH_2PO_4$, and 10 D-glucose, pH 7.4). After ~60 s the brain was hemisected and trimmed to contain a block of tissue surrounding the hippocampus. This block was submerged in ice-cold buffer and the ventral two-thirds was cut horizontally into 400-$\mu$m sections, which were immediately placed on a nylon net in an interface-type recording chamber; there they were perfused with buffer (1–2 ml/min), oxygenated (95% $CO_2$-5% $O_2$), and maintained at 31–33°C. After 30 min in the chamber, the buffer was changed to artificial cerebrospinal fluid, which contained the same constituents as the buffer except that NaCl was substituted equimolar for sucrose. The substitution of sucrose for NaCl has been used by several other laboratories because it appears to protect neurons from the damaging procedures of slice preparation. After the dissection and switch to NaCl-containing buffer, there appear to be no differences from slices prepared in NaCl-containing buffer entirely (Aghajanian and Rasmussen 1989; Lipton et al. 1995; Richerson and Messer 1995).

### *Recording and stimulation*

Extracellular recordings were made with borosilicate glass filled with artificial cerebrospinal fluid and pulled to 1–5 MΩ resistance. Intracellular electrodes were filled with 1 M potassium acetate; resistance was 75–100 MΩ. Recordings were made with a two-channel intracellular amplifier (Axoclamp 2A, Axon Instruments). Data were digitized at 22 kHz (Neurocorder Model #DR-484, Neurodata Instruments) and stored on tape for analysis off-line. All extracellular recording sites were 50 $\mu$m below the surface of the slice.

Stimulating electrodes were fabricated from Teflon-coated stainless steel wire (~75 $\mu$m diam, including Teflon). Current pulses (50–100 $\mu$A, 10–100 $\mu$s) were triggered by an interval generator (Model #1830; World Precision Instruments) for stimulation of afferent pathways. To test for responses to single stimuli, the stimulus frequency was 0.016–0.33 Hz. The stimulation site for the recordings in the dentate gyrus granule cell layer was located in the outer molecular layer just below the hippocampal fissure, adjacent to the subiculum. The recording site was ~400–500 $\mu$m away, on the border of the granule cell layer and the hilus of the upper blade. For recordings in the area CA3 pyramidal cell layer, the stimulation site was either in the outer molecular layer, the granule cell layer (at the crest of the dentate gyrus), or the hilus (150 $\mu$m from the granule cell layer at the crest). For stimulation of the fimbria, the electrode was placed in the ventral aspect of the fimbria, on the border of the alveus. For Schaffer collateral stimulation, the electrode was placed in stratum radiatum, 100–150 $\mu$m from the pyramidal cell layer, near the CA1/CA2 border. The recording site in the pyramidal cell layer was either in CA3b/c or CA3b.

For medial entorhinal cortical recordings, only those slices in the ventral third of the hippocampus were used. To stimulate the white matter of the medial entorhinal cortex, the stimulating electrode was placed on the border of layer VI and the white matter in the center of the medial entorhinal cortical area (Scharfman 1996). Placement of recording electrodes in cortical layers was aided by an ocular micrometer, so that the distances from the pia that correspond to the different layers could be estimated. When the effects of BDNF on responses to single white matter stimuli were examined, recordings in layer III and VI were made simultaneously and electrodes were maintained in the same locations throughout the first 90 min of drug application. In the same experiments, responses were sampled in each layer before and after recordings were made in layer III/VI, to obtain a laminar profile before and >90 min after drug application (Fig. 6). For such laminar analyses, attempts were made to reposition the recording electrode in the same sites. Repositioning was guided with the use of micrometer readings and landmarks in the tissue (such as blood vessels). Indeed, it appeared that recording electrodes were placed in similar locations, because amplitudes and latencies of short-latency evoked potentials were similar throughout those experiments (Fig. 6). However, it is acknowledged that recording sites for these laminar profiles were not identical. Therefore it was important that the data obtained from recordings made simultaneously in layers III and VI, in which recording sites were not moved, agreed with the data obtained from laminar analyses, where electrodes were moved.

The effects of repetitive stimulation in the dentate gyrus and area CA3 were tested with the use of paired pulses (40-ms interval) at 1 Hz for up to 10 s. In the medial entorhinal cortex, repetitive stimulation was similar except that the interval was 100 ms. These intervals were chosen because in previous experiments such frequencies produced epileptiform activity in the presence of convulsants but not in naive slices (Scharfman 1996).

### *Criteria for accepting slices for study*

Immediately after the dissection, all slices were placed in the recording chamber and remained there for the entire experiment. Viability was tested 1 h after the dissection, and only areas of slices that were acceptable were studied.

The dentate gyrus and area CA3 were acceptable if responses met the following criteria. First, the response recorded in the granule cell layer to a molecular layer stimulus was composed of a positivity >5 mV. Second, population spikes were completely inhibited when two identical stimuli were triggered with a 20-ms interval (Fig. 5; ''paired-pulse inhibition''). Third, a stimulus to the border of the granule cell layer and hilus produced a population spike in the CA3b/c pyramidal cell layer that was ≥2 mV.

The entorhinal cortex was accepted for study if the maximal response to white matter stimulation, recorded in layer VI, included an initial antidromic population spike (amplitude >5 mV) and a subsequent orthodromic population spike (>1 mV). In addition, it was required that the same stimulus that produced a maximal response in layer VI also produced a slow negative wave in layer III (>2 mV in amplitude; Fig. 6).

These criteria were chosen because in other experiments such slices had many neurons in the dentate gyrus, in area CA3, and throughout the deep and superficial layers of the medial entorhinal cortex that were healthy when recorded intracellularly; slices without such field potentials lacked neurons that were healthy when impaled intracellularly.

### *Drug application*

Recombinant human BDNF was supplied by Amgen-Regeneron Partners. It was supplied as a 1-mg/ml solution in 150 mM NaCl and 10 mM sodium phosphate buffer, pH 7.0. It was diluted in sterile phosphate-buffered saline (pH 7.4) or 0.3% bovine albumin and frozen in aliquots (−20°C) until use. The final concentration of bovine albumin that was bath applied to slices was <0.01%, and perfusion of 0.01% bovine albumin without BDNF had no effects (see RESULTS, *Controls*). There were no detectable differences between BDNF diluted in phosphate-buffered saline or bovine albumin. BDNF was used no later than 4 mo after initial dilutions were used.

Kainic acid (Sigma, 0.5–1 $\mu$M) was added to the perfusate immediately before use. Cytochrome C (from *Saccharomyces cerevisiae*, Sigma) was dissolved in sterile $H_2O$ as a concentrated stock solution; an aliquot was added to the perfusate immediately before use to reach a final concentration of 100 ng/ml. K252a (Alexis, 2 $\mu$M) was dissolved in 0.3% dimethyl sulfoxide (DMSO); 0.3% DMSO alone did not have any effects and did not block the effects of BDNF (see RESULTS, *Controls*).

### *Data analysis*

Measurements for comparison of pre- and post-BDNF responses to single stimuli used two time points: 0–15 min before BDNF bath application and 60–90 min after the start of BDNF bath application. To assess hyperexcitability, responses to multiple stimuli were tested 0–15 min before and 2 h after the start of BDNF bath application. These time periods were chosen because slices that had potentiated responses exhibited potentiation before 90 min of exposure to BDNF, and potentiation did not appear to change if perfusion with BDNF was continued; slices that became hyperexcitable showed evidence of hyperexcitability before 2 h had elapsed and did not appear to become more hyperexcitable if perfusion with BDNF was prolonged. Furthermore, slices that did not exhibit potentiation or were not clearly hyperexcitable by 2 h did not develop potentiation or hyperexcitability by perfusion with BDNF for longer periods (up to 4 h).

Evoked responses were measured as shown in Figs. 1 and 5. For area CA3 responses, the amplitude of the volley and the population spike was measured from baseline to peak. The volley was only measured in those cases where its peak was distinct from the orthodromic population spike (Fig. 1, *A2* and *C*). The latency of the population spike was measured from the stimulus artifact to the peak of the population spike. For responses recorded in the dentate gyrus, the positivity on which the population spike was superimposed is referred to as the population EPSP and was measured from baseline to peak (Fig. 5). The population spike amplitude was defined as the average amplitudes of the two deflections composing the population spike (initial, negativegoing phase and the later, positivegoing phase; Fig. 5). To assess responses to paired pulses, measurements were made from slices exposed to the highest concentration (100 ng/ml). The amplitude of the population spike evoked by the second stimulus was divided by the amplitude of the response to the first stimulus to produce a ratio (Table 1). Interstimulus intervals between 10 and 20 ms were chosen to assess paired-pulse inhibition because inhibition is strong when such interstimulus intervals are used. A 70-ms interval was chosen to assess paired-pulse facilitation because paired-pulse facilitation is near maximal when this interval is used.

## RESULTS

### *Area CA3*

EFFECTS OF BDNF ON RESPONSES TO SINGLE AFFERENT STIMULI. The effects of bath-applied BDNF (10–100 ng/ml) in area CA3 were examined on 46 slices (10 ng/ml, $n = 7$; 25 ng/ml, $n = 15$; 50 ng/ml $n = 12$; 100 ng/ml, $n = 12$). Responses were recorded extracellularly in the pyramidal cell layer of area CA3b or CA3b/c, and the stimulating electrode was placed in the dentate gyrus (molecular layer, 3 slices; granule cell layer, 32 slices; hilus, 4 slices). Responses to stimulation of the Schaffer collaterals ($n = 3$) or fimbria ($n = 5$) were also monitored in eight of the experiments.

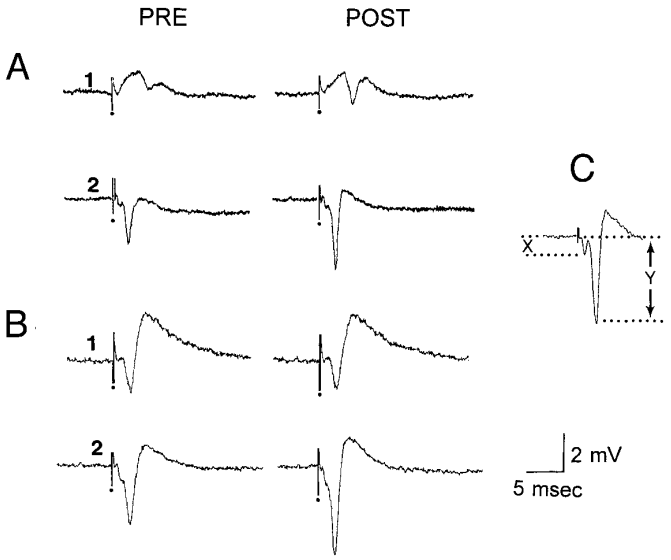


FIG. 1. Potentiation of area CA3 evoked responses to stimulation in dentate gyrus after application of brain-derived neurotrophic factor (BDNF). *A*: stimulation in areas of the slice that activate mossy fiber axons of granule cells evokes responses that increase in amplitude after BDNF bath application. *A1*: stimulation of the outer molecular layer before (PRE) and 1 h after (POST) bath application of 50 ng/ml BDNF. Recording site: CA3b/c. In this and other figures, dots mark stimulus artifacts, which are truncated. *A2*: stimulation of the granule cell layer before and 1 h after BDNF bath application. Recordings in *A1* and *A2* were made at same site. *B*: stimulation of areas of the slice that do not activate mossy fibers does not evoke responses that are increased by BDNF. *B1*: stimulation of the fimbria in another slice before (PRE) and 75 min after (POST) bath application of 50 ng/ml BDNF. Recording site: CA3b. *B2*: stimulation of hilus before and 75 min after application of BDNF. Same recording site as in *B1*. *C*: recording of the evoked response to mossy fiber stimulation illustrates that the volley (*X*) and population spike (*Y*) amplitude were measured from baseline to peak.

Initial experiments focused on responses to single stimuli. The higher concentrations of BDNF (25–100 ng/ml) increased population spike amplitude in response to molecular layer, granule cell layer, or hilar stimulation in many but not all slices. Of the 36 slices tested with granule cell layer or hilus stimulation, there were increases in 40% (6/15) tested with 25 ng/ml, 55% (6/11) tested with 50 ng/ml, and 80% (8/10) tested with 100 ng/ml. When an increase occurred, the increase was apparent regardless of the stimulus intensities chosen, which included weak (<2-mV population spike), intermediate, and high (>4-mV population spike) intensities. The mean increase in amplitude for the 100 ng/ml concentration, measured 90 min after the start of BDNF bath application, was 235 ± 35% (mean ± SE; range 130–303%). In slices where the responses were increased, there were no detectable changes in population spike latency-to-peak or amplitude of the volley (Fig. 1; Table 1). There

TABLE 1. *Effects of BDNF bath application on responses to mossy fiber stimulation recorded in area CA3*

| | Mean Population Spike Amplitude, mV | Mean Population Spike Latency, ms | Mean Volley Amplitude, mV | Responses to Paired Pulses, % Interstimulus Interval | |
|---|---|---|---|---|---|
| | | | | 10 ms | 70 ms |
| Pre-BDNF | 3.1 ± 0.31 | 2.5 ± 0.11 | 0.40 ± 0.09 | 22 ± 10 | 390 ± 25 |
| Post-BDNF | 7.1 ± 1.0 | 2.4 ± 0.09 | 0.46 ± 0.10 | 25 ± 8 | 350 ± 39 |
| *N* | 7 | 7 | 4 | 7 | 7 |

Pre- and post-brain-derived neurotrophic factor (BDNF) values are means ± SE. *N*, number of slices. Dose: 100 ng/ml. Stimulation site: granule cell layer. Recording site: pyramidal cell layer. Stimulus strength was approximately half the stimulus used to evoke a maximal response. Pre-BDNF: mean of measurements taken 15, 10, and 5 min and immediately before the start of BDNF bath application. Post-BDNF: mean of measurements taken between 1 h, 50 min and 2 h, 10 min after the start of BDNF bath application. For measurement of population spike amplitude, latency, and volley, see METHODS. For Responses to Paired Pulses, the amplitudes of the population spike evoked in response to the 1st and 2nd stimuli were compared. A ratio of the 2 population spikes was calculated by dividing the 2nd by the 1st (i.e., amplitude of the population spike in response to the 2nd stimulus divided by the amplitude of the population spike in response to the 1st stimulus).

also were no apparent changes in paired-pulse inhibition or facilitation (Table 1). Regardless of the dose, effects were never apparent until ≥30 min after the start of bath application. There were no effects of BDNF on responses to Schaffer collateral or fimbria stimulation in slices where there were clear effects on responses to mossy fiber stimulation (50 ng/ml: $n = 1$ slice tested with Schaffer stimulation, $n = 3$ fimbria; 100 ng/ml: $n = 2$ Schaffer, $n = 2$ fimbria).

EFFECTS OF BDNF ON RESPONSES TO REPETITIVE AFFERENT STIMULATION. Responses to repetitive 1-Hz stimulation of the mossy fibers were also enhanced after BDNF. The repetitive train was not very long (<10 s) but was extremely effective in producing abnormal responses consisting of multiple population spikes and spreading depression. Such responses never occurred in control slices, even when high stimulus strengths, higher frequencies of stimulation, and longer trains were tested.

A representative example of responses to repetitive stimulation is shown in Fig. 2. After four pairs of stimuli to the granule cell layer at 1 Hz, multiple population spikes occurred in area CA3 in response to each stimulus. After <1 s, small population spikes occurred spontaneously, followed by a spreading depression episode (Fig. 2). In all slices where spreading depression occurred, there was a large negative DC shift (range −16 to −22 mV). At the peak of the DC shift there were no spontaneous or evoked responses to any stimuli (Fig. 2). Recovery from spreading depression included a slow return of the DC potential and a slow recovery of responses to stimulation. In some slices, spontaneous negative potentials occurred intermittently during the recovery period (Fig. 2*B5*). Full recovery of evoked responses required up to 5 min. The slice from which data shown in Fig. 2 were taken was exposed to 25 ng/ml BDNF and required four pairs of stimuli at 1 Hz to evoke spreading depression; other slices required one to eight pairs of stimuli. There was no evidence that shorter periods of stimuli were required in slices that were exposed to higher doses of BDNF.

As was the case for potentiation of responses to single stimuli, hyperexcitability was not observed in all slices. In addition, some slices exhibited multiple population spikes in response to repetitive stimulation but did not have a spreading depression episode thereafter. When spreading depression did follow multiple population spikes, the interval between the last stimulus and onset of the DC shift (start of spreading depression) was <5 s. The values are as follows. After bath application of 25 ng/ml for 2 h, repetitive stimulation (1-Hz paired stimuli with 40-ms interstimulus intervals for <10 s) led to multiple population spikes in 40% (4/10) of slices, and spreading depression occurred thereafter in 33% (3/10) of slices. The three slices with spreading depression had multiple population spikes immediately before the spreading depression episodes. After 50 ng/ml, multiple population spikes occurred in 55% (5/9) of slices, and 33% (3/9) of slices exhibited spreading depression thereafter. The three slices with spreading depression all had multiple population spikes immediately before spreading depression. After 100 ng/ml, 80% (8/10) of slices exhibited multiple population spikes, and all eight slices exhibited spreading depression immediately thereafter. In the two slices treated with 100 ng/ml that did not exhibit multiple population spikes, repetitive stimulation led to spreading depression. In these two slices, the field potentials decreased until no response was evoked, and the typical large DC shift heralding spreading depression occurred immediately thereafter. In summary, there was no strong evidence of dose dependence in the number of stimuli required to evoke hyperexcitability. However, there was some evidence for dose dependence because hyperexcitability occurred in more slices as dose was increased.

SELECTIVITY OF BDNF'S EFFECTS. The data above indicated that the effects of BDNF on responses to single and repetitive stimuli were relatively specific for the mossy fiber pathway. No potentiation of responses to single stimuli occurred if the Schaffer collaterals or fimbria were stimulated, but potentiation did occur if the molecular layer, granule cell layer, or hilus was stimulated. In addition, hyperexcitability was only observed in response to repetitive stimulation of the dentate gyrus mossy fibers; similar stimulation of the Schaffer collaterals or fimbria did not have these effects, even after the highest dose of BDNF.

Figure 3 shows an example of this selectivity. The data were recorded from a slice that was exposed to BDNF for 3 h and was first stimulated in the hilus at 1 Hz; multiple population spikes and spreading depression occurred shortly thereafter (Fig. 3*A, left*). Approximately 15 min later (a period much longer than the recovery period from

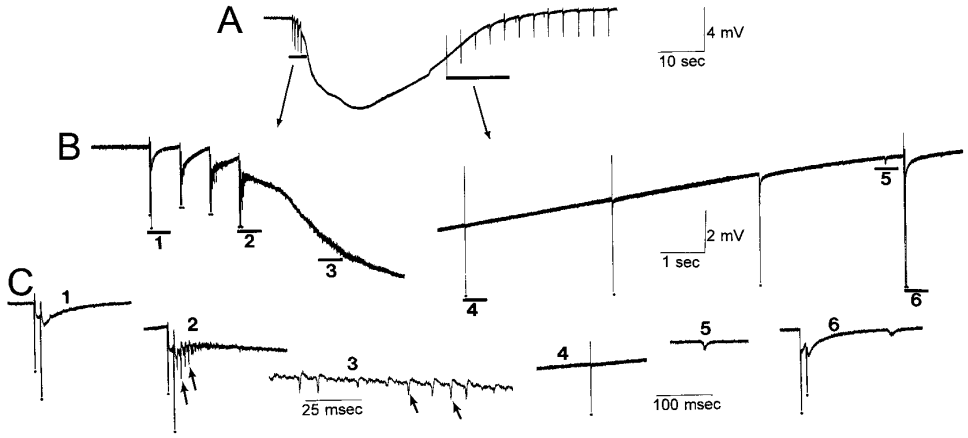


FIG. 2. Repetitive stimulation in the dentate gyrus leads to spreading depression in area CA3 after BDNF bath application. *A*: responses to repetitive stimulation (granule cell layer stimulation, 4 pairs of pulses with 40-ms intervals at 1 Hz) 2 h after the start of BDNF bath application led to spreading depression. Areas marked by horizontal bars are expanded in *B* as indicated by arrows. *B, left*: start of spreading depression shown in *A* is illustrated with higher gain. Spreading depression episode began with negative (DC) shift and multiple population spikes. *B1–B3* are illustrated at faster time base in *C. B, right*: part of the recovery of the spreading depression episode in *A.* During this period, test stimuli demonstrated gradual recovery of the evoked response. In addition, there were spontaneous negative potentials that occurred between stimuli (*B5*). *B4–B6* are illustrated at a faster time base in *C. C*: parts of *B* indicated by horizontal bars are illustrated with a different time base. *C1*: initial responses to a pair of stimuli (2 identical stimuli, 40-ms interval) were 2 large population spikes superimposed on a negativity. *C2*: 4th pair of stimuli evoked responses that were followed by several small population spikes (arrows). *C3*: during the onset of spreading depression, small population spikes (arrows) occurred spontaneously. *C4*: during the initial period of recovery from spreading depression there was no response to a single stimulus. Same stimulus strength as in *C1. C5*: spontaneous negative potential that occurred during the recovery period. *C6*: during the late part of the recovery period there was partial recovery of evoked responses to a pair of stimuli. Eventually the response recovered completely (not shown). Same stimuli as in *C1.* Vertical calibration in *B* applies to *B* and *C.* Horizontal calibration under *trace 3* only applies to *trace 3.* Horizontal calibration for *traces 1, 2, 4, 5,* and *6* is 100 ms, as shown below *trace 5.*

the 1st spreading depression episode), the stimulating electrode was moved to the Schaffer collaterals of the same slice. The recording electrode remained in the same location. A repetitive stimulus train to the Schaffer collaterals was triggered at an intensity that produced a near maximal response. This train did not evoke multiple population spikes and it did not evoke spreading depression, even though stimulus strength was relatively high and more stimuli were triggered (Fig. 3*A, middle*). Minutes later, the stimulating electrode was moved back to the hilus, and a shorter, weaker stimulus train evoked spreading depression (Fig. 3*A, right*). (The 3rd stimulus train was used to ensure that the mechanisms underlying hyperexcitability had remained intact, indicating that deterioration of the slice was not the reason for the inability of Schaffer collateral stimulation to evoke abnormal activity.) In all eight slices tested for responses to more than one input, dentate gyrus stimulation could evoke hyperexcitability but fimbria stimulation ($n = 5$) or Schaffer collateral stimulation ($n = 3$) could not.

COMPARISON OF THE EFFECTS OF BDNF AND KAINIC ACID. The effects of BDNF were similar to those produced by bath application of 500–750 nM kainic acid, which, at such low doses, appears to modulate glutamate release from mossy fiber terminals (Chitajallu et al. 1996; de Montigny et al. 1987; Ferkany et al. 1982; Malva et al. 1995; Okazaki et al. 1988). Figure 4*A* illustrates the effects of BDNF and kainic acid on extracellularly recorded activity in the pyramidal cell layer. In each case, there was an increase in ''noise'' and the appearance of small positive potentials and small population spikes. Figure 4*C* illustrates that kainic acid, like BDNF, increased the amplitude of responses to mossy fiber stimulation. Also as with BDNF, paired-pulse inhibition was maintained (Fig. 4*C*). There was no consistent effect of kainic acid on paired-pulse facilitation (data not shown), similar to the lack of effect of BDNF on paired-pulse facilitation. Kainic acid was also similar to BDNF because it appeared to affect CA3 pyramidal cells more than granule cells; there were no clear effects of 750 $\mu$M kainic acid on intracellularly recorded granule cell responses to molecular layer stimulation ($n = 8$; data not shown). Furthermore, both BDNF and kainic acid did not always produce an increase in responses to mossy fiber stimulation. Of 12 slices exposed to 500 ($n = 4$) or 750 ($n = 8$) nM kainic acid for 10 min, area CA3 population spikes evoked by hilar stimulation increased in amplitude in 4 slices. The appearance of positive potentials and small, spontaneous population spikes occurred in 7 of the 12 slices exposed to kainic acid. (The fact that kainic acid did not have universal effects could have been due to the low dose, although higher concentrations such as 1 $\mu$M also had effects only in a subset of slices tested.) Another similarity between the effects of BDNF and kainic acid was that repetitive stimulation of the dentate gyrus, in the presence of either compound, produced spreading depression in area CA3. Furthermore, spreading depression was only evoked by dentate gyrus stimulation. In the presence of kainic acid, three to eight single stimuli at 1 Hz produced spreading depression in all six pyramidal cells tested with molecular layer stimulation, but spreading depression did not follow

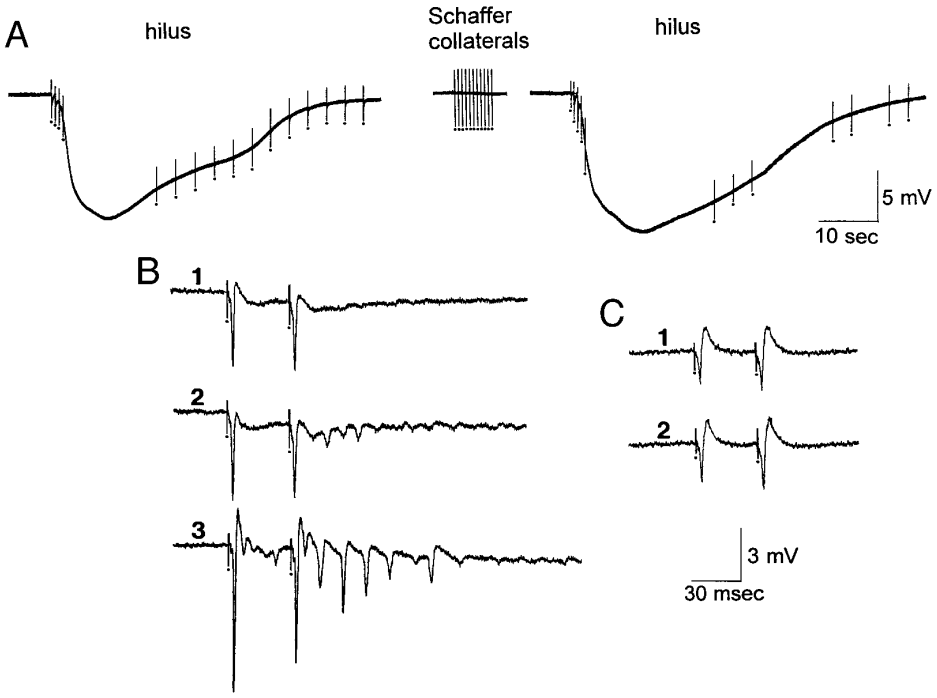


FIG. 3. Effects of BDNF on responses to repetitive stimulation of mossy fibers or fimbria. *A, left*: repetitive stimulation of the hilus led to a spreading depression episode. *A, middle*: 15 min after recovery from spreading depression, the fimbria was stimulated at the same frequency and for a longer period, but there was no indication of hyperexcitability. Same recording site as at *left*. *A, right*: 15 minutes subsequent to fimbria stimulation (*middle*), the granule cell layer was stimulated again, at the same frequency, and spreading depression ensued. Same recording site as at *left*. *B*: some of the responses to stimulation in *A, left* (hilus stimulation site) are illustrated with higher gain. *B1*: 1st pair of stimuli produced 1 population spike after each stimulus. *B2*: 2nd pair of stimuli produced additional population spikes. *B3*: 3rd pair of stimuli produced more population spikes that were larger in amplitude. *C*: some of the responses to stimulation in *A, middle* (Schaffer collateral stimulation site). *C1*: responses to the 1st pair of stimuli. *C2*: responses to the 10th pair of stimuli illustrate that multiple population spikes were not evoked. Note that stimulus strengths for each stimulation site were set at an intensity that produced ~75% of the maximal response to single stimulus; other stimulus strengths for the Schaffer collateral site were tested as well. All stimulus trains were composed of paired pulses (40-ms interval) at 1 Hz. Regardless of stimulus strength, Schaffer collateral stimulation never produced multiple population spikes or spreading depression.

this stimulus train when the fimbria was the stimulation site ($n = 4$ of the 6 pyramidal cells).

### *Dentate gyrus*

The effects of BDNF on responses recorded in the granule cell layer to molecular layer stimulation were tested in 22 slices (10 ng/ml, $n = 4$; 25 ng/ml, $n = 7$; 50 ng/ml, $n = 7$; 100 ng/ml, $n = 4$). Unlike area CA3, there were no detectable effects of up to 50 ng/ml BDNF on responses recorded in the granule cell layer, even after prolonged application (>3 h). Neither responses to single stimuli or paired stimuli (20- or 70-ms interval) were effected. Repetitive stimulation at 1 Hz for 10 s led to depression of evoked responses in slices not exposed to BDNF ($n = 22$), and the same occurred after BDNF. However, after higher-frequency stimulation (100-Hz single stimuli for 1 s, twice in succession with a 10-s interval), spreading depression occurred in 43% of slices exposed to BDNF (25- and 50-ng/ml data pooled, 14 slices total). In slices not exposed to BDNF, exposed to 10 ng/ml BDNF, or exposed to 25–50 ng/ml BDNF for <30 min, such stimulation did not lead to spreading depression.

Although spreading depression episodes in the dentate gyrus indicated that there was abnormal excitability in that subfield, it was possible that spreading depression episodes merely spread to the dentate gyrus but originated in area CA3. This possibility was raised by the results described above showing that molecular layer stimulation could activate area CA3 pyramidal cells and that BDNF potentiated these responses (Fig. 1*A*). It was supported by additional experiments in which two recording sites were employed in the same slice (1 site in the granule cell layer and 1 site in the pyramidal cell layer). In these experiments ($n = 3$), repetitive 1-Hz hilar stimulation caused spreading depression in area CA3 whenever it was tested. In two of the slices, spreading depression occurred in the dentate gyrus as well, and when it did, it always followed spreading depression in area CA3. Repetitive stimulation of the molecular layer at 100 Hz produced the same results as 1-Hz hilar stimulation.

After prolonged exposure to 100 ng/ml BDNF (>30 min) there were effects on responses recorded in the granule cell layer to single stimuli of the molecular layer. There was an increase in the positivity that the population spike rides on, the ''population EPSP'' (Fig. 5; $n = 4$ of 4 slices tested; mean increase in amplitude 155 ± 13%). This occurred regardless of stimulus intensity, including stimuli too weak to evoke a population spike, as well as those that reached

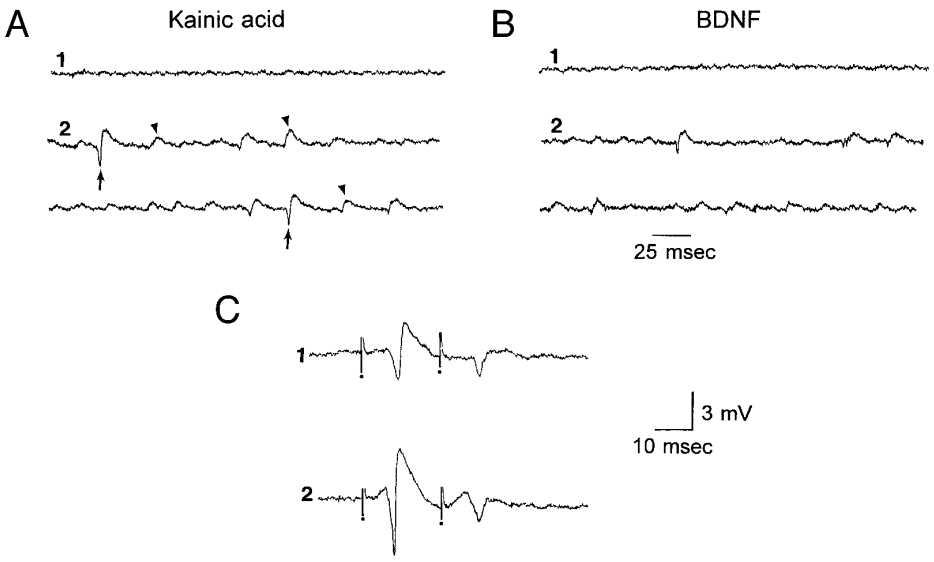


FIG. 4. Effects of BDNF and kainic acid on area CA3 baseline activity and evoked potentials were similar. *A* and *B*: both kainic acid (*A*) and BDNF (*B*) increased ''noise'' in extracellular recordings from the CA3 pyramidal cell layer. In addition, small positivities (arrowheads) or population spikes (arrows) were observed in some slices. *A*: extracellular recording of spontaneous activity in pyramidal cell layer before (*A1*) and 10 min after (*A2*) perfusion with 750 nM kainic acid. *B*: extracellular recording of spontaneous activity in pyramidal cell layer before (*B1*) and 2 h after (*B2*) perfusion with 100 ng/ml BDNF. *C*: kainic acid increased the amplitude of responses to stimulation of the hilus without impairing paired-pulse inhibition. *C1*: responses recorded in CA3b pyramidal cell layer to 2 identical stimuli to the hilus (20-ms interval) before bath application of kainic acid (750 nM). *C2*: responses to the same stimuli as in *C1*, 10 min after bath application of 750 nM kainic acid.

threshold (Fig. 5). In all of these slices there also were increases in population spike amplitude (Fig. 5; mean increase in amplitude 136 ± 11%). There were no detectable changes in responses to paired pulses; when interstimulus interval was 20 ms, the population spike was completely inhibited both before and after BDNF (Fig. 5). When interstimulus interval was 70 ms, the mean ratio (population spike evoked by 2nd stimulus vs. 1st population spike) was 270 ± 42% pre-BDNF and 281 ± 34% post-BDNF.

Although 100 ng/ml BDNF potentiated responses to single stimuli, it did not appear to produce hyperexcitability. Even after 4 h of perfusion with 100 ng/ml BDNF, repetitive paired (40-ms interval) 1-Hz stimulation did not evoke multiple population spikes, even after 20 stimuli ($n = 4$ slices). Spreading depression was triggered by 100-Hz stimulation in two of four slices, similar to the effects of lower doses.

### *Entorhinal cortex*

To examine the effects of BDNF on evoked potentials in the medial entorhinal cortex, responses to a white matter stimulus were recorded simultaneously in layer III and layer VI during the initial 90 min of exposure to BDNF. The stimulus strength was fixed at the intensity that evoked a near-maximal response in layer VI. In addition, both before and after recordings were made in layers III/VI, recordings in other layers in response to the same stimulus were made to examine a laminar profile (see METHODS; Fig. 6).

Responses recorded in the medial entorhinal cortex to single and repetitive stimulation of the white matter changed after all doses of BDNF (10 ng/ml, $n = 8$; 25 ng/ml, $n = 10$; 50 ng/ml, $n = 13$; 100 ng/ml, $n = 7$). These changes were qualitatively similar to those observed after exposure of slices to convulsants such as bicuculline (Jones 1989), pilocarpine (Nagao et al. 1996), or amino-oxyacetic acid (Scharfman 1996). That is, there was an increase in the amplitude and duration of long-latency, negative-going components of field potentials, with no clear change in antidromic potentials or other short-latency components of field potentials (Fig. 6). The effects on responses recorded in layer III were usually the largest (Fig. 6). In this layer, and sometimes in layer II, there also was an increase in noise associated with the late part of the field potential, indicating that a subpopulation of neurons depolarized or discharged synchronously (Fig. 6; note that recording electrode resistance did not change, so this was not likely to be responsible for the noise). As was the case in the hippocampus, the effects on responses to single stimuli were not observed in all slices and were observed more often after higher doses. Whenever the responses in one layer were effected, so were responses in other layers. For the 25-ng/ml dose, 2/10 (20%) of slices were effected; for 50 ng/ml, 7/13 (54%) of slices were effected; for the 100 ng/ml dose, 5/7 (71%) of slices were effected.

Hyperexcitability was evident in responses to repetitive stimulation following perfusion with 25–100 ng/ml BDNF for >30 min. Paired pulses (100-ms interval) at 1 Hz for <10 s produced spontaneous potentials that outlasted the stimuli and sometimes were followed by spreading depression (Fig. 7). Figure 7 shows examples of hyperexcitability recorded in layer III, but repetitive field potentials and spreading depression could also be recorded in the other layers. The percentage of slices exhibiting spontaneous potentials and spreading depression increased as dose was increased, but never reached 100%. For the 25-ng/ml dose, 20% (2/10) of slices exhibited spontaneous potentials, and there was no subsequent spreading depression (slices were examined for 2–10 min after the end of spontaneous field potential activity to confirm that delayed spreading depression did not occur). For the 50-ng/ml dose, 46% (6/13) of slices had spontaneous activity, and there was no spreading depression. For the 100-ng/ml dose, there were spontaneous potentials in 57% (4/7) of slices, and spreading depression occurred in three of the four slices within seconds of the spontaneous activity.

One possible reason for the abnormal activity in the entorhinal cortex was that abnormal activity occurred in the hippocampus and spread to the entorhinal region via known synaptic pathways. To rule out this possibility, the effects

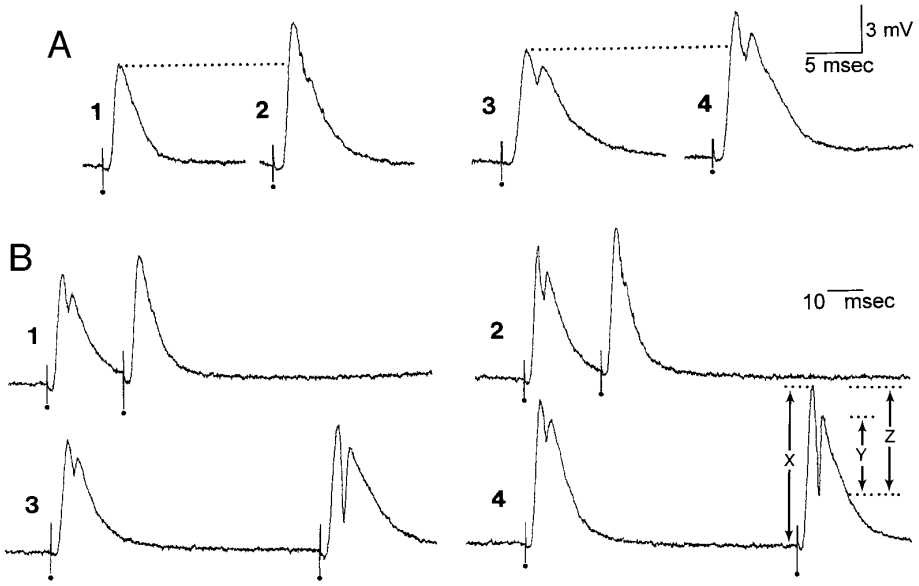


FIG. 5. Effects of BDNF on extracellularly recorded responses in the granule cell layer to outer molecular layer stimulation were apparent only at the maximal dose of BDNF (100 ng/ml). *A*: when BDNF was bath applied for 45 min (100 ng/ml) there was an increase in amplitude of evoked responses recorded in the granule cell layer. *A1*: response to a single stimulus recorded before BDNF application. *A2*: response of the same stimulus 45 min after BDNF bath application. *A3*: response to a higher-intensity stimulus before BDNF exposure. *A4*: response to the same stimulus as in *A3* 45 min after BDNF bath application. Note that although these traces were sampled at 45 min, all measurements were made 60–90 min after BDNF application (see RESULTS). *B*: BDNF bath application had little effect on responses to paired pulses. Same recording and stimulation sites as in *A*. *B1*: responses to a pair of identical stimuli, 20 ms apart, before BDNF exposure. *B2*: responses to the same stimuli as in *B1* 45 min after bath application of BDNF. *B3*: responses to a pair of identical stimuli, 70 ms apart, before BDNF exposure. *B4*: responses to the same stimuli as in *B3* 45 min after BDNF bath application. Next to the response to the 2nd stimulus is an illustration of how potentials were measured. Population excitatory postsynaptic potential (EPSP) was defined as amplitude *X*. Population spike amplitude was measured as the average of amplitudes *Y* and *Z*.

of BDNF were tested in additional experiments with the use of slices that were cut between the hippocampus and the entorhinal region. A cut was made immediately after slices were placed in the recording chamber. The cut passed through the subiculum, from the white matter to the hippocampal fissure, completely separating the entorhinal area from the hippocampus. In these slices ($n = 3$), the effects of BDNF in the entorhinal cortex persisted. Therefore the site of action of BDNF that promoted hyperexcitability was likely to reside within the entorhinal region rather than the hippocampus.

### *Controls*

No effects of BDNF on responses to single or repetitive stimuli were observed when 10 ng/ml BDNF was perfused,

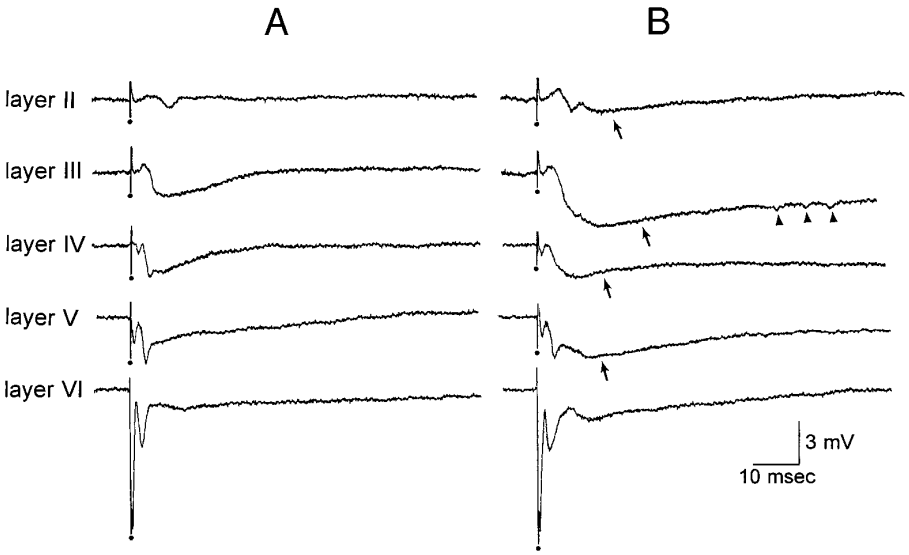


FIG. 6. Epileptiform activity in the medial entorhinal cortex evoked by BDNF bath application. Responses to single stimuli of the white matter were recorded sequentially in all layers of the medial entorhinal cortex before (*A*) and after (*B*) BDNF (25 ng/ml) was bath applied for 1 h. Note the prolonged responses and increase in amplitude of a long-latency, negative-going component of the field potential (arrows). Small spontaneous potentials appeared in responses recorded in layer III (arrowheads).

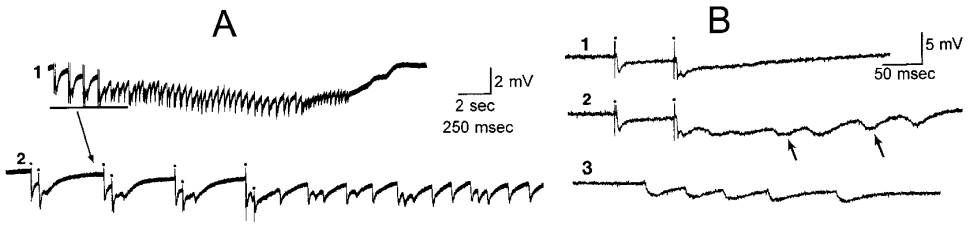


FIG. 7. Epileptiform activity in the medial entorhinal cortex evoked by BDNF bath application. *A1*: after BDNF bath application (25 ng/ml, 90 min), repetitive stimulation of the white matter (4 pairs of stimuli, 100-ms interstimulus interval, at 1 Hz), produced spontaneous activity that was recorded in layer III. Spontaneous activity included negative potentials that were superimposed on a negative DC shift. DC shift gradually recovered after spontaneous potentials stopped. Calibration: 2 mV, 2 s. *A2*: initial period of stimulation and spontaneous activity that is marked by bar in *A1* is illustrated (calibration in *A1*: 2 mV, 250 ms). *B*: in a different slice, BDNF bath application had a similar effect. *B1*: the 1st pair of stimuli of the train (paired pulses with 100-ms interstimulus intervals at 1 Hz). *B2*: 3rd pair of stimuli led to repetitive negative potentials (↘). *B3*: representative sample of spontaneous activity occurring immediately after stimulus train ended.

even after 4 h of bath application (area CA3, $n = 7$; dentate gyrus, $n = 4$; entorhinal cortex, $n = 8$). There were no effects of prolonged application of 10 ng/ml BDNF on paired-pulse inhibition (10- or 20-ms interval) or facilitation (70-ms interval) tested in area CA3 and the dentate gyrus. There also were no effects of bath application of dilute bovine albumin, used to dilute BDNF (0.01%, $n = 5$ slices). Bath application of BDNF that was boiled for 5 min had no effect (100 ng/ml; $n = 3$ slices), nor did exposure to 100 ng/ml cytochrome C ($n = 8$ slices). Cytochrome C was used to control for the effects of adding a large protein to slices, because the molecular weight of cytochrome C is similar to that of BDNF (~13,000). Cytochrome C also has physical chemical properties similar to those of BDNF (Yan et al. 1992).

The nonspecific tyrosine kinase antagonist K252a (2 $\mu$M) blocked the effects of BDNF. K252a was bath applied for 60 min before and during bath application of 100 ng/ml BDNF, and there was no potentiation of responses to single stimuli (mossy fiber responses recorded in area CA3, $n = 4$ slices; layer III and VI responses to white matter stimulation, $n = 4$ slices) and no hyperexcitability in any subfield in any of the 32 slices tested. During the initial period of K252a application, before exposure to BDNF, responses in layer III and VI to white matter stimulation were monitored simultaneously, or responses to mossy fiber stimulation were monitored in area CA3. There was no detectable effect of K252a in layer III or area CA3. The only effects were in layer VI, where the late, slow negativity was decreased in two of four slices. However, the larger components of that field potential, antidromic and orthodromic population spikes, were not affected detectably. Note that the late slow negativity in layer VI is not always present under control conditions. The final concentration of DMSO that was bath applied (0.03%; 3% DMSO was used to dissolve K252a) did not block effects of 100 ng/ml BDNF ($n = 10$ slices).

## DISCUSSION

### *Summary*

The results demonstrate that BDNF application had two major effects that were selective for certain subfields and certain afferent pathways. The first effect was to increase responses to orthodromic stimulation. This occurred in area CA3 in response to mossy fiber stimulation, and in the entorhinal cortex in response to white matter stimulation, after prolonged exposure to 25–100 ng/ml BDNF. Only at the highest dose tested, 100 ng/ml, was there an increase in the response of dentate gyrus granule cells to perforant path stimulation, indicating regional variability of this effect. The second effect of BDNF was hyperexcitability evoked by repetitive stimulation, including multiple population spikes and spreading depression. This was similar to the first effect in that it was only apparent in area CA3 after mossy fiber stimulation, in the entorhinal cortex after white matter stimulation, and the dentate gyrus was less affected.

Although striking when they occurred, the effects did not occur in all slices, and they were not always dose dependent. In addition, there was a long time-to-onset. These aspects of the results could reflect that BDNF does not diffuse well into tissue (Altar et al. 1994; Anderson et al. 1995). In addition, BDNF may have a complex, multistep mechanism of action that is dependent on labile receptor-effector systems or afferent pathways that are not maintained well in slices. The lack of dose dependence of some of BDNF's effects may reflect that BDNF acts in an all-or-none fashion to a certain extent and that its effects are limited more by glutamate stores or available ATP than by the concentration of BDNF. Further experiments will be necessary to address the extent to which BDNF diffusion, limitations of the slice preparation, or other factors were responsible for the results.

### *Comparison with other studies of BDNF's actions*

ELECTROPHYSIOLOGICAL STUDIES IN AREA CA1. The potentiation of responses to single afferent stimuli was similar to the effects that have been reported in area CA1 previously, which include potentiation of extracellularly recorded EPSPs and facilitation of LTP (Figurov et al. 1996; Kang and Schuman 1995; Patterson et al. 1996). Therefore it appears that at several different glutamatergic synapses, not just in area CA1, BDNF promotes excitatory transmission. However, there were some differences that indicate that BDNF's actions are not necessarily identical in the different areas. First, prolonged incubation was required in area CA3 and the dentate gyrus; such long periods of exposure were not necessary for the potentiation of extracellularly recorded EPSPs (Kang

and Schuman 1995), although prolonged periods of application were required by others (Figurov et al. 1996; Patterson et al. 1996). The disparities may be due to technical differences rather than differences in BDNF's actions, because in studies requiring prolonged exposure, BDNF was applied slowly, but in the study that required short exposure times, BDNF was perfused rapidly (Figurov et al. 1996; Kang and Schuman 1995; Patterson et al. 1996). Indeed, Kang et al. (1996) have reported recently that the rate of application of BDNF is an important variable.

Another difference between the present study and the studies performed in area CA1 was the effect of BDNF on paired-pulse facilitation. Paired-pulse facilitation was consistently depressed in the studies of Kang and Schuman (1995), indicating a possible presynaptic locus of action. However, there was no such effect of BDNF in the present study either in area CA3 or the dentate gyrus. There also is some discrepancy in studies of paired-pulse facilitation in slices from BDNF knockouts (the slices were not exposed to exogenous BDNF). Patterson et al. (1996) found that paired-pulse facilitation was depressed compared with that in wild-type mice, but Korte et al. (1995) did not. There could be several reasons why the present experiments did not demonstrate changes in paired-pulse facilitation. For example, the stimulation site employed in the dentate gyrus was not completely selective for mossy fiber inputs, so contamination by other inputs could have obscured a change in facilitation of the mossy fiber input. In addition, it is important to consider that the CA1 studies used recording sites in the dendritic layer (stratum radiatum) and measured the slope of the rising phase of the extracellularly recorded EPSP. The responses reported here were recorded in the cell layers and population spikes or population EPSP amplitudes were measured. If recordings were closer to the site of synaptic input and slopes of EPSPs were measured, a different degree of facilitation might have been recorded. However, in the CA3 pyramidal dendritic layer where mossy fibers innervate pyramidal cells (stratum lucidum), the population EPSP usually merges with a large presynaptic volley and the EPSP slope is obscured. This made extracellular EPSP slope difficult to measure in pilot experiments.

It is also important to point out that processes that are partly dependent or completely dependent on presynaptic mechanisms, such as LTP, are not necessarily accompanied by changes in paired-pulse facilitation (Schulz et al. 1994, 1995). This may be due to the fact that responses to paired-pulse stimulation reflect not only presynaptic function but also activity in polysynaptic inhibitory and excitatory circuits. These circuits differ in area CA1, the dentate gyrus, and area CA3, providing a possible explanation for the discrepancy between the present results and those of Kang and Schuman (1995). In the dentate gyrus, the effects of inhibitory circuits appear to have a stronger influence than in area CA1, because the test response to a conditioning stimulus is usually inhibited for interstimulus intervals of 5–40 ms; in area CA1 facilitation can be observed at these intervals. Thus a potential change in presynaptic function might be obscured by inhibitory circuits in the dentate gyrus more than in area CA1. This could explain why there was no change in paired-pulse facilitation when dentate granule cell responses were enhanced by the highest dose of BDNF. An attempt was made to circumvent this potential problem by examining responses to paired pulses with long intervals (70 ms), but that approach is imperfect because, although $\gamma$-aminobutyric acid-A ($GABA_A$)-receptor-mediated inhibition has waned by 70 ms, $GABA_B$-receptor-mediated inhibition is present. In area CA3, responses to paired-pulse stimulation are difficult to interpret because recurrent excitation is particularly strong relative to the dentate gyrus and area CA1. Therefore the effects of polysynaptic circuits in area CA3 could mask presynaptic effects of BDNF on responses to mossy fiber stimulation.

STUDIES OF BDNF'S EFFECTS ON EXCITABILITY. Hyperexcitability produced by BDNF in the present study is consistent with an earlier report concerning mutant mice that lack the BDNF gene. Although homozygotes were not viable, heterozygotes survived, and kindling was suppressed in the heterozygotes relative to wild-type mice (Kokaia et al. 1995). In addition, Binder et al. (1996) showed that animals receiving intraventricular infusions of trkB immunoadhesin (which scavenges BDNF) required more stimulations to reach a fully kindled state than control rats.

In contrast, two other groups have shown that under some conditions BDNF appears to decrease excitability. One laboratory showed that infusion of BDNF suppressed kindling (Larmet et al. 1995; Reibel et al. 1996). Those researchers also found that the number of tonic-clonic seizures occurring after injection of the convulsant pentylenetetrazol was reduced in animals that had received BDNF infusions (Reibel et al. 1996). Another group infused BDNF into the hilus chronically and found that animals required more stimulations to reach the fully kindled state relative to controls (Osehobo et al. 1996). One possible explanation for the discrepancy between the studies of chronic BDNF infusion (Larmet et al. 1995; Osehobo et al. 1996; Reibel et al. 1996) and the other studies (Binder et al. 1996; Kokaia et al. 1995; present study) is that chronic infusion of BDNF may have altered the BDNF system (BDNF message, protein, or trkB) or affected other neurotrophins or modulators (such as neuropeptides) (Croll et al. 1994; Nawa et al. 1993). Indeed, it has been shown that mRNA for BDNF or trkB can be increased or decreased by a variety of experimental manipulations (Arai et al. 1996; Ballarín et al. 1991; Beck et al. 1993; Cosi et al. 1993; Falkenberg et al. 1992; Frank et al. 1996; Humpel et al. 1993; Kokaia et al. 1993; Lindefors et al. 1992; Lindvall et al. 1992; Merlio et al. 1993; Mudò et al. 1993, 1996; Nibuya et al. 1995; Schmidt-Kastner et al. 1996a; Zafra et al. 1991).

Until more experiments address this issue, the conditions required for BDNF to promote or protect against seizure activity will remain unclear. However, it is notable that the effects on excitability usually require higher concentrations ($>$15 ng/ml) than do the effects on growth and maintenance of neurons ($<$15 ng/ml). Therefore the trophic role of BDNF may be the more potent one, and effects on excitability might require different and perhaps unusual conditions (see below).

### *Possible mechanism(s) of action of BDNF*

Although anatomic studies of BDNF localization have not demonstrated selectivity in the distribution of BDNF in the

different subfields of hippocampus (Dugich-Djordjevic et al. 1995; Kawamoto et al. 1996), recent studies in which a different antibody and fixation procedure were used have shown that BDNF is particularly prominent in the mossy fibers of the dentate granule cells relative to other afferents to area CA3 pyramidal cells (Conner et al. 1997; Yan et al. 1997). This is consistent with the results of the present study, which indicated a selective physiological effect of BDNF on CA3 pyramidal cells after mossy fiber stimulation versus stimulation of other inputs.

The evidence of specificity with respect to the mossy fibers, taken together with previous hypotheses that BDNF acts presynaptically (DiStefano et al. 1992; Kang and Schuman 1995; Lindsay et al. 1994), suggests that BDNF might act on mossy fiber terminals to enhance release of glutamate on area CA3 pyramidal cell dendrites. At the present time one cannot rule out the possibility that there are additional postsynaptic effects of BDNF (Levine et al. 1995), additional effects of BDNF release from nonneuronal populations (Zhou et al. 1993), or effects of BDNF that are not mediated by trkB (Rodriguez-Tébar et al. 1990).

Several other aspects of the results support the hypothesis that BDNF acts presynaptically. First, the effects of BDNF in area CA3 were similar to the effects of 500–750 nM kainic acid, which is thought to act presynaptically at mossy fiber terminals (Chitajallu et al. 1996; de Montigny et al. 1987; Ferkany et al. 1982; Malva et al. 1995; Okazaki et al. 1988). Second, the lack of effect of BDNF on the latency of the population spike and amplitude of the volley suggests that there were no effects on axon conduction or the time course of synaptic transmission. The preservation of paired-pulse inhibition after BDNF application suggests that BDNF did not cause hyperexcitability by impairing GABAergic inhibition. However, a recent presentation (Tanaka et al. 1996) described depressive effects of BDNF on area CA1 pyramidal cell inhibitory postsynaptic currents, raising the possibility that BDNF may indeed affect some aspect of GABAergic function in area CA1.

One argument against the hypothesis that BDNF acts presynaptically is the fact that paired-pulse facilitation did not change after exposure to BDNF. Others have used paired-pulse facilitation as an index of presynaptic function (Kang and Schuman 1995; Manabe et al. 1993), although it is not always straightforward (Schulz et al. 1994, 1995). However, as mentioned above, there are several possible reasons why a change in paired-pulse facilitation might have been obscured in the present study (see *Comparison with other studies of BDNF's actions*).

The long time-to-onset of BDNF's effects indicates that its mechanism might involve a long process, such as activation of a second-messenger system, rather than an action as a simple agonist at a neurotransmitter receptor. Indeed, it has been proposed that BDNF acts to phosphorylate trkB (Frank et al. 1996; Ip et al. 1993; Knüsel et al. 1991) or synapsin I (Jovanic et al. 1996). Consistent with this hypothesis is evidence in cultured neurons that BDNF raises intracellular calcium (Berninger et al. 1993). A rise in intracellular calcium might be a trigger in a cascade of events that ends in, for example, protein phosphorylation or dephosphorylation. Protein synthesis has been demonstrated to play a role in BDNF's actions in area CA1 (Kang et al. 1996), and a similar role may be involved in the effects of BDNF shown in the present study. However, caution is necessary in making interpretations based on the time course of BDNF's actions because, as mentioned above, the long time-to-onset of the effects of BDNF might be due to poor diffusion of BDNF into slices.

### *Implications for understanding potential endogenous actions of BDNF*

ISSUES TO CONSIDER. When relating the present results to potential effects of endogenous BDNF, it is important to consider that the manner and concentration in which BDNF is released from neurons is still a subject in its infancy. It has only recently been shown that BDNF can be transported in the anterograde direction and might be released from nerve terminals (Gall et al. 1996; Zhou and Rush 1996). Therefore one cannot predict whether the concentrations and conditions used to produce effects in the present study reflect what occurs in vivo. One can argue that it is unclear whether BDNF would ever be released in sufficient concentrations to produce the type of potentiation or hyperexcitability observed in this study.

CONDITIONS THAT COULD LEAD TO ENHANCED EXCITABILITY BY BDNF. Because BDNF might not be produced in sufficient concentration under normal conditions to have effects on excitability, it is important to consider the situations in which BDNF and trkB receptors become ''up-regulated.'' Numerous events trigger increases in BDNF message and protein and message-encoding trkB. These include focal mechanical injury (Mudò et al. 1993), seizures (Ballarín et al. 1991; Ernfors et al. 1991; Humpel et al. 1993; Isackson et al. 1991; Merlio et al. 1993; Mudò et al. 1996; Nawa et al. 1995; Nibuya et al. 1995; Schmidt-Kastner et al. 1996a), increased activity in the entorhinal cortex (Falkenberg et al. 1992) or medial septal nucleus (Lindefors et al. 1992), spreading depression (Kokaia et al. 1993), and hypoglycemic coma or cerebral ischemia (Arai et al. 1996; Lindvall et al. 1992). BDNF mRNA can also be modulated by substances such as excitatory amino acids (Zafra et al. 1991), *N*-methyl-D-aspartate (NMDA) (Gwag and Springer 1993), the inhibitory neurotransmitter GABA (Zafra et al. 1991), muscarinic agonists (Knipper et al. 1994), glucocorticoids (Cosi et al. 1993; Smith et al. 1996), and antidepressants (Nibuya et al. 1995). Increases in BDNF mRNA and protein are most striking in the dentate granule cells, but increases have also been described in the entorhinal cortex following injection of MK-801 (Castrén et al. 1993). Unpublished data also show that seizures enhance BDNF in the entorhinal cortex (J. Rudge and S. Wiegand, personal communication), as does cortical spreading depression (N. Kawahara, S. Croll, S. Wiegand, and I. Klatzo, personal communication). Thus, after insult or injury, the endogenous effects of BDNF predicted by the present study may be facilitated because its concentration and receptors become up-regulated.

IMPLICATIONS FOR UNDERSTANDING MECHANISMS OF EPILEPTOGENESIS. Although counterintuitive given BDNF's neuroprotective actions (Acheson et al. 1995; Beck et al. 1994;

Cheng and Mattson 1994; Hofer and Barde 1988; Kokaia et al. 1994; Morse et al. 1993; Yan et al. 1992), it is possible that the up-regulation of the BDNF system after an initial insult could actually have adverse effects by increasing excitability. If the initial insult were a seizure, and BDNF was enhanced subsequently, further release of BDNF might increase excitability and lead to repeated seizures. Such repeated seizures might have excitotoxic effects on the target cells of afferents containing BDNF, particularly if they involve neuronal subpopulations that appear to be vulnerable to excitotoxicity. Indeed, it has been shown that 24 h of pretreatment with BDNF potentiates necrotic cell death of cortical cultures by oxygen-glucose deprivation or NMDA (Koh et al. 1995). Pretreatment with BDNF also potentiated NMDA-induced neuronal degeneration in cultured hippocampal neurons (Prehn 1996). Thus it may be no coincidence that the neuronal subpopulations that are effected by BDNF are also the ones that are usually lost in patients with temporal lobe epilepsy (Du et al. 1993; Margerison and Corsellis 1966; Meldrum and Bruton 1992), i.e., hippocampal pyramidal neurons and entorhinal cortical neurons.

Regardless of the hypothetical link between BDNF and temporal lobe epilepsy, BDNF administration may offer insights into epileptogenesis by providing a novel experimental model of epilepsy. This is indicated by the differences in hyperexcitability produced by BDNF and hyperexcitability observed in other experimental models of epilepsy, such as those that use bath application of convulsants (Swann et al. 1986; Wong and Traub 1983). For example, paired-pulse inhibition in the dentate gyrus appeared to be unaffected by BDNF, although it is impaired in most other models of epilepsy. Perhaps because of this difference, BDNF will allow insights into mechanisms of epileptogenesis that have previously been elusive.

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