Acute Intrahippocampal Infusion of BDNF Induces Lasting Potentiation of Synaptic Transmission in the Rat Dentate Gyrus

ELHOUCINE MESSAOUDI, KJETIL BÅRDSEN, BOLEK SREBRO, AND CLIVE R. BRAMHAM
Department of Physiology, University of Bergen, N-5009 Bergen, Norway

Messaoudi, Elhoucine, Kjetil Bårdsen, Bolek Srebro, and Clive R. Bramham. Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. *J. Neurophysiol.* 79: 496–499, 1998. The effect of acute intrahippocampal infusion of brain-derived neurotrophic factor (BDNF) on synaptic transmission in the dentate gyrus was investigated in urethan-anesthetized rats. Medial perforant path-evoked field potentials were recorded in the dentate hilus and BDNF-containing buffer was infused (4 μl, 25 min) immediately above the dentate molecular layer. BDNF led to a slowly developing increase of the field excitatory postsynaptic potential (fEPSP) slope and population spike amplitude. The potentiation either reached a plateau level at ~2 h after BDNF infusion or continued to increase for the duration of experiment; the longest time point recorded was 10 h. Mean increases at 4 h after BDNF infusion were 62.2 and 224% for the fEPSP slope and population spike, respectively. No changes in responses were observed in controls receiving buffer medium only or buffer containing cytochrome C. BDNF-induced potentiation developed in the absence of epileptiform activity in the hippocampal electroencephalogram or changes in recurrent inhibition on granule cells as assessed by paired-pulse inhibition of the population spike. We conclude that exogenous BDNF induces a lasting potentiation of synaptic efficacy in the dentate gyrus of anesthetized adult rats.

INTRODUCTION

The neurotrophin family of signaling proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5 are recognized as playing critical roles in the survival, differentiation, and outgrowth of select peripheral and central neurons during development (Lewin and Barde 1996; Lindsay 1996). The functions of neurotrophins in the adult brain are less well understood, although a number of studies suggest that neurotrophins continue to exert growth-promoting or survival-promoting effects into adulthood (Lewin and Barde 1996; Prakash et al. 1996; Thoenen 1995).

Perhaps more surprisingly, recent neurophysiological studies in the CA1 region of the in vitro hippocampal slice preparation have provided evidence directly linking neurotrophins to long-term changes in synaptic strength, effects that may contribute to learning and memory. In a seminal report by Kang and Schuman (1995), exogenously applied BDNF and NT-3 induced a rapid and long-lasting enhancement of synaptic transmission at Schaffer collateral-CA1 pyramidal cell synapses. Furthermore, BDNF appears to be necessary for long-term potentiation (LTP), an extremely long-lasting increase in synaptic efficacy induced by high-frequency afferent stimulation. Thus maintenance of LTP in the CA1 region is impaired in slices obtained from BDNF

knockout mice (Korte et al. 1995; Patterson et al. 1996) as well as in slices pretreated with TrkB-IgG, a BDNF-scavenging fusion protein (Figurov et al. 1996).

One important, and largely unexplored issue, is whether BDNF regulates long-term synaptic efficacy in the hippocampus in vivo. Using freely moving rats, we previously found that LTP induction at medial perforant path-granule cell synapses triggers a delayed and sustained increase in BDNF mRNA expression in granule cells (Bramham et al. 1996). Enhanced BDNF production and release therefore may contribute to LTP's late maintenance phase, which is both mRNA and protein synthesis-dependent (Nguyen and Kandel 1996). Here, acute intrahippocampal infusion of BDNF was used to assess directly the effect of this neurotrophin on synaptic efficacy in the medial perforant path. The results provide evidence for BDNF-induced long-lasting potentiation of synaptic transmission in intact, adult rats.

METHODS

Twenty-three male Mol:SD rats (Møllegaards Avls-laboratorium, Denmark) weighing between 250 and 350 g were anesthetized with urethan (1.5 g/kg ip) and placed in a stereotaxic apparatus. Rectal temperature was maintained at 37°C by a servo-heating pad. Electrophysiological methods for obtaining selective stimulation of the medial perforant path have been described in detail previously (Bramham et al. 1991). Stereotaxic coordinates relative to Bregma were 7.9 mm posterior, 4.2 mm lateral for stimulation and 3.9 mm posterior, 2.2 mm lateral for recording. A pair of twisted Teflon-coated stainless steel wire recording electrodes (coated diam = 112 μm) were glued to the outer wall of a metal guide cannula (24 gauge, Plastics One, Roanoke, VA), and the electrodes cut so that the longest tip extended 1 mm from the end of the cannula. A bipolar stimulating electrode was lowered into the dorsomedial aspect of the angular bundle for stimulation of the medial perforant path. After making a small slit in the dura, the guide cannula and attached recording electrodes were slowly lowered into the dorsal hippocampus until a positive-going field excitatory postsynaptic potential (fEPSP) of maximum slope was obtained in the dentate hilus. The final depth of the recording electrode ranged between 200 and 300 μm below the level of the maximum negative-going fEPSP sink recorded in the middle third of the dentate molecular layer. An inner ''infusion'' cannula (31 gauge) then was inserted so that it protruded 300 μm below the end of the guide. The tip of the infusion cannula was located in deep stratum lacunosum moleculare of field CA1, 700 μm above the hilar recording site and 300–400 μm above the medial perforant synapses. Cannula placement was verified in two rats by dye injection.

Human recombinant met-BDNF (a generous gift of Amgen-Regeneron Partners) was obtained as a concentrated stock solution in phosphate buffered saline [PBS: 150 mM NaCl, 10 mM sodium phosphate buffer (pH 7.0), and 0.004% Tween-20], aliquoted in

small volumes, and stored at −80°C until use. Recombinant cyto-chrome C from yeast (Sigma, St. Louis), a protein with similar physical and chemical properties as BDNF, was used as a protein control. Solutions were infused through polyethylene tubing connected to a Hamilton syringe controlled by a microinfusion pump. The main dose of BDNF used in this study (4 μg) was comparable with the total dose perfused across hippocampal slices in the experiments of Schuman and colleagues (Kang and Schuman 1995; Kang et al. 1996); for example, 50 ng/ml BDNF perfused at a rate of 200 ml/h for 30 min = 5 μg BDNF.

Biphasic pulses of 150-μs pulse duration were applied every 30 s throughout the experiment (except during paired-pulse tests described later). Current intensity for test pulses was set on the steep part of the stimulus intensity-response function and elicited a population spike amplitude of 1–3 mV. A minimum 15-min period of stable field potential recording was established before starting the experiment, consisting of baseline recording (20 min), BDNF infusion (4 μg BDNF in 4 μl PBS, 25 min), and from 4 to 10 h of postinfusion recording. Control infusion consisted of PBS alone or PBS containing cytochrome C (4 μg). The effect of BDNF on recurrent inhibition of granule cells was assessed by paired-pulse inhibition of the population spike using a 15-ms interpulse interval. Paired-pulse tests were performed immediately before baseline recording and 2 h after terminating infusion.

Signals from the dentate hilus were amplified, filtered (1 Hz to 3 kHz), digitized [25 kHz for field potentials, 1 kHz for the electroencephalogram (EEG)], and stored to computer disk. Acquisition and analysis of field potentials were accomplished using DataWave Technologies WorkBench software (Longmont, CO). The maximum slope of the f EPSP and the amplitude of the population spike measured from its negative-going apex to the tangent line joining the first two positive peaks were measured, and averages of four consecutive responses were obtained. Analysis of variance for repeated measures followed by a post hoc Scheffe's test was used for statistical analysis of group effects, and a t-test for dependent samples was used for analysis of individual effects (STATISTICA package, StatSoft, Tulsa, OK). Statistics were based on values obtained during baseline and 4 h after terminating infusion.

RESULTS

BDNF infusion (25 min, 4 μl, 4 μg) led to a slowly developing increase of the medial perforant path-evoked f EPSP slope and population spike amplitude (Fig. 1; n = 7). Group time course plots based on 4 h post-BDNF recordings are shown in Fig. 1A. The potentiation started between 20 and 30 min after BDNF infusion and either reached a plateau level at ∼2 h or continued to increase for the duration of experiment. At 4 h after BDNF infusion, the f EPSP slope and population spike amplitude exhibited mean increases above baseline of 62.2 and 224%, respectively (P < 0.05). The effect was highly reliable, with significant potentiation occurring in seven of seven experiments (P < 0.05). The maximum duration of BDNF-induced potentiation recorded was 10 h, at which time the experiment was terminated (n = 3). A representative plot illustrating the slowly developing character of BDNF potentiation during 10 h is shown in Fig. 1B. In another series of experiments, 20 (n = 2) and 2 μg (n = 2) doses of BDNF both elicited robust potentiation equivalent to that obtained with 4 μg, whereas 0.2 μg had submaximal effects, eliciting a mean increase in the population spike of 103% at 4 h postinfusion (n = 3). In contrast to BDNF, infusions of PBS alone (4 μl, n = 5) or PBS containing cytochrome C (4 μg, n = 4) had no effect on the f EPSP or population spike responses (Fig. 2; P > 0.05).
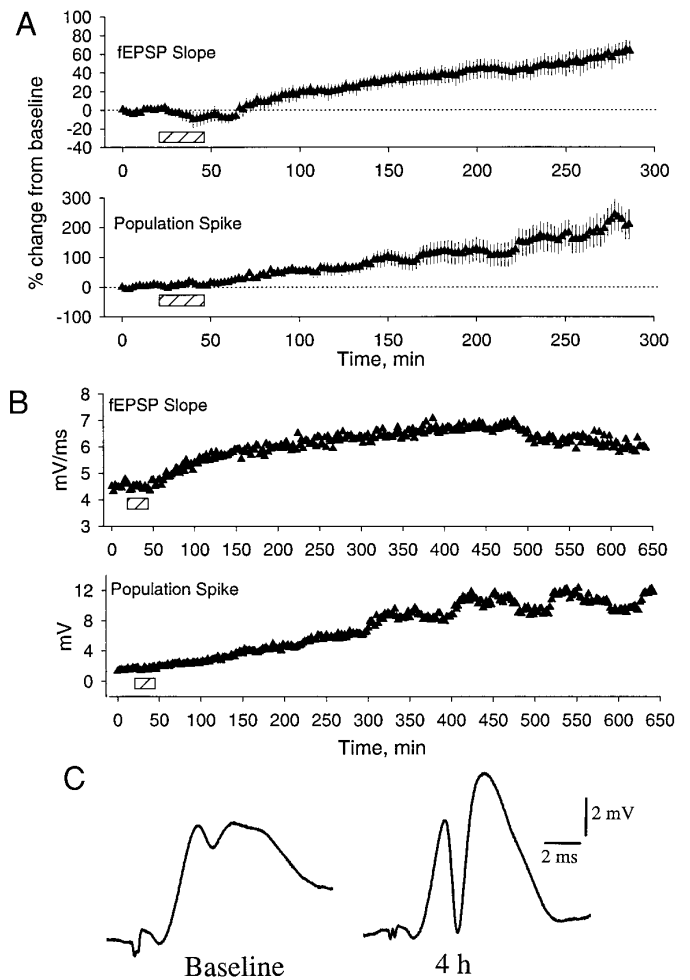

FIG. 1. Effect of acute brain-derived neurotrophic factor (BDNF) infusion on medial perforant path-evoked field potentials in the dentate gyrus. A: group mean changes in the field excitatory postsynaptic potential (f EPSP) slope and population spike amplitude. Values are expressed in percent of baseline. BDNF (4 μl, 4 μg) was infused into the CA1 region immediately above the dentate molecular layer during the period (25 min) indicated by the hatched bar (n = 7). B: representative individual time course plot from a 10-h post-BDNF recording session. Note the slowly developing character of the potentiation. C: sample recordings of field potentials (average of 4 sweeps) obtained immediately before BDNF infusion and 4 h after terminating infusion.

In tests of paired-pulse inhibition, the population spike was abolished almost completely during baseline recording and during expression of BDNF-induced potentiation, arguing against an effect of BDNF on recurrent inhibition (Fig. 3A). Finally, BDNF-induced potentiation was not associated with afterdischarges or other overt epileptiform activity as assessed by off-line viewing of the continuously recorded hippocampal EEG (Fig. 3B).

DISCUSSION

The present results show that acute intrahippocampal infusion of BDNF potentiates transmission at medial perforant path-granule cell synapses in the dentate gyrus of anesthetized adult rats. This is the first in vivo demonstration of neurotrophin-induced potentiation of synaptic transmission. The potentiation is reliable and robust, it persists for ≥10 h
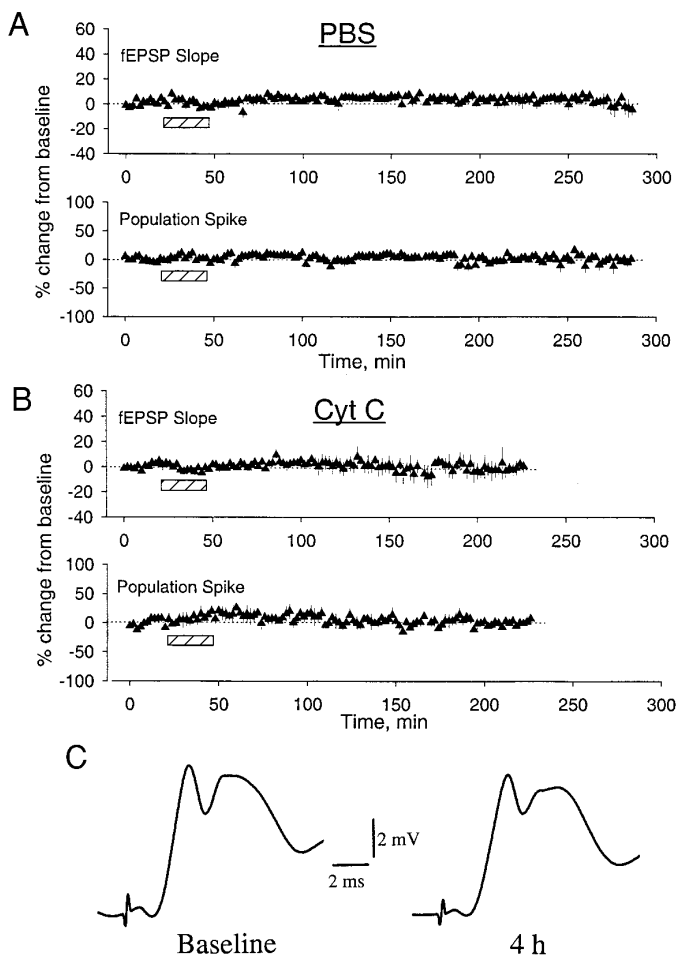
FIG. 2. *A*: group mean changes in the medial perforant-path evoked fEPSP slope and population spike amplitude after acute infusion of phosphate buffered saline (PBS). Values are expressed in percent of baseline. PBS (4 μl) was infused into the CA1 region immediately above the dentate molecular layer during the period (25 min) indicated by the hatched bar ($n = 5$). *B*: group mean changes in the fEPSP slope and population spike amplitude after cytochrome C (Cyt C; 4 μg in 4 μl PBS) infusion ($n = 4$). *C*: sample recordings of field potentials (average of 4 sweeps) obtained immediately before PBS infusion and 4 h after terminating infusion.

(maximum time point examined), and it develops in the absence of epileptiform activity.

The present findings in the dentate gyrus in vivo corroborate Kang and Schuman's (1995) original demonstration of BDNF-induced potentiation in the CA1 region of the hippocampal slice preparation. This is particularly important in light of two reports of failure to obtain BDNF-induced potentiation in the CA1 region of hippocampal slices (Figurov et al. 1996; Patterson et al. 1996). In the study of Patterson et al. (1996), the discrepancy was suggested to be due to insufficient penetration of BDNF into the slice. Substantiating this view, Kang et al. (1996) showed that BDNF-induced potentiation requires high perfusion rates and correlated this with more rapid penetration of BDNF into the tissue slice. In the present in vivo study, potentiation was elicited by slow infusion (0.16 μl/min) of BDNF at a dose comparable with the total amount perfused in the work of Schuman and colleagues. The minimum diffusion distance from the site of infusion in stratum lacunosum-moleculare of field CA1 to medial perforant path-granule cell synapses

is 300–400 μm, at least twice the minimum distance in hippocampal slices. Consistent with the longer diffusion path, the onset of the potentiation was delayed relative to that observed by Kang and Schuman (1995). In contrast, however, the in vivo potentiation often continued to increase for hours without reaching a stable plateau (in some cases for 10 h; Fig. 1*B*). Whether this ascending time course reflects potentiation of synapses more distant from the infusion site or a unique property of in vivo potentiation remains to be determined.

BDNF exerts most if not all of its cellular effects through activation of the transmembrane receptor tyrosine kinase TrkB. In cultured hippocampal pyramidal cells, transient enhancement of excitatory transmission by BDNF is considered to involve both pre- and postsynaptic actions (Lessmann et al. 1994; Levine et al. 1995). Relatively little is
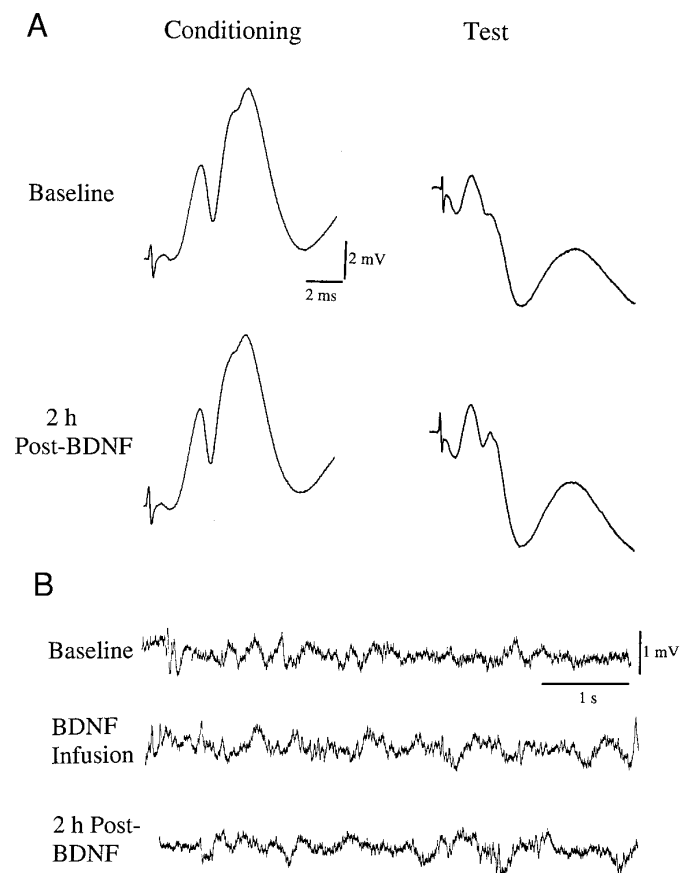

FIG. 3. Effect of BDNF on paired-pulse inhibition of the population spike and the hippocampal electroencephalogram (EEG). *A*: field potentials (mean of 4 sweeps) from paired-pulse tests carried out before baseline recording and 2 h after BDNF-infusion. Conditioning stimulation (*left*) was followed 15 ms later by test stimulation (*right*) at the same stimulus intensity. To compensate for the increase in the population spike amplitude after BDNF infusion, the stimulus intensity was lowered from 250 μA (pre-BDNF) to 200 μA (post-BDNF). Strong inhibition of the population spike was observed before and during expression of BDNF-induced potentiation. Similar results were obtained in 6 rats. *B*: EEG recordings from the dentate hilus collected during baseline recording, at the end of BDNF infusion, and 2 h postinfusion from the same experiment depicted in *A*. During urethan anesthesia, the hippocampal EEG oscillates between theta wave activity and a sleep-like EEG dominated by slow waves and intermittent fast activity. EEG segments displayed are all from the non-theta state. Off-line viewing of the continuously recorded EEG showed that BDNF-potentiation developed in the absence of afterdischarges or other overt epileptiform activity.

known about the cellular targets of BDNF in the dentate gyrus. In support of a postsynaptic target, immunocytochemical studies have demonstrated strong TrkB immunostaining on granule cell dendrites and cell bodies, and weaker staining on unidentified hilar neurons (Yan et al. 1997). With regard to possible effects of BDNF on interneurons (Tanaka et al. 1997), our results indicate that loss of recurrent GABAergic inhibition does not account for BDNF-induced potentiation in the dentate gyrus. Electron microscopic studies are needed to assess localization of TrkB receptors at excitatory synapses of the perforant path.

A key question is this: is BDNF rapidly inactivated after transient activation of TrkB receptors or is it retained in the extracellular space, providing long-lasting TrkB activation? BDNF-induced potentiation in the CA1 field in vitro was suggested to be due to transient TrkB activation because K252a (a general kinase inhibitor with known Trk inhibitory activity) blocks induction but not maintenance of potentiation (Kang and Schuman 1995). This may not be the case in vivo, however; Knusel et al. (1996) have demonstrated enhanced Trk phosphorylation lasting $\geq$1 day after a single 1 μg injection of BDNF into the hippocampus of adult rats.

We have shown previously that N-methyl-D-aspartate receptor-dependent LTP induction in the medial perforant path leads to an increase in BDNF mRNA levels in granule cells at 6 and 24 h (but not 2 h) after stimulation, suggesting a delayed and sustained enhancement in BDNF synthesis during the late, protein synthesis-dependent phase of LTP (Bramham et al. 1996). On the basis of the present results, enhanced secretion of BDNF during LTP, should it occur, would be expected to induce a lasting increase in synaptic efficacy. We currently are examining the role of endogenously released BDNF in LTP using the BDNF scavenger TrkB-IgG and have obtained evidence that BDNF does not contribute to the first 4 h of LTP (Messaoudi et al. 1997). The available data is therefore most compatible with a model of delayed BDNF release during LTP, possibly contributing to long-term stabilization of synaptic strength and structure (Geinisman et al. 1996). BDNF has been localized postsynaptically to granule cell dendrites and cell bodies as well as presynaptically in the medial perforant path (Conner et al. 1997; Dugich-Djordjevic et al. 1995). Unlike classical neurotransmitters, BDNF may be in a position to regulate long-term synaptic efficacy through both presynaptic and postsynaptic sites of release.

REFERENCES

BRAMHAM, C. R., MILGRAM, N. W., AND SREBRO, B. Activation of AP5-sensitive NMDA receptors is not required to induce LTP of synaptic transmission in the lateral perforant path. *Eur. J. Neurosci.* 3: 1300–1308, 1991.

BRAMHAM, C. R., SOUTHARD, T., SARVEY, J. M., HERKENHAM, M., AND BRADY, L. S. Unilateral LTP triggers bilateral increases in hippocampal neurotrophin and trk receptor mRNA expression in behaving rats: evidence for interhemispheric communication. *J. Comp. Neurol.* 368: 371–382, 1996.

CONNER, J., LAUTERBORN, J., YAN, Q., GALL, C., AND VARON, S. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J. Neurosci.* 17: 2295–2313, 1997.

DUGICH-DJORDJEVIC, M. M., PETERSON, C., ISONO, F., OHSAWA, F., WIDMER, H. R., DENTON, T. L., BENNETT, G. L., AND HEFTI, F. Immunohistochemical visualization of brain-derived neurotrophic factor in the rat brain. *Eur. J. Neurosci.* 7: 1831–1839, 1995.

FIGUROV, A., POZZO MILLER, L. D., OLAFSSON, P., WANG, T., AND LU, B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* 381: 706–709, 1996.

GEINISMAN, Y., DETOLEDO MORRELL, L., MORRELL, F., PERSINA, I. S., AND BEATTY, M. A. Synapse restructuring associated with the maintenance phase of hippocampal long-term potentiation. *J. Comp. Neurol.* 368: 413–423, 1996.

KANG, H. AND SCHUMAN, E. M. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* 267: 1658–1662, 1995.

KANG, H., JIA, L. Z., SUH, K., TANG, L., AND SCHUMAN, E. M. Determinants of BDNF-induced hippocampal synaptic plasticity: role of the Trk B receptor and the kinetics of neurotrophin delivery. *Learn. Memory* 3: 188–196, 1996.

KNUSEL, B., KAPLAN, D. R., AND HEFTI, F. Intraparenchymal NGF injections in adult and aged rats induce long-lasting Trk tyrosine phosphorylation. *Exp. Neurol.* 139: 121–130, 1996.

KORTE, M., CARROLL, P., WOLF, E., BREM, G., THOENEN, H., AND BONHOEFFER, T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. USA* 92: 8856–8860, 1995.

LESSMANN, V., GOTTMANN, K., AND HEUMANN, R. BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurones. *Neuroreport* 6: 21–25, 1994.

LEVINE, E. S., DREYFUS, C. F., BLACK, I. B., AND PLUMMER, M. R. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proc. Natl. Acad. Sci. USA* 92: 8074–8077, 1995.

LEWIN, G. R. AND BARDE, Y. A. Physiology of the neurotrophins. *Annu. Rev. Neurosci.* 19: 289–317, 1996.

LINDSAY, R. M. Role of neurotrophins and trk receptors in the development and maintenance of sensory neurons: an overview. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351: 365–373, 1996.

MESSAOUDI, E., BÅRDSEN, K., SREBRO, B., AND BRAMHAM, C. R. Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. *Soc. Neurosci. Abstr.* In press.

NGUYEN, P. V. AND KANDEL, E. R. A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *J. Neurosci.* 16: 3189–3198, 1996.

PATTERSON, S. L., ABEL, T., DEUEL, T. A., MARTIN, K. C., ROSE, J. C., AND KANDEL, E. R. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* 16: 1137–1145, 1996.

PRAKASH, N., COHEN CORY, S., AND FROSTIG, R. D. Rapid and opposite effects of BDNF and NGF on the functional organization of the adult cortex in vivo. *Nature* 381: 702–706, 1996.

TANAKA, T., SAITO, H., AND MATSUKI, N. Inhibition of GABA$_A$ synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J. Neurosci.* 17: 2959–2966, 1997.

THOENEN, H. Neurotrophins and neuronal plasticity. *Science* 270: 593–598, 1995.

YAN, Q., RADEKE, M. J., MATHESON, C. R., TALVENHEIMO, J., WELCHER, A. A., AND FEINSTEIN, S. C. Immunocytochemical localization of TrkB in the central nervous system of the adult rat. *J. Comp. Neurol.* 378: 135–157, 1997.