The Role of Brain-Derived Neurotrophic Factor Receptors in the Mature Hippocampus: Modulation of Long-Term Potentiation through a Presynaptic Mechanism involving TrkB

Baoji Xu,1 Wolfram Gottlebach,3 Ana Chow,2 Rachel I. Wilson,2 Eric Schnell,2 Keling Zang,1 Denan Wang,1 Roger A. Nicoll,2 Bai Lu,3 and Louis F. Reichardt1

1Howard Hughes Medical Institute, Program in Neuroscience and Department of Physiology, and 2Program in Neuroscience and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143, and 3Unit on Synapse Development and Plasticity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

The neurotrophin BDNF has been shown to modulate long-term potentiation (LTP) at Schaffer collateral-CA1 hippocampal synapses. Mutants in the BDNF receptor gene trkB and antibodies to its second receptor p75NTR have been used to determine the receptors and cells involved in this response. Inhibition of p75NTR does not detectably reduce LTP or affect presynaptic function, but analyses of newly generated trkB mutants implicate TrkB. One mutant has reduced expression in a normal pattern of TrkB throughout the brain. The second mutant was created by cre-loxP-mediated removal of TrkB in CA1 pyramidal neurons of this mouse. Neither mutant detectably impacts survival or morphology of hippocampal neurons. TrkB reduction, however, affects presynaptic function and reduces the ability of tetanic stimulation to induce LTP. Postsynaptic glutamate receptors are not affected by TrkB reduction, indicating that BDNF does not modulate plasticity through postsynaptic TrkB. Consistent with this, elimination of TrkB in postsynaptic neurons does not affect LTP. Moreover, normal LTP is generated in the mutant with reduced TrkB by a depolarization–low-frequency stimulation pairing protocol that puts minimal demands on presynaptic terminal function. Thus, BDNF appears to act through TrkB presynaptically, but not postsynaptically, to modulate LTP.

Key words: TrkB; conditional mutant; CA1; long-term potentiation; presynaptic; neuronal survival

The neurotrophins promote survival of neurons from both the CNS and PNS in cell culture (for review, see Reichardt and Fariñas, 1997). These four closely related proteins (NGF, BDNF, NT-3, and NT-4) interact with Trk receptor tyrosine kinases. TrkA is activated by NGF; TrkB is activated by BDNF and NT-4; and TrkC is activated by NT-3. In some cells, NT-3 is able to activate all three Trk receptors (Huang et al., 1999). Engagement of the Trk receptors results in activation of several intracellular signaling pathways, including ras, phosphatidylinositol-3 kinase, and phospholipase Cyl, which promote survival and differentiation. All four neurotrophins also bind to the unrelated receptor p75NTR, which activates ceramide turnover and the jun kinase cascade, promoting either cell motility or apoptosis, depending on cell type.

Both the neurotrophins and their receptors are expressed in the developing and adult CNS, and each of the neurotrophins has been shown to support survival and/or differentiation of CNS neurons in cell culture (for review, see Korsching, 1993). Despite this, comparatively few deficits have been seen in the brains of mice lacking individual neurotrophins or Trk receptors (for review, see Reichardt and Fariñas, 1997). In the hippocampus, the deficits observed include a small increase postnatally in granule cell apoptosis and striking reductions in expression of calbindin, parvalbumin, and neuropeptide Y in GABAergic interneurons (Jones et al., 1994; Minichiello and Klein, 1996; Alcántara et al., 1997). Except for the NT-4 mutant, all of the neurotrophin- and Trk-deficient mice have quite limited postnatal life spans, seldom surviving beyond a couple of weeks. Consequently, it has been not possible to determine the requirements for these molecules during the entire span of CNS development or to use these animals to examine neurotrophin functions in adults.

The neurotrophins have been shown to modulate many aspects of synaptic transmission and neural plasticity (Lohof et al., 1993) (for review, see Thoenen, 1995; McAllister et al., 1999). Mechanisms underlying establishment of long-term potentiation (LTP) in the CA1 region of the hippocampus have been the subject of many studies (for review, see Malenka and Nicoll, 1999). LTP at these synapses is greatly reduced in BDNF homozygous and heterozygous mutant mice and can be rescued by exogenous BDNF (Korte et al., 1996; Patterson et al., 1996). Consistent with these results, LTP is also strongly inhibited in slices by application of the BDNF and NT-4 scavenger TrkB-IgG (Figurov et al., 1996; Kang et al., 1997).

Which cells and receptors are involved in the BDNF signaling circuit important for modulating LTP? Using cultured hippocampal neurons as a model system, BDNF has been shown to enhance transmitter release via a mechanism inhibitable by expression of a dominant negative variant of TrkB in presynaptic cells (Li et al., 1998), suggesting a presynaptic locus via the receptor TrkB. In contrast, BDNF has been shown by different groups to enhance not only presynaptic transmitter release but also postsynaptic transmission through NMDA channels in cultured hippocampal neurons (Levine et al., 1995, 1998). Interneurons are also a potential locus for BDNF action, because BDNF deficiency clearly inhibits the differentiation of these neurons (Jones et al., 1994), and acute application of BDNF has been shown to decrease inhibition in slices from adult animals (Tanaka et al., 1997; Freking et al., 1998). Thus, it is not certain whether the targets of BDNF relevant...
for LTP are presynaptic CA3 afferents, postsynaptic CA1 pyramidal cells, interneurons, or all three. It is similarly uncertain whether the relevant signaling important for modulating synaptic plasticity in vivo occurs through the endogenous TrkB receptor, the neurotrophin receptor p75NTR, or perhaps both. To analyze the cells and receptors important in modulating synaptic plasticity in the CA1 region of the hippocampus, we have used both antibody inhibition and genetic techniques to interfere with p75NTR and TrkB functions, respectively. Our results implicate TrkB but not p75NTR. Our data also indicate that the most important site of TrkB action is the presynaptic axons of the CA3 pyramidal neurons.

MATERIALS AND METHODS

Transgenic mouse production. The cre gene with a nuclear localization signal was removed from plasmid pMML7 (kindly provided by Dr. Gail Martin, University of California, San Francisco, CA) and inserted into pNN265 (kindly provided by Drs. Mark Mayford and Eric Kandel, Columbia University, New York, NY) at its EcoRI site to generate pNN265-cre. The 2.5 kb NorI fragment of pNN265-cre was composed of the cre transgene, an exon–intron splicing signal, and an SV40 polyadenylation signal and inserted into pNN279 (kindly provided by Drs. Mark Mayford and Eric Kandel) to generate a cDNA fragment containing the promoter for the α subunit of Ca²⁺/calmodulin-dependent protein kinase II (αCaMKII) and the cre transgene, was released by SacI digestion and purified from vector DNA. The cre fragment of 3.0 kb was transferred into the 1.8 kb BamHI fragment of SV40 genomic DNA (CA12 strain of mice). The 10 kb XbaI–BamHI genomic fragment covering the first coding exon (exon S) was used to construct the tkβ targeting vector (Fig. 1A). Exon S covers a 346 bp 5’ untranslated region (UTR) and a 344 bp coding region including 31 coding codons for the signal peptide of TrkB. A Clal site, which is located at the 19th bp of exon S, and a KpnI site, which is 112 bp downstream of exon S, were two critical restriction sites for construction of the targeting vector. The targeting vector was made by replacement of the Clal–KpnI fragment that covers a 248 bp 5’ UTR and a 344 bp coding region downstream of the sequence with an ~14 kb DNA fragment that includes a 4.4 kb tkβ cDNA fragment, a 4 kb PGKneo-tr selection cassette, and a 5 kb reporter gene, tau-lacZ, as well as three loxP sites (Fig. 1B). The tkβ cDNA unit was generated by ligating a 4.3 kb SV40 polyadenylation signal sequence to the 3’ end of a 4.2 kb Clal–EcoRI tkβ cDNA fragment that was isolated from pFRK4 (a gift from Dr. Rudiger Klein, European Molecular Biology Laboratory, Heidelberg, Germany). The 2.5 kb cDNA fragment covers the open reading frame for the full-length TrkB receptor tyrosine kinase and a 1 kb 3’ untranslated region. The tkβ cDNA unit was fused into exon S at the Clal site, which contains a loxP site and a BamHI site was inserted subsequently. The PGKneo-tr cassette flanked by two loxP sites was derived from plasmid pBS-lox-neo-tr-lox (kindly provided by Dr. Nigel Killeen, University of California, San Francisco, CA), where a PGK-I promoter is followed by a neo gene, an internal ribosome entry sequence of encephalomyocarditis virus, a herpes simplex virus 2 gene, and a FV3 promoter signal sequence. A 125 bp BclI–BamHI reporter gene fragment (a gift from Dr. Chris Callahan, Salk Institute, San Diego, CA). The targeting vector contains 7 kb of homologous DNA (left arm) upstream of the first loxP site and 2.5 kb of homologous DNA (right arm) downstream of the tau-lacZ reporter gene.

The linearized targeting vector was electroperforated into J1M1 embryonic stem cells (Muller et al., 1997) grown on mitotically inactivated STO cells. A 8–10-d-old selective medium (300 μg/ml G418, 1000 μg/ml Ganciclovir) was used to select targeted ES cells, which were expanded, and screened for homologous recombination by Southern blotting using probes A and B as depicted in Figure 1A. The targeted embryonic stem (ES) cells were expanded and transfected with cre expressing plasmid pMMTV-Cre (kindly provided by Dr. Virginie Rajewsky, University of Cologne, Cologne, Germany) by electroporation to transiently express Cre recombinase to remove the PGKneo-tr cassette. Colonies were picked, expanded, and screened for cre-mediated recombination by X-gal staining and probing probe C. Among more than 400 screened ES clones, 18 clones had both the tkβ DNA unit and the PGKneo-tr cassette removed, and one clone was identified to have a floxed recombination between the second and the third loxP sites to generate a floxed tkβ locus termed fBZ. The targeted ES cells with one fBZ lucos were injected into C57Bl/6 blastocysts. Chimeric male mice were mated to C57Bl/6 females to obtain germ line transmission (F1). The F1 heterozygous fBZ mice were bred with CaMKcre transgenic mice. The F2 offspring were genotyped for both the floxed and the Cre allele (fBZ+/+; CaMKcre+/+) were mated with heterozygous fBZ mice (fBZ+/++; CaMKcre+/+) to obtain tkβ conditional knock-outs and their control animals. Animals heterozygous for both fBZ and CaMKcre were also used to analyze the pattern of Cre expression in tkβ knock-out mice. A 5’-Flank probe for the chloro-3-indolyl-β-D-galactosidase (X-gal) staining and β-galactosidase immunocytochemistry.

Histological method. For X-gal staining, animals were anesthetized and transcardially perfused with 4% paraformaldehyde in PBS, 40 ml of PBS in 20 ml of PBS. The brains were cryoprotected in 30% sucrose, embedded in O.C.T. medium, and stored at −80°C. The frozen brain sections were obtained using a cryotome and collected in PBS and allowed to air dry. Sections of 10 μm were mounted on slides and treated with X-gal staining solution as described (Farinas et al., 1996).

Electrophysiological recording. Transverse hippocampal slices (400 μm) were prepared from fBZ/fBZ mutants, tkβ CA1-KO mutants, and their wild-type littermates (young adult, 2–3 months old). The slices were perfused with ACSF at an interface temperature of 34°C and were exposed to an artificial atmosphere of 95% O₂ and 5% CO₂, as previously described (Pozzo-Miller et al., 1999). Perfusion medium [artificial CSF (ACSF), 34°C] contained (in mM): NaCl, 124; KCl, 3; CaCl₂, 2.5; MgCl₂, 1.5; NaHCO₃, 26; KH₂PO₄, 1.25; glucose, 10; and ascorbic acid, 2, pH 7.4. The perfusion rate was 15 ml/hr. TrkB-IgG (kindly provided by Regeneron Pharmaceuticals, Inc, Tarrytown, NY) and the p75NTR antibody were added directly into the chamber and perfused for 60 min in a closed circle of ~3 ml at final concentrations of 1 and 50 μg/ml, respectively. Field EPSPs were evoked by stimulation of Schaffer collaterals with twisted bipolar nichrome electrodes and recorded with ACSF-filled glass pipettes (~5 MΩ) using Axoclamp-2B amplifiers (Axon Instruments, Foster City, CA). Test stimuli consisted of 25 Hz, 1 sec, 200 μsec pulses. Monosynaptic EPSCs were recorded using the same test stimulus intensity. In each recording, synaptic efficacy (calculated as the ratio of the EPSP EPSP amplitude in the presence of fBZ/fBZ mutants, tkβ CA1-KO mutants, and their wild-type littermates (young adult, 2–3 months old). The slices were perfused with ACSF at an interface temperature of 34°C and were exposed to an artificial atmosphere of 95% O₂ and 5% CO₂, as previously described (Pozzo-Miller et al., 1999). Perfusion medium [artificial CSF (ACSF), 34°C] contained (in mM): NaCl, 124; KCl, 3; CaCl₂, 2.5; MgCl₂, 1.5; NaHCO₃, 26; KH₂PO₄, 1.25; glucose, 10; and ascorbic acid, 2, pH 7.4. The perfusion rate was 15 ml/hr. TrkB-IgG (kindly provided by Regeneron Pharmaceuticals, Inc, Tarrytown, NY) and the p75NTR antibody were added directly into the chamber and perfused for 60 min in a closed circle of ~3 ml at final concentrations of 1 and 50 μg/ml, respectively. Field EPSPs were evoked by stimulation of Schaffer collaterals with twisted bipolar nichrome electrodes and recorded with ACSF-filled glass pipettes (~5 MΩ) using Axoclamp-2B amplifiers (Axon Instruments, Foster City, CA). Test stimuli consisted of 25 Hz, 1 sec, 200 μsec pulses. Monosynaptic EPSCs were recorded using the same test stimulus intensity. In each recording, synaptic efficacy (calculated as the ratio of the EPSP EPSP amplitude in the presence of...
in which the series or input resistances changed by >25% during the duration of the experiment were discarded.

RESULTS
Generation of mice expressing reduced levels of TrkB kinase
To reveal the roles of TrkB signaling on hippocampal structure and function in adult animals, we have used the bacteriophage cre/loxP recombination system to generate viable, cell type-specific 

\[ \text{trkB} \]

mutant mice that can grow into the adulthood (Gu et al., 1994; Tsien et al., 1996). These mice can be used to examine the consequences of deletion of 

\[ \text{trkB} \]

in defined subpopulations of adult hippocampal cells. As the first step in this procedure, we designed a mutant 

\[ \text{trkB} \]

allele in which the first coding exon of the 

\[ \text{trkB} \]

gene (exon S) is replaced with a 

\[ \text{trkB} \]

cDNA unit followed by an SV40 polyadenylation signal. This unit was flanked by two loxP sites (floxed), followed in turn by a 

\[ \text{tau-lacZ} \]

reporter gene (Fig. 1A). This allele, named 

\[ \text{fBZ} \]

, was designed so that the 

\[ \text{trkB} \]

cDNA unit would be transcribed under normal control of the 

\[ \text{trkB} \]

promoter–enhancer complex. Transcription starts at least one exon upstream of exon S, because exon S does not cover all 5' untranslated sequences of 

\[ \text{trkB} \]

mRNAs. Except for a 112 bp sequence immediately downstream of exon S, no sequence in the 

\[ \text{trkB} \]

gene was deleted in the 

\[ \text{fBZ} \]

allele (Fig. 1A, middle). The SV40 poly(A) signal at the 3' end of the 

\[ \text{trkB} \]

cDNA unit was included to terminate transcription before the 

\[ \text{tau-lacZ} \]

sequence. In the rare event that messages escape termination, translation will be stopped by the multiple stop codons in the 1.4 kb 3' untranslated sequence. Thus, the 

\[ \text{tau-lacZ} \]

sequence will not be expressed before the floxed 

\[ \text{trkB} \]

cDNA unit is deleted by Cre-mediated recombination. In contrast, after the floxed 

\[ \text{trkB} \]

cDNA unit is deleted, the

Figure 1. Targeting disruption of the 

\[ \text{trkB} \]

gene. A, Schematic diagrams of the 

\[ \text{trkB} \]

gene, the targeting construct, and the targeted 

\[ \text{trkB} \]

locus. The probes used for screening and the expected Southern blot fragments are indicated. The homology arms are represented as thick lines. B, 

\[ \text{BamHI} \]; Bs, multiple 

\[ \text{BamHI} \] sites; C, 

\[ \text{ChlI} \]; 

\[ \text{HindIII} \]; K, 

\[ \text{KpnI} \]; X, 

\[ \text{XhoI} \]. B, Southern blot analyses of representative tail DNA sample. DNA was digested with 

\[ \text{BamHI} \] and blotted with probe A or probe C. Using probe A, 10.5 and 7.5 kb bands are generated by digestion of the wild-type 

\[ \text{trkB} \] and the targeted 

\[ \text{trkB} \] alleles, respectively. Probe C does not detect any band from the wild-type allele but detects a 1.3 kb band from the floxed 

\[ \text{trkB} \] allele. C, Northern blot analysis of 

\[ \text{trkB} \] mRNAs. Fifteen micrograms of total brain RNA were loaded onto each lane. +/-, Wild-type; 

\[ \text{fBZ/fBZ} \] homoygous for the 

\[ \text{fBZ} \] allele. Note the presence of a single RNA from the floxed 

\[ \text{trkB} \] allele. D, Western blot analysis of TrkB protein. Protein extracts were prepared from the brains of wild-type and 

\[ \text{fBZ/fBZ} \] heterozygous mice. Forty micrograms of protein were loaded onto each lane.
tau-lacZ will be fused into the 5’ end of exon S and will be expressed under the control of the trkB promoter (Fig. 1A, bottom). Therefore, the expression of the tau-lacZ product tau-β-galactosidase makes it possible to identify cells that in control animals would express TrkB but in an fBZ homozygote lose TrkB expression after Cre-mediated recombination. The tau sequence was fused to lacZ in an effort to target β-galactosidase to the axons and apical dendrites in addition to the cell soma (Callahan et al., 1994), facilitating comparisons of the morphologies of neurons in the presence and absence of TrkB.

Using ES cell technology, the fBZ allele was introduced into mice where it can be identified by Southern blot analyses with probe A, which detects a 10.5 kb BamHI fragment from the wild-type allele and a 7.5 kb BamHI fragment from the fBZ allele, as well as with probe C, which only detected the 1.3 kb BamHI fragment of the fBZ allele (Fig. 1B). As expected, only a single trkB mRNA (5.5 kb) was detected in homozygous fBZ mice (fBZ/fBZ) instead of the multiple mRNAs encoded by the wild-type trkB locus (Fig. 1C). Surprisingly, the amount of trkB mRNA in the fBZ/fBZ brain was only 33% of the sum of the two mRNAs (5.5 and 9.0 kb) encoded by the wild-type allele, which has been shown to encode the kinase-containing isoform of TrkB (Klein et al., 1990). Similarly, the level of full-length TrkB protein in fBZ/fBZ mice is only 24.1 ± 4.4% (n = 3) of the kinase-containing isoform in wild-type mice (Fig. 1D). Immunocytochemical analyses using anti-TrkB antibodies indicate that TrkB is expressed at reduced levels but in a normal pattern of expression throughout the brain (data not shown). As predicted, no expression of truncated isoforms of TrkB was observed in fBZ/fBZ mutants. Mice homozygous for the fBZ allele are viable and can live >3 months.

**Generation of mice lacking TrkB in hippocampal CA1 pyramidal neurons**

To create a cell- and region-specific mutation of the trkB gene, we used the promoter for αCaMKII to generate a transgenic mouse line in which the promoter drives expression of the cre transgene (termed CaMKcre) in the forebrain (Burgin et al., 1990; Mayford et al., 1995). Crossing of fBZ/+ and CaMKcre mice led to deletion of the floxed trkB cDNA in cre-expressing cells. The deletion of the trkB cDNA in TrkB-expressing cells results in the expression of tau-β-galactosidase, which can be easily identified by the X-gal staining or anti-β-galactosidase antibodies. To determine which cells were affected, mice heterozygous for both CaMKcre and fBZ (fBZ/+;CaMKcre/) were used to examine in detail the specific regions and cell types in which cre recombination has occurred. The CaMKcre transgenic line used in this study mediates deletion of the fBZ allele in many cells of the neocortex, the hippocampus, the striatum (caudate and putamen), the amygdala, and the substantia nigra (Fig. 2; data not shown).

In the hippocampus, tau-β-galactosidase expression is almost exclusively limited to the CA1 region (Fig. 2C). Very few cells in the CA3 region and dentate gyrus are positive for X-gal staining (Fig. 2C), although the trkB gene is expressed in all regions of the hippocampus (Altar et al., 1994; Yan et al., 1997). In previous work, different αCaMKII-cre transgenes have been shown to differ significantly in their expression patterns (Tsien et al., 1996), so it is not surprising that expression of this transgene does not match perfectly the endogenous expression pattern of αCaMKII. As assessed using tau-β-galactosidase expression, significant recombination does not begin before postnatal day 14 (P14), because at that age no tau-β-galactosidase is seen in the hippocampus (Fig. 2A). At P29 (Fig. 2B), the pattern of tau-β-galactosidase expression is very similar to the pattern observed at P68 (Fig. 2C).

To determine which neurons in the CA1 region lose TrkB as a result of CaMKcre-mediated recombination, antibodies to various cell-specific markers were used together with antibodies to β-galactosidase. In recent work, CaMKII has been shown to be expressed exclusively in excitatory pyramidal neurons within the CA1 region (Sik et al., 1998; Zhang et al., 1999). Co-staining with anti-αCaMKII and anti-β-galactosidase demonstrates that trkB has been deleted in these neurons with high efficiency (Fig. 3A–C). In sections from fBZ/+;CaMKcre/+ mice, which contain only one copy of fBZ, 91 of 95 αCaMKII-positive CA1 pyramidal neurons also expressed tau-β-galactosidase (96%). In similar sections from mice containing two copies of fBZ (fBZ/fBZ;CaMKcre/+), at least one allele of fBZ was deleted in all neurons expressing αCaMKII (76 of 76 αCaMKII-positive neurons expressed tau-β-galactosidase). Assuming that different alleles are targeted independently within these neurons, both copies of the fBZ allele must be deleted in 92% (0.96 × 96%) of these neurons. If targeting of different alleles within the same cell is linked, the efficiency of fBZ deletion would be even higher. These results indicate that the fBZ allele is deleted in essentially all CA1 pyramidal neurons of the fBZ/fBZ;CaMKcre/+;CaMKcre/+ mutant (trkB CA1-KO). Importantly, no examples of cells expressing β-galactosidase in the absence of αCaMKII were seen, so action of this CaMKcre transgene appears to be restricted to pyramidal neurons in the CA1 region. Consistent with results from X-gal staining (Fig. 2), only 7% (6 of 83) of αCaMKII-positive CA3 pyramidal neurons also expressed tau-β-galactosidase, indicating that the majority of CA3 pyramidal neurons continue to express TrkB.

To confirm that these CA1 neurons no longer expressed trkB mRNA, the patterns of expression of trkB mRNA were analyzed by in situ hybridization of sections of control (fBZ/fBZ) and CA1-KO (fBZ/fBZ;CaMKcre/+;CaMKcre) hippocampi. Results, presented in Figure 3, D and E, demonstrate that there is strong expression of trkB mRNA in the CA3 and CA1 regions of the control. In CA1-KO, however, expression of trkB mRNA is almost entirely eliminated in the CA1 region, although it continues to be expressed normally in the CA3 region. These data provide independent evidence that cre derived from this CaMKcre transgene is active in CA1 but not in CA3. The results are also consistent with evidence described above, indicat-
ing that trkB expression is very efficiently eliminated from CA1 pyramidal neurons.

Pyramidal neurons are not the only neurons in the hippocampus that express TrkB. In addition to pyramidal neurons, the CA1 region also contains scattered GABAergic interneurons, a majority of which express calbindin (Shetty and Turner, 1998). GABAergic interneurons have been shown to express TrkB and to be responsive to BDNF (Ip et al., 1993; Tanaka et al., 1997; Vicario-Abejon et al., 1998). Because these neurons do not express CaMKII (Sik et al., 1998; Zhang et al., 1999), they are unlikely to be affected by expression of the CaMKcre transgene. Indeed, examination of trkB mRNA expression in CA1-KO reveals that in the ventral portion of CA1 there are a few TrkB-expressing cells, which are most likely interneurons (Fig. 3E, arrow). To determine the identity of the tau-β-galactosidase-negative neurons in the ventral CA1, brain sections were stained with calbindin antibodies. Immunohistochemistry on sections from wild-type and trkB mutant mice shows that some neurons in the CA1 ventral layer express calbindin (Fig. 4G–I). In the fBZ+/CaMKcre/+ mouse, double immunofluorescence staining for calbindin and β-galactosidase indicates that all calbindin-positive interneurons in the CA1 region are negative for β-galactosidase (data not shown). Because hippocampal interneurons express the TrkB receptor (Altar et al., 1994), these neurons would have expressed the tau-β-galactosidase reporter after cre-mediated recombination. Consequently, these results indicate that the trkB cDNA is not deleted in interneurons in CA1. Taken together, our studies using cell-specific markers argue that, in this transgenic line, the trkB cDNA is only deleted in pyramidal neurons and not in other cells within the CA1 region.

**Effects of reduced expression of TrkB on hippocampal anatomy**

In studies reported elsewhere (Xu et al., 2000), deletion of the fBZ allele in neocortical pyramidal neurons has been shown to have dramatic effects on cortical anatomy, including alterations in dendritic arbors, loss of pyramidal neurons, and reductions in thickness of cortical layers II/III and V. In contrast, results presented in Figures 4 and 5 indicate that reducing expression of TrkB within the entire hippocampus or deletion of trkB within CA1 pyramidal neurons does not affect the gross morphology of the hippocampus or the morphologies of CA1 pyramidal cells and interneurons. As revealed by Nissl staining (Fig. 4A–C), the overall structures of the hippocampi of adult mice were not altered in the fBZ/fBZ hypomorphic mutant or the trkB CA1-KO. When the hippocampi of adult mice were examined using antibodies to the interneuron markers parvalbumin and calbindin (Shetty and Turner, 1998), interneurons appeared to be present in normal numbers and to have normal morphologies in each of these mutant strains (Fig. 4, compare D with E,F; G with H,I). Dendritic morphologies were examined in the CA1 regions of wild-type, fBZ/fBZ hypomorphic mutant, and trkB CA1-KO mice at P75, using antibodies to the dendritic marker MAP2. Again, no obvious morphological differences were seen in either mutant mouse strain (Fig. 5, compare A with B,C). Tau was intentionally fused to the β-galactosidase reporter with the expectation that it would facilitate detection of differences in axonal or dendritic morphology in mutant animals (Callahan and Thomas, 1994). In studies on the neocortex, trkB deletion has been shown to alter pyramidal cell morphology, as assayed with this reporter or with biocytin injections (Xu et al., 2000). In the CA1 region of the hippocampus, though, deletion of trkB has no effect on the dendritic morphologies of targeted pyramidal neurons, as assessed using this reporter (Fig. 5, compare D with E). Thus, the morphology of the hippocampus is not obviously affected by a reduction in TrkB or by specific elimination of TrkB within CA1 pyramidal neurons. Consequently, these two lines of animals with perturbed TrkB expression provided valuable reagents for studying mechanisms of BDNF modulation of synaptic transmission and plasticity.

**Effects of TrkB reduction on CA1 long-term potentiation**

Recent studies have demonstrated that BDNF can modulate hippocampal LTP (Korte et al., 1995; Figurov et al., 1996; Patterson et al., 1996; Kang et al., 1997). It is not clear whether the effects of BDNF on LTP depend on activation of the TrkB receptor tyrosine kinase or instead requires activation of p75NTR. Compared with wild-type littermates, the level of the TrkB receptor tyrosine kinase is only 24% in fBZ/fBZ mice (Fig. 1D). Thus, this line of mice can be used to determine whether the level of TrkB can limit either the magnitude or efficiency of generation of hippocampal CA1 LTP. To examine these possibilities, we used standard extracellular field recording techniques to monitor field EPSPs and applied tetanic stimulation (two 1 sec trains at 100 Hz, 20 sec apart) to Schaffer collaterals to induce LTP in the CA1 region. In the first series of experiments, we examined whether the magnitude of LTP was reduced in fBZ/fBZ mice. Hippocampal slices from wild-type mice exhibited a robust potentiation of synaptic efficacy, lasting to the end of the recordings (n = 5 mice; Fig. 6A). The same tetanic stimulation was able to induce LTP in fBZ/fBZ mice, but the magnitude of LTP was reduced significantly (n = 4 mice; Fig. 6A). Next we performed recordings on a larger number of slices and animals to determine whether the percentage of slices exhibiting LTP in the fBZ/fBZ mice was also reduced when compared with the
wild-type animals (Fig. 6B). Among all the recordings we obtained, 78.8% of slices from +/+ mice exhibited LTP (n = 33 slices, eight mice), whereas 50% of slices derived from fBZ/fBZ mice showed LTP (n = 34 slices, six mice). Moreover, the mean slope of the EPSPs at 45 min after tetanus was 149 ± 2.9% of baseline in wild type but only 131 ± 7.1% in fBZ/fBZ mice (Fig. 6D; p < 0.05, two-tailed t-test). Consistent with previous reports demonstrating that reductions in the ligand BDNF impair LTP (Korte et al., 1995; Patterson et al., 1996; Pozzo-Miller et al., 1999), these results indicate that tetanus-induced hippocampal LTP is significantly impaired by reducing the level of TrkB protein.

Effects of p75NTR inhibition on CA1 long-term potentiation
Besides the TrkB receptor, BDNF can also interact with p75NTR. p75NTR immunoreactivity has not been detected in CA3 and CA1 neurons (Pioro and Cuello, 1990), but low levels of p75NTR may have escaped detection. To examine whether p75NTR signaling contributes to CA1 LTP, we incubated hippocampal slices from wild-type animals with anti-p75NTR IgG (REX IgG). In previous work, REX IgG has been used by several groups to inhibit p75NTR-mediated responses, such as apoptosis, in vivo (Lucidi-Philipi et al., 1996). Treatment of hippocampal slices with this antibody does not significantly impair LTP (Fig. 6C; n = 4 for control and 5 for REX IgG-treated slices). Therefore, BDNF effects on LTP do not appear to be mediated through p75NTR.

Site of TrkB signaling important for modulating CA1 long-term potentiation
Results presented above have demonstrated that BDNF modulation of LTP at CA1 synapses is dependent on TrkB, not p75NTR, but have not identified the cells in which TrkB signaling is required. Considerable debate exists regarding the site at which BDNF acts to modulate synaptic efficacy in the hippocampus. BDNF has been reported to potentiate basal excitatory synaptic transmission via a presynaptic mechanism in cultured hippocampal neurons (Lessmann et al., 1994; Li et al., 1998) and in hippocampal slices (Kang and Schuman, 1995) (but see Figurov et al., 1996; Patterson et al., 1996; Tanaka et al., 1997; Frerking et al., 1998; Gottschalk et al., 1998). Additionally, BDNF increases the ability of the presynaptic terminal to release transmitter repetitively at high frequency (Figurov et al., 1996; Gottschalk et al., 1998). In contrast, a number of studies have also demonstrated that BDNF can act postsynaptically by enhancing NMDA receptor-mediated currents in mixed hippocampal neurons in culture (Levine et al., 1995, 1998; Jarvis et al., 1997). To test the role of postsynaptic TrkB in modulating LTP at
the Schaffer collateral→CA1 synapses, we used hippocampal slices from the 

\[ \text{tkkB CA1-KO} \] mice, in which the TrkB receptor has been deleted only in the postsynaptic CA1 pyramidal neurons and not in the CA3 pyramidal neurons, which are the source of the presynaptic Schaffer collaterals (Figs. 2, 3). As documented in Figure 6, A and D, the magnitude of LTP in slices from CA1-KO mice was markedly reduced compared with that from wild-type mice. However, the CA1 synapses from CA1-KO mice exhibited essentially the same LTP magnitude as those from \[ \text{fBZ/fBZ} \] mice (Fig. 6D, n = 8 mice). Moreover, 52% of the slices from the \[ \text{tkkB CA1-KO} \] mice showed LTP (defined as EPSP slope > 125% of baseline) in response to tetanus (n = 50 slices, eight mice), very similar to the value of 50% obtained using slices from the \[ \text{fBZ/fBZ} \] mice (n = 34 slices, six mice) (Fig. 6B). Thus, although TrkB is required for modulation of LTP by BDNF at Schaffer collateral→CA1 synapses, deletion of TrkB in the postsynaptic cells does not reduce or eliminate the BDNF effect.

The above results imply that BDNF acts on TrkB receptors in presynaptic CA3 afferent neurons or in interneurons to modulate LTP at the CA1 synapses. Alternatively, one might argue that the reduced level of TrkB in the \[ \text{fBZ/fBZ} \] mice makes residual CA1 LTP unresponsive to changes in endogenous BDNF levels, so that deletion of TrkB within the postsynaptic neurons in the \[ \text{tkkB CA1-KO} \] mice would not have a further effect. To determine whether LTP in the \[ \text{tkkB CA1-KO} \] mice remains dependent on TrkB activation by endogenous BDNF, we applied the BDNF and NT-4 scavenger TrkB-IgG to the \[ \text{tkkB CA1-KO} \] hippocampal slices. As shown in Figure 6D, the average magnitude of LTP was further reduced to 120 ± 1.1% of baseline after application of TrkB-IgG to slices from the \[ \text{tkkB CA1-KO} \] (n = 4 mice). Because the TrkB receptor was completely absent from >90% of the postsynaptic cells of CA1 synapses in these mice, TrkB-IgG can only have inhibited the effect of BDNF on presynaptic CA3 afferent neurons or interneurons.

At the presynaptic sites, BDNF could act directly on CA3 afferents to enhance high-frequency excitatory transmission during the tetanus (Gottschalk et al., 1998; Pozzo-Miller et al., 1999). Alternatively, BDNF might act indirectly on interneurons to attenuate inhibitory transmission (Tanaka et al., 1997; Freking et al., 1998). Either or both could contribute to the facilitation of LTP. To distinguish between these possibilities, we analyzed synaptic responses to LTP-inducing HFS (100 Hz, 1 sec; termed “response to HFS”), a parameter that directly reflects the properties of the presynaptic CA3 terminals (Dobrunz and Stevens, 1997). Compared with wild-type animals, the average response to HFS was reduced by ~25% at CA1 synapses in both \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] groups, p < 0.05. E, Synaptic responses to HFS at CA1 synapses in \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] mice. The slope of the 100th EPSP in the train is presented as the percentage of the first EPSP slope. *Significantly different from wild-type, Student’s t test, p < 0.01; #Significantly different from \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] groups, p < 0.05.

The above results imply that BDNF acts on TrkB receptors in presynaptic CA3 afferent neurons or in interneurons to modulate LTP at the CA1 synapses. Alternatively, one might argue that the reduced level of TrkB in the \[ \text{fBZ/fBZ} \] mice makes residual CA1 LTP unresponsive to changes in endogenous BDNF levels, so that deletion of TrkB within the postsynaptic neurons in the \[ \text{tkkB CA1-KO} \] mice would not have a further effect. To determine whether LTP in the \[ \text{tkkB CA1-KO} \] mice remains dependent on TrkB activation by endogenous BDNF, we applied the BDNF and NT-4 scavenger TrkB-IgG to the \[ \text{tkkB CA1-KO} \] hippocampal slices. As shown in Figure 6D, the average magnitude of LTP was further reduced to 120 ± 1.1% of baseline after application of TrkB-IgG to slices from the \[ \text{tkkB CA1-KO} \] (n = 4 mice). Because the TrkB receptor was completely absent from >90% of the postsynaptic cells of CA1 synapses in these mice, TrkB-IgG can only have inhibited the effect of BDNF on presynaptic CA3 afferent neurons or interneurons.

At the presynaptic sites, BDNF could act directly on CA3 afferents to enhance high-frequency excitatory transmission during the tetanus (Gottschalk et al., 1998; Pozzo-Miller et al., 1999). Alternatively, BDNF might act indirectly on interneurons to attenuate inhibitory transmission (Tanaka et al., 1997; Freking et al., 1998). Either or both could contribute to the facilitation of LTP. To distinguish between these possibilities, we analyzed synaptic responses to LTP-inducing HFS (100 Hz, 1 sec; termed “response to HFS”), a parameter that directly reflects the properties of the presynaptic CA3 terminals (Dobrunz and Stevens, 1997). Compared with wild-type animals, the average response to HFS was reduced by ~25% at CA1 synapses in both \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] groups, p < 0.05. E, Synaptic responses to HFS at CA1 synapses in \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] mice. The slope of the 100th EPSP in the train is presented as the percentage of the first EPSP slope. *Significantly different from wild-type, Student’s t test, p < 0.01; #Significantly different from \[ \text{fBZ/fBZ} \] and \[ \text{tkkB CA1-KO} \] groups, p < 0.05.
EPSCs; 50 EPSCs from mice of each genotype. Calibration: 20 pA, 10 msec.

EPSCs were recorded at 0.1 Hz from CA1 pyramidal cells clamped at 0 mV, and low-frequency stimulation was resumed. Wild type, fBZ/fBZ and wild-type mice. Cells were clamped at +30 mV, and afferent fibers were stimulated to evoke dual-component EPSCs; 50 μm t-AP-5 was then added to the perfusion medium, and afferent stimuli were applied at the same intensity. The average NMDA-only EPSC was derived by subtracting the average AMPA-only EPSC from the average dual-component EPSC. Wild type, n = 8; fBZ/fBZ, n = 7. B, Magnitude of the ratio of NMDA current to AMPA current in CA1 pyramidal cells from fBZ/fBZ and wild-type mice. Cells were clamped at +30 mV, and afferent fibers were stimulated to evoke dual-component EPSCs. Inset: Representative examples of NMDA-only and AMPA-only EPSCs from mice of each genotype. Calibration: 20 pA, 10 msec. C, Time course of synaptic potentiation induced by a “pairing” protocol. Evoked EPSCs were recorded at 0.1 Hz from CA1 pyramidal cells clamped at −60 mV in whole-cell mode. At time 0, the cell was depolarized to 0 mV while afferent fibers were stimulated 100 times at 1 Hz, after which the cell was repolarized to −60 mV, and low-frequency stimulation was resumed. Wild type, n = 8; fBZ/fBZ, n = 10.

Figure 7. Postsynaptic contributions to LTP are normal in fBZ/fBZ mice. A, Input–output relations for wild-type and fBZ/fBZ mutant mice. Field EPSPs were recorded from the stratum radiatum of hippocampal slices at a range of stimulus intensities. Fiber volley amplitudes were binned, and corresponding EPSP slopes were averaged between slices. Measurements were obtained in ACSF containing 100 μM t-AP-5. Each point represents the mean ± SEM for each bin. Wild-type, n = 8; fBZ/fBZ, n = 7. B, Magnitude of the ratio of NMDA current to AMPA current in CA1 pyramidal cells from fBZ/fBZ and wild-type mice. Cells were clamped at +30 mV, and afferent fibers were stimulated to evoke dual-component EPSCs; 50 μM t-AP-5 was then added to the perfusion medium, and afferent stimuli were applied at the same intensity. The average NMDA-only EPSC was derived by subtracting the average AMPA-only EPSC from the average dual-component EPSC. Wild type, n = 9; fBZ/fBZ, n = 13. Insets: Representative examples of NMDA-only and AMPA-only EPSCs from mice of each genotype. Calibration: 20 pA, 10 m sec. C, Time course of synaptic potentiation induced by a “pairing” protocol. Evoked EPSCs were recorded at 0.1 Hz from CA1 pyramidal cells clamped at −60 mV in whole-cell mode. At time 0, the cell was depolarized to 0 mV while afferent fibers were stimulated 100 times at 1 Hz, after which the cell was repolarized to −60 mV, and low-frequency stimulation was resumed. Wild type, n = 8; fBZ/fBZ, n = 10.

LTP generation are not affected by the reduced levels of TrkB in the fBZ/fBZ mice. These results, together with the data demonstrating that tetanus-induced LTP is not further reduced in the TrkB CA1-KO compared with fBZ/fBZ (Fig. 6), indicate that reductions in TrkB do not perturb signaling in postsynaptic CA1 pyramidal neurons to limit generation of LTP.

**DISCUSSION**

Our results indicate that BDNF modulates LTP by activating TrkB and not p75NTR. A mouse in which TrkB expression is reduced throughout development has been used to demonstrate that hippocampal cells and anatomy are not affected by reductions in TrkB protein levels. In addition, AMPA and NMDA receptor functions and LTP can be generated normally by a paireddepolarization–low-frequency stimulation protocol. Interestingly, though, synaptic properties of Schaffer collateral terminals in CA1 and tetanus-induced LTP are clearly altered. A second mouse, in which TrkB expression is eliminated in the vast majority of CA1 pyramidal neurons during late postnatal development, has been used to demonstrate that CA1 neurons are quite resistant to deficits in TrkB-mediated signaling. Their morphologies appear normal, and their postsynaptic properties appear to be completely normal. Despite strong evidence that induction and expression of LTP at CA1 synapses are postsynaptic in origin (Bliss and Collingridge, 1993; Isaac et al., 1995; Liao et al., 1995), loss of TrkB within these neurons does not detectably inhibit synaptic plasticity. Thus BDNF signaling through TrkB appears to affect LTP indirectly by controlling the ability of presynaptic terminals to respond to LTP-inducing patterns of stimulation.

The **reporter gene tau-lacZ identifies trkB mutant neurons**

The concept behind the design of our floxed trkB allele may be generally useful. We have attached the tau-lacZ gene to the floxed trkB, resulting in expression of β-galactosidase specifically in cells in which the trkB gene has been deleted. This has allowed us to monitor the fate of trkB null neurons in a chimeric environment. Lower expression of the TrkB receptor from the fBZ allele in comparison with the wild-type allele was unexpected but made it possible to examine the phenotype resulting from TrkB reduction. The reasons for reduced TrkB expression are not clear. It is possible that insertion of a large trkB cDNA into a small exon causes a decrease in splicing efficiency. Mice containing a trkB allele and a trkB null allele had significant losses of vestibular sensory neurons and developed more slowly, undoubtedly because they expressed only 12–13% of the normal amount of TrkB (data not shown). Because mice with two copies of the fBZ allele did thrive and appeared to develop normally, all experiments in this paper used these mice. Although in theory the expression of tau-β-galactosidase does not distinguish between deletion of one or two copies of the floxed trkB allele, both copies appear to be deleted in >90% of the CA1 pyramidal neurons. First, in situ hybridization indicates that only a few scattered cells, the vast majority of which appear to be GABAergic interneurons, express detectable trkB mRNA in CA1. Second, when efficiency of recombination was assayed in a strain with one copy of the floxed trkB allele, the allele was deleted in 96% of the CA1 pyramidal neurons, as identified by expression of β-galactosidase. In a background with two copies of the floxed trkB allele, at least one of these alleles was deleted in 100% of CA1 pyramidal neurons examined. Assuming independence in recombination of alleles within the same cell, the calculated efficiency of double recombination is 92% (0.96 × 96%). With most other assumptions, it would be even higher. Thus, two independent lines of evidence indicate that targeting of CA1 pyramidal cells was almost complete. No reporter gene expression was detected in which other classes of neurons within CA1 were targeted, so recombination appears to be restricted to the CA1 pyramidal neurons.
Survival and dendritic differentiation of CA1 pyramidal neurons do not require TrkB

In the trkB CA1-KO mutant, all neurons that express eCaMKII also express tau-β-galactosidase, whose expression is controlled by the trkB promoter (Fig. 3). Consequently, all CA1 pyramidal neurons must express TrkB. Previous work has shown that BDNF does not promote survival of embryonic rat hippocampal pyramidal neurons in culture (Ip et al., 1993; Marsh and Palfrey, 1996). Previous work has also indicated that there is not a requirement for BDNF or TrkB for survival of neonatal hippocampal pyramidal neurons in vivo (Jones et al., 1994; Minichiello and Klein, 1996; Alcántara et al., 1997). Results in the present paper extend this work by providing evidence that TrkB is not required for survival of CA1 pyramidal neurons in the mature brain. Furthermore, the dendritic structure of CA1 pyramidal neurons as revealed by immunohistochemistry to MAP2 and tau-β-galactosidase is apparently not affected by TrkB removal (Fig. 5). This is in contrast to the neocortex, where many pyramidal neurons require TrkB for survival and maintenance of their dendritic structures (Xu et al., 2000).

BDNF modulates hippocampal LTP through TrkB

BDNF modulates LTP as well as synaptic responses to tetanus at Schaffer collateral→CA1 synapses (for review, see Lu and Chow, 1999). An open issue is whether BDNF interacts with TrkB or p75NTR to achieve its modulatory effects. In the fBZ/fBZ hypothorax, expression of TrkB is dramatically reduced throughout the hippocampus, resulting in a significant reduction in both synaptic responses to tetanus and LTP induced by tetanic stimulation. It is highly unlikely that the LTP reduction in the fBZ/fBZ mutant results from subtle developmental abnormalities, because the fBZ/fBZ mutant shows normal pairing-induced LTP and the trkB fBZ/fBZ mutant is sensitive to the TrkB-IgG fusion protein in induction of LTP. Moreover, the deficiencies in LTP generation observed in BDNF mutant mice are reversed upon application of BDNF (Korte et al., 1996; Patterson et al., 1996). Thus the present study clearly implicates TrkB as a mediator for BDNF modulation of synaptic plasticity in the hippocampus. In contrast, BDNF signaling through p75NTR almost certainly does not mediate synaptic plasticity, because application to slices of p75NTR-blocking antibodies does not impair LTP. These same antibodies have been shown to be effective at inhibiting p75NTR-mediated signaling in vivo (Lucidi-Phillipi et al., 1996), Using an independently generated floxed trkB mouse, Minichiello et al. (1999) have also observed reductions in LTP as a consequence of reducing or deleting trkB throughout the hippocampus.

TrkB modulates LTP signaling in presynaptic CA3 but not postsynaptic CA1 neurons

Substantial evidence supports a role for BDNF in hippocampal LTP (for review, see Lu and Chow, 1999; McAllister et al., 1999), and our results indicate that it acts through the TrkB receptor. An issue under debate is whether the TrkB-mediated signaling relevant to generation of LTP is presynaptic or postsynaptic. Previous studies have suggested that BDNF facilitates LTP by enhancing synaptic release to tetanic stimulation, possibly by promoting docking of synaptic vesicles to the presynaptic membrane at CA1 synapses (Gottschalk et al., 1998; Pozzo-Miller et al., 1999). In contrast, BDNF has been shown to enhance postsynaptic responsiveness in cultured hippocampal neurons by enhancing transmission through postsynaptic NMDA receptor channels (Levine et al., 1995, 1998). BDNF also decreases inhibitory postsynaptic currents on CA1 pyramidal cells (Tanaka et al., 1997; Ferking et al., 1998), so a reduction in inhibitory inputs to CA1 neurons may contribute to LTP generation. Furthermore, a recent paper demonstrated that BDNF, when rapidly puffed onto the CA1 pyramidal neurons, induces direct depolarization (Kaffitz et al., 1999), which suggests that a direct, excitatory effect of BDNF on the postsynaptic CA1 cells may contribute to LTP. Several results in the present paper argue that TrkB deficiency does not affect the properties of postsynaptic CA1 pyramidal neurons necessary for generation of LTP. First, despite the observed reduction in tetanus-induced LTP in the fBZ/fBZ mouse, the AMPAR and NMDAR currents in CA1 pyramidal neurons are not different from those of the same receptors in wild-type controls. Second, LTP is generated normally in fBZ/fBZ hippocampi by pairing postsynaptic cell depolarization with low-frequency stimulation of CA3 input fibers, a protocol that specifically assesses properties of postsynaptic cells. Finally, our results demonstrate that specific deletion of TrkB receptors in postsynaptic pyramidal neurons in the fBZ/fBZ background has no additional inhibitory effect on LTP generation by tetanic stimulation. LTP remains dependent on TrkB signaling in this genetic background, however, because application of the BDNF and NT-4 scavenger TrkB-IgG does have an inhibitory effect on tetanic stimulation-induced LTP. If not the postsynaptic cells, BDNF could in principle be affecting either the Schaffer collaterals or the interneurons. The impairment of synaptic responses to tetanus in both fBZ/fBZ and trkB CA1-KO mice argues for a direct modulation of CA3 afferents by BDNF activation of TrkB, although we cannot rule out an additional role of TrkB in GABAergic interneurons. Taken together, these results strongly suggest that BDNF acts presynaptically to modulate LTP in the CA1 region. Because the major locus for the induction and expression of LTP appears to be the postsynaptic cell in the CA1 region (Bliss and Collingridge, 1993; Isaac et al., 1995; Liao et al., 1995), our results suggest that BDNF signaling is not directly involved in the biochemical changes underlying LTP within the postsynaptic cells but instead modulates the competence of presynaptic neurons to generate the repetitive exocytotic events needed to modify the long-term responses of the postsynaptic neurons. Experiments that delete the trkB gene in CA3 pyramidal neurons and interneurons should confirm these conclusions.

REFERENCES


