Recombinant BDNF Rescues Deficits in Basal Synaptic Transmission and Hippocampal LTP in BDNF Knockout Mice

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Summary

Brain-derived neurotrophic factor (BDNF) is expressed at high levels in hippocampal neurons, and its expression is modulated by neural activity. Knockout mice can be used to study the roles of molecules like BDNF in synaptic plasticity with more molecular specificity than is possible using pharmacological approaches. Because in conventional knockouts the disrupted gene product is absent in all tissues throughout the life of the animal, developmental effects may complicate the interpretation of deficits in the adult. Rescue experiments can help to distinguish between developmental and acute requirements for the missing gene product. We here demonstrate that treatment of hippocampal slices from BDNF knockout mice with recombinant BDNF completely reverses deficits in long-term potentiation and significantly improves deficits in basal synaptic transmission at the Schaffer collateral–CA1 synapse. Thus, BDNF has an acute role in hippocampal synaptic function.

Introduction

Learning and memory are thought to result from activity-dependent changes in the strength of synaptic connections. Long-term potentiation (LTP), an enduring increase in synaptic strength resulting from prior synaptic activity (Bliss and Collingridge, 1993), is the best studied form of synaptic plasticity within the hippocampus, a brain structure believed to play a critical role in explicit memory. Like memory, LTP can last days to weeks, and both appear to have at least two temporal phases: a transient early phase and a late phase that lasts at least 8 hr after tetanization and requires both protein synthesis and gene transcription (Frey et al., 1993; Nguyen et al., 1994).

Portions of the brain that retain a high degree of synaptic plasticity into adulthood, such as the hippocampus, have high levels of many neurotrophic factors (Gall and Lauterborn, 1992). We are interested in determining what role one of these factors, brain-derived neurotrophic factor (BDNF), plays in activity-dependent synaptic plasticity in the hippocampus. BDNF and two other members of the neurotrophin family, nerve growth factor (NGF) and neurotrophin-3 (NT-3), are expressed at comparatively high levels in both hippocampal pyramidal cells and dentate granule cells (reviewed by Gall and Lauterborn, 1992), as are *trk*B and *trk*C (Klein et al., 1990; Lamballe et al., 1991; Valenzuela et al., 1993), the receptor tyrosine kinases through which BDNF and NT-3 signal (reviewed by Chao, 1992).

In addition to their temporal and spatial correlation with plasticity, the neurotrophins have other characteristics that make them good candidates to be modulators of activity-dependent plasticity. There is now abundant evidence that the expression of neurotrophin and neurotrophin receptor genes is modulated by neural activity in the hippocampus (reviewed by Gall and Lauterborn, 1992; Lo, 1995; Thoenen, 1995). Strong artificial stimuli, such as experimentally induced seizure and treatment with depolarizing agents, evoke increases in NGF, BDNF, and *trk*B mRNAs (reviewed by Gall and Lauterborn, 1992; Merlio et al., 1993). Stimuli more closely resembling normal brain activity also modulate neurotrophin expression. Activation of the septohippocampal and corticohippocampal afferent systems innervating the hippocampus evokes increases in NGF and BDNF mRNAs (Lindefors et al., 1992). LTP-evoking stimulation of hippocampal CA1 pyramidal neurons (Patterson et al., 1992) or dentate granule cells (Carstén et al., 1993; Dragunow et al., 1993) is sufficient to increase BDNF mRNA in the stimulated regions.

The neurotrophins, in turn, modulate neural function. At cultured neuromuscular (Lohof et al., 1993; Stoop and Poo, 1995) and hippocampal synapses (Leßmann et al., 1994; Levine et al., 1995a), neurotrophins can rapidly potentiate both spontaneous and evoked synaptic transmission. Likewise, exogenous application of either BDNF or NT-3 has been shown to potentiate synaptic transmission rapidly in the CA1 region of rat hippocampal slices (Kang and Schuman, 1995). In addition, neurotrophins can act over a longer time course to promote neuronal survival and modulate dendritic and axonal sprouting (e.g., Davies, 1994; Ghosh et al., 1994; McAllister et al., 1995). Finally, neurotrophins enhance ion channel function (Kalman et al., 1990; Lesser and Lo, 1995; Levine et al., 1995b) and increase levels of neurotransmitters (Hefti et al., 1989) and Ca^{2+}-binding proteins (Ip et al., 1993). Since these effects of neurotrophins might reasonably be expected to produce both rapid and enduring changes in synaptic efficacy, changes in neurotrophins and their receptors might be important for activity-dependent changes in synaptic strength during processes such as LTP.

The involvement of BDNF in LTP and other forms of synaptic plasticity has been difficult to investigate because of a lack of specific pharmacological and immunological reagents. The recent generation of mice with a targeted disruption of the BDNF gene (Ernfors et al., 1994; Jones et al., 1994; Korte et al., 1995) has made it possible to circumvent some of these problems. However, the advantages of this genetic approach are offset by the possibility that the absence of the targeted gene product during development may result in abnormalities whereby LTP and other forms of synaptic plasticity are reduced or lost. While this work was in progress, Korte and colleagues demonstrated that hippocampal LTP was impaired in mice lacking BDNF (Korte et al., 1995). However, the possibility of developmental abnormalities

precluded definitively assigning BDNF a direct role in LTP. Here, using hippocampal slices from BDNF knockout mice (Ernfors et al., 1994), we have identified and characterized deficits in both basal synaptic transmission and in LTP at the Schaffer collateral–CA1 synapse. Treatment of mutant slices with recombinant BDNF significantly improves the deficit in basal synaptic transmission and completely reverses the deficit in LTP. Thus, these deficits do not simply reflect developmental consequences of the BDNF knockout, but rather reflect an acute requirement for BDNF in ongoing synaptic function in the hippocampus.

Results

BDNF Knockout Mice Have Defects in Synaptic Function

We began looking for functional deficits in the BDNF mutant mice by examining basal synaptic transmission at the Schaffer collateral–CA1 synapse in hippocampal slices (Figure 1). Using extracellular field recording, we generated input–output curves in two ways. First, we plotted the stimulus voltage (Vstim) against the slope of the field excitatory postsynaptic potential (field EPSP) to provide an initial indication of differences in the response to stimuli of a given intensity. Second, we compared the size of the presynaptic fiber volley (PSFV), which is proportional to the number of presynaptic neurons recruited by stimulation, with the slope of the field EPSP to provide a more accurate indication of basal synaptic transmission.

By these means, we found that basal synaptic transmission was reduced in the BDNF mutant mice and that this reduction in basal synaptic transmission reflected gene dosage (Figure 1A). Homozygotes (on average, 37% ± 1% of wild type) were more severely compromised than heterozygotes (65% ± 1% of wild type), which were compromised relative to wild-type littermates. Presynaptic fiber volleys were smaller in the BDNF homozygous mutant mice than in the wild-type mice (Figure 1B), suggesting that the mutants had fewer presynaptic neurons firing action potentials. This may be because there are fewer neurons, although BDNF homozygous mutant mice do not suffer from significant loss of BDNF-responsive central neurons (Ernfors et al., 1994; Jones et al., 1994), or because the intrinsic properties of the presynaptic neurons are altered in such a way as to make them less likely to fire. For presynaptic fiber volleys of a given size, the resulting field EPSP slopes are smaller (Figure 1C), suggesting either that the presynaptic neurons are releasing less transmitter or that the postsynaptic neurons are less capable of responding.

We next examined two forms of synaptic plasticity at the Schaffer collateral–CA1 synapse. Paired-pulse facilitation (PPF) is a presynaptic form of short-term plasticity in which the synaptic response to the second of a pair of closely spaced stimuli is increased due to residual Ca^{2+} in the presynaptic nerve terminal from the first stimulus adding to the influx of Ca^{2+} from the second stimulus (Katz and Miledi, 1968). PPF was reduced in BDNF knockout mice relative to wild-type littermates

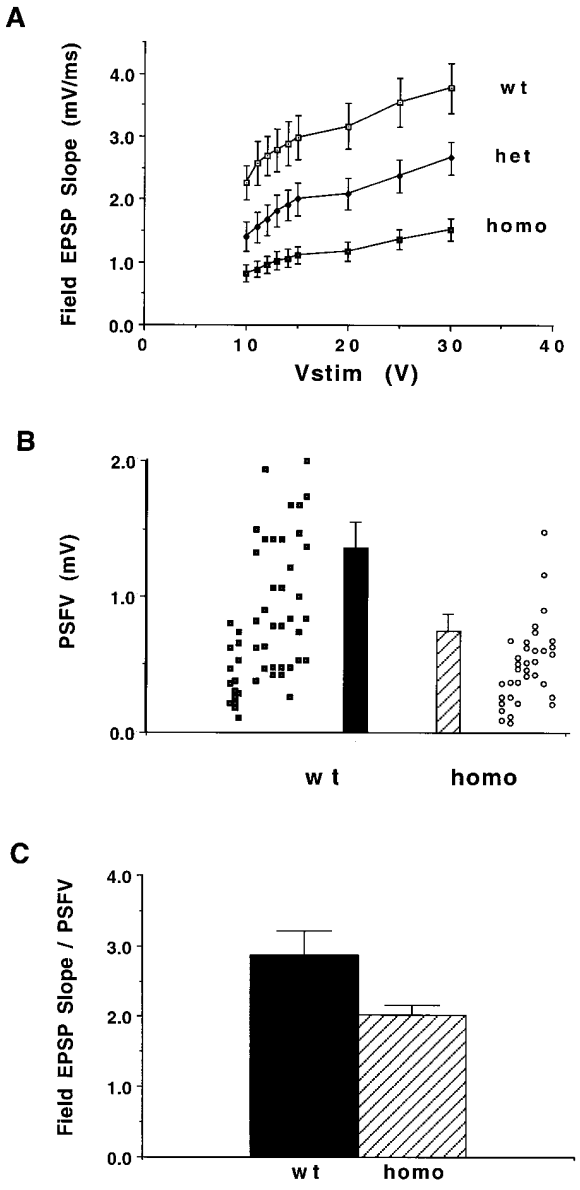

Figure 1. Basal Synaptic Transmission Is Reduced in the Schaffer Collateral–CA1 Pathway of Hippocampal Slices from BDNF Knockout Mice

(A) Stimulus voltages (Vstim) plotted against slopes of field EPSPs for 2- to 3-week-old mice (n = 25 slices, 15 mice for wild types; n = 20 slices, 14 mice for heterozygotes; n = 24 slices, 17 mice for homozygotes; values for each genotype are significantly different, $p < 0.03$).

(B) Scatter plots show PSFV amplitude in hippocampal slices from wild-type and BDNF homozygous mutant mice at various stimulus strengths. Five representative PSFV amplitudes for each slice are shown. Mean maximal PSFV size (bars) is reduced in slices from BDNF knockouts ($p < 0.03$; n = 9 slices, 8 mice for wild type; n = 7 slices, 6 mice for homozygotes).

(C) The ratio of the field EPSP slope to the PSFV amplitude over a range of stimulus strengths. Means shown are for all of the individual ratios from all slices (n = 45 ratios, 9 slices, 8 mice for wild types; n = 35 ratios, 7 slices, 6 mice for homozygotes; $p < 0.04$). Error bars indicate SEM.

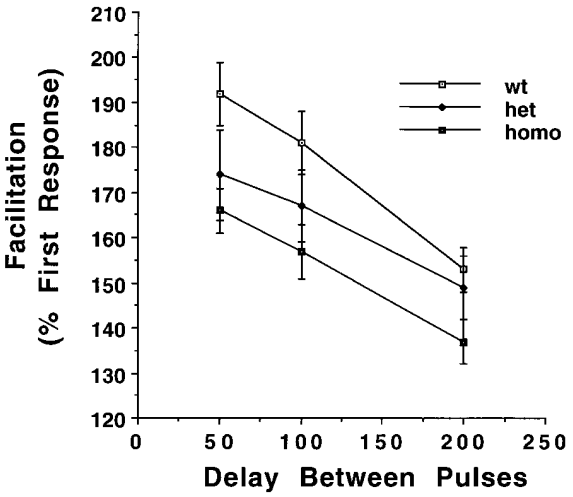

Figure 2. PPF Is Reduced in the Schaffer Collateral–CA1 Pathway of Hippocampal Slices from 2- to 3-Week-Old BDNF Knockout Mice

The plot shows the percentage of facilitation (the second field EPSP slope expressed as a percentage of the first field EPSP slope) obtained with three interstimulus intervals: 200 ms, 100 ms, and 50 ms. The differences between wild-type and homozygote values are significant for 50 and 100 ms intervals between stimuli (n = 17 slices, 7 mice for wild types; n = 13 slices, 8 mice for heterozygotes; n = 11 slices, 7 mice for homozygotes; $p < 0.03$).

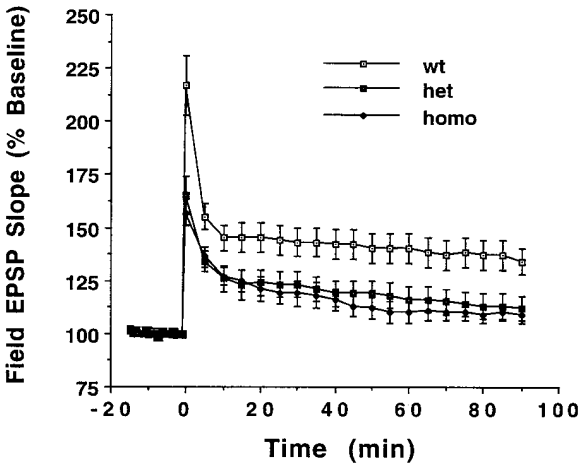

Figure 3. LTP Is Impaired in the Schaffer Collateral–CA1 Synapses of Hippocampal Slices from BDNF Mutant Mice

Field EPSP slopes plotted as percent of pretetanus baseline. Each point represents the mean ± SEM (n = 15 slices, 9 mice for wild type; n = 12 slices, 7 mice for heterozygotes; n = 11 slices, 7 mice for homozygotes).

(Figure 2) (50 ms delay; homozygotes = 166% ± 5%, heterozygotes = 173% ± 10%, wild types = 192% ± 7%, $p < 0.03$). Deficits in PPF may result from reduced mobilization of Ca^{2+} or alterations in the probability of neurotransmitter release. Both homozygotes and heterozygotes were equally deficient in posttetanic potentiation (PTP) and LTP relative to wild-type littermates (Figure 3) (immediately after stimulus trains, homozygotes = 157% ± 6%, heterozygotes = 165% ± 9%, wild types = 217% ± 14%, $p < 0.0005$; and after 1 hr, homozygotes = 110% ± 5%, heterozygotes = 116% ± 6%, wild types = 140% ± 7%, $p < 0.006$).

Neither the experiments we have described above, nor those of Korte et al. (1995) can eliminate the possibility that the observed deficits in synaptic physiology result from the lack of BDNF during development. However, the deficit in LTP is equally severe in heterozygotes and homozygotes, suggesting that BDNF may acutely influence LTP, since the heterozygotes are much less impaired than the homozygotes in basal synaptic transmission and PPF. One way to differentiate between these possibilities and gain a clearer understanding of the deficiencies in the BDNF mutants is to attempt to rescue these defects by treating mutant slices with recombinant BDNF.

Exogenous BDNF Rescues Deficits in Synaptic Function

We initially tried short bath applications of recombinant BDNF (30 min to 2 hr), since 30 min applications of BDNF or NT-3 have been found to enhance synaptic transmission in Schaffer collateral–CA1 synapses in submerged hippocampal slices from adult rats (Kang and Schuman, 1995). However, using an interface chamber, we found that BDNF treatments of this length produced no significant improvements in basal synaptic transmission or LTP in mutant slices (Figure 4A). We also failed to see an enhancement of synaptic transmission in BDNF-treated slices from 2- to 3-week-old wild-type C57BL/6J and 129/SVJ mice using our experimental conditions (data not shown).

To maximize our chances of rescuing the deficits of BDNF mutants in synaptic function, we tried significantly longer incubations with BDNF. When we incubated hippocampal slices from BDNF homozygous mutant mice with exogenous BDNF for 5–8 hr, they showed only slightly improved basal synaptic transmission (Figure 5A). However, after incubation for 12–15 hr (Figure 5A), the mean maximal field EPSP slope of the BDNF-incubated slices was significantly greater than that of the saline-incubated slices (183% ± 24%, $p < 0.05$) and was comparable to that of the naive slices from heterozygous mice ($p > 0.7$). Surprisingly, in contrast with the partial rescue of basal synaptic transmission, LTP was completely rescued by exogenous BDNF (Figure 4). Moreover, the rescue of LTP was more rapid than the rescue of basal synaptic transmission, with some improvement evident after 2–4 hr (Figure 4B), and complete rescue occurring within 5–8 hr (Figure 4C; 1 hr after tetanus, percent of baseline: BDNF-treated = 136% ± 7%, saline-treated = 108% ± 6%; $p < 0.01$; this increase was to levels comparable to those seen in wild-type slices: 140% ± 7%; $p > 0.7$).

The findings that BDNF rescues LTP completely, whereas basal synaptic transmission was only partially rescued, and that the rescue of LTP was much more rapid than the partial rescue of basal synaptic transmission suggest that the mechanisms involved are to some extent dissociable. Since LTP is completely rescued well before basal synaptic transmission shows any significant improvement, it seems unlikely that the deficit in LTP is simply a reflection of impaired basal synaptic transmission, particularly since the mice analyzed by Korte et al. (1995) had a very similar deficit in LTP with

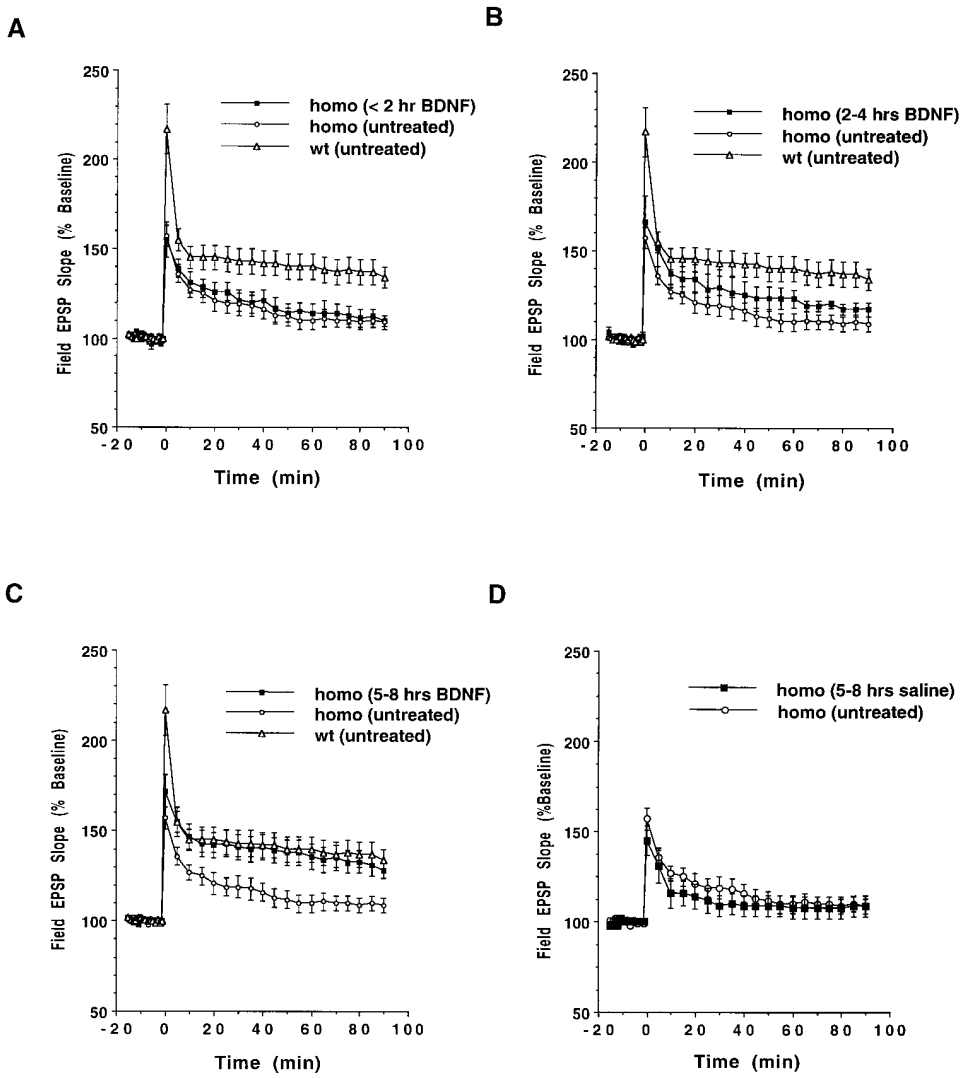

Figure 4. Deficits in Synaptic Plasticity at Schaffer Collateral–CA1 Synapses Can Be Rescued by Acute Exposure to BDNF

Mean percentages of baseline values of field EPSP slopes before and after tetanus in naive slices from wild-type (n = 15 slices, 9 mice) and homozygous BDNF mutant mice (n = 11 slices, 7 mice), and in slices from mice homozygous for the BDNF knockout after incubation of the slices with BDNF for 30–120 min (n = 5 slices, 5 mice) (A); 2–4 hr (n = 4 slices, 4 mice) (B); and 5–8 hr (n = 11 slices, 9 mice) (C).
(D) Comparison of values in slices from mice homozygous for the BDNF knockout after incubation of the slices with physiological saline for 5–8 hr (n = 7 slices, 7 mice) with those in naive slices (n = 11 slices, 7 mice).

no impairment of basal synaptic transmission. Instead, it appears that BDNF may be acutely required for some component of LTP.

The reversal of the deficits in basal synaptic transmission in the BDNF knockout mice could represent a true rescue of BDNF-dependent defects, or an enhancement of transmission via mechanisms that may be mediated by BDNF in the normal adult hippocampus, or both. Treatment with BDNF for 5–8 hr did not enhance basal synaptic transmission (Figure 5B), or LTP in slices from wild-type littermates of the BDNF mutant mice (Figure 6B). Interestingly, LTP levels in slices from heterozygotes treated with BDNF tended to be somewhat higher than those in wild-type slices (Figure 6A; 1 hr after tetanus, percent of baseline: BDNF-treated heterozygote = 161% ± 8%, BDNF-treated wild type = 125% ± 6%, untreated wild type = 140% ± 7%; $p = 0.05$). There are several possible explanations for the enhancement of LTP beyond wild-type levels in treated heterozygote but not wild-type slices. One possibility is that the slices from the heterozygotes may have an abnormal *trk*B receptor profile that renders them abnormally responsive to BDNF. Future studies will be necessary to determine whether this is the case.

Exogenous BDNF Penetrates Only Slowly into Hippocampal Slices

The long incubations (5–8 hr) of mutant slices necessary to rescue the deficits in synaptic function leave open the question of why the rescue takes so long. The delay

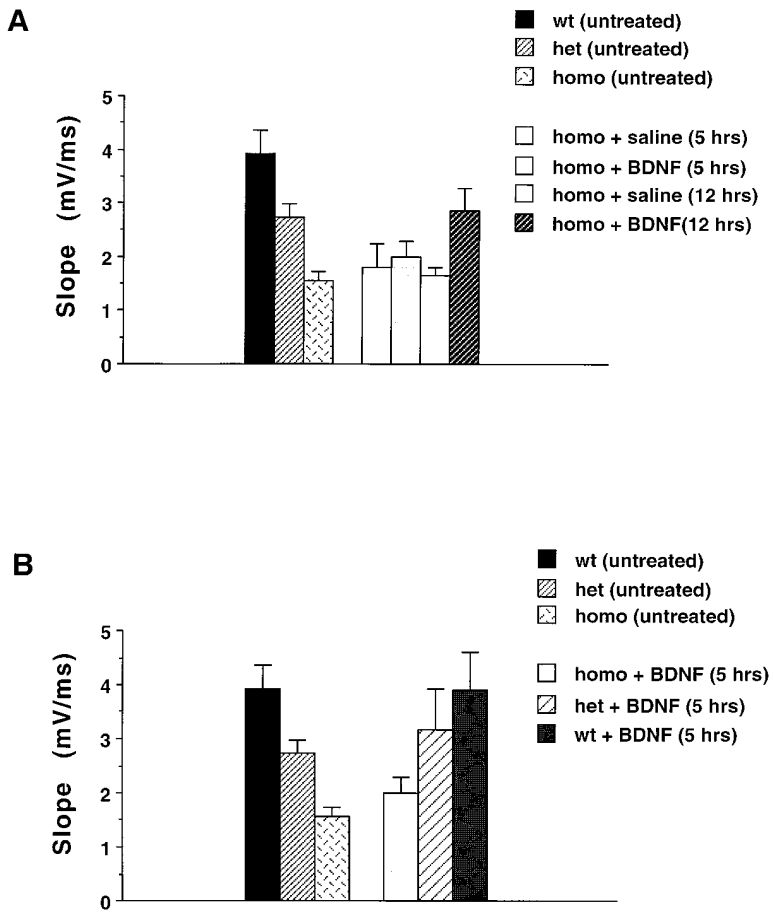

Figure 5. Deficits in Basal Synaptic Transmission at Schaffer Collateral–CA1 Synapses Can Be Partially Rescued by Acute Exposure to BDNF

(A and B) Maximum field EPSP slopes in naive slices from wild-type (n = 25 slices, 15 mice) and BDNF knockout mice (n = 20 slices, 14 mice for heterozygotes; n = 24 slices, 17 mice for homozygotes) (values for each genotype are significantly different; $p < 0.02$).

(A) Maximum field EPSP slopes in slices from homozygous BDNF mutant mice after incubation of the slices with either BDNF (n = 7 slices, 5 mice) or physiological saline (n = 9 slices, 6 mice) for 12–15 hr. Maximal field EPSP slopes in BDNF-treated slices are significantly different from those in saline-treated slices ($p < 0.05$) and are comparable to those in naive slices from heterozygotes ($p > 0.7$).

(B) Maximum field EPSP slopes in slices from homozygous (n = 8 slices, 6 mice) and heterozygous (n = 6 slices, 6 mice) BDNF mutant, and wild-type (n = 11 slices, 9 mice) mice after incubation of the slices with BDNF for 5–8 hr.

could be due to the time required for sufficient BDNF to penetrate into the slice. BDNF, like the other neurotrophins, is an unusually sticky molecule (isoelectric point = 9.99; Leibrock et al., 1989) and travels even less well than the other neurotrophins in the CNS, presumably because of the abundance of *trk*B receptors (Yan et al., 1994; Anderson et al., 1995). Alternatively, the processes that underlie the rescue may actually require a few hours to occur. To gain a clearer understanding of the delayed response to BDNF, and ultimately of the mechanisms underlying the rescue, we undertook an additional series of experiments designed to track the rate of BDNF penetration into slices.

We exposed homozygote slices to exogenous BDNF for various intervals and then stained with antibodies against BDNF and found that the penetration of BDNF into the slice is slow (Figure 7). Faint labeling for BDNF is detectable using confocal microscopy at a depth of 20 μm after 1.5 hr, but the intensity of the labeling continues to increase gradually over several hours. Thus, at least part of the time required for the rescue represents the time required for the penetration of BDNF into the slice, an observation that strengthens the case that BDNF does play a direct role in LTP. The pattern of anti-BDNF labeling appears very similar to that seen with endogenous BDNF (data not shown), suggesting that the exogenous BDNF is acting on the same systems as does endogenous BDNF in wild-type animals.

Discussion

Our data support the view (reviewed by Lo, 1995; Thoenen, 1995) that the neurotrophins play a direct role in activity-dependent synaptic plasticity in the hippocampus. We find that both basal synaptic transmission and several forms of synaptic plasticity, including LTP, are deficient at the Schaffer collateral–CA1 synapse in hippocampal slices from BDNF knockout mice. Importantly, treatment of the slices from BDNF knockout mice with recombinant BDNF significantly improves the deficits in basal synaptic transmission and completely reverses the deficits in LTP. By combining pharmacological and genetic studies, we have demonstrated the importance of BDNF for ongoing synaptic function above and beyond any developmental effect it may have in establishing the number and strength of synapses in the hippocampus. In a larger sense, our work illustrates the value of rescue experiments in distinguishing between a developmental and an acute requirement for an extracellular protein in synaptic function.

Because the rescue of LTP was complete and much more rapid than the partial rescue of basal synaptic transmission, it is unlikely that the deficit in LTP was simply a reflection of impaired basal synaptic transmission. In mice with a similar null allele at the BDNF locus, Korte et al. (1995) reported deficits in LTP similar to those we observed. They did not, however, observe any

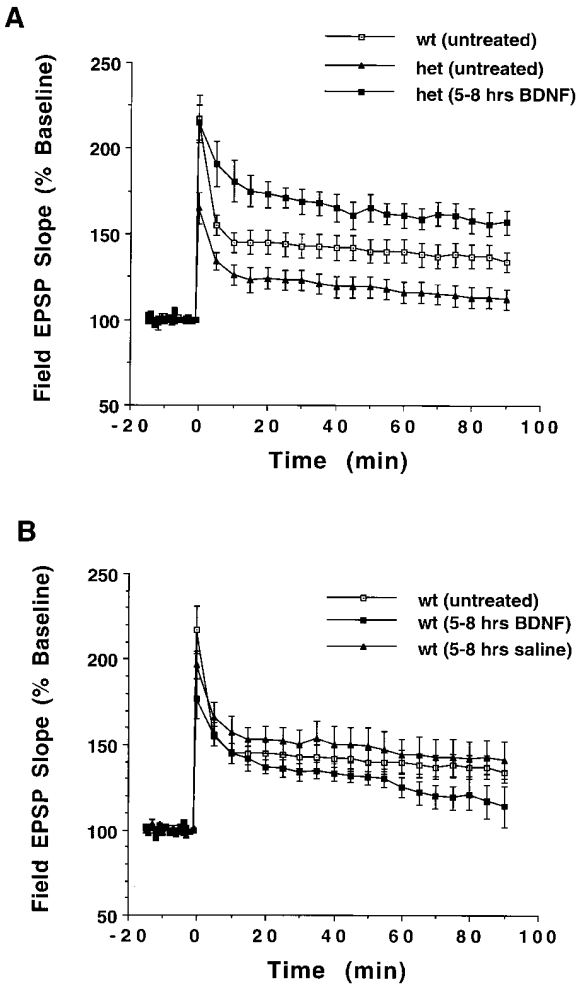

Figure 6. Effects of Exogenous BDNF on LTP in Slices from Heterozygotes and Wild-Type Mice

Mean percentages of baseline values of field EPSP slopes before and after tetanus in naive slices from wild-type (n = 15 slices, 9 mice) and heterozygous BDNF mutant mice (n = 12 slices, 7 mice), and in slices from mice heterozygous for the BDNF knockout incubated with BDNF for 5–8 hr (n = 5 slices, 5 mice) (A) and wild-type littermates of the mutant mice incubated with BDNF (n = 6 slices, 6 mice), or saline (n = 5 slices, 5 mice) for 5–8 hr (B).

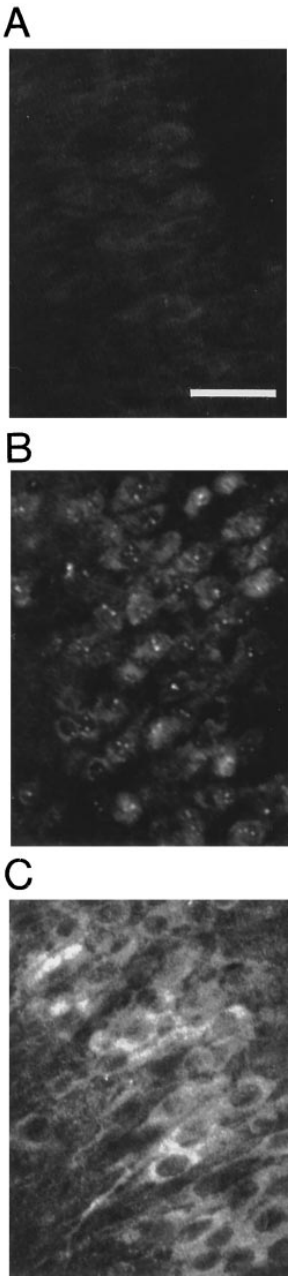

Figure 7. Penetration of Exogenous BDNF into Hippocampal Slices

Confocal micrographs of hippocampal pyramidal cells in slices from a homozygous BDNF mutant mouse labeled with antibodies against BDNF. Images were taken at a depth of 25 μm. (A) Freshly cut, untreated slice; (B) after 1.5 hr of incubation with exogenous BDNF; (C) after 5 hr of incubation with exogenous BDNF. Scale bar represents 50 μm.

deficits in basal synaptic transmission or PPF. Because both knockouts completely remove BDNF function, the observed differences are apparently due to differences in the genetic background of the mice. It is striking that genetic background can so strongly influence fundamental properties of synapses. The identification of strain-specific modifiers of BDNF function may provide important information about the mechanisms by which BDNF influences synaptic transmission. A similar effect of genetic background influencing phenotype is evident in the EGF receptor (Threadgill et al., 1995; Sibilia and Wagner, 1995) and in the PKCγ knockout mice (Abeliovich et al., 1993).

The alterations in synaptic strength that we observed in response to BDNF were slow, reflecting the gradual penetration of BDNF into our slices. Activity-dependent, localized release of endogenous BDNF might be much more effective. Although activity-dependent release of the neurotrophin NGF from hippocampal neurons has recently been reported (Blöchl and Thoenen, 1995), there is as yet very little data on subcellular localization of *trk* receptors, or on the possible localized secretion of neurotrophins. However, McAllister et al. (1995) find that pyramidal cell dendrites respond differently to individual neurotrophins in different layers of the developing

visual cortex. The exogenously applied neurotrophins did not simply enhance dendritic growth, but specifically modulated distinct patterns of dendritic arborization. These results suggest that, at least in the visual system, the morphological refinements associated with alterations in neurotrophin levels may be much more specific and complex than initially realized, suggesting localized patterns of neurotrophin release and response.

Activity-dependent changes in BDNF and *trk*B mRNA levels could contribute to localized, lasting changes in synaptic strength in the hippocampus. The induction of LTP at the Schaffer collateral–CA1 (Patterson et al., 1992) or perforant path–dentate granule cell (Carstén et al., 1993; Dragunow et al., 1993) synapses evokes increases in BDNF mRNA levels that are limited to the stimulated regions. Neural activity also increases the expression of *trk*B receptors in the hippocampus (Merlio et al., 1993). The neurotrophins can, in turn, up-regulate the expression of their receptors (e.g., Verge et al., 1992). In the hippocampus, the *trk*B receptor is extensively coexpressed with BDNF, and at least some neurons express both receptor and neurotrophin. Thus, activated neurons may produce more neurotrophic factor, which in turn up-regulates neurotrophin receptors on nearby neurons. If the neighboring neurons are also active, that activity might also up-regulate neurotrophin receptors, making them doubly efficient at competing for neurotrophin. Neurotrophin receptor levels modulated by more than one regulatory input might act as a molecular coincidence detector, responding synergistically to dual signals that arrive from independent pathways. Competition for limited quantities of neurotrophins may play a role in the refinement of cortical circuitry, since the infusion of BDNF blocks the activity-dependent formation of ocular dominance columns in the developing cat visual cortex (Cabelli et al., 1995).

Activity might also in some other way "prime" the neurons to enhance their responsiveness to neurotrophins. The survival-promoting effects of neurotrophins on cultured cerebellar Purkinje cells, cortical neurons, and purified, postnatal retinal ganglion cells are enhanced when the neurons are active (Mount et al., 1993; Ghosh et al., 1994; Meyer-Franke et al., 1995). The enhanced responsiveness of the retinal ganglion cells to neurotrophic factors was apparently mediated by an activity-evoked elevation of cAMP (Meyer-Franke et al., 1995). The effects of neurotrophins on neuronal structure are also more dramatic in some populations of active neurons. Blocking activity in cultured cortical slices blocks neurotrophin-dependent dendritic branching in these cortical neurons (L. Katz, personal communication). "Priming" PC12 cells with cholera toxin or cAMP increases the speed with which these cells sprout neurites in response to NGF (e.g., Gunning et al., 1981; Heidemann et al., 1985). Further, the synergistically evoked neurites are more stable than the neurites evoked by either treatment alone.

The apparent link between activity, increased cAMP, and increased responsiveness to neurotrophins is particularly intriguing, since it has been suggested that the neurotrophins may play an important role in stabilizing active synapses (Lo, 1995), thereby contributing to the processes believed to underlie learning and memory (Purves, 1988). Several lines of evidence have demonstrated that the cAMP pathway plays a central role in memory storage in Drosophila, Aplysia, and rodents (reviewed by Kandel and Abel, 1995). As increased cAMP levels are implicated in LTP at the Schaffer collateral–CA1 synapse (Frey et al., 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Blitzer et al., 1995; Nguyen et al., 1994), it is possible that an activity-dependent increase in cAMP also primes hippocampal neurons for enhanced responsiveness to neurotrophins. Our demonstration of a rescuable deficit in LTP in the hippocampus of BDNF knockout mice underscores the importance of BDNF in activity-dependent synaptic plasticity and highlights the potential utility of this system for further investigation of the mechanisms of BDNF action.

Experimental Procedures

BDNF Knockout Mice

The BDNF mutant mice were obtained from breeding pairs in our colony, which is derived from heterozygote mutant mice provided by P. Ernfors and R. Jaenisch (Ernfors et al., 1994; see also Jones et al., 1994). The BDNF knockout is maintained on a mixed 129/ter SV and BALB/c genetic background (Ernfors et al., 1994). Homozygous BDNF knockout mice could not be readily distinguished from their littermates at birth, but some died within a few days of birth, and all of them died within 3.5 weeks, possibly, as previously suggested, due to large scale losses of sensory neurons in the petrosal–nodose ganglia (Jones et al., 1994). The homozygous mice that did survive more than a few days were much smaller than their littermates (approximately one-third normal body weight and one-half normal brain weight) and showed deficits in balance and coordination, previously attributed to defective innervation of the vestibular portions of the inner ear (Ernfors et al., 1994). Heterozygous BDNF knockout mice gained weight at the same rate and reached the same size as their normal littermates, and were generally normal in terms of fertility, behavior, and survival.

Wild-type, heterozygote, and homozygote BDNF mutant mice were examined between 2 weeks, when the slices become capable of producing LTP, and 3 weeks, before the death of the homozygotes. The phenotype of the BDNF homozygous mice made the animals easily distinguishable from their littermates, but the BDNF heterozygotes and wild types were studied in a blind protocol. Animals were genotyped after analysis using a PCR-based assay (Ernfors et al., 1994).

Slice Preparation

Hippocampi were collected from mice following cervical dislocation. Transverse hippocampal slices (400 μm) were prepared using conventional techniques (Nguyen et al., 1994; Grant et al., 1992). Slices were maintained in an interface chamber at 28°C and perfused with an oxygenated saline solution (124.0 mM NaCl, 4.4 mM KCl, 26.0 mM $NaHCO_3$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 10 mM glucose). Slices were permitted to recover for at least 90 min before recordings. To record field excitatory postsynaptic potentials in the CA1 region of the hippocampus, both the stimulating and recording electrodes were placed in the stratum radiatum of CA1.

Stimulation Protocol

Stimuli were delivered at intensities that evoked field EPSP slopes equal to 1 mV/ms in all slices. Test stimuli were delivered every 2 min, and test responses were recorded for 15–20 min prior to beginning the experiment to assure stability of the response. Tetanization consisted of two trains of stimuli, each 1 s, at 100 Hz, delivered 20 s apart, a protocol that induces LTP lasting approximately 1.5 hr in wild-type mice of this genetic background. The same stimulus intensity was used for tetanization and evoking test responses. Responses were recorded for 1.5 hr after tetanization at 0.01 Hz.

Statistical tests included factorial ANOVAs with Fisher's Protected

Least Significant Difference and Scheffe's F procedures for post-hoc comparisons. In cases where one P value is given for multiple comparisons in the text, the stated value is the least significant result.

Treatment with Recombinant BDNF

Freshly cut slices from 2- to 3-week-old BDNF knockout mice were incubated in an oxygenated interface chamber at 28°C in either 100 ng/ml BDNF in saline solution, or saline alone for various lengths of time. Fresh recombinant BDNF (gift of Regeneron) or saline solutions were introduced 30 min prior to the start of recording, and recordings were made in these solutions.

For the longest BDNF incubations, freshly cut slices from 2- to 3-week-old homozygous BDNF mutants were treated in one of two ways. For the first method, alternate slices were incubated in an oxygenated interface chamber at 28°C in either 100 ng/ml BDNF in saline solution, or saline alone overnight (12–15 hr) prior to the start of recording. Fresh BDNF or saline solutions were introduced 30 min prior to the start of recording, and recordings were made in these solutions. For the second method, alternate slices were incubated in a secondary oxygenated interface chamber at 28°C in either 100 ng/ml BDNF in saline solution, or saline alone overnight (12–15 hr). They were transferred to fresh solutions in the recording interface chamber at 28°C and allowed 30 min to recover before recording was begun.

Immunocytochemistry and Confocal Microscopy

Slices were fixed in 4% paraformaldehyde, quenched with 50 mM NH_4Cl, and blocked with 10% goat serum. The anti-BDNF antibody was generated in rabbits to a peptide encoding amino acids 168–177 of human BDNF and was provided by Dr. D. Kaplan. The BDNF antibody was specific as assessed by Western blotting of the pure neurotrophin and preabsorption controls (D. Kaplan and W. Friedman, personal communications). Slices were incubated overnight with the primary antisera (1:100 in 10% goat serum in PBS). After extensive washing in PBS, the slices were incubated overnight with the secondary antibody conjugated to Cy3 (1:200 in 10% goat serum in PBS). The slices were washed 6–10 hr in PBS before imaging. Images were taken using a Bio-Rad MRC 1000 confocal microscope with a 15 mV krypton/argon laser attached to a Zeiss Axiovert 100 with a 40×, 0.75 n.a. long working distance objective. Kalman averaged images of 10 scans were analyzed for Cy3 fluorescence at 568 nm with 10% laser light using Bio-Rad's imaging software Comos. The same settings were used for all images.

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