Title
A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor

Permalink
https://escholarship.org/uc/item/8mr0n3d9

Journal
Journal of Neuroscience, 24(22)

ISSN
0270-6474

Authors
Kramar, E A
Lin, B
Lin, C Y
et al.

Publication Date
2004-06-01

Peer reviewed
Brain-derived neurotrophic factor (BDNF) contributes to the induction of long-term potentiation (LTP) by theta-pattern stimulation, but the specific processes underlying this effect are not known. Experiments described here, using BDNF concentrations that have minor effects on baseline responses, show that the neurotrophin both reduces the threshold for LTP induction and elevates the ceiling on maximal potentiation. The enhanced LTP proved to be as stable and resistant to reversal as that recorded under control conditions. BDNF markedly increased the facilitation of burst responses that occurs within a theta train. This suggests that the neurotrophin acts on long-lasting events that (1) are set in motion by the first burst in a train and (2) regulate the amplitude of subsequent bursts. Whole-cell recordings established that BDNF causes a rapid reduction in the size of the long-lasting afterhyperpolarization (AHP) that follows individual theta bursts. Apamin, an antagonist of type 2 small-conductance Ca\(^{2+}\)-activated potassium (SK2) channels, also reduced hippocampal AHPs and closely reproduced the effects of BDNF on theta-burst responses and LTP. The latter results were replicated with a newly introduced, highly selective inhibitor of SK2 channels. Immunoblot analyses indicated that BDNF increases SK2 serine phosphorylation in hippocampal slices. These findings point to the conclusion that BDNF-driven protein kinase cascades serve to depress the SK2 component, and possibly other constituents, of the AHP. It is likely that this mechanism, acting with other factors, promotes the formation and increases the magnitude of LTP.

**Key words:** calcium-dependent potassium channels; afterhyperpolarization; phosphorylation; apamin; Lei-Dab\(^{2}\); hippocampus; neurotrophin

### Introduction

A considerable body of evidence indicates that brain-derived neurotrophic factor (BDNF) facilitates the formation of long-term potentiation (LTP) and that this effect is separate from its more broadly studied actions on gene expression. Brief applications of BDNF, but not other neurotrophins, to hippocampal or cortical slices allow suboptimal bursts of afferent stimulation to induce robust and stable potentiation (Akaneya et al., 1997; Huber et al., 1998; Kovalchuk et al., 2002). Consistent with this, function-blocking antibodies against the TrkB receptor for BDNF disrupt the formation of stable LTP when applied before or immediately after the delivery of theta-burst stimulation (TBS) but not tetanic stimulation (Kang et al., 1997; Chen et al., 1999; Kossel et al., 2001). The results with antagonists strongly suggest that endogenous BDNF contributes to LTP induction by naturalistic patterns of afferent activity. Work with mutant mice confirms the link between BDNF and LTP. The magnitude and frequency of potentiation are significantly reduced by deletions in the BDNF coding sequence (Korte et al., 1995) in a manner that is reversed by BDNF infusions (Patterson et al., 1996) or viral-mediated gene transfer (Korte et al., 1996).

There are conflicting results regarding the mechanisms by which BDNF promotes potentiation. LTP is impaired in mice with a generalized reduction in TrkB expression but not when reduced expression is restricted to postsynaptic neurons (Xu et al., 2000). In addition, LTP is readily generated in TrkB-deficient mice using stimulation paradigms that do not require presynaptic mobilization of the transmitter (Xu et al., 2000). Other work indicates that BDNF influences the frequency-following characteristics of synapses in immature slices (Gottschalk et al., 1998; Pozzo-Miller et al., 1999). Together, these results lead to the conclusion that BDNF, via TrkB, sustains release during repetitive stimulation, thereby generating postsynaptic depolarization of sufficient magnitude to unblock NMDA receptors. However, the argument is complicated by reports that BDNF also acts on postsynaptic targets with links to LTP (Manabe, 2002). Postsynaptic densities are enriched in full-length TrkB receptors (Aoki et al., 2000), and exogenous BDNF increases NMDA receptor phos-
phorylation (Lin et al., 1998; Di Luca et al., 2001) and currents (Song et al., 1998; Crozier et al., 1999; Levine and Kolb, 2000). Although there is disagreement about the magnitude of the effect, BDNF is also reported to decrease IPSCs (Tanaka et al., 1997; Frerking et al., 1998; Cheng and Yeh, 2003), an effect that would be expected to promote LTP induction (Larson et al., 1986). Finally, recent work using applications restricted to small dendritic fields indicates that the neurotrophin increases spine calcium levels via a set of postsynaptic actions (Kovalchuk et al., 2002; Manabe, 2002).

The present study reexamined the effects of BDNF on theta burst-induced LTP, first by asking whether the neurotrophin lowers the threshold and/or raises the ceiling for potentiation. Beyond its significance in arguments about the computational significance of enhancing LTP (Ambros-Ingerson et al., 1990; Carpenter and Milenova, 2002), the generally unexamined issue of threshold versus ceiling is of importance because of differences in the factors that regulate the two variables (Arai and Lynch, 1992; Arai et al., 1994, 2004). Following from these results, additional experiments tested predictions regarding the effects of BDNF on particular physiological and biochemical events associated with LTP induction. This effort led to the identification of a novel and specific mechanism whereby low concentrations of BDNF can potently influence LTP.

Materials and Methods

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with protocols approved by the Institutional Animal Care and Use Committee of the University of California at Irvine. This includes efforts to minimize animal suffering and numbers of rats used in the work described.

Slice preparation and recording techniques. All studies used young adult (30- to 42-d-old) male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). For slice preparation, animals were deeply anesthetized with halothane (Sigma, St. Louis, MO) and then decapitated. The brain was removed quickly and placed in ice-chilled oxygenated dissected medium containing the following (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 2.5 MgSO4, 3.4 CaCl2, 26 NaHCO3, and 10 glucose (maintained at 31°C). Following preheating, artificial CSF (aCSF) composed of the following (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 3.4 CaCl2, 26 NaHCO3, and 10 glucose was introduced into the infusion line. To determine the LTP threshold and ceiling effect in BDNF-treated slices, the number of theta bursts delivered to induce LTP varied from 2, 5, 10, or 20, with each burst consisting of four pulses at 100 Hz and the bursts themselves separated by 200 msec (i.e., TBS). The stimulation intensity was not increased during TBS. In some experiments, attempts to reverse potentiation were initiated 30 min after LTP induction to determine the degree of LTP consolidation. Reversal stimulation involved delivering low-frequency theta pulses at 3 Hz [theta-pulse stimulation (TPS)] for 1 min. Three trains were used with an intertrain interval set at 1 min. Data were collected and digitized by NAC 2.0 Neurodata Acquisition System (Theta Burst, Irvine, CA) and stored on a disk.

For whole-cell recording, hippocampal slices were prepared using a vibrating tissue slicer (VT1000; Leica, Bannockburn, IL) and placed in a holding chamber for at least 2 hr before being transferred to a recording chamber. The slices were submerged in oxygenated aCSF containing the following (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 3.4 CaCl2, 2.5 MgSO4, 26 NaHCO3, and 10 glucose. Except when noted, 50 μM picrotoxin (PTX) was added to block GABA A receptors (n = 6). PTX did not amplify the effects of BDNF on the size or the waveform of the response. Lowercase letters indicate when during the recording period the traces were collected. Calibration: 0.4 mV, 5 msec.

Figure 1. Effects of BDNF on baseline synaptic transmission. A, BDNF at 2.0 nM was applied to the bath for 60 min (horizontal bar) after a stable 20 min baseline. The field EPSP slope for each slice was expressed as a percentage of the mean for the baseline period for that slice; shown is the mean ± SEM for a group of 11 slices. Field responses after 60 min of infusion showed no significant increase above baseline. Comparison of waveforms during baseline and at the 60 min time point indicated that BDNF caused a significant increase in the duration of the field EPSPs. Insets, Lowercase letters on the graph indicate when during the testing period the illustrated traces were collected. Calibration: 0.5 mV, 5 msec. B. Same as in A except that BDNF was infused along with 5 μM PTX, an antagonist of GABA A receptors (n = 6). PTX did not amplify the effects of BDNF on the size or the waveform of the response. Lowercase letters indicate when during the recording period the traces were collected. Calibration: 0.4 mV, 5 msec.
solution and stored at −8°C until further use. On the day of the experiment, spamin was further diluted in aCSF to a final working concentration containing 0.005–0.01% acetic acid. This working concentration did not influence the pH (7.35) of aCSF. The specific SK2 calcium-activated potassium channel antagonist Leiotoxin 1 with dianinobutanoic acid substituting for a met7 (Lei-Dab 7) (Shakkottai et al., 2001) was prepared in aCSF containing 0.1% bovine serum albumin and was given as a generous gift by Dr. George Chandy (University of California, Irvine, CA).

For whole-cell recording, BDNF was applied through a glass micropipette (pipette concentration, 20 nM) placed within stratum radiatum at the same distance from the cell-body layer as the stimulation electrode. Drug application pipettes had a tip diameter of 25 μm and were prepared by gently tapping the tips of regular patch electrodes. The drug solution was ejected once every 2–3 sec, at a pressure of 2–4 psi and with a pulse duration of 56 msec, using a Picospritzer (General Valve, Fairfield, NJ). Phenol Red dye was included in the pipette to monitor the spread of the solution.

Data analysis. All electrophysiological data in the text are presented as means ± SD, and the fEPSP slope was measured at 10–90% fall of the slope. Data in figures representing drug effects on LTP were normalized to the last 10 min of drug infusion. A Student’s t test was used to compare groups unless otherwise noted. The level of significance was assessed at p < 0.05. Data were collected from control slices in parallel with experimental tissue for each treatment tested. At the end of the study, measurements from control slices were pooled and used as the comparison to drug-treated slices. Measurements recorded from individual bursts within a train of 10 burst responses included burst area, duration of the burst response, and amplitude of the afterpositivity of the burst response. Areas of bursts 2–10 were expressed as a percentage change from the initial theta burst. The duration of each burst response was measured by the time (in milliseconds) it took for the repolarization phase of each burst in a train to return to a baseline established during the 10 msec period preceding stimulation. The durations of bursts 2–10 were then normalized to the duration of the first theta burst. The amplitude of the afterpositivity (in millivolts) was calculated by subtracting the last 10 msec of each burst from a 10 msec baseline period preceding stimulation. The sample size for all experiments represents the number of animals used.

Immunoprecipitation and immunoblotting. Coimmunoprecipitation techniques were used to evaluate the effects of BDNF on serine phosphorylation of SK2 channel proteins. Acute hippocampal slices were prepared and treated with 2 ng/ml BDNF or vehicle as described above. After 1 hr, paired groups of slices treated with BDNF or vehicle (n = 10 slices per group per experiment) were harvested and rapidly frozen. At the time of processing, the slices within a given treatment group were pooled, homogenized in radioimmunoprecipitation assay (RIPA) buffer containing 10 mM Tris, pH 7.2, 158 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, Complete Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, IN), and Phosphatase Inhibitor Cocktails 1 and 2 (P2850 and 5726; Sigma). After this, the samples were assayed (Bio-Rad, Hercules, CA), normalized for protein content, and then incubated with rabbit anti-phosphoserine (2.5 μg/ml; AB1603; Chemicon) or rabbit anti-SK2 (2 μg/ml; APC-028; Alomone Labs, Jerusalem, Israel) overnight at 4°C. Immunocomplexes were harvested by the addition of protein A agarose (80 μl/ml; Upstate Biotechnology, Lake Placid, NY) for 2 hr at 4°C and centrifugation within Spin Filters and Collection Tubes (CytoSignal, Irvine, CA). After two washes with RIPA buffer and one with Tris-buffered saline (in mM: 150 NaCl and 10 Tris-Cl, pH 7.4), the immunoprecipitates were eluted in reducing sample buffer, separated by 4–12% SDS PAGE, and processed for immunoblot analysis of SK2 immunoreactivity using the ECL-plus system from Amersham Biosciences (Arlington Heights, IL), as described in detail previously (Kramár et al., 2002). Two different SK2 antisera were used to probe Western blots: rabbit anti-SK2 from Alomone Labs (APC-028) (Desai et al., 2000) and rabbit anti-SK2 provided by Hans-Günther Knaus (University of Innsbruck, Innsbruck, Austria).
Figure 3. Effects of BDNF on theta-burst responses. A, The percentage change in amplitude (relative to the first field EPSP) of the second, third, and fourth field EPSP within a single stimulus train of four pulses at 100 Hz in control and in BDNF-treated slices (mean ± SEM). The measures are shown for bursts 1–4 of a train of 10 theta bursts. The overall shape of the response changes slightly across successive bursts; BDNF did not reliably affect burst topography. B, Traces collected after the delivery of the first and fourth theta bursts in control and BDNF-treated slices. Only the initial portions of the 200-msec-long responses are shown. Calibration: 1 mV, 10 msec. C, Percentage increase in response area across a series of 10 theta bursts in control and BDNF-treated slices. Within-train facilitation was significantly greater for each of the nine responses in the BDNF group (p < 0.01).
The effects of BDNF on LTP threshold are described in Figure 2, C and D. A single pair of theta bursts did not reliably induce stable potentiation in control slices but resulted in a significant increase in fEPSP slope in BDNF-treated cases compared with controls (30 min after TBS, 0.0 ± 6.6% for controls vs +13 ± 4.6% for BDNF; p < 0.01). Results for a suprathreshold but submaximal train of five bursts are summarized in Figure 2D. Five bursts caused a small but reliable degree of LTP 30 min after TBS in controls (+17 ± 6%) and a somewhat larger effect in the BDNF-treated slices (+38 ± 5%; p = 0.003). It is of interest that five theta bursts produced approximately half-maximal potentiation in each case; this similarity would arise if the neurotrophin simply increased the size of each increment of LTP generated by successive bursts. Figure 2E summarizes the results obtained from slices receiving different numbers of bursts in the stimulation train.

Changes in individual theta-burst responses

Potential effects of BDNF on facilitation within a theta burst were assessed by normalizing the amplitudes of the second through the fourth responses to that of the first fEPSP as shown in Figure 3A. In agreement with previous reports (Arai and Lynch, 1992; Bahr et al., 1997), the second of the four fEPSPs in each burst under control conditions was larger than the first, with significant decrements occurring in the third and fourth evoked potentials. The magnitude of these changes varied across experiments, but the pattern was reliable (Fig. 3A). There were no evident differences between the within-burst changes in fEPSPs in control and BDNF-treated slices (Fig. 3A). This similarity extended to the slight changes in burst topography that occur across successive bursts within the train (Fig. 3B, compare burst responses 1, 4).

Figure 3C describes the magnitude (area) of successive burst responses as a fraction of the area of the first burst response. This measure normalizes differences between slices in the area of the first burst, thereby allowing for the detection of any effects that develop during the course of the stimulation train. The pattern of facilitation (increasing and then decreasing) has been described in previous reports (Arai and Lynch, 1992; Bahr et al., 1997) and was not noticeably different for the two groups. However, the growth of the burst responses within a train was increased by 60–100% over control in the BDNF group, with the largest percentage effect occurring in the later bursts (Fig. 3C). The mean increase in burst-response area for bursts 2–10 over burst 1 was 55 ± 16% for control slices and 88 ± 29% for BDNF-treated slices (p < 0.01). A similar pattern, with equivalent BDNF versus control differences, was obtained if bursts 2–10 were normalized to the area of the first fEPSP in the initial burst response rather than to the area of the entire first burst (data not shown). In all, BDNF had much larger effects on the within-train facilitation of theta bursts than it did on the topography of individual bursts.

Although amplitude of the field EPSPs is the dominant variable in the area of theta-burst responses, the afterpositivity could also make a contribution (Arai et al., 1994). As shown in Figure 4A, theta-burst responses in control slices are followed by a positive wave that begins after the fourth fEPSP (afterpositivity) and is near maximal at 200 msec, the time point at which the succeeding burst arrives. The absolute amplitude of the afterpositivity, at 100–200 msec after the burst response, grew larger across successive bursts in parallel with the increase in the area of the burst responses (Figs. 3C, 4B, compare open bars). Examination of this relationship in control slices indicated that the afterpositivity correlated with the area of the burst that preceded it in comparisons across bursts 3–10 (r = 0.66; p < 0.04; mean of eight responses). Given these observations, the positive influence of BDNF on burst area would be expected to cause a parallel increase in the amplitude of the afterpositivity. However, as summarized in Figure 4B, BDNF significantly reduced the absolute amplitude of the afterpositivity (bursts 2–10, 0.025 ± 0.02 mV) relative to controls (bursts 2–10, 0.045 ± 0.05 mV; p = 0.03). This was associated with a significant increase in the durations of bursts 2–10 (mean percentage change in burst duration for
control, 180 ± 5% vs 212 ± 9% for BDNF; $p < 0.03$) (Fig. 4C,D).

**Effects of BDNF on calcium-dependent AHPs**
The above pattern of results suggests that BDNF influences a variable that is essentially missing from the first burst in a series but modulates the magnitude (but not the internal organization) of later bursts. The various AHPs set in motion by high-frequency spiking are reasonable candidates for this variable. Whole-cell recording in the presence of the GABA<sub>A</sub> receptor antagonist PTX was used to test this hypothesis. Theta bursts were followed by a large hyperpolarization that reached its maximum at ~200 msec and then decayed to baseline over the subsequent 2 sec (Fig. 5A, control). Changing the membrane potential to −90 mV eliminated the post-burst afterpotentials (Fig. 5B). In agreement with previous studies (Schwartzkroin and Stafstrom, 1980; Lynch et al., 1983), the AHPs were greatly reduced in recordings collected with electrodes containing the calcium-chelating agent BAPTA (20 mM) (Fig. 5A). There is good evidence that both the BK-type (fast-activating) and SK-type (medium- and slow-activating) calcium-activated potassium channels contribute to AHPs in forebrain neurons (Sailer et al., 2002; Weiger et al., 2002). In the particular case of field CA1 pyramidal cells, SK2-type channels are present in high concentrations and are reported to make a sizeable contribution to local AHPs (Stocker et al., 1999). This was confirmed in the present studies with apamin (50 nm), a toxin that preferentially blocks SK2 channels (Fig. 5C).

To test the effects of BDNF on the AHP, single theta bursts were applied at widely spaced intervals (4 min) to prevent the interactions that lead to LTP. Under control conditions, the amplitudes (percentage change from baseline) of AHPs that followed individual burst responses were stable across 90 min or longer (Fig. 5D, top, C). Locally applied BDNF (20 nm) reduced the amplitude of the AHPs as shown in Figure 5D (bottom). This effect was evident within the first 100 msec of the response and was pronounced at 200 and 400 msec, suggesting that the neurotrophin influences both medium and slow components of the AHP (Stocker et al., 1999; Shah and Haylett, 2000). Reductions in AHPs developed within 15 min of BDNF infusion, as shown for a group of 10 cells in Figure 5F (−15 ± 21% at 16 min; $p = 0.04$), and then leveled off at ~25% below baseline over the next 30 min (repeated-measures ANOVA; $p = 0.001$ for baseline to 55 min after BDNF application). Longer infusions of BDNF appeared to initiate a second decline in the AHP, as seen for a subgroup of six cells that remained stable for over 1 hr (Fig. 5G). The responses began a second decline at ~1 hr after the start of perfusion, ultimately decreasing to ~80% below baseline. It is of interest that the small changes in field responses described previously emerged after ~1 hr of treatment with BDNF.

**Effects of SK2 channel blockers on theta-burst responses**
The above results raise the possibility that reductions in AHPs, in part generated by SK2-mediated potassium currents, contribute to the enhanced theta bursts produced by BDNF. If so, then apamin would also be expected to increase the area of the burst responses that occur during a theta-burst train. The results illustrated in Figure 6, A and B, confirms this prediction. Slices treated with apamin (50 nm) had burst responses that were much larger than those found in control slices, with the change appearing on burst 2 and reaching its greatest magnitude in later bursts. The mean percentage increase in area of bursts 2–10 over burst 1 for
apamin-treated slices was $98 \pm 23\%$. This is almost twice the value obtained without the toxin ($p < 0.001$) and approximately the same as that described above for BDNF (+89%). Slices infused with apamin also displayed a significant decrease in the mean amplitude of the afterpositivity found between bursts 2–10 (apamin, 0.02 $\pm$ 0.02 mV vs control, 0.05 $\pm$ 0.03 mV; $p = 0.008$) and a considerable increase in the duration of individual bursts (mean percentage increase in duration for bursts 2–10, 103 $\pm$ 13.4% for apamin vs 80 $\pm$ 4.5% for controls; $p = 0.003$) (Fig. 6C,D, respectively). The changes in burst responses were also accompanied by an elevation of the LTP ceiling. Apamin infusions of 50 min enhanced baseline fEPSP slope by 20 $\pm$ 11%.

Using the augmented responses as a baseline, a train of 10 theta bursts produced a significant increase in fEPSP slope of $+76 \pm 24\%$ at 30 min after induction compared with $+41 \pm 8\%$ for controls ($p < 0.001$) (Fig. 6E). Previous studies using other stimulation paradigms have also shown that apamin enhances LTP (Behnisch and Reymann, 1998; Stackman et al., 2002).

Although apamin has a clear preference for SK2 channels, there is evidence that it also affects other calcium-activated potassium channels (Ishii et al., 1997; Shah and Haylett, 2000). Accordingly, a newly introduced SK2 antagonist, Lei-Dab$^7$, with a high degree of selectivity for the SK2 channel (Shakkottai et al., 2001) was used to test the conclusion that blocking the SK2 channel increases burst responses and enhances LTP. Lei-Dab$^7$ incubations at 100 nM for 50 min did not reliably affect the slope of fEPSPs (3 $\pm$ 4%), but, as shown in Figure 6, A (far right) and B, Lei-Dab$^7$ significantly increased the area of theta-burst responses ($+89 \pm 24\%$) relative to controls ($+55 \pm 16\%; p = 0.001$). Slices treated with Lei-Dab$^7$ also displayed a significantly smaller afterpositivity and a marked increase in burst duration compared with control slices ($p = 0.001$, $p = 0.01$, respectively) (Fig. 6C,D). These changes in burst-response characteristics for Lei-Dab$^7$ were accompanied by a significant increase in LTP ($+77 \pm 25\%$) at 30 min after TBS ($p = 0.002$ vs controls) (Fig. 6E).

Although both apamin and Lei-Dab$^7$ had pronounced effects on the way in which burst responses grew during a train, they did not detectably affect the first burst in the train. Figure 6F summarizes the within-burst changes for the fEPSPs during burst 1; response 2, but not response 3, was modestly facilitated over response 1, whereas response 4 was depressed. These characteristics were still present in apamin- or Lei-Dab$^7$-treated slices and in magnitude not detectably different from controls (ANOVA; $p > 0.2$).

Finally, the parallel effects of the SK2 inhibitors and BDNF across different burst parameters strongly suggest that the different compounds operate on a common endpoint. If so, then the combined effects of the neurotrophin and inhibitor should be no greater than the effect obtained with either alone. Results of a test of this point using BDNF and apamin are summarized in Figure 7. Increases in burst-response area (Fig. 7A) were virtually identical for the combined treatment relative to that obtained with BDNF alone, as were the increases in burst magnitude (Fig. 7B) and the magnitude of LTP (Fig. 7D). The reduction (from control values) of the amplitude of the afterpositivity (Fig. 7C) tended to be greater with the combined treatment than with BDNF alone, but this effect did not approach statistical significance.
significance. It should be noted that other treatments that increase theta bursts (e.g., positive modulators of AMPA-type glutamate receptors) (Arai et al., 2004) add to the effects of BDNF, resulting in responses substantially larger than those described here. Therefore, the absence of an additive effect between BDNF and apamin in the present experiments does not appear to be attributable to a ceiling on the size of the response measure but instead constitutes evidence that the two treatments act on a final common pathway to promote within-train facilitation of theta bursts.

**BDNF triggers the phosphorylation of SK2 potassium channels**

AHP currents are known to include fast, medium, and slow components that are attributable to different classes of potassium channels; large-conductance, calcium-activated potassium (BK) channels account for fast and medium responses, whereas small-conductance, calcium-activated SK channels account for medium (apamin-sensitive) and slow aspects of the response (Sailer et al., 2002; Weiger et al., 2002). BDNF binding to TrkB initiates a variety of signaling cascades (Patapoutian and Reichardt, 2001) that in some instances include serine kinases that recognize calcium-potassium channels (Holm et al., 1997; Rogalski et al., 2000; Tian et al., 2001; Weiger et al., 2002). Because this provides a route through which BDNF could exert its above-described effects, the effects of BDNF treatment on serine phosphorylation of SK2 protein were evaluated.

Figure 8, A and B, shows immunoblots of homogenates from BDNF-treated and control hippocampal slices that had been immunoprecipitated with anti-phosphoserine and then probed for SK2 immunoreactivity; the blots in A and B were processed with the normal SK2 antiserum compared with anti-SK2 that had been preabsorbed with the antigen peptide provided by the vendor, respectively. As seen in Figure 8A, in control hippocampal homogenate (lane 1), immunoreactive bands are present at >250 kDa, at slightly less than 160 kDa, at ~105 kDa, and at 45–50 kDa overlying the large deposition of heavy chain IgG at the bottom of the gel. The lower bands, at 105 kDa and at 45–50 kDa, correspond with sharp bands of SK2 immunoreactivity within straight Western blots of COS7 cell lysates (Fig. 8A, lanes 3, 4) and hippocampal homogenates (data not shown). When blots were probed with antigen-preabsorbed antibody (Fig. 8B), the density of these bands was markedly reduced in immunoblots of phosphoserine precipitates from hippocampal homogenates and eliminated in blots of COS7 cell lysates. In contrast, preabsorption did not reliably affect the densities of the larger (upper) immunoreactive bands, suggesting that they represent cross-reactivity with non-SK2 proteins.

As shown in Figure 8A (lane 2), BDNF treatment markedly increased the abundance of serine-phosphorylated SK2 immunoreactivity at 105 and 45–50 kDa (i.e., Fig. 8A, bottom dash). The enhanced SK2 immunoreactivity within both bands was eliminated by antisera preabsorption with SK2 antigen (Fig. 8B).

The larger molecular weight (>105 kDa) immunostained bands were not affected by BDNF treatment. Comparable results were obtained in three experiments. In two experiments, with anti-phosphoserine precipitates probed with anti-SK2 from Alomone Labs, the 105 kDa band was 72% and 59% more dense in BDNF-treated samples compared with control samples. In a third experiment using anti-SK2 from Knaus and colleagues (Sailer et al., 2002), the 105 kDa band was 25% more dense with BDNF treatment.

BDNF-induced changes in levels of serine phosphorylated SK2 immunoreactivity were not accompanied by changes in total SK2 immunoreactivity. Immunoprecipitation of hippocampal homogenates with anti-SK2 followed by Western blots probed with anti-SK2 yielded just one immunoreactive doublet at ~105 kDa; this SK2-IR doublet was of comparable density in control and BDNF-treated samples (Fig. 8C).

**Discussion**

The present results confirm previous reports that exogenous BDNF facilitates the induction of LTP. The use of different variants of the theta-burst paradigm established that the neurotrophin lowered the induction threshold and increased the maximum degree of potentiation elicited by trains of bursts. Previous studies have shown that the first of these effects, but not the second, can be produced by increasing the amplitude of synaptic responses, through either increased neurotransmitter release or enhanced postsynaptic responses (Arai and Lynch, 1992). It is unlikely that such effects were responsible for the increased potentiation described here. BDNF had minimal effects on the size of single synaptic responses, and these were qualitatively similar.
The facilitation of theta-burst responses that occurs over the course of a theta train was markedly enhanced by BDNF. The increase in the area and duration of the burst was accompanied by a reduction in the absolute amplitude of the slow positive response that follows the bursts. The greater than normal depolarization with attendant effects on NMDA receptors on the second burst explains how a single pair of theta bursts administered in the presence of BDNF elicits robust LTP (i.e., the lower LTP threshold). Regarding the mechanisms responsible for enhanced burst responses, transmission dynamics in the later responses were not markedly changed because within-burst facilitation of fEPSPs was similar to control in between-group comparisons. An alternative is that BDNF changes burst-response parameters through effects on the long-duration AHPs initiated by individual burst responses. Whole-cell recordings confirmed that BDNF reduces the size of the calcium-dependent AHP that follows theta bursts. This represents a previously unrecognized effect of the neurotrophin and one that logically relates to its effects on theta burst-induced LTP. That is, previous studies have shown that suppressing the AHP both lowers the threshold and raises the ceiling for LTP in CA1 pyramidal cells (Arai and Lynch, 1992; Arai et al., 1994; Sah and Bekkers, 1996; Stackman et al., 2002).

The AHP recorded in CA1 pyramidal cells is strongly blocked by calcium-chelating agents. High concentrations of SK channels are found in hippocampal neurons, and a combination of these channels generates a composite AHP with slow, medium, and fast components. The SK2 channel is among those expressed at high concentrations in CA1 pyramidal cells and makes a significant contribution to the AHP, as indicated by the results obtained with apamin (Stocker et al., 1999), which preferentially blocks SK2 function. This observation allowed an additional test of the idea that reductions in AHPs enhance theta-burst responses. Apamin reproduced each of the effects of BDNF on burst responses and, as reported previously (Behnisch and Reymann, 1998; Stackman et al., 2002), enhanced LTP. Apamin also had significant effects on baseline responses but these were absent in slices treated with the recently introduced and highly selective SK2 antagonist Lei-Dab7. Despite this, the antagonist increased theta-burst responses and enhanced LTP.

The results obtained with apamin and Lei-Dab7 point to the specific hypothesis that BDNF, at least in part, enhances theta-burst responses by reducing SK2 conductance. As predicted from this conclusion, combining BDNF and apamin had no greater effect on theta bursts or LTP than either alone. How the neurotrophin might exert its effects are unclear. Little is known about modulation of SK2 in the adult hippocampus but there is evidence that it (Lu and Wang, 1996; Kong et al., 2000; Lu et al., 2002), along with other potassium channels (Levitan, 1994; Hall and Armstrong, 2000; Rogalski et al., 2000), is regulated by phosphorylation. Given that BDNF initiates signaling cascades that in some instances include kinases that recognize potassium channels (Holm et al., 1997; Hoffman and Johnston, 1998; Rogalski et al., 2000; Gallo et al., 2002; Yuan et al., 2002), phosphorylation constitutes a potential link between the neurotrophin and the reduction in AHPs. In support of this, the same BDNF treatments that reduced the AHP and enhanced theta-burst responses increased serine phosphorylation of SK2 channels. MAPK (mitogen-activated protein kinase) has been shown recently to phosphorylate the Kv4 domain of voltage-sensitive potassium channels in apical dendrites of field CA1 via activation of protein kinases A and C (Yuan et al., 2002). The latter kinases recognize SK2 (Lu and Wang, 1996; Lu et al., 2002) and provide plausible effectors for the observed biochemical and physiological effects of BDNF.

The link between BDNF and theta bursts may be reciprocal in that the latter trigger the rapid release of the neurotrophin from...
presynaptic terminals (Balkowiec and Katz, 2002). This effect is
most probably too slow to affect the 10 burst (2 sec) trains used to
induce LTP but, from the present results, would amplify trains of
two or more bursts occurring minutes afterward. Assuming that
the highest BDNF concentrations occur near release sites, then
theta bursts would have the effect of priming (through AHP re-
duction) their target neurons for the induction of LTP by subse-
quient bursts. This could relate to the common observation that
memory in many paradigms is best encoded with significant de-
lays between acquisition sessions. In any event, it will be of inter-
est to test whether the time course for the effects of BDNF on
theta bursts predicts an optimal spacing of weak theta trains in
the production of stable LTP.

References
Akayeva Y, Tsumoto T, Kinoshita S, Hatanaka H (1997) Brain-derived neu-
rotrophic factor enhances long-term potentiation in rat visual cortex.
J Neurosci 17:6707–6716.
tion of brain-derived neurotrophic factor and TrkB receptors to postsyn-
Arai A, Lynch G (1992) Factors regulating the magnitude of long-term po-
Arai A, Black J, Lynch G (1994) Origins of the variations in long-term po-
tentiation between synapses in the basal versus apical dendrites of hip-
differentially influences synaptic plasticity in the hippocampus. Neuro-
science 123:1011–1024.
Arg-Gly-Asp-Ser-selective adhesion and the stabilization of long-term
potentiation in the CA1 region of rat hippocampus in vitro. Neurosci Lett
235:91–94.
Barker D, Carpenter G, Milenova B (2002) Redistribution of synaptic efficacy sup-
ports stable pattern learning in neural networks. Neural Comput
14:873–888.
Baker CR, Kolbeck R, Barde YA, Bonhoeffer T, Kossel A (1999) Relative con-
tribution of endogenous neurotrophins in hippocampal long-term po-
Cheng Q, Yeh HH (2003) Brain-derived neurotrophic factor attenuates
mouse cerebellar granule cell GABA(A) receptor-mediated responses via
NMDA receptors prevents BDNF enhancement of glutamatergic trans-
mission in hippocampal neurons. Learn Mem 6:257–266.
Di Luca M, Gardoni F, Finardi A, Pagliardini S, Cattabeni F, Battaglia G,
Missale C (2001) NMDA receptor subunits are phosphorylated by activa-
tion of neurotrophin receptors in PSD of rat spinal cord. NeuroReport
12:1301–1305.
lization of synaptic transmission and plasticity by brain-derived neurotro-
phic factor in the developing hippocampus. J Neurosci 18:6830–6839.
Hall SK, Armstrong DL (2000) Conditional and unconditional inhibition of
calcium-activated potassium channels by reversible protein phosphory-
Holm NR, Christophersen P, Olesen SP, Gammeltoft S (1997) Activation of
calcium-dependent potassium channels in rat brain neurons by neurotro-
Huber KM, Sawtell NB, Bear MF (1998) Brain-derived neurotrophic factor
alters the synaptic modification threshold in visual cortex. Neuropharma-
cology 37:571–579.
Ishii TM, Maylie J, Adelman JP (1997) Determinants of apamin and t-
tubocurarine block in SK potassium channels. J Biol Chem
272:23195–23200.
Kang HK, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time:
different roles for TrkB signaling in hippocampal long-term potentia-
-activated K+ channels are regulated by Ca2+-calmodulin-dependent
protein kinase II in murine colonic myocytes. J Physiol (Lond) 524:331–337.
cocampal long-term potentiation is impaired in mice lacking brain-
Korte M, Griesbeck O, Gravel C, Carroll P, Staiger V, Thoenen H, Bonhoeffer T
(1996) Virus-mediated gene transfer into hippocampal CA1 region restores
long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc Natl Acad Sci USA
93:12547–12552.
reveals an immediate/instructive effect of BDNF during hippocampal
contribute to the consolidation of long term potentiation. Neuroscience
-activated K+ channels are regulated by Ca2+-calmodulin-dependent
protein kinase II in murine colonic myocytes. J Physiol (Lond)
524:331–337.
cocampal long-term potentiation is impaired in mice lacking brain-
Korte M, Griesbeck O, Gravel C, Carroll P, Staiger V, Thoenen H, Bonhoeffer T
(1996) Virus-mediated gene transfer into hippocampal CA1 region restores
long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc Natl Acad Sci USA
93:12547–12552.
reveals an immediate/instructive effect of BDNF during hippocampal
contribute to the consolidation of long term potentiation. Neuroscience
contribute to the consolidation of long term potentiation. Neuroscience
contribute to the consolidation of long term potentiation. Neuroscience
contribute to the consolidation of long term potentiation. Neuroscience
Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16:1137–1145.


Song DK, Choe B, Bae JH, Park WK, Han IS, Ho WK, Earm YE (1998) Brain-derived neurotrophic factor rapidly potentiates synaptic transmis-

sion through NMDA, but suppresses it through non-NMDA receptors in rat hippocampal neuron. Brain Res 799:176–179.


