Activity- and Ca\(^{2+}\)-dependent Modulation of Surface Expression of Brain-derived Neurotrophic Factor Receptors in Hippocampal Neurons

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Abstract. Brain-derived neurotrophic factor (BDNF) has been shown to regulate neuronal survival and synaptic plasticity in the central nervous system (CNS) in an activity-dependent manner, but the underlying mechanisms remain unclear. Here we report that the number of BDNF receptor TrkB on the surface of hippocampal neurons can be enhanced by high frequency neuronal activity and synaptic transmission, and this effect is mediated by Ca\(^{2+}\) influx. Using membrane protein biotinylation as well as receptor binding assays, we show that field electric stimulation increased the number of TrkB on the surface of cultured hippocampal neurons. Immunofluorescence staining suggests that the electric stimulation facilitated the movement of TrkB from intracellular pool to the cell surface, particularly on neuronal processes. The number of surface TrkB was regulated only by high frequency tetanic stimulation, but not by low frequency stimulation. The activity-dependent modulation appears to require Ca\(^{2+}\) influx, since treatment of the neurons with blockers of voltage-gated Ca\(^{2+}\) channels or NMDA receptors, or removal of extracellular Ca\(^{2+}\), severely attenuated the effect of electric stimulation. Moreover, inhibition of Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII) significantly reduced the effectiveness of the tetanic stimulation. These findings may help us to understand the role of neuronal activity in neurotrophin function and the mechanism for receptor tyrosine kinase signaling.

Key words: TrkB receptors • tetanic stimulation • calcium influx • Ca\(^{2+}\)/calmodulin-dependent kinase II • synaptic transmission

Introduction

Brain-derived neurotrophic factor (BDNF),1 a member of the neurotrophin family, is a potent neurotrophic protein that regulates neuronal survival and differentiation (Lewin and Barde, 1996). Signal transduction and neurotrophic functions of BDNF are mediated primarily by TrkB, a high affinity receptor tyrosine kinase (Kaplan and Stephens, 1994). BDNF is also capable of binding to the low affinity receptor p75 (p75NR) and eliciting apoptotic function in certain populations of neurons (Casaccia-Bonnefil et al., 1996, 1998; Frade et al., 1996). Evidence accumulated in the last few years suggests that BDNF is involved in synapse development and plasticity, in addition to its traditional role in neuronal survival and differentiation (Thoenen, 1995; Bonhoeffer, 1996; Berninger and Poo, 1996; Lu and Chow, 1999; McAllister et al., 1999). BDNF has been shown to exert complex modulation of dendritic and axonal growth in the brain, particularly in the visual system (Cohen-Cory and Fraser, 1996; Cohen-Cory and Lom, 1999; McAllister et al., 1995, 1996, 1997). BDNF is also involved in activity-dependent synaptic competition and formation of ocular dominance columns in the visual cortex (Maffei et al., 1992; Gu et al., 1994; Cabelli et al., 1995; Riddle et al., 1995; Galuske et al., 1996; Huang et al., 1999). BDNF is capable of rapidly regulating synaptic transmission at the neuromuscular junction and central nervous system (CNS) synapses (Lohof et al., 1993; Knipp et al., 1994; Korte et al., 1995, 1996; Takei et al., 1997). In the hippocampus, BDNF promotes tetanus-induced long-term potentiation (LTP; Korte et al., 1995, 1996; Figurov et al., 1996; Patterson et al., 1996; Kang et al., 1997). Moreover, BDNF selectively enhances high frequency but not low frequency synaptic transmission (Tanaka et al., 1997; Frerking et al., 1998; Gottschalk et al., 1998). Recent experiments using BDNF knockout

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1Abbreviations used in this paper: AIP, autocamtide-2-related inhibitory peptide; BDNF, brain-derived neurotrophic factor; CaMKII, Ca\(^{2+}\)/calmodulin-dependent kinase II; CNS, central nervous system; CNQX, 6-cyano-7-nitroquinozaline-2,3-dione; kyn, kynurenic acid; LTD, long-term depression; LTP, long-term potentiation; p75NR, low affinity receptor p75; TBS, theta burst stimulation; TTX, tetrodotoxin.
mice demonstrate that BDNF enhances high frequency synaptic transmission by facilitating synaptic vesicle docking in the hippocampus, possibly by increasing the levels of the vesicle protein synaptobrevin in the presynaptic terminals of CA1 synapses (Pozzo-Miller et al., 1999).

Based on the above discoveries, BDNF has been proposed to participate in several forms of activity-dependent plasticity in the CNS (Thoenen, 1995; Bonhoeffer, 1996; Lu and Chow, 1999). A critical element of such proposition is that BDNF acts preferentially on active neurons. Indeed, blockade of neuronal activity and synaptic transmission prevents the increase of dendritic arborization induced by BDNF (McAllister et al., 1996). BDNF cannot enhance the survival of retinal ganglion neurons unless they are depolarized by high K\(^+\) or glutamate agonists, or their intracellular cAMP is increased (Meyer-Franke et al., 1995). Presynaptic depolarization greatly facilitates the BDNF modulation of synaptic transmission at the neuromuscular junction (Boulanger and Poo, 1999). In the hippocampus, the effect of BDNF on CA1 synapses is observed only when presynaptic neurons are stimulated at high frequency (Gottschalk et al., 1998). These results support the notion that certain levels of neuronal activity are required for neuronal responsiveness to BDNF.

As a diffusible molecule, how does BDNF distinguish active and inactive neurons or synapses, and restrict its action preferentially on active neurons/synapses? One possible mechanism is that cellular responsiveness of neurons to BDNF is enhanced by neuronal activity. Thus, whether or how well a neuron can respond to BDNF may depend on its activity levels. It is unclear, however, how activity-dependent regulation of BDNF responsiveness is achieved. Neuronal activity could increase the number of BDNF receptors on the cell surface, facilitate the internalization of BDNF–receptor complex, or facilitate the signaling mechanisms for BDNF. Depolarization or cAMP elevation has been shown to increase the levels of the BDNF receptor TrkB on the cell surface of retinal ganglion cells and spinal neurons (Meyer-Franke et al., 1998). Here we investigate whether physiologically relevant stimuli such as electric stimulation can modulate the BDNF receptors on the cell surface of neurons in the hippocampus, where activity-dependent plasticity is most commonly observed. Using three independent approaches (biotinylation, receptor binding, and immunocytochemistry), we show that high frequency tetanic stimulation, but not low frequency stimulation or simple depolarization, can rapidly enhance the insertion of TrkB into the cell surface. We also demonstrate that the activity-dependent modulation requires Ca\(^{2+}\) influx through NMDA and Ca\(^{2+}\) channels, and involves Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII). Only near these findings provide insights into the mechanistic link between activity-dependent and neurotransrophic modulation of CNS neurons and synapses, but they may also have general implications in the cell biology of growth factor signaling.

**Materials and Methods**

**Culture Preparations**

Cultures of hippocampal neurons were prepared according to the published procedure (Feng et al., 1999) with minor modifications. In brief, hippocampus was dissected from embryonic day 18 rats, dissociated in Ca\(^{2+}\)- and Mg\(^{2+}\)-free HBSS containing 0.125% trypsin for 15 min, triturated in DMEM/10% FBS, and plated at 2 × 10\(^5\) cells per well in 12-well plates. Cells were grown at 37°C, 5% CO\(_2\) and 95% humidity, first in 10% FBS/DMEM, and 1 d later switched to serum-free medium Neurobasal plus B27 (Life Technologies). Cultures were grown in serum-free medium for 11–14 d before being used for experiments, and the medium was changed every 3 d. Fresh medium was applied 24 h before each experiment. These cultures yielded virtually pure neurons (data not shown). Drugs were applied immediately before electric stimulation. In some cases, serum-free medium was replaced with Ca\(^{2+}\)-free medium (Ca\(^{2+}\)-free DMEM; Life Technologies) for 30 min before electric stimulation.

**Electric Stimulation of Neuronal Cultures**

Hippocampal neurons were stimulated using a method similar to one that has been described previously (Bito et al., 1996; Deisseroth et al., 1996; Fields et al., 1997). Field electric stimulation was applied across a 12-well dish through a homemade lid, which contained platinum wires contacting the medium in each well. Each stimulation pulse (1 msec, 2–8 V) was sufficient to elicit action potentials in these cultured neurons (see Fig. 1 A). The entire electric stimulation was performed in a 37°C, 5% CO\(_2\) incubator. The following stimulation paradigms were used. (i) TrkB: each episode consisted of four bursts, each with five biphasic pulses at 100 Hz (10-msec interval), separated by an interburst interval of 200 msec. One episode was given every 5 s throughout the whole incubation period. (ii) Tetanic stimulation: 1 s, 100 Hz, given every 10 min for 30 min. (iii) Low frequency stimulation: 0.16 Hz during the entire incubation period. (iv) Long-term depression (LTD)-inducing stimulation: 4 min, 5 Hz. Whole-cell recording was performed under the current-clamped conditions as previously described (Kim et al., 1994). Data were collected by an Axopatch 200B amplifier, filtered at 5 kHz, digitized at 10 kHz, and analyzed by P-clamp software (Axon Instruments).

**Surface Biotinylation and Western Blot Analysis of TrkB**

Surface TrkB receptors were measured by biotinylation followed by Western blot using either a TrkB antibody or a p75NR antibody, as described elsewhere (Meyer-Franke et al., 1998). In brief, various blockers were added to the hippocampal cultures, and electric stimulation was applied immediately in a 37°C incubator. At the end of electric stimulation (60 min), ice-cold PBS, pH 7.4, with Ca\(^{2+}\) and Mg\(^{2+}\), pH 7.4; Life Technologies) was added to the cultures to prevent receptor internalization. After three washes with ice-cold PBS, cells were incubated in Sallo-NHS-LC-biotin (0.25 mg/ml in cold PBS; Pierce) for 30 min. The surface biotinylation was stopped by removing the above solution and incubating the cells in 10 mM ice-cold glycine in PBS for 20 min. Cells were then washed three times with cold PBS and lysed by RIPA buffer, which contains 20 mM Hepes, pH 7.4, 100 mM NaCl, 1 mM EGTA, 1 mM Na\(_2\)VO\(_4\), 50 mM NaF, 1% NP-40, 1% deoxycholate, 0.1% SDS, 1 mM [4-(2-aminophenyl)-benzenesulfonyl fluoride hydrochloride], 10 μg/ml leupeptin, and 1 μg/ml aprotinin. Biotinylated proteins (160 μg) were precipitated with 100 μl of ImmunoPure Immobilized Streptavidin (Pierce). Western blots were performed by separating the biotinylated protein precipitates on SDS-PAGE gel and transferring the proteins to Immobilon P membrane. The membranes were probed with a monoclonal anti-TrkB antibody (1:250; Transduction Laboratories), or an anti-p75NR antibody (1:250; Upstate Biotechnology), followed by peroxidase-conjugated goat anti–rabbit IgG (1:10,000; Vector Laboratories). Immunoreactive bands were visualized by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech). The ECL signal intensities were quantified by NIH Image program. To measure the total amount of TrkB, cultured hippocampal neurons were simply harvested by RIPA buffer and processed for Western blot. Quantification for each experimental condition was based on three to six independent experiments (samples), each was repeated at least two to three times. The results were pooled and averaged, and presented as mean ± SE.

**BDNF Receptor Binding**

Binding assays were performed in hippocampal cultures in a 37°C, 5% CO\(_2\) incubator in quadruplicates. In brief, cells were washed three times with warm DMEM, and then incubated in binding buffer (DMEM plus 0.5
mg/ml protamine sulfate and 10 mM Hepes, pH 7.4) containing 135-
BDNF (2,200 Ci/mmol, 5 × 1011 M; NEN Life Science Products) with or
without excess cold BDNF (5 × 10−8 M; provided by Regeneron Pharma-
ceuticals, Inc.) for 30 min. During the entire period of incubation, the hip-
 pocampal neurons were electrically stimulated in the incubator in the
 presence or absence of various blockers. After incubation, the 12-well
dishes were placed on ice to prevent receptor internalization. Nonspecif-
ically bound BDNF was removed by washing three times with 1 ml of ice-
cold PBS. The 125I-BDNF bound to cell surface was obtained by a 10-min
acid wash on ice (0.2 M acetic acid, pH 2.2, 0.5 M NaCl, 0.5 ml), and the
counts were used as the measure for BDNF surface binding. An LKB γ
counter was used to count the radioactivity. Raw data (quadruplicates)
from a specific experimental condition were normalized to the mean in
control condition. The results in several experiments were pooled and av-
averaged, and presented as mean ± SE.

Immunofluorescence Staining of TrkB Receptors

To visualize surface TrkB, cultured hippocampal neurons were fixed with
2% paraformaldehyde, 120 mM sucrose in PBS at room temperature for 3 min.
After paraformaldehyde was quenched with 0.1 M glycine in PBS, the nonspecific binding was blocked with 50% goat serum, 1% BSA, and
100 mM lysine in PBS for 40 min. The cells were then incubated with a
chicken antibody against extracellular domain of TrkB (a gift from Dr.
Louis Reichardt, University of California, San Francisco, CA) in blocking
solution overnight at 4°C, or in room temperature for 40 min. The second-
ary antibody was Cy3-conjugated goat anti-chicken Y antibody (1:100;
Jackson ImmunoResearch Laboratories). After several washes, cells were
mounted with mounting medium Vectashield (Vector Laboratories). To
visualize both surface and intracellular TrkB, the cells were fixed with 4%
paraformaldehyde, 120 mM sucrose in PBS for 20 min at room tempera-
ture, followed by quenching with 0.1 M glycine in PBS. The cells were per-
meabilized and nonspecific binding was blocked with 10% goat serum,
0.4% Triton X-100 in PBS for 40 min at room temperature. The cells
were stained with rabbit anti-TrkB (1:50; Chemicon) overnight at 4°C.
After several washes the cells were incubated with Cy3-conjugated anti-
rabbit antibody (1:200; Jackson ImmunoResearch Laboratories) in 5%
goose serum in PBS for 1 h at room temperature. The cells were washed
three times and then mounted with Vectashield. Fluorescence images
were acquired by a MicroMax 1300 cool CCD camera mounted on a Ni-
kon Eclipse E800 microscope, and assigned to a pseudo color (green or
red). In some cases, immunostained cells were examined using a confocal
microscope (MRC1024; Bio-Rad). The images were processed by IPLab
software. Each experimental condition was repeated at least three times.

Antennapedia Fusion Peptide Experiments

The autacotide-2–related inhibitory peptide (AIP) was as a specific in-
hibitor for CaMKII (Ishida et al., 1995). To facilitate the translocation of
AIP across the cell membrane, we fused AIP to a 16-residue antennapedia
homeopeptide (antp-AIP; Prochiantz, 1996; Passafaro et al., 1999). A control
peptide was made using antp and scrambled AIP sequences (antp-
AIPscm). This scrambled sequence was analyzed by Program Blastp, and
no significant similarity was found in the current database. The sequences
of the peptides are listed below:

     [H]-ROIKWFQQRRMKKWLARRAVEDAL–Antp-AIP
     [H]-ROIKWFQQRRMKKWLAAADLVERR–Antp-AIPscm

The peptides were synthesized and HPLC-purified by Princeton Biomole-
cules. To allow sufficient penetration of the peptides into the cytoplasm of
hippocampal neurons, the cells were pretreated with Antp-AIP or Antp-
AIPscm (20 μM) 3 h before the biotinylation or binding assay was per-
formed.

Results

BDNF receptors on the surface of cultured hippocampal
neurons were determined by a biotinylation assay. All
membrane proteins were biotinylated, followed by precip-
itation with ImmunoPure Immobilized Streptavidin
and Western blot analysis using antibodies against the high af-
finity TrkB receptors or the p75NFRs. Field electric stimu-
lation was applied to the culture dishes in a 37°C incubator
to induce neuronal firings (Bito et al., 1996; Deisseloth et
al., 1996; Fields et al., 1997). Whole-cell current clamp re-
registering that field stimulation reliably elicited action
potentials (Fig. 1 A). The firing patterns of the neu-nrons followed well with either TBS (Fig. 1 A) or 100 Hz
tetanus (data not shown). When equal amounts of biotin-
ylated proteins were loaded into the SDS gel, significant
differences in the amount of both full-length (145 kD, or
p145) and truncated (95 kD, or p95) forms of TrkB recep-
tors on the cell surface were observed between active and
inactive hippocampal neurons (Fig. 1, B and C). Neurons
stimulated with TBS exhibited significantly more surface
TrkB as compared with those in unstimulated cultures
(Fig. 1, B1 and C). To control for any nonspecific effects of
electric stimulation on surface expression of TrkB, we per-
formed most of our experiments in cultures stimulated with
TBS either alone (active) or in the presence of activ-
ity blockers (inactive). The stimulation alone group is re-
ferred to as control (Ctr). Tetrodotoxin (TTX; 1 μM),
which blocks Na+ channels and therefore all neuronal ac-
tion potentials, significantly reduced the amount of surface
TrkB (Fig. 1 C). TBS had no effect on surface expression
of p75NFRs (Fig. 1 D), suggesting that the effect of TBS is
specific for TrkB receptors. In these cultures, neurons
were well connected, and electric stimulation often elicited
excitatory synaptic transmission (data not shown). Inhib-
ition of excitatory transmission, either by the general
 glutamate receptor antagonist kynurenic acid (kyn; 1 mM)
or a combination of the non-NMDA receptor antagonist
6-cyano-7-nitroquinozaline-2,3-dione (CNQX; 100 μM)
and the NMDA receptor antagonist MK801 (80 μM), sig-
nificantly attenuated the TBS-induced increase in surface
TrkB (Fig. 1, B1 and C). Blockade of high frequency trans-
mission by kyn had no effect on surface p75NFR (Fig. 1 D).
Thus, high frequency neuronal activity modulates TrkB,
but not p75NR, on the surface of hippocampal neurons,
and this effect appears to require action potentials coupled
to excitatory synaptic transmission.

The increase of surface TrkB could be due to an in-
crease in TrkB insertion into the cell surface, a decrease in
TrkB internalization, or an increase in TrkB synthesis.
Several pieces of evidence argue against a general in-
crease in TrkB synthesis. First, there was no difference in the to-
tal amount of TrkB between active (TBS) and inactive
(TBS plus TTX or Kyn) hippocampal neurons (Fig. 1 B2).
Second, inhibition of protein synthesis by anisomycin (10
μg/ml) or cycloheximide (10 μg/ml) did not decrease the
levels of surface TrkB in neurons stimulated with TBS
(Fig. 1 B3). Thus, the synthesis of TrkB receptors is not
enhanced by high frequency neuronal activity. Finally,
 electric stimulation of hippocampal neurons resulted in an
increase, rather than a decrease in TrkB internalization
(data not shown). These results, together with the immu-
nocytocchemistry experiments (see below), suggest that
TBS facilitates the insertion of TrkB onto the surface
membrane, rather than its production, in hippocampal
neurons.

A previous study showed that depolarization induced by
high concentration of K+ (50 mM) resulted in a significant
increase in the surface TrkB in retinal ganglion cells
(Meyer-Franke et al., 1998). In our study, we found that
simple depolarization by high K+ did not affect the
amount of surface TrkB in hippocampal neurons. The lev-
in the presence of indicated agents as follows: TTX (1 μM); kyn (1 mM); Q/M (0.1 mM CNQX plus 80 μM MK801); anisomycin (10 μg/ml); cycloheximide (10 μg/ml); Na⁺ (50 mM) and K⁺ (50 mM). (B1) High frequency electric stimulation increases surface expression of TrkB. Both full length (145 KD) and truncated (95 KD) TrkB receptors in stimulated (stim) or unstimulated (unstim) cultures are shown. (B2) Blockade of excitatory synaptic transmission prevents the TBS-induced increase in surface TrkB. The surface TrkB was measured in cultures in the presence or absence of blockers for excitatory transmission, kyn or Q/M. (B3) The total levels of TrkB are not changed by electric stimulation. Hippocampal neurons were stimulated with TBS in the presence or absence of TTX or kyn, harvested by RIPA buffer. Total amount of TrkB were measured directly by Western blot. (B4) TBS-induced increase in surface TrkB does not require protein synthesis. Cultures were stimulated by TBS in the presence or absence of the protein synthesis inhibitor anisomycin or cycloheximide. The surface TrkB were determined by biotinylation. (B5) Simple depolarization induced by high K⁺ does not change the levels of surface TrkB. Biotinylation was used to determine the levels of surface TrkB in cultures that were treated with 50 mM of either control agent Na⁺ or the depolarizing agent K⁺. (C) Summary of the biotinylation experiments for surface TrkB (full length, p145). (Left) The levels of surface TrkB in TBS-stimulated cultures (set as 100%) are compared with unstimulated cultures (unstim) and cultures stimulated with TBS in the presence of TTX, kyn, or Q/M; n = 7. (Right) Simple depolarization by high K⁺ has no effect; n = 4. The surface TrkB levels were determined in cultures that were treated with Na⁺ (50 mM, set as 100%) and K⁺ (50 mM). Asterisk indicates statistically different results (P < 0.05, ANOVA followed by post hoc tests). (D) Summary of the biotinylation experiments for surface p75NR. TTX and kyn have no effect on surface p75NR; n = 3. In this and all other bar graph figures, data from a specific experimental condition (e.g., TBS plus TTX) were normalized to the mean in control (TBS stimulation alone) groups. The results in several independent experiments (n) were pooled and averaged, and presented as mean ± SE.
frequency tetanic stimulation. TBS reliably elicited an increase in I^{125}-BDNF surface binding as compared with nonstimulated controls (Fig. 2 B). Another tetanic stimulation (three times, 100 Hz, 1 s every 10 min), which elicited a train of high frequency action potentials, resulted in an increase in BDNF binding similar to the result from TBS (Fig. 2 B). In contrast, low frequency stimulation, such as the LTD-inducing stimuli (5 Hz, 4 min) or a constant low frequency train (0.16 Hz), had no effect on I^{125}-BDNF surface binding (Fig. 2 B). It is worth pointing out that the same number of pulses was delivered during 30 min of stimulation in both the 100 Hz tetanic stimulation and the 0.16 Hz stimulation. Thus, the modulation of surface BDNF receptors appears to depend on the stimulation frequency, rather than the number of pulses. Again, simple depolarization induced by high K^+ had little effect on surface binding (Fig. 2 B).

An immediate consequence of tetanus-induced neuronal activity is Ca^{2+} influx through voltage-gated Ca^{2+} channels or NMDA receptors. To determine the mechanisms underlying TBS-induced increase in the surface expression of TrkB, we studied effects of a number of manipulations known to interfere with Ca^{2+} influx. Using the biotinylation assay, we found that blockade of Ca^{2+} influx by the NMDA receptor blocker MK801 (80 μM) markedly reduced the amount of both full-length and truncated TrkB receptors on the surface of hippocampal neurons stimulated with TBS (Fig. 3 A). Inhibition of Ca^{2+} influx by the general Ca^{2+} channel blockers Cd^{2+} (0.2 mM) had similar effects (Fig. 3 A). These results were further confirmed by the I^{125}-BDNF surface binding assays. The surface binding was reduced when TBS was applied together with the general Ca^{2+} channel blockers Cd^{2+} (Fig. 3 B) or

Figure 2. Activity-dependent modulation of the surface binding of BDNF receptors. Cultured hippocampal neurons were incubated with I^{125}-labeled BDNF (5 × 10^{-11} M) with or without cold BDNF (5 × 10^{-8} M) for 30 min while stimulated with TBS. Surface binding is defined as acid washable radioactivity at the end of I^{125}-BDNF incubation. The numbers associated with each column represent the total number of experiments. (A) Blockade of neuronal activity or excitatory synaptic transmission prevents the TBS-induced increase in BDNF surface binding. Hippocampal cultures were stimulated with TBS in the presence or absence of TTX, CNQX plus MK801, or kyn. All drug-treated groups were significantly lower than their paired stimulation alone (control) groups, which were set as 0% (P < 0.01, Student’s t test). (B) Modulation of BDNF receptor binding by patterned electric stimulation. Percentage of changes is presented. The data in control (no stimulation at all) are set as 0%. TBS and tetanic stimulation (three times, 100 Hz, 1 s every 10 min), but not continuous low frequency stimulation (0.16 Hz) or high K^+ (50 mM) stimulation, elicited much higher BDNF receptor binding as compared with control. LTD-inducing stimulus (5 Hz, 4 min) also had no effect. Asterisk indicates statistically different results (P < 0.05; ANOVA followed by post hoc tests).

Figure 3. Role of Ca^{2+} influx in activity-dependent modulation of cell surface TrkB receptors. Western blot analysis of cell surface TrkB receptors (A) and surface binding of I^{125}-BDNF (B) were performed under conditions that affect Ca^{2+} influx. TBS was applied in all conditions. Asterisk indicates statistically different results (P < 0.05, ANOVA followed by post hoc tests). (A) Summary of the effect of Ca^{2+} influx on surface TrkB. Inset shows an example of biotinylation analysis of surface TrkB receptors showing that MK801 and Cd^{2+} reduce both p145 and p95 TrkB proteins in TBS-stimulated cultures. The amount of full length TrkB (p145) in control conditions (TBS in regular medium) was set as 100%. Ca^{2+} channels blocked by Cd^{2+} (0.2 mM) and NMDA receptors blocked by MK801 (80 μM) all inhibited BDNF receptor on the cell surface; n = 7. (B) Summary of the effect of Ca^{2+} influx on BDNF surface binding. Controls (TBS in regular medium) were set as 0%. In Ca^{2+}-free condition, culture medium was replaced and pretreated for 30 min with Ca^{2+}-free DMEM before experiments were performed. The numbers associated with each column represent the total number of experiments.
Ca\textsuperscript{2+} (3 mM, not shown), or NMDA antagonists MK801 (80 μM; Fig. 3 B) or 2-amino-5-phosphonovalerate (50 μM; data not shown). Moreover, surface BDNF receptors were significantly reduced in neurons stimulated by TBS in Ca\textsuperscript{2+}-free medium, as compared with that in regular medium (Fig. 3 B). Thus, the TBS modulation of surface expression of BDNF receptors appears to depend on Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channels and/or NMDA receptors.

To determine the changes in the distribution of TrkB receptors on the cell surface, we performed immunocytochemistry under nonpermeable (no detergent) conditions. Cultured hippocampal neurons were fixed in 2% paraformaldehyde for 3 min, and TrkB antibodies were incubated with the fixed cells in buffers containing no detergent. Under these conditions, an antibody against intracellular domains of the TrkB did not stain hippocampal neurons, suggesting that antibodies or proteins can not penetrate the cells (Fig. 4, A and B). The same antibody was able to detect a substantial amount of TrkB if the staining was performed in the presence of detergent (Fig. 4 C). Under the nonpermeable (no detergent) conditions, surface TrkB were detected using an antibody against extracellular domains of the TrkB. In cultures stimulated with TBS, many TrkB receptors were found on the surface of the cell body. More importantly, a large number of TrkB receptors were distributed along the neuronal processes (Fig. 4 D). In contrast, TrkB receptors were mainly clustered on the cell body and there were very few receptors on the neuronal processes in cultures stimulated with TBS in the presence of kyn and Cd\textsuperscript{2+} (Fig. 4 E). The reduction in surface TrkB in the cultures treated with kyn and Cd\textsuperscript{2+} was not due to a decrease in the number of neuronal processes, which were obviously observed under phase contrast microscopy (data not shown). To better visualize the surface TrkB, we used confocal microscopy. Thin section (2 μm) confocal images revealed a substantial increase in the amount of surface TrkB receptors in active (TBS alone) neurons as compared with the inactive (TBS plus kyn and Cd\textsuperscript{2+}) neurons (Fig. 4, F and G). These experiments were repeated many times and striking differences in surface TrkB were always observed between active and inactive neurons. Unstimulated neurons also exhibited less surface TrkB receptors than stimulated neurons (data not shown).

To further investigate the changes in the TrkB receptors inside the cells, we stained the hippocampal neurons under permeable (with detergent) conditions using the antibody against the intracellular domain of TrkB. Both surface and intracellular TrkB were detected. In the stimulated cultures, it appeared that majority of TrkB receptors were on the cell membrane (arrows) and only small amounts of the receptors were inside the cells (Fig. 5 A). Significantly fewer TrkB receptors were found on the cell surface but a lot more receptors were detected inside cells (arrowheads) in cultures stimulated with TBS in the presence of kyn and Cd\textsuperscript{2+} (Fig. 5 B). Confocal microscopy was again used to better separate surface and cytoplasmic TrkB. Indeed, active neurons exhibited a “ring” pattern of staining in sections across the middle of the cell body region, with a lot of TrkB receptors on the cell surface but very little in the cytoplasm (Fig. 5 C). In contrast, a great deal of cytoplasmic TrkB was observed in inactive neurons in similar sections (Fig. 5 D). These results further support the notion that high frequency neuronal activity facilitates the insertion of TrkB receptor into the cell surface.

Ca\textsuperscript{2+} influx is known to activate CaMKII, which has been implicated in the activity-dependent insertion of AMPA-type glutamate receptors into the postsynaptic membrane during LTP (Hayashi et al., 2000). To determine whether CaMKII is also involved in the insertion of the tyrosine kinase receptor TrkB, we measured the amount of surface TrkB in cultures stimulated with TBS in the presence or absence of CaMKII inhibitors. Biotinylation experiments demonstrated that inhibition of CaMKII by either KN62 or KN93 significantly reduced the amount of TrkB receptors in cultures stimulated with TBS, whereas inactive compound KN92 had no effect (Fig. 6, A and B). KN62 and KN93 also significantly inhibited BDNF surface binding on hippocampal neurons (Fig. 6 C). Since KN62 and KN93 have been shown to inhibit other CaM kinases and may cause some nonspecific effects in certain conditions, we used AIP, a peptide known to selectively inhibit CaMKII (Ishida et al., 1995). The NH\textsubscript{2} terminus of AIP or a control peptide with scrambled sequence (AIPscm) was fused to the antennapedia homeopeptide (antp, 16 residues) derived from antennapedia gene to facilitate the translocation of the peptide across the cell membrane of hippocampal neurons (Prochiantz, 1996; Passafaro et al., 1999). To determine whether the peptides can penetrate into the hippocampal neurons, we labeled the peptides with biotin, and treated the cells with the biotinylated peptides for a few hours. Cy3-conjugated streptavidin detected the biotinylated peptides inside the hippocampal neurons 3 h after peptide incubation (data not shown). In cultures stimulated with TBS in the presence of antp-AIP, significantly lower levels of surface TrkB were detected as compared with those in cultures stimulated with TBS alone (Fig. 6, A and B). The control peptide antp-AIPscm had no effect (Fig. 6, A and B). Similar results were obtained using the I\textsuperscript{125}-BDNF surface binding assay (Fig. 6 C). These results strongly suggest the involvement of CaMKII in the activity-modulation of surface TrkB.

Discussion

A critical but unresolved question in the neurotrophin research is how a diffusible molecule such as BDNF achieves preferential regulation of active neurons or synapses. In our study, we have investigated whether neuronal responsiveness to BDNF is dependent on, or modified by, neuronal activity. Using three independent approaches, we demonstrate that several forms of tetanic stimulation, but not low frequency stimulation or simple depolarization, promotes the insertion of the BDNF receptor TrkB into the cell surface of hippocampal neurons. We also show that excitatory synaptic transmission, Ca\textsuperscript{2+} influx, and activation of CaMKII are important for the cell membrane insertion of TrkB. Thus, activity-dependent increase in the number of surface TrkB receptors may explain why BDNF acts preferentially on active neurons. This study reveals a novel mechanism by which neurotrophin signaling and function may be regulated, and provides a potential link between activity-dependent and BDNF-induced mod-
Figure 4. Immunocytochemistry of TrkB on the surface of hippocampal neurons. (A and B) Phase and fluorescence images of neurons stained with an antibody against intracellular domain of TrkB under nonpermeabilizing conditions. (C) Immunofluorescence images of neurons stained with the same antibody permeabilizing conditions. There is no staining in B but good staining in C, indicating that the antibody cannot penetrate inside cells under nonpermeabilizing conditions. (D–G) Immunocytochemistry staining using an antibody against the extracellular domain of TrkB under non-permeabilizing conditions. Hippocampal neurons were stimulated with TBS in the presence (E and G) or absence (D and F) of Cd2⁺ (0.2 mM) and kyn (1 mM) for 30 min. Cells were fixed with 2% paraformaldehyde, 120 mM sucrose in PBS at room temperature for 3 min, followed by conventional immunofluorescence procedure without any detergent. D and E are conventional immunofluorescence images, and F and G are confocal immunofluorescence images. Arrows indicate surface TrkB stainings on neuronal processes and arrowheads indicate those on cell body. Note that far more surface TrkB receptors are seen in cultures stimulated with TBS alone, especially in neuronal processes. Bar, 5 μm.
ulation of neuronal and synaptic function in the hippocampus.

In this study, hippocampal neurons were grown for 11–14 d. Electrophysiological recording indicated that these neurons were well connected synaptically (data not shown). We show that excitatory synaptic activity plays an important role in regulating surface expression of the TrkB receptor tyrosine kinase. Electric stimulation is a more physiological form of stimulation that has been successfully used to study activity-dependent regulation of signal transduction and gene transcription in cultured hippocampal neurons (Bito et al., 1996; Deisseroth et al., 1996; Fields et al., 1997). LTP-inducing tetanic stimuli enhanced surface expression of TrkB, whereas blockade of excitatory synaptic transmission inhibited the tetanus-induced insertion. Immunocytochemical studies demonstrate that the increase in surface TrkB induced by TBS occurred mostly on neuronal processes rather than cell bodies. Remarkably, low frequency stimulation such as those used to induce LTD, or continuous 0.16 Hz (which delivers the same number of pulses as the 100 Hz tetanus), had no effect. These results suggest that temporal pattern of neuronal activity and the kinetics of changes in intracellular Ca²⁺ concentrations, rather than the number of action potentials or the total amount of Ca²⁺ influx, are the critical factors for the activity-dependent insertion of TrkB receptors. Consistent with this idea, simple depolarization by high K⁺ (Figs. 1 and 2) or veratridine (data not shown) had no effect on the number of surface TrkB. Using freshly dissociated retinal ganglion cells as a model, Barres and colleagues demonstrated that depolarization by high K⁺ or glutamate agonists increases the number of cells expressing surface TrkB (Meyer-Franke et al., 1998). The mechanisms underlying the apparent discrepancy re-

Figure 5. Immunocytochemistry of hippocampal neurons stained by an antibody against the intracellular domain of TrkB under permeabilizing conditions. TBS was applied to the hippocampal neurons in the presence (B and D) or absence (A and C) of Cd²⁺ and kyn for 30 min. The cultures were then fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.4% Triton X-100 for 60 min, and processed for immunofluorescence staining of TrkB. A and B are conventional immunofluorescence images, and C and D are confocal immunofluorescence images. Arrows indicate cell surface and arrowheads indicate cytoplasmic stainings, respectively. Note that active cells (stimulated by TBS) exhibit TrkB receptors mostly on the cell surface, whereas inactive cells (TBS plus Cd²⁺ and kyn) show more cytoplasmic staining of TrkB.
main unclear. A simple explanation is that different types of neurons may use different mechanisms to regulate surface expression of TrkB. In the retinal ganglion cells, simple depolarization by high K⁺ may increase intracellular cAMP concentrations, which in turn facilitate the incorporation of TrkB into the surface through a number of unknown steps (Meyer-Franke et al., 1998). In the hippocampal neurons, high frequency stimulation may induce Ca²⁺ influx that is qualitatively different from that induced by high K⁺, leading to the activation of CaMKII. Alternatively, freshly dissociated retinal ganglion neurons and synaptically connected hippocampal neurons (grown for 11–14 d) may respond differently to high K⁺. In retinal ganglion cells, high K⁺ may be sufficient to generate the intracellular signals needed to facilitate surface expression of TrkB (Meyer-Franke et al., 1998). In the hippocampal neurons, however, high K⁺ could not produce the specific temporal pattern of neuronal activity and Ca²⁺ influx and subsequent CaMKII activation required for the modulation.

Our results suggest that the modulation of TrkB receptors by tetanic stimulation is mediated, at least in part, by high frequency excitatory synaptic transmission. We have also demonstrated that the activity-dependent modulation of TrkB requires Ca²⁺ influx. Since Ca²⁺-free medium and Cd²⁺ not only prevent Ca²⁺ influx into the postsynaptic neurons through Ca²⁺ channels but also inhibit transmitter release from presynaptic terminals, it is unclear whether blockade of Ca²⁺ influx directly affects the insertion of TrkB receptor into the cell surface, or indirectly by blocking excitatory synaptic transmission. However, blockade of NMDA receptors, which are primarily localized in the postsynaptic cells rather than presynaptic nerve terminals, also attenuates surface expression of TrkB (Fig. 3). Moreover, CaMKII appears to be involved in the activity-dependent insertion of TrkB (Fig. 6). Given that CaMKII has been shown to be important for the insertion of AMPA-type receptors postsynaptically during hippocampal LTP (Hayashi et al., 2000), it is conceivable that similar postsynaptic mechanisms may be used for the insertion of the tyrosine kinase receptor TrkB. It is important to point out, however, that tetanic stimulation does not increase all surface molecules. The surface p75NR is not increased in neurons stimulated with TBS (Fig. 1 D).

One of the remarkable features of the nervous system is that neuronal activity can modulate synaptic efficacy and connectivity in a local and synapse-specific manner (Stent, 1973; Goodman and Shatz, 1993; Katz and Shatz, 1996; Constantine-Paton et al., 1990). Recent studies strongly implicate a role of BDNF in activity-dependent synaptic modulation, such as the formation of ocular dominance columns in the visual cortex and hippocampal LTP (Theo- nen, 1995; Lu and Chow, 1999; McAllister et al., 1999). It is important to understand how diffusible factors such as BDNF achieve local and synapse-specific modulation, and how BDNF strengthens active synapses without affecting their neighbors. One such mechanism would be a localized secretion of BDNF at the site of active synapses. Although there is some evidence for an activity-dependent secretion of BDNF (Wang and Poo, 1997; Goodman et al., 1996; Heymach et al., 1996), so far local or synapse-specific secretion of any neurotrophins has not been demonstrated. It is difficult to imagine that locally secreted factors would not spread to their neighboring, less active synapses. Our results demonstrate an alternative and more practical strategy. Active neurons may respond better to BDNF, and this is achieved by an activity-dependent control of the number of TrkB receptors on the cell surface. These results provide a molecular basis for the facilitation of BDNF-induced synaptic potentiation when coupled to presynaptic depolarization at the neuromuscular junction (Boulanger and Poo, 1999), and the restricted action of BDNF on highly active synapses in the hippocampus (Gottschalk et al., 1998). In this context, it is important to
note that the tetanic stimuli such as TBS or tetanic stimulation were capable of modulating TrkB receptors, whereas low frequency stimulation was not. Since all of our experiments were done using cultured neurons, their relevance to the BDNF modulation of hippocampal synaptic plasticity in vivo has yet to be established. Nevertheless, activity-dependent enhancement of the number of surface TrkB receptor may define an important mechanism by which the specificity of BDNF modulation is achieved.

The results in our study may have general implications in the cell biology of tyrosine kinase receptors. First, we have demonstrated an activity-dependent increase in the number of surface TrkB receptors in the hippocampal neurons. This is due to an increase in the insertion of TrkB receptors into the neuronal cell surface, rather than an increase in TrkB synthesis or decrease of TrkB internalization. The mechanisms underlying membrane insertion of TrkB receptors, and tyrosine kinase receptors in general, are largely unexplored. This study may trigger further interests in investigating the mechanisms for membrane insertion of tyrosine kinase receptors. It will be interesting to examine whether molecules important for vesicle fusion, such as NSF and SNAP, are involved in the delivery of tyrosine kinases onto cell membrane. Second, we show that the membrane insertion of the TrkB receptors is enhanced by Ca$$^{2+}$$ influx. To our knowledge, this is the first report for Ca$$^{2+}$$-dependent modulation of the number of surface tyrosine kinase receptors. Thus, our results suggest a novel mechanism for cross-talk between Ca$$^{2+}$$ and tyrosine kinase signaling pathways. Whether the tyrosine kinase activity of the TrkB receptors can be regulated by intracellular Ca$$^{2+}$$ is an interesting topic for future study. Finally, CaMKII has recently been implicated in the insertion of AMPA-type glutamate receptors onto the postsynaptic membrane of hippocampal neurons (Hayashi et al., 2000). Our study demonstrates that similar mechanisms are used for the tetanus-induced increase in the tyrosine kinase receptor TrkB on the surface of hippocampal neurons. It remains to be established whether CaMKII also regulates the membrane insertion of other tyrosine kinases in neurons and in other cell types.

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References

Berninger, B., and M.-m. Poo. 1996. Fast actions of neurotrophic factors. Curr. Opin. Neurobiol. 6:324–330.
Bito, H., K. Deisseroth, and R.W. Tsien. 1996. CREB phosphorylation and dephosphorylation: a Ca$$^{2+}$$ and stimulus duration-dependent switch for hippocampal gene expression. Cell. 87:1203–1214.
Bonhoeffer, T. 1996. Neurotrophins and activity-dependent development of the neocortex. Curr. Opin. Neurobiol. 6:119–126.
Boulanger, L., and M.m. Poo. 1999. Presynaptic depolarization facilitates neurotrophin-induced synaptic potentiation. Nat. Neurosci. 2:346–351.
Cabelli, R.J., A. Horn, and C.J. Shatz. 1995. Inhibition of ocular dominance col-
unn formation by infusion of NT-4/5 or BDNF. Science. 267:1662–1666.
Cascadia-Bonelli, P., B.D. Dobrowsky, and M.V. Chao. 1996. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature. 383:716–719.
Cascadia-Bonelli, P., H. Kong, and M.V. Chao. 1998. Neurotrophins: the biological paradox of survival factors eliciting apoptosis. Cell Death Differ. 5:357–364.
Cohen-Cory, S., and S.E. Fraser. 1996. Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. Nature. 378:192–196.
Cohen-Cory, S., and B. Lord. 1999. BDNF modulates, but does not mediate, activity-dependent branching and remodeling of optic axon arbors in vivo. J. Neurosci. 19:9996–10003.
Constantine-Paton, M., H.T. Cline, and E. Debski. 1990. Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu. Rev. Neurosci. 13:129–154.
Deisseroth, K., H. Bito, and R.W. Tsien. 1996. Signaling from synapse to nucleus: post synaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. Neuron. 16:89–101.
Feng, L., C.Y. Wang, H. Jiang, C. Oho, K. Mizuno, M. Dougich-Djordjevic, and B. Lu. 1999. Differential effects of GDNF and BDNF on cultured ventral mesencephalic neurons. Mol. Brain Res. 66:62–70.
Fields, R.D., F. Eshete, B. Stevens, and K. Itoh. 1997. Action potential-dependent regulation of gene expression: temporal specificity in Ca$$^{2+}$$, cAMP-responsive element binding proteins, and mitogen-activated protein kinase signaling. J. Neurosci. 17:7252–7266.
Figueroa, A., L. Pozzo-Miller, P. Ofilsson, T. Wang, and B. Lu. 1996. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature. 381:706–709.
Frad, J.M., A. Rodriguez-Tebar, and Y.A. Barde. 1996. Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature. 385:166–168.
Freking, M., R.C. Malenka, and R.A. Nicoll. 1998. Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. J. Neurophysiol. 80:3383–3386.
Galuste, R.A., D.S. Kim, E. Castren, H. Thoenen, and W. Singer. 1996. Brain-derived neurotrophic factor reversed experience-dependent synaptic modifications in kitten visual cortex. Eur. J. Neurosci. 8:1554–1559.
Goodman, C.S., and C.J. Shatz. 1993. Developmental mechanisms that generate precise patterns of neuronal connectivity. Neuron. 10:77–98.
Goodman, L.J., J. Valverde, F. Lim, M.D. Geschwind, H.J. Federoff, A.I. Geller, and P. Hefti. 1996. Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol. Cell. Neurosci. 7:223–228.
Gottschalk, W., L.D. Pozzo-Miller, A. Figurov, and B. Lu. 1998. Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. J. Neurosci. 18:6853–6839.
Gu, Q., Y. Liu, and M.S. Cynader. 1994. Nerve growth factor-induced ocular dominance plasticity in adult cat visual cortex. Proc. Natl. Acad. Sci. USA. 91:8408-8412.
Hayashi, Y., S.H. Shi, J.A. Esteban, A. Piccini, J.C. Poncer, and R. Malinow. 2000. Driving AMPA receptors into synapses by LTP and CaMKII requirement for Glur1 and PDZ domain interaction. Science. 287:2262–2267.
Heymach, J.V., Jr., A. Kruttgen, U. Suter, and E.M. Shooter. 1996. The regulation of septal and cortical targeting of neurotrophins in neocortical and epithelial cells. J. Biol. Chem. 271:25430–25437.
Huang, Z.J., A. Kirkwood, T. Pizzorusso, V. Porcatti, B. Morales, M.F. Bear, L. Malenka, and S. Tonegawa. 1999. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell. 98:7379–755.
Ishida, A., I. Kameshita, S. Okuno, T. Kitani, and H. Fujisawa. 1995. A novel highly specific and potent inhibitor of calmodulin-dependent protein kinase II. Biochem. Biophys. Res. Commun. 212:906–912.
Kang, H., A.A. Welcher, D. Shelton, and E.M. Schuman. 1997. Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. Neuron. 19:653–664.
Kaplan, D.R., and R.M. Stephens. 1994. Neurotrophin signal transduction by the Trk receptor. J. Neurobiol. 25:1404–1417.
Katz, L.C., and C.J. Shatz. 1996. Synaptic activity and the construction of cortical circuits. Science. 274:1133–1138.
Kim, H.G., T. Wang, P. Ofilsson, and B. Lu. 1994. Neurotrophin 3 potentiates neuronal activity and inhibits g-aminobutyric acid synaptic transmission in cortical neurons. Proc. Natl. Acad. Sci. USA. 91:12341–12345.
Klein, M., M. da Penha Benzaghi, A. Blochl, H. Breer, H. Thoenen, and D. Lindholm. 1994. Positive feedback between acetylcholine and the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the rat hippocampus. Eur. J. Neurosci. 6:668–671.
Korte, M., P. Carroll, E. Wolff, G. Brem, H. Thoenen, and T. Bonhoeffer. 1995. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. USA. 92:8856–8860.
Korte, M., G. Grisebeck, C. Gravel, P. Carroll, V. Stager, H. Thoenen, and T. Bonhoeffer. 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.
Lessmann, V., K. Gottmann, and R. Heumann. 1994. BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurons.
Neuroreport. 6:21–25.
Levine, E.S., C.F. Dreyfus, I.B. Black, and M.R. Plummer. 1995. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc. Natl. Acad. Sci. USA. 92:8074–8077.
Lewin, G.R., and Y.-A. Barde. 1996. Physiology of the neurotrophins. Annu. Rev. Neurosci. 19:289–317.
Lohof, A.M., N.Y. Ip, and M.M. Poo. 1993. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature. 363:350–353.
Lu, B., and A. Chow. 1999. Neurotrophins and hippocampal synaptic plasticity. J. Neurosci. Res. 58:76–87.
Maffei, L., N. Berardi, L. Domenici, V. Parisi, and T. Pizzorusso. 1992. Nerve growth factor (NGF) prevents the shift in ocular dominance distribution of visual cortical neurons in monocularly deprived rats. J. Neurosci. 12:4651–4662.
McAllister, A.K., D.C. Lo, and L.C. Katz. 1995. Neurotrophins regulate dendritic growth in developing visual cortex. Neuron. 15:791–803.
McAllister, A.K., L.C. Katz, and D.C. Lo. 1996. Neurotrophin regulation of cortical dendritic growth requires activity. Neuron. 17:1057–1064.
McAllister, A.K., L.C. Katz, and D.C. Lo. 1997. Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron. 18:767–778.
McAllister, A.M., L.C. Katz, and D.C. Lo. 1999. Neurotrophins and synaptic plasticity. Annu. Rev. Neurosci. 22:295–318.
Meyer-Franke, A., M.R. Kaplan, F.W. Pfrieger, and B.A. Barres. 1995. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron. 15:805–819.
Meyer-Franke, A., G.A. Wilkinson, A. Kruttgen, M. Hu, E. Munro, M.G. Hanson, Jr., L.F. Reichardt, and B.A. Barres. 1998. Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. Neuron. 21:681–693.
Passafaro, M., C. Sala, M. Niethammer, and M. Sheng. 1999. Microtubule binding by CRIP2 and its potential role in the synaptic clustering of PSD-95. Nat. Neurosci. 2:1063–1069.
Patterson, S.L., T. Abel, T.A. Deuel, K.C. Martin, J.C. Rose, and E.R. Kandel. 1996. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron. 16:1137–1145.
Pozzo-Miller, L., W.A. Gottschalk, L. Zhang, K. McDermott, J. Du, R. Gopalakrishnan, C. Oho, Z. Shen, and B. Lu. 1999. Impairments in high frequency transmission, synaptic vesicle docking and synaptic protein distribution in the hippocampus of BDNF knockout mice. J. Neurosci. 19:4972–4983.
Prochiantz, A. 1996. Getting hydrophilic compounds into cells: lessons from homopeptides. Curr. Opin. Neurobiol. 6:629–634.
Patterson, S.L., T. Abel, T.A. Deuel, K.C. Martin, J.C. Rose, and E.R. Kandel. 1996. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron. 16:1137–1145.
Pozzo-Miller, L., W.A. Gottschalk, L. Zhang, K. McDermott, J. Du, R. Gopalakrishnan, C. Oho, Z. Shen, and B. Lu. 1999. Impairments in high frequency transmission, synaptic vesicle docking and synaptic protein distribution in the hippocampus of BDNF knockout mice. J. Neurosci. 19:4972–4983.
Prochiantz, A. 1996. Getting hydrophilic compounds into cells: lessons from homopeptides. Curr. Opin. Neurobiol. 6:629–634.
Riddle, D.R., D.C. Lo, and L.C. Katz. 1995. NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation. Nature. 378:189–191.
Stent, G. 1973. A physiological mechanism for Hebb’s postulate of learning. Proc. Natl. Acad. Sci. USA. 70:997–1001.
Takei, N., K. Sasaoka, K. Inoue, M. Takahashi, Y. Endo, and H. Hatanaka. 1997. Brain-derived neurotrophic factor increases the stimulation-evoked release of glutamate and the levels of exocytosis-associated proteins in cultured cortical neurons from embryonic rats. J. Neurochem. 68:370–375.
Tanaka, T., H. Saito, and N. Matsuki. 1997. Inhibition of GABAa synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. J. Neurosci. 17:2959–2966.
Thoenen, H. 1995. Neurotrophins and neuronal plasticity. Science. 270:593–596.
von Bartheld, C.S., R. Williams, F. Lefcort, D.O. Clary, L.F. Reichardt, and M. Bothwell. 1996. Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. J. Neurosci. 16:2995–3008.
Wang, X.H., and M.M. Poo. 1997. Potentiation of developing synapses by postsynaptic release of neurotrophin-4. Neuron. 19:825–835.