Neuron, Vol. 17, 1057–1064, December, 1996, Copyright ©1996 by Cell Press

Neurotrophin Regulation of Cortical Dendritic Growth Requires Activity

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Summary

Neurotrophins have been proposed to mediate several forms of activity-dependent competition in the central nervous system. A key element of such hypotheses is that neurotrophins act preferentially on active neurons; however, little direct evidence supports this postulate. We therefore examined, in ferret cortical brain slices, the interactions between activity and neurotrophins in regulating dendritic growth of layer 4 pyramidal neurons. Inhibition of spontaneous electrical activity, synaptic transmission, or L-type calcium channels each prevented the otherwise dramatic increase in dendritic arborizations elicited by brain-derived neurotrophic factor. In developing cortex, this requirement for conjoint neurotrophin signaling and activity provides a mechanism for selectively enhancing the growth and connectivity of active neurons.

Introduction

Neuronal activity profoundly influences the remodeling of axons and dendrites during brain development (axons: Wiesel, 1982; reviewed by Goodman and Shatz, 1993; dendrites: Valverde, 1968; Volkmar and Greenough, 1972; Harris and Woolsey, 1981; Katz et al., 1989; Bailey and Kandel, 1993). Patterns and levels of activity are thought to form the basis of competition for postsynaptic targets by stabilizing and elaborating coincident inputs and weakening and removing noncoincident inputs (Wiesel, 1982; Goodman and Shatz, 1993). Several lines of evidence implicate neurotrophins as mediators of activity-dependent structural plasticity in cortex. Neurotrophins and their receptors are present in neurons of the developing cortex (Merlio et al., 1992; Ringstedt et al., 1993; Allendoerfer et al., 1994; reviewed by Lindsay et al., 1994), and neurotrophin production and secretion are regulated by electrical and synaptic activity (Castren et al., 1992; Lindholm et al., 1994; Schoups et al., 1995; Blochl and Thoenen, 1995; Blochl and Thoenen, 1996; Goodman et al., 1996). In addition, exogenous neurotrophins attenuate the activity-dependent segregation of lateral geniculate axons into ocular dominance columns (Cabelli et al., 1995; Berardi et al., 1994; Gu et al., 1994; Galuske et al., 1996), ameliorate the effects of monocular deprivation (Domenici et al., 1991; Maffei et al., 1992; Riddle et al., 1995), and regulate dendritic growth of pyramidal neurons in striate cortex (McAllister et al., 1995). Finally, both neurotrophic factors and electrical activity can promote survival of central neurons in vitro (Cohen-Cory et al., 1991; Meyer-Franke et al., 1995; Ghosh et al., 1994). These observations have led to the hypothesis that neurotrophins are factors that selectively augment active connections during cortical development (Purves, 1988; Fox and Zahs, 1994; Snider, 1994; Thoenen, 1995; Lo, 1995; Bonhoeffer, 1996).

A general feature of such models is that neurotrophins must act preferentially on active neurons. We investigated this potential requirement for electrical activity by examining interactions between neurotrophins and electrical activity in regulating growth of dendrites of layer 4 cortical neurons. As the Trk ligands cause the most pronounced enhancement of layer 4 neuron dendritic arborizations (McAllister et al., 1995), interactions between brain-derived neurotrophic factor (BDNF) and electrical activity were examined. We focused on three forms of activity: spontaneously generated action potentials and action potential–evoked synaptic transmission, glutamatergic synaptic transmission, and activation of L-type calcium channels (which have been implicated in synaptic transmission as well as signal transduction events triggered by receptor tyrosine kinases [Rusanescu et al., 1995; Rosen and Greenberg, 1996; reviewed by Finkbeiner and Greenberg, 1996]). We found that specific inhibitors of these forms of activity blocked the otherwise dramatic dendrite growth elicited by BDNF, indicating that the regulation of dendritic growth by BDNF requires electrical activity.

Results

Postnatal day 14 (P14) organotypic cortical slices were treated with either BDNF alone or an activity inhibitor alone, or with a combination of BDNF and each inhibitor. The inhibitors used were tetrodotoxin (TTX, 5 μM) to block Na^+-dependent action potentials and action potential–dependent synaptic activity, CNQX (10 μM) to inhibit non-NMDA receptor-type glutamate receptors, APV (50 μM) to inhibit NMDA receptors, or nifedipine (2 μM) to block L-type calcium channels. Slices were cultured for 36 hr and neurons were visualized by particle-mediated gene transfer of *lacZ* and subsequent immunocytochemistry (Figure 1) (see Experimental Procedures and McAllister et al., 1995).

BDNF and Inhibiting Activity Elicit Distinct Patterns of Dendritic Growth

Application of BDNF alone to slices strongly enhanced dendritic growth of layer 4 pyramidal neurons (also as described previously in McAllister et al., 1995). BDNF treatment increased the extent and complexity of both apical and basal dendrites (Figures 1 and 2), roughly doubling dendritic length and branching (Table 1). BDNF also doubled the number of primary basal dendrites, resulting in a "halo" of short dendrites and protospines protruding from the cell body (Figure 2).

Inhibiting activity in slices also enhanced basal dendritic growth, but these effects were substantially different from those elicited by BDNF (Table 2). Inhibiting activity caused modest increases in elongation and branching of existing basal dendrites, but in marked contrast to BDNF, no sprouting of new dendrites (Figures 1 and 2; Table 1). Basal dendritic complexity increased by 30%–55% with the greatest effects caused

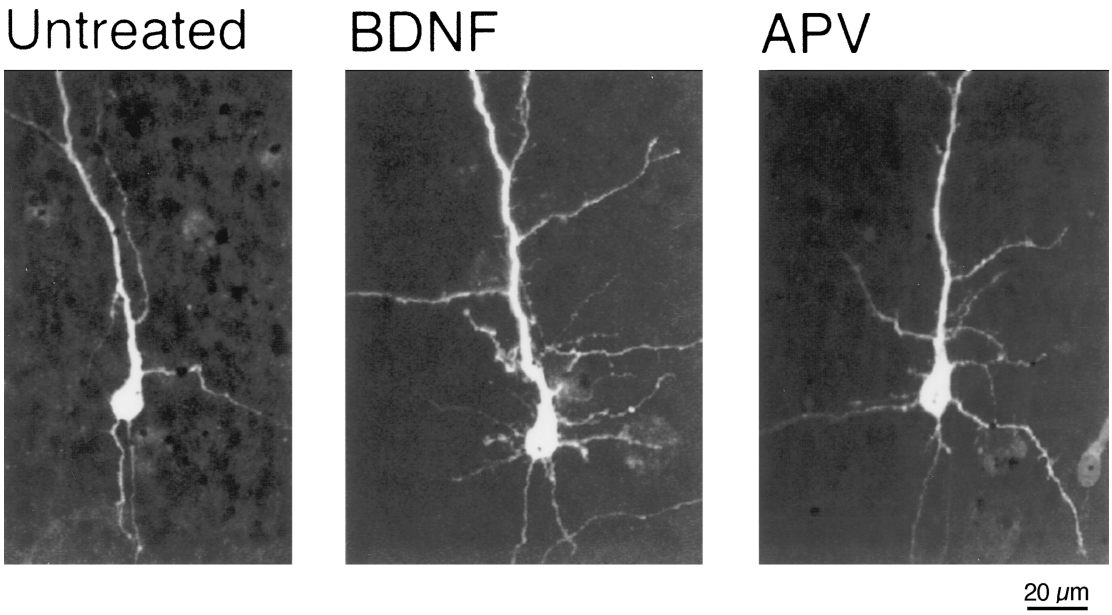

Figure 1. Both BDNF Treatment and Inhibition of Spontaneous Activity Cause Basal Dendritic Elaboration, but in Distinct Patterns

Layer 4 pyramidal neurons are shown from slices of P14 ferret visual cortex: untreated, treated with 200 ng/ml BDNF, or treated with 50 μM APV for 36 hr. BDNF caused a near doubling of the number of primary dendrites and dendritic branches while APV increased only total dendritic length and the number of branches without affecting the number of primary dendrites. Note the small gold particle present in the cell body of each of these neurons. Images were collected with a scanning laser confocal microscope (Bio-Rad) at 1 μm intervals and compiled into a Z-series.

by CNQX (Figure 3). Apical dendritic arborizations were unaffected by inhibiting activity (Figure 3 and Table 2). These observations are consistent with previous studies that have demonstrated axonal elongation resulting from inhibiting activity (Reh and Constantine-Paton, 1985; Lipton and Kater, 1989; Sretavan et al., 1988).

Activity Is Required for Neurons to Respond to the Growth-Promoting Actions of Exogenous BDNF

The strong growth-promoting effects of neurotrophins and the elongation induced by activity inhibitors were not additive; instead, blocking electrical and synaptic activity abolished the effects of BDNF on dendritic growth (Figure 3). For apical dendrites, all of the activity inhibitors prevented the 140% increase in complexity elicited by BDNF alone (Figure 3A). The apical dendrites of neurons treated with BDNF in the presence of APV or nifedipine were indistinguishable from those of untreated neurons, while TTX and CNQX blocked all but a small increase in the number of apical dendritic branches (Table 1). Thus, electrical and synaptic activity are required for the growth-promoting effects of BDNF on apical dendrites.

Neurons must also be active to respond to the growth-promoting effects of BDNF on basal dendrites. Although BDNF and activity inhibitors each independently enhanced basal dendritic growth (Table 2), glutamate receptor blockade and blockade of L-type calcium chan-

Table 1. Average Values of Each Parameter of Dendritic Growth for Apical and Basal Dendrites of Neurons in Each Treatment

Parameter	UT (191)	CNQX (62)	APV (48)	TTX (47)	Nif (49)	BDNF (52)	CNQX plus BDNF (40)	APV plus BDNF (41)	TTX plus BDNF (41)	Nif plus BDNF (40)
Basal dendrites										
TBD (μm/10)	21.7 ± 1.0	**1.5**	**1.5**	**1.4**	**1.4**	**1.9**	*1.0*	*0.9*	***1.4***	*1.2*
#Prims	3.7 ± 0.1	1.2	1.0	1.2	1.2	**1.6**	***0.7***	*1.1*	**1.6**	*1.2*
#Brs	3.2 ± 0.2	**1.7**	**1.7**	**1.8**	**1.4**	**2.3**	*1.0*	*0.9*	**2.3**	*1.2*
#Pspines	14.2 ± 0.8	**2.2**	**1.7**	**1.8**	**1.7**	**2.1**	*0.9*	*0.8*	***1.3***	*1.1*
Apical dendrites										
TAD (μm/10)	61.8 ± 2.0	1.0	1.0	1.0	1.1	**2.2**	*0.9*	*0.9*	*1.2*	*1.2*
#Brs	13.6 ± 0.5	0.9	0.9	1.1	1.1	**2.6**	***1.5***	*1.2*	***1.3***	*1.3*
#Pspines	30.0 ± 1.2	**1.6**	1.3	**1.6**	**1.5**	**2.2**	***0.7***	*0.9*	***1.3***	***1.5***

Average values for each of the parameters are tabulated for untreated cells. The ratio of the average value for treated cells versus the untreated average value is tabulated for each parameter of the experimental treatments. The data are presented in this way to compare litter-matched controls with each of the treatments. The number of neurons included in each average calculation is in parentheses below the treatment name. Abbreviations: TBD, total length of basal dendrites; TAD, total length of apical dendrites; #Prims, number of primary dendrites; #Brs, number of dendritic branches; #Pspine, number of protospines; Nif, nifedipine. Bold numbers indicate values that were statistically significantly different from litter-matched untreated control values, and italicized numbers indicate values statistically significantly different from BDNF-treated values by single-factor ANOVA with a significance level of $p < 0.05$.

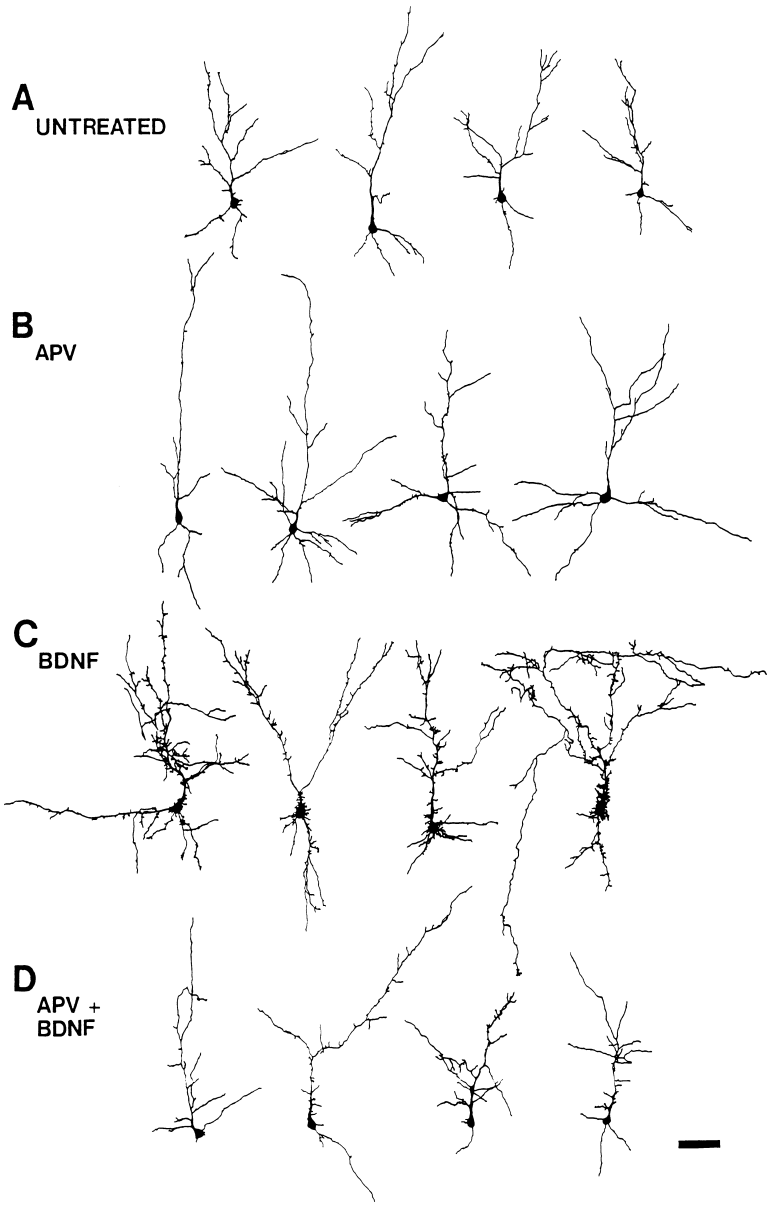

Figure 2. Neurons Must Be Active to Respond to BDNF

Camera lucida reconstructions of layer 4 neurons are shown from untreated slices (A), and slices treated with 50 μM APV (B), 200 ng/ml BDNF (C), and APV and BDNF together (D). Neurons were selected to represent the range of morphologies across the sampled populations. The range of dendritic form for APV treatment is representative for neurons in all activity-inhibited slices and the range of forms for APV plus BDNF treatment is representative for most combinations of activity blockade plus BDNF. Note the increase in length of basal dendrites resulting from inhibition of spontaneous activity (B) in contrast to the increase in the number of primary dendrites and protospines for BDNF-treated neurons (C). Inhibiting spontaneous activity blocked the strong effects of BDNF on increasing dendritic complexity (D). Scale bar, 50 μm.

nels prevented BDNF from inducing new basal dendrites (Figure 3B). In contrast, TTX had only a minor inhibitory influence on the effects of BDNF (Figure 3B), indicating that BDNF-induced growth of basal dendrites may not require Na^+-dependent action potentials. Instead, spontaneous synaptic release of glutamate may be sufficient to allow the actions of BDNF on basal dendrites. In addition, BDNF prevented the elongation of basal dendrites caused by inhibiting activity alone (Figure 3B), suggesting a dynamic balance between BDNF and activity in regulating dendritic morphology.

Inhibiting Activity and Blocking Endogenous BDNF Have Opposite Effects on Dendritic Growth

One explanation for these results is that BDNF exerts its growth-promoting effects entirely through regulating the electrical activity of target neurons and that alterations in activity levels, in turn, leads to changes in dendritic growth. This is unlikely in the case of apical dendrites, as blocking activity has no effect on dendrites. Moreover, if this idea is correct, then blocking endogenous BDNF should have qualitatively similar effects to blocking endogenous electrical activity. We found, in contrast, that blocking endogenous BDNF with TrkB receptor body (TrkB–IgG, 20 μg/ml; Shelton et al., 1995) had the opposite effect to inhibiting activity on dendritic growth (Figures 4A and 4B). While inhibiting synaptic activity with CNQX caused a 50% increase in dendrite modification index (DMI), neutralizing endogenous BDNF with TrkB–IgG caused a 50% decrease in DMI. Moreover, CNQX and TrkB–IgG affected distinct (though overlapping) aspects of dendritic growth (Table 3).

Inhibiting Activity Enhances Dendritic Growth in the Absence of BDNF

The mechanism by which activity inhibitors themselves produce modest dendritic growth (Figures 2 and 3; Table 2) remains unclear, but two lines of evidence presented thus far suggest that this regulation does not involve

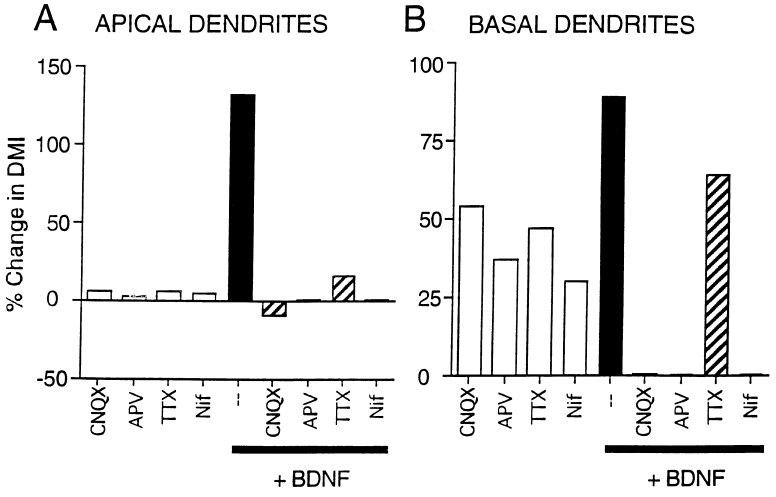

Figure 3. Inhibition of Electrical and Synaptic Activity Blocks the Effects of BDNF on Dendritic Complexity

The percent change in DMI is shown for each of the activity inhibitors alone, for BDNF alone, and for combinations. A zero value on these graphs indicates no change from control values for dendritic form. (A) For apical dendrites, inhibiting activity caused only minimal growth whereas BDNF caused a 140% increase in complexity. Inhibition of activity completely blocked the growth caused by BDNF alone. (B) For basal dendrites, both the activity inhibitors and BDNF caused increases in dendritic complexity. However, treatment with both BDNF and glutamate receptor or L-type calcium channel blockers (nifedipine, Nif) was not additive; instead, neurons treated with these combinations were similar to untreated cells. TTX did not prevent the effects of BDNF on basal dendritic complexity. Statistical significance was included in the DMI calculation at $p \leq 0.05$ by ANOVA (see McAllister et al., 1995).

BDNF. First, exogenous BDNF and activity-inhibitors regulated dendritic growth in distinct ways (Table 2). Second, the growth-promoting effects of activity inhibitors were blocked rather than enhanced by exogenous BDNF (Figure 3). We next used TrkB–IgG to determine if endogenous BDNF might be involved in the dendritic growth induced by activity inhibitors. In the presence of levels of TrkB–IgG that significantly antagonized endogenous BDNF (unpublished data), we found that CNQX and TTX were still able to elicit substantial dendritic growth (Figure 4C). In fact, the relative amounts of dendritic growth induced by CNQX and TTX were greater in the absence (Figure 4C) than in the presence of endogenous BDNF (Figure 4B). These results strongly suggest that the growth-promoting effects of CNQX and TTX do not depend directly on endogenous BDNF.

Discussion

Our results indicate that neurons in cortical slices must be electrically active to respond to the growth-promoting effects of BDNF. Similar interactions between activity and neurotrophins have been implicated in the survival of several neuronal cell types in dissociated cultures (Cohen-Cory et al., 1991; Birren et al., 1992; Franklin and Johnson, 1994; Meyer-Franke et al., 1995; Ghosh et al., 1994). For example, the survival-promoting effects of nerve growth factor (NGF) on dissociated Purkinje cells (Cohen-Cory et al., 1991) and those of BDNF on dissociated retinal ganglion cells (Meyer-Franke et al., 1995) are greatly enhanced by depolarizing agents such as high potassium, and BDNF is required for activity-dependent survival of dissociated cortical neurons (Ghosh et al., 1994). Although the mechanisms for neuronal survival in dissociated culture and dendritic outgrowth in intact brain slices are likely to be divergent, it appears that both developmental processes require interactions between activity and neurotrophins.

Table 2. Activity Inhibitors and BDNF Modify Dendritic Growth in Distinct Ways

Parameter	CNQX (62)	APV (48)	TTX (47)	Nif (49)	BDNF (52)
TBD	+	+	+	+	++
#Prims	0	0	0	0	++
#Brs	++	++	++	+	++

Inhibiting activity caused elongation and increased branching of existing dendrites, whereas BDNF increased the number of primary dendrites as well as the total length and number of dendritic branches. The magnitude of change in the average value for each parameter of dendritic growth was tabulated: 0 indicates no change from the untreated value, + indicates 40%–50% increase from the untreated value, ++ indicates 50%–140% increase. The number of neurons included in the calculation of the average for each parameter is in parentheses. For abbreviations, see Table 1.

Inhibiting activity at several levels blocked the growth-promoting effects of BDNF. Inhibiting action potentials and action potential–evoked synaptic transmission by TTX, glutamatergic synaptic activity by APV and CNQX, and L-type calcium channel activity by nifedipine could each block the enhancement of apical and/or basal dendritic growth by BDNF. These forms of activity could act as a sequence of electrical events; blocking this sequence at any point would be sufficient to block responsiveness to BDNF. Alternatively, these forms of activity may act through independent pathways that must be active to confer full BDNF responsiveness. For example, even in the absence of action potential activity, spontaneous synaptic transmission is sufficient to support basal dendritic responses to BDNF (Figure 3B). In addition, calcium entry through L-type calcium channels activates signal transduction cascades that become independent of electrical activity; these cascades overlap and may interact with those activated by receptor tyrosine kinases (Rusanescu et al., 1995; Rosen and Greenberg, 1996; reviewed by Finkbeiner and Greenberg, 1996).

While the locus of interaction between BDNF and electrical activity remains to be determined, it is unlikely that neurotrophins and activity regulate dendritic growth in a simple, ordered relationship. There are two such linear schemes, one in which activity regulates the production

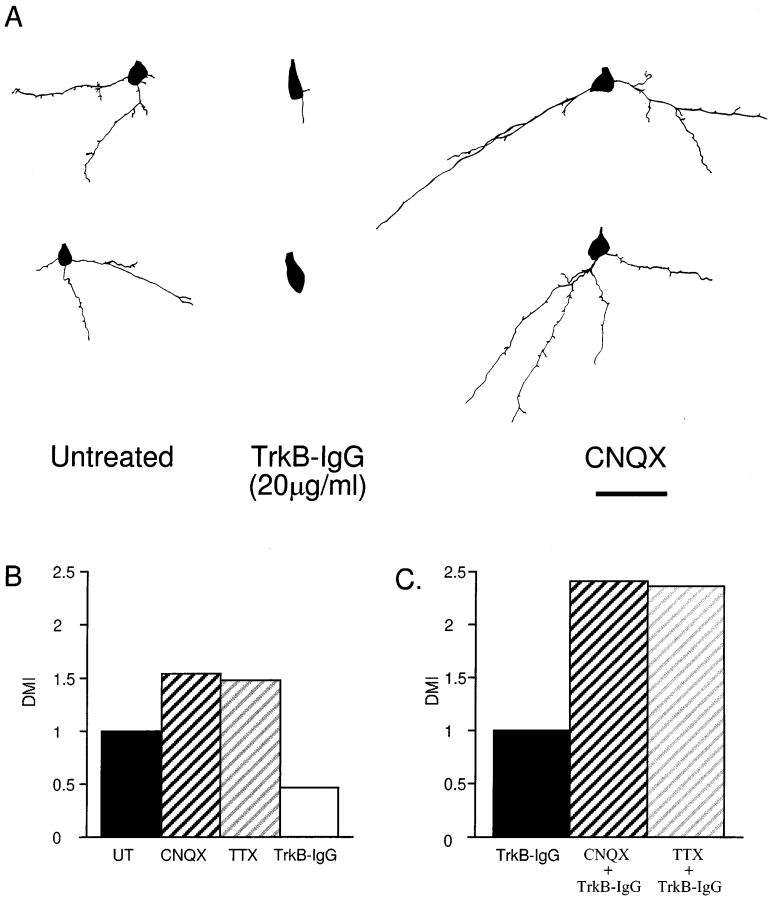

Figure 4. Inhibiting Activity and Neutralizing BDNF Have Opposite Effects on Dendritic Growth

(A) Camera lucida reconstructions of basal dendritic arborizations of representative layer 4 pyramidal neurons from untreated slices or slices treated with either TrkB–IgG (20 μg/ml) or CNQX (10 μM). Neutralizing endogenous BDNF markedly decreased basal dendrites while inhibiting activity caused elongation. Scale bar, 50 μm.

(B) Effects on the DMI for layer 4 basal dendrites, untreated or treated with CNQX, TTX, or TrkB–IgG, are shown; a DMI of 1.0 indicates no significant difference from untreated neurons. Inhibiting activity with either CNQX or TTX caused an approximately 50% increase in dendritic complexity while neutralizing endogenous BDNF with TrkB–IgG caused a 50% decrease in dendritic complexity.

(C) Inhibiting activity enhanced dendritic growth in the absence of endogenous BDNF. Changes in dendritic complexity are shown for layer 4 neurons treated with TrkB–IgG or with TrkB–IgG and either CNQX or TTX. The DMI in this case was calculated with respect to values for TrkB–IgG, i.e., the average values for TrkB–IgG treatment were used as the control values in the DMI formula (see Experimental Procedures).

of BDNF, and BDNF, in turn, regulates dendritic growth (Figure 5A). In this scheme, inhibiting activity should have no effect on the ability of exogenous BDNF to regulate dendritic growth; this scheme is contradicted by our observations (Figure 3). In the alternative linear scheme, BDNF regulates electrical activity, and electrical activity in turn regulates dendritic growth (Figure 5B). If so, then inhibiting endogenous BDNF and electrical activity should have similar effects on dendritic growth. We found, to the contrary, that neutralizing endogenous BDNF had the opposite effect than inhibiting electrical activity (Figure 4). In fact, activity inhibition clearly enhanced dendritic growth even in the nominal absence of endogenous BDNF (Figure 4C). Thus, BDNF and electrical activity must interact in a more complex way, perhaps by each exerting direct effects on target neurons, to produce the requirement that cortical neurons be electrically active to respond to the growth-promoting effects of neurotrophins (Figure 5C).

Several models of activity-dependent synaptic plasticity propose activity-dependent competition for a trophic factor, such as a neurotrophin (Purves, 1988; Fox and Zahs, 1994; Snider, 1994; Thoenen, 1995; Lo, 1995; Bonhoeffer, 1996), as a mechanism for structural remodeling. In such models, active synapses could be affected by activity-dependent postsynaptic neurotrophin secretion that strengthens synapses and stabilizes connections. Alternatively, such trophic interactions could proceed in an anterograde direction, as recently observed in the visual system (Von Bartheld et al., 1996). Many studies have demonstrated upregulation of neurotrophin mRNA in response to increased synaptic activity

Table 3. Activity Inhibitors and Blocking Endogenous BDNF Modify Basal Dendritic Growth in Opposite Ways

Parameter	Untreated (191)	CNQX (62)	TTX (47)	TrkB–IgG (40)	TrkB–IgG plus CNQX (40)	TrkB–IgG plus TTX (40)
Total basal dendrite (μm/10)	21.7 ± 1.0	**1.5**	**1.4**	**0.5**	1.2	1.1
Number of primary dendrites	3.7 ± 0.1	1.2	1.2	**0.5**	1.2	1.0
Number of dendritic branches	3.2 ± 0.2	**1.7**	**1.8**	**0.5**	1.1	**1.4**

Inhibiting activity caused increases in dendritic growth, whereas blocking endogenous BDNF with TrkB–IgG caused marked decreases in all parameters of dendritic growth. The average values ± SEM are tabulated for neurons in untreated slices. The ratio of the average values for treated neurons versus untreated cells is shown for each of the treatments. The number of neurons analyzed is in parentheses below the treatment name. Bold values were statistically significantly different from litter-matched control values by single-factor ANOVA with a significance level of $p \leq 0.01$.

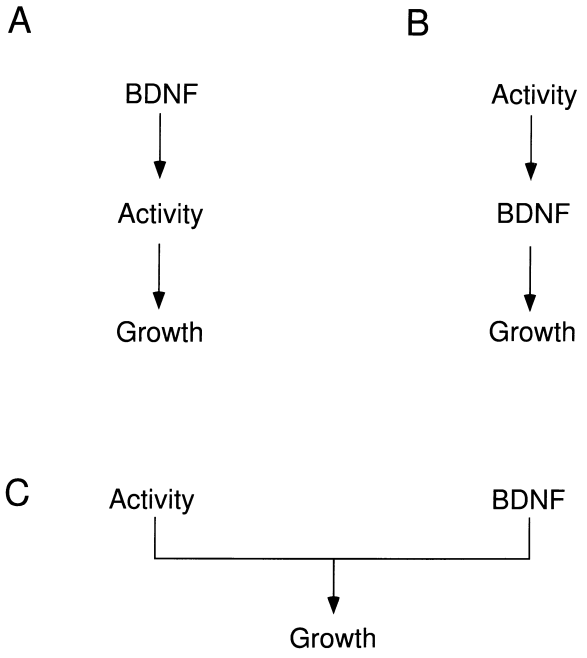

Figure 5. Three Possible Schemes for the Interaction of BDNF and Electrical Activity in Regulating Dendritic Growth in Developing Cortex

(Castren et al., 1992; Lindholm et al., 1994; Schoups et al., 1995; Thoenen, 1995; Lo, 1995; Bonhoeffer, 1996); however, it has not been clear how nonspecific release of neurotrophin in either direction could selectively stabilize only active inputs.

Our observations suggest that such specificity can be conferred by selective responsiveness to neurotrophins as determined by levels of synaptic and electrical activity. Thus, activity and neurotrophins must interact at at least two levels: activity increases neurotrophin production and secretion and also regulates responsiveness to these factors. It will be important in the future to determine whether the activity inhibitors in these experiments acted on the same neurons that responded to BDNF. Although this issue is central to determining the intracellular mechanisms by which specific forms of activity and BDNF interact, it does not detract from the general conclusion that neuronal circuits in the neocortex must be active to respond to BDNF. Altering electrical or molecular signals at any component of this circuit is likely to alter the function of other members of the circuit because all cortical neurons are connected in a complex network through specific intracortical connections. This requirement for conjoint activity and neurotrophin signaling provides a pathway through which electrical activity in the developing neocortex can be transduced into regulation of growth of cortical neurons.

Experimental Procedures

Ferrets

Ferrets were obtained from Marshall Farms (North Rose, NY). At P14, the visual system is immature; afferents from the LGN are just invading layer 4 and neurons destined for layers 2/3 are migrating through the cortical plate (McConnell, 1988). Layer 4 neurons are pyramidal at P14; the apical dendrite retracts later in development to form the spiny stellate cells characteristic of adult layer 4 (L. C. K., unpublished data). Eye-opening does not occur until 2 weeks later and retinal photoreceptors are not yet born at P14 (Allendoerfer et al., 1994).

Preparation of Visual Cortex Slice Cultures

Cultures of visual cortical slices were used in which connections between cells and thus patterns and levels of activity and gene expression are organotypic (Bolz et al., 1990; Stoppini et al., 1991; Molnar and Blakemore, 1991; Yamamoto et al., 1992); 400 μm coronal slices of P14 ferret visual cortex were prepared and cultured as previously described (McAllister et al., 1995). In brief, slices were prepared under sterile conditions in artificial cerebrospinal fluid (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 24 mM dextrose, 10 mM HEPES, 1 mM $CaCl_2$ [pH 7.4]) and then placed onto culture inserts in 6-well culture plates (0.4 μm pore size; Falcon). Culture medium (1 ml; 50% BME, 25% HBSS, 25% horse serum [Hyclone], 330 mM dextrose, 10 mM HEPES, and 10 U/ml penicilin–streptomycin [GIBCO]) was placed under each insert and the plates were incubated in 5% CO_2 at 37°C for 36 hr.

Neurotrophins, Activity Inhibitors, and Receptor Bodies

Human recombinant rHu-met-BDNF and TrkB–IgG were provided by Regeneron Pharmaceuticals (Tarrytown, NY) and were added to the medium of cortical slices to a concentration of 200 ng/ml or 20 μg/ml, respectively. This concentration of TrkB–IgG was experimentally determined to have a saturating effect on the inhibition of dendritic growth (unpublished data). The activity inhibitors were CNQX (10 μM; Research Biochemicals International), APV (50 μM), TTX (5 μM), and nifedipine (2 μM; Sigma). BDNF and/or inhibitors were added to the medium immediately following particle-mediated gene transfer and slices were returned to the incubator for 36 hr before visualization of transfected neurons.

Particle-Mediated Gene Transfer

Detailed procedures for particle-mediated gene transfer have been previously described (Lo et al., 1994). Particle-mediated gene transfer of EF β gal (*lacZ* driven by the Xenopus EF1α promoter; Krieg et al., 1989) was performed within 1–4 hr after slice preparation using a biolistics device (Bio-Rad PDS-1000/He). After bombardment, slices were immediately returned to the incubator and maintained for 36 hr in the presence or absence of BDNF and/or activity blockers.

Immunocytochemistry

Transfected neurons were then visualized by immunostaining using an affinity-purified rabbit antibody to β-galactosidase (5′–3′) and a Cy3-conjugated goat anti-rabbit secondary antibody (Chemicon) as previously described (McAllister et al. 1995). Slices were counterstained with 100 μM DAPI (4′,6-diamidino-2-phenylindole).

Cell Selection, Reconstruction, and Analysis

For each experimental treatment (activity blocker and/or BDNF), approximately 50 cells from two ferrets were used from each of two different litters; 40–60 control and BDNF-treated cells were always obtained from one to two animals from each litter to facilitate comparison with activity-blocked and combination treated cells.

We have previously demonstrated that biolistic transfection does not alter the health of neurons; there were no differences in dendritic form from cells visualized by intracellular injections and biolistic transfection, or in cell number and density in 1 μm thick, Nissl-stained plastic sections or in MAP2 staining in 400 μm thick transfected and control slices (see McAllister et al., 1995). BDNF treatment also did not change any of the measures of neuron health described above (unpublished data). Based on Lucifer Yellow intracellular fills of neurons at P14 before culturing and at P14 plus 36 hr in vitro, BDNF treatment appears to cause growth rather than selection of subpopulations of neurons (unpublished data).

Layer 4 was delineated by counter staining with DAPI, and the first 50 pyramidal neurons that were stained sufficiently to distinguish the tips of the smallest processes were reconstructed with a camera

lucida using a 63× objective and a standard rhodamine filter on a Zeiss Axiophot microscope. From these drawings, four parameters of dendritic growth were directly counted or measured: the total length of dendrite (μm), and the numbers of primary dendrites, branches of each dendrite, and protospines (spinelike processes less than 0.5 μm) as previously described (McAllister et al., 1995).

Calculation of the DMI

The dendrite modification index, or DMI, summarizes the average of the four parameters for each treatment into a value that conveys the direction and magnitude of resulting dendritic changes. This index is simply a weighted sum of the ratios for each of the four measures of dendritic growth in treated versus untreated cells calculated as:

$$\mathrm{DMI} = \sum_{i=1} C_i T_i / UT_i$$

where T_i is the value of a given parameter i after treatment, UT_i is the value in untreated slices, and C_i is a normalized weighting constant whose magnitude depends on the parameter (for more details, see McAllister et al., 1995). An increase in DMI indicates an increase in dendritic complexity, and a decrease in DMI indicates a decrease in dendritic complexity as compared with neurons in untreated slices.

Acknowledgments

Correspondence should be addressed to A. K. M. We thank Regeneron Pharmaceuticals for their generous gift of neurotrophins and receptor bodies and D. Fitzpatrick, D. Riddle, and N. Tang for critical reading of the manuscript. Supported by a Ruth K. Broad Biomedical Research Foundation Fellowship (A. K. M.), the Alfred P. Sloan Foundation (D. C. L.), National Institutes of Health grants NS32742 and EY11553 (D. C. L.) and EY07690 (L. C. K.), the McKnight Endowment Fund for Neuroscience (D. C. L.), and the Howard Hughes Medical Institute (L. C. K.).

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Received September 11, 1996; revised November 4, 1996.

References

Allendoerfer, K.L., Cabelli, R.J., Escandon, E., Kaplan, D.R., Nikolics, K., and Shatz, C.J. (1994). Regulation of neurotrophin receptors during the maturation of the mammalian visual system. J. Neurosci. *14*, 1795–1811.

Bailey, C.H., and Kandel, E.R. (1993). Structural changes accompanying memory storage. Annu. Rev. Physiol. *55*, 397–426.

Berardi, N., Cellerino, A., Domenici, L., Fagiolini, M., Pizzorusso, T., Cattaneo, A., and Maffei, L. (1994). Monoclonal antibodies to nerve growth factor affect the postnatal development of the visual system. Proc. Natl. Acad. Sci. USA *91*, 684–688.

Birren, S.J., Verdi, J.M., and Anderson, D.J. (1992). Membrane depolarization induces $p140^{trk}$ and NGF responsiveness, but not $p75^{LNGFR}$, in MAH cells. Science *257*, 395–397.

Blochl, A., and Thoenen, H. (1995). Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. Eur. J. Neurosci. *7*, 1220–1228.

Blochl, A., and Thoenen, H. (1996). Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. Mol. Cell. Neurosci. *7*, 173–190.

Bolz, J., Novak, N., Gotz, M., and Bonhoeffer, T. (1990). Formation of target-specific neuronal projections in organotypic slice cultures from rat visual cortex. Nature *346*, 359–362.

Bonhoeffer, T. (1996). Neurotrophins and activity-dependent development of the neocortex. Curr. Opin. Neurobiol. *6*, 119–126.

Cabelli, R.J., Hohn, A., and Shatz, C.J. (1995). Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. Science *267*, 1662–1666.

Castren, E., Zafra, F., Thoenen, H., and Lindholm, D. (1992). Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc. Nat. Acad Sci. USA *89*, 9444–9448.

Cohen-Cory, S., Dreyfus, C.F., and Black, I.B. (1991). NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. J. Neurosci. *11*, 462–471.

Domenici, L., Berardi, N., Carmignoto, G., Vantini, G., and Maffei, L. (1991). Nerve growth factor prevents the amblyopic effects of monocular deprivation. Proc. Natl. Acad. Sci. USA *88*, 8811–8815.

Finkbeiner, S., and Greenberg, M.E. (1996). Ca^{2+}-dependent routes to ras-mechanisms for neuronal survival, differentiation, and plasticity. Neuron *16*, 233–236.

Fox, K., and Zahs, K. (1994). Critical period control in sensory cortex. Curr. Opin. Neurobiol. *4*, 112–119.

Franklin, J.L., and Johnson, Jr., E.M. (1994). Block of neuronal apoptosis by a sustained increase of steady-state free Ca^{2+} concentration. Phil. Trans. Roy. Soc. (Lond.) B *345*, 251–256.

Galuske, R.A.W., Kim, D.-S., Castren, E., Thoenen, H., and Singer, W. (1996). Eur. J. Neurosci., in press.

Ghosh, A., Carnahan, J., and Greenberg, M.E. (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. Science *263*, 1618–1623.

Goodman, C.S., and Shatz, C.J. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell *72*, 77–98.

Goodman, L.J., Valverde, J., Lim, F., Geschwind, M.D., Federoff, H.J., Geller, A.I., and Hefti, F. (1996). Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol. Cell. Neurosci. *7*, 222–238.

Gu, Q., Liu, Y., and Cynader, M.S. (1994). Nerve growth factor-induced ocular dominance plasticity in adult cat visual cortex. Proc. Natl. Acad. Sci. USA *91*, 8408–8412.

Harris, R.M., and Woolsey, T.A. (1981). Dendritic plasticity in mouse barrel cortex following postnatal vibrissa follicle damage. J. Comp. Neurol. *196*, 357–376.

Katz, L.C., Gilbert, C.D., and Wiesel, T.N. (1989). Local circuits and ocular dominance columns in monkey striate cortex. J. Neurosci. *9*, 1389–1399.

Krieg, P.A., Varnum, S.A., Wormington, W.A., and Melton, D.A. (1989). The mRNA encoding elongation factor 1α (EF-1α) is a major transcript at the midblastula transition in *Xenopus*. Dev. Biol. *133*, 93–100.

Lindholm, D., Castren, E., Berzaghi, M., Blochl, A., and Thoenen, H. (1994). Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain: implications for neuronal plasticity. J. Neurobiol. *25*, 1362–1372.

Lindsay, R.M., Wiegand, S.J., Altar, C.A., and DiStefano, P.S. (1994). Neurotrophic factors: from molecule to man. Trends Neurosci. *17*, 182–190.

Lipton, S.A., and Kater, S.B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. Trends Neurosci. *12*, 265–270.

Lo, D.C. (1995). Neurotrophic factors and synaptic plasticity. Neuron *15*, 979–981.

Lo, D.C., McAllister, A.K., and Katz, L.C. (1994). Neuronal transfection in brain slices using particle-mediated gene transfer. Neuron *13*, 1263–1268.

Maffei, L., Berardi, N., Domenici, L., Parisi, V., and Pizzorusso, T. (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., Lo, D.C., and Katz, L.C. (1995). Neurotrophins regulate dendritic growth in developing visual cortex. Neuron *15*, 791–803.

McConnell, S.K. (1988). Fates of visual cortical neurons in the ferret

after isochronic and heterochronic transplantation. J. Neurosci. *8*, 945–974.

Merlio, J.-P., Ernfors, P., Jaber, M., and Persson, H. (1992). Molecular cloning of rat *trk*C and distribution of cells expressing messenger RNAs for members of the *trk* family in the rat central nervous system. Neuroscience *51*, 513–532.

Meyer-Franke, A., Kaplan, M.R., Pfrieger, F.W., and Barres, B.A. (1995). Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron *15*, 805–819.

Molnar, Z., and Blakemore, C. (1991). Lack of regional specificity for connections formed between thalamus and cortex in coculture. Nature *351*, 475–477.

Purves, D. (1988). Body and Brain (Cambridge, Massachusetts: Harvard University Press).

Reh, T.A., and Constantine-Paton, M. (1985). Eye-specific segregation requires neural activity in three-eyed *Rana pipiens*. J. Neurosci. *5*, 1132–1143.

Riddle, D.R., Lo, D.C., and Katz, L.C. (1995). NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation. Nature *378*, 189–191.

Ringstedt, T., Lagercrantz, H., and Persson, H. (1993). Expression of members of the *trk* family in the developing postnatal rat brain. Dev. Brain Res. *72*, 119–131.

Rosen, L.B., and Greenberg, M.E. (1996). Stimulation of growth factor receptor signal transduction by activation of voltage-sensitive calcium channels. Proc. Natl. Acad. Sci. USA *93*, 1113–1118.

Rusanescu, G., Qi, H., Thomas, S.M., Brugge, J.S., and Halegoua, S. (1995). Calcium influx induces neurite growth through a src-ras signaling cassette. Neuron *15*, 1415–1425.

Schoups, A.A., Elliott, R.C., Friedman, W.J., and Black, I.B. (1995). NGF and BDNF are differentially modulated by visual experience in the developing geniculocortical pathway. Dev. Brain Res. *86*, 326–334.

Shelton, D.L., Sutherland, J., Gripp, J., Camerato, T., Armanini, M.P., Phillips, H.S., Carroll, K., Spencer, S.D., and Levinson, A.D. (1995). Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. J. Neurosci. *15*, 477–491.

Snider, W.D. (1994). Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell *77*, 627–638.

Sretavan, D.W., Shatz, C.J., and Stryker, M.P. (1988). Modification of retinal ganglion cell axon morphology by prenatal infusion of tetrodotoxin. Nature *336*, 468–471.

Stoppini, L., Buchs, D.A., and Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. J. Neurosci. Meth. *37*, 173–182.

Thoenen, H. (1995). Neurotrophins and neuronal plasticity. Science *270*, 593–598.

Valverde, F. (1968). Structural changes in the area striata of the mouse after enucleation. Exp. Brain Res. *5*, 274–292.

Volkmar, F.R., and Greenough, W.T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science *176*, 1145–1147.

Von Bartheld, C.S., Byers, M.R., Williams, R., and Bothwell, M. (1996). Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. Nature *379*, 830–833.

Wiesel, T.N. (1982). Postnatal development of the visual cortex and the influence of environment. Nature *299*, 583–591.

Yamamoto, N., Yamada, K., Kurotani, T., and Toyama, K. (1992). Laminar specificity of extrinsic cortical connections studied in coculture preparations. Neuron *9*, 217–228.