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BDNF Has Opposite Effects on the Quantal Amplitude of Pyramidal Neuron and Interneuron Excitatory Synapses

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Summary

Recently, we have identified a novel form of synaptic plasticity that acts to stabilize neocortical firing rates by scaling the quantal amplitude of AMPA-mediated synaptic inputs up or down as a function of neuronal activity. Here, we show that the effects of activity blockade on quantal amplitude are mediated through the neurotrophin brain-derived neurotrophic factor (BDNF). Exogenous BDNF prevented, and a TrkB–IgG fusion protein reproduced, the effects of activity blockade on pyramidal quantal amplitude. BDNF had opposite effects on pyramidal neuron and interneuron quantal amplitudes and modified the ratio of pyramidal neuron to interneuron firing rates. These data demonstrate a novel role for BDNF in the homeostatic regulation of excitatory synaptic strengths and in the maintenance of the balance of cortical excitation and inhibition.

Introduction

During development, cortical synaptic connections undergo activity-dependent refinement that is crucial for generating the fine structure and response properties of cortical circuits (Shatz, 1990; O'Leary et al., 1994). Synapse-specific changes in synaptic strength such as long-term potentiation (LTP) and depression (LTD) are thought to play an important role in this process (Bienenstock et al., 1982; Miller et al., 1989; Kirkwood and Bear, 1994, 1995), but global mechanisms that stabilize neuronal activity by regulating the total synaptic strength of a neuron are also thought to be necessary for synaptic refinement and competition (von der Malsberg, 1973; Miller and MacKay, 1994; Miller, 1996; Davis and Goodman, 1998; Turrigiano et al., 1998). Recently, we have identified a novel form of synaptic plasticity that scales the quantal amplitude of cortical pyramidal neuron excitatory synapses up or down in response to changes in activity. This quantal scaling has the necessary characteristics to stabilize pyramidal neuron firing rates during periods of intense change in synapse number and strength, and allows neurons to adjust the total amount of excitatory current they receive while preserving relative differences between inputs (Turrigiano et al., 1998). While the characteristics and mechanism of quantal scaling appear to be fundamentally different from LTP and LTD, the nature of the signal linking changes in activity to the scaling of synaptic strengths has not yet been determined.

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In order to promote stability in firing rates, such a signal must be well correlated with activity and must be capable of coordinately and differentially regulating different classes of intracortical synapses. Cortical pyramidal neurons are embedded in complex networks with extensive recurrent excitatory and inhibitory feedback (Figure 1A). Pyramidal neuron firing rates reflect not just excitatory drive but the balance between excitatory inputs received from other pyramidal neurons and inhibitory inputs received from GABAergic interneurons. Activity in the interneurons is, in turn, driven by excitation from the pyramidal neurons. These interactions suggest that an increase in pyramidal neuron firing rates requires increased excitation onto pyramidal neurons and either decreased or constant excitation onto interneurons. Conversely, a reduction in pyramidal neuron firing rates requires decreased excitation onto pyramidal neurons and either constant or increased excitation onto interneurons. How might such differential regulation of the strength of different classes of intracortical synapses be accomplished? An attractive model is that pyramidal neuron activity is well correlated with the release of a substance that can act at many sites in the cortical circuit to coordinately adjust the strength of different classes of synaptic connections.

A signaling molecule with the necessary characteristics to play such a role is the neurotrophin brain-derived neurotrophic factor (BDNF). BDNF is produced by cortical pyramidal neurons, and the high affinity BDNF receptor TrkB is present on both pyramidal neurons and interneurons (Kokaia et al., 1993; Miranda et al., 1993; Cabelli et al., 1996; Cellarino et al., 1996). BDNF expression is regulated by activity (Isackson et al., 1991; Zafra et al., 1991; Castrén et al., 1992; Ghosh et al., 1994), and there is evidence that release of neurotrophins may also be activity dependent (Wetmore et al., 1994; Blochl and Thoenen, 1995). Long-term manipulations of BDNF in visual cortex have been shown to disrupt ocular dominance column segregation in vivo (Cabelli et al., 1995, 1997; Galuske et al., 1996) and to influence dendritic growth (McAllister et al., 1995, 1996, 1997) and cortical inhibition (Marty et al., 1997; Rutherford et al., 1997) in vitro, suggesting that BDNF plays an important role in activity-dependent cortical development and in the regulation of cortical excitability. Recently, neurotrophins have been shown to influence synaptic transmission in a variety of preparations. Acute application of neurotrophins can modulate synaptic transmission in hippocampus and cortex (Kim et al., 1994; Kang and Schuman, 1995; Figurov et al., 1996; Akaneya et al., 1997; Carmignoto et al., 1997). In addition, neurotrophins can influence the development of synaptic transmission over longer time scales at the neuromuscular junction (Wang et al., 1995; Liou and Fu, 1997; Wang and Poo, 1997) and at sympathetic synapses (Lockhart et al., 1997) and can regulate the expression of synaptic proteins in cortical neurons (Takei et al., 1997). The long-term effects of neurotrophins on intracortical synaptic strengths have not been examined.

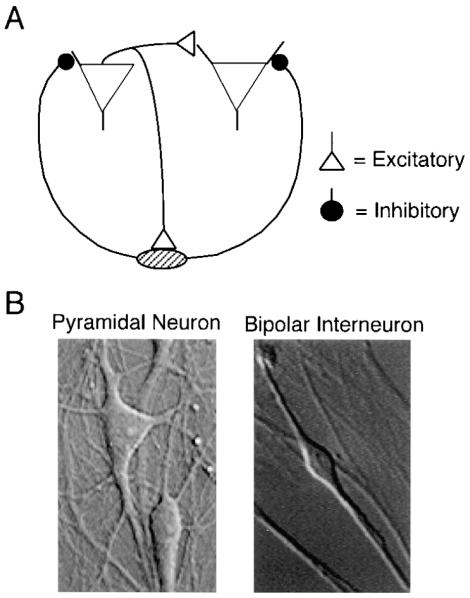

Figure 1. Differences in the Morphology and Connectivity of Cultured Cortical Pyramidal Neurons and Interneurons

(A) A diagram illustrating the recurrent connections between cortical pyramidal neurons and interneurons. Pyramidal neurons are shown as large open triangles, and interneurons as hatched ovals. Pyramidal neurons form excitatory connections (small open triangles) onto other pyramidal neurons and interneurons, and interneurons form inhibitory connections (closed circles) onto pyramidal neurons.

(B) Differential interference contrast images of pyramidal neurons and interneurons, showing the differences in morphology.

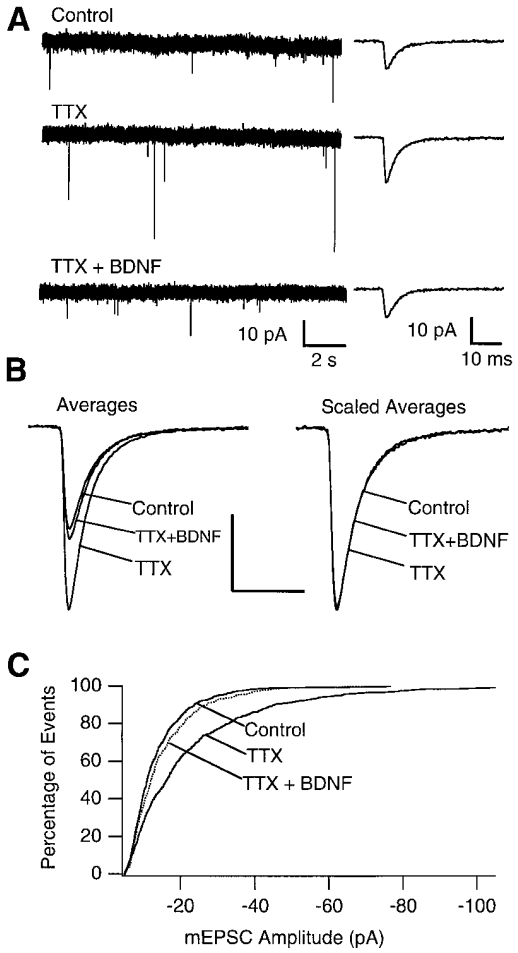

Figure 2. In Pyramidal Neurons, BDNF Prevents the Increase in Quantal Amplitude Produced by Activity Blockade

(A) Representative recordings from pyramidal neurons from sister cultures grown for 48 hr under control conditions, in TTX, or in TTX + BDNF (25 ng/ml).

(Left) A stretch of raw data with rapid AMPA-mediated mEPSCs.

(Right) The average mEPSC for the cell on the left at a faster time base.

(B) Average or scaled average mEPSC waveforms from control (n = 15) , TTX-treated (n = 17), or TTX + BDNF–treated (25 ng/ml, n = 16) pyramidal neurons. Vertical scale bar, 10 pA; horizontal scale bar, 10 ms.

(C) Cumulative amplitude histograms for control, TTX-treated, and TTX + BDNF–treated (25 ng/ml) neurons. Histograms were constructed by randomly selecting 40 mEPSCs from each neuron and pooling the mEPSCs from each condition.

Here, we demonstrate that BDNF differentially regulates the quantal amplitude of the AMPA-mediated synapses formed between pyramidal neurons and two of their targets: other pyramidal neurons and inhibitory interneurons. Exogenous BDNF opposes the effects of activity blockade on pyramidal quantal amplitude by decreasing quantal amplitude back to control levels, and scavenging endogenous TrkB ligands increases quantal amplitude to a similar extent as activity blockade. In contrast, exogenous BDNF increases interneuronal quantal amplitude, and these differential effects of BDNF contribute to differential effects on pyramidal neuron and interneuron firing rates. These data demonstrate that BDNF participates in the activity-dependent scaling of cortical synaptic strengths and adjusts the relative balance of cortical excitation and inhibition by coordinately regulating the strengths of different classes of cortical synapses.

Results

Regulation of Pyramidal Neuron Quantal Amplitude by BDNF

Chronic activity blockade in cortical cultures increases the amplitude of pyramidal neuron miniature excitatory postsynaptic currents (mEPSCs) without changing mEPSC kinetics, whereas raising firing rates decreases mEPSC amplitude. These effects are accompanied by changes in the postsynaptic responsiveness to glutamate and occur through a multiplicative scaling of synaptic strengths that occurs slowly over tens of hours (Turrigiano et al., 1998). Chronic decreases in activity are known to decrease BDNF expression both in vivo and in vitro (Isackson et al., 1991; Zafra et al., 1991; Castrén et al., 1992; Bozzi et al., 1995), suggesting that the increase in mEPSC amplitude produced by activity blockade could be due to reduced release of BDNF and a consequent reduction in the activation of TrkB receptors. To test this, we determined whether exogenous BDNF prevents the increase in pyramidal quantal amplitude produced by activity blockade.

To sample the quantal amplitude at a large number

Table 1. mEPSC Frequencies and Rise Times

Condition	mEPSC Frequencies		mEPSC Rise Times	
	Pyramidal	Interneuron	Pyramidal	Interneuron
Control	0.43 ± 0.05	0.44 ± 0.13	0.73 ± 0.06	0.81 ± 0.05
TTX	0.58 ± 0.22	0.32 ± 0.05	0.71 ± 0.05	0.85 ± 0.04
TTX + BDNF (25 ng/ml)	0.51 ± 0.11	0.42 ± 0.07	0.77 ± 0.04	0.89 ± 0.07
TrkB–IgG	0.73 ± 0.20	0.30 ± 0.05	0.70 ± 0.05	0.75 ± 0.06
TrkA–IgG	0.66 ± 0.12	ND	0.82 ± 0.05	ND
BDNF (25 ng/ml)	0.56 ± 0.11	0.38 ± 0.06	0.81 ± 0.05	0.82 ± 0.05

Average mEPSC frequencies and rise times for pyramidal or interneurons grown for 48 hr in the different experimental conditions. ANOVAs and individual t tests reveal no significant differences between conditions for values from pyramidal neurons or interneurons.

of synapses, we measured mEPSCs arising from activation of AMPA receptors. Data were obtained from visual cortical cultures prepared from P4–P6 rat pups and maintained in culture for 7–9 days prior to recording. These cultures contain ~75% pyramidal neurons and 25% GABAergic interneurons (Rutherford et al., 1997). Over the first few day in culture, these neurons extend processes, form excitatory and inhibitory connections, and become spontaneously active (Rutherford et al., 1997; Turrigiano et al., 1998). Whole-cell voltage clamp recordings were obtained from cultured cortical pyramidal neurons (Figure 1B) with spike-mediated transmission, NMDA receptors, and $GABA_A$ receptors blocked.

For pyramidal neurons grown under control conditions, the average mEPSC amplitude was −15.2 ± 0.7 pA (measured at −70 mV, n = 64). In accord with previous results (Turrigiano et al., 1998), growing cultures for 48 hr in tetrodotoxin (TTX) to block spontaneous activity produced a significant increase in the AMPA quantal amplitude of pyramidal neurons, to 177.6% ± 12.2% of values from sister control cultures (Figures 2A and 2B; TTX different from control, corrected t test, $p < 0.0001$, n = 17). Cumulative amplitude histograms showed that the entire distribution of amplitudes was shifted toward larger values following 48 hr of TTX treatment (Figure 2C). BDNF completely blocked the increase in pyramidal neuron mEPSC amplitude produced by TTX treatment. In the presence of exogenous BDNF (25 ng/ml), 48 hr of TTX treatment had no significant effect on mEPSC amplitude (Figures 2A and 2B; n = 16). Amplitudes from cultures treated with TTX + BDNF were 108.5% ± 7.4% of control values and were significantly different from TTX alone ($p < 0.005$, corrected t test) but were not significantly different from control. Cumulative amplitude histograms showed that the amplitude distribution following TTX + BDNF treatment was similar to that recorded from control cultures (Figure 2C).

To compare the kinetics of mEPSCs under the different conditions, average mEPSC waveforms were generated for all neurons recorded under each condition (Figure 2B). Normalizing the peak amplitudes of the average mEPSCs to one and overlaying them demonstrated that the average kinetics of the mEPSCs were not influenced by treatment with either TTX or TTX + BDNF. Individual measurements of rise times confirmed that there were no significant differences between conditions (Table 1). This indicates that the changes in mEPSC amplitude produced by these different treatments are not the result of differences in electrotonic filtering. In accord with previous results (Rutherford et al., 1997; Turrigiano et al., 1998), none of the treatments described here or below had significant effects on mEPSC frequencies or on cellular properties such as input resistance (R_{in}), whole cell capacitance, and resting potentials (V_m) (Table 2; one way ANOVAs revealed no significant differences in these parameters between experimental conditions, unless otherwise noted).

Regulation of Interneuronal Quantal Amplitude by BDNF

In culture as in vivo, cortical pyramidal neurons form AMPA-mediated synapses onto both other pyramidal neurons and onto inhibitory interneurons. TTX treatment for 48 hr does not increase the amplitude of AMPA mEPSCs recorded from GABAergic interneurons (Turrigiano et al., 1998), suggesting that the role activity plays in regulating the synapses formed by pyramidal neurons depends on the nature of the target neuron. To determine whether BDNF influences AMPA synapses on interneurons differently from those on pyramidal neurons, we recorded AMPA mEPSCs from a readily identifiable class of interneurons, the bipolar interneurons (Figure 1B) (Rutherford et al., 1997; Turrigiano et al., 1998). Control AMPA mEPSCs from bipolar interneurons were similar in amplitude to those from pyramidal neurons (−17.5 ± 1.1 pA, n = 43) but had significantly faster time constants of decay, as expected (Hestrin 1992, 1993) (Table 2). In striking contrast to the decrease in pyramidal neuron mEPSC amplitude produced by BDNF, application of exogenous BDNF (25 ng/ml) for 48 hr produced a significant increase in mEPSC amplitude in

Table 2. Control Values of Cellular and mEPSC Properties of Pyramidal Neurons and Interneurons

	Pyramidal Neurons (n = 64)	Interneurons (n = 43)
V_m (mV)	−60.1 ± 1.2	−60.3 ± 0.7
R_{in} (MΩ)	603 ± 71	656 ± 70
WCC (pF)	36.6 ± 3.3	26.1 ± 2.3*
R_s (MΩ)	8.8 ± 0.7	8.7 ± 0.5
mEPSC rise time (ms)	0.73 ± 0.06	0.81 ± 0.03
mEPSC τ decay (ms)	3.29 ± 0.15	2.80 ± 0.13*

Average values for pyramidal neurons and interneurons grown under control conditions. V_m, membrane potential; R_{in}, input resistance; WCC, whole cell capacitance; R_s, series resistance; τ decay, time constant of decay of the mEPSCs. *Interneuron values significantly different from pyramidal neurons ($p < 0.03$).

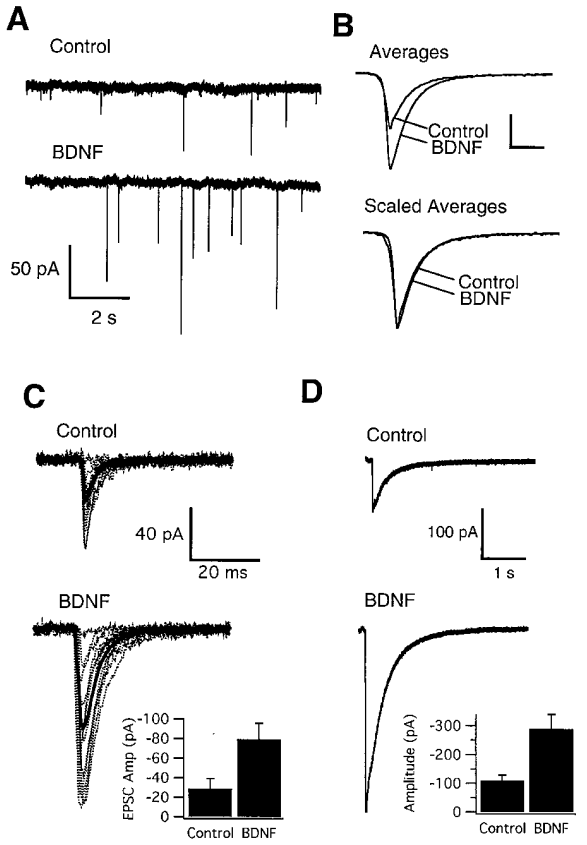

Figure 3. In Bipolar Interneurons, BDNF Increases the Quantal Amplitude of AMPA mEPSCs

(A) Representative recordings from bipolar interneurons from sister cultures grown under control conditions or in the presence of 25 ng/ml BDNF for 48 hr.

(B) Average or scaled average mEPSC waveforms from control (n = 15) or BDNF-treated (25 ng/ml, n = 15) bipolar interneurons. Vertical scale bar, 10 pA; horizontal scale bar, 5 ms.

(C) Paired recordings between pyramidal neurons (presynaptic) and interneurons (postsynaptic) showing the amplitude of monosynaptic EPSCs recorded at −70 mV. Shown are representative monosynaptic EPSCs from cultures grown under control conditions (Control) or in the presence of BDNF for 48 hr (BDNF). Dotted traces are individual EPSCs, and the thick solid lines are the average EPSCs for each pair.

(Inset) EPSC amplitude averaged across cells; n = 4 for each condition.

(D) BDNF increases the postsynaptic sensitivity of bipolar interneurons to glutamate. Representative responses of interneurons grown under control conditions (Control), or in the presence of BDNF (BDNF), to a brief application of glutamate. Each trace shows the average of five responses elicited from the same site.

(Inset) Response amplitude averaged across cells for control (n = 6) and BDNF-treated (n = 5) neurons.

interneurons, to 190% ± 30% of control values (Figures 3A and 3B; corrected t test, $p < 0.002$, n = 15). Cumulative amplitude distributions showed that BDNF shifted the entire amplitude distribution toward higher values (data not shown). BDNF had no significant effect on mEPSC kinetics (Figure 3B). These data indicate that AMPA quantal amplitudes in pyramidal neurons and interneurons are regulated in the opposite direction by BDNF.

BDNF Increases Spike-Mediated Transmission between Pyramidal Neurons and Interneurons and Increases the Postsynaptic Responsiveness to Glutamate

The increase in pyramidal neuron quantal amplitude produced by activity blockade results in an increase in spike-mediated transmission between pyramidal neurons (Turrigiano et al., 1998). To verify that the increase in interneuronal quantal amplitude produced by BDNF also translates into a change in spike-mediated synaptic transmission, we obtained paired recordings between pyramidal neurons (presynaptic) and interneurons (postsynaptic) and determined the effect of BDNF on AMPA-mediated transmission at this synapse. Paired recordings were obtained from sister cultures grown under control conditions, or in the presence of 25 ng/ml BDNF for 48 hr. BDNF significantly increased the amplitude of monosynaptic EPSCs onto interneurons, from 28.9 ± 10.0 to 79.6 ± 16.3 pA (measured at −70 mV) (Figure 3C; n = 4 in each condition, $p < 0.04$). BDNF had no effect on EPSC reversal potential (BDNF, 0.0 ± 1.1 mV; control, 2.8 ± 2.4 mV). This increase in spike-mediated transmission is similar in magnitude to the increase in quantal amplitude produced by BDNF (272% ± 56% and 190% ± 30% of control values, respectively).

We have shown previously that the activity-dependent change in pyramidal-to-pyramidal neuron synaptic strength is accompanied by a change in the postsynaptic responsiveness to glutamate (Turrigiano et al., 1998). To ask whether this is also true of the regulation of pyramidal-to-interneuron synaptic strength by BDNF, we grew cultures under control conditions or in the presence of 25 ng/ml BDNF for 48 hr and examined the responses of interneurons to applied glutamate. Bipolar interneurons were voltage clamped to −70 mV in the presence of TTX, APV, and bicuculline, and postsynaptic currents were elicited by application of brief puffs of glutamate (0.5 mM for 20 ms) from a patch pipette. Glutamate administered in this way to the soma or 40 μm along the dendrite of bipolar interneurons produced currents that were highly reproducible from trial to trial in amplitude and time course. BDNF treatment significantly increased the amplitude of the glutamate currents recorded from the soma, from 109.1 ± 19.4 to 288.9 ± 51.2 pA (Figure 3D; measured at −70 mV, $p < 0.02$), and had a similar effect on dendritic currents. Control and BDNF responses had similar latencies (15.8 ± 3.5 and 15.3 ± 3.1 ms, respectively) and times to peak (25.1 ± 5.4 and 22.4 ± 4.0 ms, respectively). These data suggest that BDNF regulates mEPSC amplitude through a postsynaptic change in AMPA receptor number or function.

The Effects of BDNF on Quantal Amplitude Are Dose Dependent

To determine the concentration of exogenous BDNF required to prevent the increase in pyramidal quantal amplitude produced by activity blockade, we coincubated cultures for 48 hr in TTX and different concentrations of BDNF, ranging from 0.01 to 25 ng/ml. The effect of BDNF on pyramidal quantal amplitude was dose dependent and saturated at concentrations of BDNF as

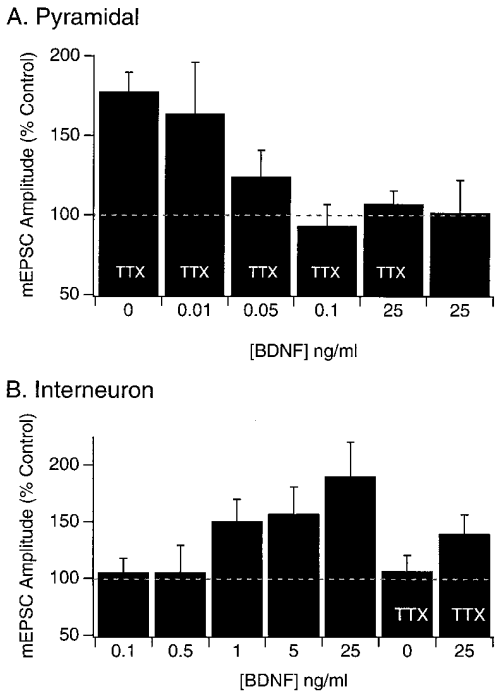

Figure 4. The Effects of BDNF on Quantal Amplitude Are Dose Dependent

(A) Pyramidal neurons. The quantal amplitudes for AMPA mEPSCs from cultures treated with TTX, or TTX and the indicated concentration of BDNF, expressed as a percentage of control values. The number of neurons per condition, from left to right, were 21, 7, 8, 7, 16, and 15. BDNF had a significant effect on mEPSC amplitude (one way ANOVA, $p < 0.0001$). The dashed gray line indicates control values (100%).

(B) Interneurons. Average quantal amplitude for bipolar interneurons grown for 48 hr in the indicated concentration of BDNF or in TTX or TTX + BDNF (25 ng/ml). BDNF had a significant effect on mEPSC amplitude (one way ANOVA, $p < 0.002$). The number of neurons per condition, from left to right, were 7, 9, 10, 6, 15, 15, and 17. The dashed gray line indicates control values (100%).

low as 0.1 ng/ml (Figure 4A; one way ANOVA, $p < 0.0001$). This concentration is much lower than those generally needed to elicit acute modulatory effects of BDNF on synaptic transmission (generally 10–200 ng/ml) (Kang and Schuman, 1995; Figurov et al., 1996; Stoop and Poo, 1996; Akaneya et al., 1997; Carmignoto et al., 1997) and is in the range of concentrations sufficient to support neuronal survival and to influence GABA expression and inhibition in cortical cultures (0.05–1 ng/ml) (Pinon et al., 1995; Cohen et al., 1996; Rutherford et al., 1997).

BDNF either in the presence of TTX or alone did not reduce mEPSC amplitude significantly below control values (Figure 4A). This suggests that under control conditions the endogenous concentration of BDNF is saturating for the effect on pyramidal quantal amplitude. Additionally, these data indicate that the decrease in mEPSC amplitude below control values observed following elevation of firing rates (Turrigiano et al., 1998) is not due solely to increased activation of TrkB receptors by BDNF, although it is possible that BDNF interacts with additional unidentified factors to regulate quantal amplitude in the high activity regime. This suggests that there may be multiple signals that interact to regulate pyramidal quantal amplitude over the whole range of firing rates exhibited by cortical pyramidal neurons.

The effect of BDNF on interneuron mEPSC amplitude was also dose dependent. While doses of BDNF of 0.5 ng/ml and lower had no effect, at concentrations between 1 and 25 ng/ml BDNF progressively increased interneuronal mEPSC amplitude (Figure 4B; BDNF has a significant effect on mEPSC amplitude, one way ANOVA, $p < 0.002$). As reported previously (Turrigiano et al., 1998), 48 hr of TTX treatment had no significant effect on interneuron mEPSC amplitude. BDNF (25 ng/ml) significantly elevated mEPSC amplitude when applied in the presence of TTX, although this increase was smaller than that produced by BDNF alone (Figure 4B).

The dose of BDNF required to first see an effect on interneuron mEPSC amplitude (1 ng/ml) is at least an order of magnitude higher than that needed to completely block the effects of TTX on pyramidal neuron mEPSC amplitude (0.1 ng/ml). These data indicate that, in addition to regulating pyramidal neuron and interneuron quantal amplitude in opposite directions, BDNF operates over different concentration ranges at these two sites.

Regulation of Quantal Amplitude by Endogenous TrkB Ligands

What role do endogenous ligands of TrkB play in the regulation of these two classes of synapse? To address this, cultures were incubated for 48 hr with a TrkB–IgG fusion protein (Shelton et al., 1995). This protein binds both BDNF and NT-4/5 with high affinity, thus preventing activation of TrkB receptors by endogenous release of neurotrophins. We have shown previously that blockade of Trk receptors for 48 hr does not influence neuronal survival in these postnatal cortical cultures (Rutherford et al., 1997). Incubation with the TrkB–IgG fusion protein (2 μg/ml) produced a significant increase in the amplitude of pyramidal neuron mEPSCs, to 168.1% $\pm$ 22.9% of control values (Figure 5; $p < 0.012$, corrected t test, $n = 16$), indicating that activation of endogenous TrkB receptors acts to keep pyramidal quantal amplitudes low under control conditions. In contrast, incubation with a TrkA–IgG fusion protein (2 μg/ml) (which binds nerve growth factor [NGF] with high affinity) had no effect on mEPSC amplitude (98.5% $\pm$ 10.1% of control values, $n = 16$). Comparisons of the average mEPSC waveforms showed no significant difference in kinetics between conditions, and this was confirmed with individual measurements of rise times (Table 1). These data show that scavenging endogenous TrkB ligands mimics the effects of activity blockade on pyramidal neuron quantal amplitude. Taken together with the observation that low concentrations of exogenously applied BDNF prevent the effects of activity blockade, these data strongly suggest that reduced activity adjusts the strength of pyramidal-to-pyramidal neuron excitatory synaptic connections through a reduction in activation of TrkB receptors.

In contrast to the effects of the TrkB–IgG fusion protein on pyramidal mEPSC amplitude, the TrkB–IgG had

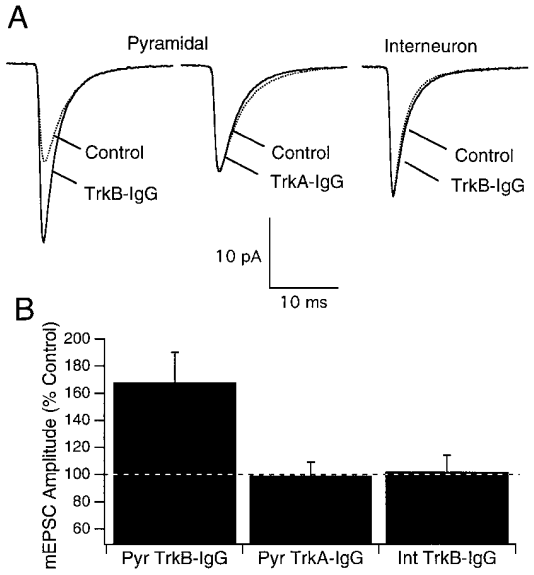

Figure 5. The TrkB–IgG Fusion Protein Increases the Quantal Amplitudes of Pyramidal Neuron AMPA Synapses but Not Those from Interneurons

Cultures were treated with either the TrkB–IgG or TrkA–IgG fusion protein (2 μg/ml) for 48 hr and mEPSC amplitudes measured relative to amplitudes from sister control cultures. N = 16 for each condition. For pyramidal neurons, TrkB–IgG is significantly different from control values, but TrkA–IgG is not (one way ANOVA, $p < 0.001$; TrkB–IgG different from control, $p < 0.012$, corrected t test).

no effect on the amplitude of mEPSCs in bipolar interneurons (Figure 5; 102.4% ± 11.7% of control, n = 16). These data suggest that under control conditions endogenous levels of TrkB ligands in these cultures are not sufficient to regulate the quantal amplitude of interneuronal AMPA synapses. This is consistent with the requirement for much higher concentrations of exogenous BDNF to regulate quantal amplitude at these synapses than at pyramidal neuron synapses (Figure 4). Interestingly, the TrkB–IgG fusion protein produced a significant reduction in the whole cell capacitance of bipolar interneurons, from 26.1 ± 2.3 to 19.4 ± 1.6 pF, and a concomitant increase in the input resistance from 0.656 ± 0.070 to 1.116 ± 0.186 GΩ. These effects are consistent with reports that BDNF increases the soma size and growth of cortical and hippocampal interneurons and suggest that although endogenous levels of TrkB ligands are not sufficient to regulate bipolar quantal amplitude, they are sufficient to regulate the growth of these neurons (Widmer and Hefti, 1994; Marty et al., 1996a, 1996b, 1997).

Differential Regulation of Pyramidal Neuron and Interneuron Firing Rates by Activity and BDNF

Our data indicate that when activation of TrkB receptors is reduced, excitation onto pyramidal neurons increases (Figure 6A, TTX/TrkB–IgG), whereas raising the BDNF concentration increases excitation onto interneurons (Figure 6A, BDNF). These data suggest that the relative activity of interneurons and pyramidal neurons will be differentially regulated by manipulations that increase or

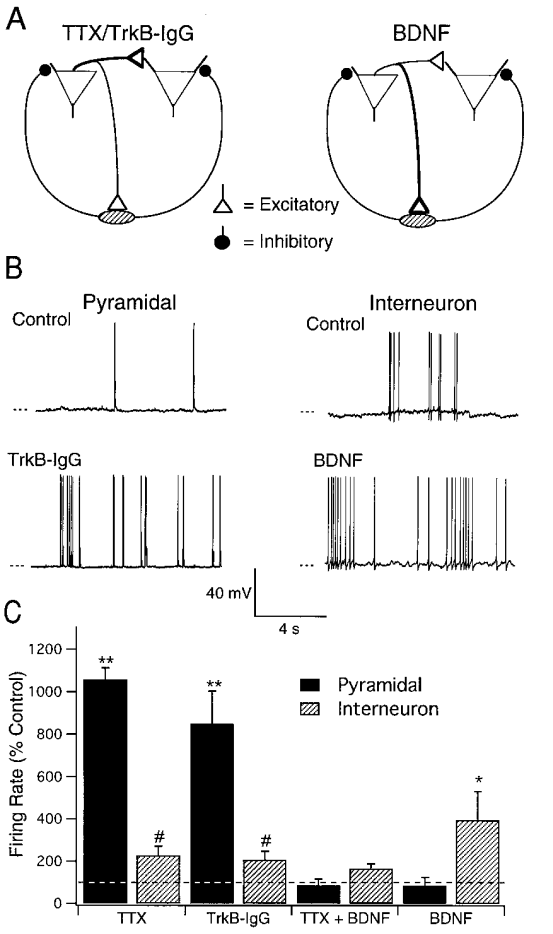

Figure 6. BDNF Differentially Regulates Pyramidal and Interneuronal Firing Rates

(A) Diagram of changes in quantal amplitude produced by different experimental conditions.

(Left) Activity blockade, or the TrkB–IgG fusion protein, increases quantal amplitude of pyramidal-to-pyramidal connections (indicated by thick lines) while leaving pyramidal-to-interneuron connections unchanged.

(Right) BDNF increases the quantal amplitude of pyramidal-to-interneuron synapses.

(B) Representative current clamp recordings from pyramidal neurons (left) and interneurons (right) showing spontaneous activity from neurons grown under the indicated conditions for 48 hr. Dashed lines indicate −60 mV.

(C) The effects of treatment with TTX, BDNF, and the TrkB–IgG fusion protein for 48 hr on the spontaneous firing rates of pyramidal neurons and interneurons. Data are expressed as a percentage of control. Cultures were treated for 48 hr with either TTX, TTX + BDNF (25 ng/ml), BDNF (25 ng/ml), or the TrkB–IgG fusion protein (2 μg/ml). The number of neurons per condition, from left to right, were 23, 16, 16, 16, 13, 13, 10, and 10. Recordings were made in regular ACSF following washout of the different pharmacological agents. All data are compared to spontaneous firing rates from pyramidal neurons or bipolar interneurons from sister control cultures. Pyramidal and interneuronal firing rates varied significantly as a function of treatment condition (one way ANOVAs, $p < 0.0001$). Unpaired corrected t tests for experimental condition versus control: #$p < 0.03$, *$p < 0.01$, and **$p < 0.001$.

decrease the activation of TrkB receptors. Specifically, growing cultures under conditions of low activity or in the TrkB–IgG fusion protein should enhance pyramidal

neuron firing rates, while growing cultures under conditions of high BDNF should enhance interneuronal firing rates. Consistent with this, we and others have shown that chronic activity blockade raises pyramidal neuron firing rates upon washout of TTX (Ramakers et al., 1990; Rutherford et al., 1997), and this can be prevented by coincubation with BDNF but not NGF or neurotrophin-3 (NT-3) (Rutherford et al., 1997).

To ask whether manipulations of BDNF produce selective changes in the relative activity of pyramidal neurons and interneurons, we grew cultures for 48 hr in either TTX, the TrkB–IgG fusion protein, TTX + BDNF (25 ng/ml), or BDNF alone (25 ng/ml) and measured the spontaneous firing rates of pyramidal neurons and bipolar interneurons relative to controls following washout of the pharmacological agents (Figures 6B and 6C). The average control firing rates for pyramidal neurons and interneurons were 0.1 ± 0.06 Hz and 0.28 ± 0.24 Hz, respectively. TTX treatment elevated pyramidal neuron firing rates by a factor of 10.6 but elevated bipolar neuron firing rates by only a factor of 2.3 (Figure 6C), thus shifting the balance of activity toward pyramidal neuron firing. The small elevation seen in interneuronal firing rates is likely to result from an increase in synaptic drive from more rapidly firing pyramidal neurons. Treatment with the TrkB–IgG fusion protein had effects on firing rates similar to TTX treatment. Cotreatment with BDNF and TTX prevented the increase in pyramidal neuron firing rates produced by TTX alone and produced a small elevation in interneuronal firing rates, in keeping with the small increase in AMPA quantal amplitude in interneurons under these conditions (Figure 4). Finally, BDNF alone significantly elevated interneuron firing rates by a factor of 4.0, while pyramidal neuron firing rates were unaffected, thus shifting the balance of activity to favor inhibition. These data demonstrate that chronically reducing TrkB activation reorganizes these cultured cortical networks to favor activity in excitatory neurons, whereas chronically increasing TrkB activation with BDNF reorganizes the networks to favor firing in inhibitory interneurons.

Discussion

Our results demonstrate that long-term manipulations of endogenous and exogenous BDNF produce dramatic changes in the quantal amplitude of intracortical synapses. BDNF regulates the quantal amplitude of AMPA synapses onto pyramidal neurons and interneurons in opposite directions and has opposing effects on pyramidal neuron and interneuron firing rates. In addition, the effects of BDNF on pyramidal and interneuronal quantal amplitude occur over different concentration ranges, suggesting that the sites of action of this neurotrophin shift as the concentration increases. These data suggest that activity-dependent changes in the release of BDNF adjust the synaptic connectivity of in vitro cortical networks to promote stability in pyramidal neuron firing rates. When activity is low and BDNF release is reduced, synaptic strengths are adjusted to promote excitation onto pyramidal neurons, and when activity is high and BDNF release increases, synaptic strengths are adjusted to promote excitation onto interneurons, which should in turn recruit more inhibition onto pyramidal neurons. This suggests that BDNF plays a key role in the activity-dependent stabilization of cortical activity.

Role of BDNF in the Activity-Dependent Regulation of Pyramidal Neuron Quantal Amplitude

Chronic changes in activity can bidirectionally scale the quantal amplitude of AMPA-mediated pyramidal neuron synapses. Activity blockade scales quantal amplitude up, whereas raising firing rates with bicuculline scales quantal amplitude down (Turrigiano et al., 1998). Our data strongly suggest that the increase in quantal amplitude produced by activity blockade occurs through activity-dependent changes in the activation of TrkB receptors. Exogenous BDNF prevents the increase in quantal amplitude produced by activity blockade, and reducing activation of endogenous TrkB receptors with the TrkB–IgG fusion protein mimics the effects of activity blockade. In addition, the ability of BDNF to antagonize the effects of activity blockade are dose dependent, demonstrating that mEPSC amplitude varies in a continuous manner as a function of BDNF concentration. Numerous studies both in vitro and in vivo have demonstrated that BDNF expression is regulated by activity (Isackson et al., 1991; Zafra et al., 1991; Castrén et al., 1992; Ghosh et al., 1994). If release of BDNF is also activity dependent, as has been suggested (Wetmore et al., 1994; Blochl and Thoenen, 1995; Bonhoeffer, 1996), then our data support a model in which activity continuously modifies pyramidal quantal amplitude by modulating release of BDNF.

Glutamatergic Synapses onto Pyramidal Neurons and Interneurons Are Regulated Differently

Pyramidal neurons form glutamatergic synapses onto both other pyramidal neurons and inhibitory interneurons. Our data show that the effect of BDNF on the AMPA component of these synapses is a function of the postsynaptic target. Whereas BDNF decreases the AMPA quantal amplitude of pyramidal neurons, BDNF increases the AMPA quantal amplitude of interneurons. These differential effects must arise either from differential regulation of pyramidal neuron synaptic terminals formed onto the two classes of neuron or through differences in the postsynaptic responses to BDNF. The latter interpretation is supported by the observation that the scaling of quantal amplitude in both pyramidal neurons (Turrigiano et al., 1998) and interneurons (Figure 3D) is accompanied by changes in the postsynaptic responsiveness to glutamate, suggesting that it occurs through a postsynaptic change in the number or function of AMPA receptors. This suggests that BDNF acts directly on both classes of cortical neuron to regulate the postsynaptic expression or function of AMPA receptors, although our data do not rule out additional presynaptic effects on pyramidal synaptic terminals.

Whereas both interneurons and pyramidal neurons express TrkB receptors on their somas and dendrites, neuronal production of BDNF appears to be restricted to pyramidal neurons (Kokaia et al., 1993; Miranda et al., 1993; Cabelli et al., 1996; Cellarino et al., 1996). This

suggests that interneuronal quantal amplitudes are regulated by release of BDNF from pyramidal neurons, as a function of pyramidal neuron activity. Interestingly, BDNF begins to increase interneuronal quantal amplitude at concentrations that are much higher ($\sim$1 ng/ml) than concentrations that have maximally reduced pyramidal quantal amplitude ($\sim$0.1 ng/ml). This suggests that the sites of action of BDNF shift as pyramidal neuron activity rises. The ability of BDNF to regulate interneuronal quantal amplitudes at higher concentrations may represent a mechanism for recruiting additional inhibition onto pyramidal neurons under very high activity regimes. This may allow pyramidal neuron firing rates to be stabilized without reducing the strength of excitatory connections between pyramidal neurons so low as to compromise synaptic transmission.

Regulation of Circuit Activity

When firing rates in these in vitro cortical circuits are raised, the circuit adjusts over time to bring the firing rates of pyramidal neurons back to control values (Turrigiano et al., 1998). This stabilization of firing rates may serve several important functions in circuits that utilize correlation-based rules for the refinement of synaptic connectivity. It may prevent the runaway synaptic potentiation of synapses that obey Hebbian correlation-based rules for synaptic strengthening (Miller, 1996), allow neurons to remain responsive to their inputs as the number of synapses change during development, and promote competition between inputs.

The opposite effects of BDNF on pyramidal and interneuronal excitatory synapses are consistent with a model in which activity-dependent release of this neurotrophin contributes to the homeostatic regulation of cortical firing rates (Figure 6A). The changes we observe in excitatory synaptic strengths correlate well with the changes in pyramidal and interneuron firing rates produced by increased or decreased TrkB signaling and are likely to be a significant factor in their production. However, in recurrent networks the firing rate of a neuron will result from many factors, including the relative strengths of excitatory and inhibitory inputs, the rate of firing of those inputs, the number of synaptic connections formed between different classes of neurons, and the intrinsic excitability of the neurons. In addition to the ability of BDNF to directly regulate AMPA quantal amplitude demonstrated here, and to regulate the amount of inhibition received by pyramidal neurons (Rutherford et al., 1997), BDNF is likely to influence a number of other properties of cortical circuits. For example, BDNF produces selective changes in the dendritic growth of some classes of visual cortical pyramidal neurons, suggesting that BDNF may influence the pattern and extent of cortical connectivity (McAllister et al., 1995, 1996, 1997). How these different factors interact to produce the observed shift in the balance of excitatory and inhibitory activity remains to be determined.

Implications for Cortical Development and Plasticity

Prior work has emphasized two key mechanisms for the activity-dependent refinement of connections in the developing visual system: Hebbian, or correlation-based synaptic plasticity between cortical neurons and their thalamic afferents, and competition between thalamic afferents for the formation and maintenance of synapses onto postsynaptic targets. BDNF and its high affinity receptor TrkB have been implicated in both mechanisms through a possible role in long-term potentiation (Kang and Schuman, 1995; Figurov et al., 1996; Akeneya et al., 1997) and their ability to disrupt the segregation of thalamic afferents into ocular dominance columns (Cabelli et al., 1995, 1997; Riddle et al., 1995; Galuske et al., 1996). Here, we demonstrate that BDNF also plays a crucial role in adjusting the activity of intrinsic cortical circuits by regulating the balance of excitation and inhibition. This may be important for allowing cortical neurons to participate effectively and selectively in Hebbian plasticity. If activity levels are too high, there will be a tendency to potentiate all inputs, and selectivity will be lost. Conversely, if activity levels are too low, even otherwise effective inputs will remain subthreshold and will fail to be potentiated. Our results suggest that BDNF acts postsynaptically to ensure that cortical neurons are optimally responsive to afferent activity.

One way to promote competition between inputs is to control the total synaptic strength of the postsynaptic neuron (Miller, 1996; Turrigiano et al., 1998), so that when some synapses are strengthened, others are weakened and fall below some threshold for maintenance (Lo and Poo, 1991; Colman et al., 1997). In addition to regulating postsynaptic excitability, activity-dependent synaptic scaling may generate competition by scaling down all synaptic strengths in response to the strengthening of selective inputs through Hebbian mechanisms (Turrigiano et al., 1998). Our data suggest that BDNF may influence cortical synaptic competition by globally scaling synaptic strengths up or down in response to changes in activity.

Experimental Procedures

Methods were followed as previously described (Rutherford et al., 1997; Turrigiano et al., 1998). Briefly, rat visual cortical cultures were prepared from P4–P6 rat pups, and whole cell recordings were obtained in artificial cerebrospinal fluid (ACSF). Experiments were performed after 7–9 days in vitro. All data were obtained in parallel on treated and age-matched sister control cultures. For mEPSC measurements, data were obtained from pyramidal neurons (64 control and 93 experimental neurons) and bipolar interneurons (43 control and 89 experimental neurons). Recordings with resting potentials of >-50 mV, series resistances of >20 MΩ, or fewer than 40 mEPSCs were excluded. The average number of mEPSCs per neuron was 145. Series resistance and input resistance were continuously monitored throughout data collection and neurons in which these parameters changed by $>10\%$ were excluded. To record mEPSCs, neurons were held in voltage clamp at -70 mV using an Axopatch 1D in the presence of TTX (1 μM), bicuculline (10 μM), and APV (50 μM). In-house software was used to detect and measure mEPSCs; detection criteria included amplitudes of >5 pA and 20%–80% rise times of <3 ms. To construct cumulative amplitude histograms, 40 events were randomly selected from each neuron in a given condition. Inclusion of all events from each neuron gave quantitatively and qualitatively similar results (data not shown). To generate the average mEPSC for each neuron, all events recorded from that neuron were aligned on the peak and averaged. To generate the average mEPSC for a given experimental condition, the average mEPSCs for each neuron recorded in that condition were averaged. All data are reported as mean $\pm$ SEM for the number of neurons indicated. Statistical comparisons were made using one way ANOVAs

followed by unpaired two-tailed t tests using a Bonferoni correction factor for multiple comparisons (corrected t tests); p values of <0.05 were considered significant. Where data were presented normalized to control data, all statistics were performed prior to normalization.

Pyramidal and bipolar interneurons were identified morphologically as previously described (Rutherford et al., 1997). Bipolar interneurons have oval somata with processes extending from both ends (Figure 1B). Neurons with this morphology are observed to be GABA-positive when double labeled against GABA and the neuronal marker MAP2 (Rutherford et al., 1997) and to form inhibitory connections with pyramidal neurons (N. Desai and G. G. T., unpublished data). They fire rapid action potentials with large rapid afterhyperpolarizations, have significantly lower whole cell capacitance than pyramidal neurons, and have AMPA mEPSCs with significantly faster time constants of decay than pyramidal neurons (Table 2).

Paired recordings were obtained in ACSF + 50 μM APV, and the internal solution for the postsynaptic cell contained cesium and QX-314. Spikes were elicited in the presynaptic neuron at a frequency of 0.2 Hz, and PSCs were obtained at a variety of potentials to allow determination of the reversal potential. Monosynaptic EPSCs were considered to be those with latencies under 4 ms, <1.5 ms of jitter, a monotonic rising phase, and reversal potentials of close to 0 mV. To determine reversal potentials, PSC amplitudes were plotted against voltage over the range of -50 to -10 mV, and the voltage where current equaled zero determined from the equation for the best fit of a straight line to the data. A picospritzer was used to deliver puffs of 0.5 mM glutamate (using puffer pipettes of 2 μm in diameter) while holding pyramidal neurons at -70 mV in whole cell voltage clamp. Puffs were repeated at least five times at each site, and the results averaged.

Firing rates were measured by obtaining whole cell recordings using an Axoclamp 2B in bridge mode. Neurons with V_m of >-55 mV, R_{in} of <200 MΩ, or that were not capable of generating overshooting action potentials upon injection of depolarizing current were excluded. At least 4 min of spontaneous activity was averaged for each neuron.

Human recombinant BDNF was obtained from Promega. For firing rate experiments, BDNF from Amgen was also tested and had identical effects to BDNF from Promega. TrkB–IgG and TrkA–IgG were obtained from Genentech. BDNF and the Trk–IgGs were aliquoted and stored at -80°C and thawed just prior to use.

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