demonstrated that the concentration of antigen required for tolerance of double positive thymocytes is below the concentration required to detect a mature T cell response (*20*). It has also been shown that presentation of particular antigens in the thymus can delete a defined population of thymocytes, whereas the same antigens presented to mature T cells expressing the same TCR do not elicit a response (*21*). Although some of the differences may be attributed to the comparison of immature and mature T cells, these experiments also suggest that the "avidity" required for T cell activation is stronger than negative selection.

Collectively, these models suggest that differences in the affinity between the TCR and its ligand exist for positive selection, negative selection, and T cell activation. One way to explain the different developmental fates of the T cell has been to invoke the presence of two sets of peptides, one for low-affinity and one for high-affinity interactions, that mediate positive or negative selection, respectively (*22*). Our model has shown that no special peptides are involved in discriminating between positive or negative selection, since the viral peptide antigen that is recognized by mature T cells will mediate both positive and negative selection. Further experiments with unrelated H-2D^b–binding peptides will define how precise the interaction must be for positive selection. Our findings suggest that the avidity of the interactions between the thymocyte and stromal elements is reflected by the number of TCRs engaged with MHC molecules at a given time. This in turn may alter the strength of intracellular signals. It is likely that other molecules influence the stability of the TCR:peptide-MHC interactions and directly affect the signals generated through the TCR. Further studies with this model will help define the key molecules and interactions required for selection in the thymus.

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23. Thymocytes (1 × 10^6) were stained with phycoerythrin (PE)-conjugated antibody to CD4 (anti-CD4) (Becton Dickinson), fluorescein isothiocyanate (FITC)–conjugated anti-CD8 (Becton Dickinson), or supernatant from hybridomas KT3 (anti-CD3), KJ16 (anti-V_β8.1,8.2), or B20.1 (anti-V_α2) (*24*), followed by staining with FITC-conjugated goat antibody to rat immunoglobulin G (TAGO). Ten thousand events were collected in list mode storage with FACScan software (Becton Dickinson), and samples were gated on live cells by a combination of forward and side scatter and analyzed with LYSYS software (Becton Dickinson).
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26. Spleen cells (1 × 10^5) from TCR transgenic mice were incubated with 2 × 10^4 macrophages that were prepulsed with various concentrations of p33-41 (Lys-Ala-Val-Tyr-Asn-Phe-Ala-Thr-Met) for 3 hours at 37°C. After 48 hours of cocultivation, cells were pulsed with 1 μCi of [^{3}H]thymidine (Amersham) for 16 hours. Cells were harvested and counted on a Matrix 96 direct beta counter (Canberra Packard). Background proliferation for T cells incubated with macrophages in the absence of peptides was 300 cpm.
27. Day 16 fetal thymic lobes were cultured on 0.8-μm polycarbonate filters (Costar) in serum-free media [Iscove's modified Dulbecco's media, 2% Ultroser (Gibco), human β_2M (2.5 μg/ml; Sigma), glutamine, and antibiotics]. Peptides were added daily at a concentration of 10^{-6} or 10^{-12} M. After 8 days in culture, the lobes were harvested and stained with monoclonal antibodies as described (*23*).
28. The V_α2 used in two-color analysis was B20.1 conjugated to PE (Pharmingen). For three-color fluorescence, biotinylated B20.1 (Pharmingen) was detected with streptavidin-red 670 (Gibco/BRL).

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Requirement for BDNF in Activity-Dependent Survival of Cortical Neurons

Anirvan Ghosh, Josette Carnahan, Michael E. Greenberg*

Cultured embryonic cortical neurons from rats were used to explore mechanisms of activity-dependent neuronal survival. Cell survival was increased by the activation of voltage-sensitive calcium channels (VSCCs) but not by activation of *N*-methyl-D-aspartate receptors. These effects correlated with the expression of brain-derived neurotrophic factor (BDNF) induced by these two classes of calcium channels. Antibodies to BDNF (which block intracellular signaling by BDNF, but not by nerve growth factor, NT3, or NT4/5) reduced the survival of cortical neurons and reversed the VSCC-mediated increase in survival. Thus, endogenous BDNF is a trophic factor for cortical neurons whose expression is VSCC-regulated and that functions in the VSCC-dependent survival of these neurons.

The patterning of connections in the developing nervous system is determined by the axonal and dendritic growth of individual neurons as well as by naturally occurring cell death. There is considerable evidence that both the morphology and survival of neurons can be influenced by neuronal activity (*1–4*). The survival of peripheral neurons in vitro is enhanced by the activation of VSCCs. In central neurons another major source of calcium entry is the *N*-methyl-D-aspartate (NMDA) subtype of

A. Ghosh and M. E. Greenberg, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA.
J. Carnahan, Amgen, Thousand Oaks, CA 91320, USA.

*To whom correspondence should be addressed.

glutamate receptors. It is not known if calcium influx through these two different calcium channels can similarly promote the survival of central neurons. Moreover, beyond a requirement for calcium influx (4), the mechanism by which neuronal activity leads to enhanced survival is not known. We now show that the survival of embryonic cortical neurons is differentially regulated by the activation of distinct calcium channels and provide evidence that the effect of calcium channel activation on neuronal survival is mediated by the enhanced expression of BDNF, which acts as a trophic factor for these neurons.

Enhanced activity in cortical cultures from rats at embryonic day 17 or 18 (E17–E18) (5) was attained by membrane depolarization (with elevated extracellular KCl) or by the stimulation of glutamate receptors with L-glutamate, NMDA, or kainic acid. To examine the effectiveness of these and other agents in stimulating cortical neurons, we used an assay that allowed us to determine if intracellular signaling pathways were being activated in individual cultured neurons. The cyclic adenosine monophosphate–responsive, element-binding protein (CREB) becomes rapidly phosphorylated on Ser^{133} when cells are exposed to a variety of extracellular stimuli. This phosphorylation event can be detected in single neurons by immunostaining with an antibody that specifically recognizes only phosphorylated CREB (PCREB) (6). In unstimulated cultures, less than 5% of the neurons showed PCREB immunoreactivity (Fig. 1A). After an 8-min treatment with 50 mM KCl or 10 μM glutamate, over 85% of the neurons showed PCREB immunoreactivity, which indicates that both KCl and glutamate can effectively activate in these neurons intracellular signaling pathways that lead to CREB phosphorylation (Fig. 1, B and C).

To determine the effects of depolarizing stimuli on the survival of embryonic cortical neurons, we stimulated cells 1 day after their plating and examined them 48 hours after the onset of the treatment (7). Depolarizing concentrations of KCl (25 and 50 mM) led to a two- to threefold increase in cell survival as well as increased neurite outgrowth (Fig. 1, D, E, and G). In contrast, glutamate (10 μM) and NMDA (25 μM) did not have an effect on the survival of embryonic cortical neurons (Fig. 1, F and G). Kainate (30 μM) treatment resulted in a small but significant ($P < 0.01$) increase in cell survival, but was much less effective than elevated concentrations of extracellular KCl (Fig. 1G) (8). Even at 10 times higher concentrations, glutamate, NMDA, and kainate did not significantly promote cell survival. Consistent with previous observations (4), we prevented the enhanced survival brought about by 50 mM KCl by chelating extracellular calcium with 1 mM EGTA and reduced the KCl-induced survival with the L-type VSCC blocker nifedipine, which suggests that the survival effects of increased KCl concentrations require calcium influx.

Because neurogenesis is not over at E17–E18 (9), we examined whether the increased cell number after KCl treatment reflected enhanced cell survival or increased proliferation. Cell counts at different times after plating indicated that untreated cultures underwent significant cell loss during the experimental window of 48 hours and that this decrease in cell number was prevented by KCl (10). In separate experiments, we labeled cortical subplate neurons in vivo at E14 with an injection of bromodeoxyuridine (BrDU) (9). By E17–E18, all the BrDU-labeled neurons were postmitotic. In cortical cultures from BrDU-injected animals, treatment with KCl but not glutamate led to increased survival of labeled neurons (10). Thus, prolonged depolarization enhances the survival of postmitotic cortical neurons, and the survival of subplate neurons is specifically increased by this treatment.

Because neurotrophic factors have been implicated in the survival of several neuronal populations, we considered the possibility that the distinct survival effects of KCl and glutamate might involve differential regulation of the expression of a neurotrophin. Because BDNF is expressed widely in the cortex (11) and can be regulated by neuronal activity (12), we examined the regulation of BDNF by KCl and glutamate in primary cortical cultures. Although several early signaling events, such as CREB phosphorylation and expression of c-*fos*, were regulated similarly by KCl and gluta-

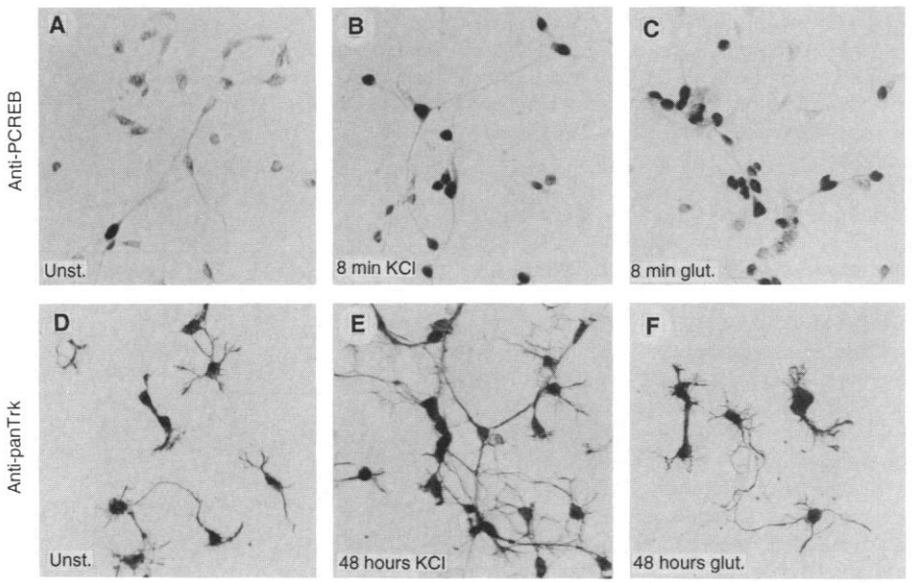


Fig. 1. Enhancement of neuronal survival by depolarizing stimuli. (**A** through **C**) KCl and glutamate treatment stimulate intracellular signaling in cultured cortical neurons. Cells from E17 cortical cultures were treated for 8 min with either 50 mM KCl (KCl) or 10 μM glutamate (glut.), fixed with 4% paraformaldehyde, and immunostained with an antibody against the phosphorylated form of CREB (anti-PCREB). In unstimulated (Unst.) cultures (A), very few (<5%) cells showed PCREB immunoreactivity. After stimulation with KCl (B) or glutamate (C), over 85% of the cells showed nuclear PCREB immunoreactivity, indicating that both agents effectively stimulated signaling in these neurons. (**D** through **F**) The survival of cortical neurons is enhanced by KCl but not by glutamate. Cells were plated at E17, treated with 50 mM KCl or 10 μM glutamate at E17 + 1 day in vitro for 48 hours, fixed with 4% paraformaldehyde, and immunostained with an antibody that recognizes all members of the Trk receptor family (anti-panTrk). Cultures treated with KCl showed about a threefold increase in survival compared to controls and cells treated with glutamate. (**G**) Quantitative analysis of the effects of a 48-hour treatment with KCl (50 mM), glutamate (Glut.) (10 μM), NMDA (30 μM), or kainic acid (KA) (25 μM) on the survival of cortical neurons under the conditions described above (D through F). C, control. Cell numbers (in Figs. 1, 4, and 5) are indicated for a 100 μm by 100 μm field of view (mean ± SEM) (7). The effects of KCl ($P < 0.001$) and kainic acid ($P < 0.01$) are statistically significant by the *t* test.

mate, BDNF expression showed a difference in its regulation by these stimuli. Whereas glutamate led to a transient induction of BDNF expression that peaked after 1 hour and subsequently decreased rapidly, the induction by KCl was delayed, reached peak expression at 3 hours, and remained elevated over a longer duration (Fig. 2A). Moreover, the peak BDNF expression induced by KCl was significantly higher than that induced by glutamate (Fig. 2A). In both cases, the induction of BDNF expression was blocked by 2 mM EGTA, which indicates that the increased expression required extracellular calcium (Fig. 2, B and C).

The difference in the pattern of BDNF expression in response to KCl and glutamate suggested that the induction of BDNF by these stimuli may be mediated by the activation of distinct calcium channels. Induction of BDNF expression by KCl was completely blocked by the L-type VSCC blocker nifedipine but was not affected by the NMDA receptor blocker D-2-amino-5-phosphonovaleric acid (APV) or by the non-NMDA glutamate receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Fig. 2C). In contrast APV, but not nifedipine or CNQX, effectively blocked BDNF induction by glutamate (Fig. 2B). The minor inhibitory effects of nifedipine on glutamate stimulation of BDNF expression (Fig. 2B) were not seen consistently. These results indicate that KCl and glutamate induced BDNF expression by the activation of L-type channels and NMDA receptors, respectively.

The kinetics and level of BDNF expression induced by KCl and glutamate indicated a correlation between BDNF mRNA levels induced by these stimuli and the effectiveness of the stimuli in promoting survival. To determine if BDNF was a likely candidate to mediate the survival of cortical neurons, we examined the response of cortical neurons to BDNF and other members of the neurotrophin family. Immunostaining with an antibody that specifically recognizes the phosphorylated form of CREB indicated that an 8-min stimulation of cortical cultures with BDNF, NT3, or NT4/5, but not nerve growth factor (NGF), led to CREB phosphorylation in virtually every neuron (>95%) (*10*). Similarly, stimulation with BDNF, NT3, or NT4/5 led to the induction of c-*fos* expression (Fig. 3, A and C), whereas NGF was ineffective. Thus, BDNF, NT3, or NT4/5 can lead to the activation of a variety of signaling molecules that may be involved in mediating physiological responses in cortical neurons.

By blocking the effects of BDNF with specific antibodies to BDNF (anti-BDNF), we examined the possibility that endogenous BDNF regulates the survival of cortical neurons. Anti-BDNF was generated to full-length human recombinant BDNF (*13*), and the specificity of these antibodies was confirmed by immunoblot. Antibodies to BDNF recognized BDNF but did not show detectable cross-reactivity to the related neurotrophins NGF and NT3 (Fig. 3D) (*14*). The ability of these antibodies to block the survival effects of BDNF was examined in primary cultures of chick dorsal root ganglion (DRG) cells (*15*), whose survival is enhanced by the presence of neurotrophins (*16*). Compared to untreated cultures (4% survival), cultures treated with BDNF (60%), NGF (70%), or NT3 (33%) showed increased cell survival (Table 1). When these cultures were treated with neurotrophins in the presence of anti-BDNF (*17*), BDNF-dependent survival was blocked, but the NGF- and NT3-dependent survival was unaffected (Table 1). In addition, preimmune serum did not affect the BDNF-dependent survival of DRG neurons.

The specific inhibition of BDNF-induced signaling by anti-BDNF was also confirmed in cortical neurons. Stimulation with BDNF as well as with NT3 or NT4/5 leads to a robust induction of c-*fos* expression in these cultures (Fig. 3, B and C). Preadsorption (12 hours at 4°C) of BDNF, NT3, or NT4/5 with anti-

Table 1. Effects of BDNF antibodies on the neurotrophin-dependent survival of chick DRG cells.

Treatment	Cell survival at 48 hours (%)		
	No serum	Pre-immune sera	BDNF antisera
BDNF (20 ng/ml)	60 ± 5	58 ± 3	5 ± 1
NGF (10 ng/ml)	70 ± 5	64 ± 4	65 ± 4
NT3 (20 ng/ml)	33 ± 4	36 ± 4	30 ± 6
Untreated	4 ± 2		

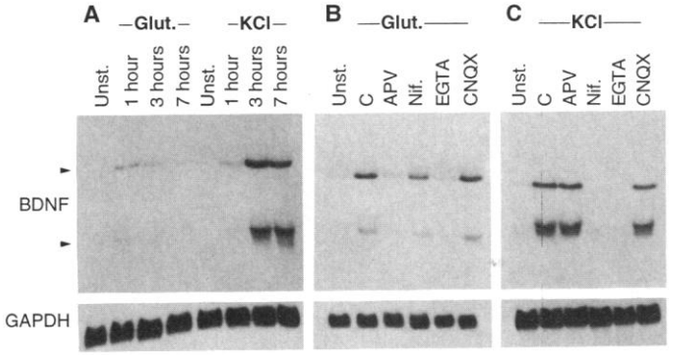
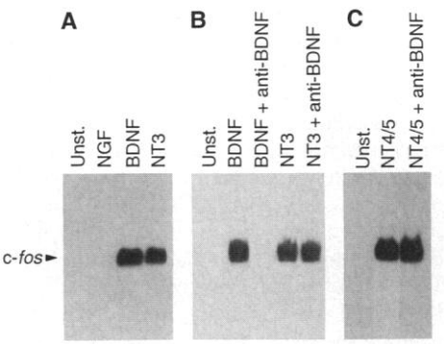


Fig. 2. KCl and glutamate (Glut.) induce BDNF expression with distinct kinetics and by means of the activation of different calcium channels. Northern (RNA) blots containing approximately 10 μg of total RNA in each lane were hybridized with a probe to the coding region of BDNF (exon 5). This probe recognizes BDNF transcripts of 4 and 1.5 kb (indicated by arrowheads). (**A**) Kinetics of BDNF mRNA expression after stimulation of cortical neurons with 10 μM glutamate or 50 mM KCl. Unst., unstimulated. (**B** and **C**) BDNF mRNA amounts after stimulation with glutamate (1 hour) (B) or KCl (4 hours) (C) in the presence of various ion channel blockers and a calcium chelator. The pharmacological agents used were H_2O (control; C), APV (100 μM), nifedipine (Nif.) (5 μM), EGTA (2 mM), and CNQX (40 μM); they were added 10 min before stimulation. After being probed for BDNF mRNA, the blots were stripped and hybridized with a probe to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to indicate that equivalent amounts of RNA were loaded in the various lanes.



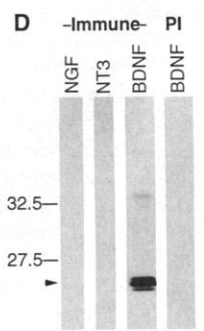


Fig. 3. Responsiveness of cortical neurons to neurotrophins and specificity of antibodies to BDNF (anti-BDNF). In all of these experiments, the final concentration of each of the growth factors is 50 ng/ml. In (A) through (C), Northern blots containing 10 μg of total RNA per lane were hybridized with a probe to the coding region of c-*fos*. Unst., unstimulated. (**A**) Expression of c-*fos* in cortical cultures 1 hour after stimulation with NGF, BDNF, or NT3. BDNF and NT3, but not NGF, lead to a robust induction of c-*fos* expression. (**B** and **C**) Expression of c-*fos* in cortical neurons stimulated for 1 hour with BDNF, NT3, or NT4/5 before and after preadsorption with anti-BDNF. Northern blots (*25*) show that anti-BDNF blocks BDNF-induced c-*fos* expression but does not affect c-*fos* induction by NT3 or NT4/5. (**D**) Anti-BDNF (immune) recognizes recombinant BDNF on a protein immunoblot (arrowhead) but not NGF or NT3 (200 ng of protein per lane). The preimmune serum (PI) does not recognize recombinant BDNF. Molecular size standards are indicated to the left in kilodaltons.

BDNF completely blocked BDNF induction of c-*fos* expression but had no effect on the induction of c-*fos* by NT3 or NT4/5 (Fig. 3, B and C). Thus, anti-BDNF blocked signaling by BDNF without interfering with signaling by even closely related neurotrophins.

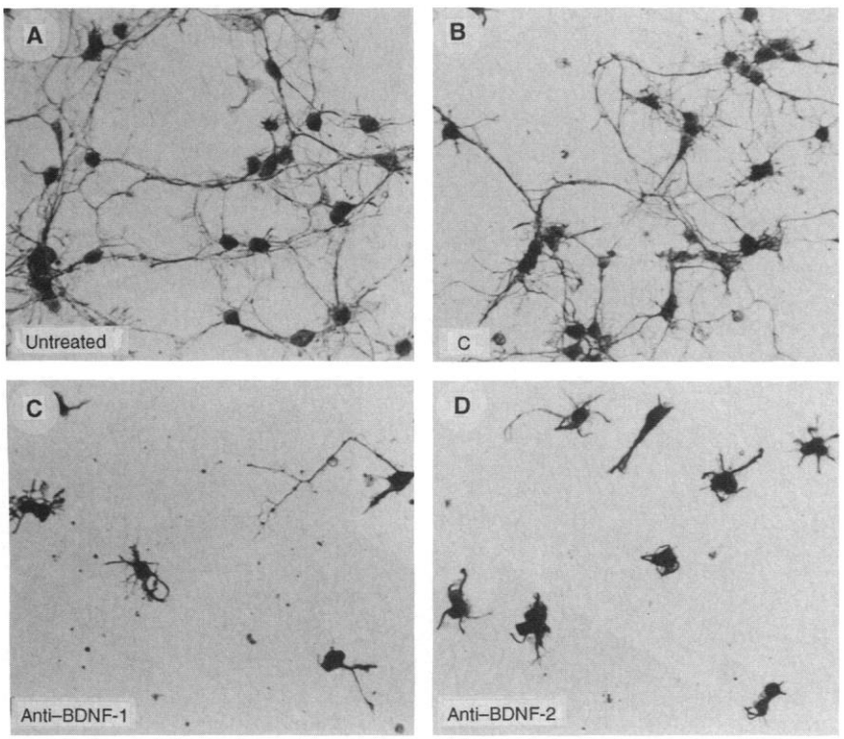
The importance of endogenous BDNF in the survival of cortical neurons was examined by growing cultures in the presence of anti-BDNF. Although control turkey sera and function-blocking antibodies to NT3 (anti-NT3) had insignificant effects on the survival of cortical neurons, two independently derived antibodies to BDNF led to significant cell loss in these cultures (Fig. 4) (*18*). The effects of anti-BDNF on cell survival were visible within 24 hours, at which point there was some neuronal loss and reduced neurite outgrowth from many of the remaining neurons. By 48 hours, over 80% of the cortical neurons were lost from cultures treated with anti-BDNF (Fig. 4E). The fact that virtually every neuron in these cultures can initiate intracellular signaling (CREB phosphorylation) in response to BDNF suggests that most neurons are directly dependent on BDNF for survival. However, the possibility that only some neurons are directly BDNF-dependent and that their death leads to secondary loss of other neurons cannot be ruled out. These observations indicate that BDNF is a trophic factor for cortical neurons and that endogenous BDNF participates in the survival of embryonic cortical neurons in culture.

Because treatment of cortical cultures with KCl leads to a prolonged increase in BDNF expression, we next examined whether the increased cell survival after treatment with KCl was also dependent on BDNF function. Cortical cultures from E18 embryos were depolarized with 50 mM KCl after 1 day in culture. The cultures were also treated with one of two antibodies to BDNF, anti-NT3, or control sera. Only anti-BDNF completely prevented the survival effects of KCl upon examination 48 hours after treatment (Fig. 5). In the presence of anti-BDNF, cell survival was comparable to that of unstimulated cultures treated with anti-BDNF. This probably reflects an inhibition by anti-BDNF of basal as well as KCl-induced BDNF. Accordingly, simultaneous treatment of the cultures with excess recombinant BDNF (1 μg/ml) inhibited the effects of anti-BDNF on KCl-induced cell survival (Fig. 5E). These experiments indicate that increased cell survival that occurs as a consequence of VSCC activation requires BDNF function.

A number of previous studies, both in vivo and in vitro, have shown that neuronal activity can regulate cell survival (*1–3*). Recent evidence suggests that calcium influx through VSCCs is a critical primary event in this response (*4*). Here we have extended these observations by showing that the expression of BDNF induced by the activation of different calcium channels correlates with their ability to promote the survival of embryonic cortical neurons. We also provide evidence that endogenous BDNF acts as a trophic factor for these neurons (*19*) whose function is required for VSCC-dependent cell survival. Together, these experiments suggest that one mechanism of activity-dependent neuronal survival involves the VSCC-mediated increase in BDNF production, which in turn acts as a trophic factor.

The differential regulation of BDNF expression by NMDA receptors and VSCCs also suggests a mechanism that might explain the observation that calcium influx through NMDA receptors is more excitotoxic than calcium influx through VSCCs (*20*). It is possible that calcium influx through either channel represents an excitotoxic assault; however, the robust increase in BDNF expression after VSCC activation could act as a neuroprotectant, whereas the transient and weaker induction of BDNF after NMDA receptor activation may be ineffective in protecting the neurons.

These experiments provide the first evidence that BDNF is a trophic factor for cortical neurons. The widespread expression of BDNF as well as its receptor, TrkB, in the cerebral cortex supports a trophic role for BDNF during cortical development (*11*, *21*). Cortical neurons in vivo are likely to experience the kinds of stimulation that might lead to prolonged increases in BDNF expression and neuronal survival. The activity of retinal ganglion cells during late embryonic development consists of rhythmic bursts of action potentials (*22*). Such patterns of activity when transmitted to the cerebral cortex would



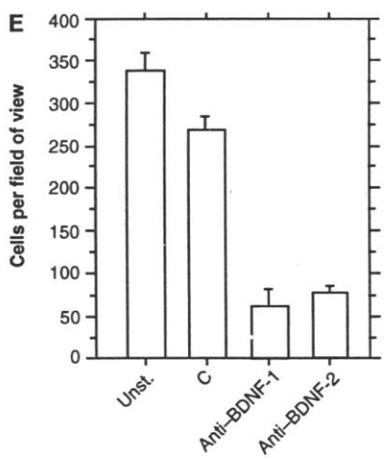
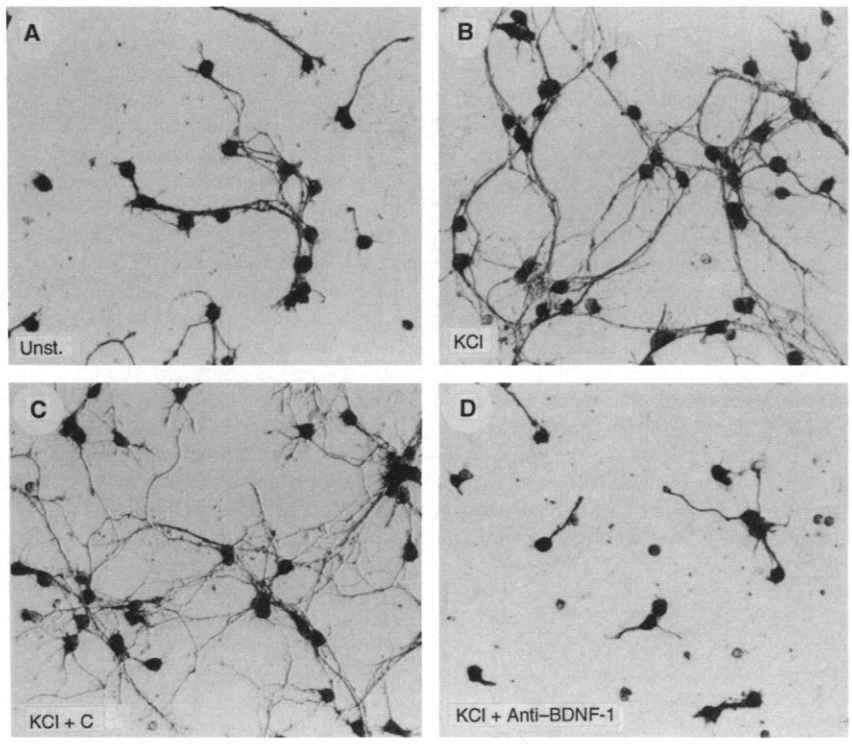


Fig. 4. Survival of cortical neurons requires BDNF function. (**A** through **D**) Cortical cultures from E17 animals were treated 1 day after plating with control sera (C) or with one of two BDNF antibodies (anti–BDNF-1 or anti–BDNF-2) for 48 hours. Compared to controls (B), cultures treated with anti-BDNF showed severe cell loss (C and D). Many of the remaining cells in anti-BDNF–treated cultures appeared atrophied and had substantial neurite loss. (**E**) Quantitative analysis of the effects of BDNF antibodies on neuronal survival indicates that over 80% of the neurons die when cultured in the presence of anti-BDNF. Unst., unstimulated; C, control.



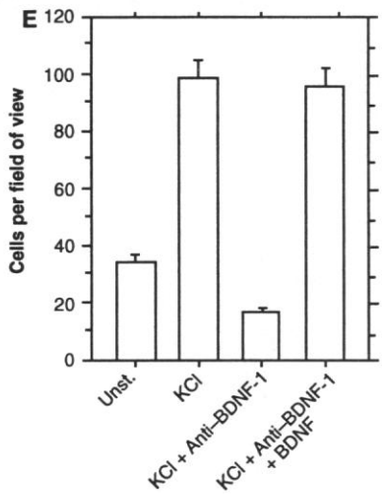


Fig. 5. Increased survival of cortical neurons under depolarizing conditions is prevented by anti-BDNF. (**A** through **D**) Cells were left untreated (Unst.) or treated for 48 hours with 50 mM KCl (KCl) in the presence of control sera (C) or anti-BDNF (anti–BDNF-1). The increase in cell survival after KCl treatment (B) [compared to that of untreated cultures (A)] was not affected by control sera (C) but was completely prevented by anti-BDNF (D). (**E**) Quantitative analysis of the effects of anti-BDNF on the survival of cortical neurons under depolarizing conditions. The effects of anti-BDNF were abolished by preadsorbing the antibody with excess BDNF (1 μg/ml).

be expected to lead to prolonged depolarization of cortical neurons, which would allow for the activation of calcium channels. Calcium imaging studies in acute slices have also shown evidence for spontaneous bursts of calcium currents in ensembles of neurons in the developing cortex (*23*). It has also recently been shown that retinal activity in adult animals regulates BDNF expression in the visual cortex (*24*). One possibility suggested by our experiments is that during early development cortical neurons might produce BDNF that acts locally by autocrine or paracrine mechanisms to promote survival. The activity-dependent regulation of BDNF expression, together with these possible modes of neurotrophin action, suggest a mechanism by which synaptic stimulation might regulate the survival of postsynaptic neurons.

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5. Cortical neurons from E17–E18 rat embryos were cultured essentially as described [H. Bading and M. E. Greenberg, *Science* **253**, 912 (1991)] in either F12 medium–Dulbecco's minimum essential medium with 5% rat serum or in L15 medium with 10% fetal bovine serum and 5% horse serum. Immunostaining with antibodies to microtubule-associated protein 2 (MAP2), growth-associated protein 43 (GAP43), neuron-specific enolase, and glial fibrillary acidic protein (GFAP) indicates that over 95% of the cells in these cultures are neurons. Experiments with cells grown in either medium gave comparable results.
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7. In every experiment cells were treated in triplicate and each experiment was repeated two to four times. In survival assays, cells were counted from 20 to 30 different randomly selected fields of view for each treatment. To visualize the complete morphology of the cells, we fixed the cultures with a solution of 4% paraformaldehyde and 4% sucrose and immunostained them with an antibody that recognized all members of the Trk receptor family (panTrk). This antibody intensely labeled all neurite-bearing cells in the culture, which is consistent with the observation that virtually every neuron in the cultures was responsive to neurotrophin stimulation.
8. These survival effects of KCl are in contrast to the known excitotoxic effects of KCl and glutamate [reviewed in D. W. Choi, *Trends Neurosci.* **11**, 465 (1988)]. This apparent discrepancy is probably due to the fact that immature neurons are much less susceptible to excitotoxicity than older neurons. Consistent with other studies, we have found the KCl and glutamate are excitotoxic to cortical neurons that have been cultured for longer periods of time.
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13. Human recombinant BDNF expressed in CHO cells was used as an antigen preparation to immunize four adult turkeys. The titer of the antisera was monitored by an enzyme-linked immunosorbent assay, and the animals were boosted once a month. The specificity of the antibodies was determined by protein immunoblot with recombinant BDNF, NGF, and NT3; each test (bleed) showed highly specific recognition of BDNF.
14. For the protein immunoblots, 200 ng of recombinant BDNF, NGF, or NT3 was run on a 10 to 20% gradient polyacrylamide-SDS gel and subsequently transferred to nitrocellulose membranes. After a blocking step to reduce nonspecific binding [10% goat serum in phosphate-buffered saline (PBS) for 1 hour], the blots were incubated overnight with immune or preimmune antisera (1:100 in PBS with 1% goat serum). After several washes with PBS, the blots were incubated in PBS with horseradish peroxidase–conjugated rabbit antibodies to turkey immunoglobulin G (1:500) and visualized with diaminobenzidine as a chromagen.
15. Dorsal root ganglia were dissected from chick embryos at E7, trypsinized, and dissociated as described (*16*). Two preplating steps were used to enrich for neuronal cells that were then plated at 100 to 200 cells per well in eight-well plates coated with polyornithine-laminin. Cultures were maintained in F14 medium with 10% horse serum (*16*). For survival assays, growth factors were added in the presence or absence of preimmune or BDNF antisera at the time of plating. Neurons were counted once at 4 hours and again at 48 hours after plating; the effects of the treatments are indicated in terms of the

percentage of cells present at 4 hours that survive to 48 hours.
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17. In all experiments, BDNF antisera as well as control sera were used at a concentration of 10 μl per 1 ml of medium.
18. Antibodies to NT3 were generated in turkeys with human recombinant NT3 as antigen. By the same criteria described for BDNF antibodies, NT3 antibodies specifically block NT3 function without recognizing or interfering with signaling by NGF, BDNF, or NT4/5. Antibodies to BDNF are not generally cytotoxic to cells: anti-BDNF has no apparent morphological effects on NGF-differentiated PC-12 cells or on cortical neurons cultured from neonatal animals.
19. Preliminary experiments suggest that recombinant BDNF can promote the survival of embryonic cortical neurons (*10*).
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Long-Range Cis Preference in DNA Homology Search over the Length of a *Drosophila* Chromosome

William R. Engels,* Christine R. Preston, Dena M. Johnson-Schlitz

P element–induced chromosome breakage on the X chromosome of *Drosophila melanogaster* was repaired six times more frequently when a homologous template was located anywhere on the X chromosome rather than on an autosome. Cis-trans comparisons confirmed that recombinational repair was more frequent when the interacting sequences were physically connected. These results suggest that the search for homology between the broken ends and a matching template sequence occurs preferentially in the cis configuration. This cis advantage operates over more than 15 megabases of DNA.

Most genetic recombination events are preceded by a homology search to align the interacting DNA sequences. This alignment can take place on the level of whole chromosomes, such as when two homologs synapse for meiosis, but it can also occur on a much smaller scale involving short sequence matches. Only a few hundred base pairs of homology are needed for efficient homology searching in *Drosophila* and mammalian cells (*1–4*), and fewer than 100 base pairs (bp) are required in *Escherichia coli* and yeast (*5–7*). One way to study this process is provided by P transposons in *D. melanogaster*. Excision of these elements initiates recombinational repair, which requires a genome-wide homology search for a template (*1*, *8–10*). This template is normally the corresponding gene on the sister chromatid or homolog, but it can also be an ectopic sequence carried on a transposable element anywhere in the genome (*9*). In the experiments reported here, we use ectopic templates to examine the process of genome-wide homology searching.

A comparison of gene conversion frequencies for 10 template sites on the X chromosome and 28 on the two major autosomes is shown in Fig. 1. For each site, we measured the rate at which a P element insertion mutation at the *white* locus was restored to the wild type in the presence of a *white* sequence template at the indicated genomic position (*11*). Such reversions occur when the P element in *white* excises and sequences from the template are copied into the vacated site, replacing some of the flanking sequence (*9*). These events, which occur in the premeiotic germ cells when P transposase is present, are interpreted as repair of double-strand DNA

Genetics Department, University of Wisconsin, Madison, WI 53706, USA.

*To whom correspondence should be addressed.

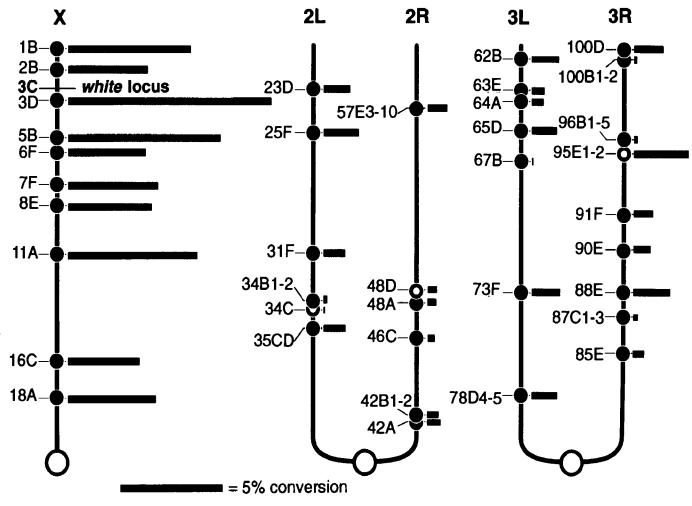

Fig. 1. Conversion rates for template sites. The five major chromosome arms (X, 2L, 2R, 3L, and 3R) of *Drosophila* are shown with template sites indicated according to their cytological position (*21*). Bars represent estimated conversion rates associated with each template site, defined as the proportion of X-bearing gametes in which the w^{hd} allele had reverted to the wild type and had acquired sequences from the template gene (*11*). For sites marked with a solid dot (●), the template was P[*w*alter], a modified *white* gene carried on a P element (*9*). The *white* gene in P[*w*alter] has 12 single-base modifications that alter restriction sites but do not prevent expression of the gene (*9*). We confirmed conversion events by verifying that at least one of these marker sites had been copied into the reverted w^{hd} gene (*11*). Three templates (○) carried a slightly modified form of P[*w*alter] that had lost its mobility or in which some of the markers had been substituted (*9*). (The immobile element at 34C is missing part of its 5′ P end.) All reversion events occurred in the germ line of w^{hd} males with a single copy of one of the template elements and the Δ2-3 (99B) element to provide transposase (*14*). A total of 3298 single-male crosses were performed to yield 209,057 potential revertant progeny. The results were pooled with an earlier data set obtained by identical methods and reported previously (*9*), accounting for 43% of the data shown here. All data for sites 1B, 2B, 3D, 5B, 7F, 8E, 11A, 16C, 18A, 23D, 48A, and 57E3-10 are new, and data from sites 25F, 31F, 35CD, 64A, 65D, 73F, 78D4-5, 85E, 91F, 96B1-5, 100B1-2, and 100D include both experiments. The remaining sites were tested only in the first experiment. The new P[*w*alter] insertion sites were obtained by mobilizing previous insertions with transposase and selecting for jumps (*14*). Cytological positions were determined by in situ hybridization to polytene chromosomes with a labeled *white* DNA probe (*23*).