Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8074–8077, August 1995
Neurobiology

Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors

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Communicated by L. L. Iversen, University of Oxford, Oxford, United Kingdom, May 26, 1995 (received for review March 23, 1995)

ABSTRACT **Although neurotrophins are primarily associated with long-term effects on neuronal survival and differentiation, recent studies have shown that acute changes in synaptic transmission can also be produced. In the hippocampus, an area critically involved in learning and memory, we have found that brain-derived neurotrophic factor (BDNF) rapidly enhanced synaptic efficacy through a previously unreported mechanism—increased postsynaptic responsiveness via a phosphorylation-dependent pathway. Within minutes of BDNF application to cultured hippocampal neurons, spontaneous firing rate was dramatically increased, as were the frequency and amplitude of excitatory postsynaptic currents. The increased frequency of postsynaptic currents resulted from the change in presynaptic firing. However, the increased amplitude was postsynaptic in origin because it was selectively blocked by intracellular injection of the tyrosine kinase receptor (Ntrk2/TrkB) inhibitor K-252a and potentiated by injection of the phosphatase inhibitor okadaic acid. These results suggest a role for BDNF in the modulation of synaptic transmission in the hippocampus.**

Neurotrophins are important regulators of the survival, development, and differentiation of multiple neuronal populations (1–3). These effects generally occur over the course of hours or even days. Recently, evidence has accumulated that neurotrophins can also modulate transmitter release and synaptic transmission (4–8). Such effects could underly a role for these factors in synaptic plasticity. The potential effects of neurotrophins on synaptic transmission in the hippocampus were studied with primary cultures of embryonic hippocampal neurons. We report that brain-derived neurotrophic factor (BDNF) rapidly enhanced the strength of synaptic connections in these neurons. Both the amplitude and the frequency of excitatory postsynaptic currents (EPSCs) were increased within 2–3 min of neurotrophin application. Moreover, the potentiation of synaptic current amplitude occurred via a previously undescribed postsynaptic mechanism in which BDNF increased the responsiveness of the postsynaptic neuron to the excitatory input. These results indicate a previously unreported role for this neurotrophin in the hippocampus.

MATERIALS AND METHODS

Cell Culture. Hippocampal cultures were grown as described (9). Briefly, hippocampi were obtained from embryonic day 18 Sprague–Dawley rats, and cells were plated on poly(D-lysine)-coated Petri dishes at a final density of 10^6 cells per 35-mm dish. Cultures were maintained in serum-free medium (SFM) at 37°C in an incubator with humidified 95% air/5% CO_2. SFM consisted of a 1:1 (vol/vol) mixture of Ham's F-12 and Eagle's minimal essential medium supplemented with insulin at 25 μg/ml, transferrin at 100 μg/ml, 60 μM putrescine, 20 nM progesterone, 30 nM selenium, glucose at 6 mg/ml, and 0.5 units of penicillin and 0.5 mg of streptomycin per ml. These cultures contained virtually pure neurons, as judged by neuron-specific enolase immunocytochemistry.

Electrophysiological Recordings. Whole-cell current-clamp and voltage-clamp recordings were obtained after 12–16 days *in vitro* with standard techniques (10). Recorded cells had large, pyramidal-shaped cell bodies and two or three major processes. The external bath solution contained 1.67 mM Ca^{2+}, 1 mM Mg^{2+}, 5.36 mM K^+, 137 mM Na^+, 17 mM glucose, 10 mM Hepes, and 50 mM sucrose. The pipette solution contained 112 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 11 mM EGTA, 10 mM Hepes, and 2 mM ATP. Signals were recorded with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA), sampled at 2.5 kHz (IDA15125 interface; Indec Systems, Capitola, CA), and filtered at 5 kHz. For voltage-clamp experiments, cells were held at a membrane potential of −60 mV.

Data Analysis. Data were analyzed by integrating the synaptic current for each sweep during the baseline and test periods and converting these values to synaptic charge. The charge measurements for all of the sweeps in a particular period were averaged, and the percent baseline increases for the individual experiments were calculated by dividing the average synaptic charge during the test period by the average synaptic charge from the baseline period. Statistical comparisons used a Student's *t* test and were based on 2-min periods immediately prior to and 3 min after the onset of drug application.

Materials. Purified BDNF was provided by Cephalon (West Chester, PA). Nerve growth factor (NGF) was purchased from Boehringer Mannheim. K-252a was obtained from Kamiya Pharmaceutical (Thousand Oaks, CA). All other chemicals were from Sigma.

RESULTS

Patch-clamp recordings were made from virtually pure neuronal cultures obtained from embryonic rat hippocampus grown in serum-free, fully defined medium. Under these conditions, neurons formed extensive connections and fired action potentials in response to suprathreshold depolarization from synaptic inputs. In the first set of studies, action potentials were recorded by using the whole-cell current-clamp configuration. Bath application of BDNF at 50 ng/ml increased the firing rate of hippocampal neurons within 2–3 min of application (Fig. 1*A*), and a gradual recovery to baseline levels occurred over 10–15 min after wash-out of BDNF (Fig. 1*B*). On average, firing rate increased >2-fold during BDNF exposure (Fig. 1*C*) [the mean baseline firing rate = 10.61 ± 0.3

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Abbreviations: BDNF, brain-derived neurotrophic factor; EPSC, excitatory postsynaptic current; NGF, nerve growth factor.

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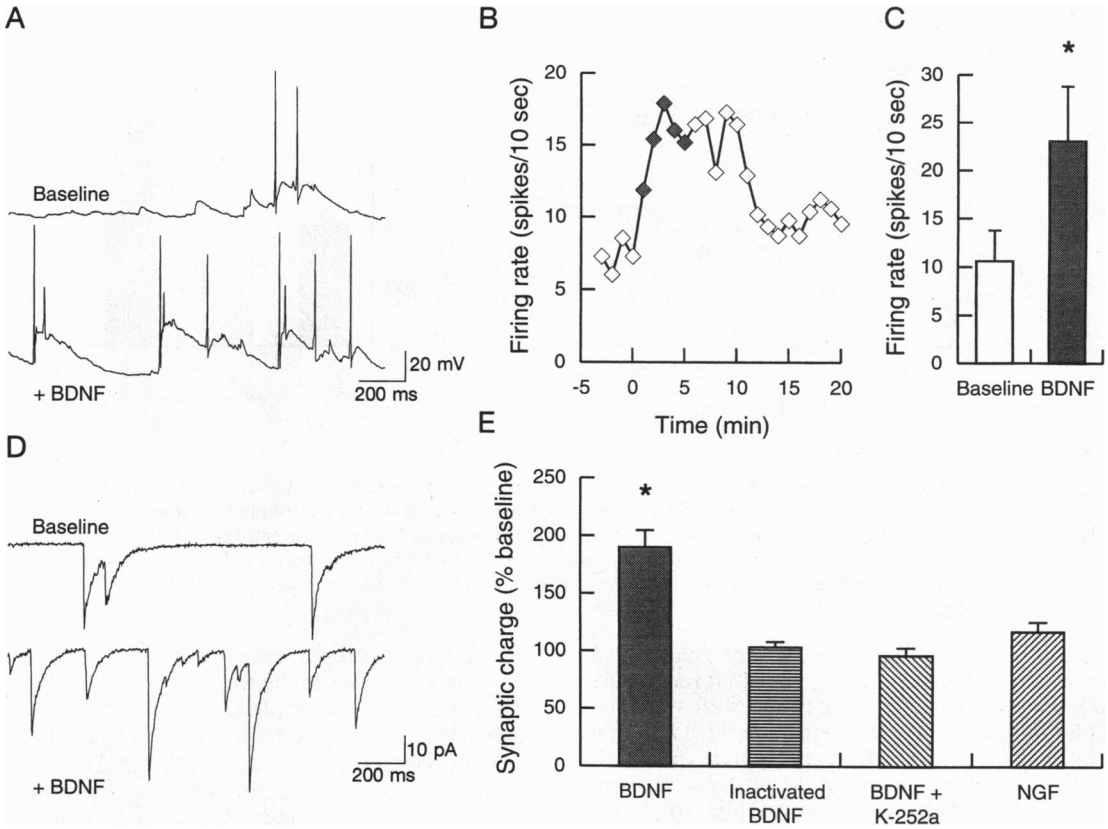

FIG. 1. Potentiation of synaptic transmission by BDNF. (*A*) Current clamp recordings from a hippocampal neuron before and 3 min after bath application of BDNF (at 50 ng/ml). (*B*) Time course for the effect of BDNF on the firing rate of the same neuron as shown in *A*. BDNF was applied during the time represented by the filled symbols. (*C*) Mean group data for the effect of BDNF on firing rate ($n = 8$ cells; $P < 0.01$). (*D*) Whole-cell voltage-clamp recordings from a hippocampal neuron before and 3 min after exposure to BDNF ($V_{hold} = -60$ mV). (*E*) Effects on synaptic charge after bath application of BDNF ($n = 10$ cells), heat-inactivated BDNF ($n = 9$ cells), or NGF ($n = 13$ cells; 50 ng/ml). In some experiments, cultures were pretreated for 1 hr with 200 nM K-252a in the bath solution ($n = 7$ cells). *, $P < 0.001$ compared with baseline levels for that condition. Recordings were obtained from multiple platings.

spikes per 10 sec; the mean firing rate during BDNF application = 23.09 ± 0.6 spikes per 10 sec (mean ± SEM; $n = 9$; $P < 0.01$)]. Similar results were also obtained when using BDNF at 10 ng/ml ($n = 5$; data not shown).

The effect of BDNF was further characterized in a series of whole-cell voltage-clamp experiments. Recordings of EPSCs revealed a marked enhancement in response to BDNF application (Fig. 1*D*). These synaptic currents were not spontaneous miniature potentials and were action potential-dependent, as their occurrence was eliminated by 1 μM tetrodotoxin, a sodium channel blocker. EPSCs were quantified by measuring the integrated current, or synaptic charge. BDNF application produced a rapid increase in synaptic charge [baseline value = 14.51 ± 3.2 pC; value 5 min after BDNF application = 25.26 ± 5.1 pC (mean ± SEM, $n = 10$; $P < 0.001$)], with a time course similar to the increase in action potential activity. In contrast to BDNF exposure, treatment with heat-inactivated BDNF had no effect on synaptic charge (Fig. 1*E*; $n = 9$). The actions of BDNF are mediated by the neurotrophic tyrosine kinase receptor type 2 (Ntrk2; previously named TrkB), which is expressed throughout the hippocampus (11–15). Bath application of 200 nM K-252a, the specific Ntrk/Trk inhibitor which prevents biological responses to neurotrophins (16–20), completely blocked the effect of BDNF (Fig. 1*E*; $n = 7$). In addition, the related neurotrophin NGF had no significant effect on synaptic charge (Fig. 1*E*; $n = 13$).

In the population of cells tested above, we observed that some neurons did not respond to BDNF application, thus underestimating the magnitude of the effect. To compare changes in the magnitude of the BDNF effect under various conditions, we defined a response as a maintained increase at least two standard errors over baseline within 5 min of application. Using these criteria, 80% of the neurons tested with BDNF responded with an increase in synaptic charge. In addition, ≈20% of the neurons tested with NGF appeared to respond. Because of the small proportion of neurons that responded, however, this effect of NGF was not examined further.

The next series of studies focused on the role of postsynaptic changes in the effect of BDNF. For some recordings, either K-252a or the phosphatase inhibitor okadaic acid was added to the internal solution of the patch pipette, thereby injecting the drug directly in the postsynaptic neuron. Fig. 2*A* shows individual examples of the time course of the BDNF effect on synaptic charge under various conditions. Intracellular injection of 200 nM K-252a significantly decreased the magnitude of the effect compared with BDNF treatment alone (Fig. 2*B*; $n = 8$; $P < 0.05$). Conversely, intracellular injection of 0.5 μM okadaic acid significantly increased the magnitude of the effect (Fig. 2*B*; $n = 8$; $P < 0.05$). Baseline synaptic charge was not affected by inclusion of either K-252a or okadaic acid in the internal solution (control vs. K-252a, $P > 0.6$; control vs. okadaic acid, $P > 0.1$). These results suggest that a component of the BDNF effect on synaptic charge involved modulation of the postsynaptic response.

Further analysis revealed that the increase in synaptic charge consisted of increases in both the frequency and the amplitude of the EPSCs. The frequency of synaptic currents was increased in all of the neurons that showed an increase in synaptic charge. Intracellular injection of 200 nM K-252a did

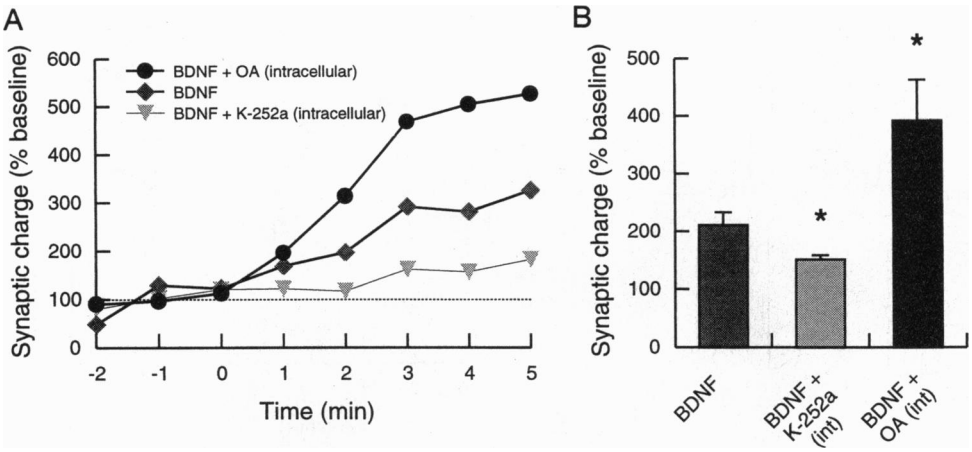

FIG. 2. Effect of BDNF on synaptic charge is modified by postsynaptic manipulations. (*A*) Time courses of recordings from individual neurons for the effect of either bath-applied BDNF alone at 50 ng/ml (diamonds) or in combination with intracellular injection of 200 nM K-252a (triangles) or 0.5 μM okadaic acid (OA) (circles). Recordings were obtained from sister cultures. Each point represents the average synaptic charge for a 1-min period. (*B*) Effects of BDNF on synaptic charge. Intracellular injection of K-252a (n = 8 cells) decreased the magnitude of the charge effect compared with BDNF treatment alone, while intracellular injection of okadaic acid (n = 8 cells) increased the effect. *, $P < 0.05$. Recordings were obtained from multiple platings.

not alter the magnitude of the frequency effect compared with BDNF treatment alone (Fig. 3*A*; n = 8), nor did it reduce the number of cells that responded. Similar results were obtained with intracellular injection of 0.5 μM okadaic acid (Fig. 3*A*; n = 8). This indicates that the effect of BDNF on EPSC frequency is independent of changes in the postsynaptic neuron and most likely reflects increased presynaptic firing.

The median EPSC amplitude was increased in ≈60% of the cells that responded to BDNF. Intracellular injection of okadaic acid increased the magnitude of the amplitude effect compared with BDNF treatment alone (Fig. 3*B*), with a similar proportion of neurons responding. Conversely, intracellular injection of K-252a completely blocked the effect of BDNF on EPSC amplitude. Under this condition, none of the neurons tested showed an increase (Fig. 3*B*; $P < 0.05$; χ^2 test). These data suggest that the effect of BDNF on EPSC amplitude resulted from a selective effect on the postsynaptic neuron.

DISCUSSION

The present results indicate that BDNF rapidly and reversibly potentiates synaptic transmission in hippocampal neurons. This effect involves an increase in action-potential firing rate, as well as increases in the frequency and amplitude of EPSCs. The effect on EPSC amplitude results, at least in part, from an increased postsynaptic responsiveness to excitatory input, via a phosphorylation-dependent pathway. These larger postsynaptic currents may be responsible for the increase in action-potential firing rate and subsequent increase in EPSC frequency.

Neurotrophin activation of Ntrk/Trk tyrosine kinase receptors engages multiple second messenger pathways that may underly these synaptic effects. This includes activation of many serine-threonine protein kinases (21, 22). These kinases may in turn act on postsynaptic neurotransmitter receptors to enhance ligand-gated synaptic currents. For example, a number of studies have shown that the magnitude of glutamate-evoked currents through α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptors can be increased by activators of cAMP-dependent protein kinase, protein kinase C, and calcium/calmodulin-dependent protein kinase (23–25). Such effects are generally rapid and occur along a time course similar to that seen in the present study. Recently it has also been

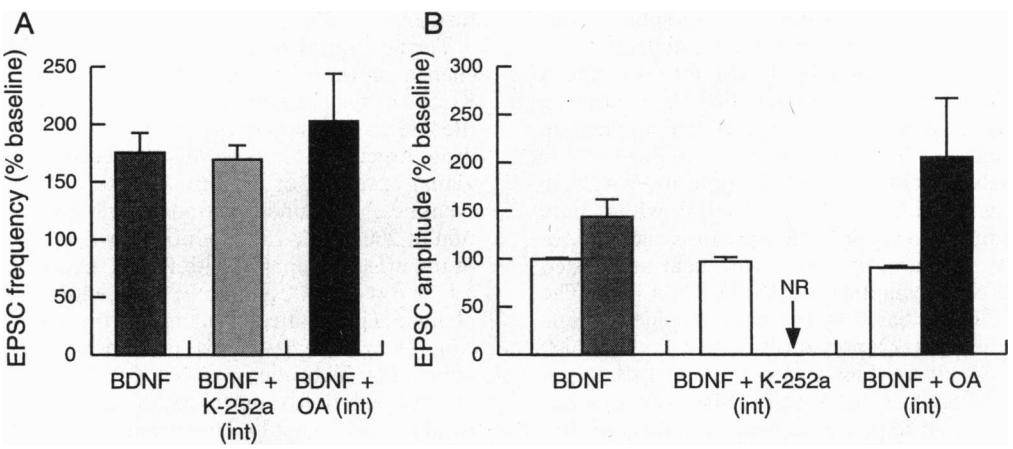

FIG. 3. Effect of BDNF on the frequency and median amplitude of EPSCs. (*A*) Changes in EPSC frequency for the same group of neurons shown in Fig. 2. Cultures were treated with either bath-applied BDNF alone at 50 ng/ml or in combination with intracellular injection (int) of 200 nM K-252a or 0.5 μM okadaic acid (OA). EPSC frequency was increased in all three conditions, with no significant differences between groups. (*B*) Changes in median EPSC amplitude for the same group of neurons as in *A*. Filled bars represent the neurons with at least a 20% increase in amplitude over baseline (BDNF treatment alone, four of seven cells; BDNF treatment + OA injection; five of eight cells). None of the neurons injected with K-252a showed an increase in EPSC amplitude (BDNF treatment + K-252a injection, zero of eight cells; NR, no cells responded). Open bars represent the neurons that did not show an increase in amplitude.

shown that the degree of phosphorylation of the GluR1 subunit of the AMPA receptor correlates with the level of synaptic activity in cortical neuron cultures (26), further supporting the notion that modulation of postsynaptic receptor sensitivity is an important mechanism for regulating synaptic strength.

In addition to this effect on postsynaptic currents in hippocampal neurons, it has been shown that neurotrophins can modulate presynaptic neurotransmitter release in other systems. BDNF and neurotrophin-3 enhance activity at developing synapses in the *Xenopus* neuromuscular junction by increasing acetylcholine release (4). BDNF also potentiates synaptic activity in cultured cortical neurons, an effect that was attributed to a reduction in GABAergic transmission (7). Additionally, a recent study has shown neurotrophin effects on synaptic transmission in hippocampal slices from adult rat (8). Together with the present results, these findings suggest that neurotrophins act through multiple mechanisms to enhance synaptic efficacy. Reciprocally, neurotrophin gene expression in the hippocampus is regulated by neuronal activity and exposure to neurotransmitters (27–31) as well as by stimulation that induces long-term potentiation, considered a model for learning and memory (32, 33). Thus, neurotrophins may modulate synaptic activity in response to specific neuronal signals, effects that could play a crucial role in both synaptogenesis and dynamic control of synaptic strength.

We thank Cephalon, Inc., for the BDNF. We also thank Dr. Robin Davis for a critical reading of the manuscript. This work was supported by a National Research Service Award to E.S.L., a National Institute of Child Health and Human Development grant to I.B.B., and grants from the American Heart Association and Hoechst Celanese to M.R.P.

1. Levi-Montalcini, R. & Angeletti, P. U. (1968) *Physiol. Rev.* **48,** 534–569.
2. Thoenen, H. (1991) *Trends Neurosci.* **14,** 165–170.
3. Davies, A. M. (1994) *J. Neurobiol.* **25,** 1334–1348.
4. Lohof, A. M., Ip, N. Y. & Poo, M. M. (1993) *Nature (London)* **363,** 350–353.
5. Knipper, M., da Penha Berzaghi, M., Blochl, A., Breer, H., Thoenen, H. & Lindholm, D. (1994) *Eur. J. Neurosci.* **6,** 668–671.
6. Knipper, M., Leung, L. S., Zhao, D. & Rylett, R. J. (1994) *NeuroReport* **5,** 2433–2436.
7. Kim, H. G., Wang, T., Olafsson, P. & Lu, B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12341–12345.
8. Kang, H. & Schuman, E. M. (1995) *Science* **267,** 1658–1662.
9. Lu, B., Yokoyama, M., Dreyfus, C. F. & Black, I. B. (1991) *J. Neurosci.* **11,** 318–326.
10. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100.
11. Klein, R., Martin-Zanca, D., Barbacid, M. & Parada, L. F. (1990) *Development (Cambridge, U.K.)* **109,** 845–850.
12. Klein, R., Nanduri, V., Jing, S. A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F. & Barbacid, M. (1991) *Cell* **66,** 395–403.
13. Merlio, J. P., Ernfors, P., Jaber, M. & Persson, H. (1992) *Neuroscience* **51,** 513–532.
14. Berninger, B., Garcia, D. E., Inagaki, N., Hahnel, C. & Lindholm, D. (1993) *NeuroReport* **4,** 1303–1306.
15. Ringstedt, T., Lagercrantz, H. & Persson, H. (1993) *Dev. Brain Res.* **72,** 119–131.
16. Berg, M. M., Sternberg, D. W., Parada, L. F. & Chao, M. V. (1992) *J. Biol. Chem.* **267,** 13–16.
17. Knusel, B. & Hefti, F. (1992) *J. Neurochem.* **59,** 1987–1996.
18. Knusel, B., Kaplan, D. R., Winslow, J. W., Rosenthal, A., Burton, L. E., Beck, K. D., Rabin, S., Nikolics, K. & Hefti, F. (1992) *J. Neurochem.* **59,** 715–722.
19. Nye, S. H., Squinto, S. P., Glass, D. J., Stitt, T. N., Hantzopoulos, P., Macchi, M. J., Lindsay, N. S., Ip, N. Y. & Yancopoulos, G. D. (1992) *Mol. Biol. Cell* **3,** 677–686.
20. Tapley, P., Lamballe, F. & Barbacid, M. (1992) *Oncogene* **7,** 371–381.
21. Keegan, K. & Halegoua, S. (1993) *Curr. Opin. Neurobiol.* **3,** 14–19.
22. Kaplan, D. R. & Stephens, R. M. (1994) *J. Neurobiol.* **25,** 1404–1417.
23. Greengard, P., Jen, J., Nairn, A. C. & Stevens, C. F. (1991) *Science* **253,** 1135–1138.
24. Keller, B. U., Hollmann, M., Heinemann, S. & Konnerth, A. (1992) *EMBO J.* **11,** 891–896.
25. Tan, S., Wenthold, R. J. & Soderling, T. R. (1994) *J. Neurosci.* **14,** 1123–1129.
26. Blackstone, C., Murphy, T. H., Moss, S. J., Baraban, J. M. & Huganir, R. L. (1994) *J. Neurosci.* **14,** 7585–7593.
27. Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H. & Lindholm, D. (1990) *EMBO J.* **9,** 3545–3550.
28. Lu, B., Yokoyama, M., Dreyfus, C. F. & Black, I. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6289–6292.
29. Zafra, F., Castren, E., Thoenen, H. & Lindholm, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10037–10041.
30. Lindefors, N., Ernfors, P., Falkenberg, T. & Persson, H. (1992) *Exp. Brain Res.* **88,** 78–90.
31. Zafra, F., Lindholm, D., Castren, E., Hartikka, J. & Thoenen, H. (1992) *J. Neurosci.* **12,** 4793–4799.
32. Patterson, S. L., Grover, L. M., Schwartzkroin, P. A. & Bothwell, M. (1992) *Neuron* **9,** 1081–1088.
33. Castren, E., Pitkanen, M., Sirvio, J., Parsadanian, A., Lindholm, D., Thoerren, H. & Riekkinen, P. J. (1993) *NeuroReport* **4,** 895–898.