trols the situation and will catch the ball, but he does not know when. This explains why fielders run into walls chasing uncatchable fly balls and why they do not rush ahead to the ball destination point, choosing instead to catch the ball while running. The LOT model explains why balls hit to the side are easier to catch. Fielders can use their robust ability to discriminate curvature rather than resorting to their weak ability to discriminate acceleration (*11*, *12*). It is also an error-nulling method that compensates for minor perceptual distortion or flight trajectory irregularity. In short, the LOT strategy provides a simple and effective way to pursue and catch a target traveling with approximately parabolic motion in three-dimensional space.

REFERENCES AND NOTES

1. M. K. McBeath, *Perception* **19**, 545 (1990).
2. D. Regan, K. I. Beverly, M. Cynader, *Sci. Am.* **241**, 136 (July 1979); D. N. Lee, D. Young, P. Reddish, S. Lough, T. Clayton, *Q. J. Exp. Psychol.* **35**, 333 (1983); G. J. P. Savelsbergh and H. T. A. Whiting, *Ergonomics* **31**, 1655 (1988).
3. B. V. H. Saxberg, *Biol. Cybern.* **56**, 159 (1987a); *ibid.*, p. 177.
4. P. J. Brancazio, *Am. J. Phys.* **53**, 849 (1985); R. K. Adair, *The Physics of Baseball* (Harper and Row, New York, 1990); N. de Mestre, *The Mathematics of Projectiles in Sport* (Cambridge Univ. Press, New York, 1990); R. G. Watts and A. T. Bahill, *Keep Your Eye on the Ball* (Freeman, New York, 1990).
5. S. Chapman, *Am. J. Phys.* **36**, 868 (1968).
6. T. Babler and J. Dannemiller, *J. Exp. Psychol. Hum. Percept. Perform.* **19**, 15 (1993).
7. C. F. Michaels and R. R. D. Oudejans, *Ecol. Psychol.* **4**, 199 (1992); P. McLeod and Z. Dienes, *Nature* **362**, 23 (1993); Z. Dienes and P. McLeod, *Perception* **22**, 1427 (1993); P. McLeod and Z. Dienes, *J. Exp. Psychol. Hum. Percept. Perform.*, in press.
8. J. Todd, *J. Exp. Psychol. Hum. Percept. Perform.* **7**, 795 (1981).
9. J. R. Tresilian, *Perception* **19**, 223 (1990).
10. D. A. Rosenbaum, *J. Exp. Psychol. Hum. Percept. Perform.* **1**, 395 (1975).
11. R. M. Gottsdanker, J. W. Frick, R. B. Lockhard, *Br. J. Psychol.* **52**, 31 (1961); S. Runeson, *Psychol. Res.* **37**, 3 (1974); J. Schmerler, *Perception* **5**, 167 (1976); D. M. Regan, L. Kaufman, J. Lincoln, in *Handbook of Perception and Human Performance*, vol. 1, *Sensory Processes and Perception*, K. R. Boff, L. Kaufman, J. P. Thomas, Eds. (Wiley, New York, 1986), pp. 19.1–19.46; J. B. Calderone and M. K. Kaiser, *Percept. Psychophys.* **45**, 391 (1989).
12. L. A. Riggs, *Science* **181**, 1070 (1973); J. R. Hopkins, J. Kagan, S. Brachfeld, S. Hans, S. Linn, *Child Dev.* **17**, 1166 (1976); R. J. Watt and D. P. Andrews, *Vision Res.* **22**, 449 (1982).
13. M. K. Kaiser and L. Mowafy, *J. Exp. Psychol. Hum. Percept. Perform.* **19**, 1028 (1993).
14. F. M. Toates, in *Control Theory in Biology and Experimental Psychology* (Hutchinson Educational, London, 1975), pp. 151–257; C. D. Wickens, in *Engineering Psychology and Human Performance* (HarperCollins, New York, 1992), pp. 466–481; J. E. Cutting, P. A. Braren, P. M. Vishton, *Psychol. Rev.*, in press.
15. J. A. Adams, *Psychol. Bull.* **58**, 55 (1961); S. N. Roscoe, *Hum. Factors* **10**, 321 (1968).
16. T. S. Collett and M. F. Land, *J. Comp. Physiol.* **99**, 1 (1975); B. S. Lanchester and R. F. Mark, *J. Exp. Biol.* **63**, 627 (1975).

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Potentiated Necrosis of Cultured Cortical Neurons by Neurotrophins

Jae-Young Koh, Byoung J. Gwag, Doug Lobner, Dennis W. Choi*

The effects of neurotrophins on several forms of neuronal degeneration in murine cortical cell cultures were examined. Consistent with other studies, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 all attenuated the apoptotic death induced by serum deprivation or exposure to the calcium channel antagonist nimodipine. Unexpectedly, however, 24-hour pretreatment with these same neurotrophins markedly potentiated the necrotic death induced by exposure to oxygen-glucose deprivation or *N*-methyl-D-aspartate. Thus, certain neurotrophins may have opposing effects on different types of death in the same neurons.

Four related members of the neurotrophin family of growth factors have been identified to date: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (*1*). These neurotrophins act on a set of high-affinity receptor tyrosine kinases (*2*)—TrkA, TrkB, and TrkC—to promote survival, differentiation, and neurite extension in many types of mammalian central neurons. In health, the survival-promoting effects of neurotrophins are probably mediated by the antagonism of naturally occurring programmed cell death. This death generally occurs by apoptosis, characterized by cell volume loss, membrane blebbing, chromatin condensation, and DNA fragmentation (*3*). Some programmed cell death can be inhibited by transcription or translation inhibitors, which suggests that expression of active "death proteins" is required (*4*).

Neurotrophins can also attenuate the pathological neuronal death induced by different insults. For example, they inhibit several forms of axotomy-induced death, an apoptotic death that most likely reflects the failure of target-supplied trophic factors to reach the cell body. The degeneration of basal forebrain cholinergic neurons that results from fimbria-fornix lesions can be blocked by administration of BDNF or NGF (*5*), and the degeneration of axotomized spinal motoneurons can be blocked by administration of BDNF (*6*).

In addition, neurotrophins (*7*) as well as other growth factors (*8*) can reduce the neuronal death induced by exposure to excitotoxins, glucose deprivation, or ischemia. These deaths are thought to occur by necrosis, a process morphologically distinguishable from apoptosis and characterized by prominent early cell swelling (*3*). Thus, it is widely held that the survival-promoting properties of neurotrophins are extensive, perhaps involving interference with injury mechanisms common to both apoptosis and necrosis (*9*).

However, recent studies suggest that apoptosis itself may occur in paradigms involving excitotoxins or oxygen-glucose deprivation. Morphological changes and DNA fragmentation consistent with apoptosis have been described in 3-day-old cultured cortical neurons exposed to glutamate (*10*) and adult cortical neurons at the periphery of focal ischemic insults in vivo (*11*). In addition, the protein synthesis inhibitor cycloheximide has been shown to reduce hypoxic neuronal death in rodents and in cortical cultures in which excitotoxicity has been pharmacologically blocked (*12*). We hypothesized therefore that the neuroprotective effects of neurotrophins may be restricted to apoptosis. To test this, we determined the effects of neurotrophins on murine cortical cell cultures exposed to stimuli that induced apoptosis or necrosis.

To induce neuronal apoptosis, we transferred near-pure neuronal cultures (Fig. 1A) to serum-deficient medium (*13*), resulting in widespread neuronal degeneration over 24 hours (Fig. 1, B and F). This type of neuronal death showed three features typical of apoptosis. (i) The neurons exhibited gradual cell body shrinkage (Fig. 1B); (ii) death was almost completely abrogated by the addition of cycloheximide (Table 1); and (iii) death was accompanied by the appearance of a DNA "ladder" upon agarose gel electrophoresis (Fig. 1E) (*14*). Addition of BDNF, NT-3, or NT-4/5 to the bathing medium (all at 100 ng/ml) markedly reduced neuronal degeneration (Fig. 1, C and F). In contrast, NGF did not show any neuroprotective effect (Fig. 1, D and F); a control experiment documented the ability of our NGF sample to rescue PC-12 cells from serum deprivation–induced death (*15*).

We also induced neuronal apoptosis by exposing mixed neuron-glia cultures to the dihydropyridine calcium channel antagonist nimodipine, which resulted in neuronal de-

Center for the Study of Nervous System Injury and Department of Neurology, Box 8111, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed.

generation evolving slowly over 48 hours (*16*). Addition of BDNF, NT-3, or NT-4/5 (all at 100 ng/ml) substantially reduced the frequency of this death, whereas NGF had no effect (Fig. 1G).

To induce necrosis, we used oxygen-glucose deprivation (*17*). Exposure of mixed neuron-glia cultures to 60 min of oxygen-glucose deprivation produced acute swelling of neuronal cell bodies, followed by widespread neuronal death; no DNA ladder (Fig. 1E) or protection with cycloheximide was detected (Table 1). A deprivation period of 40 min induced little neuronal injury in control cultures (Fig. 2, A and D) but produced marked acute neuronal swelling (Fig. 2B) and subsequent death in sister cultures treated with BDNF, NT-3, or NT-4/5 (Fig. 2D). Again, NGF treatment had no effect (Fig. 2, C and D).

Because toxic Ca^{2+} influx has been shown to be a critical event in excitotoxic necrosis (*18*), we tested whether the necrosis-potentiating effect of neurotrophins is accompanied by increases in Ca^{2+} influx, as measured by the $^{45}Ca^{2+}$ uptake assay (*19*). Although 24-hour pretreatment of cultures with BDNF (100 ng/ml) did not change the baseline $^{45}Ca^{2+}$ uptake, it increased the $^{45}Ca^{2+}$ uptake induced by 40 min of oxygen-glucose deprivation (Fig. 3A). The selective NMDA antagonist MK-801 (10 μM) blocked this BDNF-induced increase in $^{45}Ca^{2+}$ uptake (Fig. 3A). BDNF-potentiated neuronal death after oxygen-glucose deprivation was also blocked by the addition of MK-801 (10 μM), but it was not reduced by 6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (NBQX; 50 μM) (Fig. 3B), which is a selective α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–kainate antagonist. Similar neurotrophin-mediated potentiation of necrosis was observed when NMDA was added directly to the cultures (Fig. 3C) (*20*).

Table 1. Effect of cycloheximide on neuronal death, shown as the mean percent neuronal loss (± SEM, $n = 4$) estimated by cell counting (serum deprivation) or LDH efflux assay (nimodipine, oxygen-glucose deprivation, NMDA). Cycloheximide was present during and after the indicated insults.

Treatment	Neuronal loss (%)	
	−Cycloheximide	+Cycloheximide (1 μg/ml)
Serum deprivation (24 hours)	66 ± 0.4	10 ± 1.4*
Nimodipine (50 μM, 48 hours)	65 ± 3.4	7 ± 2.5*
Oxygen-glucose deprivation (60 min)	52 ± 3.9	53 ± 3.8
NMDA (500 μM, 5 min)	69 ± 2.5	67 ± 3.5

*Differences from the cultures without cycloheximide ($P < 0.05$).

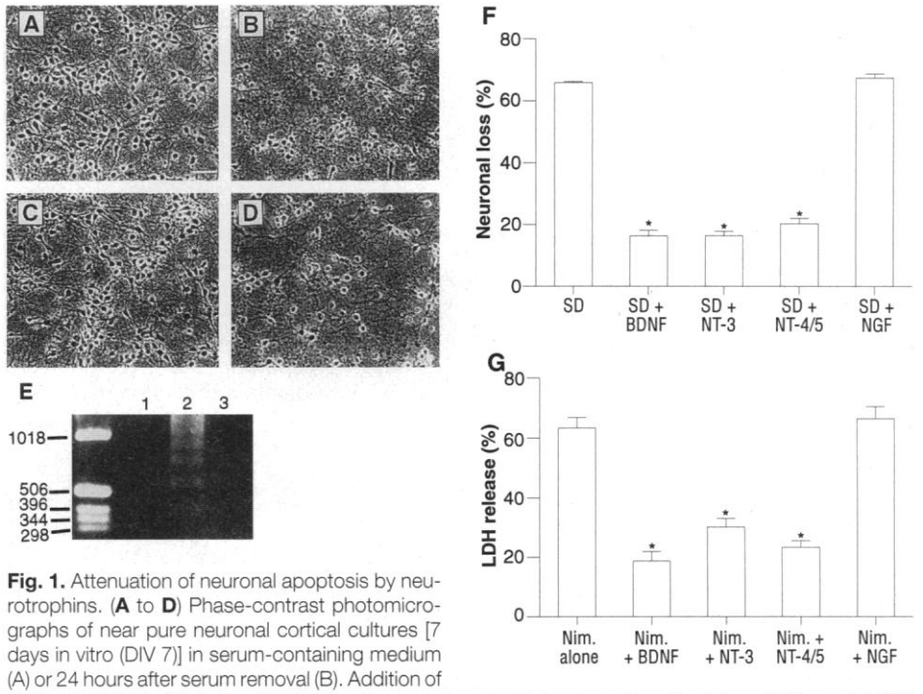

Fig. 1. Attenuation of neuronal apoptosis by neurotrophins. (**A** to **D**) Phase-contrast photomicrographs of near pure neuronal cortical cultures [7 days in vitro (DIV 7)] in serum-containing medium (A) or 24 hours after serum removal (B). Addition of 100 ng/ml BDNF (C), but not NGF (D), attenuated neuronal degeneration. Scale bar, 100 μm. (**E**) DNA agarose gel electrophoresis reveals a ladder pattern 24 hours after serum deprivation (lane 2), but not after sham wash (lane 1) or oxygen-glucose deprivation (OGD) for 60 min (lane 3). DNA size markers were purchased from Gibco. (**F**) Percentage neuronal loss (mean + SEM, $n = 16$) 24 hours after serum deprivation (SD), without or with the indicated neurotrophins (all at 100 ng/ml). (**G**) Percentage LDH release (mean + SEM, $n = 8$) in mixed neuron-glia cultures (DIV 12) after 48-hour exposure to 50 μM nimodipine (Nim.) without or with the indicated neurotrophins (all at 100 ng/ml). Asterisks indicate differences from controls ($P < 0.05$, here and subsequently with a two-tail t test with Bonferroni correction). NGF (2.5S NGF-β) was purchased from Boehringer-Mannheim.

The observation that BDNF, NT-3, and NT-4/5 can protect cultured cortical neurons from apoptosis is consistent with many previous observations. However, we document here that the same neurotrophins can potentiate excitotoxic necrosis in the same neurons. The lack of an effect with NGF is consistent with the expression of TrkB and TrkC, but not of TrkA, in the mammalian neocortex (*21*). The concentrations of

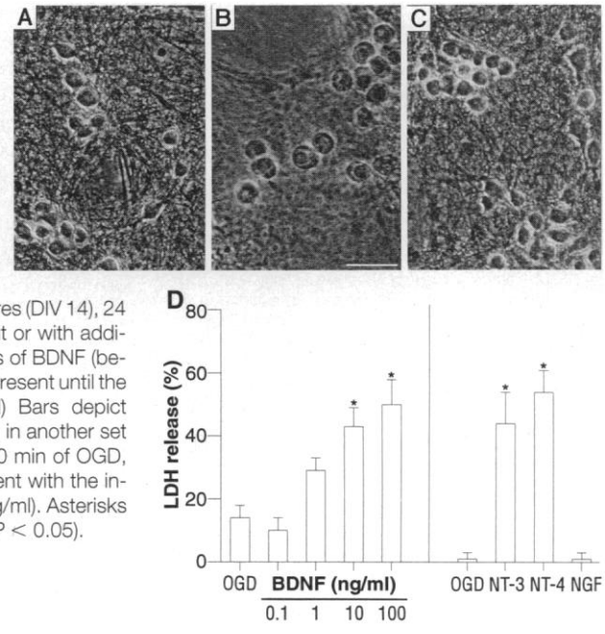

Fig. 2. Potentiation of neuronal necrosis by neurotrophins. (**A** to **C**) Phase-contrast photomicrographs of mixed cultures (DIV 14) immediately after a 40-min exposure to OGD, without (A) or with 24-hour pretreatment with 100 ng/ml BDNF (B) or NGF (C). Note the markedly swollen neuronal cell bodies in (B). Scale bar, 100 μm. (**D**) (Left panel) Bars denote LDH release (mean + SEM, $n = 10$ to 12) in sister cultures (DIV 14), 24 hours after 40 min of OGD, without or with addition of the indicated concentrations of BDNF (beginning 24 hours before OGD and present until the LDH measurement). (Right panel) Bars depict LDH release (mean + SEM, $n = 8$) in another set of sister cultures, 24 hours after 40 min of OGD, without or with 24-hour pretreatment with the indicated neurotrophins (all at 100 ng/ml). Asterisks denote differences from controls ($P < 0.05$).

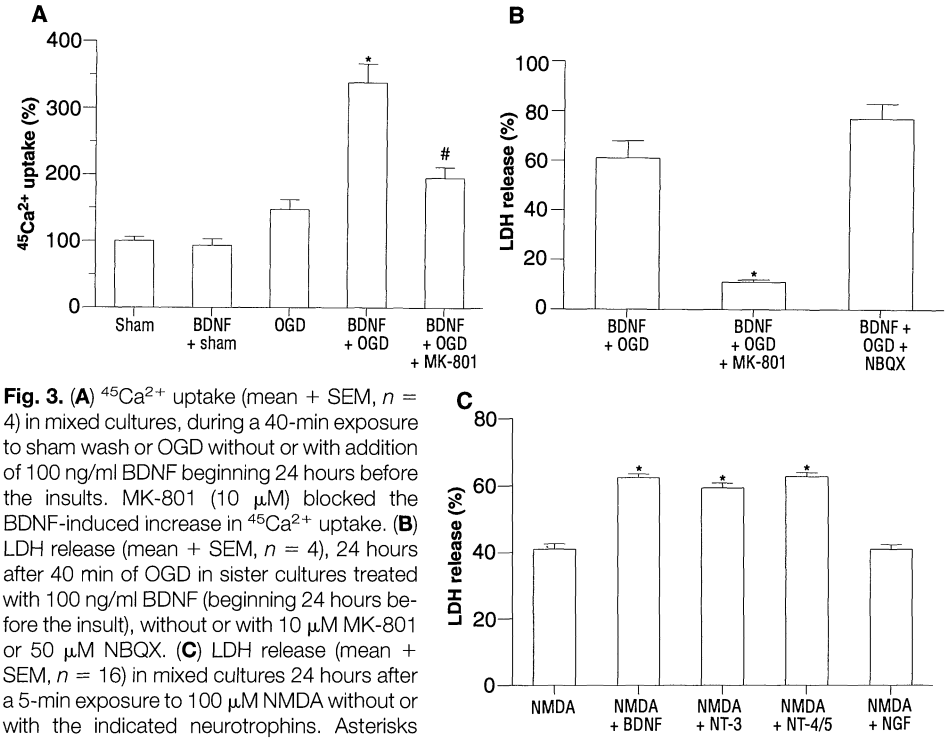

Fig. 3. (**A**) $^{45}Ca^{2+}$ uptake (mean + SEM, n = 4) in mixed cultures, during a 40-min exposure to sham wash or OGD without or with addition of 100 ng/ml BDNF beginning 24 hours before the insults. MK-801 (10 μM) blocked the BDNF-induced increase in $^{45}Ca^{2+}$ uptake. (**B**) LDH release (mean + SEM, n = 4), 24 hours after 40 min of OGD in sister cultures treated with 100 ng/ml BDNF (beginning 24 hours before the insult), without or with 10 μM MK-801 or 50 μM NBQX. (**C**) LDH release (mean + SEM, n = 16) in mixed cultures 24 hours after a 5-min exposure to 100 μM NMDA without or with the indicated neurotrophins. Asterisks denote differences from controls [(A), OGD; (B), BDNF + OGD; and (C), NMDA] at $P < 0.05$. In (A), # denotes difference from BDNF + OGD, but no difference from OGD.

BDNF, NT-3, and NT-4/5 that potentiated necrosis have been shown previously to produce selective trophic effects in other systems (*22*).

Although further study will be needed to delineate the mechanisms responsible for the necrosis-potentiating effect of neurotrophins, our results suggest that this potentiation is at least partly due to increased NMDA receptor–mediated Ca^{2+} influx. Other plausible mechanisms might include increases in phosphoinositide hydrolysis (*23*), Ca^{2+} currents through voltage-gated Ca^{2+} channels (*24*), or intracellular free Ca^{2+} (*25*). Increased concentrations of intracellular free Ca^{2+} might be beneficial to cells undergoing apoptosis (*26*), but may also be detrimental to cells already overloaded with Ca^{2+} because of excitotoxic NMDA receptor overstimulation (*18*). Yet another possible mechanism might be increased cell metabolism and glucose utilization (*27*), an effect that could be beneficial under certain circumstances but that could increase excitotoxicity under conditions of energy depletion (*28*).

Regardless of the underlying mechanism, careful consideration should be given to the therapeutic application of neurotrophins for the treatment of certain forms of brain injuries, in particular hypoxic-ischemic injury. Even when net beneficial effects are observed, there is the possibility that neuroprotection is limited by the concurrent potentiation of excitotoxic necrosis.

REFERENCES AND NOTES

1. H. Thoenen, *Trends Neurosci.* **14**, 165 (1991); W. D. Snider, *Cell* **77**, 627 (1994).
2. D. Martin-Zanca, R. Oskam, G. Mitra, T. Copeland, M. Barbcaid, *Mol. Cell. Biol.* **9**, 24 (1989); D. S. Middlemas, R. A. Lindberg, T. Hunter, *ibid.* **11**, 143 (1991); F. Lamballe, R. Klein, M. Barbcaid, *Cell* **66**, 967 (1991).
3. J. F. R. Kerr, A. H. Wyllie, A. R. Currie, *Br. J. Cancer* **26**, 239 (1972); M. J. Arends, R. G. Morris, A. H. Wyllie, *Am. J. Pathol.* **136**, 593 (1990).
4. V. Hamburger and R. W. Oppenheim, *Neurosci. Comment* **1**, 39 (1982); D. P. Martin *et al.*, *J. Cell Biol.* **106**, 829 (1988).
5. F. Hefti, *J. Neurosci.* **6**, 2155 (1986); M. H. Tuszynski, H. U. Sang, D. G. Amaral, F. H. Gage, *ibid.* **10**, 3604 (1990); H. R. Widmer, B. Knusel, F. Hefti, *NeuroReport* **4**, 363 (1993).
6. Q. Yan, J. Elliott, W. D. Snider, *Nature* **360**, 753 (1992).
7. T. Shigeno *et al.*, *J. Neurosci.* **11**, 2914 (1991); S. W. Davies and K. Beardsall, *Neurosci. Lett.* **140**, 161 (1992); D. M. Frim *et al.*, *NeuroReport* **4**, 367 (1993); B. Cheng and M. P. Mattson, *Brain Res.* **640**, 56 (1994).
8. M. P. Mattson, M. Murrain, P. B. Guthrie, S. B. Kater, *J. Neurosci.* **9**, 3728 (1989); K. Nozaki, S. P. Finkelstein, M. F. Beal, *J. Cereb. Blood Flow Metab.* **13**, 221 (1993); J. H. Prehn, C. Backhaus, J. Krieglstein, *ibid.*, p. 521; M. T. Fernando-Sanchez and A. Novelli, *FEBS Lett.* **335**, 124 (1993).
9. D. W. Choi *et al.*, in *Neurodegenerative Disorders: Mechanism and Prospect for Therapy*, D. L. Price, H. Thoenen, A. J. Aguayo, Eds. (Wiley, Chichester, UK, 1990), pp. 233–248.
10. S. Kure, T. Tominaga, T. Yoshimoto, K. Tada, K. Narisawa, *Biochem. Biophys. Res. Commun.* **179**, 39 (1991).
11. M. D. Linnik, R. H. Zobrist, M. D. Hartfield, *Stroke* **24**, 2002 (1993).
12. T. Shigeno *et al.*, *Neurosci. Lett.* **120**, 117 (1990); B. J. Gwag, D. Lobner, J. Koh, M. B. Wie, D. W. Choi, *Soc. Neurosci. Abstr.* **20**, 248 (1994).
13. Near-pure neuronal cultures were used for serum deprivation experiments, because the presence of glia prevents neuronal degeneration after serum deprivation. Dissociated cortical cells obtained from mouse embryos (at embryonic day 15) were plated in 24-well plates (coated with poly-D-lysine and laminin) with Eagle's minimal essential medium (MEM, Earl's salt) supplemented with 15 μM glucose, 2 mM glutamine, 5% fetal bovine serum, and 5% horse serum (four cortical hemispheres per 24-well plate). Three days after plating, 10 mM cytosine arabinoside was added for 1 to 3 days. Cells were subsequently maintained in growth medium similar to the plating medium but lacking fetal serum or added glutamine. Antiglial acidic fibrillary protein immunocytochemistry revealed that less than 1% of total cells were astrocytes. We initiated serum deprivation by transferring cultures into growth medium lacking serum. Neuronal cell death was assessed by cell counts after staining with 0.4% trypan blue dye. Experiments were done in accordance with a protocol approved by our institutional animal care committee.
14. For evaluation of cellular DNA fragmentation, soluble DNA was extracted as described [D. Hockenbery, G. Nuñez, C. Milliman, R. D. Schrieber, S. J. Korsmeyer, *Nature* **348**, 334 (1990)].
15. M. I. Beherens, J. Koh, D. W. Choi, unpublished results.
16. J. Koh and C. W. Cotman, *Brain Res.* **587**, 233 (1992). Mixed neuron-glia cultures were generated as described (*13*), except that cells were plated onto pre-established cortical glia. We estimated neuronal death by measuring lactate dehydrogenase (LDH) activity released into the bathing medium. After subtraction of background LDH values obtained in sister cultures exposed to a sham wash, the resulting LDH signal was scaled to the mean value obtained in other sister cultures exposed for 48 hours to 500 μM NMDA (resulting in nearly complete neuronal death without glial death).
17. M. P. Goldberg and D. W. Choi, *J. Neurosci.* **13**, 1510 (1993). Oxygen-glucose deprivation was initiated by transferring cultures to a deoxygenated solution in an air-tight chamber flooded with 95% N_2 and 5% CO_2; cell death was assessed the next day by morphological inspection and LDH efflux.
18. D. W. Choi, *Neuron* **1**, 623 (1988); D. H. Hartley, M. C. Kurth, L. Bjerkness, J. H. Weiss, D. W. Choi, *J. Neurosci.* **13**, 1993 (1993).
19. To measure $^{45}Ca^{2+}$ uptake, we transferred sister cultures to exposure medium containing $^{45}Ca^{2+}$ (New England Nuclear; final activity, 0.5 to 1 mCi/ml) for 40 min, with and without oxygen-glucose deprivation. After thorough washout of extracellular $^{45}Ca^{2+}$, intracellularly accumulated $^{45}Ca^{2+}$ activity was measured (*18*).
20. Exposure to NMDA was performed in a physiological salt solution at room temperature (*18*). Cell death was assessed the next day by morphological inspection and LDH efflux. Previous studies had documented the necrosis-type acute cell swelling (*18*) and absence of DNA laddering in this paradigm [C. A. Csernanski, L. M. T. Canzoniero, S. L. Sensi, S. P. Yu, D. W. Choi, *J. Neurosci. Res.* **38**, 101 (1994)]. Cycloheximide did not reduce rapidly triggered neuronal death induced by NMDA (Table 1).
21. T. Ringstedt, H. Lagercrants, H. Persson, *Dev. Brain Res.* **72**, 119 (1993); D. M. Holtzman *et al.*, *J. Neurosci.* **15**, 1567 (1995).
22. N. Y. Yip *et al.*, *Neuron* **10**, 137 (1993).
23. M. Knipper, A. Beck, J. Rylett, H. Breer, *NeuroReport* **4**, 483 (1993).
24. E. S. Levine, C. F. Dreyfus, I. B. Black, M. R. Plummer, *J. Neurosci.*, in press.
25. B. Beringer, D. E. Garcia, N. Inagaki, C. Hahnel, D. Lindholm, *NeuroReport* **4**, 1303 (1993).
26. T. Koike, D. P. Martin, E. M. Johnson Jr., *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6421 (1989).
27. A. Morelli, M. Grasso, P. Calissano, *J. Neurochem.* **47**, 375 (1986).
28. R. C. Hennebury, A. Novelli, J. A. Cox, P. G. Lysko, *Ann. N.Y. Acad. Sci.* **568**, 225 (1989).

29. Supported by NIH grant NS 30337. BDNF, NT-3, and NT-4/5 were generously supplied by Genentech.

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