with LTP in CA1 (*14*). Therefore, there are likely to be at least two independent mechanisms by which synaptic strength can be enhanced at these synapses. The mRNA levels for BDNF and NT-3 are enhanced after tetanic stimulation (*3*), however, which suggests that the synaptic enhancement documented here may contribute to later phases of LTP. Taken together, these data and a previous study (*5*) suggest that one action of neurotrophins may be to alter synaptic strength acutely in the period of time preceding the long-term structural changes that underlie developmental and adult plasticity.

REFERENCES AND NOTES

1. R. Klein *et al.*, *Cell* **61**, 647 (1990); F. Lamballe *et al.*, *ibid.* **66**, 967 (1991); P. Enforrs *et al.*, *Eur. J. Neurosci.* **4**, 1140 (1992).
2. F. Zafra *et al.*, *EMBO J.* **9**, 3545 (1990).
3. S. L. Patterson *et al.*, *Neuron* **9**, 1081 (1992).
4. B. Lu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 937 (1991); P. J. Isackson *et al.*, *Neuron* **6**, 937 (1991).
5. A. M. Lohof *et al.*, *Nature* **363**, 350 (1993).
6. Hippocampal slices were prepared from young adult male Sprague-Dawley rats (mean age = 46.8 ± 1.4 days). Slices were submerged in a stream of ACSF (flow rate 250 ml/hour) (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 11.0 mM glucose) maintained at room temperature (22° to 25°C) and gassed with 95% O_2 and 5% CO_2. Field EPSPs, measured in the stratum radiatum, were evoked by stimulation of the Schaffer collateral-commissural afferents (once every 15 s); the initial (1 to 2 ms) slope was measured. Percent baseline values are reported for 1 hour after neurotrophin addition or LTP induction. Input resistance was monitored before and after neurotrophin application by current injection through intracellular recording electrodes placed in the stratum pyramidale of CA1. Ensemble average plots represent group means of each EPSP, for all experiments, aligned with respect to the time of neurotrophin application or LTP induction (four individual 100-Hz trains delivered for 1 s each at the test intensity; intertrain interval = 15 s). To assess statistical significance, paired *t* tests were done on nonnormalized data, comparing mean EPSP slope values for the 10 min preceding the application of the neurotrophin to values 50 to 60 min after application. *P* values greater than 0.05 are designated as NS.
7. Great care was taken in the application and storage of the neurotrophic factors. BDNF and NT-3 were kept at −70°C; phosphate buffer stock solutions were made every 1 to 3 days and kept at 4°C. NGF (R&D Systems, Minneapolis, MN) was kept at 4°C. New supplies of NT-3 and BDNF were obtained on a regular basis (every 2 to 4 months), as it was observed that individual stocks became less potent over time. BDNF was prepared in phosphate-buffered saline (PBS) and NT-3 was prepared in 0.5% sucrose and 4.5% mannitol. Application of vehicle alone at the appropriate dilutions (10^{-5} to 10^{-6}) had no effect on synaptic transmission. The perfusion apparatus was modified to include chemically inert materials: silicon tubing and a Teflon beaker. Bovine serum albumin was not used as a carrier, because previous work (*19*) has suggested that it has independent effects on synaptic transmission and LTP in the hippocampus. The K252 compounds (Kamiya Biochemical, Thousand Oaks, CA) were kept as 10^4 stock solutions in dimethyl sulfoxide (DMSO) at 4°C. The final concentration of DMSO in our experiments was 0.01%, which has no detectable effect on synaptic transmission.
8. M. E. Vasquez and T. Ebendal, *Neuroreport* **2**, 593 (1991).
9. A. Ghosh, J. Carnahan, M. E. Greenberg, *Science* **263**, 1618 (1994).
10. H. Kang and E. M. Schuman, data not shown.
11. The amplitude of the presynaptic fiber volley did not change significantly after treatment with BDNF (20 ng/ml) (mean percent of baseline: 106.2 ± 3.6; n = 12) or NT-3 (20 ng/ml) (mean percent of baseline: 107.0 ± 4.1; n = 11). The input resistance of CA1 neurons did not change significantly after treatment with BDNF (50 ng/ml) (mean percent of baseline: 94.0 ± 7.2; n = 6) or NT-3 (50 ng/ml) (mean percent of baseline: 87.6 ± 11.9; n = 5). Although simultaneous measurements of the population spike in the CA1 pyramidal cell layer revealed significant increase in amplitude on exposure to either BDNF or NT-3 [mean percent of baseline: BDNF, 2530.0 ± 60.5% (n = 3); NT-3, 230.7 ± 47.0% (n = 3)], multiple population spikes were never observed as would be expected if the slice were exhibiting epileptic activity or large increases in neuronal excitability.
12. At 200 nM, K252a potently inhibits receptor tyrosine kinases, whereas a fivefold greater concentration of K252b is required to achieve similar levels of inhibition. B. Knusel and F. Hefti, *J. Neurochem.* **57**, 955 (1991); M. M. Berg, D. W. Sternberg, L. F. Parada, M. V. Chao, *J. Biol. Chem.* **267**, 13 (1992); P. Tapley, F. Lamballe, M. Barbacid, *Oncogene* **7**, 371 (1992).
13. B. Katz and R. Miledi, *J. Physiol. (London)* **195**, 481 (1968).
14. T. Manabe, D. J. A. Wyllie, D. J. Perkel, R. A. Nicoll, *J. Neurophysiol.* **70**, 1451 (1993).
15. PPF was examined at three interstimulus intervals: 100, 50, and 25 ms. BDNF decreased PPF to 92.0, 77.4, and 66.9% of control levels (n = 7), respectively, without reducing the stimulus strength, and to 93.7, 78.0, and 67.8% of control levels (n = 3) when the stimulus strength was reduced to match the size of the field EPSP to pre-BDNF levels. PPF after NT-3 application was 103.0, 90.0, and 67.7% of control levels (n = 6) without stimulus readjustment, and 108.6, 89.4, and 74.9% of control levels (n = 2) with stimulus readjustment. PPF measurements were made from 30 to 90 min after neurotrophin washout. PPF after LTP was 108.1, 104.4, and 100.3% of control levels (n = 4) without stimulus readjustment.
16. To maximally induce LTP, we delivered two to four sets of tetanic stimulation (one set = four trains of 100-Hz stimulation delivered for 1 s; inter-train interval = 15 s, inter-set interval = 5 to 20 min) until the field EPSP had reached its apparent maximum value and no further potentiation could be elicited. This stimulation protocol resulted in LTP of the following magnitude: mean percent of baseline ± SEM: 183.6 ± 23.4% (n = 10). The magnitude of this potentiation was not significantly different from the potentiation obtained with the use of normal induction protocols (one set of tetanic stimulation) [170.5 ± 8.4% (n = 16)].
17. U. Frey, M. Krug, K. G. Reymann, H. Matthies, *Brain Res.* **452**, 57 (1988).
18. P. V. Nguyen, T. Abel, E. R. Kandel, *Science* **265**, 1104 (1994).
19. F. P. Bellinger, S. Madamba, G. R. Siggins, *Brain Res.* **628**, 227 (1993).

20. We thank D. Anderson, M. Kennedy, G. Laurent, P. Patterson, and members of the Schuman lab for helpful discussions and comments and C. Hung for writing data acquisition and analysis software. We thank Amgen for supplying BDNF and NT-3. E.S. is a John Merck Scholar and an Alfred P. Sloan Fellow.

27 October 1994; accepted 10 February 1995

Inhibition of Ocular Dominance Column Formation by Infusion of NT-4/5 or BDNF

Robert J. Cabelli,* Andreas Hohn, Carla J. Shatz

During the development of the visual system of higher mammals, axons from the lateral geniculate nucleus (LGN) become segregated into eye-specific patches (the ocular dominance columns) within their target, layer 4 of the primary visual cortex. This occurs as a consequence of activity-dependent synaptic competition between axons representing the two eyes. The possibility that this competition could be mediated through neurotrophin-receptor interactions was tested. Infusion of neurotrophin-4/5 (NT-4/5) or brain-derived neurotrophic factor (BDNF) into cat primary visual cortex inhibited column formation within the immediate vicinity of the infusion site but not elsewhere in the visual cortex. Infusion of nerve growth factor, neurotrophin 3 (NT-3), or vehicle solution did not affect column formation. These observations implicate TrkB, the common receptor for BDNF and NT-4/5, in the segregation of LGN axons into ocular dominance columns in layer 4. Moreover, they suggest that in addition to their better known roles in the prevention of cell death, neurotrophins may also mediate the activity-dependent control of axonal branching during development of the central nervous system.

One of the principal mechanisms thought to drive the refinement of specific sets of neural connections during development is activity-dependent competition between presynaptic axons for postsynaptic target neurons (*1*). Perhaps the best studied example of this competition in the central nervous system is the formation of ocular dominance columns in the visual cortex of highly binocular mammals such as carnivores and primates. The anatomical basis for the ocular dominance columns is the segregation of axonal inputs from the LGN into alternating, eye-specific patches within layer 4 (*2*). Early in development, however, LGN axons from the two eyes are intermixed with each other in layer 4. The eye-specific patches emerge gradually over a postnatal period of 4 to 6 weeks in the cat as LGN axons remodel and restrict their terminal arbors (*3*, *4*). Experimental perturbations of neural activity have given rise to the idea that competitive interactions be-

Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed.

tween inputs from the two eyes control the balance of ocular dominance within the cortex (*1*, *5*). However, the molecular basis for this competition is poorly understood.

Here, we examine the hypothesis that members of the neurotrophin family, which is comprised of nerve growth factor (NGF), BDNF, NT-3, and NT-4/5 (*6*, *7*), may be involved in aspects of the competitive interactions between LGN axons that control the eye preference of cortical neurons. It is generally thought that developing neurons compete for limiting amounts of target-derived factors. Although neurotrophins have been implicated as factors secreted by target neurons leading to the selective survival of subpopulations of peripheral neurons during development (*8*), recent data suggest that neurotrophins may also modulate other aspects of neuronal development, including synaptic transmission (*9*) and axonal and dendritic arborization (*10*). Moreover, neurotrophins are expressed in the cortex (*7*, *11–13*), and the expression of BDNF in the visual cortex (*12*), and of NGF and BDNF in the hippocampus (*14*), is regulated by activity, as might be expected for signaling molecules postulated to participate in activity-dependent competition. In addition, our previous developmental analysis of neurotrophin receptors in the cat and ferret visual systems (*15*) indicated that TrkB, the specific receptor for BDNF and NT-4/5, and TrkC, the specific receptor for NT-3 (*16*), are present in certain neuronal subpopulations in the LGN and visual cortex during the period in which LGN axons segregate to form ocular dominance patches in layer 4. These observations suggested that LGN neurons might compete for one or more ligands of TrkB or TrkC during the eye-specific segregation of LGN axons. If so, we reasoned that supplying an excess of one of these neurotrophins would remove the basis for competition and therefore should block the formation of ocular dominance patches within layer 4.

To determine whether excess exogenous neurotrophins would prevent ocular dominance columns from forming, we infused neurotrophins by means of osmotic minipumps (*17*) into cat visual cortex from postnatal day 28 (P28) to P42. The ocular dominance patches in layer 4 are clearly visible by P42, whereas at P28 axons have just barely begun to segregate and distinct patches are not yet evident (*3*, *18*). The consequences of the infusions for the segregation of LGN axons into ocular dominance patches within layer 4 were assessed by tracing the transneuronal transport of radioactive label into LGN axon terminals after an intraocular injection of [^{3}H]proline ipsilateral to the cortical hemisphere receiving the neurotrophin infusion (*3*, *17*). Comparison of normal animals (n = 4) with animals infused with the vehicle solution (*17*) (n = 2) demonstrated that vehicle infusion and tissue damage caused by cannula implantation did not alter the normal formation of eye-specific patches or cortical plate cytoarchitecture.

Next, each of the four neurotrophins was tested for its effects on column formation during the height of the segregation period. Representative examples of the pattern of transneuronally transported radioactive label within layer 4 are shown in Fig. 1. After infusion of NGF for 2 weeks, well-defined ocular dominance patches were present in layer 4 throughout the visual cortex (Fig. 1A) (and in all three other animals treated with NGF), regardless of proximity to the tip of the cannula. In marked contrast, when NT-4/5 was infused, silver grains representing the injected eye were distributed much more uniformly within layer 4 in the area close to the infusion site (Fig. 1B), without the patchlike pattern indicative of the pres-

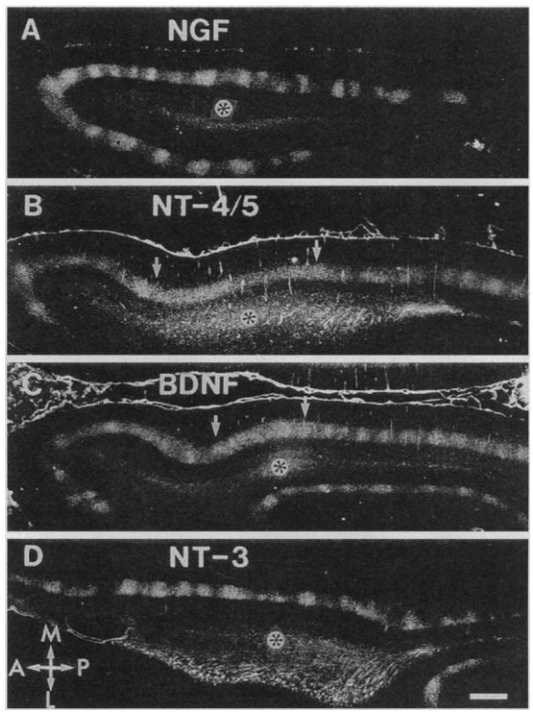

Fig. 1. Infusion of NT-4/5 or BDNF, but not NGF or NT-3, prevents the formation of ocular dominance patches. Cats were administered neurotrophins by intracortical infusion (*16*, *17*). Geniculocortical afferents were labeled with the transneuronal transport of [^{3}H]proline, injected intraocularly ipsilateral to the infused hemisphere. Radioactively labeled LGN axons were visualized by autoradiography and are revealed as bright areas in the dark-field photomicrographs. Photographs were taken from horizontal sections, located immediately below the tip of the cannula, which is indicated by the asterisk. (**A**) NGF (2.4 μg/day) was infused from P28 to P42. The section shown was ~0.75 mm below the tip of the cannula. (**B**) NT-4/5 (2.4 μg/day) was infused from P27 to P42. The section shown was 1.3 mm below the tip of the cannula. (**C**) BDNF (1.2 μg/day) was infused from P27 to P35. The section shown contains the hole generated by the tip of the cannula (**D**) NT-3 (2.4 μg/day) was infused from P27 to P43. The section shown was ~0.35 mm below the tip of the cannula. Scale bar, 1 mm. A, anterior; P, posterior; M, medial; L, lateral. White arrows denote the approximate borders of the area affected by neurotrophin infusion.

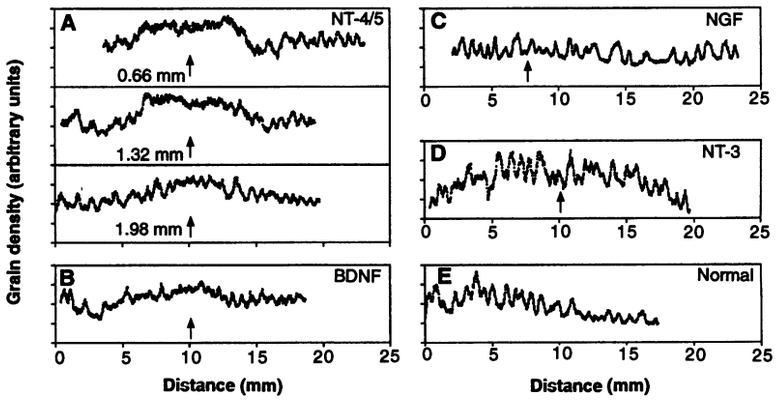

Fig. 2. Quantitative determination of the effects of neurotrophin infusion on silver grain density in layer 4. (**A**) Infusion of NT-4/5. Three sections located at different distances below the tip of the cannula (0.66 mm, as in Fig. 3B; 1.32 mm, as in Fig. 1B; and 1.98 mm) were analyzed. Profiles are aligned on the basis of the position of the cannula in the anterior-posterior axis. (**B**) Infusion of BDNF (as in Fig. 1C). (**C**) Infusion of NGF (as in Fig. 1A). (**D**) Infusion of NT-3 (as in Fig. 1D). (**E**) Control (noninfused animal) at P42. The arrows point to the approximate position of the cannula in the anterior-posterior axis. Note that the grain density profiles in layer 4 of NT-4/5– and BDNF-treated cortex are almost flat in the regions close to the cannula tip, relative to the larger oscillations elsewhere in the same section and in the NT-3– and NGF-treated brains.

ence of normal ocular dominance columns. Relatively uniform labeling within layer 4 was observed within an area 2 to 3 mm anterior and posterior of the infusion site, whereas ocular dominance patches were clearly present in layer 4 beyond these distances from the infusion site (Fig. 1B). Similar results were obtained in all four experiments in which NT-4/5 was infused. Infusion of BDNF, the other ligand for TrkB, also prevented the formation of ocular dominance patches (n = 4) (Fig. 1C); the effect was even more localized than that seen with NT-4/5 infusion because uniform labeling was only apparent in primary visual cortex within 1 to 2 mm of the infusion site (see Fig. 2) (*19*). Infusion of NT-3 had no effect on the pattern of ocular dominance patches (n = 2) (Fig. 1D) despite the fact that its receptor, TrkC, is known to be present at comparable times in the developing ferret visual cortex and LGN (*15*). Thus, infusion of NT-4/5 and BDNF but not of other neurotrophins apparently either arrested or prevented the segregation of LGN axons into eye-specific patches within cortical layer 4.

To quantify the effects of the neurotrophin infusions on ocular dominance column formation, we performed an analysis of the distribution of silver grains within cortical layer 4 on representative sections (*20*) (Fig. 2). The grain density profiles from sections of an NT-4/5–infused brain at three different distances below the cannula tip (0.66 to 1.98 mm) confirmed our impression that the columns had failed to form near the infusion site (Fig. 2A). Fluctuations in grain density in the region close to the infusion site were of very small amplitude, reminiscent of or even smaller than those observed to be present at P28, the time when the infusions were begun (*3*). Furthermore, grain density remained essentially flat over at least 5 mm in the central region of the infusion. At further distances, clear oscillations in grain density, indicative of the presence of ocular dominance columns in layer 4, are evident. Moreover, at the furthest distance below the cannula tip, periodic fluctuations of modest amplitude in grain density are apparent in the affected region, which is significantly smaller in size than that closer to the cannula tip. The grain density profile was similar in BDNF-infused brains, although the region of diminished oscillations in grain density was smaller than in the NT-4/5–treated cortex (Fig. 2B) (*21*). We also noted that the average grain density in the region close to the cannula tip was higher than that in surrounding areas (Fig. 2, A and B), which suggests that an increase in axonal branching may have occurred. However, regional variations in average grain density were also present in other animals, including untreated controls (Fig. 2E), which suggests that nonuniform uptake of [^{3}H]proline within the retina or other factors might account for this observation. Finally, NGF and NT-3 had no effect on fluctuations in silver grain density in layer 4 at any distance from the infusion site (Fig. 2, C and D) and, on average, the grain density profiles were indistinguishable from those in normal (Fig. 2E) and control brains in terms of both distinctness and periodicity (which ranged from 0.35 to 0.5 mm) of ocular dominance columns.

The differences found between the various neurotrophins in their ability to prevent segregation could be due to differences in their intrinsic biochemical roles in the developing visual cortex; alternatively, they could reflect differences in their ability to diffuse from the site of administration. To distinguish between these possibilities, we performed an immunohistochemical analysis. Antibodies specific for recombinant human NT-4/5 (Fig. 3A) or NT-3 (Fig. 3C) were used to detect these particular infused neurotrophins in tissue sections at the completion of the experiments (*22*). In each case, infused neurotrophins were detected at some distance from the infusion site, and there was a good correlation between the area of neurotrophin immunostaining and the region of layer 4 lacking ocular dominance patches. Diffusion within the white matter was most extensive (note, for example, the spread of NT-4/5 and NT-3 in the anterior-posterior axis in these horizontal sections), which suggests that white matter presented less of a barrier, but diffusion into

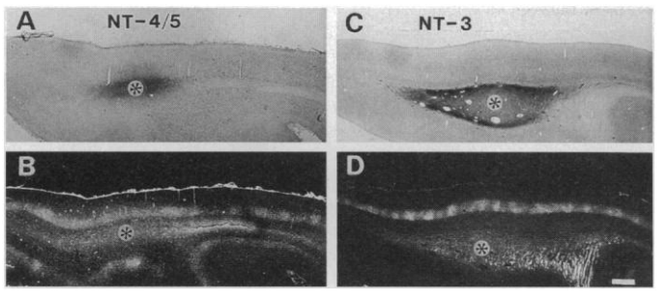

Fig. 3. Assessment of the diffusion of infused NT-4/5 and NT-3 within the visual cortex. Horizontal sections obtained after intracortical infusion of either NT-4/5 (**A** and **B**) or NT-3 (**C** and **D**) were processed for NT-4/5 (A) or NT-3 (C) immunohistochemistry (*25*). Additional sections were processed for autoradiographic analysis of geniculocortical terminals [(B) and (D)]. The position of the cannula is indicated by the asterisk in each case. Scale bar, 1 mm.

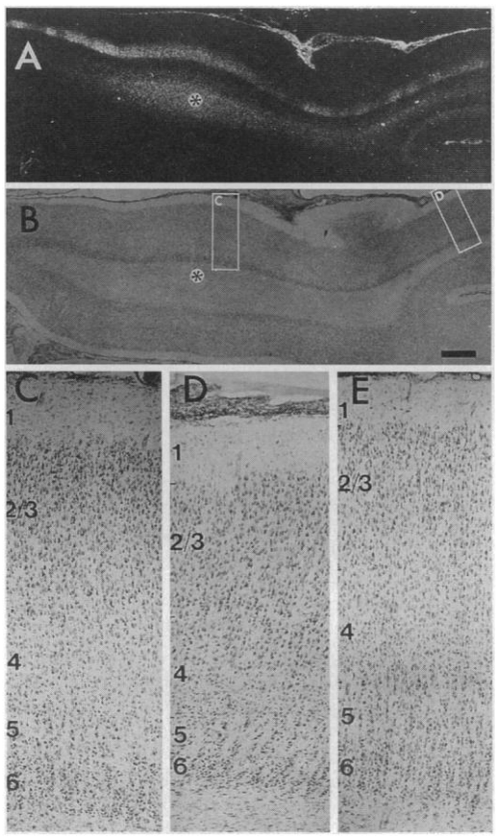

Fig. 4. Cortical cytoarchitecture is unaffected by NT-4/5 infusion. (**A**) Autoradiograph of a horizontal section shown in dark-field optics to indicate affected and unaffected ocular dominance patches in layer 4. The section shown was located ~1.3 mm below the tip of the cannula. (**B**) Bright-field photomicrograph of a section adjacent to that shown in (A) at identical magnification, stained with cresyl violet. Boxed regions corresponding to an area in which the pattern of ocular dominance columns was disrupted (**C**) and a distant area in which columns were unaffected by NT-4/5 infusion (**D**) are shown at higher magnification. (**E**) Region within the visual cortex from the untreated hemisphere corresponding in location to that shown in (C). The position of the cannula is indicated by the asterisk. Scale bar, 1 mm in (A) and (B), 0.25 mm in (C) through (E). Cortical layers are indicated by the number in (C) through (E).

the adjacent cortical plate was also observed, apparent as diffuse extracellular staining at higher magnification in the same sections. These observations and the likelihood that the neurotrophins were provided in considerable excess (*23*) indicate that the effect of NT-4/5 and BDNF on the segregation of LGN axons in layer 4 and the lack of effect of NT-3 and NGF are likely due to specific differences in their biochemical roles, rather than to differences in their bioavailability after infusion.

The absence of clear ocular dominance patches within layer 4 was not due to any obvious effect of neurotrophin infusion on the gross histological organization of the cortex. Cresyl violet staining of sections adjacent to those analyzed for the presence of ocular dominance columns (Fig. 4) revealed that the cortical layers appeared essentially normal, regardless of their proximity to the infusion site (*24*). Moreover, comparison of adjacent dark-field (Fig. 4A) and nissl-stained (Fig. 4B) sections indicated that the restriction of LGN axon terminals to layer 4 was not altered by the treatment; only the segregation into eye-specific patches was affected. In addition, the fact that there was a high grain density, indicative of robust innervation by geniculocortical afferents, in those areas of cortex in which columns failed to form suggests that the axonal pathways were not themselves damaged or disrupted (Fig. 4A).

This study reveals an effect of neurotrophins on the anatomical organization of the system of ocular dominance columns within the mammalian visual cortex. It is important that these effects were seen at the level of the geniculocortical connections, because it is the LGN axons themselves that are involved in the activity-dependent competition. Moreover, the experiments reported here contrast in several important respects with previous studies of the effect of neurotrophins on the ocular dominance of cortical neurons (*25*, *26*). Intraventricular infusion of NGF during the analogous critical period in the rat or the cat can prevent the physiological shift toward the open eye produced by monocular eye closure (*26*). In those experiments, the effects of NGF on the anatomical segregation of LGN axons in layer 4 were not assessed. The mechanism of this effect is not interpretable currently because neither visual cortical neurons nor LGN axons are known to express TrkA, a specific receptor for NGF. However, it is possible that other neuronal systems sensitive to NGF (for example, the basal forebrain cholinergic projections to the cortex) might be involved in monocular deprivation plasticity (*27*). NGF infusion into visual cortex also can cause a physiological shift in responsiveness toward the open eye after monocular occlusion in the adult cat, when there is normally little plasticity (*25*). As seen here, the lack of effect of NGF on the segregation of LGN axons into ocular dominance columns is not necessarily at odds with the results of these other studies, given major differences in the age of infusion and experimental model (initial formation versus later plasticity of ocular dominance columns) and the method of assay of the effect (anatomical versus electrophysiological).

These observations demonstrate that the presence of excess amounts of BDNF or NT-4/5 in visual cortex disrupt the formation of ocular dominance columns, either by arresting or permanently blocking segregation of LGN axons. Because both of these neurotrophins are ligands of TrkB, it seems likely that TrkB is the receptor through which the disruption of ocular dominance columns is mediated. In the carnivore, TrkB is present in both the LGN and the visual cortex at developmental times comparable to the period covered by the experiments described here (*15*). These observations suggest that LGN axons are likely targets of BDNF or NT-4/5 (*28*, *29*). If so, infused TrkB ligands could directly alter the development of LGN axons through binding to TrkB on the axon terminals, triggering signal transduction cascades that modulate axon remodeling. Moreover, the fact that segregation of LGN axons is prevented by flooding visual cortex with excess BDNF or NT-4/5 suggests that neurotrophins are normally present in limiting amounts, thereby creating a true competitive situation. A specific role for endogenous TrkB ligands in the competitive remodeling of axon terminals requires a source of these neurotrophins. Although the presence of small amounts of BDNF mRNA in layer 4—subject to regulation by activity—has been demonstrated in the rat visual system (*12*) and we have also detected BDNF mRNA in the developing cat visual cortex (*13*), it is not known whether NT-4/5 is expressed by layer 4 neurons in any species. An important test of whether endogenous neurotrophins function during the normal formation of ocular dominance columns is a demonstration that blocking the function of the relevant neurotrophins prevents segregation in layer 4.

REFERENCES AND NOTES

1. C. S. Goodman and C. J. Shaltz, *Cell* **72**, 77 (1993).
2. S. LeVay, D. H. Hubel, T. N. Wiesel, *J. Comp. Neurol.* **159**, 559 (1975).
3. S. LeVay, M. P. Stryker, C. J. Shatz, *ibid.* **179**, 223 (1978).
4. A. Antonini and M. P. Stryker, *J. Neurosci.* **13**, 3549 (1993).
5. C. J. Shatz, *Neuron* **5**, 745 (1990); D. H. Hubel, T. N. Wisel, S. LeVay, *Philos. Trans. R. Soc. London Ser. B* **278**, 377 (1977); C. J. Shatz and M. P. Stryker, *J. Physiol. (London)* **281**, 267 (1978).
6. S. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 302 (1960); Y.-A. Barde *et al.*, *EMBO J.* **1**, 549 (1982); J. Leibrock *et al.*, *Nature* **341**, 149 (1989); P. C. Maisonpierre *et al.*, *Science* **247**, 1446 (1990); A. Rosenthal *et al.*, *Neuron* **4**, 767 (1990); K. Jones and L. Reichardt, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8060 (1990); F. Hallböök, C. F. Ibánez, H. Persson, *Neuron* **6**, 845 (1991); L. R. Berkemeier *et al.*, *ibid.* **7**, 857 (1991); N. Ip *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3060 (1992).
7. A. Hohn, J. Leibrock, K. Bailey, Y.-A. Barde, *Nature* **344**, 339 (1990).
8. R. W. Oppenheim, *Annu. Rev. Neurosci.* **14**, 453 (1991).
9. A. M. Lohof, N. Y. Ip, M.-m. Poo, *Nature* **363**, (1993).
10. D. Purves, W. D. Snider, J. T. Voyvodic, *ibid.* **336**, 123 (1988); S. Cohen-Cory, C. F. Dreyfus, I. B. Black, *J. Neurosci.* **11**, 462 (1991); J. Diamond, M. Holmes, M. Couglin, *ibid.* **12**, 1454 (1992); L. G. Isaacson, B. N. Saffran, K. A. Crutcher, *J. Comp. Neurol.* **326**, 327 (1992); L. Schnell, R. Schneider, R. Kolbeck, Y.-A. Barde, M. E. Schwab, *Nature* **367**, 170 (1994).
11. M. Hayashi, A. Yamashita, K. Shimizu, *Neuroscience* **36**, 683 (1990); T. Timmusk *et al.*, *Eur. J. Neurosci.* **5**, 605 (1993).
12. E. Castren, F. Zafra, H. Thoenen, D. Lindholm, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9444 (1992).
13. A. Hohn, E. Lein, C. J. Shatz, paper presented at the 24th Annual Meeting of the Society for Neuroscience, Miami, FL, 13 to 18 November 1994.
14. P. Ernfors *et al.*, *Neuron* **7**, 164 (1991); C. M. Gall and P. J. Isackson, *Science* **245**, 758 (1989); F. Zafra *et al.*, *EMBO J.* **9**, 3545 (1990); P. J. Isackson *et al.*, *Neuron* **6**, 937 (1991).
15. K. Allendoerfer *et al.*, *J. Neurosci.* **14**, 1795 (1994); R. J. Cabelli *et al.*, paper presented at the 23rd Annual Meeting of the Society for Neuroscience, Washington, DC, 7 to 12 November 1993.
16. M. V. Chao, *Neuron* **9**, 583 (1992).
17. Intracortical minipump implantations were carried out under aseptic surgical conditions, and the cats were anesthetized with a mixture of isoflurane and nitrous oxide throughout all procedures, in accordance with institutional guidelines. For the infusions, the tip of the minipump cannula, a 28-gauge stainless steel needle that was unbeveled, was placed in the white matter, approximately 2 mm below the dorsal surface of the lateral gyrus, 12 mm anterior of lambda and 2 mm lateral to the sagittal suture. Neurotrophins (Genentech, South San Francisco, CA) at a concentration of 0.2 mg/ml in 10 mM sodium phosphate buffer, pH 7.4, 140 mM NaCl, and 3.7 mM KCl (PBS), containing bovine serum albumin (1 mg/ml), were infused at a rate of 2.4 μg/day unless otherwise noted. To reveal the ocular dominance patches in layer 4, we labeled geniculocortical afferents with the transneuronal tracer [^{3}H]proline injected intraocularly, ipsilateral to the cortical hemisphere receiving the neurotrohpin infusion. The projection from the ipsilateral eye was studied because it is not confounded by the spillover of radioactive label from fibers of passage into the LGN layers receiving input from the uninjected eye (*3*). Experiments were terminated 7 to 10 days after the intraocular injections. The cats were aesthetized with pentobarbitol (15 grains per kilogram of body weight) and perfused with 250 ml of 4% paraformaldehyde in PBS. Brains were sectioned and sections dipped for emulsion autoradiography, and adjacent sections were stained with cresyl violet to assess the overall histological appearance of the cortex (*3*).
18. In the cat's visual system, geniculocortical axons begin to segregate into ocular dominance columns within layer 4 at about P21; by P50 segregation is nearly like that found in adults (*3*). The neurotrophins were infused for a 2-week period because the osmotic minipumps are active for only 2 weeks and because of concerns about the biological activity of the neurotrophins for longer periods of time at 37°C. P42 was chosen as an endpoint because columns should be clearly observable at this time under nor-

mal circumstances using the technique of transneuronal transport.

19. The presence in some instances of ocular dominance patches in the cortical plate lateral to the cannula site in area 18 as in, for example, Fig. 1C, might be a result of variable or asymmetric diffusion of the neurotrophin or an inability of neurotrophin infusion at this age to affect the formation of ocular dominance patches in area 18, which is known to occur earlier than in area 17.
20. Dark-field images were directly imported from microscope slides by means of a video camera with the use of Metamorph (Universal Imaging). For each slide, layer 4 was subdivided into a series of overlapping linear segments. A window was drawn around each segment and a grain density profile was generated with Scion 1.55 (National Institutes of Health). Grain density profile data were spliced to create the continuous linear profiles shown (Fig. 2).
21. Measurements of grain density in the BDNF- or NT-4/5–treated brains indicate that the average diameter of the region over which grain density fluctuation was clearly diminished was 3.4 ± 0.4 mm for BDNF (n = 4 animals) and 5 ± 0.8 mm for NT-4/5 (n = 4 animals).
22. The specificity of these antibodies has been demonstrated biochemically, on protein immunoblots, and immunohistochemically, through the visualization of neurotrophins injected into rat brain and after preabsorption with antigen (J. Dugich-Djordjevic *et al.*, unpublished results). After perfusion, brains were dissected, postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer, cryoprotected in 25% sucrose in 0.1 M phosphate buffer, frozen, and stored at −80°C. Sections were cut on a freezing microtome, washed in PBS three times, and incubated with 3% normal serum in PBS containing 0.5% bovine serum albumin and 0.1% Tween-20 (blocking buffer) for 2 hours at room temperature. Sections were then incubated with primary antibodies overnight at 4°C, washed three times for 10 min with blocking buffer, incubated with secondary antibodies for 2 hours at room temperature, washed, and visualized with the ABC kit (Vector) using diaminobenzidine (0.05%) with hydrogen peroxide (0.006%) and nickel ammonium sulfate (0.05%) as chromogen.
23. Although it is not possible to relate directly the immunohistohemical staining of neurotrophins to their free effective concentrations in the tissue (the micrographs shown in Fig. 3 probably represent a minimum estimate of the diffusion distance of the infused neurotrophins), it is likely that an excess of all four neurotrophins over the amount necessary to saturate their receptors has been provided. First, the rate of infusion of neurotrophins in this study (2.4 μg/day) is identical to the rate of infusion of NGF used by Gu *et al.* (*25*) to induce monocular deprivation plasticity in the adult cat. It is also well within the range of rates of ventricular NGF infusion that prevent the physiological shift in responsiveness after monocular deprivation (*26*). Second, assuming that the daily dosage of neurotrophin was able to diffuse throughout the affected area or, in the case of NT-3, the area in which it is detectable by immunohistochemistry, approximately 24 μg of neurotrophin would diffuse into 1 ml of cortex each day. Given that half-maximal neurotrophin responses typically occur at concentrations less than 1 ng/ml, we almost certainly provided a significant excess, even if concentrations within the cortical plate were smaller than those in the white matter and with some metabolism of the infused neurotrophins. Finally, the neurotrophins maintain at least 80% of their activity upon storage for 2 weeks at 37°C [L. R. Williams, *Neurobiol. Aging* **2**, 39 (1991); F. Hefti, personal communication]. Thus, it is unlikely that differences in the effective concentrations, diffusion properties, or stability of the different neurotrophins could account for the lack of effect of NGF and NT-3.
24. In several animals, the infusion itself created a large hole in the white matter. However, in control animals this did not have any effect on cortical plate morphology or on the formation of ocular dominance columns, even in close proximity to the infusion site. Furthermore, the presence of a hole at the site of infusion did not affect the diffusion of neurotrophins into the surrounding white matter and cortex, as determined immunohistochemically. Cortical plate morphology was indistinguishable from that of controls in two of the four BDNF-infused brains and in two of the four NT-4/5–infused brains (as well as in those brains in which NGF or NT-3 had been infused). In the remaining brains (two with BDNF; two with NT-4/5), some disorganization and decrease in cell number was observed in cortical layers 4 and 5, but only in very close proximity to the site of infusion and over a much smaller area than that in which ocular dominance patches were absent.
25. Q. Gu, Y. L. Liu, M. S. Cynader, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8408 (1994).
26. L. Domenici *et al.*, *ibid.* **88**, 8811 (1991); L. Maffei *et al.*, *J. Neurosci.* **12**, 4651 (1992); G. Carmignoto *et al.*, *J. Physiol. (London)* **464**, 343 (1993).
27. The cholinergic inputs from the basal forebrain to the cortex express TrkA [M. E. Vazquez and T. Ebendal, *NeuroReport* **2**, 593 (1991); D. M. Holtman *et al.*, *Neuron* **9**, 465 (1992)] and are thought to allow ocular dominance plasticity, as assessed physiologically [M. F. Bear and W. Singer, *Nature* **320**, 172 (1986)]. Thus, it is possible that the NGF infusions could have acted to alter these inputs.
28. As an alternative, it is possible that the effects of the infused NT-4/5 and BDNF are mediated indirectly, through interactions with TrkB-expressing cortical neurons. For example, it is known that many pyramidal neurons in cortical layers 3 and 5 are also immunoreactive for TrkB (*16*). However, given the absence of direct connections between LGN axons and layer 3 or layer 5 neurons, we consider it unlikely that the responses of the neurons to neurotrophins would have a direct effect on the behavior of LGN axons.
29. Subplate neurons have been shown to be required for the formation of ocular dominance columns [A. Ghosh and C. J. Shatz, *J. Neurosci.* **14**, 3862 (1994)]. However, it is unlikely that the results presented here reflect a modulation of subplate neuron survival, which might in turn have altered column formation. The major phase of subplate cell death is already over by P28 [J. J. M. Chun and C. J. Shatz, *J. Comp. Neurol.* **282**, 555 (1989)], the time at which these infusions were begun, and preliminary analysis does not indicate significant changes in subplate neuron survival that are a result of neurotrophin infusion at this particular age.
30. We wish to thank F. Hefti, G. Burton, C. Schmelzer, and G. Bennett at Genentech for their generous gifts of neurotrophins and antibodies to NT-3 and NT-4/5. We also acknowledge the able technical assistance of S. Tavazoie. Supported by NIH grants EY02858 and MH 48108 (C.J.S.), NIH postdoctoral fellowship EY06327 (R.J.C.), and the Swiss National Foundation (A.H.). C.J.S. is an investigator of the Howard Hughes Medical Institute.

27 October 1994; accepted 23 February 1995

TECHNICAL COMMENTS

The Body Temperature of *Tyrannosaurus rex*

R. E. Barrick and W. J. Showers (*1*) purport to show that the oxygen isotope signature of *Tyrannosaurus rex* bone phosphate indicates that this species was homeothermic. A statistical analysis of their results based on our knowledge of bone remodelling processes shows that the data is consistent with a widely varying body temperature for *T. rex*.

Living compact bone is remodeled by the construction of individual Haversian systems, so that the shortest event that could possibly be investigated for palaeothermophysiology is the time taken for this process. The same applies to other techniques including palaeodietary reconstruction (*2*). In adult animals this process does not take place in adjacent Haversians in sequence, but essentially randomly throughout the bone. Samples taken across a bone do not, therefore, "record physiological conditions over time," as Barrick and Showers state, but events randomly scattered through an individual creature's life. Moreover, samples which include mineral from more than one Haversian system will produce results averaging different times in the individual's life. The method used by Barrick and Showers (*3*) uses a sample of some 30 mg of phosphate, which is therefore a volume of at least 1 mm^3 of bone (at an overestimated density for bone of 3 g cm^{-3}). Haversian systems are typically cylinders of diameter 200 μm, which if a 1 mm length of a system is taken has a volume of 0.031 mm^3. I therefore estimate that each sample of bone taken represented a minimum of 32 Haversian systems, and therefore that each of the measurements made was the average of the values for 32 Haversian systems.

Taking a series of measurements, each of which is the average of 32 individual events, will lead to the same mean value, but the variance of the measurements will be 32 times smaller than the true variance for those events, and thus the spread of the data will be $\sqrt{32} = 5.7$ times smaller than the true spread. Using this factor to multiply up the ranges observed for single skeletal elements of 1.7° to 3.8°C gives ranges of 9.7° to 21.7°C. These ranges must be regarded as minimum estimates, because of the small value taken for the sample volume.

Such large temperature variations preclude the classification of *T. rex* as a homeotherm, but they are concordant with Barrick and Showers' estimate of 20°C as the annual variation in the core body temperature of a 5000-kg bradymetabolic hadrosaur. It must be concluded that *T. rex* was an ectotherm, not a homeotherm.

Andrew R. Millard
Research Laboratory for Archaeology and the History of Art, Oxford University, 6 Keble Road, Oxford, OX1 3QJ, United Kingdom