Neurotrophins strongly affect visual system development and plasticity. However, the mode of delivery and targets of neurotrophin action are still under debate. For instance, cortical NT-4/5 (neurotrophin 4/5; Ntf4/5) was shown to rescue lateral geniculate nucleus (LGN) neurons from monocular deprivation-induced atrophy suggesting a retrograde action on thalamic afferents. It is still unclear whether LGN neurons respond to NT-4/5 and other neurotrophins during development in animals with normal vision. We now show that infusions of NT-4/5 and NGF (nerve growth factor) into visual cortex at the onset and the peak of the critical period accelerated LGN neuron growth. BDNF (brain-derived neurotrophic factor) was ineffective. The effects of neurotrophin on LGN development were clearly dissociated from the effects at cortical level because soma growth of cortical layer IV and VI neurons was strongly promoted by BDNF. NT-4/5 was only effective at the onset, but no longer at the peak of the critical period suggesting a switch in neurotrophin dependency for these cortical cell classes. To dissociate retrograde and anterograde effects of the TrkB ligands, we analyzed the stratum griseum superficiale (SGS) of the superior colliculus, a target of visual cortical efferents. Indeed, TrkB-expressing inhibitory SGS neurons responded to cortical NT-4/5 infusion with somatic growth. Strikingly, the TrkB-expressing excitatory tectothalamic calbindin neurons in the SGS did not respond. This demonstrated for the first time a selective cell type-specific anterograde action of NT-4/5 and suggested for the LGN that anterograde as well as retrograde effects contribute to soma size regulation. Strikingly, cortical infusion of the cytokine LIF, which affects development of visual cortex neurochemical architecture, transiently inhibited growth of neurons in LGN, cortical layer IV and VI and SGS. In summary, the study presents three important results. First, central neurons regulate soma size development in an age- and ligand-specific fashion. Second, NT-4/5 and NGF accelerate LGN development in rats with normal vision while LIF delays growth. Third, anterogradely transported NT-4/5 effectively promotes neuronal maturation. These differential actions on subcortical neurons may contribute to the different effects of neurotrophins on visual system development and plasticity.

Key words: BDNF, NT-4/5, NGF, LIF, TrkB, TrkC, Rat visual cortex, synRAS-TG mice

INTRODUCTION

Neurotrophins regulate visual cortical development and plasticity (Maffei et al., 1992; Cabelli et al., 1995; Cabelli et al., 1997; Galuske et al., 1996; Berardi et al., 1994; Huang et al., 1999; McAllister et al., 1999; Berardi and Maffei, 2000). Different neurotrophins have different mechanisms of action (Lodovichi et al., 2000; McAllister et al., 1999; Minichiello et al., 1998) possibly due to a difference in their cellular cortical and subcortical targets and/or the cell type-specific activation of signalling pathways (McAllister et al., 1999; Riddle et al., 1995; Wirth et al., 1998a; Wahle et al., 2000). Particularly intriguing is the case of NT-4/5, in that its effects on cortical plasticity clearly differ from those of the other TrkB ligand, BDNF (Lodovichi et al., 2000). Local supply of NT-4/5, but not other neurotrophins, into ferret primary visual cortex rescued LGN neurons from monocular deprivation induced shrinkage (Riddle et al., 1995). This shrinkage results from the loss in competition between LGN fibers driven by the deprived and nondeprived eye at cortical level (Guillery, 1972). Deprived neurons become less effective in evoking release of target-derived factors (Maffei et al., 1992; Berardi et al., 1994; Riddle et al., 1995). Thus, thalamic neurons could be one of the targets for the action of NT-4/5.

However, a factor that prevents LGN atrophy after monocular deprivation is not necessarily the factor that neurons
depend on during development. For instance, a factor could act at the cortical level preventing the ocular dominance shift as is the case for NT-4/5 (Lodovichi et al., 2000; Gillespie et al., 2000), thereby allowing LGN neurons to maintain their normal connections and size without this factor being trophic for LGN neurons. Furthermore, it is unclear whether the effect of cortical NT-4/5 (and other neurotrophins) on thalamic neurons is purely mediated by retrograde transport, since LGN is also target of a corticothalamic projection.

To clarify the issue of the effects of cortical neurotrophins on thalamic neuron development we analyzed LGN soma size development after intracranial infusion of neurotrophins in rats with normal vision. The choice of the rat was prompted by the wealth of molecular data present for the action of neurotrophins in rat visual system (McAllister et al., 1999) and by the fact that the action of all four neurotrophins on ocular dominance plasticity and visual cortical neuron electrical activity have been compared in this species (Lodovichi et al., 2000). Cortical layer IV and VI neurons and synRAS transgenic mice were analyzed to assess whether neurotrophin action on LGN neurons was secondary to an action on their cortical target neurons. To test whether anterograde transport of neurotrophins may contribute to soma growth, we analyzed the superior colliculus (SC) taking advantage of the fact that neurons in the ipsilateral stratum griseum superficiale (SGS) of the SC receive only corticofugal projections. We also investigated the effects of the cytokine leukemia inhibitory factor, LIF, because we recently found that exogenous LIF has effects similar to NT-4/5 on neuropeptide Y mRNA expression in the neocortex, while the endogenous LIF expression is negatively regulated by thalamic afferents (Wirth et al., 1998a; Wahle et al., 2000).

MATERIAL AND METHODS

Animals and surgery

A total of 29 pigmented Long-Evans rats were used (Table 1); 25 were implanted with osmotic minipumps (model 1007D; Alzet, Palo Alto, CA) in the left hemisphere, two animals served as untreated controls, and two untreated animals were used for single- and double-labeling in the SC. As previously described, minipumps were connected via PE tubing to a stainless steel 30-gauge cannula implanted 1 mm lateral to lambda into medial area 18b (secondary visual cortex) of the left visual cortex (Pizzorusso et al., 1999; Lodovichi et al., 2000) leaving area 17 (primary visual cortex) intact. In rat, area 17 is the primary target of the LGN afferents; they project to a lesser extent to secondary visual cortex, which receives its principal input from the thalamic lateralis posterior nucleus. Layer VI pyramidal neurons of areas 17, 18a and 18b project back to the LGN, while layer V pyramidal neurons of these visual fields project to the SC (Sefton and Dreher, 1995).

Osmotic minipumps (pumping rate 0.5 μl/hour for about 6-7 days) were filled with: mouse NGF1 μg/μl (2.5 S, kind gift from Dr D. Mercanti); human recombinant NT-4/5 1 μg/μl (Regeneron); human recombinant BDNF 8.3 μg/μl (Regeneron); LIF 0.08 μg/μl (Alomone Labs, Israel); and cytochrome C 8.3 μg/μl (Sigma) as control for nonspecific effects of the infusion protocol. The efficacy of the LIF batches was proved in organotypic cultures in the assay previously described (Wirth et al., 1998a). Factors were either infused from P12-20, which is the beginning of the critical period in rat (Fagiolini et al., 1994), or from P20-28 (peak of the critical period) and animals were either killed at the end of the infusion period or survived until P45 (end of critical period) in order to analyze long-term effects (Table 1).

In addition, eight age-matched pairs of wild-type and synRAS-TG mice (heterozygous line 50) were analyzed at P13 (2), P30 (1), P40 (3) and 3 months (5) of age. These transgenic mice have activated valin-12 mutant p21 ras under synapsin-I promoter control and were used previously to obtain our morphometric data of neocortical pyramidal neurons (Heumann et al., 2000).

Histology

Animals were killed with an overdose of pentobarbital and transcardially perfused with saline solution followed by 4% paraformaldehyde in 0.1 phosphate buffer, pH 7.4. Brains were postfixed for 2 hours and cryoprotected in 25% buffered sucrose overnight. The right hemispheres were marked by cuts in the ventral cortex and brain base. Blocks containing cortex, LGN and SC were frozen in Tissue Tek. Serial coronal sections of 30 μm thickness were cut with a cryostat. One series of sections was mounted on gelatin-coated slides and stained with thionin. Alternating series were processed for immunohistochemistry and in situ hybridization employing previously described protocols (Wirth et al., 1998a). Immunohistochemistry was performed with antibodies against the Ga2+ -binding proteins parvalbumin (PV, 1:1000, Swant) and calbindin D28K (CB, 1:1000, Swant), and the pan-neuronal marker NeuN (1:1500; Chemikon) then developed by the ABC-horseradish peroxidase method using diaminobenzidine as the chromogen. For in situ hybridization, digoxigenin-UTP or biotin-UTP (Roche) labeled antisense riboprobes were synthetized by in vitro transcription from linearized plasmid cDNA encoding GAD67 (Gad1), GAD65 (Gad2), LIF β-receptor, TrkB kinase domain and TrkC. Hybridization, color development and fluorescent double-labeling was performed as described previously (Wirth et al., 1998b; Gorba and Wahle, 1999).

Analysis

In thionin-stained sections of LGN, SC and visual cortex, all neuronal somatic outlines (nucleus present in the plane of the section) in several randomly chosen fields of interest were reconstructed at 1000× magnification with a camera lucida. In the LGN, neuronal somata in at least 10 such fields throughout the nucleus were reconstructed. Since we did not employ a retrograde tracer to select for thalamocortical relay neurons, we sampled all neurons. In the SGS we reconstructed ‘total’ neurons in thionin-stained sections, and CB-immunoreactive and PV-immunoreactive subsets from ABC-horseradish peroxidase-stained sections. In the visual cortex, thionin-stained neurons were sampled in layers IV and VI of area 17 starting about 0.5 mm lateral to the infusion site boundary. The drawings were digitalized and soma area was determined. For every hemisphere at least 150 somata per animal were reconstructed from at least 3 non-adjacent sections in many cases by operators familiar with soma reconstruction but blind with respect to the hemisphere analyzed or factor infused. Ipsilateral (left infused hemisphere) and contralateral (right untreated hemisphere) values were compared by a non-

Table 1. Animals and infusion protocols

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Factor infused</th>
<th>Infusion period</th>
<th>Age of analysis</th>
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<tr>
<td>3</td>
<td>Cytochrome C</td>
<td>P20-28</td>
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<tr>
<td>4</td>
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<td>Untreated</td>
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parametric rank sum test (Mann-Whitney U-test; significance level accepted $P<0.001$. In addition, KS-tests and ANOVA were run. Animals were first analyzed separately by comparing ipsilateral to contralateral values. To construct the size frequency histograms the data rows of ipsi- and of contralateral hemispheres, respectively, of animals of the same age and treatment were pooled. The size shifts observed in the individual animals were indicated as ‘percentage median shift’ in small insets in the figures while the median shifts for the pooled data were given in the text together with the total cell numbers analyzed. Positive shifts indicate a larger ipsilateral side, negative shifts indicates a smaller ipsilateral side. The cytochrome C-infused control animals delivered the biological variability, it was found to be no larger than $\pm 5\%$ median size shift.

Lack of published information on Trk receptor expression in SC cells types required single- and double-labeling techniques. In defined regions of interest in the SGS we counted the total number of neurons, using NeuN as a marker, the number of GAD65 (Gad2), TrkB (Ntrk2) and TrkC (Ntrk3) mRNA expressing neurons, the CB- and PV-immunoreactive neurons, and the proportion of CB- and PV-immunoreactive neurons expressing GAD65, TrkB and TrkC mRNA. The overlap of GAD65 and TrkB mRNA was analyzed with double-in situ hybridization with a DIG-UTP-labeled GAD riboprobe and a biotin-UTP-labeled TrkB riboprobe (Gorba and Wahle, 1999).

RESULTS

 NT-4/5 and NGF, but not BDNF, increase LGN neuron soma size

In P28 untreated animals LGN neurons in the left and right hemisphere displayed the same variance of soma size (not shown). This was also true after cytochrome C control infusion (A; $n=3$, 543 ipsi/570 contra cells analyzed for the ipsi and the contralateral side, respectively; MWU-test, $P>0.05$) ruling out nonspecific growth-stimulatory effects on LGN neurons by cannula implantation medial to area 17. The size shifts for each animal were calculated and we obtained a mean size difference of $-2.2\%$ for cytochrome C-treated animals.

BDNF infusion did not induce somatic growth in LGN (Fig. 1B, 516 ipsi/520 contra cells, MWU-test $P>0.05$; mean size shift $-0.3\%$, not significantly different from cytochrome C data, one-way ANOVA Tukey post-hoc test). Immunostaining for the infused neurotrophins has revealed the diffusion range (Lodovichi et al., 2000) indicating that the lack of effect of BDNF on thalamic neurons cannot be attributed to a failure of diffusion of BDNF at cortical level. Furthermore, a strong

Fig. 1. Neurotrophins affect LGN neuron size. White bars represent contralateral and black bars ipsilateral values. Size frequency histograms reveal that (A) cortical cytochrome C control and (B) BDNF infusions were not effective. In contrast, (C) cortical NT-4/5 and (D) NGF infusions consistently induced shifts to larger sizes. In the absence of a retrograde tracer all neuronal somata had to be sampled including small-sized cells, which presumably correspond to non-reactive interneurons (representing 20% of LGN neurons) (Sefton and Dreher, 1995). However, the significance levels remained the same when running the tests on only the largest 80% of the neurons. The small inset icons indicate the median shifts $[\text{ipsilateral median size} - \text{contralateral median size}] / \text{contralateral median size}$ in the individual animals analyzed. Positive shifts indicate larger ipsilateral neurons, negative shifts indicates smaller ipsilateral neurons; shifts of up to $\pm 5\%$ were due to biological variation.
effect of BDNF on cortical expression of neuropeptide Y (Engelhardt et al., 2001) and on the soma sizes of cortical neurons was detected in these animals throughout the entire area 17 (see Fig. 5).

In contrast, the infusion of NT-4/5 (Fig. 1C; 661 ipsi/638 contra cells) and NGF (Fig. 1D; 543 ipsi/581 contra cells) clearly induced somatic growth. Neurons in the LGN ipsilateral to the infused cortex displayed dramatic shifts towards larger sizes. The mean size shift with NT-4/5 was +19.2% and with NGF +14.9% (both MWU-tests P<0.0001; significantly different from that with cytochrome C, one way ANOVA P<0.0001, Tukey post hoc test). Thus, at the peak of the critical period thalamic neurons in rats with normal vision responded to the infused cortex displayed dramatic shifts towards larger sizes. (C,D) When (C) early and (D) late infusion periods were followed by survival to P45, the contra- and the ipsilateral population histograms reveal that cortical NT-4/5 infusions (A) at the beginning and (B) at the peak of the critical period consistently induced shifts to larger sizes. (C,D) When (C) early and (D) late infusion periods were followed by survival to P45, the contra- and the ipsilateral population showed the same size variation and for both sides the peaks occurred at larger sizes than in the younger animals. The small insets indicate the median shifts in the individual animals analyzed.

NT-4/5 accelerates LGN soma size development

To obtain a more complete picture of NT-4/5 action on LGN development we compared the effects of cortical infusion at P20-28 with those of an infusion at the beginning of the critical period, P12-20. This early NT-4/5 infusion on LGN soma size was also effective (Fig. 2A: 502 ipsi/540 contra cells, MWU-test P<0.0001; mean size shift +9.6%; different from that with cytochrome C and from NT-4/5 at P28, one way ANOVA P<0.0001), although slightly less than infusions at the peak of the critical period at P20-28 (Fig. 2B; shown again here for direct comparison).

It has been shown that cortical NT-4/5 can induce long lasting changes in cortical neurochemical architecture and in cortical neurons soma size (Wahle et al., 2000) (P. W., unpublished). We therefore asked whether the LGN neuron soma size increase induced by NT-4/5 was transient or permanent. NT-4/5 was infused from P12-20 and P20-28, respectively, and animals were allowed to survive until P45 (Fig. 2C,D). In both cases, there was no difference between ipsilateral and contralateral LGN neurons (Fig. 2C: MWU-test P>0.05; mean size differences +3.2%, 343 ipsi/333 contra cells; Fig. 2D: MWU-test P>0.05, mean size difference +0.6%, 535 ipsi/513 contra cells, one way ANOVA for both data sets P>0.05). When comparing the peak ranges of contralateral and ipsilateral LGN neurons in Fig. 2B-D, it became evident that the NT-4/5 infusion had precociously induced the adult size variation in ipsilateral neurons.

Next, the medians of all ipsilateral data at P20 (NT-4/5, n=2) and P28 (NT-4/5 and NGF, combined n=7) and P45 (NT-4/5, combined n=4, untreated age-matched animals, n=2) were compiled and compared to the medians of the contralateral

Fig. 2. NT-4/5 accelerates growth of LGN neurons. White bars represent contralateral and black bars ipsilateral values. Size frequency histograms reveal that cortical NT-4/5 infusions (A) at the beginning and (B) at the peak of the critical period consistently induced shifts to larger sizes. (C,D) When (C) early and (D) late infusion periods were followed by survival to P45, the contra- and the ipsilateral population showed the same size variation and for both sides the peaks occurred at larger sizes than in the younger animals. The small insets indicate the median shifts in the individual animals analyzed.
sides (Fig. 3). The NGF-infused animals were included because the growth effects produce by the two factors were the same. It became evident that there was a continuous soma size development of contralateral LGN neurons between P20 and P45. In contrast, on the ipsilateral hemispheres NT-4/5 and NGF had accelerated this development such that LGN neurons had acquired their normal adult size already at P28 (Fig. 3; two way ANOVA side x age; age is a significant factor: size at P45 was significantly different from contra at P28 (P<0.01), whereas size at P45 was not different from ipsi at P28 (P>0.05)).

Superior colliculus neurons grow in response to cortically infused NT-4/5

The growth-promoting effect of NT-4/5 on LGN neurons might be mediated solely by retrograde axonal transport (Riddle et al., 1995). However, neurotrophins in the visual system can be anterogradely transported, synthetically released and transferred to postsynaptic neurons (VanBartheld and Butowt, 2000; Kohara et al., 2001; Spalding et al., 2002). Anterogradely delivered neurotrophins have functional effects because BDNF injected into the eye affects gene expression in retinal target structures and promotes survival of axotomized LGN neurons (Caleo et al., 2000). The growth promoting effects in the LGN could thus also be influenced by anterograde transport, since layer VI projects back to the LGN. To test whether anterograde transport affects cell growth we analyzed neurons in an efferent projection (Lane et al., 1997) and do not receive direct synaptic input from the visual cortex. The hypothesis was clear: if cell volume regulation is mediated only by retrogradely transported neurotrophins, SC neurons should not grow.

The two populations (total and subset) responded differentially. In control animals no significant size difference was observed between ipsi- and contralateral total SGS neurons (Fig. 4A; mean size difference +4.5%, 569 ipsi/522 contra cells). Also CB neurons were not different (Fig. 4A inset; mean size difference +1.0%, 330 ipsi/340 contra cells). Cortically infused NT-4/5 induced significant size increases of the total population indicating that anterogradely delivered neurotrophins contribute to volume regulation (Fig. 4B; mean size shift of +10.6%, MWU-test P<<0.001, KS-test P<<0.05; 833 ipsi/972 contra cells). Surprisingly, neither CB nor PV neurons were significantly different (for CB: Fig. 4B inset; mean size difference +2.4%, MWU-test P>0.05; 873 ipsi/746 contra cells; for PV: mean size difference +1.1%, MWU-test P>0.05, 707 ipsi/609 contra cells; not shown and only evaluated in the NT-4/5 infused material). Unexpectedly, BDNF infusion caused a highly significant shrinkage of the total SGS neurons (Fig. 4C; mean size shift -11.8%, MWU-test P<0.0001, 917 ipsi/943 contra cells). Again, the CB neurons were not significantly different (Fig. 4C inset; mean size difference -2.4%, 582 ipsi/529 contra cells). NGF had no effect (not shown).

Hypertrophy of cortical target neurons does not elicit growth of afferent LGN neurons

It is important to understand whether the specific neurotrophin effects on LGN neurons are related to the action of neurotrophins on cortical neurons. We therefore assessed, in
area 17, the soma size distribution of neurons in layers IV and VI where LGN afferents terminate. Cytochrome C control had no effect (Fig. 5A; layer IV: 357 ipsi/364 contra cells, mean size difference -0.1%, MWU-test P >0.05; layer VI: 499 ipsi/472 contra cells, mean size difference +1.3%, MWU-test P >0.05). However, a striking difference between effects in cortical layers and action on LGN development was found for the TrkB ligands. Although BDNF was ineffective for the LGN, it produced a dramatic size increase of neurons in layers IV and VI (Fig. 5B). Layer IV displayed a mean size shift of +26.2% (747 ipsi/696 contra cells, MWU-test P >0.0001); layer VI displayed a mean size shift of +26.7 (705 ipsi/676 contra cells, MWU-test P <0.0001; both significantly different from cytochrome C, one way ANOVA P <0.0001).

Surprisingly, the effects of NT-4/5 were age-dependent. During P20-28 infusions, when LGN neurons responded dramatically, layer IV and VI neurons did not respond with growth (Fig. 5C; layer IV: 681 ipsi/683 contra cells, mean size difference +1.8%, MWU-test P >0.05, not different form cytochrome C; layer VI: 661 ipsi/615 contra cells, mean size difference +4.1%, MWU-test P >0.05). However, the P12-20 NT-4/5 infusions produced significant somatic growth in both layers (Fig. 5C; layer IV: 427 ipsi/452 contra cells, mean size shift +14.7%, MWU-test P <0.0001; layer VI: 300 ipsi/312 contra cells, mean size shift +17.8%, MWU-test P <0.0001). This suggests a switch in neurotrophin dependency of the two neuron types.

NGF was not effective (Fig. 5D; layer IV: 728 ipsi/737 contra cells, mean size difference +2.9%, MWU-test P >0.05; layer VI: 400 ipsi/400 contra cells, mean size difference +1.3%, MWU-test P >0.05). This suggested that the action of NT-4/5 on LGN development is not an indirect consequence of effects at the cortical level and that BDNF is a specific and highly potent factor for layer IV and VI neurons.

To further rule out indirect effects at the cortical level, we examined synRAS-TG mice, which overexpress constitutively activated V12-Ha ras. Ras is a signalling mediator of neurotrophins. The transgene expression is driven by the synapsin-I promoter and developmentally regulated, starting to increase from the first week onwards. synRas-TG is expressed in all cortical layers as is synapsin I (Melloni et al., 1993), but not in thalamic sensory nuclei (Heumann et al., 2000). We previously reported that synRAS-TG mice displayed hypertrophic cortical pyramidal neurons and increased neuropeptide expression indicating that activated p21ras acts as an ‘intracellular neurotrophin’ (Heumann et al., 2000). The question was clear: if the target neurons in the cortex grow larger, does the afferent population react with a size increase?

Five sibling pairs of adult wild-type and transgenic animals (heterozygous line 50) aged P40 and 3 months were analyzed. Layer IV neurons displayed a dramatic hypertrophy with highly significant larger sizes in synRAS-TG mice (Fig. 6A; 750 TG/729 WT neurons, mean size shift WT versus TG +22.4%, MWU-test P <0.0001; similar size shifts occurred in layer VI, not shown). However, we found no systematic size shifts in the LGN (Fig. 6B; 749 TG/779 WT neurons, mean...
size difference +0.2%, MWU-test \( P > 0.05 \)). This indicated that LGN neuron size was neither affected by the constitutively active p21ras in cortical neurons in general nor by the larger size of their cortical target neurons. We further analyzed 3 pairs of mice aged P13 and P30, ages comparable to neurotrophin-infused animals, and that overlapping the critical period of plasticity. However, no size shifts in the LGN were detected (Fig. 6B; 373 TG/374 WT neurons, mean size difference –1.9%, MWU-test \( P > 0.05 \)) suggesting that no developmental acceleration occurs in LGN neurons at times when V12-ras expression causes the hypertrophy in cortical neuron classes. Together, these results suggested that somatic growth of the cortical target cell population (produced by BDNF or in the transgenic model) does not elicit compensatory growth of the afferent LGN neurons.

**Increase in cortical availability of LIF inhibits growth of LGN and SGS neurons**

In organotypic cortex cultures, the cytokine LIF promotes NPY mRNA expression like NT-4/5, and the endogenous LIF expression is downregulated by thalamic fiber ingrowth (Wirth et al., 1998a,b; Wahle et al., 2000). While cortical neurons express LIF β-receptor mRNA, we failed to detect it in LGN and SC neurons (not shown). We therefore compared the effects of intracortical NT-4/5 and LIF infusion. Unexpectedly, LIF inhibited growth (Fig. 7). After the P12-20 infusion period the LGN neurons in the infused hemisphere were very significantly smaller than the contralateral hemisphere (Fig. 7A; 841 ipsi/893 contra cells, mean size shift –9.6%, MWU-test \( P < 0.0001 \); significantly different from cytochrome C, one way ANOVA \( P < 0.05 \)), but without symptoms of degeneration. Two animals were allowed to survive the P12-20 infusion period until P45. At this stage, LGN neurons were of equal size variation (Fig. 7B; 422 ipsi/396 contra cells, mean size difference +1.4%, MWU-test \( P > 0.05 \)). This indicated that the LIF-induced inhibition of LGN somatic growth is transient and that the neurons manage to grow to adult control size variation after the end of the LIF infusion.

In addition, P12-20 LIF infusions affected cortical layer IV and VI neurons, which underwent dramatic shifts to smaller sizes (Fig. 7C; layer IV: 867 ipsi/784 contra cells, mean size shift of –19.1%, MWU-test \( P < 0.0001 \); layer VI: 791 ipsi/691 contra cells, mean size shift –11.0%, MWU-test \( P < 0.0001 \)). Furthermore, the P12-20 LIF infusions caused a dramatic

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**Fig. 5.** Effects of neurotrophins on soma size in primary visual cortex layer IV neurons, and layer VI neurons given in the small insets. White bars represent contralateral and black bars ipsilateral values. (A) Size frequency histograms reveal that cytochrome C control was not effective. (B) BDNF, however, consistently induced dramatic shifts to larger sizes (MWU-test, ipsi versus contra, \( P < 0.0001 \)). (C) In contrast, NT-4/5 did not induce growth of layer IV neurons (diamonds) and layer VI neurons (circles) during the P20-28 infusions. However, the early P12-20 infusions did result in a significant growth of layer IV (triangles up) and layer VI neurons (triangles down), indicating a developmental time window of responsiveness to NT-4/5. (D) NGF did not evoke any growth. The small insets indicate the percentage of median shift in the individual animals analyzed. At P20-28, only the values for BDNF infused animals differed from controls (one way ANOVA, Tukey’s post hoc test).
also depend on cortical neurotrophins during their normal deprivation (Domenici et al., 1993; Riddle et al., 1995) and LGN neurons from shrinkage caused by monocular termination fields regress. Cortical NT-4/5 and NGF rescue supply (Maffei, 1992; Riddle et al., 1995) and their axon level (Guillery, 1972) in eliciting sufficient trophic factor LGN shrink as a result of competitive disavantage at cortical is sensitive to the balance of activity between the two eyes. In developmental growth period of LGN neurons, their soma size termination fields in the cortex. During the protracted 1995). Larger somata may support larger dendritic trees with the backprojection from visual cortex (Sefton and Dreher, 1995). Only these neurons project to visual cortex and receive neurons representing 80% of all LGN neurons (Riddle et al., 1995). However, there was no consistent trend to larger sizes in LGN neurons indicating that they do not adjust with larger sizes to the hypertrophic cortical neurons which develop postnatally in synRAS-TG mice. The small inset indicates the percentage of median shift observed in the five sibling pairs aged P40 and 3 months (diamonds) and in the three sibling pairs aged P13 and P30 (circles).

**DISCUSSION**

**Volume regulation in the LGN by NT-4/5 and NGF**

Employing a classical neurotrophin assay we found that in rats with normal binocular vision LGN neurons reach their adult soma size earlier when given cortical infusion of NT-4/5 and NGF, but not BDNF. The affected neurons are projection neurons representing 80% of all LGN neurons (Riddle et al., 1995). Only these neurons project to visual cortex and receive the backprojection from visual cortex (Sefton and Dreher, 1995). Larger somata may support larger dendritic trees with more afferent synapses and may also support larger axonal termination fields in the cortex. During the protracted developmental growth period of LGN neurons, their soma size is sensitive to the balance of activity between the two eyes. In animals with vision in only one eye, neurons in the affected LGN shrink as a result of competitive disadvantage at cortical level (Guillery, 1972) in eliciting sufficient trophic factor supply (Maffei, 1992; Riddle et al., 1995) and their axon termination fields regress. Cortical NT-4/5 and NGF rescue LGN neurons from shrinkage caused by monocular deprivation (Domenici et al., 1993; Riddle et al., 1995) and functionally maintain the binocular input to cortical neurons (Lodovichi et al., 2000). An indication that thalamic neurons also depend on cortical neurotrophins during their normal development comes from experiments in rats with normal binocular vision where the action of endogenous NGF was antagonized by the intraventricular implant of hybridoma cells secreting anti-NGF antibodies. The LGN neurons shrink, cortical connectivity is altered and the critical period is prolonged. However, since the implant was intraventricular it was impossible to conclude whether the action on LGN neurons was due to a neutralization of cortical NGF. Our present results clarify that the cortical neurotrophins NT-4/5 and NGF specifically accelerate LGN neuron growth during normal development, and do not exclusively act in the deprived condition (Riddle et al., 1995). Both factors cause LGN neurons to reach their adult size variation by P28, whereas they would normally only reach this size by the end of the critical period.

**Retrograde and anterograde actions of NT-4/5**

The NT-4/5 effect could be due to a direct action on LGN terminals because full length TrkB receptors are expressed by LGN neurons and their cortical axon terminals (Silver and Stryker, 2001). Riddle et al. (Riddle et al., 1995) showed a retrograde action, because in their study neurotrophins were coupled to latex beads allowing only retrograde transport. The failure of P20-28 NT-4/5 infusion to evoke a size increase in layer IV and VI neurons indicates that a specific trophic response of LGN neurons can be produced in the absence of a similar effect on their target neurons. This suggests that at the peak of the critical period of visual cortical plasticity, layer IV/VI neurons release NT-4/5 as a trophic factor for their afferent neurons without responding themselves to NT-4/5. However, since these neurons responded during the P12-20 infusion period, they must have selectively downregulated the responsiveness to NT-4/5 (but conserved the responsiveness to BDNF) towards the peak of the critical period.

Our results on SGS neurons now change the interpretation of a solely retrograde action. Selectively those neurons contacted by cortical fibers (Mize, 1996; Mize, 1988) respond with somatic growth, and likely the GAD-65/TrkB mRNA expressing inhibitory neurons receiving somatic synapses are Fig. 6. Size variation of cortical layer IV and LGN neurons in wild-type (WT) and synRAS-TG mice. White bars represent wild-type and black bars synRAS-TG values. (A) Layer IV neurons were consistently larger in the synRAS-TG mice (MWU-test, WT versus TG, P<0.0001). (B) However, there was no consistent trend to larger sizes in LGN neurons indicating that they do not adjust with larger sizes to the hypertrophic cortical neurons which develop postnatally in synRAS-TG mice. The small inset indicates the percentage of median shift observed in the five sibling pairs aged P40 and 3 months (diamonds) and in the three sibling pairs aged P13 and P30 (circles).
the responsive neurons. Indeed, the corticotectal pathway exerts inhibitory actions on the activity produced by the retinotectal pathway (McIllwain and Fields, 1971), and it activates SC interneurons and inhibits LTP through GABAergic mechanisms (Hirai and Okada, 1993). In contrast, the tectothalamic CB neurons were not responsive, despite the fact that these neurons also express TrkB in addition to TrkC mRNA. These neurons receive retinal, but not cortical input, respond to enucleation with a decrease in CB immunoreactivity, and most are excitatory (Mize, 1996; Dreher et al., 1996) (present study). The excitatory PV neurons are also targeted predominantly by retinal input (Mize, 1996).

Since SGS neurons do not project to cortex, this finding reveals a clear anterograde trophic effect of NT-4/5 during postnatal development. Spalding et al. (Spalding et al., 2002) recently showed that ocular injections of NT-4/5 in newborn rats was anterogradely transported and reduced cell death in the SC. Moreover, our NT-4/5 effect appears extraordinarily cell type-specific and input-specific, as if NT-4/5 is delivered by cortical afferents exclusively to SGS inhibitory neurons without affecting nearby excitatory neurons. We must therefore conclude that the strong effects on LGN development in animals with normal vision could stem both from anterograde and retrograde actions of cortical NT-4/5.

**BDNF fails to accelerate LGN development**

BDNF had no effects on LGN soma size development and cannot prevent the structural defects caused by monocular deprivation (Riddle et al., 1995). However, LGN projection neurons are able to respond to BDNF derived from another source, since BDNF injected into the eye rescues LGN neurons from death after visual cortex ablation (Caleo et al., 2000). Thus, for LGN survival the loss of target-derived cortical neurotrophins can be compensated for by an excess of retinal BDNF, whereas cortex-derived NT-4/5 appears to play a dominant role in controlling LGN soma size development. Thus, BDNF and NT-4/5 clearly have a different profile of action in the visual system despite the fact that both converge on the TrkB receptor.

The failure of BDNF was not due to a lack of diffusion or...
biological activity, because on cortical neuron populations analyzed concurrently in these animals BDNF altered gene expression (Engelhardt et al., 2001) and induced a hypertrophy of layer IV and VI neurons throughout area 17. This is in line with the data from the synRAS-TG mice. The lack of LGN soma growth in these mice confirmed that boosting the size of layer IV and VI target neurons during the critical period and in adulthood does not trigger a growth of afferent neurons. It further suggests that the specific neurotrophin effects on LGN development do not depend on activation of the ras pathway in cortical neurons.

The BDNF-induced shrinkage of SGS neurons was an unexpected result which is difficult to explain. Having identified anterograde NT-4/5 as a major mediator for volume regulation, we suggest two scenarios. BDNF infusion might reduce cortical NT-4/5 expression followed by a reduced delivery to SGS inhibitory neurons contacted by the fibers, followed by shrinkage. Alternatively, BDNF is transported anterogradely (Caleo et al., 2000; Kohara et al., 2001), and the excess of cortically infused BDNF might compete with endogenous NT-4/5 for anterograde transport, thus reducing the amount of NT-4/5 delivered to SGS neurons. Exogenous BDNF can apparently not compensate for the loss of NT-4/5 in the afferent axons. However, BDNF did not shrink LGN neurons, either because these neurons manage to acquire sufficient quantities of NT-4/5 by retrograde mechanisms or the suggested BDNF-induced downregulation of NT-4/5 is confined to corticotectal pyramidal neurons.

NGF accelerates LGN development

It has been demonstrated that antagonizing endogenous NGF action during development causes shrinkage of LGN neurons (Berardi et al., 1994). We now show that in the rat cortical NGF, like NT-4/5, accelerates LGN cell growth. This effect is likely mediated by activation of cortical TrkA, since the specific TrkA-activating antibody RTA also rescues LGN neurons from monocular deprivation-induced shrinkage (T. Pizzorusso, N. B. and L. M., unpublished) and prevents monocular deprivation effects in visual cortex (Pizzorusso et al., 1999). TrkA is not expressed by LGN neurons (Merlio et al., 1992; Holtzman et al., 1995), and LGN neurons do not retrogradely transport cortically infused NGF or RTA (Domenici et al., 1994; Pizzorusso et al., 1999). Rather, TrkA is expressed by cholinergic afferents (Merlio et al., 1992; Sobreviela et al., 1994; Holtzman et al., 1995; Pizzorusso et al., 1999; Rossi et al., 2002) and some cortical glutamatergic terminals (Sala et al., 1998). NGF could act on cholinergic and glutamatergic transmission in the visual cortex, causing the release of TrkB ligands, which then act directly on LGN afferents. LGN fibers in cat and rat have nicotinic receptors (Prusky et al., 1988a; Prusky et al., 1988b). An attractive hypothesis is that NGF, by increasing acetylcholine release (Rylett and Williams, 1994; Sala et al., 1998) facilitates transmission of LGN fibers, thus stimulating release of NT-4/5 in layers IV and VI, or alternatively increase the amount of NT-4/5 delivered to the LGN by anterograde transport. Muscarinic m1 receptors in turn are in layer IV neurons during the critical period (Prusky and Cynader, 1990); they can stimulate the MAP kinase pathway (Rosenblum et al., 2000) involved in the control of gene expression, growth and plasticity. This scenario makes sense when considering the trophin dependency of the thalamocortical target neurons. At the peak of the critical period layer IV and VI neurons respond selectively to BDNF, but no longer to NT-4/5 which they probably supply to their LGN afferents. The absence of NGF effects on SGS neurons (at best, positive trends were observed) as compared to its unequivocal effect for LGN neurons could suggest that the NGF-induced mobilization of NT-4/5 is confined to the thalamocortical target layers and/or the backprojecting layer VI pyramidal neurons.

LIF delays maturation

Specific LIF β-receptors are expressed by cortical excitatory neurons, and to variable degrees by interneuronal populations (Wirth et al., 1998b), but not by LGN or SGS neurons. LIF infusions cause cortical neuropeptide expression (similar to NT-4/5 and BDNF) (Wahle et al., 2000) (P. W., unpublished), but a transient delay in soma size development of cortical neuron populations (P. W., unpublished). LIF has the same growth-delaying action on SGS and LGN, although both populations recover quickly after the end of the LIF infusion. An important result is that ingrowth of thalamic fibers in the cortex in vitro downregulates cortical LIF mRNA (Wahle et al., 2000): possibly, low LIF levels might be necessary for the thalamocortical fibers to form functional connections able to sustain their growth while increasing cortical LIF levels could be detrimental for LGN development. One explanation compatible with the interpretation of the cortical and subcortical effects is that an excess of exogenous LIF transiently reduces production, release or action of cortical TrkB ligands, this way reducing the anterograde or retrograde supply of the growth-promoting NT-4/5 to LGN and SGS neurons. For instance, NGF and LIF have antagonizing roles in the maturation of olfactory receptor neurons with LIF inhibiting differentiation (Moon et al., 2002). The explanation requires LIF receptors on cortical neurons, but not on the subcortical populations, and no axonal transport of LIF. Alternatively, the observation that LIF induces a severe shrinkage of layer IV and VI neurons suggests that a smaller cortical target cell population may cause a delay in maturation of afferent neurons. Support comes from the observation that LIF was the only factor affecting also the tectothalamic CB neurons in the SGS. The growth delay of LGN neurons has apparently caused a growth delay in their tectothalamic afferent population in a transneuronal way.

Conclusions

The study presents three important results. First, central neurons regulate soma size in an age- and ligand-specific fashion. Second, NT-4/5 and NGF accelerate LGN development in rats with normal vision while LIF delays growth. Third, NT-4/5 mediates growth of inhibitory SGS neurons via anterograde axonal transport. It is interesting that NGF and NT-4/5, which in rat most effectively prevent ocular dominance shifts after monocular deprivation (Lodovichi et al., 2000), are also the only factors able to promote LGN development. This would suggest that in addition to their effects on cortical targets, a direct or indirect action on subcortical neurons is an important component of the role these factors play in cortical plasticity. In contrast, BDNF-responsive targets clearly differ from those of NT-4/5, and are primarily located at the cortical level.
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