Neuron, Vol. 36, 623–634, November 14, 2002, Copyright ©2002 by Cell Press

Neurotrophin-3 Is Required for Appropriate Establishment of Thalamocortical Connections

Long Ma,[1] Takayuki Harada,[1] Chikako Harada,[1] Mario Romero,[1] Jean M. Hebert,[2] Susan K. McConnell,[2] and Luis F. Parada[1,3]
[1]Center for Developmental Biology and Kent Waldrep Foundation Center for Basic Research on Nerve Growth and Regeneration
University of Texas Southwestern Medical Center
Dallas, Texas 75390
[2]Department of Biological Sciences
Stanford University
Stanford, California 94305

Summary

In the vertebrate brain, the thalamus serves as a relay and integration station for diverse neuronal information en route from the periphery to the cortex. Formation of the thalamocortical tract occurs during pre- and postnatal development, with distinct thalamic nuclei projecting to specific cortical regions. The molecular forces that underlie the invasion by axons into specific cortical layers followed by activity-dependent maturation of synapses are poorly understood. We show that genetic ablation of neurotrophin-3 (*NT-3*) in the mouse neocortex results in reduction of a set of anatomically distinct axonal bundles projecting from thalamus through cortical white matter. These bundles include thalamocortical axons that normally establish connections with retrosplenial and visual cortex, sites of early postnatal *NT-3* expression. These results implicate neurotrophins in the critical stage of precise thalamocortical connections.

Introduction

The thalamocortical tract is the major relay pathway for peripheral sensory information to reach the cortex. In mice, dorsal thalamic neurons are generated between E10 and E13 (Angevine, 1970), and the first thalamocortical axons reach the neocortex by E14 (Bicknese et al., 1994; Metin and Godement, 1996). Upon reaching the subplate, thalamocortical axons grow tangentially and pause before invading the cortical plate (Kostovic and Rakic, 1990; Shatz, 1992: Catalano et al., 1991). The molecular interactions taking place at the subplate and neocortex that enable thalamocortical axons to invade the cortical plate, target precise neuronal layers, and reinforce synaptic contacts in an activity dependent manner, are not well defined.

The neurotrophins exert numerous important functions in the development and maintenance of both the peripheral and central nervous systems (Bibel and Barde, 2000; Patapoutian and Reichardt, 2001; Snider, 1994). Their roles in the PNS, as survival and differentiation factors, have been well established (Bibel and Barde, 2000; Snider, 1994). In CNS, a complete picture for neurotrophin function continues to emerge. Analysis of neurotrophin and Trk family receptor null mice has indicated some limited changes in CNS neuron populations at neonatal stages, suggesting a potential functional redundancy between TrkB and TrkC in promoting the survival of subgroups of CNS neurons (Minichiello and Klein, 1996). However, such insights are only partial and have been hindered by the perinatal lethality of the mutant mice (Liebl et al., 1997; Snider, 1994).

Experiments using slice cultures and gene transfer have demonstrated BDNF, TrkB, and NT-3 function in regulating the arborization of pyramidal neuron dendrites in visual cortical layers 4 and 6 (McAllister et al., 1995, 1997). Additionally, in vitro and in vivo studies suggest that BDNF is involved in the formation of hippocampal long-term potentiation (LTP) in the CA1–CA3 region, as well as in the regulation of eating behavior (Kernie et al., 2000; Korte et al., 1995; Patterson et al., 1996). Compared with BDNF, NT-3 seems to have a minor and controversial role in hippocampal LTP (Kang and Schuman, 1995; Kokaia et al., 1998; Ma et al., 1999).

Both BDNF and NT-3 are expressed in the cerebral cortex during the critical period when thalamocortical axons invade the cortical plate and establish layer-specific synaptic connections with cortical neurons (Lein et al., 2000; Vigers et al., 2000). Studies in cat visual cortex have examined the role of these factors. Infusion of the exogenous TrkB ligands BDNF or NT-4 inhibits the formation of ocular dominance columns, whereas infusion of NGF or NT-3 has no effect (Cabelli et al., 1995). Similarly, blockade of TrkB signaling with TrkB-IgG inhibits formation of ocular dominance columns, while TrkA-IgG or TrkC-IgG had no effect (Cabelli et al., 1997). However, the specific expression of NT-3 in layer 4 of cat visual cortex, both before and during the critical period of thalamocortical synapse formation (Lein et al., 2000), makes this neurotrophin an attractive candidate to regulate early stages in the development of connections between thalamocortical axons and target cortical neurons.

In the mouse, application of *Cre/loxP* gene targeting technology to the neurotrophins has permitted selective study of function beyond that of their well-established developmental roles (Bates et al., 1999; Xu et al., 2000). To further understand the function of NT-3 in the CNS, we have generated conditional mouse models in which *NT-3* is completely deleted in the cerebral cortex. We find that the development of a specific group of thalamocortical axons is disrupted in these mice. Our results provide evidence for a novel target-derived function of NT-3 in the establishment of synaptic interactions between thalamic axons and cortical neurons.

Results

Ablation of *NT-3* in Cortex

It has been documented that *NT-3* transcripts and proteins are present at high levels in the CNS from E17 until

[3]Correspondence: luis.parada@utsouthwestern.edu

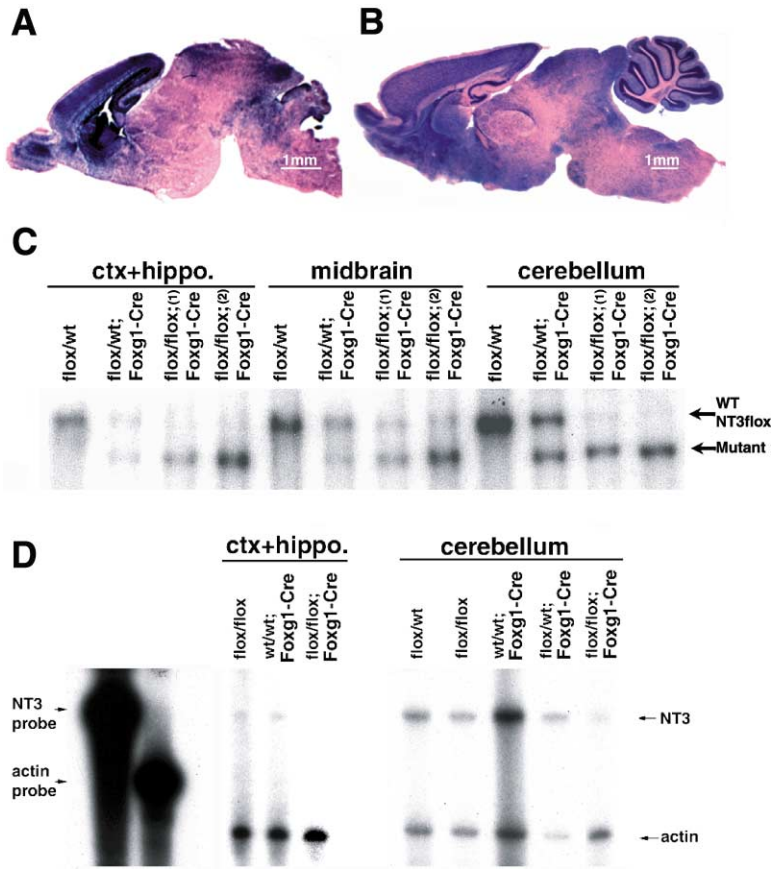

Figure 1. *Cre*-Mediated Recombination and Cortical Ablation of *NT-3*

The pattern of Cre-mediated recombination in *Foxg1-Cre* mice in the P0 (A) and adult (B) brains was revealed by crossing animals to a *ROSA-26* reporter mouse. β-galactosidase is expressed only after Cre-mediated recombination. (C) Southern blot and (D) RNase protection assay of different regions of adult brain with indicated genotypes show that the *NT-3flox* allele is completely deleted, and *NT-3* mRNA is totally absent in cortex and hippocampus. Southern blot (C) of two *NT3^{flox};Foxg1-Cre* mice (labeled flox/flox;Foxg1-Cre [1] and [2]) showing complete deletion of the *NT-3^{flox}* allele in cortical DNA. Cre activity is also present in the cerebellum of *NT3$^{flox/flox}$;Foxg1-Cre* mice.

adulthood in the rodent cerebral cortex, hippocampus, and cerebellum (Maisonpierre et al., 1990; Zhou and Rush, 1994). Analysis of a mouse strain in which *lacZ* was targeted to the *NT-3* locus has demonstrated that *NT-3* expression is abundant in specific cortical subregions from P0 until early adulthood (8 weeks), whereupon it is greatly reduced (Vigers et al., 2000). The coincidence of *NT-3* cortical expression during the critical period for the formation of thalamocortical projections suggests a potential role in this process (Lein et al., 2000; Vigers et al., 2000). *NT-3* knockout mice die perinatally due to peripheral nervous system defects (Ernfors et al., 1994; Tessarollo et al., 1994). To enable study of *NT-3* function beyond this period, we generated a "conditional mutant" mouse strain in which the single coding exon of *NT-3* is flanked by bacteriophage P1 *loxP* sites. This *NT-3* allele, *NT-3^{flox}*, is labile in vivo to Cre recombinase-specific deletion (Ma et al., 1999).

We crossed *NT-3$^{flox/flox}$* mice with *Foxg1-Cre* mice, in which the *Cre* recombinase has been targeted to the endogenous *Foxg1* locus (Hebert and McConnell, 2000). The expression of the *Foxg1-Cre* transgene has been well characterized and demonstrated to be present in embryonic telencephalon beginning at E9 (Hebert and McConnell, 2000). Thus, *Cre*-mediated *loxP* recombination is expected to efficiently disrupt the *NT-3* gene in all cells (neurons and glia) derived from the embryonic telencephalon. To confirm the scope of *Cre* activity in the postnatal brain of *Foxg1-Cre* mice, we crossed these mice to a *ROSA-26* β-*galactosidase* reporter mouse strain (Soriano, 1999). X-gal staining of resulting P0 and adult progeny verified that *Foxg1-Cre*-mediated *loxP* recombination was strongest in forebrain and cerebellum, with some activity in midbrain (Figures 1A and 1B). Genomic Southern blot analysis of *NT3$^{flox/flox}$;Foxg1-Cre* mice confirmed the LacZ analysis, demonstrating deletion of the *NT-3* coding exon in cerebral cortex and hippocampus (Figure 1C, *flox/flox;Foxg1-Cre*). Finally, sensitive RNase protection assays corroborated the absence of *NT-3* transcripts in adult forebrain of *NT3$^{flox/flox}$;Foxg1-Cre* mice (Figure 1D).

We conclude that *NT3$^{flox/flox}$;Foxg1-Cre* mice lack *NT-3* expression in cerebral cortex. The genomic configuration of the *Foxg1-Cre* mice, generated by the knock in of *Cre* into the endogenous *Foxg1* locus, renders one allele of the *Foxg1* gene null. Therefore, in all studies described here, we include the *Foxg1-Cre* transgene in either wild-type or heterozygous (*NT3$^{flox/+}$*) background as littermate controls.

Abnormal Cortical White Matter in Mutant Mice

Ablation of *NT-3* from the cortex and hippocampus did not affect survival or cause overt behavioral changes. Analysis of adult whole-brain sections using various histological methods, including H&E, Nissl, DAPI, and neuron-specific antibody immunostaining, indicated no detectable morphological changes (see Supplemental

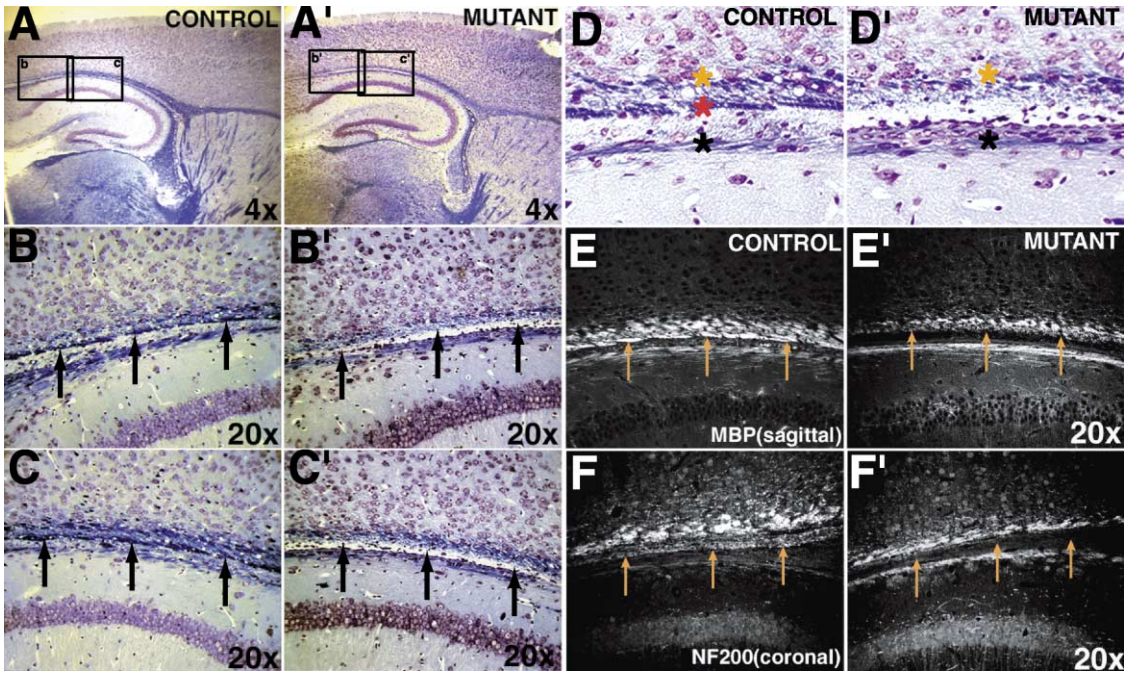

Figure 2. Reduction of Axonal Bundles in Cortical White Matter of *NT3*$^{flox/flox}$*;Foxg1-Cre* Mice

Low (A and A′) and high (B–C′) magnification of sagittal sections through the adult brain stained with Luxol Fast Blue and Nissl show that a major group of axonal bundles in the cortical white matter, normally present in control mice (B and C, arrows), is missing in *NT-3*$^{flox/flox}$*;Foxg1-Cre* mice (B′ and C′, arrows). Higher magnification pictures of the axonal bundles are shown in D (control) and D′ (mutant), showing that axonal bundles marked by a red star (D) in control are missing in mutant (D′). MBP (E and E′, sagittal) and Neurofilament-200 (NF-200) (F and F′, coronal) immunostaining of subcortical white matter. n = 6 for control and mutant mice

Figure S1 at http://www.neuron.org/cgi/content/full/36/4/623/DC1).

We next examined whether axonal projections emanating from, or invading, the cortex might be affected. These include projections from cortex: to spinal cord (corticospinal tracts); to thalamus (corticothalamic tracts); to ipsilateral and contralateral cortex (corticocortical tracts); and projections from the thalamus to cortex (thalamocortical tracts). To visualize the major axonal fibers for these various projections in the subcortical region, we employed Luxol Fast Blue to highlight myelin and thus, axonal bundles. Compared to littermate controls, the pattern of major CNS axonal fibers is similar in *NT3*$^{flox/flox}$*;Foxg1-Cre* mice (n = 6; Figures 2A and 2A′). However, close examination of the cortical white matter revealed a persistent alteration in the tracts of *NT3*$^{flox/flox}$*;Foxg1-Cre* mice. As illustrated in Figures 2B–2D, three anatomically distinct myelin bundles can be discerned beneath the cortex of control mice. *NT3*$^{flox/flox}$*;Foxg1-Cre* mice have only two white matter bundles (Figures 2B′ and 2C′), and higher magnification suggests that the middle bundle (Figure 2D, red star) is greatly reduced in the mutant white matter (Figure 2D′). Antibodies against myelin basic protein confirmed the reduction in myelin bundles (MBP; Figures 2E and 2E′). To determine whether axons were present in the absence of myelination, a neurofilament antibody was employed (NF200; Figures 2F and 2F′). The combination of myelin and axon detection methods confirms the absence of the axon bundles and not merely of the myelin ensheathing glia.

To independently assess the phenotype observed by *Foxg1-Cre*-mediated ablation of *NT-3*, we utilized a second transgenic line in which the *Cre* transgene was placed under control of the *GFAP* promoter. The *GFAP* promoter drives expression in several neuroepithelial regions including telencephalon and thus intersects with *Foxg1-Cre* in telencephalic-derived structures (L.M., Y. Zhu, A. Messing, and L.F.P., unpublished data; Zhuo et al., 2001). The broad ablation of *NT-3* in the CNS, including the cortex, resulted in loss of the same specific subcortical tract as assessed by Luxol Fast Blue, MBP, and NF200 staining (see Supplemental Figure S2 at http://www.neuron.org/cgi/content/full/36/4/623/DC1, and data not shown). Taken together, the data demonstrate that cortical loss of *NT-3* during embryogenesis results in a reduction of anatomically distinct axonal bundles in subcortical white matter.

In addition to the cortical relays described above, the subcortical white matter also contains axons of the corpus callosum and cingulate bundle. Histological examination of the corpus callosum revealed no significant differences between control and mutant mice. We also examined the cingulum, a collection of axonal fibers connecting frontal and parietal cortices with parahippocampal and adjacent temporal cortices (Carpenter, 1985), and found it to be unaffected in mutant mice (see Supplemental Figure S2 at http://www.neuron.org/cgi/

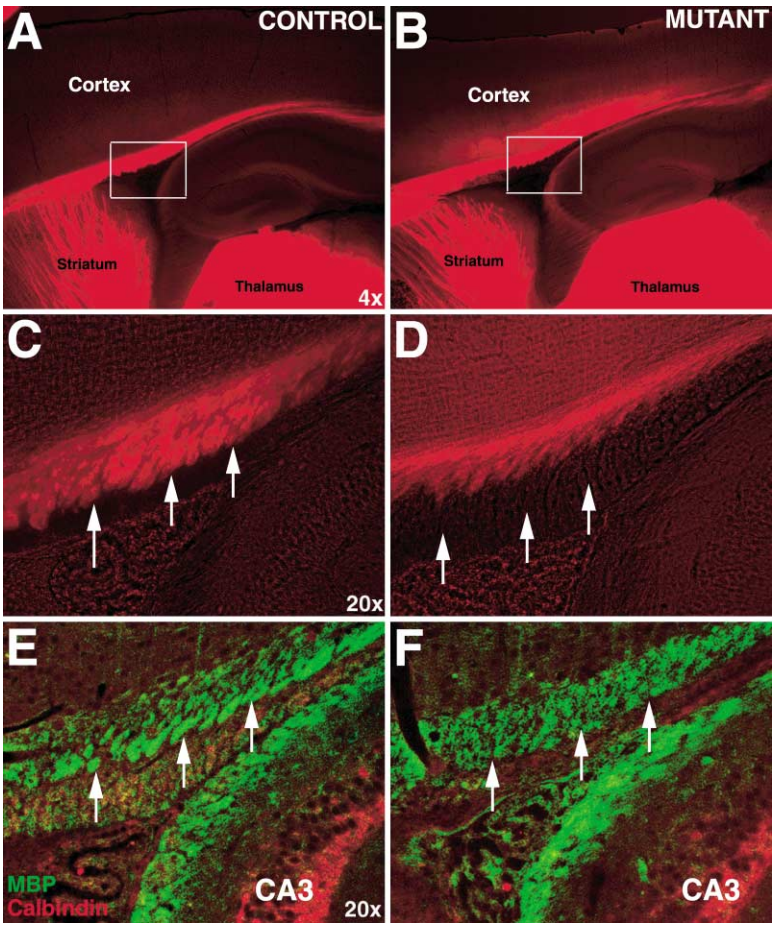

Figure 3. Reduction in Axonal Projections between Cortex and Thalamus in Mutant Mice

Low (A and B) and high (C and D) magnification of saturating DiI application to thalamus of adult mice. The control cortical white matter ([C], arrows) has greater number of axonal bundles labeled by DiI compared to mutant mice ([D], arrows). This area of missing axonal bundles occupies the same position as the MBP-positive (green) axonal bundles ([E], for control, arrows), which are missing in mutant mice ([F], arrows). ([E and F], red) Calbindin immunostaining defines the CA3 region of the hippocampus. n = 5 for control and n = 4 for mutant mice.

content/full/36/4/623/DC1). Thus, mutant corticocortical connections do not exhibit overt morphological alterations and leave the cortico-subcortical and thalamocortical projections as remaining candidates to be affected by loss of cortical NT-3.

Thalamocortical Projection Defects

To test the hypothesis that the missing cortical white matter axonal bundles in both strains of *NT-3* cortical mutant mice (*NT3$^{flox/flox}$;Foxg1-Cre* and *NT3$^{flox/flox}$;GFAP-Cre*) consist of cortico-subcortical and/or thalamocortical axons, we used the lipophilic tracer DiI as an anterograde and retrograde tracer (Figures 3A and 3B). Comparison of cortical white matter traced with DiI placed in the thalamus from control (n = 5) and mutant (n = 4) brains revealed a reduction of labeled white matter in mutant brains (Figures 3C and arrows in 3D). The region in which DiI labeling is absent in the mutant brain coincides with the previously identified intermediate subcortical axonal bundle that is absent in mutants (compare Figures 3C and 3D, red, to 3E and 3F, green). These results suggest that the missing white matter tract in *NT3$^{flox/flox}$;Foxg1-Cre* and *NT3$^{flox/flox}$;GFAP-Cre* mice is composed primarily of axons projecting between the cortex and the thalamus.

To examine the status of thalamocortical projections, we retrogradely labeled thalamic neurons by injecting BDA into two cortical regions that exhibit high-level *NT-3* expression during early postnatal development: retrosplenial cortex (Figures 4A and 4A′) and visual cortex (Figures 4F and 4F′) (Lein et al., 2000; Vigers et al., 2000). As described below, deficiencies in thalamic projections to these two cortical regions were found.

Retrosplenial Cortex

The major thalamic nuclei projecting to retrosplenial cortex are the anterodorsal (AD), anteroventral (AV), anteromedial (AM), and laterodorsal (LD) nuclei (Shibata, 1993, 1998, 2000; Sripanidkulchai and Wyss, 1986; Van Groen and Wyss, 1995). Following BDA injections into retrosplenial cortex, the labeling of anterodorsal and anteromedial nuclei neurons by BDA was similar in control and mutant mice (n = 4; Figures 4B, 4B′, and 4C, and "AD" and "AM" in 4C′). In contrast, mutant mice showed a dramatic reduction in the labeling of neurons in the dorsal region of the anteroventral nucleus which was devoid of label (compare Figures 4B′ and 4C′, "AV", with 4B and 4C, "AV"). Similarly, the laterodorsal nucleus of mutant mice showed a considerable reduction in labeling (Figures 4D and 4D′). Control mice show dense and strong BDA labeling (Figures 4D, "LD," and 4E) while only a few labeled neurons could be found in the laterodorsal nucleus of mutant mice (Figure 4D′, "LD," and

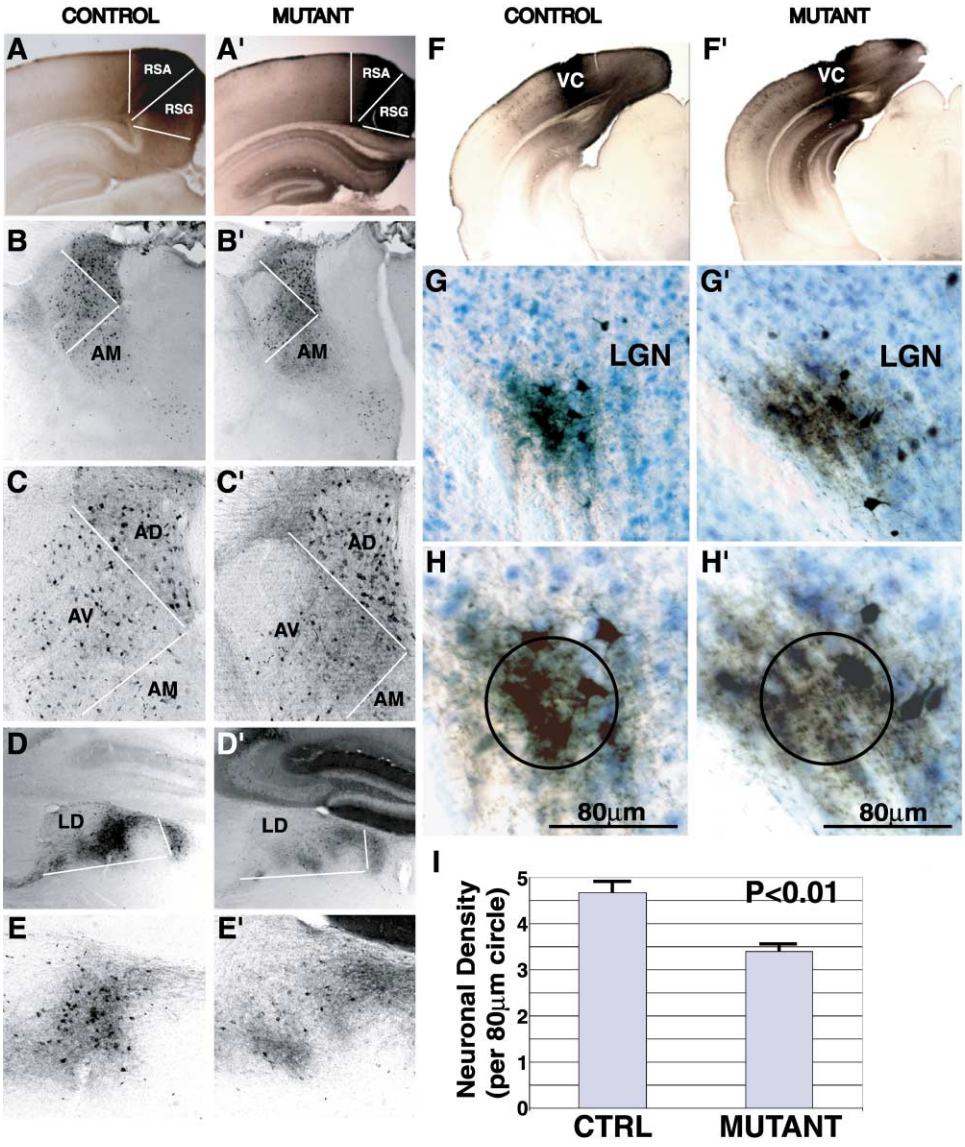

Figure 4. Specific Thalamocortical Projections to the Target Cortex Are Dramatically Reduced

(A and A′) BDA injection site in the retrosplenial cortex in control and mutant mice, respectively. Low (B) and high (C) magnifications of anterior nuclei in control mice indicating normal labeling of neurons in AD, AV, and AM nuclei. Low (B′) and high (C′) magnifications of mutant anterior nuclei demonstrating normal AD and AM but abnormal AV labeling. Mutant mice (D′ and E′) have dramatically reduced labeling in LD nucleus compared with control mice (D and E). BDA injection sites in the visual cortex of control and mutant mice (F and F′), respectively. Low and high magnification of retrogradely labeled LGN neurons in control mice (G and H) and mutant mice (G′ and H′). Neuronal density within an 80 μm diameter circle placed in the center of the labeling field (I). AD, anterodorsal nucleus; AV, anteroventral nucleus; AM, anteromedial nucleus; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; RSA, retrosplenial cortex agranular; RSG, retrosplenial cortex granular; VC, visual cortex. n = 4 for control and mutant littermates.

4E′). These data indicate that a subset of thalamic projections to the retrosplenial cortex is reduced in mice that lack cortical *NT-3* expression.

Visual Cortex

We next placed BDA injections into the visual cortex and examined retrogradely labeled neurons in the lateral geniculate nucleus (LGN) (Figures 4F and 4F′). Significantly reduced numbers of labeled neuronal somata were identified in mutant mice (Figures 4G′ and 4H′). Compared with control mice, a 28% reduction of labeled neurons was found in mutant mice (n = 4). (Figure 4I, $p < 0.01$). These data indicate that loss of NT-3 causes reduction in thalamocortical projections to the visual cortex.

Reduction in number of lateral geniculate nucleus projections to visual cortex (Figures 4F–4I) and anteroventral and laterodorsal nuclei projections to retrosplenial cortex should produce a concomitant reduction in the

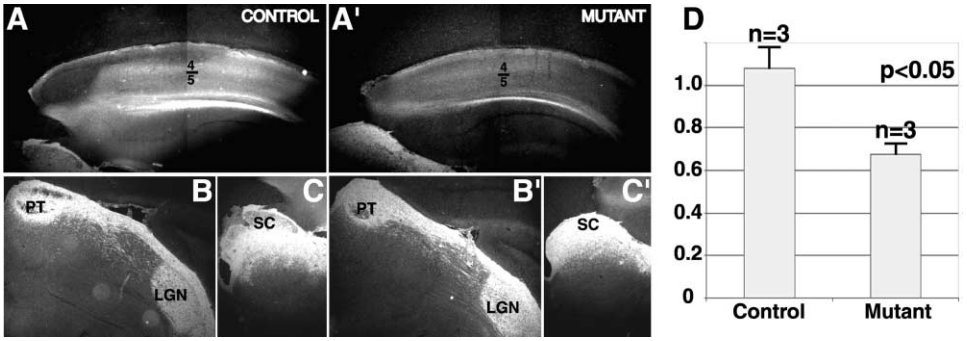

Figure 5. Reduced Geniculocortical Projection Demonstrated with [^{3}H]-Proline Transneuronal Tracer

Control (A) and mutant (A′) visual cortex transneuronally labeled with [^{3}H]-proline injected into one eye of P40 mice. LGN, pretectum (B and B′), and superior colliculus of control and mutant mice (C and C′) indicating equivalent amount of labeling by [^{3}H]-proline. (D) Semiquantification of [^{3}H]-proline signal in visual cortex of control and mutant mice. LGN, lateral geniculate nucleus; PT, pretectum; SC, superior colliculus. n = 3 for control and mutants.

number of synapses and in cortical synaptic activity. Consistent with this prediction, the retrosplenial and visual cortices of adult mutant mice showed reductions in cytochrome oxidase activity (Wong-Riley, 1989) (see Supplemental Figure S4 at http://www.neuron.org/cgi/content/full/36/4/623/DC1). We next examined the density of thalamocortical synapses in visual cortex by transneuronal labeling following application of [^{3}H]-proline to the retinas of 40-day-old mice (Grafstein, 1971; Reinis and Goldman, 1984). The radiolabeled amino acid is anterogradely projected from retinal ganglion cells to the pretectum, lateral geniculate nucleus, and to superior colliculus. In lateral geniculate nucleus, the amino acid is secreted and taken up by dendrites of thalamic neurons, which then anterogradely transport the labeled molecule to the visual cortex. Comparison of control and mutant mice (n = 3) indicates equally efficient labeling of lateral geniculate nucleus, pretectum, and superior colliculus (Figures 5B–5C′). However, examination of the visual cortex reproducibly demonstrated a substantial reduction of label in mutant brains (Figures 5A and 5A′). Comparison of the relative digital intensity indicates a 30% reduction of [^{3}H]-proline signal in mutant visual cortex (Figure 5D; $p < 0.05$; see Experimental Procedures).

To determine if thalamocortical projections from ventral posterior nucleus to somatosensory cortex are affected in mutant mice, we injected BDA into somatosensory cortex and retrogradely labeled ventral posterior thalamic nucleus. Ventral posterior thalamic neurons from mutant and control mice were similarly labeled (n = 4; data not shown). Consistent with absence of *NT-3* expression during early postnatal periods, loss of cortical *NT-3* has no discernible effect on thalamocortical projections into somatosensory cortex.

To determine whether reduction of thalamocortical projections coincided with loss of thalamic neurons, we performed histological examination (Nissl stain): in situ hybridization using a *trkC* probe; parvalbumin, calbindin, GABA, and glutamate immunohistochemistry; and cytochrome oxidase activity histochemistry of mutant brains (data not shown). No differences between control and mutant thalamic nuclei were observed. Thus, in adult mice, absence of *NT-3* in the retrosplenial and visual cortex results in a reduction of synaptic input from the thalamus to specific regions and is not associated with loss of thalamic neurons.

We also examined the status of corticothalamic and corticospinal projections in the mutant mice, and no qualitatively discernible deficiencies were observed in these tracts (see Supplemental Figure S3 at http://www.neuron.org/cgi/content/full/36/4/623/DC1).

Neonatal Defects in Thalamocortical Projections

Analysis of *NT-3* expression in a β-*galactosidase* knockin mouse indicates that *NT-3* is specifically expressed in layers 2 and 3 of the P8 retrosplenial cortex and layer 4 of the visual cortex (data not shown) (Wilkinson et al., 1996; Vigers et al., 2000). To investigate if the thalamocortical projection defects observed in the mutant mice resulted from embryonic or postnatal abnormalities, we applied DiI to the thalamus of mutant and control pups at various time points between P0 and P10. At P0, thalamocortical axons in rodents have reached the subplate and the earliest invasion of somatosensory cortex layer 4 commences (Auladell et al., 2000; Catalano et al., 1991). We found normal development in mutant thalamocotical projections, showing appropriate arrival in subplate and early invasion of the somatosensory cortex (Figures 6A1–6A2′, and data not shown for other cortical regions). Thus, prior to P0, ablation of *NT-3* in the CNS has no apparent effect on the formation of thalamocortical projections.

At P3, neither control (n = 3) nor mutant (n = 1) thalamocortical axons have reached the subplate of retrosplenial cortex (Figures 6B1 and 6B1′, arrowheads) while invasion of the visual cortex has commenced. In both control and mutant mice (Figures 6B2 and 6B2′) thalamocortical axons are present at the cortical subplate. Control mice exhibit clear projection of collaterals into the cortical plate (Figure 6B2, arrows), while mutant mice show reduced collateral branching from axons that extend into the visual cortical plate (Figure 6B2′, arrows). Thus, *NT-3*-dependent deficiencies are discernible in visual cortex as early as P3.

At P5, thalamocortical axons have reached the subplate of retrosplenial cortex (Figures 6C1 and 6C1′, red arrowheads). Control mice exhibit robust collateral inva-

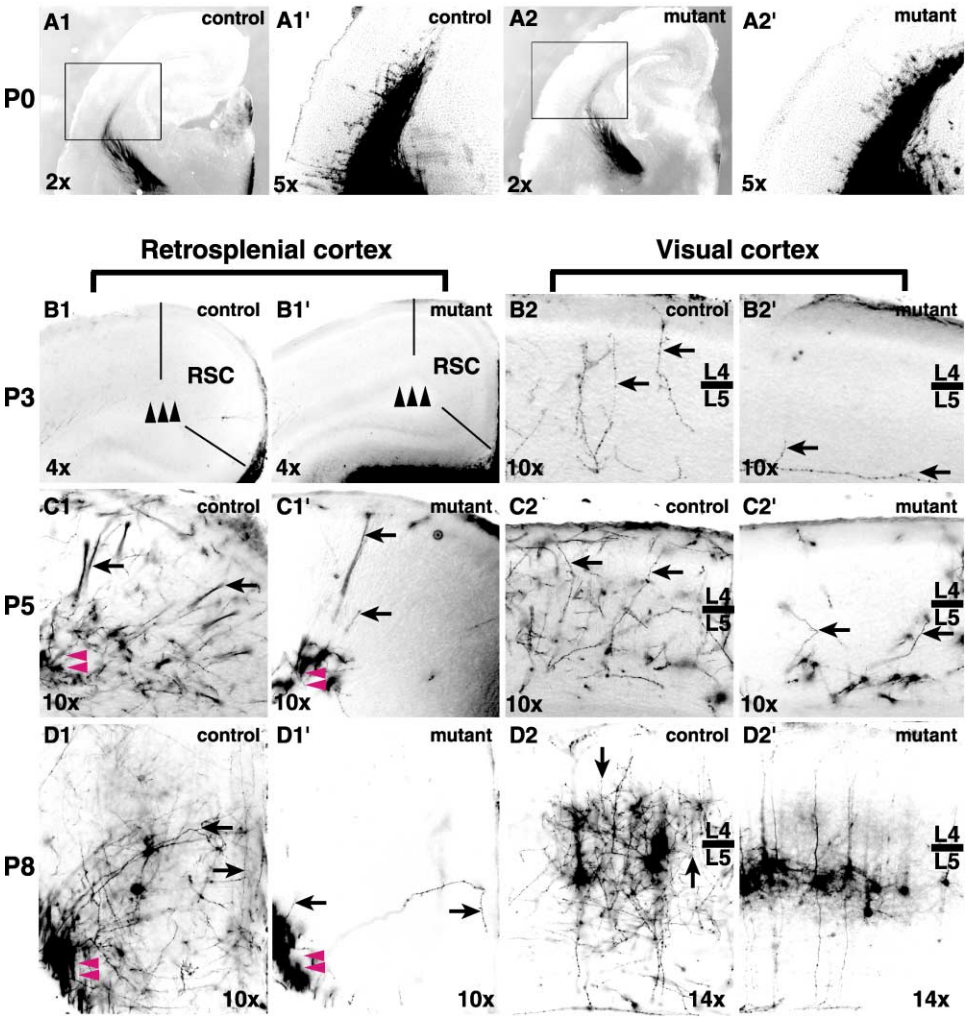

Figure 6. DiI Tracing Indicates Defective Thalamocortical Projection in Neonatal Mutant Mice

(A1–A2′) Low- and high-magnification images of DiI tracing from thalamus of P0 mouse brains indicating similar thalamocortical projections between control (n = 5) and mutant (n = 5) mice, respectively.

(B1 and B1′) P3 control (n = 3) and mutant (n = 1) retrosplenial cortex showing absence of thalamocortical axons in the subplate (dark arrowheads).

(B2 and B2′) P3 control (n = 3) and mutant (n = 1) visual cortex showing normal initial collateral invasion into the cortical plate in control mice ([B2], arrows) but failed invasion of collaterals in mutant ([B2′], arrows).

(C1 and C1′) P5 control (n = 2) and mutant (n = 2) retrosplenial cortex showing extensive collateral invasion in control mice ([C1], arrows) but significantly reduced invasion in mutant mice ([C1′], arrows). Notice both control ([C1], red arrowheads) and mutant ([C1′], red arrowheads) have accumulated thalamocortical axons in the subplate.

(C2 and C2′) P5 control (n = 2) and mutant (n = 2) visual cortex showing normal collateral invasion in control mice ([C2], arrows) but greatly reduced invasion in mutant ([C2′], arrows).

(D1 and D1′) P8 control (n = 5) and mutant (n = 5) retrosplenial cortex showing continued collateral invasion and elaboration in control mice ([D1], arrows) but few invasion in mutant ([D1′], arrows). Notice the accumulation of thalamocortical axons in the subplate ([D1 and D1′], red arrowheads).

(D2 and D2′) P8 control (n = 5) and mutant (n = 5) visual cortex showing extensive collateral elaboration into the cortical plate in control mice ([D2], arrows) but significantly reduced invasion and elaboration in mutant (D2′). RSC, retrosplenial cortex.

sion into the cortical plate (Figure 6C1, arrows); while few mutant collaterals could be found (n = 2; Figure 6C1′, arrows). Similar results were observed in P5 visual cortex (Figures 6C2 and 6C2′). In all cases, control littermates had extensive collateral growth into the cortical plate (Figure 6C2, arrows), while mutants had extremely limited collateral projections (Figure 6C2′, arrows).

At P8, when *NT-3* expression in the retrosplenial and visual cortex peaks (Vigers et al., 2000), continued collateral invasion persists in retrosplenial and visual cortex of control mice (n = 5; Figure 6D1, arrows, and 6D2, arrows, respectively). However, in mutant brains, only few collaterals are visible in retrosplenial (Figure 6D1′, arrows) and visual cortex (Figure 6D2′). In both control and mutant littermates, apparent accumulation of thalamocortical axons is visible in the subplate of retrosplenial cortex (Figures 6D1 and 6D1′, red arrowheads). To

rule out artifacts of differential labeling, we ensured that control and mutant mice acquired equal DiI saturation of all nuclei (AD, AV, AM, LD, and LGN; data not shown).

We examined *TrkC* mRNA expression in the thalamus at P7 and found concordance between the sites of receptor expression and the observed thalamocortical deficiencies (see Supplemental Figure S5 at http://www.neuron.org/cgi/content/full/36/4/623/DC1). Thus, TrkC is expressed in thalamic nuclei that project to retrosplenial and visual cortex but not somatosensory cortex. Consistent with this, we saw no evidence of alterations in the presence or pattern of thalamocortical projections to somatosensory cortex (data not shown).

Impaired Visual Function in Mutant Mice

To assess the functional consequences of disrupted geniculocortical projections in *NT3$^{flox/flox}$;Foxg1-Cre* or *NT-3$^{flox/flox}$;GFAP-Cre* mice, we studied mouse behavior in a paradigm for vision, the visual cliff avoidance test (Lione et al., 1999; Lore and Sawatski, 1969) (see Experimental Procedures for details). The percentage of time that mice spend in the open side of the box (Figure 7A)is used as an indicator of visual function impairment. We selected 6-week-old mice for this test to permit full development of the visual system (Antonini et al., 1999; Gordon and Stryker, 1996). As expected, wild-type mice hesitate to cross the cliff edge (Figure 7A; left column) and prefer the covered side of the visual cliff box. In contrast, mutant mice often crossed the cliff edge without hesitation (Figure 7A; right column) and showed no preference for either side (Figure 7B; see Supplemental Movie at http://www.neuron.org/cgi/content/full/36/4/623/DC1). These data are comparable to results obtained with retinal degeneration (*rd*) mice, which are functionally blind due to photoreceptor cells loss (Chang et al., 1993). *rd* mice showed no preference for either side of the box (Figure 7B). Thus, this behavioral assay is consistent with the anatomical data indicating that geniculocortical connectivity is perturbed in *NT-3* mutant mice.

NT-3 expression is negligible in lateral geniculate nucleus, pretectum, and superior colliculus from P0 to adult (Vigers et al., 2000), suggesting that *NT-3* does not have a role in establishing connections between these CNS structures and the retina. Consistent with this, the [^{3}H]-proline tracing experiments indicate that retinal projections to lateral geniculate nucleus, pretectum, and superior colliculus are similar in mutant and control mice (Figure 5). To rule out possible additional abnormalities in the retino-tectal axis that might contribute to the reduced visual function in the mutant mice, we examined the retinas from *NT3$^{flox/flox}$;Foxg1-Cre* and *NT3$^{flox/flox}$;GFAP-Cre* mice. No difference could be found between control and mutant (n = 3) mice as to the number, morphology, and alignment of photoreceptors (data not shown).

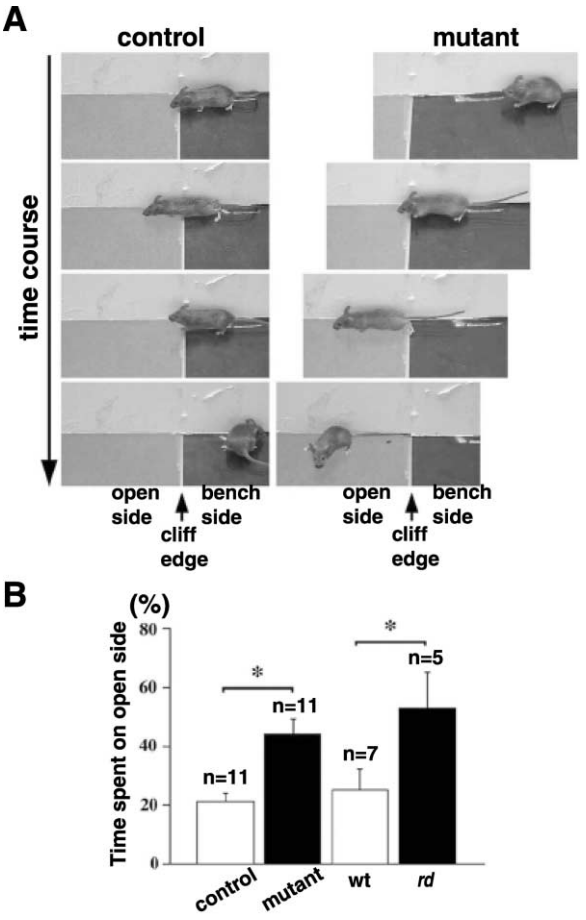

Figure 7. Impaired Visual Function in Mutant Mice

(A) Sequential pictures of a control mouse (left column) and a mutant mouse (right column) responding to the open field in visual cliff assay (see Supplemental Movie at http://www.neuron.org/cgi/content/full/36/4/623/DC1).

(B) Quantitative analysis of the percentage of time spent in open field. Notice that mutant mice, like *rd* mice (n = 5; 53% ± 12%), spend equal time on both sides (44% ± 5%). Control mice (Cre positive) behave like wild-type (wt, n = 7; 25% ± 7%) mice, spending only about 20% of the time on the open side (21% ± 9%). *rd*, retinal degeneration. n = 11 for control and mutants.

Discussion

Synaptic connections form between the thalamus and the cortex with exquisite precision. In visual cortex, thalamic afferents form synapses mainly with neurons in layer 4, whereas in retrosplenial cortex, thalamic inputs localize extensively to layers 2/3 (Robertson and Kaitz, 1981). Much progress has been made in understanding the forces that regulate cortical development and the sequential and inverse formation of precise layers (McConnell, 1995). Similar progress has been made in understanding the embryonic mechanisms that regulate the formation of thalamic projections to the subplate (Allendoerfer and Shatz, 1994; Braisted et al., 1999; Ghosh and Shatz, 1992). Once thalamic neurons are born, they project laterally to the hypothalamic border where they abruptly turn course to enter the internal capsule and finally reach the cortical subplate (Braisted et al., 1999). Each of these steps is apparently governed by specific cues (Bagri et al., 2002; Braisted et al., 1999, 2000). However, the molecular cues that induce the timely invasion of the cortical plate and the establishment of precise layer-specific synaptic connections have remained elusive.

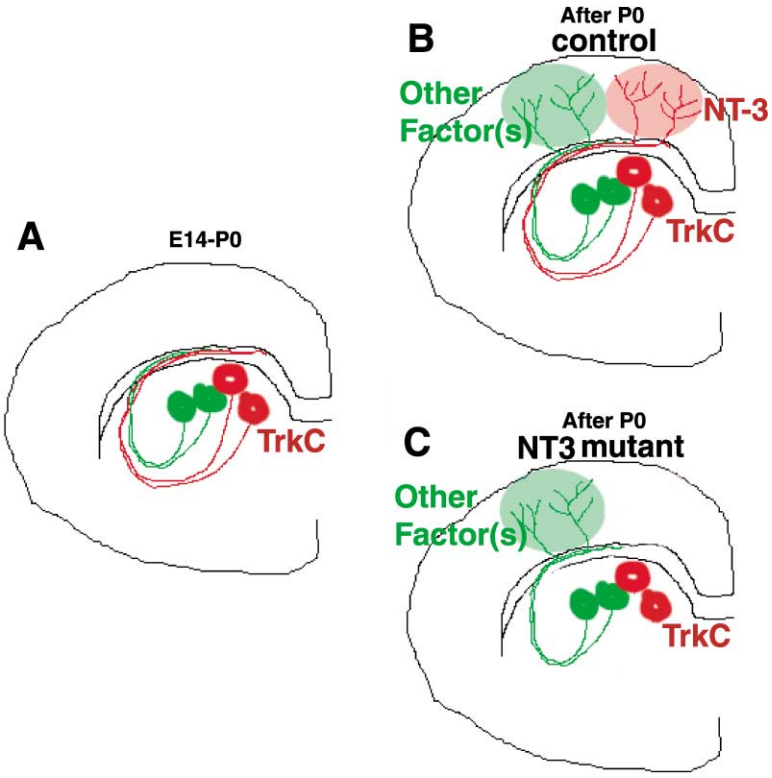

Figure 8. Diagram Illustrating Thalamocortical Projections in the Absence of Cortical NT-3

(A) During development, thalamocortical axons from distinct thalamic nuclei enter the subplate and wait for invading signals.

(B) In the presence of NT-3, and possibly other factors, distinct collaterals invade the cortical plate and elaborate in respective cortical regions.

(C) Without NT-3, NT-3-dependent thalamocortical axons failed to invade the cortical plate and finally retract while other thalamocortical axons persist.

The neurotrophins have gained considerable attention in this regard. In the present study, we utilized reverse genetics to examine the role of *NT-3* in cortical development. We find that *NT-3* is critical for the formation of specific connections between thalamic nuclei, which express *TrkC* during early postnatal life, and the retrosplenial and visual cortices, which transiently express *NT-3* during the critical period (Lein et al., 2000; Tessarollo et al., 1993; Vigers et al., 2000). Our data indicate that this process is not regulated by the classical role of *NT-3* in neuronal survival, as no evidence of neuronal loss was observed in the relevant thalamic nuclei. In addition, studies of neonatal animals indicate that thalamic axons were able to progress normally from the thalamus to the cortical subplate (see model in Figure 8A), although reduced synaptic connections resulted. Thus, *NT-3* does not appear to exert distant tropic effects on thalamocortical axons. Instead, the defect must lie either in signaling the invasion of the cortex and/or the elaboration of dense layer-specific synaptic contacts within appropriate layers (Figure 8B). The anatomic consequence of *NT-3* loss is a reduced innervation of the visual and retrosplenial cortices, which is reflected in the reduction of mitochondrial activity in these regions.

As outlined in Figure 8C, we consider the likeliest explanation for these results to reside in an eventual retraction of thalamic projections rather than inappropriate projection to alternative cortical regions. Consistent with this, thalamocortical tracing experiments, including [^{3}H]-proline transneuronal tracing, did not reveal evidence of aberrant projections.

Recent studies suggest that *P75* expressing subplate neurons are required for the pathfinding of thalamocortical axons and that *NT-3* could enhance the neurite length of the subplate neurons in vitro (Allendoerfer et al., 1994; DeFreitas et al., 2001; McQuillen et al., 2002). Our data indicate that thalamocortical axons reach the subplate normally in NT-3 cortically deficient mice. We also examined the expression of *NT-3* pre- and postnatally using a β-*galactosidase* knockin reporter mouse (Wilkinson et al., 1996) and found no detectable *NT-3* expression in the subplate, subventricular zone, or internal capsule at E14.5, E16.5, P0, P7, and P14 (data not shown). Based on these criteria, we consider the observed phenotype to be the direct consequence of loss of cortical *NT-3* expression rather than a secondary result of subplate neuron defects.

Several studies have noted the function or expression of *BDNF*, *NT4/5*, *and NT-3* in the visual cortex during the "critical period" of synaptic strengthening (Cabelli et al., 1995, 1997; Lein et al., 2000; Vigers et al., 2000). In addition, slice culture and in vivo experiments have supported a function for neurotrophins in dendritic arborization, the segregation of ocular dominance columns, maintenance of thalamic neuronal cell somata, and the maturation of visual acuity (Bibel and Barde, 2000; Cabelli et al., 1995, 1997; Cohen-Cory and Fraser, 1995; Huang et al., 1999; Lom and Cohen-Cory, 1999; McAllister et al., 1995, 1996, 1997). Interestingly, the activities revealed by these studies have been primarily attributed to TrkB and its ligands, BDNF and NT-4, and not to NT-3 (Katz, 1999). While apparently paradoxical, we consider these preceding studies to be congruent with the present findings. Disruption or enhancement of TrkB signaling at the visual cortex in those studies related directly to the synaptic strengthening of already established connections. Our genetic study indicates that for compartments of visual cortex and retrosplenial cortex, *NT-3*

likely acts at an earlier developmental stage when thalamocortical projections are instructed to invade the cortex and/or establish initial connections. We therefore propose that thalamic axon development of these cortical regions is regulated by a sequential set of events that depend first on NT-3 activity and later on BDNF and/or NT4 activity.

Thalamocortical deficiencies were found in only a subset of cortical areas, which correspond with specific thalamic targeting and *TrkC* expression, coupled with specific cortical *NT-3* expression during early postnatal life. We consider it likely that additional thalamocortical deficiencies may exist in these mice, corresponding to additional sites of thalamic *TrkC* and cortical *NT-3* expression. A broader question arises that relates to unaffected thalamocortical projections in cortical *NT-3*-deficient mice. One compelling possibility is that other neurotrophins, such as NGF, BDNF, and NT-4, may mediate similar functions. Studies are underway to consider this hypothesis.

The anatomic consequences of loss of cortical *NT-3* are reduced thalamocortical synaptic interactions. In the visual cortex, we can assess the behavioral sequel, which is one of relative cortical blindness. Cortical blindness could be caused by defects of any portion of the visual pathway following the lateral geniculate nucleus (Aldrich et al., 1987; Baker-Nobles and Rutherford, 1995; Birch and Bane, 1991; Wong, 1991). The thalamocortical projection defects of *NT3$^{flox/flox}$;Foxg1-Cre* and *NT3$^{flox/flox}$;GFAP-Cre* mice fit into this definition. The precise molecular interactions that are affected by *NT-3* loss remain to be defined. Although neurotrophins can regulate the dendritic arborization of neurons (McAllister et al., 1995, 1997), as assessed by Cox-Golgi staining, we saw no overt changes in the arrangement or arborization of dendrites among neurons in the visual or retrosplenial cortices of the mutant mice (data not shown). However, developmental analysis consistently revealed deficient collateralization of subcortical axons into the retrosplenial and visual cortices, respectively. These findings are most consistent with short distance tropic defects. Additional, more detailed examination will be required to assess this properly. These mice should prove valuable in the understanding of genetic events that integrate peripheral sensory information in the cortex.

Experimental Procedures

Southern Blot and RNase Protection Assay

Southern blots were performed as described (Ma et al., 1999). RNase protection assays were performed as described using the HybSpeed RPA kit (Ambion). The probe for *NT-3* was a 650 base ^{32}P-labeled antisense RNA covering a sequence from ATG to a SmaI site of the mouse *NT-3* cDNA.

Immunohistochemistry and β-Galactosidase Staining

Immunohistochemistry was performed as described (Zhu et al., 2001) using the following antibodies and dilutions: rabbit anti-calbindin (1:1000), rabbit anti-parvalbumin (1:1000), rabbit anti-GFAP (1:500), monoclonal NeuN (1:500), monoclonal anti-MBP (1:500), and rabbit anti-NF200 (1:1000). For fluorescence double labeling, secondary antibodies (1:400) labeled with either Cy2 or Cy3 fluorescent modules (Jackson Laboratory) were used to reveal the different primary antibodies.

For β-galactosidase staining, P0 or adult mice brains were perfused and fixed with 4% paraformaldehyde (PFA) and then cut into 50 μm sections with a vibrotome. The sections were then stained in a solution containing 2 mM $MgCl_2$, 40 mg/ml X-gal, 5 mM potassium ferri/ferrocyanide in 1× PBS at 37°C overnight, as described previously (Ma et al., 2000).

DiI Tracing

DiI filters were prepared by soaking nylon membranes (Hybond-N+, Amersham) with 2.5 mg/ml DiI (Molecular Probes) solution in N,N′-dimethylformamide (Sigma). The filters were then dried, cut into 0.5 mm square pieces, and inserted in parallel into the whole thalamus with no distinction of specific thalamic nuclei. Brain tissues were then incubated in 4% paraformaldehyde, 1× PBS at 37°C in the dark for 4 weeks (P0), 5 weeks (P3–P8) or 6 weeks (adult). The brains were then cut into 100 μm sections with a vibrotome and visualized using fluorescence microscopy.

Biotinylated Dextran Amine and Horseradish Peroxidase Tracing

Anesthetized adult mice (9 vol ketamine:1 vol xylazine, 1:1 in H_2O) received unilateral stereotaxic injections of 10% biotinylated dextran amine (BDA; Molecular Probes) directly into the retrosplenial cortex, visual cortex, sensorimotor cortex, and somatosensory cortex, or 20% horseradish peroxidase (HRP) type 4 (Sigma) directly into the ventral posterior thalamic nucleus using a glass micropipette attached to a Nanoject injector (World Precision Instruments). The identification of cortical regions or thalamic nuclei is based on Franklin and Paxinos (1997). After 5 days, mice were perfused with 4% paraformaldehyde. Brains and spinal cords were postfixed and sectioned at 50 μm using a vibratome. Sections were then developed immunohistochemically for BDA or HRP signals as described (Yokoyama et al., 2001). Neuronal counting was performed using a double-blind protocol. Two-tailed student's t test was used to determine the difference between two groups of data.

[^{3}H]-Proline Transneuronal Tracing

Monocular injections of [^{3}H]-proline (2 μl containing 0.14 mCi, concentrated from NEN Cat. NET483) were made into 40-day-old mice. The mice were allowed to survive for 5 more days before sacrifice. 4% paraformaldehyde fixed mouse brains were sectioned at 50 μm with a vibratome, mounted onto slides, and processed for autoradiography using Kodak emulsion. The slides were exposed for 2–3 months and were then developed and fixed using Kodak Developer D-19 and Fixer (Kodak). Dark field pictures were then taken under the same exposure, and silver particle intensity (histogram) was quantified using Adobe Photoshop 5.5 software (Adobe). We calculated the relative intensity of labeling by normalizing pixel intensity of visual cortex to that of the LGN from the same sections.

Visual Cliff Avoidance

Visual cliff avoidance was tested in an open-topped box (60 × 60 cm square × 30 cm high) as previously reported (Lione et al., 1999; Lore and Sawatski, 1969) with minor modifications. The four walls of the box were made from white plywood, and the base was made from clear Perspex. The box was positioned on the edge of a laboratory bench so that half of the base sat directly on the bench ("bench side"), while the other half was suspended 1 m above the floor ("open side"), thus creating a visual cliff. Mice were placed in the middle of the base at the edge of the cliff, and their activity was recorded for 5 min using a video camera. Representative results of the video movie are attached as a file of Quick Time Player. The videos were subsequently analyzed to measure the percentage of time each mouse spent in the open side of the box.

Acknowledgments

We thank Dr. Hideshi Shibata for helpful suggestions on the identification of different thalamic nuclei. Yijia Chen, Yajuan Liu, Penny Houston, Steven McKinnon, Tingwan Sun, and Ari Nandi provided excellent animal husbandry and technical support. We thank members of the Parada Lab for helpful discussions, particularly Yuan Zhu, Mark Lush, Lei Lei, and Steve Kernie. We thank Albee Messing for providing GFAP-Cre mice. L.F.P. is supported by the NINDS and

the Christopher Reeve Paralysis Foundation. T.H. is supported by a Human Frontier Science Program, and C.H. is supported by the Uehara Memorial Foundation.

Received: March 11, 2002
Revised: September 3, 2002

References

Aldrich, M.S., Alessi, A.G., Beck, R.W., and Gilman, S. (1987). Cortical blindness: etiology, diagnosis, and prognosis. Ann. Neurol. *21*, 149–158.

Allendoerfer, K.L., and Shatz, C.J. (1994). The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu. Rev. Neurosci. *17*, 185–218.

Allendoerfer, K.L., Cabelli, R.J., Escandon, E., Kaplan, D.R., Nikolics, K., and Shatz, C.J. (1994). Regulation of neurotrophin receptors during the maturation of the mammalian visual system. J. Neurosci. *14*, 1795–1811.

Angevine, J.B., Jr. (1970). Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. J. Comp. Neurol. *139*, 129–187.

Antonini, A., Fagiolini, M., and Stryker, M.P. (1999). Anatomical correlates of functional plasticity in mouse visual cortex. J. Neurosci. *19*, 4388–4406.

Auladell, C., Perez-Sust, P., Super, H., and Soriano, E. (2000). The early development of thalamocortical and corticothalamic projections in the mouse. Anat. Embryol. (Berl.) *201*, 169–179.

Bagri, A., Marin, O., Plump, A.S., Mak, J., Pleasure, S.J., Rubenstein, J.L., and Tessier-Lavigne, M. (2002). Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. Neuron *33*, 233–248.

Baker-Nobles, L., and Rutherford, A. (1995). Understanding cortical visual impairment in children. Am. J. Occup. Ther. *49*, 899–903.

Bates, B., Rios, M., Trumpp, A., Chen, C., Fan, G., Bishop, J.M., and Jaenisch, R. (1999). Neurotrophin-3 is required for proper cerebellar development. Nat. Neurosci. *2*, 115–117.

Bibel, M., and Barde, Y.A. (2000). Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. *14*, 2919–2937.

Bicknese, A.R., Sheppard, A.M., O'Leary, D.D., and Pearlman, A.L. (1994). Thalamocortical axons extend along a chondroitin sulfate proteoglycan- enriched pathway coincident with the neocortical subplate and distinct from the efferent path. J. Neurosci. *14*, 3500–3510.

Birch, E.E., and Bane, M.C. (1991). Forced-choice preferential looking acuity of children with cortical visual impairment. Dev. Med. Child Neurol. *33*, 722–729.

Braisted, J.E., Tuttle, R., and O'Leary, D.D. (1999). Thalamocortical axons are influenced by chemorepellent and chemoattractant activities localized to decision points along their path. Dev. Biol. *208*, 430–440.

Braisted, J.E., Catalano, S.M., Stimac, R., Kennedy, T.E., Tessier-Lavigne, M., Shatz, C.J., and O'Leary, D.D. (2000). Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. J. Neurosci. *20*, 5792–5801.

Cabelli, R.J., Hohn, A., and Shatz, C.J. (1995). Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. Science *267*, 1662–1666.

Cabelli, R.J., Shelton, D.L., Segal, R.A., and Shatz, C.J. (1997). Blockade of endogenous ligands of trkB inhibits formation of ocular dominance columns. Neuron *19*, 63–76.

Carpenter, M.B. (1985). Core Text of Neuroanatomy, Third Edition (Baltimore, MD: Williams & Wilkins).

Catalano, S.M., Robertson, R.T., and Killackey, H.P. (1991). Early ingrowth of thalamocortical afferents to the neocortex of the prenatal rat. Proc. Natl. Acad. Sci. USA *88*, 2999–3003.

Chang, G.Q., Hao, Y., and Wong, F. (1993). Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. Neuron *11*, 595–605.

Cohen-Cory, S., and Fraser, S.E. (1995). Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. Nature *378*, 192–196.

DeFreitas, M.F., McQuillen, P.S., and Shatz, C.J. (2001). A novel p75NTR signaling pathway promotes survival, not death, of immunopurified neocortical subplate neurons. J. Neurosci. *21*, 5121–5129.

Ernfors, P., Lee, K.F., Kucera, J., and Jaenisch, R. (1994). Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell *77*, 503–512.

Franklin, K.B.J., and Paxinos, G. (1997). The Mouse Brain in Stereotaxic Coordinates (San Diego, CA: The Academic Press).

Ghosh, A., and Shatz, C.J. (1992). Involvement of subplate neurons in the formation of ocular dominance columns. Science *255*, 1441–1443.

Gordon, J.A., and Stryker, M.P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. *16*, 3274–3286.

Grafstein, B. (1971). Transneuronal transfer of radioactivity in the central nervous system. Science *172*, 177–179.

Hebert, J.M., and McConnell, S.K. (2000). Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. Dev. Biol. *222*, 296–306.

Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell *98*, 739–755.

Kang, H., and Schuman, E.M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science *267*, 1658–1662.

Katz, L.C. (1999). What's critical for the critical period in visual cortex? Cell *99*, 673–676.

Kernie, S.G., Liebl, D.J., and Parada, L.F. (2000). BDNF regulates eating behavior and locomotor activity in mice. EMBO J. *19*, 1290–1300.

Kokaia, M., Asztely, F., Olofsdotter, K., Sindreu, C.B., Kullmann, D.M., and Lindvall, O. (1998). Endogenous neurotrophin-3 regulates short-term plasticity at lateral perforant path-granule cell synapses. J. Neurosci. *18*, 8730–8739.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., and Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. USA *92*, 8856–8860.

Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. J. Comp. Neurol. *297*, 441–470.

Lein, E.S., Hohn, A., and Shatz, C.J. (2000). Dynamic regulation of BDNF and NT-3 expression during visual system development. J. Comp. Neurol. *420*, 1–18.

Liebl, D.J., Tessarollo, L., Palko, M.E., and Parada, L.F. (1997). Absence of sensory neurons before target innervation in brain-derived neurotrophic factor-, neurotrophin 3-, and TrkC-deficient embryonic mice. J. Neurosci. *17*, 9113–9121.

Lione, L.A., Carter, R.J., Hunt, M.J., Bates, G.P., Morton, A.J., and Dunnett, S.B. (1999). Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. J. Neurosci. *19*, 10428–10437.

Lom, B., and Cohen-Cory, S. (1999). Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritic and axonal arborization in vivo. J. Neurosci. *19*, 9928–9938.

Lore, R., and Sawatski, D. (1969). Performance of binocular and monocular infant rats on the visual cliff. J. Comp. Physiol. Psychol. *67*, 177–181.

Ma, L., Reis, G., Parada, L.F., and Schuman, E.M. (1999). Neuronal NT-3 is not required for synaptic transmission or long-term potentiation in area CA1 of the adult rat hippocampus. Learn. Mem. *6*, 267–275.

Ma, L., Merenmies, J., and Parada, L.F. (2000). Molecular characterization of the TrkA/NGF receptor minimal enhancer reveals regulation by multiple cis elements to drive embryonic neuron expression. Development *127*, 3777–3788.

Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E., Lindsay, R.M., and Yancopoulos, G.D. (1990). NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron *5*, 501–509.

McAllister, A.K., Lo, D.C., and Katz, L.C. (1995). Neurotrophins regulate dendritic growth in developing visual cortex. Neuron *15*, 791–803.

McAllister, A.K., Katz, L.C., and Lo, D.C. (1996). Neurotrophin regulation of cortical dendritic growth requires activity. Neuron *17*, 1057–1064.

McAllister, A.K., Katz, L.C., and Lo, D.C. (1997). Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron *18*, 767–778.

McConnell, S.K. (1995). Constructing the cerebral cortex: neurogenesis and fate determination. Neuron *15*, 761–768.

McQuillen, P.S., DeFreitas, M.F., Zada, G., and Shatz, C.J. (2002). A novel role for p75NTR in subplate growth cone complexity and visual thalamocortical innervation. J. Neurosci. *22*, 3580–3593.

Metin, C., and Godement, P. (1996). The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. J. Neurosci. *16*, 3219–3235.

Minichiello, L., and Klein, R. (1996). TrkB and TrkC neurotrophin receptors cooperate in promoting survival of hippocampal and cerebellar granule neurons. Genes Dev. *10*, 2849–2858.

Patapoutian, A., and Reichardt, L.F. (2001). Trk receptors: mediators of neurotrophin action. Curr. Opin. Neurobiol. *11*, 272–280.

Patterson, S.L., Abel, T., Deuel, T.A., Martin, K.C., Rose, J.C., and Kandel, E.R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron *16*, 1137–1145.

Reinis, S., and Goldman, J.M. (1984). The transneuronal transport of proline within the mouse visual system: some characteristics of the [^{3}H]-proline containing material. Brain Res. Bull. *12*, 339–342.

Robertson, R.T., and Kaitz, S.S. (1981). Thalamic connections with limbic cortex. I. Thalamocortical projections. J. Comp. Neurol. *195*, 501–525.

Shatz, C.J. (1992). How are specific connections formed between thalamus and cortex? Curr. Opin. Neurobiol. *2*, 78–82.

Shibata, H. (1993). Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. J. Comp. Neurol. *330*, 533–542.

Shibata, H. (1998). Organization of projections of rat retrosplenial cortex to the anterior thalamic nuclei. Eur. J. Neurosci. *10*, 3210–3219.

Shibata, H. (2000). Organization of retrosplenial cortical projections to the laterodorsal thalamic nucleus in the rat. Neurosci. Res. *38*, 303–311.

Snider, W.D. (1994). Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell *77*, 627–638.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. *21*, 70–71.

Sripanidkulchai, K., and Wyss, J.M. (1986). Thalamic projections to retrosplenial cortex in the rat. J. Comp. Neurol. *254*, 143–165.

Tessarollo, L., Tsoulfas, P., Martin-Zanca, D., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., and Parada, L.F. (1993). trkC, a receptor for neurotrophin-3, is widely expressed in the developing nervous system and in non-neuronal tissues. Development *118*, 463–475.

Tessarollo, L., Vogel, K.S., Palko, M.E., Reid, S.W., and Parada, L.F. (1994). Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons. Proc. Natl. Acad. Sci. USA *91*, 11844–11848.

Van Groen, T., and Wyss, J.M. (1995). Projections from the anterodorsal and anteroventral nucleus of the thalamus to the limbic cortex in the rat. J. Comp. Neurol. *358*, 584–604.

Vigers, A.J., Baquet, Z.C., and Jones, K.R. (2000). Expression of neurotrophin-3 in the mouse forebrain: insights from a targeted LacZ reporter. J. Comp. Neurol. *416*, 398–415.

Wilkinson, G.A., Farinas, I., Backus, C., Yoshida, C.K., and Reichardt, L.F. (1996). Neurotrophin-3 is a survival factor in vivo for early mouse trigeminal neurons. J. Neurosci. *16*, 7661–7669.

Wong, V.C. (1991). Cortical blindness in children: a study of etiology and prognosis. Pediatr. Neurol. *7*, 178–185.

Wong-Riley, M.T. (1989). Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci. *12*, 94–101.

Xu, B., Zang, K., Ruff, N.L., Zhang, Y.A., McConnell, S.K., Stryker, M.P., and Reichardt, L.F. (2000). Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron *26*, 233–245.

Yokoyama, N., Romero, M.I., Cowan, C.A., Galvan, P., Helmbacher, F., Charnay, P., Parada, L.F., and Henkemeyer, M. (2001). Forward signaling mediated by ephrin-B3 prevents contralateral corticospinal axons from recrossing the spinal cord midline. Neuron *29*, 85–97.

Zhou, X.F., and Rush, R.A. (1994). Localization of neurotrophin-3-like immunoreactivity in the rat central nervous system. Brain Res. *643*, 162–172.

Zhu, Y., Romero, M.I., Ghosh, P., Ye, Z., Charnay, P., Rushing, E.J., Marth, J.D., and Parada, L.F. (2001). Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the brain. Genes Dev. *15*, 859–876.

Zhuo, L., Theis, M., Alvarez-Maya, I., Brenner, M., Willecke, K., and Messing, A. (2001). hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. Genesis *31*, 85–94.