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Altered Development of Visual Subcortical Projections Following Neonatal Thalamic Ablation in the Hamster

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ABSTRACT

Previous research has demonstrated that precise patterns of axonal connectivity often develop during a series of stages characterized by pathfinding, target recognition, and address selection. This last stage involves the focusing of projections to a precisely defined region within the target. Because thalamic projections begin to innervate cortex before the latter stages are reached, these projections may be important in the establishment of adult-like patterns of cortical connectivity. To address this issue, we examined the mature corticopontine and corticospinal projections of visual cortex deprived of early thalamic input by visual thalamic ablation. Although ablations on the day of birth in hamsters did not disrupt the targeting of appropriate subcortical structures by visual cortical axons, they did alter the organization of projections within the basilar pons and spinal cord. The density and spread of visual corticopontine connections in lesioned animals was greatly increased relative to unlesioned animals, suggesting that thalamic afferents are required during address selection, when the topographic specificity of projections is established. To determine whether early visual thalamic ablation increases connectivity by stabilizing an exuberant developmental projection, we examined the normal development of visual corticopontine connections in hamsters ages postnatal days 1–17 (P1–P17). From the earliest ages, visual cortical axons innervate the pontine nucleus in regions specific to their adult projection zones and show progressive growth within these zones. At no time during development do projections exist that are equivalent to the projections found after thalamic ablation, suggesting that removal of thalamic input does not simply stabilize a developmental projection. J. Comp. Neurol. 424: 165–178, 2000.

Indexing terms: corticopontine, corticospinal, dorsal lateral geniculate nucleus, visual cortex

Adult isocortex is divided into a number of functionally distinct regions, each characterized by a unique pattern of connections. Developmental factors that generate this precise connectivity are increasingly well-understood for intracortical projection systems (i.e., vertical and horizontal connections; Lowel and Singer, 1992; Callaway and Lieber, 1996; Ruthazer and Stryker, 1996; Castellani and Bolz, 1997; Castellani et al., 1998; Dantzker and Callaway, 1998), whereas factors contributing to the development of subcortical projection systems remain to be elucidated. Several indirect lines of evidence suggest that thalamocortical projections play a role in the patterning of subcortical projections. Thus, the present experiments were conducted to examine directly the role of thalamic input in the formation of corticopontine and corticospinal projections.

Specific connections between cortical cells and their subcortical targets emerge during a series of developmental stages (O'Leary et al., 1990). These stages correspond well to the sequential stages of pathway selection, target selection, and address selection used to describe other axonal projection systems (Goodman and Shatz, 1993). During pathway and target selection, imprecise connections between cells and their target structures are formed.

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These connections are then remodeled and refined within targets during the period of address selection.

Whether the initial axonal extension of a cortical neuron follows a subcortical, intracortical, or callosal route (i.e., pathway selection) is likely determined early in the ventricular zone, given that a neuron's birthday predicts its mature laminar position (McConnell and Kaznowski, 1991) and that laminar position is correlated with the selected projection route. Experimental manipulations that cause cells born during cortical layer V production to occupy ectopic positions demonstrate that axons from these cells project to appropriate subcortical targets (Jensen and Killackey, 1984; Terashima, 1995), providing further evidence that factors acting at the time of mitotic division, or shortly thereafter, specify initial axonal projections. The presence of subcortical cues that facilitate pathway guidance and target innervation is demonstrated by coculture studies in which diffusible chemotropic molecules attract subcortical axons toward the internal capsule and into subcortical targets (Bolz et al., 1990; Heffner et al., 1990; Molnar and Blakemore, 1991; Joosten et al., 1994; Metin and Godement, 1996; Richards et al., 1997). However, the connections formed between explants of cortex and subcortical targets lack the regional specificity found in vivo (Molnar and Blakemore, 1991; Joosten et al., 1994), implying that other factors are required for the development of precise subcortical connections.

One such factor may be thalamocortical input, as suggested by transplant studies (O'Leary and Koester, 1993). Heterotopically transplanted tissue develops subcortical connections appropriate for the cortical area into which it is transplanted: Visual cortex transplanted to motor cortex retains its normally transient corticospinal projection, whereas motor cortex transplanted to visual cortex loses its corticospinal projection but maintains its normally transient corticotectal projection (Stanfield and O'Leary, 1985; O'Leary and Stanfield, 1989). Importantly, transplanted cortices receive thalamocortical input characteristic of their new locale (Schlaggar and O'Leary, 1991; O'Leary et al., 1992). In contrast, recent experiments suggest that heterotopically transplanted cortex forms efferent connections characteristic of its site of origin (Ebrahimi-Gaillard et al., 1994), but the subcortical connections of the transplant are nonetheless appropriate for the type of thalamic input that the transplant receives (Frappe et al., 1999). Hence, input from the sensory thalamus may play a crucial role in the patterning of subcortical connections.

We have addressed this issue by examining the adult corticopontine and corticospinal connections formed by visual cortex denervated of its early thalamic input. Visual thalamic ablations were performed on the day of birth in hamsters, a time when visual cortical efferents are in the internal capsule but have not yet reached their subcortical targets (Miller et al., 1993). Based on the findings above, we hypothesized that visual projections emerging from deafferented cortex would project subcortically but fail to show specificity in 1) the selection of subcortical targets and/or 2) the arborization within targets. Initial experiments confirmed the second hypothesis; neonatal thalamic ablations resulted in an increase and spread of visual corticopontine projections beyond the normal arborization zones in the basilar pons. Based on findings that visual corticopontine projections in the rat undergo slight refinement during development (Mihailoff et al., 1984), we examined the development of normal visual corticopontine projections in the hamster to determine whether the substantial removal of thalamic input arrests subcortical projections in an immature exuberant state.

MATERIALS AND METHODS

Offspring of timed pregnant Syrian hamsters (*Mesocricetus auratus*) from our breeding colony were used for the present experiments. Animals were maintained on a 12L:12D photoperiod and fed food and water ad libitum. Throughout all experiments, animals were maintained in strict accordance with the policies and procedures set forth in The National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approved regulations of Cornell University (Institutional Animal Use Committee).

Experiment 1: thalamic ablation study

Neonatal thalamic ablations. Hamster pups aged postnatal day 0 (P0; the first 24 hours following birth) were anesthetized by hypothermia and given unilateral electrolytic ablations targeting the left visual thalamic nuclei: lateral nucleus (L), dorsal lateral geniculate nucleus (LGd), and lateral posterior nucleus (LP). Specific thalamic nuclei may be ablated at P0 without directly injuring the cortex, insofar as posterior cortex has not yet grown over the posterior thalamus. A small hole was made in the skull on the dorsal surface behind the developing cortex. An insulated stainless steel electrode with a 0.25 mm exposed tip (FHC, Brunswick, ME) was lowered at a 45° caudal to rostral angle to a depth of 2 mm and current was applied using a Grass SD9 Stimulator. Pups were rewarmed and returned to their mothers.

Tracer injections in adults. At P23 or P24, the anterograde tracer biotinylated dextran amine (BDA; 10,000 MW; Molecular Probes, Eugene, OR) was placed into the visual cortex of neonatally lesioned hamsters for the examination of visual subcortical projections. At these ages in the rat and hamster, subcortical connections from visual cortex to the pons and spinal cord are adult-like (Mihailoff et al., 1984; O'Leary and Stanfield, 1986). Pilot experiments demonstrated that more consistent and complete labeling of long-range subcortical connections was obtained using an implant of BDA-soaked filter paper (protocol based on Ding and Elberger, 1995) than with pressure injections of BDA. The implant method was therefore adopted here. Animals were anesthetized with Nembutal (0.16 cc/100 mg of 50 mg/ml). The scalp was incised, and a small piece of skull was retracted to expose underlying visual cortex ipsilateral to the lesion. The dura was incised, and a piece of filter paper (0.75 mm by 0.5 mm) that had been soaked in 20% BDA in phosphate buffer (PB; pH 7.4) and air dried was implanted into the visual cortex. The skull flap was repositioned and the scalp was sutured. Following a 9 or 10 day survival period, animals (P32 or P33) were overdosed with Nembutal and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M PB. Brains were removed and stored in fixative at 4°C until processing. Survival time was selected based on pilot data demonstrating optimal transport with this interval.

Histology. Brains were transferred to a 30% sucrose solution 24 hours prior to sectioning. The forebrain was separated from the hindbrain and spinal cord by making an angled cut through the midbrain so as not to compromise the thalamus or pontine nucleus. Both blocks of

tissue were embedded in albumin-gelatin and cut on a freezing microtome at 40 μm. The forebrain block of tissue was sectioned coronally to facilitate thalamic lesion reconstruction; the hindbrain block was sectioned sagittally for optimal visualization of corticospinal projections. Every fourth section from the forebrain block and alternate sections from the hindbrain block were processed as follows: Sections were rinsed in phosphate-buffered saline (PBS; pH 7.2), quenched in a 0.5% H_2O_2 solution for 5 minutes and incubated in an avidin-biotin complex solution (1:50; Vectastain Elite Standard Kit; Vector Laboratories, Burlingame, CA) containing 0.4% Triton X-100 (TX) for 2 hours. Tissue was rinsed in PBS and reacted in a 0.05% diaminobenzidene (DAB; Sigma Chemical Co., St. Louis, MO) solution enhanced with 1% $CoCl_2$ and 1% $NiSO_4$. After additional PBS rinses, tissue was mounted on chrom-alum-subbed slides and air dried. Sections were left unstained or stained lightly with neutral red, dehydrated, cleared in xylene, and coverslipped with Permount. The remaining series of unreacted tissue was stained with cresyl violet for visualization of cortical and thalamic cytoarchitecture.

Criteria for including experimental animals in the analyses were as follows: 1) at least 50% ablation of the visual thalamic nuclei (L, LGd, and LP combined) and 2) accurate placement of BDA into visual cortex. Experimental animals were paired with age-matched controls that had received BDA implants (1 mm by 0.5 mm) in a similar location (see Table 1). Unlesioned control brains were processed in a manner identical to that for experimental brains.

A BDA implant of smaller width was employed for lesioned animals (0.75 mm vs. 1 mm in controls) to adjust for the loss in cortical thickness produced by early thalamic ablation (owing to a specific loss of layer 4 cells; density of cells in other cortical layers remains unchanged although layer 6 shows a nonsignificant decrease; Windrem and Finlay, 1991). Pilot experiments demonstrated that implants of the sizes selected traverse the depth of the cortical gray matter without entering the white matter. Visual thalamic ablations do not cause a substantial tangential shrinkage of total cortical surface area (average reduction is 5%; M.A. Kingsbury, E.R. Graf, B.L. Finlay, unpublished observations). BDA diffusion into the white matter occurred in one experimental and two control animals. However, labeling in these animals did not differ from that found in animals with BDA confined to gray matter and they were therefore included in the analyses.

Reconstruction of thalamic ablations. Using a projection microscope, visual thalamic nuclei on the intact side and ablated side (when the nucleus was not completely ablated) were outlined in alternate coronal sections of the cresyl violet stained series (i.e., every 160 μm). Surface area measurements were obtained using NIH Image 1.60 (W. Rasband, U.S. National Institutes of Health, Bethesda, MD), and nuclear volumes were computed. The unablated volume of each remaining nucleus (L, LGd, LP) on the lesioned side was then expressed as a percentage of the volume of the nucleus on the intact side (Table 2). Given that the nuclei targeted lie on the dorsolateral surface of the thalamus, the ablations were readily recognized by gliosis and neuronal absence/reduction immediately dorsal to the external medullary lamina and superior thalamic radiation. Ablations distorted the general shape of the thalamus in some subjects. However, remaining thalamic nuclei retained many of their original characteristics and could be identified on the basis of staining intensity, cell size and density, and relative position. In cases in which remaining thalamic nuclei were unrecognizable, brains were excluded from the analysis.

Reconstruction of implantation sites. BDA implantation sites for all animals were charted in alternating coronal sections (i.e., every 80 μm) under a projection microscope. Measurements from coronal sections were used to generate a dorsal view reconstruction of each implanted hemisphere. The reconstructed hemisphere was then fitted to a standard dorsal view map of hamster isocortex (see, e.g., Windrem and Finlay, 1991; Miller et al., 1991), with adjustments made to account for the cytoarchitecture of the reconstructed brain.

The borders between visual cortical areas (V1 and V2 combined) and adjacent cortices were readily identified in both control and experimental tissue based on the following characteristics: 1) the presence of a distinct cortical layer 4 in parietal cortex but not in immediately adjacent visual cortex (corresponding to V2 in control tissue); 2) a reduction in cortical width between parietal and adjacent visual cortex (i.e., parietal cortex is wider); 3) the presence of large Betz cells in layer V of hindlimb cortex but not in adjacent medial visual cortex; 4) the specific condensation of layer II cells and the overall appearance of small, homogeneously globular cells in retrosplenial cortex but not in medial visual cortex; and 5) the presence of a well-defined layer IV and hypocellular layer Vc (but not layer Vb) in temporal cortex but not in lateral visual cortex.

In contrast, the location of the V1/V2 border could not be identified in animals from the experimental group. Whereas a well-defined cortical layer IV and hypocellular layer V distinguished V1 from V2 in controls, this distinction was not possible in lesioned animals because of the significant loss of layer IV cells (Windrem and Finlay, 1991). However, this likely does not substantially impact our results; placement of BDA into a variety of medial locations in control visual cortex produced very similar patterns of corticopontine labeling (see below under Methodological considerations). Outside of the visual cortices, the cytoarchitecture of other cortical areas appeared normal in animals with incomplete visual thalamic ablations. In animals with complete or extremely large visual thalamic ablations (i.e., animals 541.8, 593.3, and 541.10), thalamic damage encroached into nonvisual thalamic nuclei located ventral to the visual nuclei, thus affecting the cytoarchitecture of some nonvisual cortices. Specifically, we observed a decrease in the thickness of layer IV and, to a lesser extent, of layer VI, in parietal cortex ipsilateral to the ablation, compared to parietal cortex in the contralateral hemisphere. The thickness of layer IV in forelimb cortex was also slightly reduced in the ablated hemisphere. No noticeable differences were detected in the retrosplenial cortices or motor (frontal) cortices of the deafferented hemisphere compared to the control hemisphere. In the two animals with damage to the medial geniculate nucleus (i.e., animals 593.4 and 541.8), layer IV of primary auditory cortex of the denervated hemisphere was reduced in width compared to the control hemisphere.

The parcellation of the adult dorsal view map into cytoarchitectonically distinct areas has been previously described (Windrem and Finlay, 1991) and is shown in Figure 1 (right). For clarity, only the visual cortices are labeled. Implantation sites in both experimental and control brains were located primarily in medial visual cortex

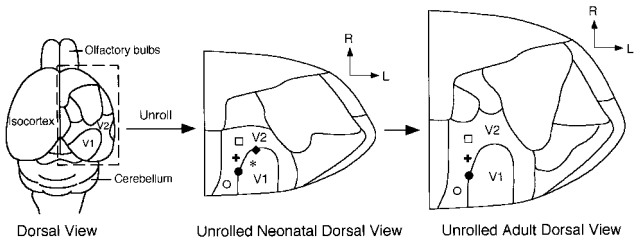


Fig. 1. Dorsal view representations of normal (**left**) and unrolled (**center** and **right**) hamster isocortex. The locations of implantation sites in developing and adult animals used in the present studies are shown on the dorsal view maps of unrolled neonatal and adult cortex, respectively. See Table 1 for the specific site location for individual subjects. V1, primary visual cortex; V2, secondary visual cortex; R, rostral; L, lateral.

TABLE 1. Description of Subjects and Tracer Placement

Animal	Neonatal treatment	Age at injection/ perfusion	Implant/Injection placement[1,2]
Lesion study			
541.8	P0 lesion	P24/P33	○
541.3	Normal	P24/P33	○
541.10	P0 lesion	P24/P33	●
507.6	Normal	P22/P32	●
593.3	P0 lesion	P24/P33	□
594.7	Normal	P23/P33	□
593.4	P0 lesion	P24/P33	✚
593.11	Normal	P24/P33	✚
594.8	P0 lesion	P24/P33	✚
594.5	Normal	P23/P33	✚
Developmental study			
678.2	Normal	P0/P1	◆
679.1	Normal	P2/P3	◆
697.3	Normal	P4/P5	◆
683.3	Normal	P6/P7	□
692.4	Normal	P8/P9	□
701.3	Normal	P8/P9	✚
750.1	Normal	P8/P9	*
750.2	Normal	P8/P9	✚
694.7	Normal	P10/P11	◆
694.8	Normal	P10/P11	□
704.5	Normal	P10/P11	✚
750.5	Normal	P10/P11	✚
750.6	Normal	P10/P11	✚
699.3	Normal	P12/P13	○
700.1	Normal	P12/P13	*
695.7	Normal	P14/P15	●
692.9	Normal	P14/P15	*
694.9	Normal	P16/P17	●

[1]See Figure 1 for symbol location.
[2]Note that tracer placement in lesioned animals was determined based on position within visual cortices (V1 and V2 combined), because the V1/V2 border could not be distinguished in these subjects.

(Fig. 1, right). Implantation site for each subject is indicated in Table 1.

Analysis of adult corticopontine projections. Axonal arborization in the pontine nucleus of five matched pairs of subjects was analyzed using NIH Image 1.60 and Adobe Photoshop 3.0. (Adobe Systems, Inc., Mountain View, CA) for the Macintosh. Brightfield images of the basilar pons were captured using a Leitz Diaplan microscope and a COHU 4910 CCD video camera. The perimeter of the pons was outlined in each cresyl violet section and then imposed on the adjacent unstained section. Labeled axons in the pons were traced in every 40 μm unstained section (i.e., every 160 μm) in experimental animals (n = 5) and control animals (n = 5) by investigators blind to the subject's status.

Pontine area and axon arborization were then calculated based on perimeter and axonal tracings. Axon arborization was expressed as a fraction of total pons area, thus correcting for individual differences in pons size. A measure of total axon spread was additionally obtained by drawing best-fit convex polygons around clusters of traced axons. The amount of pontine area innervated by axons (defined as total area of convex polygons) was then divided by total pons area to determine the percentage of pons area that contained axons.

Analysis of adult corticospinal projections. The number of BDA-labeled axons in consecutive 800 μm^2 grids was counted in the pyramidal tract (ipsilateral to BDA implantation) and in the contralateral dorsal funiculus (up to 6,400 μm^2) in three matched pairs of subjects. We focused on the projections within the pyramidal tract and upper cervical cord because a large number of transient corticospinal axons from visual cortex are present within these areas during development compared to more caudal aspects of the cord (O'Leary and Stanfield, 1986). Our measurements were conducted in every 40 μm unstained sagittal section through the hindbrain using an eyepiece reticule. Axon counts were conducted by assistants blind to the subject's status.

Photography. BDA-labeled axons in the pons and pyramidal tract of lesioned and control subjects were photographed using a Nikon Eclipse E-800 microscope and a Wratten Gelatin A filter (Friedlander and Martin, 1989).

Experiment 2: developmental study

Visual corticopontine connections were examined at several postnatal ages (P1–P17; see Table 1) to determine whether the topography of the projection at any time during development (P1–P17) was similar to that found after thalamic ablation. We focused on the projections present at P9–P11, because these ages in the hamster correspond to the period of maximum diffuseness of visual corticopontine projections described for the rat (P9–P10; Mihailoff et al., 1984). We examined corticopontine projections up to age P17 because 1) the refinement of corticopontine connections in the rat is largely complete by the middle of the third postnatal week (Mihailoff et al., 1984) and 2) adult-like corticopontine connections were observed at P17 (present study).

Tracer injections in developing animals. Biocytin was used as the anterograde tracer for the present developmental study as pilot experiments demonstrated that 1) BDA injections in neonatal animals produced poor axonal labeling and 2) biocytin yielded clear labeling of axons and growth cones in animals as young as P0 (Kingsbury et al., 1998). The use of different tracers in experiments 1 and 2 could have potentially produced quantitative differences in label, although our data do not support this assertion (compare adult control subjects in experiment 1 to P15–P17 subjects in experiment 2; Fig 5. vs. Fig. 7).

Our primary methodological concern was that biocytin injections in developing animals be placed into regions comparable to those targeted in experiment 1 adults. Because there is substantial growth of neonatal hamster cortex during the first postnatal weeks, a flat-mounted cortex was prepared for an additional unmanipulated subject of each developmental stage, allowing us to compensate for isocortical growth. Based on this compensation, injections were made into the medial posterior cortex of subjects at each selected age. We assumed in these procedures that the proportions of V1 and V2 remain constant during normal development (Duffy et al., 1998).

Animals were anesthetized by hypothermia (P0–P8) or by injections of Nembutal (P10–P16). The skull was exposed, and a hole was made in the skull overlying visual cortex with a needle (P0–P4) or drill (P6–P16), using the sagittal and transverse sinuses as guides. A solution of 5% biocytin dissolved in filtered dH_2O was picospritzed into cortex using a glass micropipette. Injections were made to span the full thickness of the cortex and were confined to the cortical gray matter in subjects of all ages except P0 and P2. Following injection, the scalp was sutured and the animal was allowed to recover under a heat lamp before being returned to its mother. Twenty-four hours after injection, subjects were overdosed with Nembutal and perfused through the heart with 0.9% saline, followed by 4% paraformaldehyde and 0.1% gluteraldehyde in 0.1 M PB. Brains were removed and stored in fixative containing 30% sucrose.

Histology. Brains were blocked into forebrain (coronal) and hindbrain (sagittal) components as described for experiment 1 but were not embedded in albumin-gelatin. Tissue blocks were cut frozen at 60 μm. All sections were processed with an enhanced histochemistry protocol (after Ding and Elberger, 1995) to facilitate visualization of labeled axons in developing brains: Sections were rinsed in PBS, quenched in 0.5–1% H_2O_2, and incubated in 1% TX. Tissue was then incubated overnight in an avidin-biotin complex solution (1:100; Vectastain Elite Standard Kit) containing 1% TX. Following additional PBS rinses, tissue was reacted with a 0.004% tetramethylbenzidine (Sigma) solution enhanced with 0.5% sodium tungstate (Sigma). Sections were then rinsed in Tris-HCl buffer (Tris; pH 7.4), enhanced in a 0.5% $CoCl_2$ solution, and reacted with a 0.05% DAB solution in Tris. Tissue was rinsed again, mounted onto chrom-alum-coated slides, and dried overnight. Sections were left unstained or were stained lightly with thionin, dehydrated, cleared in xylene, and coverslipped with Krystalon (Harleco, Gibbstown, NJ).

Reconstruction of injection sites. Biocytin injections were mapped in dorsal view reconstructions following the procedures described for experiment 1. Each reconstructed hemisphere was fitted to a standard dorsal view map of developing hamster isocortex (Fig. 1, center). The dorsal view map was divided into broad cortical areas using the cytoarchitectonic divisions identified in coronal sections of P7 brains. Primary sensory areas were identified by the presence of a well defined granular layer 4 and hypocellular layer V. Delineation of cortical areas in animals younger than age P7 is difficult in that the cortical plate is only partially differentiated (Miller et al., 1993). However, visual cortex expands isotropically during development (Duffy et al., 1998), so the reconstructed hemispheres of brains younger than age P7 were fitted to the proportions of visual cortical areas from P7 brains.

In addition to injection site mapping, anterograde label was charted in the thalamus of developing brains to verify the placement of injections in visual cortex. In animals aged P9 and younger, there was also limited retrograde transport of biocytin to the thalamus, which provided additional information on injection site placement. Corticothalamic label (and thalamocortical label for animals aged <P11) was found in the visual thalamus of the animals included in the developmental study. Injection sites were located in medial V2, medial border of V1/V2, and medial V1 (Fig. 1, center). The injection site for each subject is indicated in Table 1. Animals with injections in areas other than medial V1 or V2 were not included.

Analysis of developing corticopontine projections. Visual corticopontine label in developing animals was analyzed using a Leitz Diaplan microscope and Neurolucida 3.0 (Microbrightfield, Inc., Colchester, VT). Labeled axons located within the basilar pons were traced in alternate 60 μm sagittal sections in animals aged P5–P17. Axons in animals aged P1–P3 had not yet grown into the nucleus.

RESULTS

Experiment 1: effects of neonatal ablations on adult visual subcortical projections

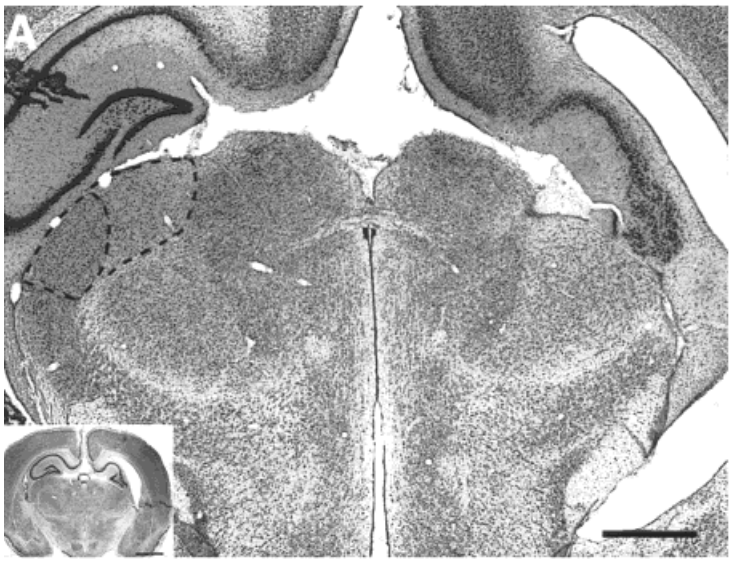
Early thalamic ablations did not alter the appropriate subcortical targeting by corticofugal fibers. As in controls, labeled axons in experimental animals projected to the striatum, remaining visual thalamus, pretectal nuclei, tectum, pontine nucleus, and spinal cord. However, visual subcortical connections within the pons and spinal cord (the two subcortical structures that were the focus of the present study) were increased in lesioned animals relative to controls.

Extent of neonatal thalamic ablations. Visual thalamic nuclei (LGd, LP, and L; Fig. 2) were successfully ablated without causing direct damage to overlying isocortex (see Fig. 2A, inset). Subjects exhibited both complete (n = 2) and incomplete (at least 50% ablation of visual thalamic nuclei; n = 3) visual thalamic ablations. A representative incomplete lesion is shown in Figure 2A–D, where L, LGd, and LP were reduced to 54%, 15%, and 31% of their normal size, respectively. Complete ablations were characterized by the removal of all three visual thalamic nuclei at all levels of the thalamus with damage often extending into neighboring thalamic nuclei (Fig. 2E–H). The percentages of visual thalamic nuclei remaining on the ablated side of lesioned animals are presented in Table 2.

The trajectory of subcortical axons was not compromised by the ablation procedure. Fibers labeled by a BDA injection in visual cortex traveled through the internal capsule and cerebral peduncle to the pons and spinal cord in a manner similar to that of labeled axons in controls. The paths taken by axons traveling to the thalamus and tectum were also comparable in lesioned and control animals.

Corticopontine projections. Experimental animals had a substantially greater amount of axonal arbor in the basilar pons compared to controls (Fig. 3A). The amount of pontine area innervated by visual corticopontine projections in experimental animals was similarly greater (Fig. 3B). On average, three times as much area was innervated in lesioned animals as in controls, and the extent of reorganization was strongly correlated with lesion size. Axonal arbor and axonal spread in animals with complete ablations averaged 554% and 498% of control values, respectively, whereas axonal arbor and axonal spread of incompletely ablated animals averaged 193% and 117% of control values, respectively.

Experimental animals also exhibited altered visual corticopontine topography. Corticopontine axons in control animals arborized in rostral and caudal patches in outer lateral pons (Fig. 4A; left columns of Fig. 5A,B). The caudal patch was found in the most lateral sections, whereas the rostral patch was located coincident with or slightly medial to the caudal patch and continued over a greater number of sections. In contrast, axonal arbor in the lateral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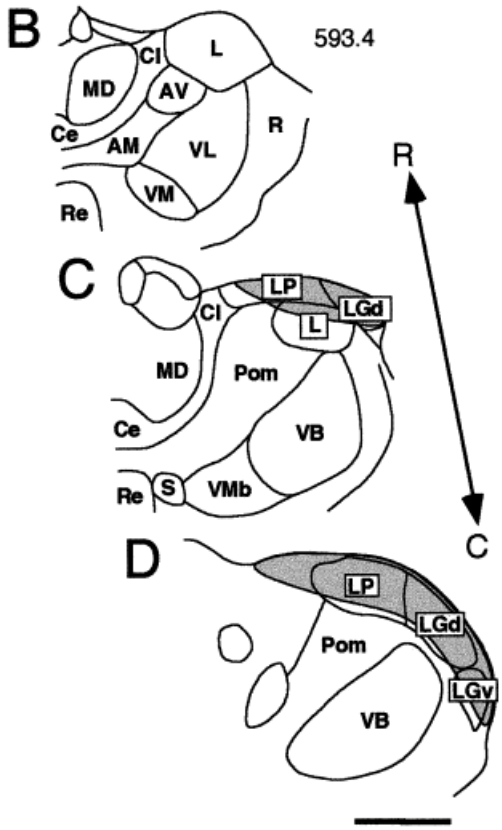
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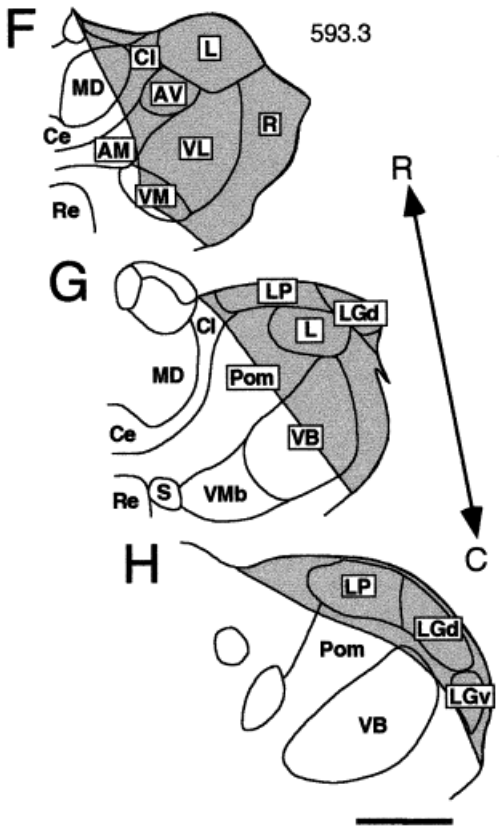
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Figure 2

TABLE 2. Percentage of Visual Thalamic Nuclei Remaining in Lesioned Animals[1]

Animal	L	LGd	LP
541.8	0	0	0
510.10	0	0	26
593.3	0	0	0
593.4	54	15	31
594.8	54	48	43

[1]Volume of lesioned nucleus/volume of intact nucleus. L, lateral nucleus; LGd, dorsal lateral geniculate nucleus; LP, lateral posterior nucleus.

pons of lesioned animals extended throughout this portion of the nucleus rather than being localized to discrete patches (Fig. 4B,C; right columns of Fig. 5A,B). Furthermore, a rostral patch of axons was found throughout the medial pons of every lesioned animal (right columns of Fig. 5A,B) compared to only one control animal (not shown). A small caudal patch of axons was also found in the medial pons of one experimental animal (right column of Fig. 5A) that was not present in controls. The increase in visual corticopontine connections in lesioned animals was not associated with a change in pontine size; there was no significant difference in volume of the nucleus between control and lesioned subjects (n = 5 matched pairs; $P = 0.187$, paired t-test).

Corticospinal connections. Although the general trajectory of axons from visual cortex to the pyramidal tract, pyramidal decussation, and contralateral dorsal funiculus was similar for the two experimental groups, the visual corticospinal projection was greater in lesioned animals compared to controls (Fig. 6B). Whereas a small number of labeled axons were present in the pyramidal tract and dorsal funiculus of adult controls, adult animals that had received neonatal thalamic ablations exhibited a substantially larger number of labeled axons within these pathways, particularly in the rostral pyramidal tract (cf. Fig. 6C and D).

Experiment 2: development of visual corticopontine projections

The growth of visual subcortical axons into the basilar pons is specific to their adult projection zones in rostrolateral and caudolateral pons. Representative corticopontine projections from each developmental age are shown in Figure 7. Data from P1 and P3 subjects are not shown because no labeled axons are found within the pons at these times. Subcortical projections from visual cortex are first seen entering the pons on P5 and are located in the rostrolateral areas of the nucleus. At this age, axons tipped with growth cones are also observed traveling through the medullary pyramidal tract above the basilar pons (not shown). The arborization pattern remains relatively unchanged from P5 to P7. A caudal patch of axons, located coincident with or slightly lateral to the rostral patch, is observed in half of the animals aged P9. A slight increase in the amount of axonal arbor within the rostral patch is also found at this age. By P11, a caudal patch of axons is observed in all animals and is characterized by a greater density of axons than at P9. The topography of the visual corticopontine projection at P11 is essentially adult-like and does not substantially change between P11 and P17 (cf. P11–P17 data in Fig. 7 to experiment 1 control data in Fig. 5). At no time during development is a projection observed that is topographically similar to the pattern observed in neonatally lesioned animals. This is illustrated in Figure 8 by the topography of the corticopontine projection in a P11 animal that had received a large and extremely dense injection of biocytin (Fig. 8A, cf. Fig. 8B). Although the density of axonal label is increased in this animal (694.7) compared to other P11 animals (compare columns in Fig. 8C), the projection resembles the normal arborization pattern rather than that found in lesioned animals (cf. Fig. 8C and lesioned data from Fig. 5A,B).

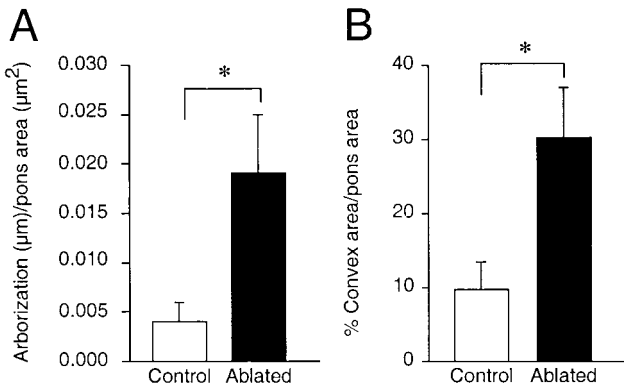


Fig. 3. Comparison of BDA-labeled corticopontine arbor in control hamsters and hamsters given neonatal visual thalamic ablations at birth (n = 5 pairs matched for size and placement of BDA in visual cortex). **A:** Visual corticopontine arbor in adults, as measured by axonal arbor (μm) corrected for pontine area (μm²; mean ± SEM; *$P = 0.032$; paired t-test). **B:** Amount of pontine area occupied by visual corticopontine axons in adults (mean ± SEM; *$P = 0.014$; paired *t*-test).

Fig. 2. Extent of thalamic damage produced by neonatal unilateral electrolytic ablations. **A,E:** Brightfield photomicrographs of Nissl-stained coronal sections through the thalamus of adult hamsters with incomplete (A) and complete (E) visual thalamic ablations. Visual thalamic nuclei on the unablated side are outlined with a broken line. **B–D,F–H:** Schematics of coronal sections at rostral, medial, and caudal levels of the thalamus, respectively, illustrating the extent of thalamic ablations. The photomicrograph in A is at the level of the thalamus depicted in D; E corresponds to the level shown in G. There was no direct mechanical damage to the isocortex from the ablations as shown by a low-power photomicrograph of a more rostral coronal section through the thalamus of animal 593.4 (**inset** in A, lesioned side shown on right). AM, anteromedial nucleus; AV, anteroventral nucleus; Ce, central nucleus; CL, central lateral nucleus; L, lateral nucleus; LGd, dorsal lateral geniculate nucleus; LGv, ventral lateral geniculate nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; Pom, posterior complex, medial nucleus; R, thalamic reticular complex; Re, reuniens nucleus; S, submedial nucleus; VB, ventrobasal nucleus; VL, ventrolateral nucleus; VM, ventromedial nucleus; VMb, ventromedial, basal nucleus; R, rostral; C, caudal. Scale bars = 1 mm in A–H, 1.5 mm in inset.

DISCUSSION

The present experiments demonstrate that early visual thalamic ablations substantially alter adult subcortical projections from visual cortex. The results of our first experiment show that, relative to controls, neonatally ablated animals exhibit increased visual corticopontine and corticospinal projections as adults. The topographic organization of visual corticopontine projections is altered such that axons are no longer confined to rostral and

caudal patches in lateral pons. These abnormal corticopontine projections in thalamic ablated animals do not resemble visual corticopontine connections observed at any stage of normal development, as shown in our second experiment, indicating that the early removal of thalamic input does not stabilize a developmental projection (summarized in Fig. 9).

Methodological considerations

The neonatal visual thalamic ablations employed here produced alterations in cortical cytoarchitecture. These alterations did not prevent the identification of boundaries between visual and nonvisual cortices but did substantially reduce the number of layer IV cells in the visual cortices, making it difficult to distinguish V1 from V2. Thus, a primary concern of the present study is whether the locations of BDA implants in lesioned and control animals are equivalent. We addressed this concern by matching lesioned and control subjects for BDA placement within overall visual cortex (V1 and V2 combined). This method might not provide exact precision if visual thalamic ablations caused differential shrinkage of V1 or V2. However, the present data suggest that different corticopontine projection topographies between lesioned and control animals cannot be explained by a difference in the placement of BDA. First, control animals with BDA implants in a variety of locations in medial visual cortex had similar corticopontine projection patterns. Thus, a broad area of visual cortex exhibits similar subcortical projection patterns, suggesting that slight differences in tracer placement should not produce striking differences in projection topography. Second, the projections in lesioned animals did not resemble those observed following any of the 23 tracer placements made in the control animals in experiment 1 and the normal animals in experiment 2.

Importance of thalamic input in the establishment of subcortical connectivity

Whereas neonatal visual thalamic ablations did not disrupt the ability of visual corticofugal axons to target appropriate subcortical structures, ablations did disrupt the topographic organization of visual projections within a subcortical target (pontine nucleus). That corticopontine projections do not require thalamic afferents for target selection is supported by coculture studies in which axons of layer V cells from cortical explants show directed growth and branching towards explants of pons, but not control tissues, in response to a diffusible chemotropic molecule (Heffner et al., 1990; Sato et al., 1994). However, it is not known whether the topographic organization of corticopontine projections is maintained in culture. We now demonstrate that a substantial portion of visual thalamocortical projections is necessary for the establishment of normal visual corticopontine topography, strongly suggesting that these projections, or the activity relayed through them, are important during the stage of address selection. This reorganization of corticopontine circuitry is most likely induced by removal of visual thalamocortical axons and not by novel or increased projections from other thalamic nuclei; previous work has shown that there is little or no reorganization of remaining thalamocortical connections to deafferented cortex following ablation (Miller et al., 1991).

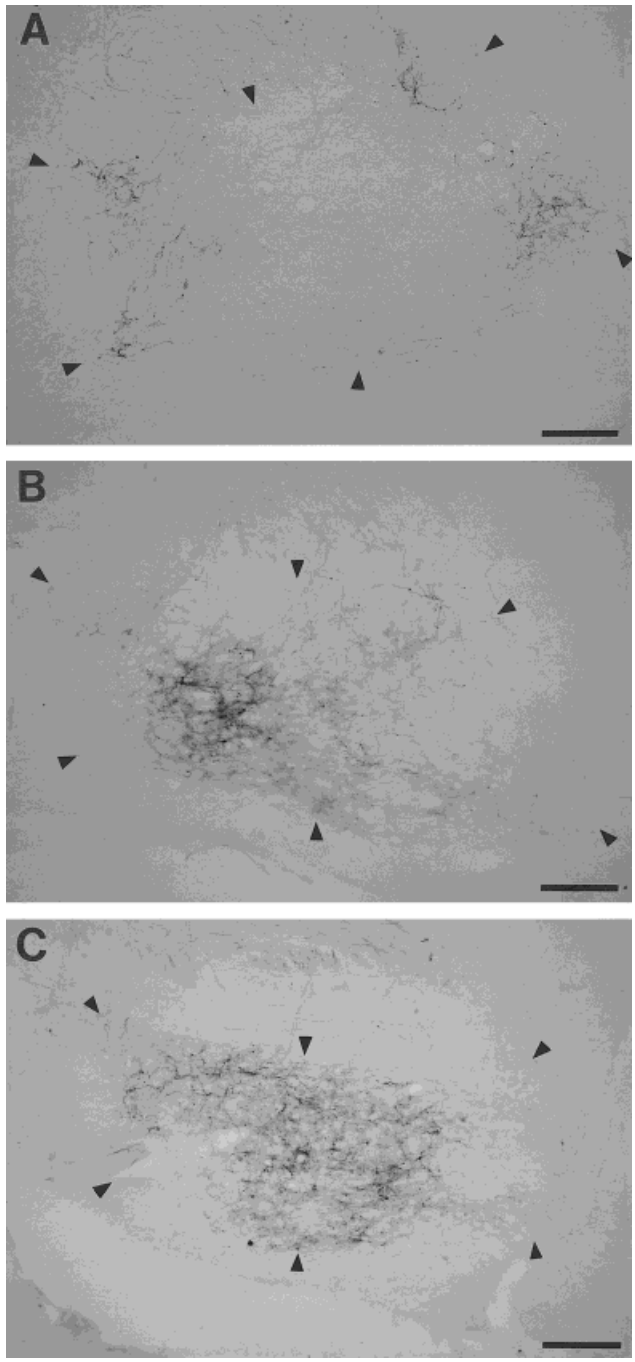


Fig. 4. Brightfield photomicrographs of axons labeled by BDA implantation in visual cortex, shown in unstained sagittal sections through the lateral pons of a P33 control hamster (**A**) and P33 hamsters that sustained neonatal visual thalamic ablations (**B,C**). Sections were photographed using a Wratten Gelatin A filter. Borders of the pontine nucleus are indicated by arrowheads (rostral, left; dorsal, top). Scale bars = 200 μm.

Adult visual subcortical connections in control and lesioned hamsters

Corticopontine projections. Projections in adult controls were characteristic of normal visual corticopontine projections, whereas those in lesioned animals showed

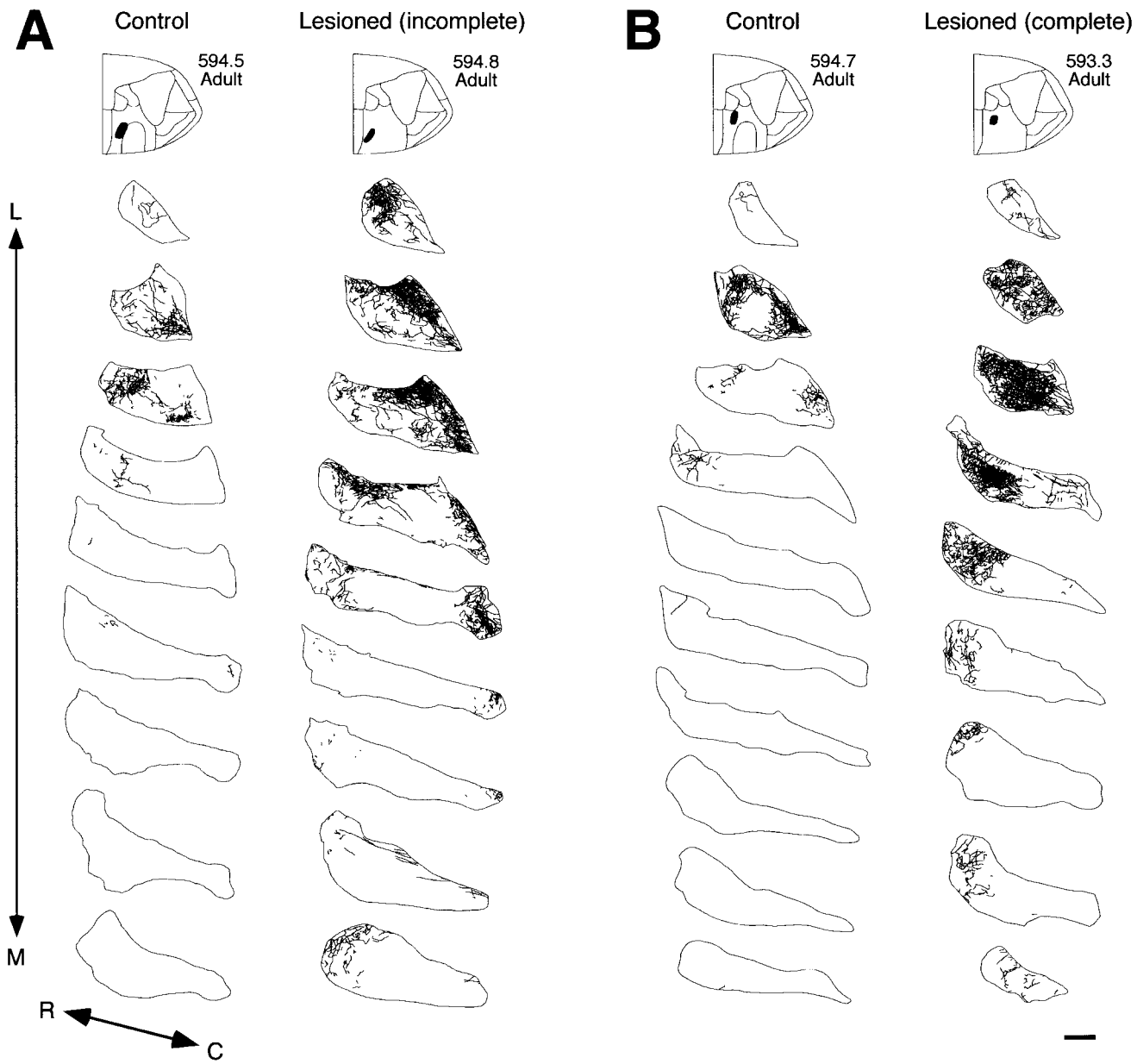


Fig. 5. **A,B:** Reconstructed sagittal sections through the lateromedial pons showing the distribution of BDA-labeled axons in control subjects and subjects with neonatal ablations of the visual thalamus. Two pairs of control and experimental animals are presented, matched for size and location of BDA implantation (shown in unrolled cortical maps, top). Experimental subject 594.8 had an incomplete visual thalamus ablation; subject 593.3 had a complete visual thalamus ablation (see Table 2). R, rostral; C, caudal; M, medial; L, lateral. Scale bar = 200 μm.

considerable reorganization. Previous experiments have shown that normal visual corticopontine connections are primarily localized to rostrolateral pons (Burne et al., 1978; Lent, 1982). The present study confirmed these findings and additionally demonstrated that visual cortex sends a minor projection to caudolateral pons. This is also in agreement with more recent studies of corticopontine circuitry (Wiesendanger and Wiesendanger, 1982; Mihailoff et al., 1984; Leergaard et al., 1995). Corticopontine axons labeled by BDA placements in visual cortex were organized into patches in external lateral pons and were often wedge-shaped, consistent with descriptions of rat visual corticopontine axons (Leergaard et al., 1995). In contrast, BDA-labeled corticopontine axons in thalamic-lesioned animals extended well beyond normal arborization zones in both rostrocaudal and lateromedial directions (Fig. 9A). Previous studies have demonstrated a clear topographic organization of normal corticopontine circuitry where anterior and lateral cortical areas project to more medial, internal and caudal pontine regions while posterior and medial cortices project to more lateral, external and rostral pontine areas (Wiesendanger and Wiesendanger, 1982; Leergaard et al., 1995). In lesioned animals, the spread of visual corticopontine connections

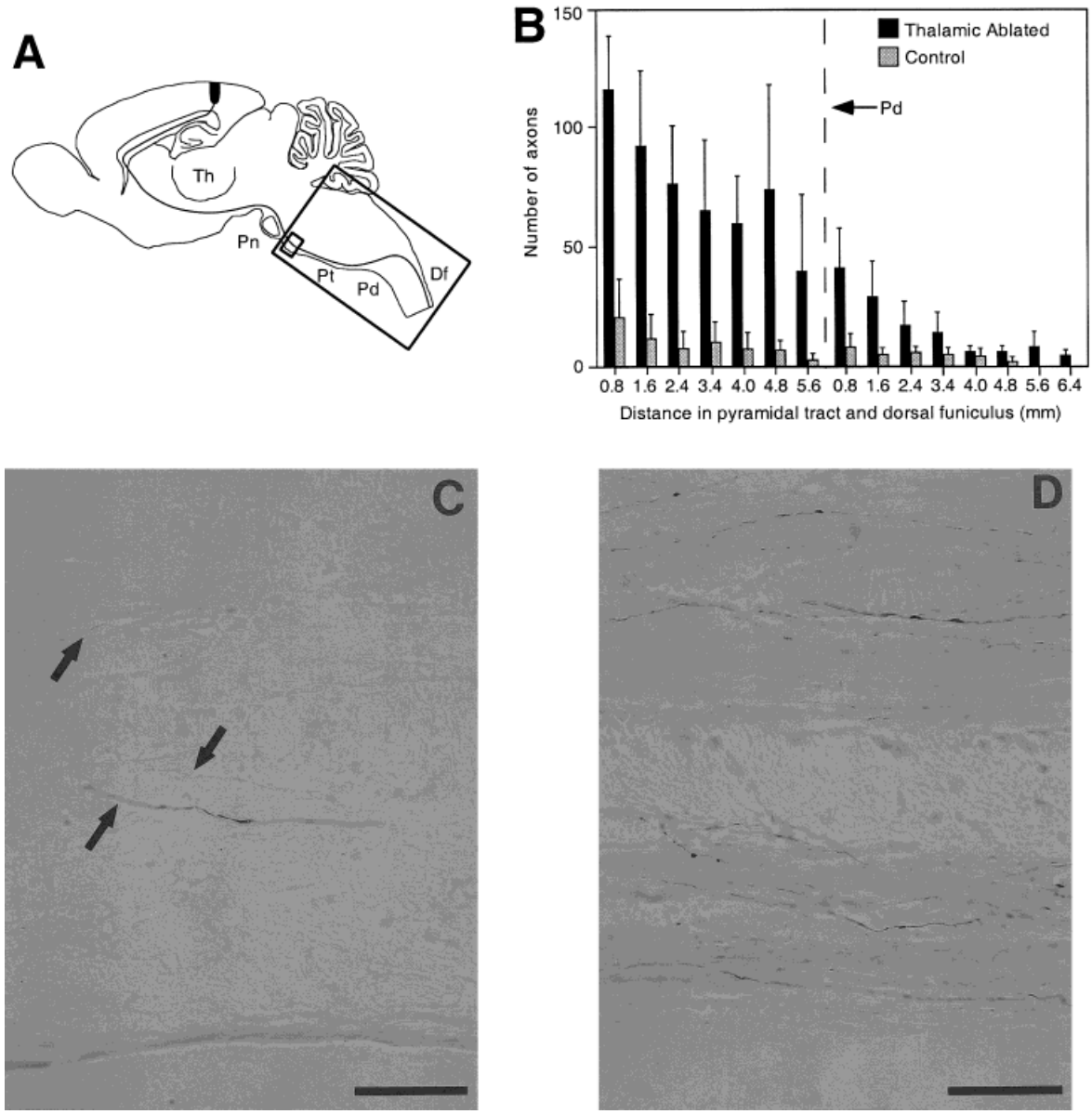


Fig. 6. **A:** Schematic of the visual corticospinal tract. The large box represents the area of concentration for quantitative analysis of visual corticospinal axons; the small box represents the location of the corticospinal axons presented in C and D. **B:** Comparison of BDA-labeled corticospinal axons in control hamsters and hamsters given neonatal visual thalamic ablations (n = 3 pairs matched for size and placement of BDA in visual cortex). Graph shows the number of axons (mean ± SEM) counted in consecutive 800 μm^2 grids in the pyramidal tract (ipsilateral to the BDA implant) and contralateral dorsal funiculus of adult animals. The pyramidal decussation is represented by a dashed line. **C,D:** Brightfield photomicrographs of BDA labeled axons in unstained sagittal sections through the rostral pyramidal tract of a P33 control hamster (C) and P33 experimental (neonatally lesioned) hamster (D) that had received BDA implants of similar size and placement in visual cortex (rostral, left; dorsal, top). Arrows in C point to the labeled corticospinal axons (in plane of focus and at slightly different depths of field) in the control animal. Df, dorsal funiculus; Pd, pyramidal decussation; Pn, pontine nucleus; Pt, pyramidal tract; Th, thalamus. Scale bars = 50 μm.

into internal and medial pontine regions may have disrupted the organization of corticopontine projections from other regions of cortex. However, it remains to be determined whether the increased projections from visual cortex overlap with these other projections or exclude them from the nucleus. In addition, it is presently unknown whether the reorganization of corticopontine connections induced by visual thalamic ablations is specific to visual cortex. Examination of corticopontine projections from non-visual cortical areas following visual thalamic ablations, as well as the examination of visual corticopontine connections following non-visual thalamic ablations, would provide insight into whether visual thalamic afferents specifically shape subcortical projections from visual cortex.

Corticospinal projections. The number of adult visual corticospinal axons in the pyramidal tract and contralateral dorsal funiculus of experimental animals was

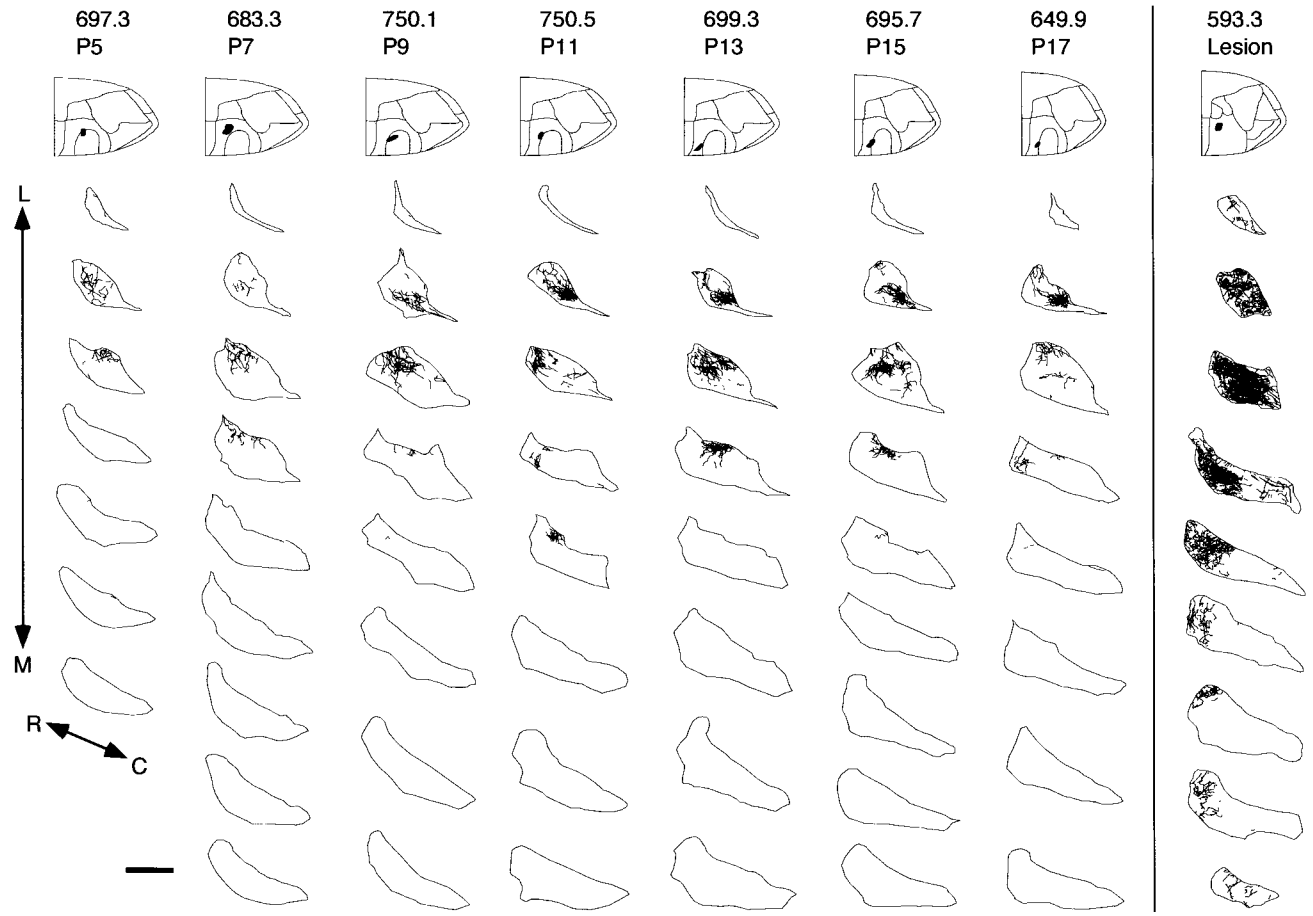


Fig. 7. Distribution of visual corticopontine projections during normal development (P5–P17), presented as in Figure 5. A representative subject from each developmental age is shown. Adult visual corticopontine axons in a neonatally lesioned animal are shown at the far right, illustrating that projections observed following thalamic ablation do not resemble projections observed during normal development. Scale bar = 400 μm.

substantially greater than that found for controls. Previous research indicates that visual corticospinal connections are not present in normal adult hamsters (O'Leary and Stanfield, 1986). However, the small number of BDA-labeled axons in our adult controls is likely due to the placement of BDA in medial V2; an area which has been shown to project sparsely to all levels of the spinal cord in adult rats (Miller, 1987). Whether the differences found between control and lesioned animals are reflected in the terminal zones of corticospinal axons (i.e., spinal gray matter) remains to be determined as only the upper cervical cord was processed here.

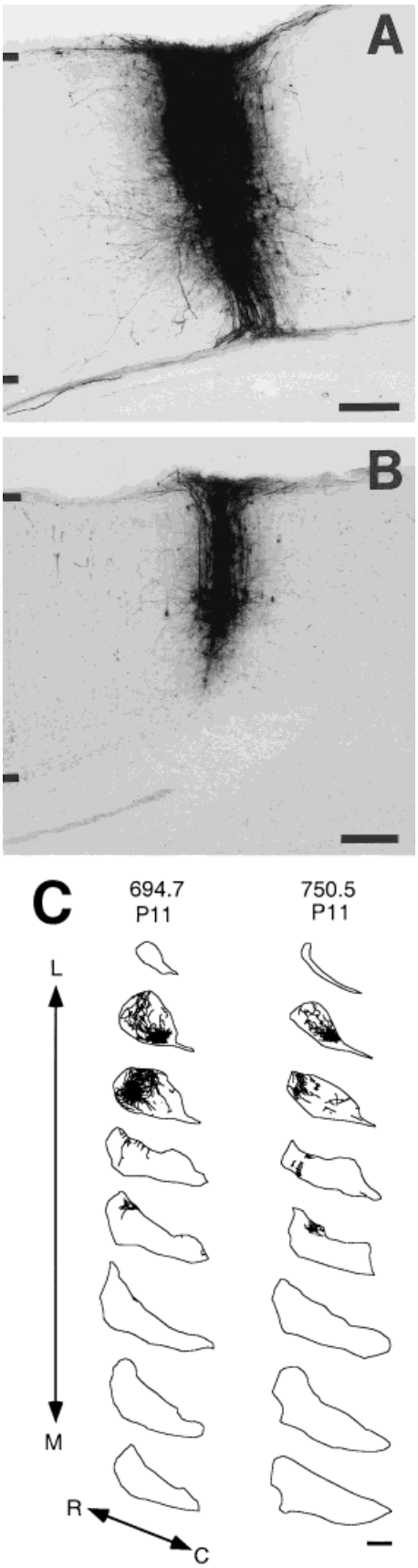
Development of corticopontine connections

Although the development of visual corticopontine connections described here is in general agreement with that described for the rat, projection refinement per se is not observed for the hamster. Both our study and those in rats demonstrate that the initial growth of visual corticopontine axons into the pons is specific to the adult projection zones in rostrolateral and caudolateral pons (Mihailoff et al., 1984; Leergaard et al., 1995). Projections within these regions also become more focused during development in both rats (Mihailoff et al., 1984) and hamsters (present study). However, whereas corticopontine projections in the rat focus through a process of constriction or fragmentation (Mihailoff et al., 1984), no such refinement of these projections was observed for the hamster. Rather, terminal fields appeared to focus only by a simple increase in axonal concentration (Fig. 9B).

Possible mechanisms of increased subcortical projections after thalamic ablation

We found no evidence to suggest that complete or partial removal of visual thalamic input stabilizes an exuberant developmental projection. No corticopontine projections were observed during development that were equal in extent or topography to the projections found in lesioned animals. Similarly, only small numbers of labeled axons were seen in the pyramidal tract beyond the pons in the animals aged P5–P17.

Although the early deafferentation of a brain area can induce axonal sprouting into the denervated area (for review see Cotman et al., 1981), such effects cannot sufficiently account for the present findings. In previous ablation studies, increases in corticopontine and corticospinal connections were produced by sprouting of corticofugal



projections from intact cortex into subcortical space that was made available by the removal of corticofugal projections originating in ablated cortex (Hicks and D'Amato, 1970; Leong and Lund, 1973; Kartje-Tillotson et al., 1986; Kuang and Kalil, 1990). In contrast, thalamic ablations in the present study directly deafferented the cortex, not subcortical targets, and the observed increases in connections projected from, rather than into, the deafferented region.

Given that thalamic ablations disrupt corticopontine address selection and that address selection is associated with activity dependent mechanisms (Goodman and Shatz, 1993), it can be proposed that the increased connectivity following ablation is due to a disruption of activity. Activity is important in shaping connectivity within the visual system (Hubel et al., 1977; Shatz and Stryker, 1978), and retinogeniculate and geniculocortical axons fail to segregate into eye-specific lamina or columns, respectively, following tetrodotoxin (TTX)-induced activity blockade (Stryker and Harris, 1986; Shatz and Stryker, 1988). Previous findings suggest that axon segregation depends on the pruning of exuberant connections via a Hebbian mechanism (termed *selectionism*; Quartz and Sejnowski, 1997). However, rather than remaining in an exuberant or frozen state as selectionism would predict, retinogeniculate, geniculocortical, and long-range visual cortical axons subjected to TTX treatment show extensive growth and/or expansion beyond their normal range, in addition to a loss of clustering and/or segregation (Sretavan et al., 1988; Antonini and Stryker, 1993; Ruthazer and Stryker, 1996). These results are consistent with the proposal that loss of activity induces axonal sprouting (Brown and Ironton, 1977; Frank, 1987) and support findings that electrical activity suppresses neurite elongation (Cohan and Kater, 1986). Thalamic-ablated animals in the present study exhibited an increase in visual corticopontine axons beyond normal projection zones and failed to develop well-defined rostral and caudal patches in lateral pons. This pattern of reorganization appears similar to that observed following TTX treatment and suggests that thalamic ablations may induce sprouting through reduction of activity.

Three alternate hypotheses may explain the increased growth and/or arborization observed in previous TTX experiments and in the present study. The first hypothesis posits that activity is instructive (Crair, 1999), and its removal therefore results in axons which no longer recognize their correct terminal zones, as evidenced by increased growth beyond terminal zones and a lack of clustering. However, evidence for an instructive role for activity may only be provided by experiments in which changing the *pattern* of activity, whereas keeping the overall *amount* of activity constant, induces subsequent changes in connectivity. Alternatively, removal of activity may simply alter cellular processes, inducing profuse ax-

Fig. 8. Large (**A**) and small (**B**) injections of biocytin in the visual cortex of normal P11 hamsters. Cortical gray matter in A and B is delineated by small lines. Dorsal is to the top. **C:** Visual corticopontine projections in P11 animals with large (left) and small (right) biocytin injections, presented as in Figure 5. Larger injections of biocytin increased the density of corticopontine label without modifying the normal projection topography. Scale bar = 200 μm.

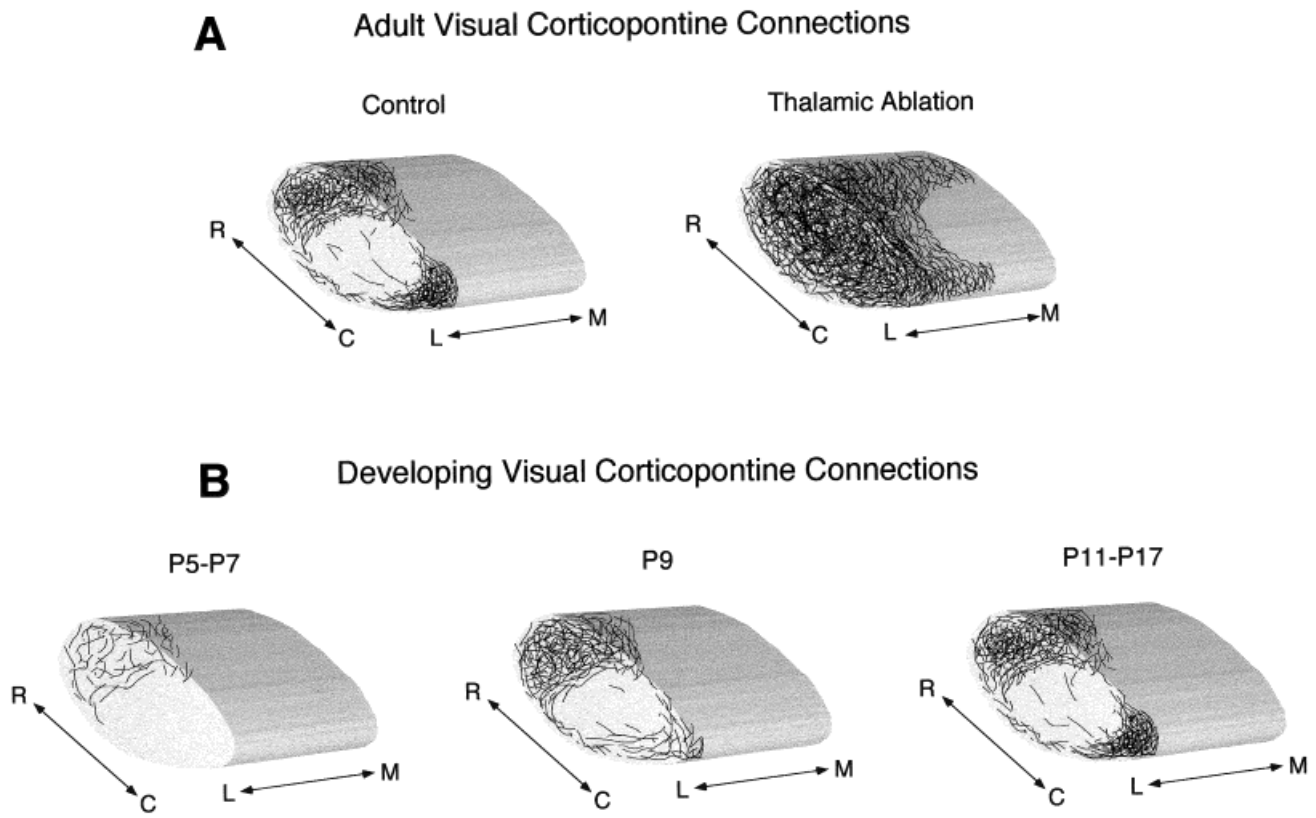


Fig. 9. Three-dimensional schematics of the pons, summarizing findings from experiment 1 (thalamic ablation study; A) and experiment 2 (developmental study; B). **A:** Visual corticopontine projections in normal adult hamsters arborize in discrete rostral and caudal patches in lateral pons (left). The topographic specificity of visual corticopontine connections is disrupted in thalamic ablated animals because of an increase in axonal arbor, both within and beyond normal arborization zones (right). **B:** During development, visual corticopontine axons grow into pontine regions specific to adult projection zones and show progressive growth within these zones. At no stage of development do visual corticopontine projections resemble those observed after neonatal thalamic ablation. Note that the typical pattern of label found for adult controls (A) is virtually identical to that found for P11–P17 animals (B).

onal growth and/or sprouting that is independent of an instructive role for activity (Frank, 1987). A third possibility is that activity patterns induce transitions from one growth stage to the next. In the corticopontine system, the pattern of visual activity relayed through thalamocortical afferents may cue axons to switch from a mode of target innervation to one of address selection (directed growth to a particular target region). With the removal of sensory information via thalamic ablations, visual corticopontine axons may remain in a stage of target innervation and thus fail to enter a stage of directed growth.

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LITERATURE CITED

Antonini A, Stryker MP. 1993. Development of individual geniculocortical arbors in cat striate cortex and effects of binocular impulse blockade. J Neurosci 13:3549–3573.

Bolz J, Novak N, Gotz M, Bonhoeffer T. 1990. Formation of target-specific neuronal projections in organotypic slice cultures from rat visual cortex. Nature 346:359–362.

Brown MC, Ironton R. 1977. Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. Nature 265:459–461.

Burne RA, Mihailoff GA, Woodward DJ. 1978. Visual corticopontine input to the paraflocculus: a combined autoradiographic and horseradish peroxidase study. Brain Res 143:139–146.

Castellani V, Bolz J. 1997. Membrane-associated molecules regulate the formation of layer-specific cortical circuits. Proc Natl Acad Sci USA 94:7030–7035.

Callaway EM, Lieber JL. 1996. Development of axonal arbors of layer 6 pyramidal neurons in ferret primary visual cortex. J Comp Neurol 376:295–305.

Castellani V, Yue Y, Gao PP, Zhou RP, Bolz J. 1998. Dual action of a ligand for Eph receptor tyrosine kinases on specific populations of axons during the development of cortical circuits. J Neurosci 18:4663–4672.

Cohan CS, Kater SB. 1986. Suppression of neurite elongation and growth cone motility by electrical activity. Science 232:1638–1640.

Cotman CW, Nieto-Sampedro M, Harris EW. 1981. Synapse replacement in the nervous system of adult vertebrates. Physiol Rev 61:684–784.

Crair MC. 1999. Neuronal activity during development: permissive or instructive? Curr Opin Neurobiol 9:88–93.

Dantzker JL, Callaway EM. 1998. The development of local, layer-specific visual cortical axons in the absence of extrinsic influences and intrinsic activity. J Neurosci 18:4145–4154.

Ding S-D, Elberger AJ. 1995. A modification of biotinylated dextran amine histochemistry for labeling the developing mammalian brain. J Neurosci Methods 57:67–75.

Duffy KR, Murphy KM, Jones DG. 1998. Analysis of the postnatal growth of visual cortex. Vis Neurosci 15:831–839.

Ebrahimi-Gaillard A, Guitet J, Garnier C, Roger M. 1994. Topographic distribution of efferent fibers originating from homotopic or heterotopic transplants: heterotopically transplanted neurons retain some of the developmental characteristics corresponding to their site of origin. Brain Res Dev Brain Res 77:271–283.

Frank E. 1987. The influence of neuronal activity on patterns of synaptic connections. Trends Neurosci 10:188–190.

Frappe I, Roger M, Gaillard A. 1999. Transplants of fetal frontal cortex grafted into the occipital cortex of newborn rats receive a substantial thalamic input from nuclei normally projecting to the frontal cortex. Neurosci 89:409–421.

Friedlander MJ, Martin KAC. 1989. Development of Y-axon innervation in area 18 of the cat. J Physiol (London) 416:183–213.

Goodman CS, Shatz CJ. 1993. Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell 72(Suppl):77–98.

Heffner CD, Lumsden AGS, O'Leary DDM. 1990. Target control of collateral extension and directional axon growth in the mammalian brain. Science 247:217–220.

Hicks SP, D'Amato CJ. 1970. Motor-sensory and visual behavior after hemispherectomy in newborn and mature rats. Exp Neurol 29:416–438.

Hubel DH, Wiesel TN, LeVay S. 1977. Plasticity of ocular dominance columns in monkey striate cortex. Phil Trans R Soc Lond [Biol] 278: 377–409.

Jensen KF, Killackey HP. 1984. Subcortical projections from ectopic neocortical neurons. Proc Natl Acad Sci USA 81:964–968.

Joosten EAJ, Gispen WH, Bar PR. 1994. Tropism and corticospinal target selection in the rat. Neurosci 59:33–41.

Kartje-Tillotson G, Neafsey EJ, Castro AJ. 1986. Topography of corticopontine remodelling after cortical lesions in newborn rats. J Comp Neurol 250:206–214.

Kingsbury MA, Clancy B, Finlay BL. 1998. Development of interhemispheric axonal connections in neonatal hamster isocortex. Soc Neurosci Abstr 24:58.

Kuang RZ, Kalil K. 1990. Specificity of corticospinal axon arbors sprouting into denervated contralateral spinal cord. J Comp Neurol 302:461–472.

Leergaard TB, Lakke AJF, Bjaalie JG. 1995. Topographical organization in the early postnatal corticopontine projection: a carbocyanine dye and 3-D computer reconstruction study in the rat. J Comp Neurol 361:77–94.

Lent R. 1982. The organization of subcortical projections of the hamster's visual cortex. J Comp Neurol 206:227–242.

Leong SK, Lund RD. 1973. Anomalous bilateral corticofugal pathways in albino rats after neonatal lesions. Brain Res 62:218–221.

Lowel S, Singer W. 1992. Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. Science 255:209–212.

McConnell SK, Kaznowski CE. 1991. Cell cycle dependence of laminar determination in developing neocortex. Science 254:285.

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

Mihailoff GA, Adams CE, Woodward DJ. 1984. An autoradiographic study of the postnatal development of sensorimotor and visual components of the corticopontine system. J Comp Neurol 222:116–127.

Miller B, Chou L, Finlay BL. 1993. The early development of thalamocortical and corticothalamic projections. J Comp Neurol 335:16–41.

Miller MW. 1987. The origin of corticospinal projection neurons in rat. Exp Brain Res 67:339–351.

Molnar Z, Blakemore C. 1991. Lack of regional specificity for connections formed between thalamus and cortex in co-culture. Nature 351:475–477.

O'Leary DDM, Stanfield BB. 1986. A transient pyramidal tract projection from the visual cortex in the hamster and its removal by selective collateral elimination. Brain Res Dev Brain Res 27:87–99.

O'Leary DDM, Stanfield BB. 1989. Selective elimination of axons extended by developing cortical neurons is dependent on regional locale: experiments utilizing fetal cortical transplants. J Neurosci 9:2230–2246.

O'Leary DDM, Bicknese AR, De Carlos JA, Heffner CD, Koester SE, Kutka LJ, Terashima T. 1990. Target selection by cortical axons: alternative mechanisms to establish axonal connections in the developing brain. Cold Spring Harb Symp Quant Biol 55:453–468.

O'Leary DDM, Schlaggar BL, Stanfield BB. 1992. The specification of sensory cortex: lessons from cortical transplantation. Exp Neurol 115: 121–126.

Quartz SR, Sejnowski TJ. 1997. The neural basis of cognitive development: a constructivist manifesto. Behav Brain Sci 20:537–596.

Richards LJ, Koester SE, Tuttle R, OLeary DDM. 1997. Directed growth of early cortical axons is influenced by a chemoattractant released from an intermediate target. J Neurosci 17:2445–2458.

Ruthazer ES, Stryker MP. 1996. The role of activity in the development of long-range horizontal connections in area 17 of the ferret. J Neurosci 16:7253–7269.

Sato M, Lopez-Mascaraque L, Heffner CD, O'Leary DDM. 1994. Action of a diffusible target-derived chemoattractant on cortical axon branch induction and directed growth. Neuron 13:791–803.

Schlaggar BL, O'Leary DDM. 1991. Potential of visual cortex to develop an array of functional units unique to somatosensory cortex. Science 252: 1156–1560.

Shatz CJ, Stryker MP. 1978. Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J Physiol (London) 281:267–283.

Shatz CJ, Stryker MP. 1988. Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. Science 242:87–89.

Sretavan DW, Shatz CJ, Stryker MP. 1988. Modification of retinal ganglion cell axon morphology by prenatal infusion of tetrodotoxin. Nature 336:468–471.

Stanfield BB, O'Leary DDM. 1985. Fetal occipital cortical neurones transplanted to the rostral cortex can extend and maintain a pyramidal tract axon. Nature 313:135–137.

Stanfield BB, O'Leary DDM, Fricks C. 1982. Selective collateral elimination in early postnatal development restricts cortical distribution of rat pyramidal tract neurones. Nature 298:371–373.

Stryker MP, Harris WA. 1986. Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J Neurosci 6:2117–2133.

Terashima T. 1995. Course and collaterals of corticospinal fibers arising from the sensorimotor cortex of the reeler mouse. Dev Neurosci 17:8–19.

Wiesendanger R, Wiesendanger M. 1982. The corticopontine system in the rat. II. The projection pattern. J Comp Neurol 208:227–238.

Windrem MS, Finlay BL. 1991. Thalamic ablations and neocortical development: alterations of cortical cytoarchitecture and cell number. Cereb Cortex 1:230–240.