Postnatal Development of the Pontine Projections from the Visual Cortex of the Mouse

By

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Summary: We studied the postnatal development of the corticopontine tract in mice by the injection of the axon tracer DiI into the visual cortex. In the postnatal day (P) 0.5 mouse, labeled pyramidal tract fibers pass through the internal capsule and cerebral peduncle, grow over the basilar pontine gray, and enter into the medullary pyramid (in this study, P0 refers to the first 24 hours after birth). Small collateral branches arise from these pyramidal tract fibers on P0.5–1.0, and elongate quickly into the basilar pontine gray around P2–4. These collateral branches give off many secondary branches on P4 and form the bright terminal zone in the rostral portion of the lateral basilar pontine gray on P9. In the P16 mouse, this terminal zone is more restricted, suggesting, on the basis of the anterograde DiI labeling technique, that the visual corticopontine projection matures by P16. DiI-labeled pyramidal tract fibers distal to the branching point of the pontine collaterals are found during the postnatal two weeks, but disappear by the latter stages. We conclude that the visual corticopontine tract develops as collateral branches of the transient pyramidal tract fibers arising from the visual cortex of the mouse, as just described in the rat (O’Leary and Terashima, Neuron 1:901-910, 1988).

Timing of innervation is a key factor in the development of the basilar pontine gray. In fact, the time of afferent arrival from the cerebral cortex is closely correlated in time with the development of neurons in the basilar pontine gray and the outgrowth of pontocerebellar axons (King et al., 1987). However, observations concerning the development of the corticopontine projection of the mouse have not been reported elsewhere. The mouse has a great advantages for understanding the mechanisms leading to the high degree of specificity of neural connectivity, because more than 100 mutations affecting the nervous system of the mouse are known (Sidman et al., 1985). Accordingly, the present study was undertaken to illustrate accurately the development of the corticopontine tract during postnatal periods of the normal mouse, which allow the future investigator to analyze morphogenetic mechanisms deranged by the mutations in the genetic loci of the neurological mutant mouse.

Materials and Methods

Adult mice and litters of the C57BL/6J strain were obtained from our breeding colony. Postnatal day (P) 0 refers to the first 24 hours after birth. We used the anterogradely transported fluorescent dye DiI for the pups. This fluorescent dye works very well in the developing brain (Simon and O’Leary, 1990; Thanos and Bonhoeffer, 1987), but not in the adult brain. Thus, the anterograde horseradish peroxidase (HRP) method instead of the DiI method was used to trace corticopontine fibers of the adult mouse.

(1) HRP method

Six adult mice at 2–3 months of age were used for HRP histochemistry. They were anesthetized with chloral hydrate (3.5 mg/10 g body weight, intraperitoneal injection) and clamped in a stereotactic apparatus. After the scalp had been retracted, a small burr hole was made directly over the primary visual cortex under visual guidance, using the map of Caviness (1975). In all experiments, 0.01–0.04 μl of a 30% aqueous solution of HRP dissolved in distilled water was injected into the bilateral primary visual cortex under an operating microscope. After 48 hours, the animals were perfused transcardially with a solution of 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, the brains were placed in the same fixative containing 10% sucrose for
12 hours, and then in a solution of phosphate buffered 30% sucrose (pH 7.4) for 36–48 hours. The brains were sagittally sectioned at a thickness of 70 μm with a freezing microtome. All sections were treated with diaminobenzidine (DAB) according to the method of LaVail et al. (1973) and tetramethyl benzidine (TMB) according to the method of Mesulam (1978). After incubation, sections were mounted on gelatin-coated slides and counterstained with 0.05% toluidine blue. Drawings were made directly on section paper with the aid of a net ocular micrometer (10 x 10 mesh, 1 x 1 mm in width) inserted into the ocular lens of an Olympus microscope. The original large drawings at x 2000 were reduced photographically.

(2) DiI labeling procedure
To examine the formation of collateral projections from the cortex to the basilar pontine gray, the fluorescent dye DiI (Molecular Probes; Eugene, OR, USA) was used as an anterograde axonal tracer in mice of ages between P0 and P28. Ages and numbers of animals injected with DiI in the visual cortex and killed after various time are shown in Table 1. Pups at P0–7 were anesthetized by hypothermia, and chloral hydrate was used for older pups (3.5 mg/10 g body weight). A small burr hole was made as described for the HRP method. In all experiments, a 0.001–0.002 μl of a 30% solution of DiI dissolved in dimethylformamide (Sigma; St. Louis, MO, USA) was bilaterally injected into the primary visual cortex under an operating microscope. After 8–72 hours, the animals were perfused transcardially with a solution of 4% paraformaldehyde in phosphate buffer (pH 7.4). Immediately after perfusion, the brains were placed in the same solution for 12–24 hours (4°C) and sagittally sectioned at a thickness of 50–100 μm with a microslicer (Dosaka E. M.; Kyoto, Japan). Sections were collected in phosphate buffer and photographed on a fluorescent microscope equipped with an RITC filter.

(3) Basilar pontine cytoarchitectonics
For descriptive purposes, the basilar pontine gray of the mouse was subdivided. Like the rat (Mihailoff et al., 1981), the mouse basilar pontine gray in Nisslstained frozen sections consists of four subdivisions referred to as lateral, ventral, medial and peripeduncular nuclei with respect to their position relative to the cerebral peduncle (Fig. 1). The peripeduncular pontine nucleus is further divided into two subdivisions, dorsal and ventral peripeduncular pontine nuclei.

Results

(1) Experiments using HRP as axonal marker
Sites of injection of HRP determined by DAB were localized in the primary visual cortex (Fig. 2D). After the injection of HRP, anterogradely labeled terminals were observed in the rostral two thirds of the lateral pontine nucleus (Fig. 2A; Fig. 3A, B). These HRP-filled terminals were also rostrally distributed in the sagittal section through the ventral pontine nucleus (Fig. 2C, D; Fig. 3C, D, E). Anterogradely labeled terminals were also localized in the dorsal peripeduncular nucleus just above the cerebral peduncle in the sagittal section through the ventral pontine nucleus (Fig. 3D). Small non-labeled spots were scattered within these HRP-labeled terminals (for example, see Fig. 2B).

(2) Experiments using DiI as axonal marker
DiI was injected into the primary visual cortex (Fig. 4). DiI-labeled axons were observed in the pyramidal tract over the basilar pontine gray on P0.5 (Fig. 5A). The caudal end of the labeled axons reached the rostral margin of the medullary pyramid on P0.5. Therefore, these labeled axons were considered as the pyramidal tract fibers. At this stage, DiI-labeled growth cones were found along the pyramidal tract from the junction of the cerebral peduncle and the pons to the rostral margin of the medullary pyramid (e.g., see white arrow in Fig. 5A). On P0.5–1.0, short collateral branches budded from the ventral side of the parent pyramidal tract fibers at a right angle (Fig. 5B; asterisks) toward the basilar pontine gray, and elongated into the ventral margin of the rostral portion of the basilar pontine gray on P2 (Fig. 6A). Thus, the parent pyramidal tract fiber and its collateral takes a T-form shape (Fig. 6A; inset). Pontine collaterals took a slightly tortuous course, bore

### Table 1. Ages and Numbers of Animals Sacrificed after Injection of DiI into the Visual Cortex

<table>
<thead>
<tr>
<th>Age*</th>
<th>Number</th>
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<tbody>
<tr>
<td>P0.5</td>
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<tr>
<td>P1</td>
<td>10</td>
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<td>P2</td>
<td>12</td>
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<td>P3</td>
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<tr>
<td>Total</td>
<td>96</td>
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*In this study, postnatal day (P) 0 refers to the first 24 hours after birth.
nodular protuberances on their sides, and ended in a beaded terminal which did not have a growth cone-like morphology. At later stages, pontine collaterals increased in length and in the complicated axonal arbors in the rostral portion of the lateral and ventral pontine nuclei, as seen on P4 (Fig. 6B).

The caudal ends of the pyramidal tract fibers entered the pyramidal decussation by around P2 (data is not shown). After P2, anterogradely labeled axons were observed along the medullary pyramidal tract during the first two postnatal weeks (Fig. 7). At later stages, these distal segments of pyramidal tract fibers caudal to the branching point of the pontine collaterals began to retract. After P9, the brightness of the fluorescence within the distal segment of the pyramidal tract fibers was decreased, and no labeling was seen in the motor decussation. After P16, no labeling could be seen in the pyramidal tract caudal to the pons, suggesting that all of the pyramidal tract fibers distal to the pons completely disappeared by P16 (Fig. 8A). With this elimination of pyramidal tract fibers, L-form pontine collaterals were found among T-form pontine collaterals in the pyramidal tract just above the basilar pons of the P9 animal (Fig. 9), but all of the pontine collaterals appeared to take an L-form shape by P16, suggesting that the L-form collaterals were made by elimination of pyramidal tract fibers distal to the branching point of the pontine collaterals. At the age of P16, DiI-labeled axonal arborizations were present in the small area of the basilar pontine gray (Fig. 8B).

In summary, on P0.5–1.0, the visual corticopontine fibers of the mouse develop as collaterals of the pyramidal tract fibers, which lose their spinal projections during the second postnatal week (Fig. 10).

**Discussion**

This HRP study revealed that the pontine projection of the visual cortex of the adult mouse terminates in the rostral portion of the lateral and ventral pontine nuclei. Although the projection from the visual cortex to the basilar pontine gray has been established in many mammals, for example, monkeys (Glickstein et al., 1980), cats (Brodal, 1972; Bjaalie, 1985, 1986; Keizer et al., 1987), rabbits (Distel and Hollander, 1980) and rats (Mihailoff et al., 1978; Wiesendanger and Wiesendanger, 1982), a study of this tract of the mouse has not been published elsewhere. These findings on the pontine projection from the visual cortex of the adult mouse are quite similar to those on the rat (Wiesendanger and Wiesendanger, 1982). Wiesendanger and Wiesendanger (1982) claimed that the visual cortex of the rat projects the rostral portion of the lateral basilar pons (compare Figure 5 in their paper with Figure 3 in the present paper). The distribution pattern of the visual corticopontine tract of the mouse is also similar to that previously found in monkeys. In this species, the occipital cortex projects the lateral part of the basilar pontine gray (Nyby and Jansen, 1951; Brodal, 1978). Because the motor corticopontine projections of the mouse end in the medial column of the basilar pons (Inoue et al., unpublished data), there is a medial-to-lateral succession of the pontine projection from the motor and visual cortices in the mouse. Thus, the major topological principle which determines the corticopontine organization in the mouse is similar to that previously described for the monkey (Nyby and Jansen, 1951; Brodal, 1978) and the rat (Wiesendanger and Wiesendanger, 1982).

The present study reveals that visual cortical neurons lose their pyramidal tract projections during the first few weeks of life. Stanfield et al. (1982) examined the distribution of neurons of the origin of the pyramidal tract during the postnatal development of the rat neocortex by a long-term dye procedure in which True Blue is injected on P2 into the pyramidal decussation and the rat is killed 3 weeks later. They succeeded in demonstrating that layer 5 cells in the occipital cortex of the rat lose their spinal projections during the early postnatal days. Their finding coincides with the decrease in the number of axons in the pyramidal tract during the early postnatal development in the hamster (Reh and Kalil, 1982). The double retrograde fluorescence method confirms that the decrease in the number of pyramidal tract fibers is due to selected loss of the spinal projection of the layer 5 neurons in the visual cortex of the rat (O’Leary and Stanfield, 1985). This phenomenon called collateral elimination, is also anterogradely demonstrated by the injection of WGA-HRP into the visual cortex of the hamster (O’Leary and Stanfield, 1986) or the injection of DiI into the visual cortex of the rat (O’Leary and Terashima, 1988). The present study shows that the spinal projection of the layer 5 neuron in the visual cortex of the mouse is eliminated during the postnatal days, suggesting that such a postnatal reorganization of the cortical projection is shared by all species of rodents.

This study of the development of the visual corticopontine projections labeled by DiI confirms that pontine projections arising from the visual cortex develop as the collaterals of the pyramidal tract fibers. Therefore, the development of the pontine projection of the visual cortex of the mouse is quite similar to that of the rat (O’Leary and Terashima, 1988). However, there is a small discrepancy between these two species. In rats, the first signs of collateral development are seen early on P2. In mice, collateral budding from the visual pyramidal tract occurs on P0.5–1.0. Except for this, the time schedule of the formation of the corticopontine projection of the mouse is quite similar to that of the rat.
References


Abbreviations

cp cerebral peduncle
CPu caudate-putamen
dP dorsal peripeduncular nucleus
FL forelimb area of cortex
Fr1 frontal cortex, area 1
GP globus pallidus
HL hindlimb area of cortex
ic internal capsule
io inferior olivary complex
LI lateral lemniscus
ML medial lemniscus
MP medial pontine nucleus
Oc1 occipital cortex, area 1
Oc2 occipital cortex, area 2
P postnatal day
Pn basilar pontine gray
PRh perirhinal cortex
Py pyramidal tract
RTg reticulotegmental nucleus of pons
TZ nucleus of trapezoid body
tz trapezoid body
VP ventral pontine nucleus

Plate I

Fig. 1. Parasagittal sections through the basilar pontine gray. A, The rectangle indicates the basilar pontine gray of the adult mouse focused on in this study. Rostral is to the left; dorsal to the top. B-D, Three parasagittal sections through the lateral pontine nucleus (B), the ventral pontine nucleus (C) and the medial pontine nucleus (D). Cytoarchitecture is not different among these three pontine nuclei. Scale bars: 1 mm (A); 0.2 mm (B, C and D). Frozen sections counterstained with toluidine blue.
Plate III

Fig. 3. Schematic illustrations showing the distribution of HRP-labeled corticopontine terminals in the basilar pontine gray after the injection of HRP into the primary visual cortex of the adult mouse. The five parasagittal sections (A-E) are arranged in lateral (A) to medial (E) sequence. The labeled terminals are seen in the rostral two-thirds of the lateral pontine nucleus (A and B), and the rostral one-third of the ventral pontine nucleus. The injection site is centered in the primary visual cortex (F).

Plate II

Fig. 2. Labeled terminals found in the lateral (A), ventral (B) and medial (C) pontine nuclei after the injection of HRP into the primary visual cortex (D) of the adult mouse. The two arrowheads in D indicate the border of the primary visual cortex. Scale bars: 0.2 mm (A, B and C); 1 mm (D). TMB method.
Fig. 4. Representative Dil injection into the primary visual cortex of a P1 mouse. The injection site is indicated by the large white arrow in A and large black arrows in B and C. The two white arrowheads in A border the primary visual cortex. These margins of the primary visual cortex are demonstrated by counterstaining the same section (B and C). The two black arrowheads in B and C indicate the margin of the primary visual cortex. Scale bars: 1 mm (A and B); 0.5 mm in C.
Fig. 5. Dil-labeled fibers in the pyramidal tract above the basilar pontine gray of a P0.5 mouse. A: Growth cones of the pyramidal tract fibers are labeled (white arrow). B: Short collateral branches (asterisks) arise from the parent pyramidal tract axons and project ventrally into the basilar pontine gray. Scale bars: 100 μm.
Fig. 6. Collateral branches arising from the parent pyramidal tract axons and elongating into the ventral margin of the rostral area of the basilar pontine gray. A: P2 mouse. The inset shows the branching point (large white arrow). B: P4 mouse. These collateral branches give off secondary and tertiary branches. Pyramidal tract fibers distal to the branching point of the pontine collaterals are also labeled by Dil. Scale bars: 100 μm.
Fig. 7. Dil-labeled pyramidal tract axons in a P6 mouse. The axons give off pontine collaterals at the rostral basilar pons and enter the medullary pyramid. Scale bar: 0.2 mm.
Fig. 8. Absence of Dil-labeled pyramidal tract axons distal to the basilar pontine gray in a P16 mouse (A) and a P30 mouse (B). Their disappearance suggest that the corticopontine tract matures by P16. Scale bars: 100 μm.
Plate IX

Fig. 9. Pontine projections in the pyramidal tract over the rostral basilar pons of a P9 mouse. An L-form pontine projection (B) is found among T-form pontine projections (A). The distal portions of pyramidal tract fibers are still observed (white arrow in C). The asterisk in C marks an original terminal field of the corticopontine projection. Scale bars: 100 μm (A); 50 μm (C).
Plate X

Fig. 10. Schematic illustration of the development of the corticopontine fibers arising from the visual cortex. On P0.5-1 small collateral buddings arise on the pyramidal tract fibers. These corticopontine collaterals form an original terminal field in the rostral area of the basilar pontine gray. Parent pyramidal tract fibers lose their spinal axons during the first two postnatal weeks. Visual corticopontine fibers mature by P16, as demonstrated by the Dil technique.