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A target-derived chemoattractant controls the development of the corticopontine projection by a novel mechanism of axon targeting

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Summary

Here, we review our studies in rats of target recognition by developing cortical axons focusing on their innervation of the basilar pons, a major hindbrain target. The corticopontine projection develops by a 'delayed interstitial budding' of collaterals from layer 5 corticospinal axons, rather than by a direct ingrowth of primary axons or by bifurcation of the growth cone. Branches form *de novo* from the axon cylinder in the pathway overlying the basilar pons and extend directly into it. Co-cultures of cortex and basilar pons in 3-dimensional collagen matrices show that a diffusible chemotropic signal released by the basilar pons directs the growth of collateral branches from layer 5 axons in a target and neuron specific manner. 'Delayed' co-cultures suggest that a diffusible, pontine-derived signal also initiates the selective branching of layer 5 axons. *In vivo* experiments support this chemotropic mechanism. First, corticospinal axons form collateral branches at novel locations directly over ectopic aggregations of basilar pontine neurons induced by X-irradiation; no branches form at positions that would normally overlie the appropriate region of basilar pons which is absent because of the X-irradiation. Thus, the basilar pons, rather than local cues in the axon pathway, appears to control the location of corticospinal axon branching. Second, in a series of experiments in which different subsets of corticospinal axons are prevented from innervating the basilar pons, remaining corticospinal axons extend collaterals in a directed manner to regions of the basilar pons deprived of cortical input, a behavior consistent with a response to a diffusible chemoattractant emanating from these regions. In conclusion, our findings suggest that a diffusible, target-derived chemotropic molecule(s) underlies target recognition in this developing system by initiating the formation and directing the growth of pontine collateral branches of primary layer 5 corticospinal axons.

Key words: axon targeting, chemotropism, collateral branching, corticospinal axons, growth cones, target recognition.

Introduction

Unraveling the mechanisms that control the formation of specific axonal connections in the developing nervous system has long piqued the interest of neurobiologists (see Cajal, 1911; Purves and Lichtman, 1985; Jacobson, 1991). The development of axonal connections involves several distinct phases, but none more crucial than target recognition, until recently a process considered to be carried out exclusively by the axonal growth cone (for review see Dodd and Jessell, 1988). Here, we consider our studies of this process in the developing corticopontine projection, a major cortical projection formed by axons arising from layer 5 neurons distributed across most areas of the neocortex (Wiesendanger and Wiesendanger, 1982; Jones, 1984). In the sensorimotor cortex of adult mammals, the corticopontine and corticospinal projections are collateral projections of the same layer 5 neurons (Cajal, 1911; Endo *et al.* 1973; Ugolini and Kuypers, 1986). For other cortical regions in the adult, for example, the visual cortex, layer 5 neurons do not have a corticospinal projection and the corticopontine projection is their most distal connection; during development, though, the same neurons transiently form both projections, but later lose that to the spinal cord (O'Leary and Stanfield, 1985). These features of the corticopontine projection make it an especially well suited model for studies of target recognition since the formation of multiple collateral projections to distant, widely separated targets is a common feature of the vertebrate nervous system (see, for example, Cajal, 1894).

The growth cone of layer 5 axons plays no role in the selection of the basilar pons as a target

As summarized in Fig. 1, we find that the corticopon-

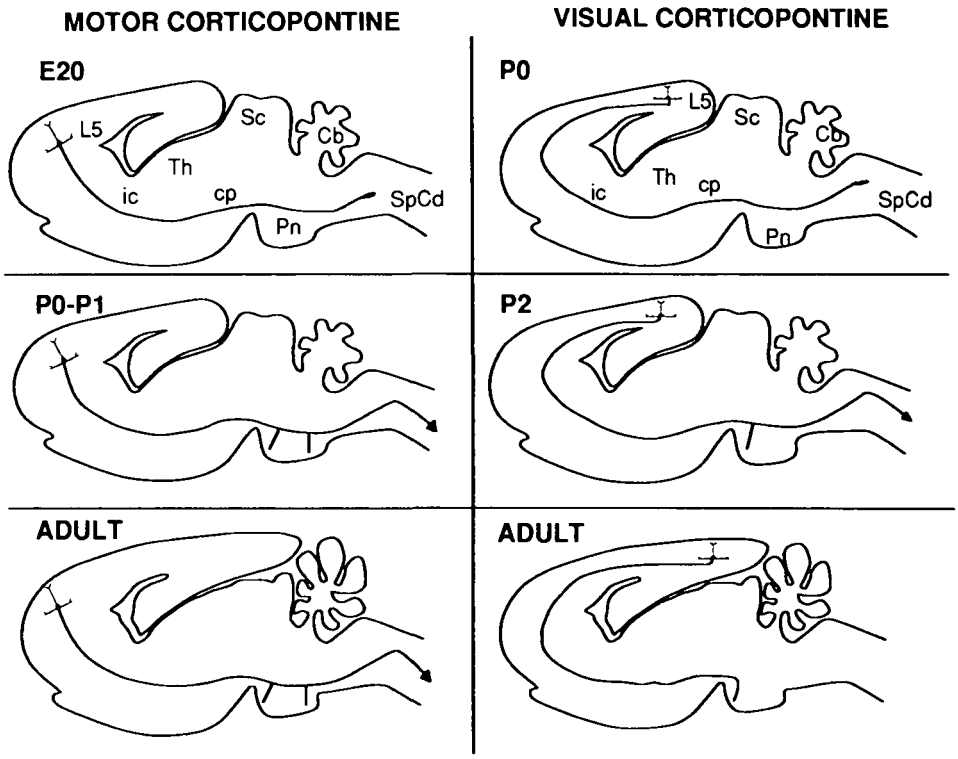

Fig. 1. The corticopontine projection develops by a delayed interstitial budding of collateral branches from layer 5 corticospinal axons (O'Leary and Terashima, 1988). Sagittal views of the rat brain show the spinally directed trajectory of developing layer 5 axons from motor cortex (left panels) and visual cortex (right panels), and the time and location of the extension of collateral branches into the basilar pons (Pn). Axons arise from layer 5 neurons, leave cortex *via* the internal capsule (ic), and continue a subcortical trajectory through the cerebral peduncle (cp) and pyramidal tract enroute to the spinal cord (SpCd). The primary axons reach the pons on embryonic day (E) 20 (motor) or postnatal day (P) 0 (visual) but grow caudally past it. About 2 days later, branches bud from the primary axons at positions in the axon tract directly overlying the basilar pons. Motor axons form a caudal branch on P0 and a rostral branch on P1; visual axons form only a rostral branch which buds on P2. The spinal segment of the primary layer 5 axons from visual cortex is later eliminated caudal to the permanent collateral branch that innervates the basilar pons; layer 5 neurons in motor cortex retain both the primary spinal axon and its pontine collaterals. Rostral is to the left, caudal to the right, ventral to the bottom. Other abbreviations: Sc, superior colliculus; Th, thalamus; Cb, cerebellum.

tine projection develops from pre-existing corticospinal axons by a delayed budding of collateral branches from the axon cylinder rather than through a direct ingrowth of the primary axons or of branches formed by the bifurcation of their growth cones (O'Leary and Terashima, 1988). Layer 5 axons extend out of cortex *via* the internal capsule, grow caudally through the cerebral peduncle, pass over the basilar pons, and continue through the pyramidal tract toward the spinal cord. About 2 days later the first overt signs of collateral budding become apparent, at which time layer 5 axons are still elongating and their growth cones are found 4 mm or more caudal to the pons. The examination of static images of DiI-labeled axons at sequential developmental stages shows that pontine collaterals bud *de novo* from smooth segments of cortical axons rather than develop from filopodia left behind by the primary growth cone. Collateral branches bud from the primary axons at stereotypic locations in the axon tract overlying the basilar pons and grow directly into it; the timing and location of axon branching differs with the cortical region supplying the layer 5 axons. The timing of collateral branching does not seem to relate to any specific behavior of the primary growth cones – for instance, a lull in axon extension, or reaching a certain point in the pathway. The corticopontine projection develops by this mechanism of 'delayed interstitial branching' regardless of whether the segment of the primary layer 5 axon beyond the basilar pons forms a permanent spinal projection, as for motor cortex, or is later eliminated, as for visual cortex. Therefore, the growth cones of layer 5 axons have a limited role in the development of the corticopontine projection: they take layer 5 axons to the vicinity of the basilar pons, but do not participate in establishing the definitive connection.

Our observations imply that the growth cones of primary layer 5 axons fail to recognize the basilar pons as a target appropriate for cortical innervation. However, we can infer that cues do mark the basilar pons as an appropriate target, since collateral branches do not form randomly but rather develop at specific locations in the axon tract (O'Leary and Terashima, 1988; O'Leary *et al.* 1990). Why, then, do the growth cones of primary layer 5 axons fail to respond to these cues? They may have a greater affinity for the environment of the axonal pathway over the targeting cues, or may be elongating too rapidly to be adequately influenced, or possibly the cues have yet to develop when the axons pass over the basilar pons. Alternatively, only the axon cylinder proximal to the growth cone, and not the growth cone itself, may be receptive to the cues.

In vitro evidence that a diffusible, pontine-derived chemotropic signal initiates and directs the growth of cortical axon branches

Our *in vivo* observations show that the selection of the

basilar pons as a target appropriate for cortical innervation is not a responsibility of the growth cone of cortical axons (O'Leary and Terashima, 1988). Thus, the process of target recognition in this system poses unique problems to be resolved. It is necessary to determine not only the cues that provide the directional guidance of pontine collateral branches to their target, the basilar pons, but also to identify the cues that mark branch points and initiate interstitial branching. The latter event can be considered as the act of target recognition. The cues that govern the budding and directed growth of pontine collaterals likely fall into one of two classes: local or diffusible. Local cues, either molecular or structural, might develop selectively in the region of the axon tract overlying the basilar pons and come in contact with cortical axons. Alternatively, a molecular signal released by the basilar pons might diffuse into the overlying axon tract and interact with cortical axons, either directly or indirectly, to induce interstitial branching and direct collateral extension.

An established method to dissociate the action of diffusible and local cues is to co-culture neuronal populations with explants of target and control tissue at a distance in 3-dimensional collagen matrices (Lumsden and Davies, 1983, 1986). In this context, any possible action of potential local cues is excluded since the explants do not contact one another; axon outgrowth consistently directed across the intervening matrix toward a target explant would require that the axons are responding to a diffusible chemotropic signal released by the target. When testing for chemotropic activities, it is important to distinguish directed axon outgrowth from selective axon stabilization. For example, the observation of axons directed to a target explant after long periods of co-culturing could result from an initially non-directed pattern of outgrowth, through the preferential survival of axons that contact the target explant. Therefore, it is necessary to make observations as axons are actively growing through the matrix.

To test the hypothesis that a pontine-derived chemoattractant controls the directed growth of collateral branches of layer 5 cortical axons, explants of cortex, extending from the pial surface to the ventricle, were taken from motor and visual cortical areas and cultured alone or co-cultured with explants of basilar pons and a control tissue (hypothalamus, olfactory bulb and pieces of cortex) for 24 to 48 h (Heffner *et al.* 1990). Explants were taken from P0 or P1 rats, the time at which pontine collaterals form *in vivo* (O'Leary and Terashima, 1988). The pontine and control explants were positioned on opposite sides of the cortical explant at a distance of 150–300 μm (Fig. 2). Phase-contrast observations of the cultures were made over the first 48 h *in vitro*, a period during which cortical axons are actively growing.

Observations of cortex cultured alone in a collagen matrix reveal that axon outgrowth is predominantly from the ventricular surface, with some growth extending from the sides of the explant; most axons grow in an inferior direction (that is, take a trajectory roughly parallel to the pial to ventricular axis of the cortical explant). However, in co-cultures, axon outgrowth is enhanced from the side of the cortical explant facing the basilar pons. Whereas axons emanating from cortical explants tend to grow in an inferior direction, the outgrowth of cortical axons on the pontine side is highly directed toward the pontine explant indicating a tropic response. The results obtained with explants of motor and visual cortex, and with different regions of basilar pons (e.g. rostral or caudal), were comparable. Quantitative measurements of actively growing axons, judged by the presence of obvious growth cones, support our qualitative observations. First, cortical axons (n=2564) were scored for a trajectory that, if continued, would intercept either the pontine or control tissue explant; 79% were directed toward the basilar pons. Second, cortical axons (n=593) were scored for a turn of 30° or more toward either the pontine or control explant; 96% were deviated toward the pontine explant. These figures underestimate the influence of the pontine explant on the directionality of cortical axon growth, since axons that had already contacted the pontine or control explants were excluded and far more axons contact the pontine explant than the control explant. These findings show that the basilar pons releases a diffusible signal that influences the directional growth of cortical axons in a target specific manner.

To visualize axon behavior in the cortical explant and to identify the cortical neurons that send axons into the collagen matrix, we used the fluorescent, lipophilic axon tracer DiI in aldehyde-fixed cultures. DiI applied in aldehyde-fixed tissue to an axon or one of its collaterals will label by diffusion the entire neuronal

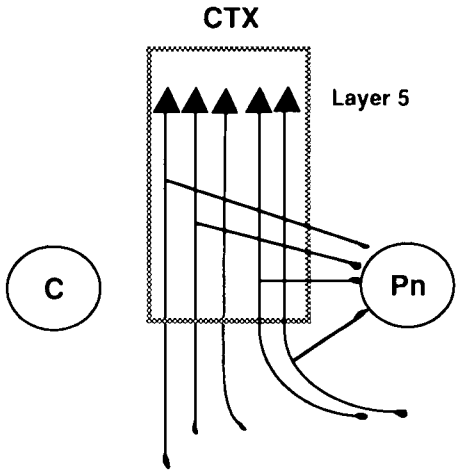

Fig. 2. Schematic illustration of 3-dimensional collagen co-culture experiments (Heffner *et al.* 1990), showing the arrangement of P0–P1 explants of cortex (CTX), basilar pons (Pn) and control tissue (C) co-cultured at a distance in 3-dimensional collagen matrices for up to 48 h. The pial surface of the cortical explant is to the top, the ventricular surface is to the bottom. Triangles represent layer 5 neurons; thin lines are axons and collaterals. The side of the cortical explant on which the pontine and control explants were placed was random. The basic findings are summarized; see text for details.

membrane, including the axon and all of its branches (Godement *et al.* 1987; Honig and Hume, 1989). DiI injected into the path of axons extending from the ventricular surface of cortical explants cultured alone labels cells scattered over much of the pial to ventricular extent of the explant. In contrast, DiI injected into the pontine explant labels cells predominantly confined to a narrow band that runs the width of the cortical explant. Therefore, diverse populations of cortical cells extend axons into the collagen matrix, but only the axons of a specific population of cortical cells respond to the diffusible, pontine-derived signal. We identified these cells as layer 5 neurons by matching their distribution with that of layer 5 corticospinal-pontine neurons prelabeled *in vivo* with the retrograde tracer RITC 24 h before the cortex was explanted into collagen matrices. DiI injected into the control explants labels few, if any, cortical cells. DiI labeling from the pontine explant shows that the majority of cortical axons that contact it are collateral branches of 'primary' axons that extend toward the ventricular surface, often continuing inferiorly into the collagen matrix. Some 'primary' cortical axons do turn in the cortical explant and emerge from the side facing the pontine explant. These findings demonstrate that a chemoattractant derived from the basilar pons can operate over a distance *in vitro* and specifically affect the directional growth of layer 5 cortical axons, either directly, or by conditioning the collagen substrate.

Does the basilar pons also control the formation of collateral branches within the axon tract overlying it? To address this issue we carried out a series of 'delayed' co-culture experiments in 3-dimensional collagen matrices (Heffner and O'Leary, 1990). P0 or P1 cortical explants were cultured alone for 24 h in the collagen matrix and examined with phase-contrast optics, then an explant of basilar pons or a control tissue (same as above) was embedded in the matrix to the side of axons that had extended inferiorly from the ventricular surface of the cortical explant. Re-examination of delayed pontine co-cultures after an additional 24 h showed that cortical axons not present at the time the pontine explant was added are directed laterally through the collagen matrix toward the pontine explant (Fig. 3). Many of these later arising cortical axons can be identified as collateral branches that form in the collagen matrix and extend orthogonal to the growth of the primary axons. This directed '*de novo*' extension of collaterals does not occur if cortex is cultured alone for 48 h, nor when a control explant is added. Therefore, these observations suggest that a diffusible pontine-derived signal promotes the collateral branching of corticospinal axons.

To quantify branch number in both the collagen matrix and the cortical explant, the directed growth of branches, and the laminar location of their parent neurons, cultures were aldehyde-fixed and DiI was injected into the delayed pontine or control explant or, in cases in which cortex was cultured alone, directly in the collagen matrix off its ventricular surface (Heffner and O'Leary, 1990; also see Table 1 in O'Leary *et al.*

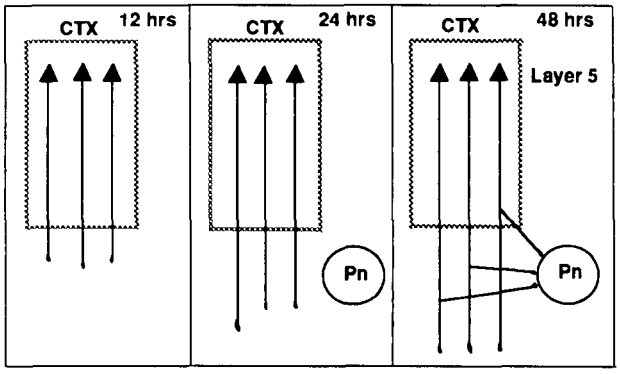

Fig. 3. Depiction of 'delayed' co-culture experiments in 3-dimensional collagen matrices (Heffner and O'Leary, 1990). Left panel: cortical explants (CTX) were cultured alone for an initial period. Middle panel: after 24 h, an explant of basilar pons (Pn) was placed to the side of the axons that had extended 'inferiorly' from the ventricular surface of the cortical explant. Right panel: after an additional 24 h, collateral branches had formed and were directed toward the pontine explant. The branches were preferentially extended by layer 5 axons. Branch formation was not stimulated when control tissue was used as the delayed explant. See text for further details.

1990). In cultures of cortex alone, branches are not found in the collagen matrix. Some branches do form in the cortical explant, but extend equally to the left or right. The retrogradely labeled parent cells are dispersed through the cortical explant. DiI injected into delayed control explants retrogradely fills only a few axons and cells, consistent with phase-contrast observations that very few axons contact the control explant. Larger DiI injections made into the control explant and the adjacent collagen matrix result in labeling more similar to that obtained in cultures of cortex alone: a small number of branches with no preferred direction are labeled in the cortical explant and branches are rarely labeled in the collagen matrix. Cells retrogradely labeled from the matrix are scattered in the cortical explant. Thus, DiI labeling confirms the phase-contrast observations that delayed addition of a control tissue explant has little influence on the directional growth or branching of cortical axons. However, the directional growth and branching of cortical axons is greatly influenced by the delayed addition of an explant of basilar pons. DiI injected into the delayed pontine explant shows that the number of branches formed in the cortical explant per labeled cell is 5- to 8-fold greater than the number labeled in cultures of cortex alone or in co-cultures with a delayed control explant. In the same delayed co-cultures, a relatively large proportion of cortical axons form branches in the collagen matrix, all of which extend toward the pontine explant, and the majority of retrogradely labeled neurons are present in the layer 5 region. These findings from delayed co-culture experiments provide further evidence that the basilar pons releases a diffusible chemoattractant that directs the growth of collateral branches of layer 5 corticospinal axons, and support the hypothesis that a diffusible, pontine-derived signal

controls the interstitial branching of layer 5 corticospinal axons.

In vivo evidence that the basilar pons controls the formation and directional growth of collateral branches

The behavior of cortical axons growing in the presence of basilar pons in 3-dimensional collagen matrices (Heffner *et al.* 1990; Heffner and O'Leary, 1990) resembles that of layer 5 corticospinal axons *in vivo*, implying that the formation of the corticopontine projection may be controlled by the basilar pons through the release of a diffusible chemotropic signal. To evaluate this possibility further, we performed three sets of *in vivo* experiments (Missias *et al.* 1990; López-Mascaraque and O'Leary, unpublished results).

In the first set of experiments, we set out to determine whether local cues that develop in the axon tract overlying the basilar pons, or alternatively the basilar pons itself, control the branching of corticospinal axons. We used X-irradiation, a treatment shown to kill proliferating cells and induce migrational defects (D'Amato and Hicks, 1980; Jensen and Killackey, 1984), to disrupt the normal development of the basilar pons. By X-irradiating pregnant rats during the period of genesis and migration of basilar pontine neurons, the basilar pons was decreased to as little as 10% of its normal volume and occupied a position that corresponded to the caudal part of the normal basilar pons, and caudal to this position ectopic cellular aggregations formed on the ventral surface of the hindbrain beneath the path of corticospinal axons (Fig. 4). Specific markers for basilar pontine neurons are not available, but an argument can be made that the ectopic cellular aggregations comprise basilar pontine neurons. First, basilar pontine neurons are the only hindbrain neurons being generated during or after the time of X-irradiation (Altman and Bayer, 1978) and thus are the only population in a state to be affected. Second, the ectopic aggregations are found along the normal migratory path of basilar pontine neurons, a path unique to these neurons (Altman and Bayer, 1978). Third, in Nissl stained sections the cells resemble basilar pontine neurons.

If local cues that develop in the axon tract overlying the basilar pons govern the branching of corticospinal axons, branches should still form at the correct locations even in the absence of the basilar pons. On the other hand, if the basilar pons controls the branching of corticospinal axons, branches should form at locations along corticospinal axons that overlie aggregations of basilar pontine neurons. Using DiI to label layer 5 axons extending from visual cortex in X-irradiated cases, we find that branches form in the axon tract directly over both the reduced basilar pons and the ectopic cellular aggregations and extend ventrally into them (Fig. 4). No ventrally directed collaterals are apparent in the axon tract rostral to the greatly reduced basilar pons, the position that would overlie rostral

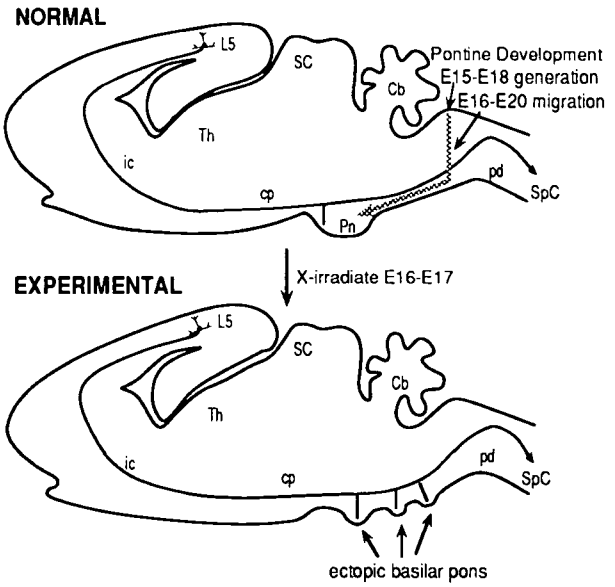

Fig. 4. Corticospinal axons extend collateral branches to ectopic aggregations of basilar pontine neurons induced by X-irradiation. Basilar pontine (Pn) neurons are generated in the lateral recess of the fourth ventricle on the dorsal aspect of the medulla from E15 to E18, with more than 90% born on E16 and later (Altman and Bayer, 1978 – as given here, these ages reflect the day of insemination as E0, to be consistent with our usage). The postmitotic neurons leave the proliferative zone, migrate ventrally around the circumference of the medulla and rostrally along its ventral surface, and aggregate beneath the pons proper. Following X-irradiation on E16 and E17, the basilar pons is substantially reduced in size and often multiple, discrete aggregations of basilar pontine neurons are found caudally at ectopic positions on the ventral surface of the hindbrain underlying the pathway of layer 5 corticospinal axons. This experiment can be done since virtually all layer 5 neurons are born on E14 and E15 (Bruckner *et al.* 1976). DiI injected into visual cortex reveals in normal P3 rats (top) that layer 5 axons form collaterals only over rostral basilar pons, but in experimental P3 rats corticospinal axons form collaterals at aberrant positions coincident with the locations of the ectopic aggregations of basilar pontine neurons. See text for further details. Rostral is to the left, caudal to the right, ventral to the bottom.

basilar pons in normal rats at which visual cortical axons normally branch. These findings imply that local cues do not control the formation of the pontine collateral and support the alternative; that the basilar pons controls the formation of the collateral branches that innervate it. These findings are not definitive, though, since we cannot exclude the possibility that the same or coincident local cues govern the aggregation of pontine neurons and cortical axon branching and that X-irradiation alters their spatial expression.

The second and third sets of *in vivo* experiments involve depriving the basilar pons of a part of its normal cortical input and assessing the response of spared layer 5 corticospinal axons (Missias *et al.* 1990; Lopez-Mascaraque and O'Leary, unpublished results). The

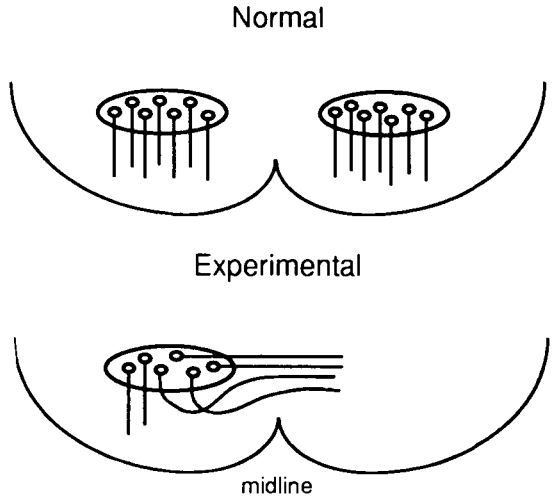

Fig. 5. Crossing of pontine collaterals of layer 5 corticospinal axons to opposite basilar pons deprived of cortical input. Schematic diagram showing coronal view through the cerebral peduncles (the pathway of layer 5 axons, cut transversely and depicted by the large ovals) and the underlying basilar pons (a bilaterally symmetrical structure). In normal development, primary layer 5 axons (small circles) extend collaterals directly into the underlying basilar pons. The projection is almost entirely ipsilateral. When the normal ipsilateral cortical input to the 'right' basilar pons is prevented from developing, it receives input from contralateral layer 5 axons in two ways: (1) collaterals that had extended into the 'left' basilar pons are redirected across the midline, and (2) collaterals form in the cerebral peduncle and extend directly across the midline. Lateral is to the left, ventral to the bottom. See text for details.

basilar pons is a bilaterally symmetrical, midline structure. DiI injections in normal rats show that the basilar pons receives predominantly an ipsilateral cortical input at all stages of development; only a sparse contralateral input, if any, is observed (Missias *et al.* 1990). If a diffusible, target-derived chemoattractant serves to promote the cortical innervation of the basilar pons, then when the latter is deprived of its normal ipsilateral cortical input, collaterals of contralateral layer 5 axons should be attracted across the midline. Such attraction could be minimized in normal development by normal inputs that limit the availability and diffusion of the chemoattractant or perhaps prevent a response by neighboring populations of pontine collaterals through axon–axon interactions. Indeed, in postnatal day 6 rats in which cortical axons were unilaterally removed at birth, prior to the extension of collateral branches into the basilar pons, DiI injected into the intact cortical hemisphere labels an abnormally large contingent of corticopontine collaterals that cross the midline and enter the opposite, 'denervated' basilar pons; the rerouted collaterals cross at the level of the basilar pons (Fig. 5). The paucity of contralateral input seen in normal development rules out the possibility that the dense labeling seen in the denervated basilar pons is due to an expansion of a normally transient contralateral projection. Given the magnitude of the axonal response and the finding that the collaterals turn and cross at the level of the basilar pons, a straightforward interpretation is that the aberrantly crossing axons are responding to a chemoattractant diffusing across the midline from the denervated basilar pons. Two additional pieces of evidence support this interpretation. First, the corticopontine projection is displaced from the midline in normal animals by medial lemniscal axons from the dorsal column nuclei that terminate densely in the most medial part of the basilar pons. This midline lemniscal input is intact in the experimental animals. Therefore, the cortical axons are being attracted around and through this intact projection. Second, in the experimental rats many collaterals form in the axon tract overlying the 'innervated' basilar pons and extend directly across the midline toward the 'denervated' basilar pons (Fig. 5) – such a trajectory is observed infrequently in normal rats and is most easily explained as a response to a diffusible chemoattractant.

In the third set of *in vivo* experiments, sensorimotor cortex was removed bilaterally at birth, thus depriving the caudal part of the basilar pons of its normal cortical input, and the response of layer 5 axons arising from visual cortex was examined at day 6 using anterograde DiI labeling (Missias *et al.* 1990). Visual cortical axons show two distinct behaviors in this situation (Fig. 6). First, collaterals that form in the axon tract at the normal location for visual layer 5 axons extend into

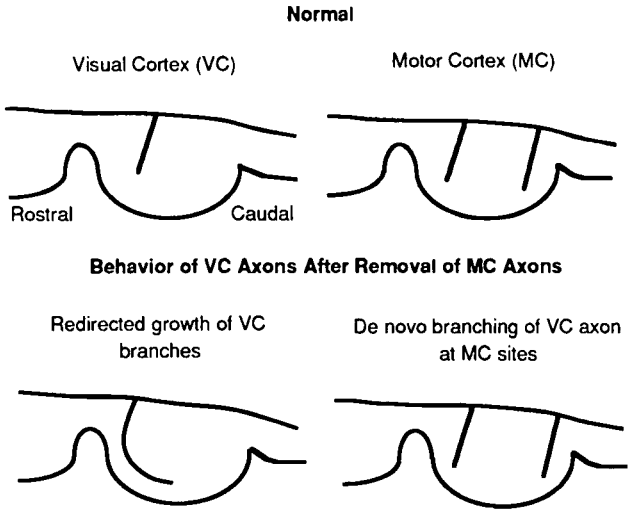

Fig. 6. Altered behavior of layer 5 axons from visual cortex in the absence of layer 5 axons from motor cortex. Schematic diagrams show a sagittal view of the basilar pons and layer 5 corticospinal axons in the overlying cerebral peduncle. In normal development, primary layer 5 axons from visual cortex extend collateral branches into rostral basilar pons (top left), and those from motor cortex extend collaterals into caudal and rostral basilar pons. When the projection from motor cortex is prevented from developing, layer 5 axons from visual cortex behave in two distinct ways. First, collaterals extended into rostral basilar pons are redirected toward the input deprived caudal part. Second, in addition to forming their normal rostral set of branches, visual layer 5 axons extend a second set of collateral branches into caudal basilar pons. See text for details.

rostral basilar pons, turn, and grow caudally into caudal basilar pons deprived of its normal input from motor cortex. Similarly to the second set of *in vivo* experiments described above, this redirected growth can be interpreted to be a response to chemoattractant diffusing rostrally from the denervated caudal basilar pons. Second, primary layer 5 axons from visual cortex form not only a set of rostrally located pontine collaterals, but also form another set of collaterals caudally which extend into caudal basilar pons. Thus, in the absence of the corticopontine projection from motor cortex, the collateral branching pattern of visual cortical axons resembles that characteristic of motor cortical axons. This finding indicates that visual cortical axons can respond to cues that control the branching of motor cortical axons. This capacity for developing similar patterns of collateral branches suggests that in normal development the basilar pons uses the same cue(s) to effect the development of pontine collaterals from cortical axons arising from diverse regions of cortex. Although such an interpretation can be made regardless of the nature of cues involved, local or diffusible, it is consistent with our *in vitro* observations that explants of visual and motor cortex show similar responses to basilar pontine explants taken from different regions of the basilar pons.

Closing remarks

The *in vitro* and *in vivo* evidence reviewed here suggests that in the developing brain the basilar pons becomes innervated by controlling at a distance the budding and directed ingrowth of layer 5 axon collaterals through the release of a diffusible molecule. The pontine-derived signal may influence cortical layer 5 axons in two ways. First, it provides directional cues for the growing collateral branches, indicative of a tropic activity. This property may be comparable to that of a target-derived diffusible signal that promotes the extension of trigeminal ganglion axons to the maxillary process (Lumsden and Davies, 1983, 1986) and to that of a different diffusible signal emanating from the spinal floor plate that acts as a directional cue for commissural axons over an intermediate part of their pathway (Tessier-Lavigne *et al.* 1988; Placzek *et al.* 1990). Second, the diffusible pontine-derived signal may initiate the budding of collateral branches from the axon cylinder; this response is a novel action for a chemotropic signal, and may be distinct from directing the growth of pontine collaterals. The potential dual action of the pontine-derived signal raises the possibility that it consists of two (or more) distinct molecules. Branch initiation, though, may prove to be an early step in a sequence of cellular events that result in the directed growth of collaterals toward a source of a diffusible, tropic signal. Therefore, the initiation and directed extension of pontine collaterals may be a response to a single chemotropic signal.

In normal development, the growth cone of the primary layer 5 axons apparently does not respond to the pontine-derived chemotropic signal and plays no role in target recognition. However, the primary growth cone does serve important roles in the establishment of the corticopontine projection. For example, it makes pathway choices and elongates the axon along a subcortical tract toward the spinal cord (Koester and O'Leary, 1989). The extension of layer 5 axons along a trajectory through the cortex and the internal capsule, the proximal part of their spinally directed course, probably reflects the response of their growth cones to local molecular cues or to cortical subplate axons, the first axons to take this trajectory (McConnell *et al.* 1989; De Carlos and O'Leary, 1990, 1992). However, target recognition in the developing corticopontine projection is controlled by a chemotropic signal emanating from the basilar pons that promotes the extension of pontine collateral branches from the cylinder of layer 5 corticospinal axons millimeters behind the growth cone.

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