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TROPISM AND CORTICOSPINAL TARGET SELECTION IN THE RAT

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Abstract—Layer V pyramidal cells in the intermediate part of the cerebral cortex enter the lumbar spinal gray, but not the cervical spinal gray matter, during the first postnatal week. To study if the ingrowth of intermediate corticospinal axons into the lumbar spinal gray is guided by a diffusible tropic factor, we co-cultured explants of the intermediate part of the sensorimotor cortex and of the lumbar spinal gray matter in 3-D collagen gels. Using this test system, a target specific directional growth of cortical axons towards the lumbar spinal gray explant can be demonstrated *in vitro*. Retrograde labeling indicates that most labeled cell bodies were located in layer V of the cortex explant and were characterized by a pyramidal cell shape. Furthermore, axon behavior of retrogradely labeled neurons within the cortical explant is considerably affected by the presence of lumbar spinal gray target tissue. In contrast to lumbar spinal gray innervation, intermediate corticospinal tract axons do not enter the cervical spinal gray *in vivo*. Is it the inability of intermediate corticospinal tract axons to respond to cervical target-derived influences? In the current study we co-cultured explants of the intermediate cortex and cervical spinal gray matter in 3-D collagen gels.

Our data indicate that *in vitro* axons from layer V neurons in the intermediate part of the cortex are capable of recognizing and responding to a diffusible factor released by the cervical spinal cord target area. This suggests that the failure of neurons within the intermediate cortex to enter the cervical spinal gray *in vivo*, is not due to their inability to respond to a target-derived diffusible factor, but probably regulated by other extrinsic factors.

The formation of adequate functional nerve connections is based upon the correct sequence of cell–cell interactions during the development of the CNS. Among these distinct yet co-ordinated events, axon outgrowth and guidance play an important role.

Growing axons may be guided by mechanical channels in the neuroepithelium,[42] by local chemical cues expressed along the pathway,[13,43] by diffusible factors from intermediate guideposts[46] or factors released directly from the target.[25,26]

The first evidence for a directed growth of axons to an intermediate or final target in response to a diffusible factor(s) emanating from cells in the target region (=chemotropism) came from studies on nerve growth factor (NGF): chick dorsal root ganglion neurites undergo a rapid reorientation of their direction of growth in response to βNGF-concentration gradients *in vitro*.[10,11] With *in vitro* explant culture techniques other chemotropic factors have been implicated in directing axon outgrowth. For example, in the embryonic rat spinal cord, floor plate cells secrete a diffusible factor that evokes the outgrowth of commissural axons from spinal cord explants and, in addition, orients these axons.[5,33] Other central fiber systems also exhibit a directed growth in such a way that they are probably guided by chemotropism: the rat corticopontine system,[14] the rat or chick visual cortex[2,43] and the rat corticospinal projection from layer V neurons, located in the anterior part of the cerebral cortex to the cervical spinal gray matter.[20]

The corticospinal tract (CST) in rodents represents a good model to study the development, guidance and axon outgrowth of a long fiber system in mammals.[17,18,20,40,41] Furthermore, the CST in rodents is characterized by its postnatal outgrowth throughout the spinal cord as well as by its convenient experimental accessibility.[6,9,36,39]

During early development neurons which give rise to corticospinal axons are found in many regions of the mammalian cortex.[19,44] During the first postnatal week the rat CST contains three components: the first having its originating neurons in the anterior part of the cortex and its termination field in the cervical intumescence, the second with its neurons originating in the intermediate part of the cortex and its termination field in the lumbar enlargement, and a third transient one originating in the posterior cortex and gradually disappearing from spinal cord levels.[19] By co-culturing anterior cortex and cervical spinal gray matter in 3-D collagen gels, a target-specific directional growth towards the cervical spinal

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Abbreviations: CST, corticospinal tract; DiI, 1,1-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate; NGF, nerve growth factor; P, postnatal day; PB, phosphate buffer.

gray explant could be demonstrated.[20] Interestingly, the axons with its neurons originating in the intermediate parts of the cortex do not respond, at least *in vivo*, to a tropic factor located in the cervical spinal gray matter.[19,20]

This study was undertaken to examine what mechanism controls the fiber ingrowth of the "intermediate" CST axons into their appropriate spinal gray target area. The possibility that the maturing lumbar spinal gray matter induces its own innervation by producing a long-range, tropic signal that acts on the corticospinal neurons in layer V of the intermediate part of the cortex was studied using *in vitro* collagen-gel co-culture techniques. In addition the question was asked why the intermediate corticospinal neurons do not respond to a diffusible factor located in the cervical spinal gray *in vivo*. To study if it is the inability of the intermediate corticospinal neurons to enter the cervical spinal gray *in vivo*, we co-cultured intermediate cortex and cervical spinal gray matter explants in 3-D collagen gels.

EXPERIMENTAL PROCEDURES

In vitro *collagen gel co-culture*

One-day-old (postnatal day 1, P1), P4 and P6 Wistar rat pups were used. The experimental design is illustrated in Fig. 1. Under sterile conditions the whole brain of a P1 rat pup or the spinal cord of a P4 or P6 animal was placed in a drop of Leibovitz (L15, Gibco) medium. A total of 64 P1, 39 P4 and 86 P6 rat pups (Wistar rats) were used in this study.

Cortical explants were obtained from "intermediate" cortex[19] from P1 rats, while normal corticospinal target tissue, i.e. cervical or lumbar spinal gray matter, was obtained from P4 and P6 rats, respectively. White matter was completely removed from the spinal cord gray matter explants, since white matter oligodendrocytes at P4 might already contain inhibitory proteins[4,40] which might affect these experiments. Control tissues used were olfactory bulb or cerebellum (both tissues do not receive direct neocortical input) from P4 and P6 animals. Coronal sections, with a thickness of about 350 μm, were either cut by hand or using a slicer with four parallel razor blades.[37]

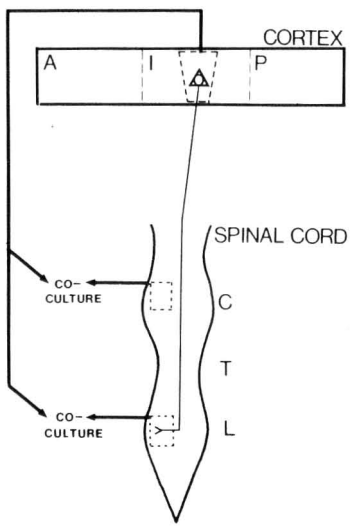

Fig. 1. Schematic representation of the experimental paradigm. The rat CST component originating in the intermediate parts of the cerebral cortex projects to lumbar spinal gray matter *in vivo*, but not to cervical spinal gray, during the first postnatal week. Explants of intermediate cortex from P1 rats were co-cultured, in a collagen matrix, with either cervical spinal gray from P4 animals or lumbar spinal gray from P6 rats.

Cortical explants were cultured alone ($n = 73$) or co-cultured ($n = 338$) in collagen gels, with explants of cervical or lumbar spinal cord, or a non-target "control" explant, or both. The explants were positioned above the floor of a plastic culture dish (Costar) within a hemisphere matrix of rat tail collagen.[25,26] Spinal cord and control explants were positioned on the flanks of the cortical explant at a distance of about 200–300 μm (see Fig. 2A). The cultures were incubated in a 50:50 mixture of HAM's F12 and Eagle's MEM (Gibco) with 5% fetal bovine serum (Hyclone) for 48 h and subsequently examined with phase-contrast optics (Nikon). Quantification of outgrowing axons was carried out according to Heffner *et al.*[14] (modified by Joosten *et al.*[20]). Briefly, outgrowing cortical axons were counted only if their growth cones were visible in areas defined by dotted lines (areas L, R). Hence, quantification of cortical axons was carried out in a defined left (L) and right (R) area (see Fig. 2A). Turning axons (in areas L and R) are those axons that have an initial trajectory when exiting from the cortical explant that would have missed the spinal cord or control explant, but which subsequently turn by $>30^\circ$ in the direction of either the target or the control explant.

The spinal cord outgrowth coefficient was calculated by dividing the number of axons growing in the area facing the spinal cord divided by the total number of outgrowing. The spinal cord turning coefficient was calculated in a similar manner.

The quantification of total amount and/or turning of axons was carried out using photomicrographs of the co-cultures (eventually at different focal planes).

Retrograde DiI labeling of cortical axons

To visualize the cortical projection neurons *in vitro*, and to study the behavior of axons within the cortical explants, small crystals of the fluorescent dye DiI [Molecular Probes, Eugene (OR)][16] were placed into the collagen co-cultures, in the axonal outgrowth areas, about 100 μm from the sides of the cortical explant. Before application of this retrograde tracer, collagen co-cultures were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.4). DiI diffuses through the aldehyde-fixed membrane and retrogradely labels the entire axon tree and cell body from uptake by the primary axon.[8] After four to six weeks (fixed and stored at 4°C) cultures were observed by fluorescence microscopy. Additionally, DiI was injected into the lumbar enlargement *in vivo*, in order to retrogradely label corticospinal neurons in layer V of the cortex. Photomicrographs were made with the use of a Zeiss universal microscope with epifluorescent equipment using the appropriate filter system for DiI and a Kodak-Tri-X film (400 ASA).

RESULTS

Intermediate cortex and lumbar spinal gray matter co-cultures

When intermediate cortex is cultured by itself in a collagen gel matrix ($n = 73$) axon outgrowth is predominantly from the ventricular surface and in an inferior direction. However, in co-cultures of cortex with lumbar spinal cord and control tissue ($n = 110$) the axonal growth is strongly directed toward the

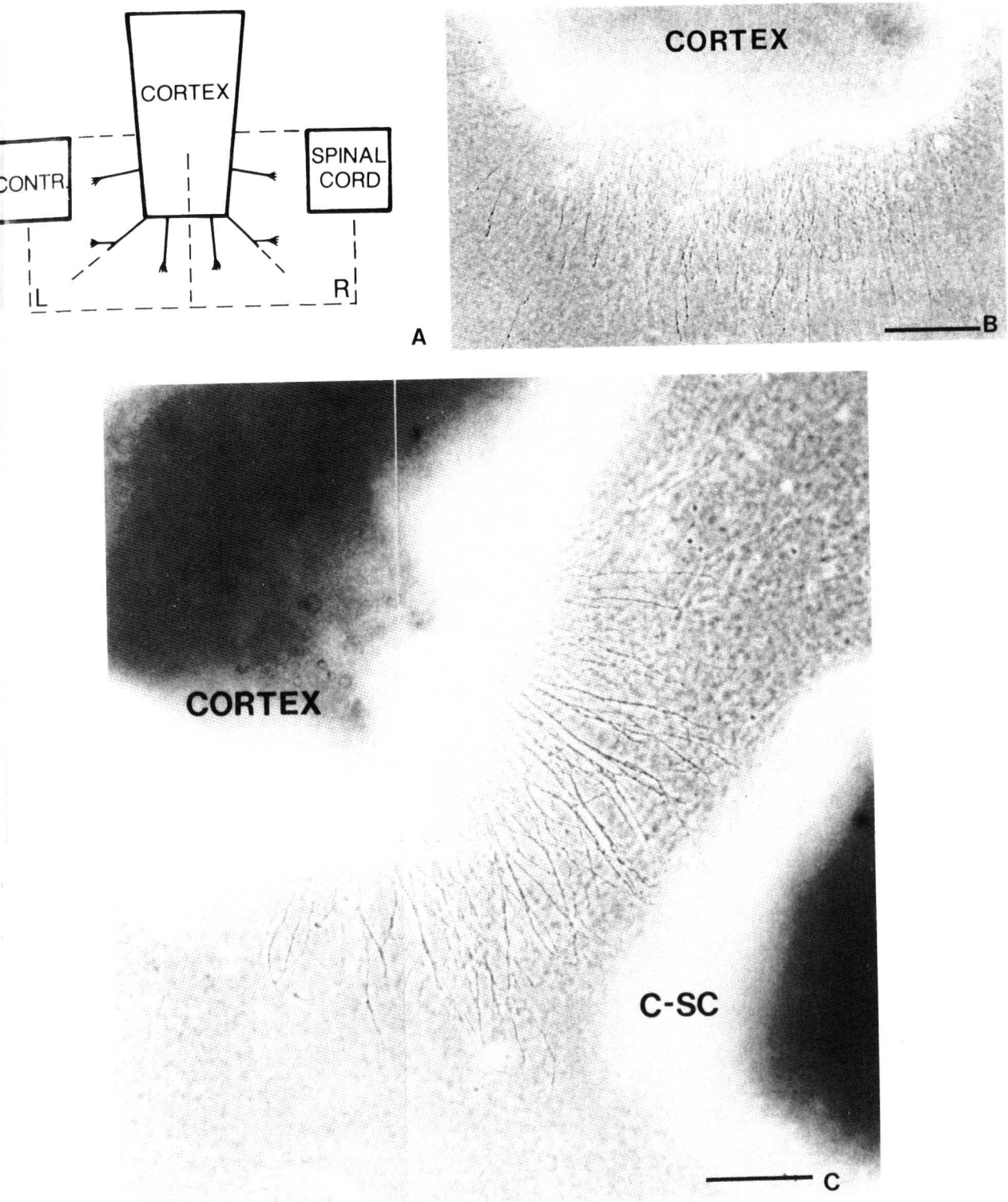

Fig. 2. Collagen gel co-cultures. (A) Schematic representation of the co-culture experiments and the quantification areas. Pial surface of the cortical explant is to the top, and ventricular side to the bottom. Quantification of intersecting and/or turning is carried out in defined areas (marked by dotted lines): L = Left; R = Right. (B) Cortex explant cultured alone. (C) Cortex explant (P1) (ventricular side to the bottom) co-cultured with cervical spinal cord (P4) tissue explant after 48 h of incubation. Most axon growth is present in the area facing the cervical spinal cord explant (C-SC). Scale bar = 100 μm.

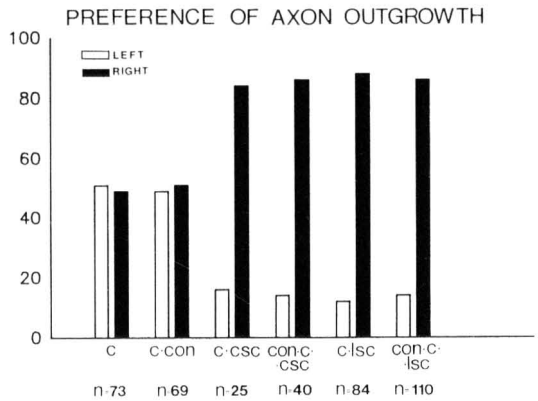

Fig. 3. Preference of cortical axon outgrowth after 48 h of incubation. C, cortex; CON, control; CSC, cervical spinal cord; LSC, lumbar spinal cord. Bars indicate the preference of cortical axon outgrowth to the left (control) or right (spinal cord) area. Cortex explant, or cortex co-cultured with control tissue: no preference of axon outgrowth can be noticed. Co-cultures with spinal cord explant: preference of cortical axon outgrowth towards the cervical or lumbar spinal cord tissue. n = number of explants used.

lumbar spinal gray explant but not the control explant (Fig. 2B). A preference of cortical axonal outgrowth toward the lumbar spinal explant can be deduced from Fig. 3: axon outgrowth extends predominantly from the side of the cortical explant facing the lumbar spinal cord in 86% of the cases and from the side facing the control explant in 5% of the cases; the remaining 9% had near equal outgrowth from two sides of the cortical explant. Using cortex–lumbar spinal cord co-cultures ($n = 84$), a preference of cortical axon outgrowth from the side facing the lumbar spinal cord explant is observed in 88% of the cases.

An inhibitory effect from the control explants (bulbus olfactorius or cerebellum) can be excluded since the presence of a control explant does not affect the total cortical axon outgrowth. In addition, no significant preference of outgrowth can be noticed in co-cultures of control and cortex explants ($n = 69$) (Fig. 3).

Quantitative measurements also support the conclusion that the lumbar spinal gray matter releases a diffusible factor that affects the directional growth of "intermediate" cortical axons. Based on the quantification of axon outgrowth a preference can be noticed towards the lumbar spinal cord tissue, when compared with controls (Table 1). In areas LR 9755 axons scored: (lumbar) spinal cord outgrowth-coefficient 0.63 ± 0.04. In the control experiments (control–cortex co-cultures or cortex alone) in areas LR 7622 axons scored: the lumbar spinal cord outgrowth-coefficient is 0.50 ± 0.03.

In addition axons are noted that would have missed the lumbar spinal cord explant but turn preferentially toward it: for 745 axons scored the spinal cord turning coefficient was 0.79 ± 0.07 (Table 1).

The origin and cellular morphology of the cortical neurons, the axons of which preferentially project to the lumbar spinal cord explant, was determined by placing a small crystal of the lipophilic dye DiI in the axonal outgrowth area on the proximal side of the cortical explant. After application of DiI in the axonal outgrowth area of the cortex explant co-cultured with lumbar spinal cord tissue 82% of the DiI-labeled cortical neurons are located in one

Table 1. Collagen co-cultures quantification of axon outgrowth

	I-cortex L-spinal cord $n = 194$	I-cortex C-spinal cord $n = 65$	A-cortex C-spinal cord $n = 49$
Spinal cord outgrowth coefficient	0.63 ± 0.04	0.64 ± 0.04	0.61
Spinal cord turning coefficient	0.79 ± 0.07	0.91 ± 0.10	0.85

Quantification of axon outgrowth and turning in the collagen co-cultures. A-Cortex, anterior cortex; I-Cortex, intermediate cortex; C, cervical; L, lumbar. A-Cortex and Cervical Spinal Cord co-cultures results are from Ref. 20. n is number of cultures used.

Table 2. Quantitative analysis of DiI labeling

		Labeled cells		Branching in cortical explant
Culture situation	No. of cultures	Total	in layer V (%)	Labeled cells containing branches
Cortex alone	32	310	29%	29 (9.4%)
Cortex + control	25	290	25%	41 (14.1%)
Cortex + spinal cord	41	407	82%	110 (27.0%)

Quantitative analysis of DiI labeling *in vitro*. Lumbar spinal cord only. Axon behavior within the cortical explant is studied using retrograde DiI labeling. The fluorescent tracer DiI is injected into the collagen matrix in the path of the axons extending from the ventricular side of the cortical explants, cultured alone ($n = 32$) or co-cultured ($n = 66$). Labeled cells containing branches are quantified. Virtually all branches in the cortex–lumbar spinal cord co-cultures are directed towards the spinal cord explant, whereas in the cortex alone or cortex–control co-cultures no preference is observed.

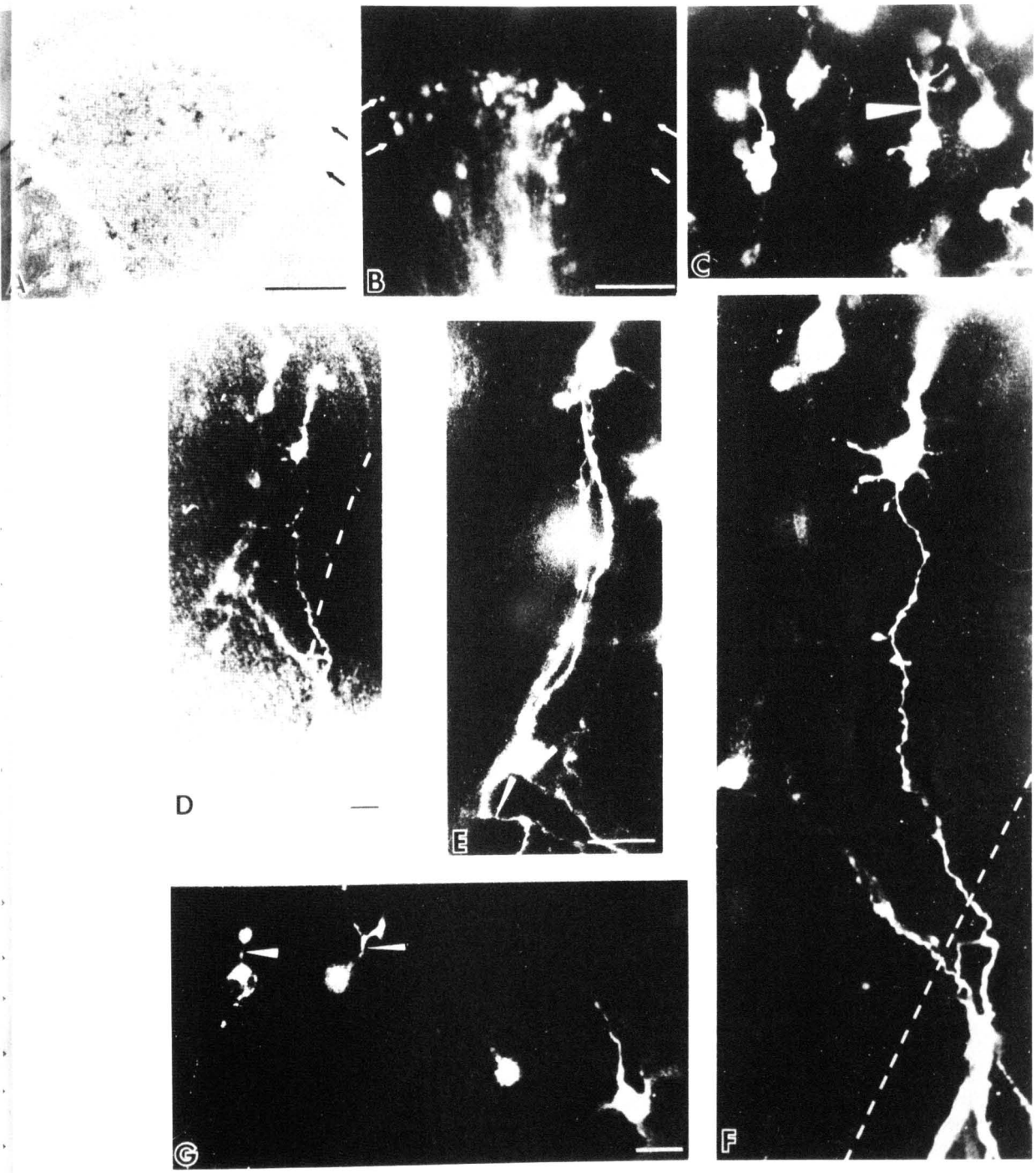

Fig. 4. Retrogradely DiI-labeled neurons in the cerebral cortex. Pial side of the cortex explant is always on top. (A) Low-magnification phase-contrast picture of cortex explant in a control–cortex–lumbar spinal gray co-culture. (B) Retrogradely DiI-labeled cell bodies are present across the entire width of the same cortical explant as shown in 4A. Most labeled cell bodies are localized in one specific layer (arrows) (compare 4A and 4B). Scale bar = 100 μm. (C) Detail of the retrogradely labeled neurons in 4B; the apical dendrite is marked (arrowheads). Scale bar = 20 μm. (D–F) Details of retrogradely DiI-labeled axons and their cells of origin. Control–cortex–spinal cord co-culture fixed after 48 h. Primary axons, initially growing towards the ventricular side of the cortex explant are turning towards the spinal cord facing surface (D, F). Besides, primary axons give off collaterals, which grow laterally towards the spinal cord explant (E, F arrowheads). Scale bar = 20 μm. (G) Labeled corticospinal neurons in layer V of the cortex after DiI injection *in vivo* in the lumbar enlargement at P5. Survival time 48 h. The typical apical dendrite is marked (arrowheads) Transverse section. Scale bar = 20 μm.

particular layer of the cortex explant (Table 2; Fig. 4A, B). The labeled cells are characterized by their pyramidal cell shape (Fig. 4C), and are localized in layer V of the cortex explant and situated across the entire width of the cortical explant (Fig. 4A, B). Using semi thin Toluidine Blue stained horizontal sections of a cortex explant it is possible to distinguish all cortical zones (not shown). Layer V is characterized by the presence of large pyramidal cells, with their apical dendrite pointed toward the pial side of the explant, and these layer V cells are more widely spaced than the neurons in the adjacent layers. If DiI is applied to the axonal outgrowth area of a cortex explant cultured alone or co-cultured with control tissue (P4 or P6), labeled neurons are located in virtually all cortical layers (29% or 25% of the labeled neurons are located in layer V: see Table 2).

After application of DiI into the lumbar enlargement *in vivo*, again most retrogradely DiI-labeled cortical neurons are located in the same band (layer V) and the morphology of the *in vivo* labeled cell bodies is similar to that of the *in vitro* labeled pyramidal-shaped neurons (Fig. 4G; compare with Fig. 4C).

Retrograde DiI labeling experiments *in vitro* reveal that the outgrowth of cortical axons is considerably affected by the presence of the lumbar spinal cord explant. Several primary axons, which initially are growing to the ventricular side of the cortical explant turn towards the lateral spinal cord-facing surface (Fig. 4D, F). In addition, back-branching *in vitro* can be observed (Fig. 4D, E): primary axons growing towards the ventricular side of the explant give off collaterals, which grow laterally toward the lumbar spinal cord explant. This development of branches (or collaterals) is specifically induced by the presence of the lumbar spinal gray explant (Table 2).

Intermediate cortex and cervical spinal gray matter co-cultures

Although CST axons originating in the intermediate parts of the cortex do not enter the cervical spinal gray matter *in vivo*,[19] our *in vitro* collagen co-culture experiments indicate that these axons are very well capable of recognizing and responding to a diffusible factor released by the cervical spinal target area.

If the cortex explants are cultured alone, no preference of outgrowth can be noted but if co-cultured with P4 cervical spinal gray matter explants, axon outgrowth preferably occurs at the side of the cortical explant facing the spinal cord explant (Fig. 2B). Quantitative measurements of axon outgrowth support this conclusion (Table 1): in 65 co-cultures (cortex–cervical spinal cord or control–cortex–cervical spinal cord) 2987 axons were scored on the ventricular side of the cortex explant: 1912 axons were located in the area facing the cervical spinal gray and are growing towards the target explant: a (cervical) spinal cord outgrowth coefficient of 0.64 ($\pm$0.04). In addition, cortical axons that would have missed the cervical spinal gray explant if they had maintained their initial trajectory, turn preferentially toward it: 315 axons scored and a (cervical) spinal cord turning coefficient of 0.91 ($\pm$0.10).

Again retrograde labeling was used to determine the origin and cellular morphology of the cortical neurons whose axons preferentially project to the cervical spinal explant in the collagen co-cultures. DiI injected into the path of axons extending from the ventricular side of the intermediate cortex explants co-cultured with cervical spinal gray matter retrogradely labels neurons mainly located in one single layer: a position comparable to that after application of DiI in co-cultures of intermediate cortex and lumbar spinal cord explants (see Fig. 4), and which corresponds to the normal layer V distribution of corticospinal neurons *in vivo*. The similarity between the DiI labeling pattern either using cervical spinal gray explants or lumbar spinal gray explants in co-cultures, strongly suggests that axons from pyramidal shaped neurons in layer V of the intermediate cortex also grow preferentially toward the cervical spinal gray, *in vitro*.

DISCUSSION

Corticospinal tract outgrowth in lumbar spinal gray matter and tropism

Our *in vitro* observations indicate the involvement of a diffusible factor during lumbar spinal gray target innervation. These observations are in line with other *in vitro* experiments which did demonstrate that chemotropic factor(s) located in the basilar pons and the cervical spinal gray matter[14,20,28] are involved in corticospinal target selection. With respect to the tropic behavior of axons in the collagen co-cultures in general, it needs to be emphasized that after 48 h of culturing only a small, although significant, percentage of the total amount of outgrowing axons (in cortex–lumbar spinal cord co-cultures 745 out of 9755: 7.6%) is turning within the collagen. This turning is directed, however, almost exclusively toward the spinal gray explant (see Table 1). This relatively low percentage probably underestimates the real turning effect of the lumbar spinal cord explant on cortical axons. With the use of DiI retrograde tracing turning axons are noticed within the cortical explants (Fig. 4). The turning within the cortical explant probably occurs already during the first 24 h.

The question arises as to how the tropic factor(s) affects the outgrowth and guidance of the CST axons into their appropriate targets, *in vivo*. Based on anterograde DiI tract tracing experiments, the corticopontine projection mainly develops by an interstitial budding of collaterals from parent axons.[32] This budding occurs one to two days after the parent axons grow spinally past the pons and thus accounts for the "waiting period" in this system.[28,30] Also at spinal cord levels a waiting period of about two days exists between the arrival of corticospinal axons

in the DF of a given spinal cord segment, and the extension into the respective spinal gray matter is observed.[9,36,48] DiI anterograde tract tracing techniques show that also at lumbar spinal cord levels the waiting period can be explained by interstitial budding of collaterals from parent CST axons (unpublished observations). Our co-culture experiments indicate that *in vitro* the lumbar spinal gray matter specifically induces CST axon-collateral formation. Both the *in vivo* and the *in vitro* observations strongly suggest that the diffusible factor released by the lumbar spinal gray is involved in the target innervation through the formation of interstitial collaterals of CST axons.

The fact that parent axons in lumbar spinal white matter grow past the spinal gray target area suggests that either these axons do not respond to cues which identify the lumbar spinal gray matter as a correct target, or that cues probably released by the target interneurons in the spinal gray have yet to be developed. Hence, the waiting period may be attributed to the developmental lag of the target neurons in the spinal gray. In the spinal gray most CST axons end on interneurons (and some on motoneurons[24]) in the dorsal horn and intermediate spinal gray regions.[21] Consequently, a thorough study on the genesis and development of these interneurons may be an important aspect in future studies concerning CST outgrowth and target finding.

Intermediate corticospinal neurons and tropism at cervical spinal cord

Tract-tracing studies have shown that the axons of anteriorly situated corticospinal neurons enter cervical spinal gray matter during the first postnatal week.[19] In contrast, intermediate corticospinal neurons do not respond to putative cues in cervical spinal gray *in vivo*, but instead grow more caudally to lumbar spinal cord levels. The "intermediate" CST axons recognize cues which identify the lumbar spinal gray as an appropriate target. The question is raised as to if and why it is the inability of these intermediate corticospinal neurons to respond to a diffusible factor located in the cervical spinal gray matter.

Our *in vitro* co-culture experiments demonstrate that axons from layer V in the intermediate part of the cortex are able to recognize and to respond to a diffusible signal released by the cervical spinal gray matter. Hence, other factors are involved in the development and regulation of the target-innervation of this "intermediate" CST projection *in vivo*. One possibility is that the intrinsic capacities of layer V neurons located in various cortical positions (anterior, intermediate and posterior) are very important in regulating the ability of a layer V neuron to respond to, for instance, a diffusible target-derived factor *in vivo*. However, transplantation experiments have demonstrated that differences among cortical regions in eventual targets of layer V neurons are not due to inherent differences in the layer V subcortical projection neurons in the various cortical areas.[34] All layer V neurons appear to have similar projectional capacities and the position differences are not predisposed by genomic qualities. Occipital (or posterior) cortex transplanted in motor (or intermediate) cortex develops output characteristic of motor cortex and vice versa.[29,31,44]

The extrinsic factors involved in the development of CST projections are still unknown. Our *in vitro* data indicate that the absence of target ingrowth of intermediate corticospinal neurons into the cervical spinal gray *in vivo* is not due to the inability of these neurons to respond to target-derived factors. The signal that causes the intermediate corticospinal axons not to enter the cervical spinal gray *in vivo* might occur at the level of the parent cell bodies. Thalamocortical fibers appear to form synapses as soon as they enter the sensorimotor parts of the cerebral cortex during the first postnatal week.[48] Their synapse formation on a certain population of layer V neurons might be the trigger for the development of projections to particular targets. Because of the use of cortical explants in our co-culture system, the possible influence or input of other brain areas on the layer V neurons does not affect the projectional capacities of these neurons.

Our *in vitro* data emphasize the importance of future experiments focused on a possible correlation between the developmental state of the parent neurons in layer V of the cortex and the input these cells receive from various other brain areas in relation to their target finding.

The diffusible tropic factor: general considerations

In vitro experiments have shown that during CST development tropism is involved in the determination of appropriate targets. The release of a diffusible tropic factor at the level of the basilar pons,[14,28] the cervical spinal gray[20] as well as the lumbar spinal gray matter target has been demonstrated. The question arises as to whether or not the tropic factor located in the pons is the same one as in the cervical and/or lumbar spinal gray matter. Comparison of the quantitative data as well as the DiI retrograde labeling observations indicate that the *in vitro* induction of cervical and/or lumbar spinal gray matter by axons stemming from intermediate and/or anterior[20] parts of the cortex are quite identical (see Table 1). These results suggest that possibly both at the cervical and lumbar spinal cord levels the same tropic factor is involved in CST target selection. However, it must be emphasized that our observations do not rule out the possibility that various different diffusible factors are acting during CST target finding at the level of the pons and spinal cord.

Nevertheless it would be interesting to know if this (or these) tropic factor(s) is identical to one of the members of the neurotrophin family, like NGF, brain-derived neurotrophic factor or neurotrophin-3.[1,12,15,23,27,38] At least NGF has been

shown to exert a tropic action on outgrowing axons *in vitro*.[10,11]

If the tropic factor as demonstrated in our *in vitro* experiments is identical to one of the above-mentioned neurotrophins, the most likely candidate seems to be neurotrophin-3. *In situ* hybridization experiments revealed that the neurotrophin-3 mRNA detected in developing rat spinal cord was derived from motoneurons,[7] and it is shown that spinal motoneurons are among the CST target cells.[24] Although developing spinal motoneurons contain also NGF-receptor-like immunoreactivity,[49] it seems unlikely that NGF is involved in CST target finding. The NGF-receptor exists in two forms having high and low affinity for NGF,[22,45] but only the high-affinity receptor can internalize NGF and mediate its function.[47] The NGFR protein detected in the spinal cord motoneurons is only in the low-affinity state.[35]

Besides the fact that the tropic guidance during CST outgrowth might be regulated by one of the well-known neurotrop(h)ic factors the possibility remains that an as yet unknown tropic molecule is involved.

Future *in vitro*-biochemical experiments might give an answer to the above-mentioned questions.

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