Antibody Neutralization of Neurite Growth Inhibitors from Oligodendrocytes Results in Expanded Pattern of Postnatally Sprouting Retinocollicular Axons

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After early postnatal ablation of one superior colliculus together with the ipsilateral eye in Syrian hamsters, retinofugal fibers abnormally cross the tectal midline and innervate the remaining superior colliculus. The fibers of this aberrant decussation are confined mainly to the superficial gray layer, with little ingrowth or termination in the deeper stratum opticum; laterally, most termination is in the superficial part of the superficial gray. Establishment of this abnormal pattern is temporally correlated with the appearance of oligodendrocytes at progressively more superficial locations in the colliculus. Oligodendrocytes express, on their surface, molecules that are inhibitory to neurite growth. This raises the possibility that their differential distribution in the superior colliculus during growth of retinal fibers is causally involved in the generation of the observed termination pattern.

We tested this hypothesis by applying the monoclonal antibody IN-1, which neutralizes this inhibitory activity, during the time of postnatal fiber growth and terminal arbor formation. We found that in the presence of IN-1, but not a control antibody, recrossing retinofugal fibers, observed at postnatal day 12, traverse the stratum opticum as well as the superficial gray, with greater depth of termination in superficial gray and stratum opticum. This pattern resembles that of the normal contralateral retinotectal projection.

The results indicate that neurite growth inhibitors expressed by oligodendrocytes are responsible for restricting the innervation of a target area in postnatal plasticity.

The reaction to lesions in the CNS of early postnatal animals is often strikingly different from that in the adult. In adult animals, axonal growth in the CNS after lesions is a relatively restricted event, whereas in neonatal animals, more extensive regrowth and sprouting of lesioned fiber systems have been demonstrated (e.g., Devor, 1976; Lund, 1978; Schneider, 1981; So et al., 1981; Kalil and Reh, 1982). One particularly striking

example of growth and rearrangement of a central pathway after damage has been described after lesions of the optic tectum in neonatal hamsters. If the outer layers of one superior colliculus are destroyed, the lesioned retinal fibers not only regrow to cover the surface of the damaged superior colliculus, but also cross the tectal midline and terminate in the contralateral superior colliculus. If the eye that would normally innervate this side is removed at the time of the lesion, the retinal fibers terminate throughout the entire mediolateral extent of the superior colliculus (Schneider, 1973; So and Schneider, 1978). Terminals of the retinal fibers, however, in this case are mainly confined to the superficial gray, whereas in the normal animal retinal terminals cover the superficial gray and the deeper stratum opticum; in the case of the recrossed projection, the latter is virtually devoid of retinofugal axons and terminals (So and Schneider, 1978).

In a recent study (Schneider et al., 1990; Jhaveri et al., 1992), the time course of the appearance and morphological differentiation of oligodendrocytes in the optic pathway of the hamster has been examined. Oligodendrocytes were identified from pre-ensheathing stages by immunostaining with the Rip antibody (Friedman et al., 1989). Pre-ensheathing oligodendrocytes were first observed in the optic tract at postnatal day 3 (P3) and appeared at the lateral aspect of the rostral superior colliculus at P5, spreading caudally and medially throughout most of the stratum opticum by P7–P9. No oligodendrocytes, however, are present in the superficial gray before P11. Oligodendrocytes thus appear in the stratum opticum at a time when recrossing fibers extend and form terminals (P3–P12) in the remaining superior colliculus (Carman et al., 1987).

This raises the possibility that the restricted ingrowth and termination pattern of retinal fibers might be caused by the presence of oligodendrocytes, for example, in the stratum opticum but not in the superficial gray. Oligodendrocytes are known to express proteins on their surface that have negative effects on growth cone motility and fiber growth (Schwab and Caroni, 1988; Fawcett et al., 1989; Bandtlow et al., 1990; Vanselow et al., 1990; Bastmeyer et al., 1991). These so-called neurite growth inhibitors from oligodendrocytes have molecular weights of 35 kDa (NI-35) and 250 kDa (NI-250) (Caroni and Schwab, 1988a). A monoclonal antibody has been raised against these proteins and was shown to neutralize their inhibitory effects both *in vitro* (Caroni and Schwab, 1988b; Bandtlow et al., 1990) and *in vivo*, allowing for long-distance fiber growth within the adult mammalian CNS (Schnell and Schwab, 1990; Cadelli and Schwab, 1991).

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We have used this antibody to test the hypothesis that the observed restriction of the distribution pattern of recrossing retinal fibers after unilateral tectal ablation is due to the effect of neurite growth inhibitors from oligodendrocytes present at the time of fiber growth and arborization in the superior colliculus. We found that in the presence of the IN-1 antibody this termination pattern is changed, and includes both the superficial gray and the stratum opticum.

Materials and Methods

Surgery. Experiments were performed on Syrian hamster pups, with the day of birth counted as P0. At P1 the animals were anesthetized by hypothermia, and cooling on ice was maintained throughout the surgical procedure. Lesions of the right superior colliculus were performed according to Schneider (1973). The superior colliculi were visualized through the thin skull after opening the overlying skin. The flattened head of a pin was heated in a flame and the upper layers of the right superior colliculus were destroyed by application of the pin to the overlying skull. Some of the animals received an injection of approximately 2×10^5 hybridoma cells in $2 \mu\text{l}$ of culture medium with a Hamilton syringe in one or both rostral hemispheres. The right eye of the animals was removed to denervate the left superior colliculus, the skin wounds closed with cyanoacrylate glue, and the animals returned to their mother.

Hybridoma cell culture. IN-1 (Caroni and Schwab, 1988b) and anti-HRP antibody-producing hybridoma cells (Schwab and Schnell, 1991) were used in the experiments. Cells were grown in Iscove's modified Dulbecco's medium with supplements as described (Caroni and Schwab, 1988b). Cells were harvested in the logarithmic growth phase, spun down, and resuspended in a small volume of culture medium immediately prior to use for injections.

Tracing of the optic projection. On P7 or P11 pups were anesthetized with Chloropent and the left eye was injected with $3 \mu\text{l}$ of a 50% HRP solution with 2% dimethyl sulfoxide. Animals were returned to their mothers overnight and killed 18–24 hr after the injection. Under deep barbiturate anesthesia, animals were perfused intracardially with 0.9% saline containing 0.25% sodium nitrite followed by 1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer. Brains were post-fixed for at least 6 hr and immersed in 30% sucrose. Coronal sections ($50 \mu\text{m}$ thick) were cut serially with a freezing microtome, collected in 0.1 M phosphate buffer, and further processed as floating sections. The HRP reaction with tetramethylbenzidine (TMB) as a chromogen and sodium nitroferricyanide as a stabilizing agent was carried out according to Mesulam (1978). Reacted sections were mounted on subbed slides, air dried, counterstained with neutral red, and coverslipped.

Analysis of the retinocollicular projection. Sections were viewed under polarized light for optimal detection of HRP-labeled axons, and under bright-field illumination for viewing the cytoarchitecture of the colliculus as revealed by the neutral red stain. The most rostral sections were excluded from the analysis, since the majority of the ipsilateral projection terminates in the rostral half of the superior colliculus (Frost et al., 1979), and it might not have been possible to assign clearly labeled fibers to the recrossed projection. On the serial sections analyzed, label could clearly be followed back to the lesioned superior colliculus through crossing fiber bundles and therefore exclusively represents the recrossed projection. According to the distribution of the HRP-TMB reaction product on the sections that showed the most extensive recrossing of retinofugal fibers, each case was then assigned to one of the following groups: A, label confined to superficial gray; B, in addition to label in superficial gray, moderate label in the stratum opticum; or C, in addition to label in superficial gray, strong or very strong label in the stratum opticum.

Care was taken not to allow a possible bias of the investigator in making these scoring decisions, and due to the clear differences, forced-choice decisions were rarely necessary. However, a blind procedure was not attempted, because those control cases without injected hybridoma cells could be identified by the absence of unspecific effects, such as hydrocephalus. We therefore supplemented the scoring data with a less subjective measurement. The extension of retinal label in the dorsal-ventral axis was measured in each case for the section showing maximal label (maximal "thickness") with the cell-measuring program of the "Neurotrace" computer system developed at MIT (Passera et al., 1988). Due to considerable shrinkage of the sections during the HRP-TMB reaction, these measured values are smaller than the real values. Nu-

merical results were analyzed using the nonparametrical Wilcoxon test (Walter, 1980). The exact number of analyzed cases is given in the Results.

Immunostaining. Each experimental case was checked for the presence of mouse hybridoma cells in the forebrain at the time of death. Coronal sections ($50 \mu\text{m}$ thick) were cut at $400 \mu\text{m}$ intervals through the brain rostral to the level of the lateral geniculate and processed with the Vector Vectastain ABC anti-mouse immunoglobulin kit. Since the mouse hybridoma cells express immunoglobulins constitutively, the procedure was carried out according to the manufacturer's instructions with omission of the incubation with the first antibody. Diaminobenzidine was used as the chromogen to visualize the avidin-peroxidase complexes. The sections were then mounted on subbed slides, air dried, counterstained with 0.1% cresyl violet, and coverslipped. Only experimental animals with detectable hybridoma cells at the time of death were included in the analysis.

A total of 45 animals was analyzed, 25 with IN-1 hybridoma cells, 7 with anti-HRP hybridoma cells, and 13 without hybridoma cells injected.

Results

Analysis of the fate of injected hybridoma cells

In each animal that received an injection of hybridoma cells, frontal sections rostral to the level of the lateral geniculate were collected and analyzed with an immunostain for anti-mouse immunoglobulin as described in Materials and Methods. Survival and growth of hybridoma cells could be confirmed in approximately 50% of the injected animals, and only these were included in the analysis. Hybridoma cells showed a tendency to settle around the ventricular borders, and also invaded tissue adjacent to the ventricles. Figure 1*A* shows a case with hybridoma cells present bilaterally at the region of the corpus callosum. In the case shown in Figure 1*B* hybridoma cells again are present at the ventricular borders and have also invaded parts of the striatum. The lining of the ventricles always showed strong immunoreactivity, indicating the presence of antibody in the cerebrospinal fluid. The presence of antibody within the superior colliculus itself was not examined in the experimental animals, because complete serial sections of this area were reacted for HRP-TMB histochemistry. In more rostral sections, however, immunoglobulin staining extended 1–2 mm into the tissue of the diencephalon, despite the decreased sensitivity of the immunostaining due to the glutaraldehyde fixation required for optimal sensitivity of the HRP-TMB procedure. In two animals that only had an injection of the IN-1 hybridoma cells without further surgery, a similar penetration of antibody staining into the tissue of the superior colliculus could be observed. It therefore appears likely that in those animals with hybridoma cells present in the rostral brain antibodies did penetrate into the superficial layers of the superior colliculus. Animals with growth of hybridoma cells usually also developed a hydrocephalus (obvious in Fig. 1*A,B*).

Termination pattern of retinal fibers in control animals (not treated with the IN-1 antibody)

After a lesion of the right superior colliculus and removal of the right eye at P1, optic tract fibers from the left eye abnormally cross the midline at the level of the superior colliculus and terminate in the denervated left superior colliculus (Schneider, 1973; So and Schneider, 1978). The tectal termination area of these fibers, however, is mostly limited to the superficial gray of the left superior colliculus, and very little fiber growth and termination can be detected in the deeper stratum opticum. A typical case is shown in Figure 2*A–C*. Despite massive recrossing

Figure 1. Settlement of IN-1 hybridoma cells injected in the neonatal hamster brain. The animal was killed at P12. Hybridoma cells are labeled by an anti-mouse immunoglobulin staining and appear *dark black*. The cells often invaded and formed tumors in structures close to the ventricles, like the corpus callosum (*A*), but they could also invade structures like the striatum (*B*). Note the considerable hydrocephalus that formed in both cases. Scale bar, 1 mm.

of retinofugal fibers, the label is confined mainly to the area of the superficial gray.

In animals that, as a control, had anti-HRP hybridoma cells injected, the same pattern of termination was found. A typical case is shown in Figure 3*A–C*. The fibers from the left retina remain confined to the superficial gray.

Termination pattern of retinal fibers in animals with IN-1 hybridoma cells

In animals with the IN-1 antibody present there was more variability in the tectal labeling pattern of retinal fibers. In the vast majority of these cases the label of the retinal fibers extended more ventrally than in the control cases, and often included the full depth of the stratum opticum. In many cases the label in the stratum opticum was as dense as in the superficial gray. One such case is shown in Figure 2*D–F*. The qualitative contrast with the control case shown in Figure 2*A–C* is obvious. Two more examples are shown in Figure 3. The case in Figure 3*D–F* is labeled very heavily. In the bright-field view of the HRP-TMB reaction product, it is thus possible to recognize fiber bundles running from medial to lateral in the stratum opticum of the left superior colliculus, whereas the label in the superficial gray is more uniform (Fig. 3*F*). This pattern mimics the normal pattern, with retinal fibers being located in the stratum opticum and only arbors penetrating into the superficial gray.

The observed expansion of the terminal area cannot be explained simply by enhanced crossing of retinal fibers in the IN-1-treated cases. Figure 3*G–I* shows an IN-1-treated case that has only few recrossing fibers, yet the weak label in the superior colliculus is distributed throughout the superficial gray and the stratum opticum with approximately the same intensity. In this case it is very unlikely that the deep fibers have been forced down only because all termination sites in the superficial gray were already occupied.

Quantitative analysis of the data

A total of 45 hamster pups with surgery on P1 and death at P12 are included in this study: 25 animals had successful forebrain injections of the IN-1 hybridoma cells, 13 were animals without

hybridoma cells injected, and 7 were animals that successfully received the anti-HRP hybridoma cells. Since the results for the uninjected control animals were very similar to those of the anti-HRP-treated animals, they are treated as a single group in the following. Animals receiving injections of hybridoma cells were only included in the study if the presence of these cells in the brain at the time of death could be confirmed by the anti-mouse immunoglobulin immunostaining (cf. Fig. 1).

The first method for quantifying the data was to use a scoring system, in which each case was assigned to one of the following three groups: A, label confined to superficial gray; B, in addition to label in superficial gray, moderate label in the stratum opticum; C, in addition to label in superficial gray, strong or very strong label in the stratum opticum. The results are shown in Figure 4. Most of the control cases fall into group A, with virtually no label in the stratum opticum (Fig. 4, left). In contrast, the majority of the IN-1-treated cases fall into group C, with strong label in the stratum opticum; fewer cases fall into group B, with only moderate label in the stratum opticum (Fig. 4, right).

We also measured the maximal dorsoventral extension of the distribution of recrossed retinal fibers and terminals in the superior colliculus. The results are shown in Figure 5. The IN-1-treated cases show a fairly wide distribution of the individual values, indicating greater variability of the results. However, compared to the control cases, the distribution is clearly shifted to the right and now centered around a mean of 230 μm , that is, about 100 μm more than in the control cases. For the control cases a narrow distribution in a band around a mean thickness of 120 μm became apparent. If the two groups are compared with the distribution independent Wilcoxon test, they are significantly different with $p < 0.001$.

Discussion

Restriction of the termination pattern in control cases

The recrossing of retinal fibers from one side of the brain to the other after a unilateral ablation of the superior colliculus soon after birth in hamsters is a dramatic example of the massive reorganization possible in the neonatal CNS. The time period

Figure 2. Tectal termination patterns of retinal fibers of a control without injected hybridoma cells (*CTR*, *A–C*) and an *IN-1*-treated (*+IN-1*, *D–F*) case: overview (*A*, *D*) and higher magnification (*B*, *C*, *E*, *F*) under polarized light (*A*, *B*, *D*, *E*) and bright-field illumination (*C*, *F*). Approximate location of the superficial gray (*SGS*) and the stratum opticum (*SO*) is indicated in *C* and *F*. In the control case the label is confined to the superficial gray (*SGS*), whereas in the *IN-1*-treated case both the superficial gray and the stratum opticum (*SO*) are labeled with equal density. Scale bars: *D*, 500 μ m for *A* and *D*; *F*, 200 μ m for *B*, *C*, *E*, and *F*.

when such changes are possible, however, ends soon in early postnatal life (Devor, 1976; So et al., 1981; Kalil and Reh, 1982). Furthermore, the innervation of the targets is often abnormal, and may resemble, but usually falls short of being identical to, the normal pattern. This is also the case for the recrossed retinotectal projection. Despite the denervation of the superior colliculus by contralateral eye removal, only the superficial gray becomes densely innervated by the retinal fibers, and the ingrowing axons seldom enter the stratum opticum, their normal principal pathway. Interestingly, a very similar termination pattern is observed when embryonic retinas are grafted to the brains

of neonatal hosts. In these cases the fibers from the grafted retina also course and terminate predominantly in the superficial gray of the host tectum (McLoon and Lund, 1980; Hankin and Lund, 1987, 1990). Both experiments have in common that retinal fibers arrive later than normal in the superior colliculus and therefore encounter an environment different from the one seen by normal fibers. One of these changes is that the corticotectal pathway starts innervating the stratum opticum of the superior colliculus at about P6 (Ramirez et al., 1990). Therefore, the available postsynaptic sites in the stratum opticum might already be occupied upon arrival of the retinal fibers. Although

Figure 3. Tectal termination patterns of retinal fibers of a case with anti-HRP control antibody (+ α -HRP, *A–C*) and two IN-1-treated cases (+IN-1, *D–F* and *G–I*): overview (*A*, *D*, *G*) and higher magnification (*B*, *C*, *E*, *F*, *H*, *I*) under polarized light (*A*, *B*, *D*, *E*, *G*, *H*) and bright-field illumination (*C*, *F*, *I*). Approximate outlines of the superficial gray (SGS) and the stratum opticum (SO) are indicated in *C*, *F*, and *I*. In the case with the control antibody directed against HRP (α -HRP), the label is confined to the superficial gray. In the IN-1-treated case shown in *D–F*, the label of the crossing retinal fibers is so strong that parallel running fibers can be recognized in the stratum opticum on the bright-field picture (*F*). The IN-1-treated case shown in *G–I*, in contrast, only shows little crossing, but still the label is equally dense in the superficial gray and the stratum opticum (*H*). Scale bars: *G*, 500 μ m for *A*, *D*, and *G*; *I*, 200 μ m for *B*, *C*, *E*, *F*, *H*, and *I*.

such an explanation cannot be completely ruled out, it is unlikely, since the corticotectal projection is much smaller than the retinotectal projection, and the recrossed fibers arrive early enough (from P3 on; Carman et al., 1987) to compete with the cortical fibers for terminal space.

Another important change in the microenvironment of the superior colliculus is that oligodendrocytes, as revealed by the Rip antibody, start to appear in the stratum opticum by about P5, first at the rostrolateral edge, and spread to all parts of the stratum opticum by P9 (Schneider et al., 1990; Jhaveri et al., 1992). Oligodendrocytes express on their surface potent inhibitors of neurite growth (Caroni and Schwab 1988a,b). Inhibitory activity in the rat optic nerve can be detected at birth, that is, 5–6 d before the onset of myelination (Caroni and Schwab, 1989). Inhibitory activity in the stratum opticum, but not in the superficial gray, therefore can be expected to be present at P5, when Rip-positive pre-ensheathing oligodendrocytes are first detected in the stratum opticum. It is possible that neurite growth inhibitors are expressed even earlier than the Rip antigen, but this possibility awaits further study. Evidence for an early presence of inhibitory activity comes from observations made in animals where the recrossed retinal projection has been analyzed

at P8. At this time point the recrossed projection does not yet always cover the whole mediolateral extension of the superior colliculus, but the restriction of the fibers to the superficial gray is already obvious, particularly in the more lateral aspects of the superior colliculus (J. P. Kapfhammer, unpublished observations). Therefore, inhibitory activity sufficient to affect the course of retinal fibers must already be present in the stratum opticum at and before P8. This inhibitory activity cannot be strong enough to prevent any fiber growth completely, however: cortical afferents are able to invade and terminate in the stratum opticum from P6 onward in normal animals (Ramirez et al., 1990). Inhibitory activity from oligodendrocytes can nevertheless be sufficient to prevent retinal fibers from invading the stratum opticum. These fibers have a major part of their normal target area, the superficial gray, still available and therefore may have a choice of where to go. The situation is different for the corticotectal fibers, which primarily synapse in the stratum opticum and only later in development invade the basal part of the superficial gray (Ramirez et al., 1990). Recrossing retinofugal fibers and cortical fibers may also differ in their sensitivity toward the neurite growth inhibitors; that is, sprouting retinal neurites could be more sensitive than the cortical neurites. At

Figure 4. Frequency of assignment of control and IN-1-treated cases to one of the following groups: *A*, label confined to superficial gray; *B*, in addition to label in superficial gray, weak or moderate label in the stratum opticum; or *C*, in addition to label in superficial gray, strong or very strong label in the stratum opticum. $n = 20$ for control; $n = 25$ for IN-1-treated cases.

P6, cortical neurites are in a developmentally more immature stage than retinal neurites. Such differences in developmental stage and origin might influence the responsiveness of nerve fibers toward neurite growth inhibitors (Wictorin et al., 1990).

Expansion of the retinal terminal area and layer specificity in the presence of IN-1 antibodies against neurite growth inhibitors from oligodendrocytes

In animals with the IN-1 antibody present recrossing retinal fibers terminate heavily in the stratum opticum, a pattern not seen in control cases without this antibody. This suggests that the observed restriction of the recrossed retinal projection to the superficial gray in cases without the IN-1 antibody is due to the activity of neurite growth inhibitors from oligodendrocytes. This interpretation is further supported by findings with the Rip antibody, indicating the presence and pattern of first appearance of oligodendrocytes in the stratum opticum (Schneider et al., 1990; Jhaveri et al., 1992). It confirms that neurite growth inhibitors NI-35 and NI-250 can be present well before myelination, and that their presence not only restricts fiber growth in white matter fiber tracts but also can have significant effects on the termination pattern of afferent fibers in a gray matter target field in early postnatal life.

Two important questions concerning antibody penetration and specificity should be addressed. Sufficient amounts of antibody need to be present in the stratum opticum. In this study antibody penetration probably was facilitated by the immaturity of the very young animals and the nearby brain lesion. Nevertheless, significant variations in antibody penetration from case to case are to be expected. This is reflected in the data, which show considerable variability of the fiber distribution in the IN-1-treated animals.

Another possibility to be considered would be that the observed changes are due to unspecific effects of the antibody treatment such as hydrocephalus and the presence of mouse immunoglobulin. This is unlikely, since animals with the control hybridoma cells present in the brain showed a termination pattern identical to that of untreated animals, despite the formation of hydrocephalus and the presence of mouse immunoglobulin.

Figure 5. Frequency distribution of the extent of label, that is, the maximal extension of retinal label in the dorsoventral axis in the superior colliculus: absolute values in μm . Note the difference between control (left) and IN-1-treated cases (right). $n = 20$ for control; $n = 25$ for IN-1-treated cases.

That the changes in the distribution of recrossing retinal fibers after IN-1 treatment might be the result of enhanced crossing is also ruled out. The extent of crossing in our experiments was similar in experimental and control cases. The amount of crossing is mainly related to the anatomy of the lesion site. It has been shown that a lesion of midline radial glial cells is both necessary and sufficient to induce crossing of retinal fibers (Wu et al., 1990; D.-Y. Wu, G. E. Schneider, and S. Jhaveri, unpublished observations). Without a lesion of the midline, there is no crossing, a finding also in IN-1-treated animals (Kapfhammer, unpublished observations). In the few cases with only little crossing in the presence of the IN-1 antibody, fibers still spread out and terminate in the stratum opticum (Fig. 3*G-I*). This makes very unlikely the hypothesis that the terminal sites in the superficial gray are simply preferred by retinal fibers and that, in the presence of the IN-1 antibody, fibers and terminals spread to the less attractive stratum opticum only because of an enhanced crossing. It rather suggests that retinal fibers are indeed excluded from sprouting into the stratum opticum by the presence of neurite growth inhibitors, and are able to do so if these inhibitors are neutralized.

We did not examine in this study whether the presence of the IN-1 antibody interferes with oligodendrocyte differentiation. In a similar study on the effects of the IN-1 antibody on the development of the corticospinal tract in neonatal rats (Schwab and Schnell 1991), however, the presence of the IN-1 antibody did not alter the developmental appearance of immunostaining for myelin basic protein (M. E. Schwab, personal communication). This suggests that oligodendrocyte differentiation does proceed normally in the presence of the IN-1 antibody, and that the observed effects are due to the neutralization of the inhibitory proteins rather than the interference with oligodendrocyte differentiation or survival.

Is there a role for neurite growth inhibitors from oligodendrocytes in the normal brain?

This study shows that neurite growth inhibitors from oligodendrocytes can have distinct effects on the termination pattern of afferent fibers in a CNS target area. It does not mean that during normal development they are involved in the formation of such patterns. The evidence from many studies points toward specific target recognition and fiber–fiber interactions as the main mechanisms involved (for review, see Udin and Fawcett, 1988). In the experiments described here, it was necessary to denervate the target area. Otherwise, retinal fibers would not have spread beyond the medial third of the superior colliculus due to competitive interactions (Schneider, 1973). This competition appears not to be affected by the presence of the IN-1 antibody (Kapfhammer, unpublished observations).

During early embryonic development, neurite growth inhibitors from oligodendrocytes have no impact (since they are not yet present), but this can be different for developmental processes that are occurring late and last until after the expression of these proteins. This is the case for the spinal growth of the corticospinal tract (Schwab and Schnell, 1991), or after early postnatal lesions resulting in postnatal fiber growth as in this study. In the adult, neurite growth inhibitors contribute to the absence of regenerative fiber growth in the CNS (Savio and Schwab, 1990; Schnell and Schwab, 1990).

This study raises questions about the differential distribution of myelin and oligodendrocytes in CNS gray matter. Oligodendrocytes appear late in the superficial gray of the superior colliculus, and even in the adult remain fairly sparse nearest the pia, despite an intense presence of myelin antigens in the deeper layers of the superior colliculus (Jhaveri et al., 1992). This corresponds to a sprouting of retinal axons in the most superficial part of the superficial gray in hamsters, induced by lesions in animals as old as P30 (Schneider et al., 1985). Similarly, the molecular layer in the cerebellum, layer I of the cerebral cortex, and several layers of the hippocampus remain almost free of oligodendrocytes and myelin even in adult stages (Jacobson, 1963; Agrawal et al., 1977; Reynolds and Wilkin, 1988). It is likely that limited amounts of sprouting are at least more easily achieved in such regions as compared to gray matter areas with considerable myelin present. The regions and layers devoid of myelin and oligodendrocytes would therefore be candidates for enhanced plasticity and sprouting even in the undamaged adult brain. The extent to which this is indeed the case still remains to be shown, but the presence or absence of neurite growth inhibitors from oligodendrocytes should be considered in situations where fiber growth or sprouting in the postnatal or adult brain is an issue.

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