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Oligodendrocytes Arrest Neurite Growth by Contact Inhibition

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We have used video time-lapse microscopy to analyze *in vitro* the interactions of growth cones of newborn rat dorsal root ganglion cells with dissociated young rat CNS glial cells present in the cultures at low density. To provide optimal conditions for neurite extension, cells were grown on laminin and in NGF-supplemented medium. Our initial observations showed that there are 2 subpopulations of growth cones differing in their growth rate on laminin (averages of 12 and 45 μm/hr). When these growth cones encountered *astrocytes,* they maintained their normal configuration and growth velocity. They subsequently grew along or on top of astrocytes. In some cases, however, fast-moving growth cones showed a slight reduction in their growth rate. When growth cones countered *oligodendrocytes,* however, firm filopodial contact was sufficient to induce a rapid and long-lasting arrest of the growth cone motility, often followed by a collapse of the growth cone structure. One third of the paralyzed growth cones were observed to retract. Growth arrest and growth cone collapse were strictly dependent on membrane contact between neurons and oligodendrocytes. This contact inhibition phenomenon was exclusively found with differentiated oligodendrocytes and could be prevented by the monoclonal antibody IN-1 directed against neurite growth inhibitors NI-35 and NI-250 (Caroni and Schwab, 1988b). These results confirm previous findings that the neurite growth inhibitor proteins are important in axon outgrowth. Further, the inhibition of neurite growth exerted by oligodendrocytes is a contact-mediated phenomenon that can be triggered by the tip of growth cone filopodia.

Neurite growth in both developing and regenerating nervous systems is crucially influenced by the location of a nerve cell and the environment encountered by its axon. Soluble molecules such as trophic and tropic factors, and direct membrane contacts with non-neuronal cells, other neurites, or with the extracellular matrix, can support and direct axonal growth in the CNS and PNS. A growing number of cell adhesion and substrate molecules have been identified in the vertebrate (reviewed by Jessel, 1988; Rutishauser and Jessel, 1988) and invertebrate nervous systems (reviewed by Anderson, 1988). *In vitro* and some *in vivo* studies have shown that antibodies against such molecules can disrupt cell migration, neurite growth, fasciculation of neurites, and interactions between specific cell types (Rutishauser et al., 1978; Edelman, 1986; Rathjen et al., 1987; Kunemund et al., 1988; Moos et al., 1988). Very recently, constituents with repulsive or inhibitory effects on neurite growth or cell adhesion are being found in the developing and adult nervous systems (Caroni and Schwab, 1988a; Cox et al., 1990; Davies et al., 1990; Raper and Kapfhammer, 1990). However, much of the *in vivo* roles played by all these molecules remain to be clarified. Their spatial and temporal patterns of expression suggest complex interacting effects, possibly also with soluble molecules.

Regeneration is, in part, a repetition of developmental processes. In adult vertebrates, lesioned nerve fibers regenerate extensively within the PNS, but poorly, if at all, within the CNS. Increasingly more is known about the factors and conditions that contribute to a successful reelongation of lesioned neurites over long distances in the PNS (Abrahamson et al., 1986; Daniloff et al., 1986; Heumann et al., 1987; Rieger et al., 1988). In contrast, absence of fiber regeneration in the CNS of higher vertebrates is poorly understood.

Transplantations of pieces of peripheral nerves into various parts of the adult rat brain and spinal cord after lesion gave evidence for the capacity of central nerve fibers to regenerate (David and Aguayo, 1981; So and Aguayo, 1985). Recent studies demonstrated the existence of trophic factors (NGF or brain-derived neurotrophic factor) in the CNS, also in the adult (Korsching et al., 1985; Davies et al., 1986; Shelton and Reichardt, 1986). In addition, a number of survival or neurite-promoting activities could be extracted from lesioned brain tissue (Whittemore et al., 1985, 1987; Nieto-Sampedro and Berman, 1987; Zhou et al., 1987). However, as shown by the absence of neurite ingrowth into optic nerve explants in culture in the presence of trophic factors (Schwab and Thoenen, 1985), a hypothetical lack of trophic factor production in the CNS (Ramon y Cajal, 1928) cannot entirely account for the absence of fiber tract regeneration in higher vertebrates. Instead, other constituents or compounds found in the microenvironment of reelongating nerve fibers in the CNS have to be postulated.

The roles of the different CNS glial cell types for neuronal development and regeneration are not well understood. In early development, neurons migrate along radial glia processes (Rakic, 1988). In cerebellar slices, this migration could be disturbed by the addition of antibodies against the cell adhesion molecules L1, N-CAM, or AMOG (Lindner et al., 1986; Antonicek et al., 1987). *In vitro,* astrocytes are good substrates for neuronal adhesion and fiber growth (Hatten et al., 1984; Fallon, 1985), in contrast to oligodendrocytes, which were shown to represent an inhibitory substrate for growing neurites (Schwab and Caroni, 1988). Subsequent observations indicated that defined com-

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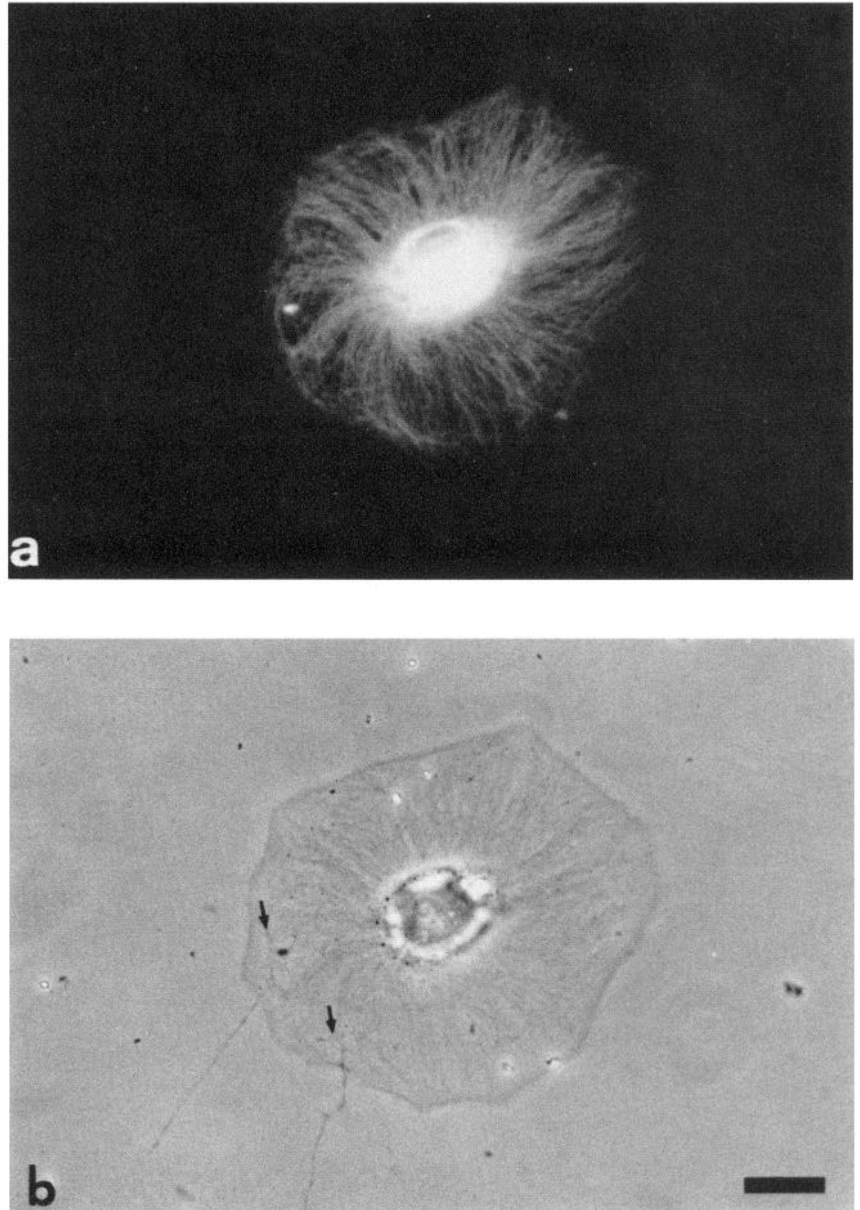

Figure 1. Immunohistochemically identified astrocyte encountered by 2 DRG growth cones (see also Fig. 2). *a,* Indirect immunofluorescence photomicrograph for GFAP. *b,* Phase-contrast photomicrograph of the same astrocyte showing growth cone (*arrows*)–astrocyte relationship. Scale bar, 100 μm.

ponents of oligodendrocyte membranes and of CNS myelin exert strong inhibitory effects on growing neurites and spreading fibroblasts (Caroni and Schwab, 1988a). Two membrane proteins of CNS myelin, NI-35 and NI-250, accounted for these effects. Antibodies against these inhibitory proteins injected into adult optic nerve explants resulted in extensive neurite ingrowth into these optic nerves *in vitro* (Caroni and Schwab, 1988b). Recently, application of these antibodies to lesioned spinal cords in rats was shown to result in regeneration of corticospinal tract fibers also *in vivo* (Schnell and Schwab, 1990). Here, as a first step towards a cell biological understanding of these inhibitory effects, we have examined growth cone interactions of dorsal root ganglion (DRG) neurons with various CNS glial cell types *in vitro* using video time-lapse microscopy. A dramatic difference in growth cone behavior could be observed upon contact with astrocytes or oligodendrocytes. Whereas growth cones interacting with astrocytes maintained their normal morphology and growth velocity, firm filopodial or lamellipodial contact with differentiated oligodendrocytes resulted in a rapid and long-lasting arrest of growth cone motility. This contact inhibition of growth cones was exclusively found with oligodendrocytes and could be prevented by the antibody IN-1, which neutralizes the effects of oligodendrocyte-associated inhibitory membrane proteins.

Materials and Methods

Cocultures of rat CNS glial cells with DRG neurons. Optic nerves were dissected from 6–12-d-old Lewis rats and dissociated as described by Raff et al. (1979). Plastic tissue-culture dishes were modified in order to film directly through the glass coverslips on which the cocultures were grown: holes were cut into the bottom of 35-mm Falcon dishes, and glass coverslips were cemented over the holes from below with paraffin. Optic nerve cells were plated at low density (15,500 cells/cm², 6000 cells/well) on a poly-L-lysine- and subsequent laminin- (10 μg/ml in phosphate buffer; Collaborative Research Inc., Bedford, MA) coated coverslips. Cells were grown in enriched L15 medium (Mains and Patterson, 1973) supplemented with 5% heat-inactivated rat serum. After 2–3 d, dissociated DRG cells of newborn Lewis rats, which had been

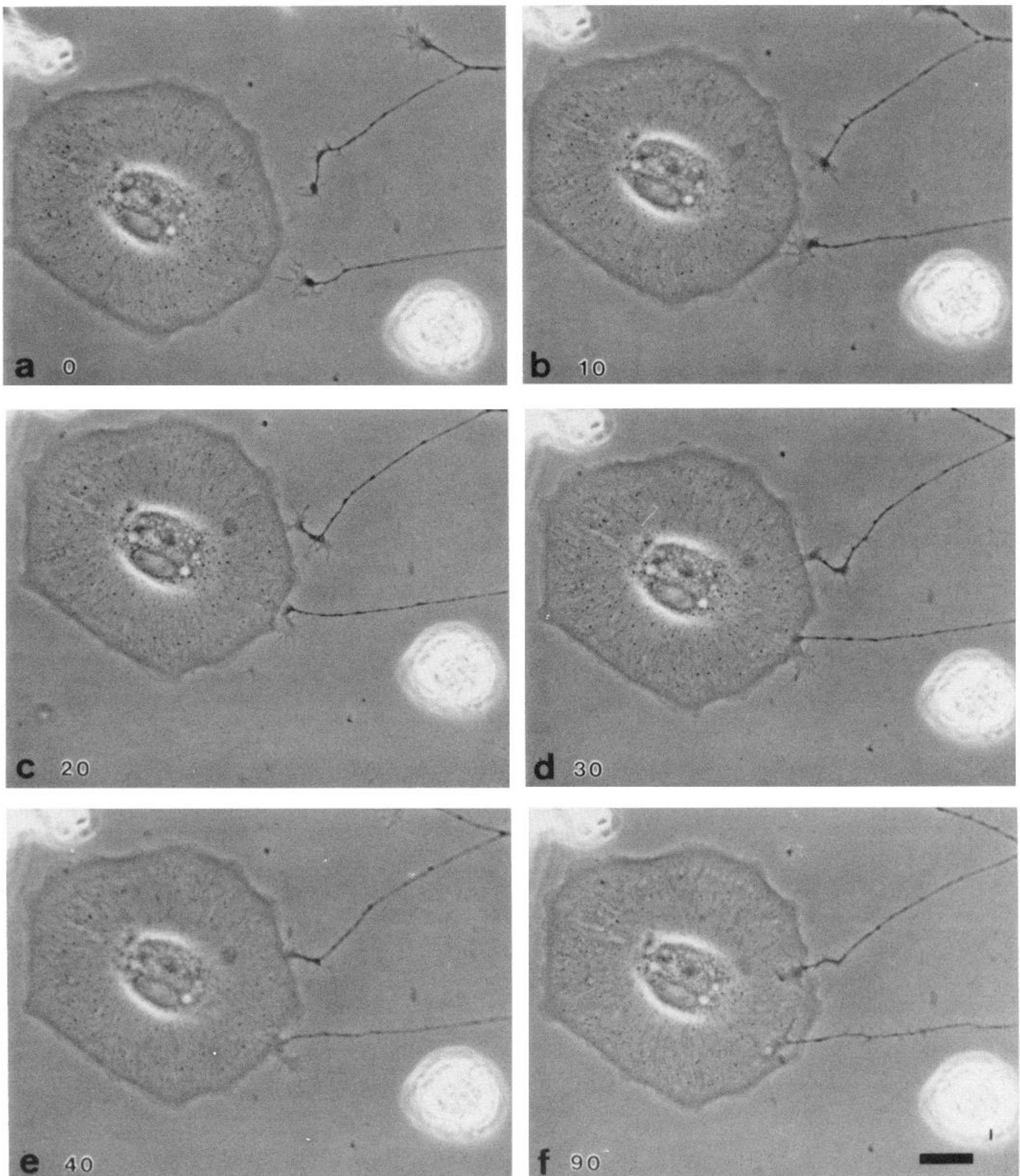

Figure 2. Photomicrographs showing specific sequences in interaction of 2 DRG growth cones with GFAP-positive astrocyte shown in Figure 1. *a,* Growth cones approaching the astrocyte. *b* and *c,* Initial contact and interaction of short filopodial branches with the astrocyte surface. *d–f,* Subsequent movement and growth of DRG growth cones on top of the astrocyte. Times are indicated in min on the *lower left* of the photomicrographs. Scale bar, 100 μm.

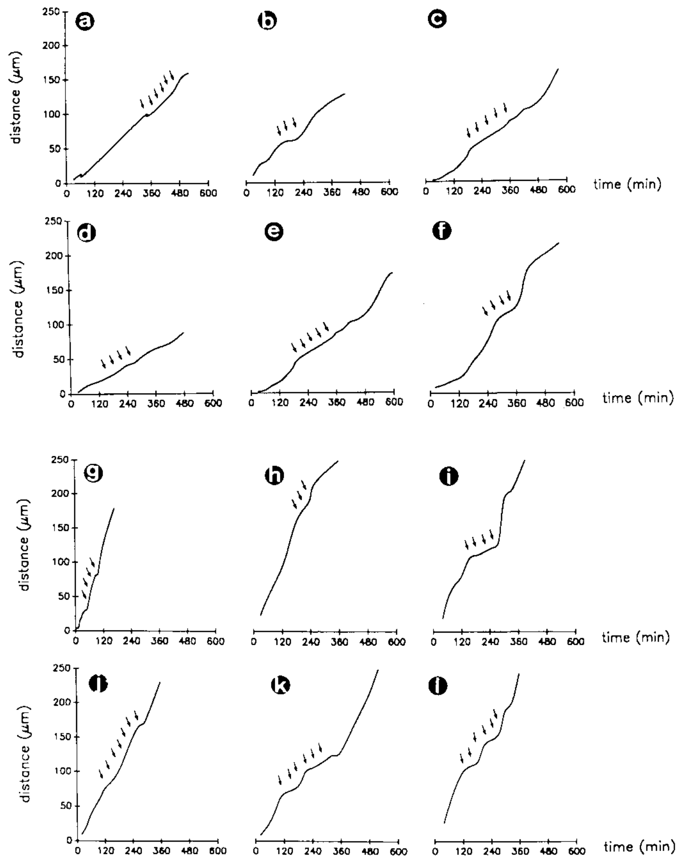

Figure 3. Velocity plots of 12 individual DRG growth cones encountering astrocytes. Positional changes of growth cones were measured in microns and plotted as distance over time. In all plots, firm lamellipodial or filopodial contacts with astrocytes are indicated by *arrows*. *a–f* represent growth cones moving along (*a–c*) or on top of (*d–f*) astrocytes with an average velocity of 12–15 μm/hr. Only minor changes in growth rates are seen upon contact with the astrocyte surface. In contrast, some faster-moving growth cones (average rate of 45–50 μm/hr) reduce their speed by up to 25% when meeting astrocytes or during their subsequent growth along (*g–i*) or on top of (*j–l*) astrocytes.

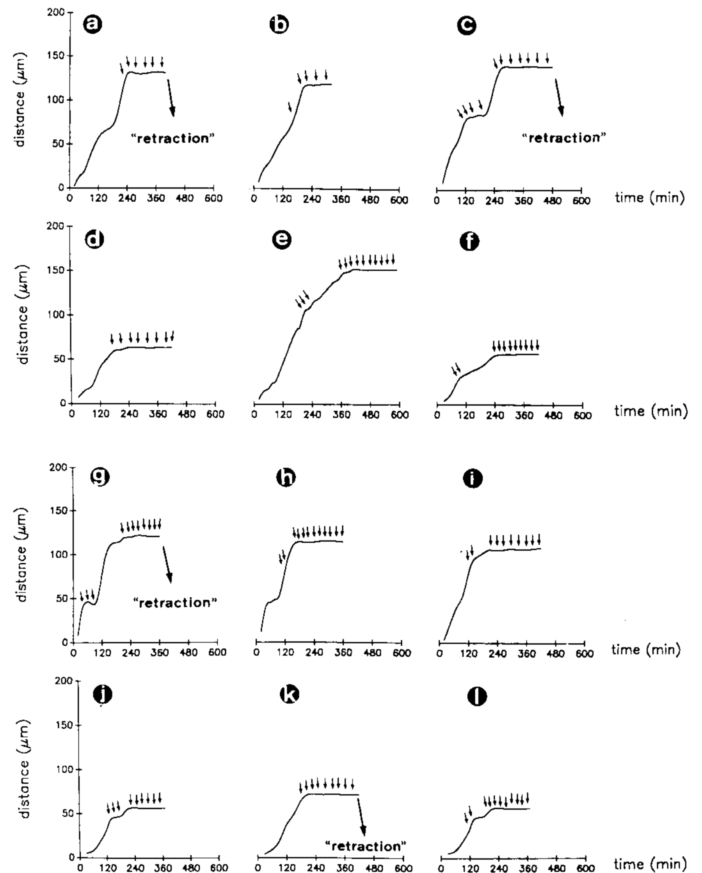

Figure 4. Velocity plots of 12 individual DRG growth cones encountering oligodendrocytes. Arrest of growth cone motility occurs when firm filopodial contacts are established with the oligodendrocytes (*small arrows*). Retraction of growth cones and neurites are indicated by *long arrows*. No obvious behavioral difference can be seen in the response of growth cones interacting with highly branched oligodendrocytes (*a–f*) or O_1-positive cells with few, thick processes (*g–l*). Fast- (*a–c, g–i*) and slow- (*d–f, j–l*) moving growth cones show the same arrest response.

preplated for 3 hr on plastic culture dishes to reduce the amount of non-neuronal cells, were added to the glial cell cultures (7800 neurons/cm^2, 3000 neurons/well). The same medium was used for the cocultures, with the addition of NGF (2.5 S NGF, 50 ng/ml; a gift from Dr. H. Thoenen) and cytosine arabinofuranoside (Ara C; 0.25 nM; Sigma).

Video time-lapse movies. Video time-lapse movies were taken after 12–24 hr of coculture. The medium level was reduced until only the hole over the coverslip was filled. To avoid evaporation, a silicon grease ring was put around the hole, filled up with medium, and sealed with a glass coverslip. These sealed chambers, containing about 70 μl fresh, previously equilibrated medium, allowed cell survival and perfect neurite growth for up to 16 hr. Individual culture dishes were transferred onto a heating stage (37°C) of an inverted Olympus IMT-2 microscope. The growth cones were viewed in phase contrast with a 40× Planapo objective (Olympus). The time-lapse video recording system consisted of an Aqua Tec SM72 CCD camera (300 × 300 pixels; Aqua Tec, Kempten, FRG), a Panasonic time-lapse video recorder (Panasonic NV-8051-E), and a VTE monitor. The sampling time for most movies was 3 frames/sec. Photomicrographs were taken either from the monitor with a Nikon FA camera on Kodak TMX 400 film or with a camera attached to the microscope on Kodak Pan 100 film.

Immunofluorescence. After recording, we identified the cells encountered by growth cones using the mouse monoclonal antibody O_1 recognizing a galactocerebroside (Sommer and Schachner, 1981) as a marker for differentiated oligodendrocytes and a rabbit antiserum against glial fibrillary acidic protein (GFAP) as a marker for astrocytes (Dahl and Bignami, 1976). Before antibody incubation, the recorded cultures were washed twice with buffer-P (0.1 M phosphate buffer, pH 7.2, containing 5% sucrose and 0.1% BSA), fixed for 15 min with 4% formalin in buffer-P, and rinsed again. Cocultures were then incubated for 30 min at room temperature with the O_1 hybridoma supernatant (a gift from Dr. M. Schachner), rinsed, and incubated for 30 min with an anti-mouse fluorescein isothiocyanate (FITC)-linked secondary antibody (Cappel, West Chester, PA; diluted 1:100). Staining for GFAP (Dakopatts, Glostrup, Denmark; diluted 1:250) was done for 30 min on cultures previously fixed and treated with 0.1% Triton X-100 for 5 min.

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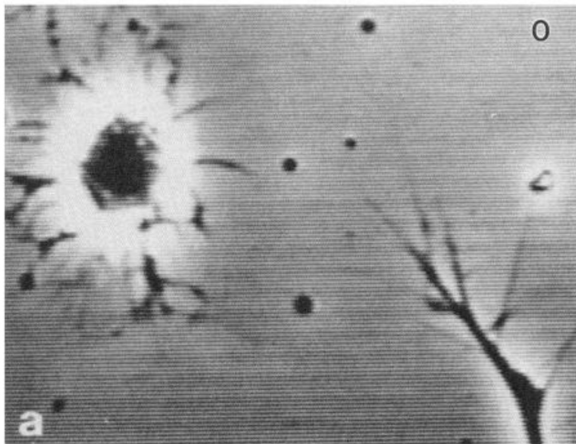
Figure 5. A sequence of time-lapse videomicrographs showing interaction of DRG growth cone and highly branched oligodendrocyte. Initially, a fast-moving growth cone, having long, thin filopodia, can be seen approaching an oligodendrocyte (*a*). Filopodia from this growth cone (*b, arrow*) touch the oligodendrocyte (*b, c*) before establishing firm contact (*d*). Additional filopodia establish permanent contact (*e* and *f, arrows*). This sometimes results in a filopodial fascicle formation (*g*). After 3 hr of immobilization, however, the growth cone collapses and retracts, while remaining in contact with the oligodendrocyte via a thin stretch of cytoplasm (*h, arrow*). Times are indicated in min in the *upper right* of each frame. Scale bar, 100 μm.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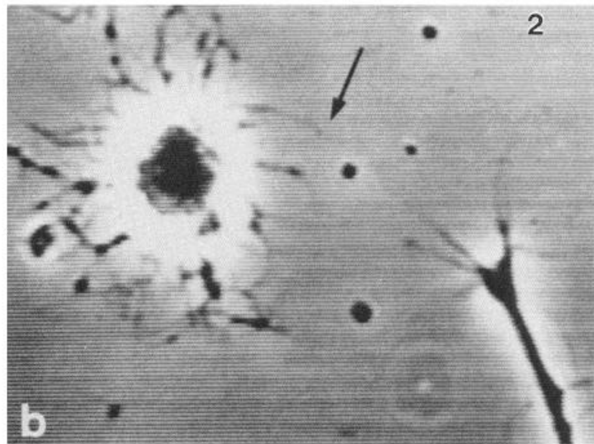
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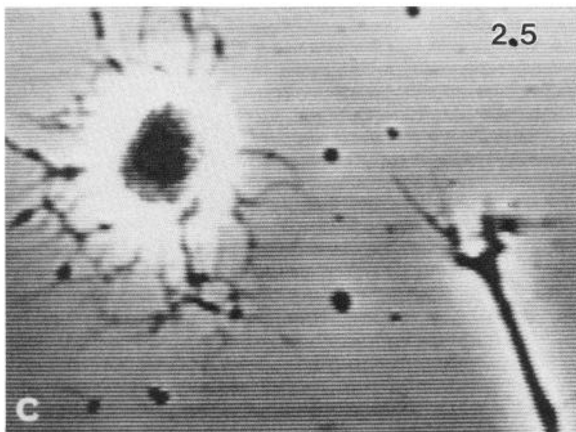
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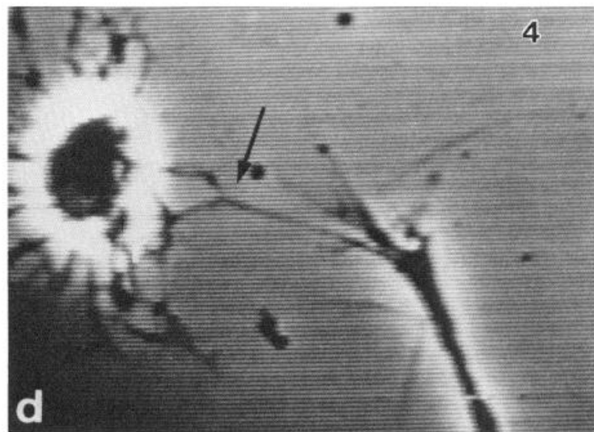
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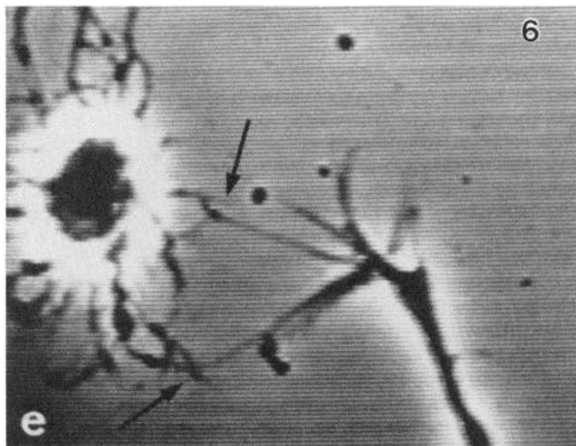
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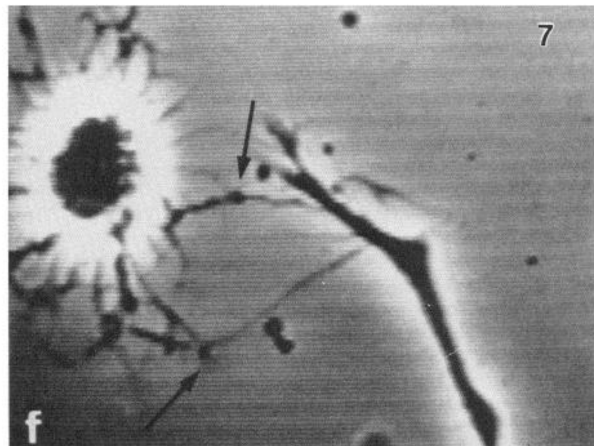
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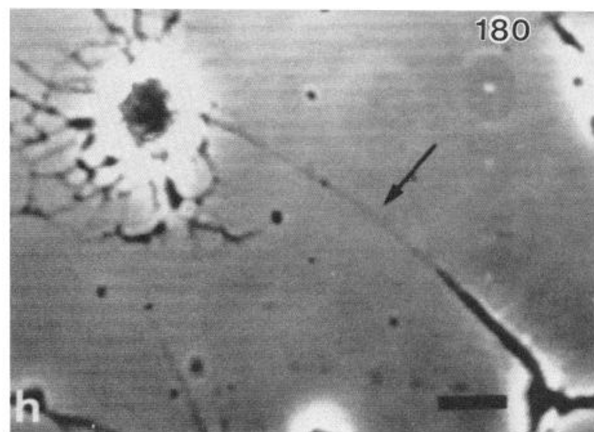
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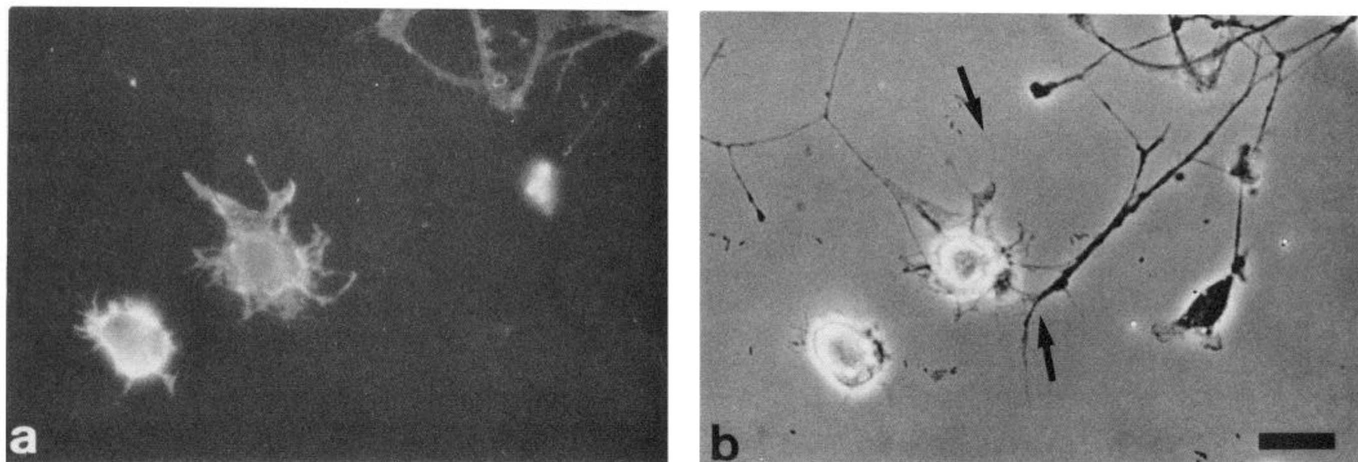

Figure 6. Immunohistochemically identified highly branched oligodendrocyte encountered by DRG growth cone. *a*, Indirect immunofluorescence photomicrograph for O_1. *b*, Phase-contrast photomicrograph of the same oligodendrocyte, showing oligodendrocyte–DRG growth cone (*arrows*) relationship. Scale bar, 100 μm.

For visualization, the cultures were incubated for 1 hr with an anti-rabbit FITC-linked secondary antibody (Miles; diluted 1:100).

Preabsorption of the cocultures with monoclonal IN-1 antibodies. Prior to the addition of DRG neurons, glial cells were preabsorbed with heat-inactivated, 1:2-diluted IN-1 hybridoma supernatant, directed against NI-35 and NI-250 (Caroni and Schwab, 1988b). The antibody was also present at the same dilution throughout recording. Controls were recorded in the presence of O_1 hybridoma supernatant, diluted 1:2. Both these antibodies are IgMs and recognize surface epitopes on living oligodendrocytes.

Quantitative evaluation of growth cone velocities. To illustrate the growth velocities of individual growth cones before and after their interactions with glial cell surfaces, we recorded the relative position of each growth cone in relation to several reference points in 5-, 10-, 20-, or 30-min intervals on transparent paper from the TV monitor. The positional changes were measured in microns and plotted as distances over time, using the graphic computer programs SIGMA-SCAN and SIGMA-PLOT (Jandel Scientific, 1987, Sausalito, CA). Specific events such as filopodial contacts or retracting neurites were also recorded and indicated in the graphs.

Results

General observations

Several cell types were present in short-term cultures of dissociated optic nerves of 6–12-d-old rats: flat cells representing astrocytes (GFAP positive) and fibroblasts, differentiated oligodendrocytes (O_1 positive) with various morphologies, and several types of precursor cells. The main oligodendrocyte subtype present under these culture conditions possessed a radial, highly branched, and anastomosing process network (Schwab and Caroni, 1988). This cell type is often myelin basic protein (MBP)-positive and can form myelinlike structures after long-term culture periods (Yim et al., 1986). In addition to radial, highly branched cells, O_1-positive oligodendrocytes with few, thick processes also occurred, especially with laminin as a substrate. These cells were distinct from the bipolar or polygonal precursor cells by their antigenic characteristics (O_1 positive, A_2B_5 and GD_3 negative).

Dissociated rat DRG neurons were added to the low-density glial cell cultures after 2–3 d. The presence of NGF in the culture medium and the laminin substrate led to a rapid fiber outgrowth. A striking difference was observed in the growth rates of the DRG growth cones. In 40% of the observed cases, the growth cones grew with an average rate of 12–15 μm/hr. These growth cones had extensive lamellipodia with few, short filopodia. Sixty percent of the growth cones advanced with an average rate of 45–50 μm/hr. These growth cones showed a thin configuration and long, thin filopodia. In cocultures with dense neurite networks, the formation of windows around differentiated oligodendrocytes was seen as described previously (Schwab and Caroni, 1988).

Growth cones encountering astrocytes

To analyze the behavioral dynamics of DRG growth cones interacting with astrocytes, we have observed 31 astrocyte–growth cone encounters. Cultures were stained after recording for GFAP to determine the identity of the cells contacted by the DRG growth cones. Fifteen of the observed growth cones followed the edge of astrocytes, 12 grew on top of the astrocytes, and 4 grew under the cells. A typical encounter between an astrocyte (GFAP positive; Fig. 1) and 2 DRG growth cones is shown in Figure 2. The growth cones approached the astrocyte with a growth rate of 15 μm/hr (Fig. 2*a*) and repeatedly touched the cell surface with their short filopodia (Fig. 2*b,c*) over a few min. Having established firm filopodial contact (Fig. 2*d*), the growth cones then moved onto the astrocyte without major changes of their extended, lamellipodial morphology (Fig. 2*e,f*). In this particular case, the growth cones did not reduce their velocity upon contact with or during subsequent growth on top of the astrocyte.

Figure 3 shows the velocity of 12 growth cones before and during their encounter with astrocytes. The initial growth rates on laminin ranged between 12 and 15 μm/hr (Fig. 3*a–f*) or between 45 and 50 μm/hr (Fig. 3*g–l*). Slow-growing growth cones, such as the ones presented in Figure 2, did not detectably change their growth during their growth along (Fig. 3*a–c*) or on top (Fig. 3*d–f*) of the astrocytes. However, some (about ⅓) of the fast-growing growth cones reduced their velocity by up to 25% after having established contact with the astrocyte's surface (Fig. 3*g–l*). Clearly, though, these results confirm the earlier reports that astrocytes are good substrates for neurite growth (Hatten et al., 1984; Fallon, 1985).

Growth cones encountering oligodendrocytes

Whereas DRG growth cones showed no or only minor changes in their motility upon interaction with astrocytes, contact with

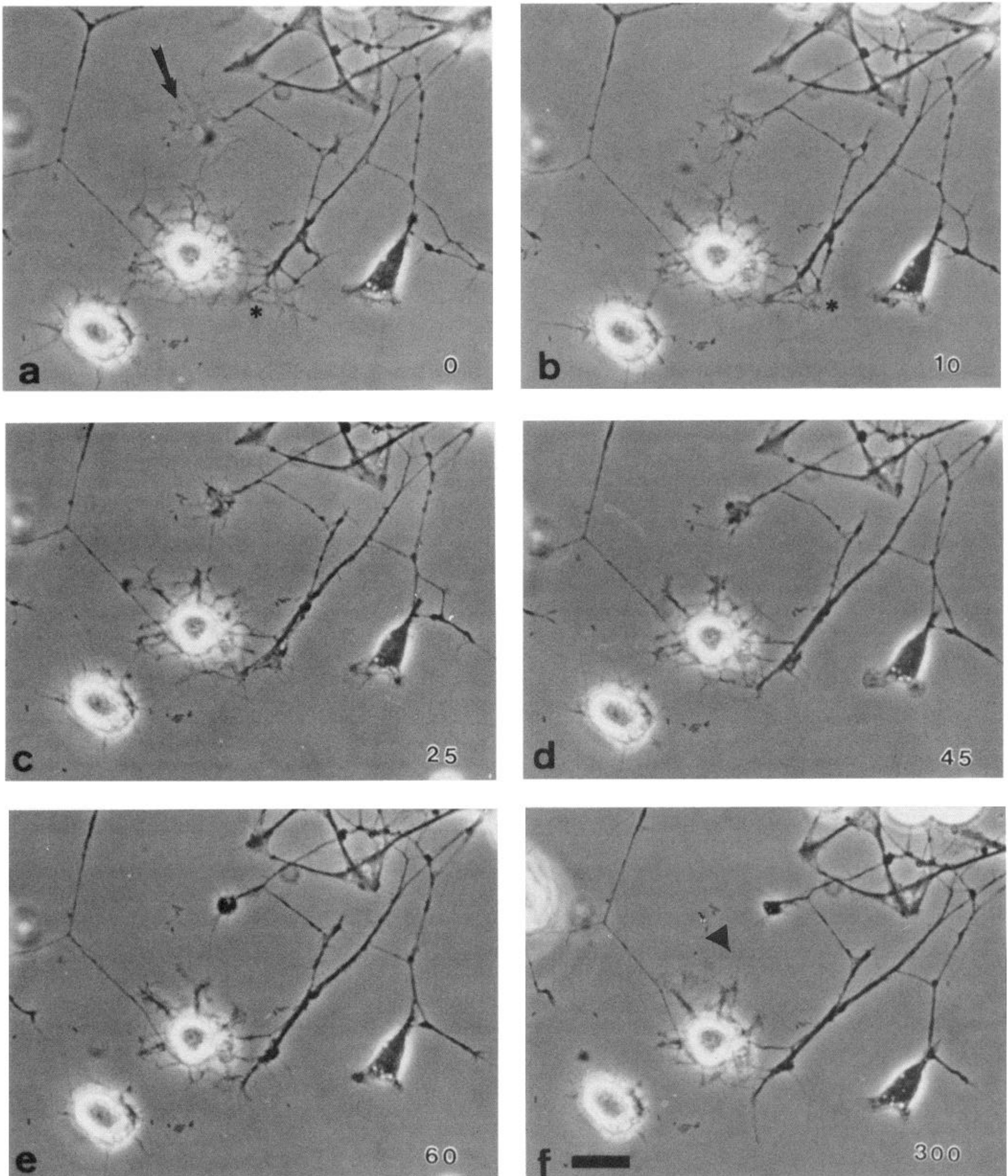

Figure 7. Sequence of phase-contrast photomicrographs showing DRG growth cones encountering oligodendrocyte shown in Figure 6. The growth cone morphologies shown in *a* represent an intermediate (*arrow*) and an advanced (*asterisk*) stage of growth cone–oligodendrocyte contact. *b–f,* A single filopodial contact is sufficient to arrest growth cone motility and collapse growth cone structure. The various growth cones remain immobilized over the entire time of observation (5 hr; *f*). Contact, however, remains between the oligodendrocyte and the collapsed growth cone (*f, arrowhead*). Times are indicated in min on the *lower right* of each frame. Scale bar, 100 μm.

differentiated, O_1- [GalC- (galactocerebroside)] positive oligodendrocytes resulted in an arrest of the growth cones over the entire period of observation (1–6 hr). We have recorded encounters of 36 growth cones with O_1-positive oligodendrocytes. In all (36/36) observed cases, stable filopodial attachment of the growth cones with the oligodendrocytes was followed by an arrest of the growth cones. In about ⅓ of the observed interactions (10/36), the growth cones and the neurites eventually retracted. In these cases, the retracting growth cones often retained contacts with the oligodendrocytes; long, stretched filopodia spanned the distance between the oligodendrocyte and the growth cone, even under the considerable tension produced by the retraction. In a few cases, retracted growth cones, which have lost the connection with the oligodendrocyte, recovered after a certain lag phase: they continued their growth, provided no further contacts with oligodendrocytes occurred. Regrowing growth cones that contacted the same oligodendrocyte, which previously caused collapse and retraction, were immobilized again (data not shown).

Velocity plots of growth cones and their reaction to oligodendrocyte contacts are shown in Figure 4. Notably, the growth cone velocity often decreased immediately after stable filopodial contacts were established (Fig. 4, arrows). No obvious differences in their inhibitory effect were seen for oligodendrocytes of different morphologies (Fig. 4*a–f*, highly branched oligodendrocytes; *g–l*, O_1-positive cells with few, thick processes). Both types of growth cones, the fast-growing ones (Fig 4*a–c*, *g–i*), as well as the slow ones (Fig. 4*d–f*, *j–l*), showed the same arrest responses after filopodial contact with the oligodendrocyte surface.

An example of an encounter between a growth cone and a highly branched oligodendrocyte is shown in Figure 5. Before contact, the growth cone with a long, thin conformation approached the oligodendrocyte with a velocity of 47 μm/hr (Fig. 5*a*). After single neuronal filopodia have surveyed the surface of the oligodendrocyte (Fig. 5*b,c*), 1 of the long, thin filopodia formed firm contact with the process network of the oligodendrocyte (Fig. 5*d*). Growth arrest (forward displacement) occurred simultaneously. Undirected filopodial movement continued for some time, contacted the oligodendrocyte (Fig. 5*e*), and formed a filopodial fascicle (Fig. 5*f*). Finally, filopodial activity ceased, and all growth cone motility was completely arrested (Fig. 5*g*). The growth cone stayed paralyzed over several (6) hours, and finally retracted. Thin processes could be seen keeping contact with the oligodendrocyte surface (Fig. 5*h*).

Another interaction between growth cones and an oligodendrocyte (O_1 positive; Fig. 6) is shown in Figure 7. A large growth cone, having a typical lamellipodial structure, advanced with a rate of 17 μm/hr until it made first filopodial contact with the cell surface (Fig. 7*a*). This contact of 1 or 2 filopodia led within 10 min to the arrest of growth cone motility and to a shrinkage of the growth cone (Fig. 7*b*). The growth cone slowly lost its lamellipodial configuration, started to round up (Fig. 7*c,d*), and completely collapsed after 60 min (Fig. 7*e*). The collapsed growth cone was still in contact with the oligodendrocyte with 1 or 2 thin filopodia after additional 4 hr and showed no signs of recovery (Fig. 7*f*).

Growth cones encountering oligodendrocytes in the presence of monoclonal antibody IN-1

In order to determine whether preadsorption of the cocultures with antibodies neutralizing the oligodendrocyte-associated neurite growth inhibitors could prevent the motility arrest of growth cones upon contact with oligodendrocytes, we observed 10 growth cone–oligodendrocyte encounters in the presence of the monoclonal antibody IN-1 (Caroni and Schwab, 1988b). Control cultures were recorded in the presence of monoclonal antibody O_1 recognizing oligodendrocyte-specific surface antigens (GalC; Sommer and Schachner, 1981). This monoclonal antibody O_1 represents an optimal control because it binds specifically and at high density to oligodendrocyte membranes and, like IN-1, belongs to the IgM class.

No obvious morphological or locomotory changes of isolated glial cells or individual growth cones were seen upon addition of the hybridoma supernatants over up to 7 hr of observation. A typical growth cone–oligodendrocyte encounter in the presence of the IN-1 antibody is shown in Figure 8. The growth cone approached the oligodendrocyte with a velocity of 13 μm/hr. While surveying the cell's surface with short filopodial processes, the growth cone shrank, became long and thin, and reduced its speed for several min (Fig. 8*a*). However, no paralysis or retraction could be seen, and the growth cone then continued to advance with its initial growth rate onto and over the process network of the oligodendrocyte (Fig. 8*b–f*). Seven of 10 growth cones interacting with oligodendrocytes in the presence of IN-1 antibodies showed no visible signs of inhibition and grew along and over oligodendrocytes without major changes in their motility (Fig. 9*a–c*). In the remaining 3 cases, the growth cones moved onto the oligodendrocyte process network at a significantly slower rate (Fig. 9*d,e*). However, this situation, too, is different from the absence of IN-1, where growth cones were stopped at the margin of the process network. In contrast to IN-1, the antibody O_1, in spite of its strong binding to the oligodendrocyte surface, could not neutralize the inhibitory surface properties of differentiated oligodendrocytes (Fig. 9*f–h*).

Discussion

We have used cocultures of young rat CNS glial cells and DRG neurons to study the growth cone behavior upon contact with individual astrocytes or oligodendrocytes by video time-lapse microscopy. Measuring the growth velocity of 56 different growth cones allowed us to distinguish between 2 subgroups of growth cones: fast-growing ones (45 μm/hr), which represent about 60% of all observed cases and have thin growth cones with many, long filopodia, and slow-growing ones (12 μm/hr), which represent about 40% of the growth cones and possess extensive lamellipodia with few, small filopodia. Both types of growth cones occurred within the same culture dish. Because the pop-

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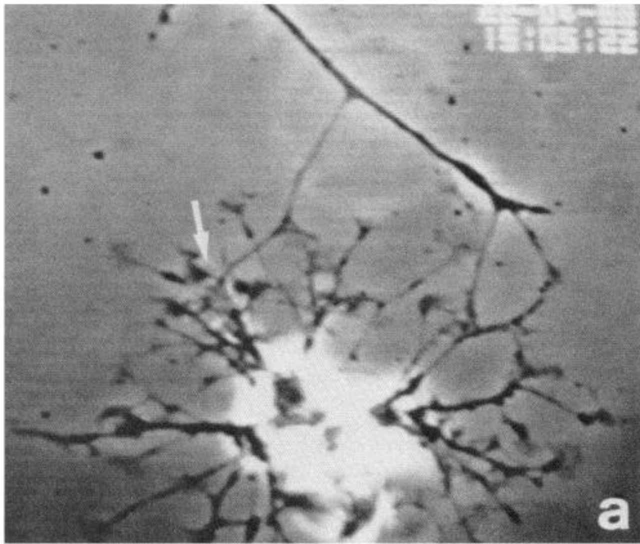
Figure 8. Sequence of time-lapse videomicrographs showing encounter of DRG growth cone with highly branched oligodendrocyte in presence of monoclonal antibody IN-1. Contact between the growth cones (*arrows*) and the oligodendrocyte (*a*), in the presence of the neutralizing antibody, does not lead to an arrest of growth cone motility (*b–f*). Within 20 min, the growth cones have moved under and over several processes of the oligodendrocyte (*b*). Two hr later, the growth cones have completely crossed the processes of the oligodendrocyte (*f*). Times are indicated in hr/min/sec at the *upper right* of each frame. Scale bar, 100 μm.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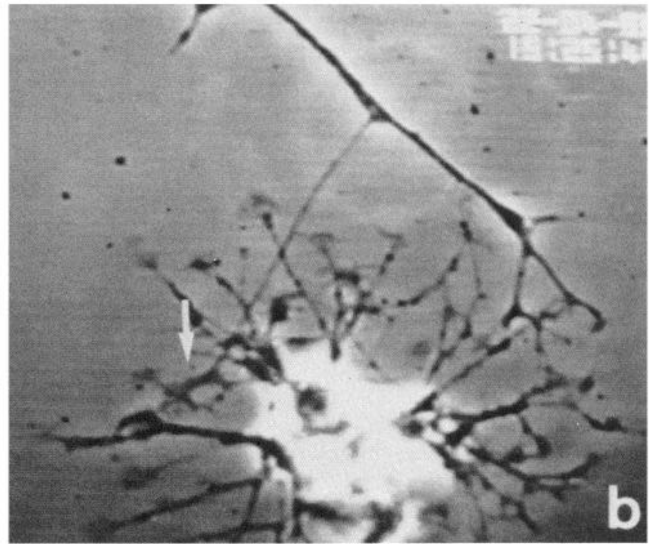
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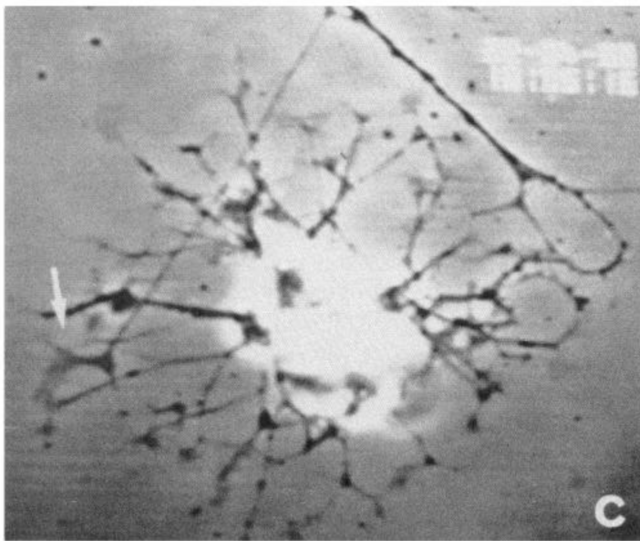
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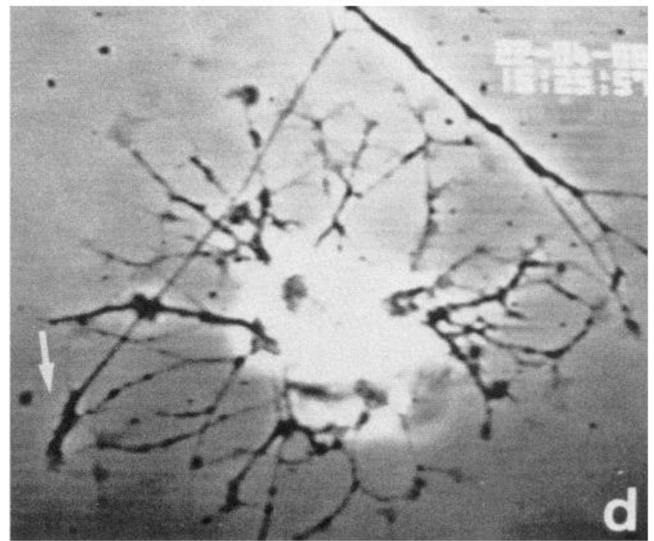
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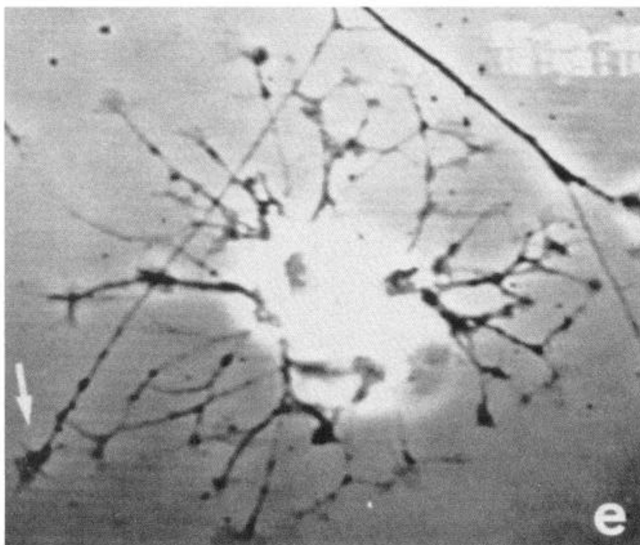
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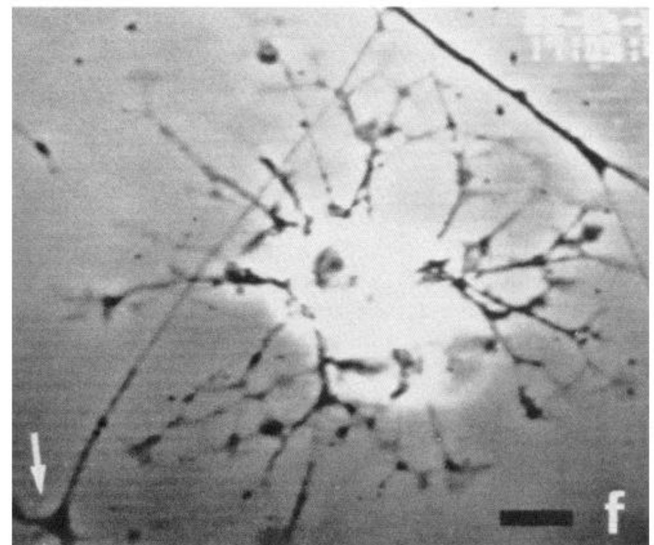
f

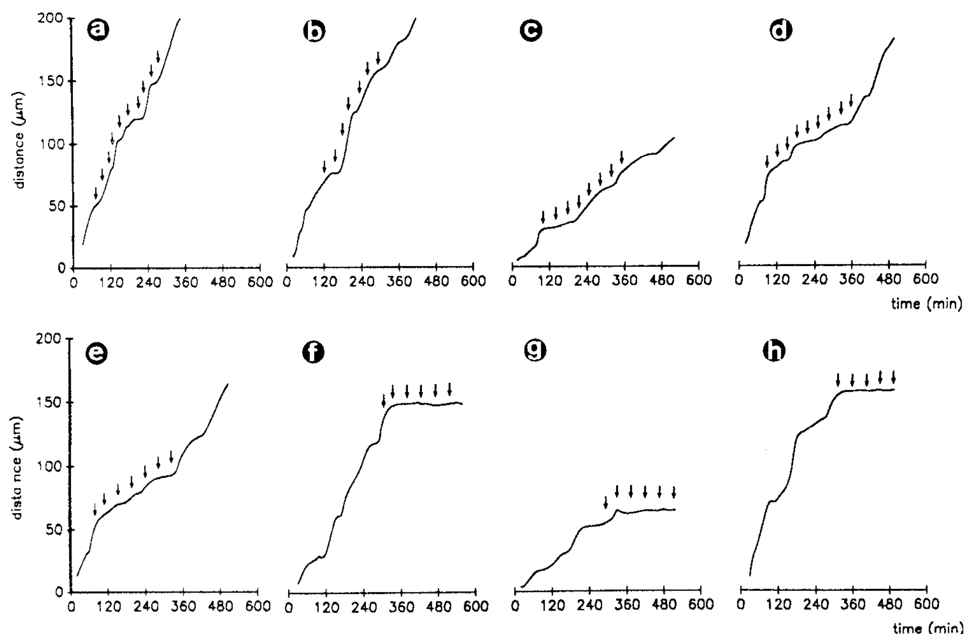

Figure 9. Eight velocity plots of DRG growth cones encountering oligodendrocytes in presence of IN-1 or O_1 antibodies. In the presence of the IN-1 antibody (*a–e*), growth cones, though sometimes reducing their growth rate (*d, e*), continue to move along oligodendrocyte processes (*a–e*). However, in the presence of the O_1 antibody (*f–h*), growth cones become arrested when in contact with the oligodendrocytes.

ulation of neurons within the DRG is heterogeneous, different kinds of growth cones might relate to different neuronal subpopulations. These growth cones could differ, for example, in their expression of laminin receptors, or by other properties.

When growth cones advancing on the laminin substrate contacted an *astrocyte,* slow-growing growth cones continued to advance without changing their velocity along or on top of the astrocytes. Thirty percent of the fast-growing growth cones, however, reduced their growth rate (by up to 25%) when meeting astrocytes and during their subsequent growth on the astrocytes. Nevertheless, our results are in agreement with earlier observations that astrocytes are favorable substrates for elongating neurites (Hatten et al., 1984; Fallon, 1985). In contrast to astrocytes, *oligodendrocytes* were found to display strong inhibitory surface properties for growing neurites. Growth cones, after formation of firm filopodial contact with these cells, underwent dramatic behavioral changes. In all cases observed, their net motility was completely blocked, an arrest that persisted throughout the period of observation. In addition, ⅓ of the arrested growth cones and neurites eventually retracted. Contact of filopodia with the oligodendrocyte surface sometimes persisted even under the considerable tension produced by the retraction. In addition, habituation or densensitization was not observed; retracted growth cones that lost their connection with the oligodendrocyte and recovered were immobilized again when contacting an oligodendrocyte a second time. Our results strongly indicate that the above-described inhibition of growth cone motility is based on a *cell-surface interaction* because the observed effect is strictly contact dependent. A diffusible factor produced by oligodendrocytes, which would act, for example, similarly to certain neurotransmitters as growth inhibitors (Haydon et al., 1984; McCobb and Kater, 1988), can therefore be excluded. The fact that growth cones are arrested or even collapse but do not simply turn away from oligodendrocytes speaks against a mechanism based on differential adhesiveness. It is particularly noteworthy that contact of the tips of very few growth cone filopodia with the oligodendrocytes was sufficient to arrest the growth cones. This suggests the involvement of an intracellular signal transferred from the filopodial tips to the body of the growth cone, where an important part of the machinery for motility and adhesion site formation is located.

Analyzing the inhibitory substrate properties of CNS myelin, Caroni and Schwab (1988a) found that 2 membrane proteins of MW 35 and 250 kDa are mainly responsible for this effect, which could be neutralized by monoclonal antibodies raised against these constituents (Caroni and Schwab, 1988b). In the presence of one of these antibodies (IN-1), our growth cones were no longer arrested upon contact with oligodendrocytes. Quite in contrast, they often maintained their normal speed and grew over the dense oligodendrocyte process network. This observation was never made in control cultures in the presence of the monoclonal antibody O_1, though O_1 antibodies strongly stain and therefore densely bind to living oligodendrocytes.

The cell biological mechanisms underlying this growth cone arrest and paralysis are still unknown. Exchange of intracellular components between growth cones and specific subsets of cells through gap junctions has been shown in the grasshopper embryo (Raper et al., 1982). However, in the rat or chick, gap junctions between growth cones and CNS glial cells have not been observed so far, neither *in vitro* nor *in vivo.* Changes of intracellular calcium in the growth cones have been related to growth cone behavior and motility, especially with regard to inhibitory effects of certain neurotransmitters (Haydon et al., 1984; McCobb and Kater, 1988). These results have demonstrated that calcium concentrations below or above a certain range greatly affect the growth cone motility (Cohan et al., 1987; Mattson and Kater, 1987; Kater et al., 1988). Whether the interaction of growth cone filopodia with oligodendrocyte membranes or with the purified inhibitory proteins NI-35 and NI-250 leads to critical changes in the intracellular Ca^{2+} levels of growth cones is currently under investigation.

The physiological functions of the potent inhibitory effects of oligodendrocytes on neurite growth remains to be investigated. The hypothesis that inhibitory membrane components could play an important role in growth cone navigation is supported by the recent findings of several growth cone collapse-inducing activities found in the developing CNS and PNS. *In vitro* experiments in the developing chick retinotectal system showed

that elongating axons from the temporal part of the retina are repulsed by components present in the membranes of the posterior tectum (Walter et al., 1987). Nasal retinal growth cones do not distinguish between anterior and posterior tectal membranes. In addition, membrane vesicles of the posterior optic tectum induce temporal growth cones to collapse and retract, whereas nasal growth cones are not affected (Cox et al., 1990). Both activities are present in the optic tectum before the generation of oligodendrocytes and disappear after embryonic day 13–14 (Arees, 1978). Kapfhammer and Raper (1987) have described the arrest and collapse of growth cones upon contact with neurites of a different type of neuron (mainly central vs. peripheral). Yet another collapse-inducing activity has been found by Davies et al. (1990) in the posterior half of somites in chicken embryos. This activity appears to be associated with components of 48 and 55 kDa. The differences in localization, cell type, and time of expression of these inhibitory and repulsive constituents indicate that they are not identical. Their functions and interactions with other inhibitory or growth-promoting components remain to be elucidated. The oligodendrocyte- and myelin-associated neurite growth inhibitors appear only late in CNS development. They exert boundary functions for a late-growing CNS tract, the corticospinal tract (Schwab and Schnell, 1991). In the adult CNS, these inhibitory proteins could play an important role in the stabilization of fiber tracts.

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