Brain Research, 459 (1988) 373–380
Elsevier

BRE 23076

Exogenous GM_1 gangliosides protect against retrograde degeneration following posterior neocortex lesions in developing hamsters

Bernhard A. Sabel, Jacqueline Gottlieb* and Gerald E. Schneider

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 (U.S.A.)

(Accepted 17 May 1988)

Key words: Ganglioside; Brain damage; Visual system; Retrograde degeneration; Lateral geniculate body

Developing and adult hamsters received unilateral neocortex aspiration lesions and were then treated daily with exogenous ganglioside (GM_1, 30 mg/kg, i.p.). When lesions were made at the age of two weeks, GM_1-treated animals had less shrinkage of the dorsal lateral geniculate nucleus of the thalamus compared to controls. Although a similar observation was made in adults, the effect was not as striking. Thus, GM_1-treatment reduces retrograde degeneration after neocortical lesions and this effect is most pronounced during early development.

Treatment of brain-damaged, mature rodents with monosialogangliosides (GM_1) or nerve growth factor (NGF) has recently been shown to reduce the severity of retrograde degenerative events, and behavioral studies of brain-damaged rats have demonstrated that such a treatment ameliorates some of the functional deficits[9,15,23–27,29,37]. For example, following hemitransections of the nigrostriatal pathway, a loss of tyrosine hydroxylase (TH), indirectly indicating a loss of dopamine-containing neurons in substantia nigra, was counteracted by systemic GM_1 treatment[35]. Evidence for ganglioside treatment-induced reduction of retrograde degenerative events has also been found in the case of lesions in other systems[5,22,33]. Given this evidence in the injured adult nervous system, the question arises as to whether GM_1 treatment has similar effects when lesions are made early in life.

That gangliosides have a special involvement during normal ontogenesis is well known[38]. A major increase in endogenous gangliosides coincides in time with neuropil proliferation, synapse formation, and myelination[7,20,36] and it immediately precedes synaptogenesis in vitro and is maintained throughout that period[8].

A first indication that GM_1 reduces injury-related degeneration in the developing CNS has recently been obtained[28]. In this study, we assessed the effects of GM_1 on axonal sprouting of retinofugal fibers by creating unilateral tectal lesions in hamster pups on the day after birth. Routine evaluation of the lesion size revealed, unexpectedly, that GM_1-treated pups had significantly less damage in the medial portion of the contralateral tectum, and that the overall size of the tectal lesion was significantly reduced. This result suggested to us that secondary degeneration of cells which were partially injured, or which were axotomized and/or severely deafferented, may have been decreased by the GM_1 treatment.

In order to further study the effects of exogenous GM_1 on secondary degenerative events, we have now examined, both in developing and in adult animals, the consequences of posterior neocortex lesions and evaluated the extent of retrograde degeneration as attested by alterations in the size of the dorsal lateral geniculate nucleus of the thalamus

* Present address: Section of Neuroanatomy, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510, U.S.A.
Correspondence: B.A. Sabel. Present address: Institute of Medical Psychology, University of Munich School of Medicine, Goethestr. 31, 8000 Munich 2, F.R.G.

(LGd). The hamster was selected for this study because previous work demonstrated most severe retrograde cell loss in the LGd after neonatal area-17 lesions, but much less severe loss after such lesions in adulthood[30].

Fifteen adult male Syrian hamsters and 26 hamster pups, 14 days old, received right posterior neocortex lesions after being anesthetized with a mixture of Valium-Nembutal (10 mg/kg Valium, 50 mg/kg Nembutal, diluted 1:1 with saline for anesthesia of the pups). In the pups, the exposure of the right posterior neocortex was achieved by cutting the cartilagenous bone with a surgical knife along the midline from lambda to half-way in between lambda and the anterior edge of frontal cortex. After making two cuts from the midline laterally, the bone flap was removed. In adult hamsters, a bone flap of equivalent size was drilled out of the skull. Posterior neocortex underlying the bone flap was removed, after opening of the dura mater, by gentle aspiration with a 21 gauge modified hypodermic needle. Dexamethasone (0.1 ml, Elkins-Sinn) was routinely administered to pups to prevent brain swelling; adults were given this treatment on an as-needed basis. Excessive bleeding was arrested with ice-cold saline. After the wound cavity was filled with gelfoam which had previously been soaked in saline, the bone flap was replaced and the wound sutured with 6.0 surgical silk.

Following surgery, the pups were returned to the mother's nest and reared normally. Adult hamsters were housed individually with food and water ad libitum and were given intensive postoperative care. Daily i.p. injections with either GM_1 ganglioside (30 mg/kg, Fidia Research Laboratories) or an equivalent volume of saline were started immediately after surgery and continued until the day of sacrifice.

Young pups (Y) or adult hamsters (A) were randomly assigned to one of the following surgical groups, identified by their respective survival time (1, 3, 4, or 8 weeks) and the treatment condition (G = ganglioside, C = Control): Y1C (n = 6), Y1G (n = 7), Y3C (n = 6, Y3G (n = 7) and A4C (n = 3), A4G (n = 4), A8C (n = 4) and A8G (n = 4).

Following the respective survival times, animals were again anesthetized and perfused transcardially with 0.9% saline followed by 4% formalin. After brains were postfixed in 30% sucrose formalin-solution, they were embedded in albumin-gelatine and cut coronally at 30 μm on a freezing microtome. Every 5th section was stained with Cresyl violet.

Using a drawing tube attached to a Nikon light microscope, we reconstructed the volume of the LGd at a magnification of 123×. The borders of LGd were identified as follows: the dorsal border was defined as the lateral margin of the lateral posterior nucleus of the thalamus (LP). When this border was not clear, the line half-way between the ventral border of the LGd and the border between the LP and the pretectal nucleus was used as an aid in estimating the dorsal LGd border. Medially, the LGd reaches the dorsolateral border of the external medullary lamina; the lateral border is identified by the presence of the optic tract; and ventrally it is delineated by the neuropil at the dorsal margin of the ventral nucleus of the lateral geniculate (LGv). The outlines of the LGd were recorded on paper, and the size of the area was determined with the aid of a graphics tablet attached to an Apple-II-plus computer.

For each animal, the volume of the right and the left LGd was determined using the following formula:

$$V = \left(\sum_{i=1}^{n} Ai\right) \times 0.15/(123)^2$$

where V is the estimated volume, n represents the total number of sections containing LGd, Ai is the area measured in the ith section in mm^2, 0.15 represents the absolute distance between consecutive sections in millimeters, and 123 is the combined linear magnification factor of the drawing tube and the microscope. Because the animals' group identity was known to the observer (with the exception of the adult cases), a second observer, who was unaware of the animal identities, independently verified 10 randomly chosen sections. Because the values of both observers varied only randomly, experimenter bias can be ruled out.

The statistical analysis of the data involved t-test for a priori, orthogonal comparisons.

Two animals had to be excluded from the analysis: case C7 (group Y3C) had direct damage to the right LGd; case G9 (group Y3G) developed severe left hydrocephalus and herniation of the right hippocampus, making identification of LGd difficult.

The lesions extended for approximately 4 mm in

the anteroposterior axis, including all of area 17 and part of immediately adjacent cortex, with some damage to underlying white matter. The portion of neocortex extending lateral to area 18a to the rhinal sulcus was left undamaged in most cases (Fig. 1). In no case did the lesion cross the midline into the other hemisphere. For animals of the same age, there were no treatment differences in the antero-posterior extents of the lesions.

When examining the LGd ipsilateral to the cortical lesion, we did not find any area with complete sparing of cell bodies. This confirms our observation that the lesion included the entire striate cortex.

The differences between the extent of retrograde degeneration in young and adult animals were readily apparent (Fig. 2b and e). As expected from previous studies, the geniculi of young lesioned hamsters shrunk dramatically to a thin, sometimes barely detectable strip on the dorsal aspect of the thalamus. Few healthy neurons could be detected in these small tissue remnants, the bulk of which was comprised of glia and optic tract fibers. In contrast, the lesioned geniculi of adult animals were easily distinguishable from neighboring structures and, although diminished in size and gliotic, they contained many healthy-looking neurons. This was generally confirmed by LGd volume estimates, showing that the lesioned geniculi shrank by 60–90% in young and by only 40–50% in adult animals (Fig. 4).

We found that ganglioside treatment significantly diminished the volume shrinkage of the right LGd in the pups with the longer survival time; Fig. 4 shows the volume loss of the lesioned relative to the healthy geniculi (percent shrinkage, calculated as (100 ×

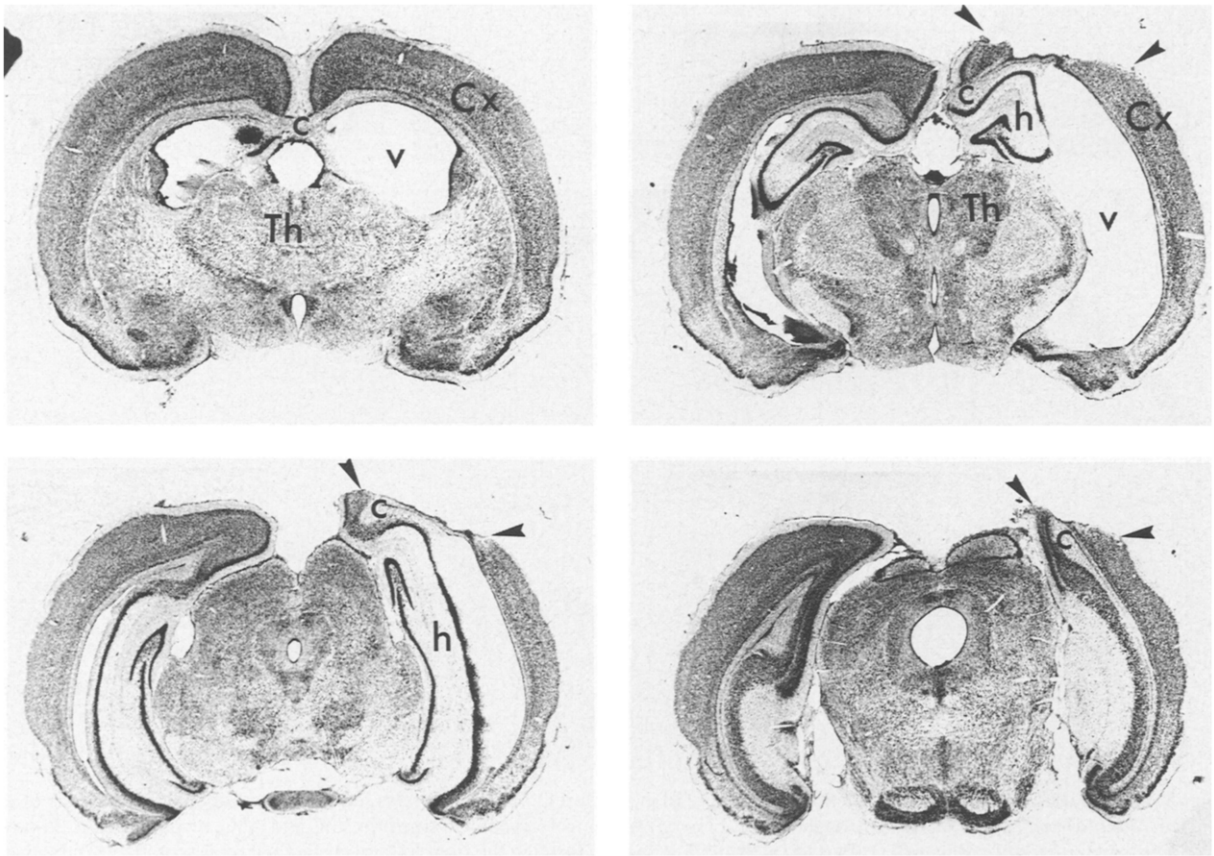

Fig. 1. Photomicrographs of coronal brain sections showing the neocortex lesion (arrowheads) of a typical case operated 14 days after birth with 3 weeks survival. Note the thinning of the neocortical mantle ipsilaterally. Cx, neocortex; Th, thalamus; v, lateral ventricle; h, hippocampus; c, corpus callossum.

(left LGd vol. – right LGd vol.)/left LGd vol.)) in the treated groups remained constant from the first to the third postoperative week (Y1G vs Y3G). Since the normal geniculi undergo considerable expansion during this period (from 0.38 to 0.50 mm^3 in average), this implies that retrograde degeneration had stopped in the treated group by the end of the first posttraumatic week, and that subsequently the lesioned geniculi expanded at the same rate as their normal counterparts. In contrast, the percent shrink-

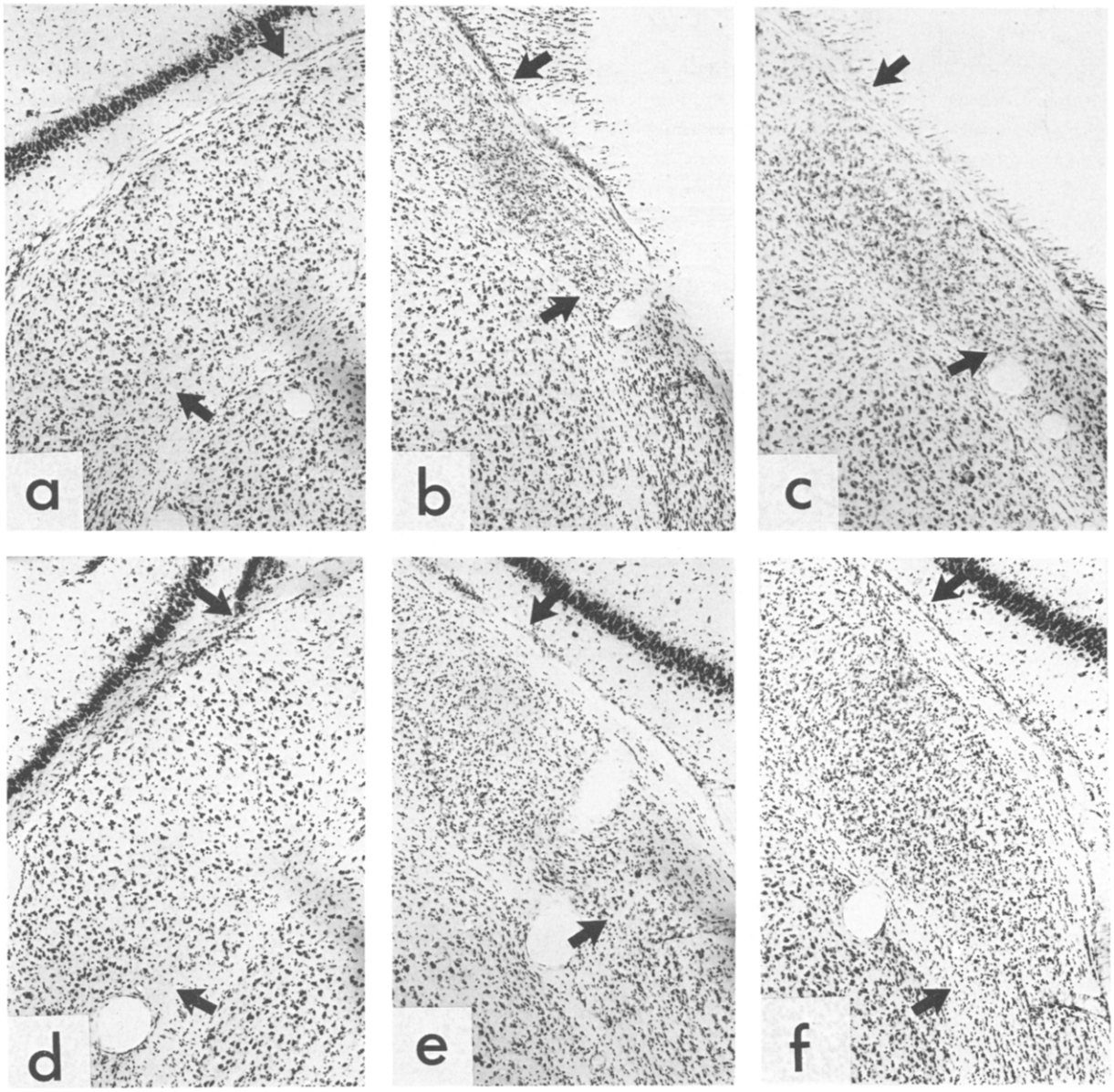

Fig. 2. Photographs of representative sections through LGd in 4 different animals. Arrows indicate dorsal and ventral borders of the nucleus. Part (a) and (d) are sections through the left LGd of two control animals from groups Y3C and A8C, respectively (a, 35-days-old hamster; d, 6-month-old hamster). Part (b) is the middle section through the right LGd of the animal represented in (a). Note extreme shrinkage in both length and width of the nucleus. Part (c) is taken from the right LGd of a 35-days-old hamster (group Y3G) treated with GM_1. Note relative sparing of LGd. Part (e) is a section through the right LGd of the animal represented in (b) (group A8C). (f) is taken from an adult treated with GM_1 for 8 weeks postlesion (group A8G). Note relative lack of difference between (e) and (f), as well as sparing of the right LGd relative to young cases (part b and c). Cresyl violet stain, ×94.

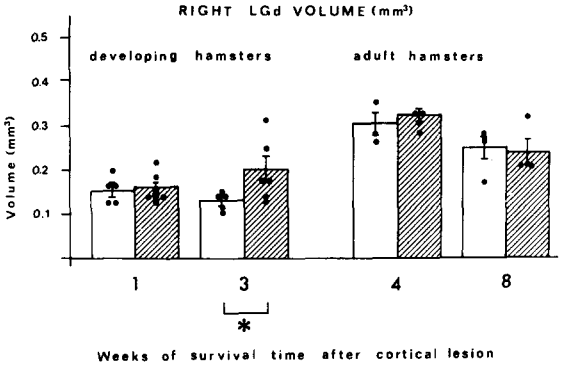

Fig. 3. Right LGd volume. Bars represent group means ± S.E.M. Black dots, individual values; white bars, control groups; hatched bars, ganglioside-treated groups. $^{*}P < 0.05$.

age (volume loss) increased significantly in the young control hamsters during the second and 3rd postoperative weeks (from 59 to 73%, $t = 5.79$, $P < 0.001$). Thus in these animals, either the retrograde reaction lasted longer than in the treated pups, or normal development of the remaining tissue was arrested, or both. This latter finding also supports the argument that the constancy of the lesioned to healthy LGd ratios in the treatment groups was not an artifact of uniform neuropil expansion.

Comparison of LGd volumes in treated and untreated pups confirmed these observations: no significant differences were found after one week of survival time (Fig. 3), but at 3 weeks, the geniculi of the treated groups were significantly larger than those of the untreated pups. Average volumes and standard deviations were 0.13 ± 0.009 mm^3 for Y3C animals and 0.2 ± 0.029 mm^3 for the Y3G group ($t = 2.13$, $P < 0.05$).

A similar, but less pronounced effect was seen in the adult animals. LGd volume loss in the control group continued after the 4th postoperative week, yielding a significant difference between A4C and A8C groups ($t = 2.15$, $P < 0.05$, Fig. 4). No such difference was noted in the ganglioside groups (A4G and A8G) suggesting that the degenerative period may have been shortened by GM_1 administration.

It is interesting to note that the *absolute* LGd volume measurements were statistically less sensitive (i.e. revealed fewer statistically significant group differences) than were the *relative* volume-loss mea-

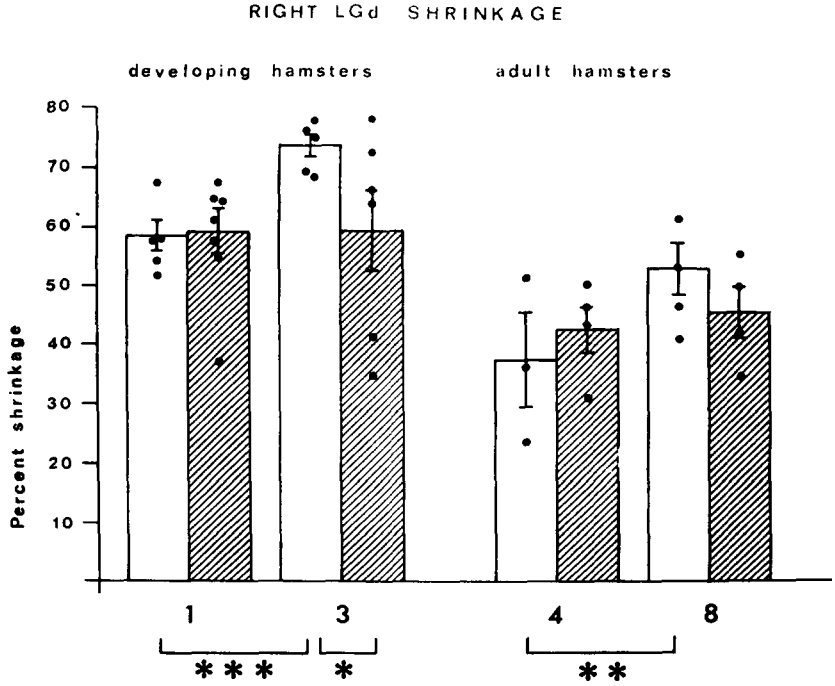

Fig. 4. Group means and S.E.M. of right LGd shrinkage, calculated as 100 × (Vl − Vr)/Vl, where Vl is left LGd volume, Vr is right LGd volume, and thus represents the percentage of LGd volume lost in each case. Bars, mean ± S.E.M.; black dots, individual values; white bars, control groups; hatched bars, ganglioside groups. $^{*}P < 0.052$; $^{**}P < 0.05$; $^{***}P < 0.001$.

sures. This is probably due to the fact that the relative measure minimizes the large within-group variability.

It is known that neuronal reaction to distal injury is an age-dependent phenomenon[1,11,14,18,30,31]. Retrograde or transsynaptic degeneration is a predominant consequence of lesions involving the axon or terminal field of a neuron and is often more severe, and much more rapid, in young animals[1,2–4,19,22]. With successive stages of development, less severe and/or slower retrograde reactions are observed, evident as limited chromatolysis and swelling of the cell body or a more gradual cell loss. The neuron progressively advances toward the adult stage in which the cell may be capable of surviving the lesion in either a significantly atrophied, or, in rarer cases, hypertrophied state[1].

These observations can be interpreted as a gradual shift of developing neurons toward independence from their target, although in many cases it has been postulated that uninjured collateral branches of the axon are reponsible for the neuron's survival (the concept of 'sustaining collaterals').

Our study confirms previous observations that retrograde degeneration after lesions is more severe in developing than in adult tissue; we found a greater percent LGd shrinkage after posterior cortex lesions in young hamsters (Fig. 4). Moreover, we have now shown that GM_1 treatment reduces the progression of LGd shrinkage in young hamsters to a much more potent degree than in adults. In these adult hamsters, we have seen only weak effects; a less severe progression of shrinkage was apparent only when percentage of shrinkage was taken as a measure and not when absolute measurements were considered. In any case, the percent shrinkage is a very sensitive measure for volume loss, since, by normalizing the volume of the affected LGd with the intact LGd in the same animal, between-animal differences, and thus the variance, are diminished.

Before accepting the conclusion that GM_1 reduces retrograde degenerative events in development, we should discuss the validity of the volume measurements of the LGd. One may argue, for example, that the volume increase in group Y3G relative to Y3C could have reflected glial proliferation or expansion of the neuropil rather than increased neuronal survival. The first suggestion is unlikely, given the small size of glial cells and the evidence that GM_1 inhibits proliferation of at least one type of glia in vitro[13]. The second possibility cannot be ruled out with the methods we used in this study, but such a phenomenon has never been reported in the neuroplasticity literature. Although quantification of cell number and cell size is needed, based on the above considerations we believe that the reduced shrinkage is a direct consequence of reduced retrograde cell shrinkage or cell death.

At this point we can only speculate as to the underlying mechanism of GM_1-reduced retrograde degeneration. Two principle events might be candidates for such a mechanism: (a) gangliosides may directly prevent progressive neuronal death of axotomized neurons or those marginally injured, and/or (b) the drug may enhance regenerative sprouting or compensatory sprouting of injured axons[32], thus providing them with essential target contact and trophic support.

On prevention of neuronal death. Whether the prevention of retrograde neuronal degeneration is a behaviorally meaningful event is still unclear. We have recently argued[27] that the prevention of retrograde neuronal death may be of little meaning to explain the fact that gangliosides are so effective in reducing behavioral deficits after brain injury (for review see refs. 9, 23). It is unlikely that a neuron, still disconnected from its target, plays any major role in behavioral sparing. In any event, prevention of neuronal death may be accomplished in two ways: (1) the treatment may have accelerated neuronal development, and (2) the drug may have a direct or indirect trophic influence on the damaged neurons.

Acceleration of neuronal development. In the 14-day-old hamster (the stage at which the pups in this experiment received the neocortical lesions) the geniculo-cortical axons have penetrated layers 5 and 6 of visual cortex, and are arborizing rapidly in layer 4 (ref. 21). Only on the 20–21st postnatal days will these neurons have reached full maturity. It is possible that, as the geniculo-cortical system matures, the neuronal population becomes increasingly composed of cells that have a greater capacity for surviving the cortical lesions. (That neurons have not yet reached full maturity in this respect is made clear by the discrepancy between the LGd volume loss in young and adult controls in this experiment (see Fig. 2b and e).

Indirect support for the hypothesis that ganglio-

sides influence developmental events comes from studies of Karpiak and his colleagues[16,17], who administered either gangliosides or anti-ganglioside antibodies to neonatal rats. The ganglioside treatment resulted in accelerated behavioral development whereas the antibodies produced behavioral deficits that lasted throughout adulthood. We did not observe an effect of GM_1 on the development of the size of LGd in the present experiment, and therefore it appears unlikely that 'reduced shrinkage' of LGd after lesions is due to accelerated brain development.

Enhancement of the neurons' ability to survive the injury. Neuronal death after early lesions is not believed to be a direct consequence of the axotomy itself, but a result of depriving the neuron of a (trophic) factor secreted by the target, which is normally retrogradely transported to the cell body and which is essential for its survival[12,18]. It has been proposed that gangliosides may act synergistically with neuronotrophic or neurite outgrowth promoting factors[6]. This hypothesis was also based on the observation that NGF-induced neurite outgrowth of cultured PC12 cells is potentiated by gangliosides[6,10]. It is conceivable that GM_1 has similar synergistic effects with neuronotrophic factors in vivo and would thus enable both the axotomized neurons as well as those marginally damaged to better survive the damage.

On regeneration and sprouting. It is known that competition among axonal populations plays a crucial role in the establishment or failure of establishment of new synaptic connections in the CNS[31]. Competition for either trophic factors or synaptic space will determine, whether a neuron lives or dies. It is conceivable that the availability of trophic factors is not solely dependent upon absolute levels in the target tissue, but that it also depends on the number of collateral branches an axon forms[32]. Because many studies advocate a stimulatory effect of GM_1 on *collateral sprouting* as attested by anatomical[26,28] and biochemical evidence[34,39], the possibility exists that improved cell survival after GM_1 treatment may be due to the fact that axons have formed more collateral branches and thus have an advantage in competing for synaptic space and/or trophic factors.

Whatever the precise mechanism may be, we have presented evidence that GM_1 gangliosides reduce the course of retrograde degeneration in developing hamsters after neocortex lesions. In adult hamsters, gangliosides appear to have a somewhat similar effect, although this is not as striking. While our present method might have been insufficiently sensitive to detect clear GM_1 effects in adulthood, the potency of GM_1 to protect against the consequences of injury appears to be greater in early stages of development.

We thank FIDIA Research Laboratories, Abano Terme, Italy, for kindly providing GM_1 gangliosides. Supported by NIH Grants EY 00126 and EY 02621.

1 Bleier, R., Retrograde transsynaptic cellular degeneration in mammilary and ventral tegmental nuclei follwing limbic decortication in rabbits of various ages, *Brain Research,* 15 (1969) 363–393.
2 Bregman, B.S. and Goldberger, M.E., Anatomical plasticity and sparing of function after spinal cord damage in neonatal cats, *Science,* 217 (1982) 553–555.
3 Bregman, B.S. and Goldberger, M.E., Infant lesion effect: III. Anatomical correlates of sparing and recovery of function after spinal cord damage in newborn and adult cats, *Dev. Brain Res.,* 9 (1983) 137–154.
4 Brodal, A., Modification of the Gudden method for study of cerebral localization, *Arch. Neurol. Psychiat.,* 43 (1940) 46–58.
5 Cuello, A., Stephens, P., Tagari, P., Sofroniew, M. and Pearson, R., Retrograde changes in the nucleus basalis of the rat are prevented by exogenous ganglioside GM_1, *Brain Research,* 376 (1986) 373–377.
6 Dal Toso, R., Skaper, S.D., Ferrari, G., Toffano, G. and Leon, A., Ganglioside involvement in membrane-mediated transfer of trophic information: relationship to GM_1 effects following CNS injury. In D.G. Stein and B.A. Sabel (Eds.), *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury,* Plenum, New York, 1988, pp. 143–165.
7 Dreyfus, H., Urban, P.F., Edel-Harth, S. and Mandel, P., Developmental patterns of gangliosides and of phospholipids in chick retina and brain, *J. Neurochem.,* 25 (1975) 245–250.
8 Dreyfus, H., Harth, S., Massarelli, R. and Louis, J.C., Mechanisms of differentiation in cultured neurons: involvement of gangliosides. In M. Rapport and A. Gorio (Eds.), *Gangliosides in Neurological and Neuromuscular Function, Development and Repair,* Raven, New York, 1981.
9 Dunbar, G.L. and Stein, D.G., Gangliosides and functional recovery from brain injury. In D.G. Stein and B.A. Sabel (Eds.), *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury,* Plenum, New York, pp. 195–218.
10 Ferrari, G., Fabris, M. and Gorio, A., Gangliosides enhance neurite outgrowth in PC12 cells, *Dev. Brain Res.,* 8 (1983) 215–221.
11 Guillery, R.W., Quantitative studies of transneuronal atrophy in the dorsal lateral geniculate nucleus of cats and kittens, *J. Comp. Neurol.,* 149 (1973) 423–438.
12 Hamburger, V. and Oppenheim, R., Naturally occuring

neuronal death in vertebrates, *Neurosc. Comm.*, 1 (1982) 39–55.
13 Hefti, F., Hartikka, J. and Frick, W., Gangliosides alter morphology and growth of astrocytes and increase the activity of choline acetyltransferease in cultures of dissociated septal cells, *J. Neurosci.*, 5 (1985) 2086–2094.
14 Jonsson, G., Gorio, A., Hallman, H., Janigro, D., Kojima, H. and Zanoni, R., Effect of GM_1 ganglioside on neonatally neurotoxin induced degeneration of serotonin neurons in the rat brain, *Dev. Brain Res.*, 16 (1984) 171–180.
15 Karpiak, S.E., Ganglioside treatment improves recovery of alternation behavior after unilateral entorhinal cortex lesion, *Exp. Neurol.*, 81 (1983) 330–339.
16 Karpiak, S., Vilim, F. and Mahadik. S., Ganglioside accelerate rat neonatal learning and level of acetylcholinesterase, *Dev. Neurosci.*, 6 (1984) 1–7.
17 Kasarskis, E., Karpiak, S., Rapport, M., Zu, R.K. and Bass, N., Abnormal maturation of cerebral cortex and behavioral deficit in adult rats after neonatal administration of antibodies to ganglioside, *Dev. Brain Res.*, 1 (1985) 25–35.
18 LaVelle, A. and LaVelle, F., Neuronal reaction to injury during development. In S. Finger and R. Almli (Eds.), *Early Brain Damage,* Academic, 1984, pp. 3–16.
19 Lieberman, A.R., The axon reaction: a review of the principle features of perikaryal response to axon injury, *Int. Rev. Neurobiol.*, 14 (1971) 49–124.
20 Merat, A. and Dickerson, J.W.T., The effect of development on the gangliosides of rat and pig brain, *J. Neurochem.*, 20 (1983) 873–880.
21 Naegele, J., *Map Formation During Development: Studies of Topography and Axonal Arbors in the Visual Cortex of the Syrian Hamster,* PhD Dissertation, Massachussetts Institute of Technology, 1984.
22 Prendergast, J. and Stelzner, D.J., Changes in the magnocellular portion of the red nucleus following thoracic hemisection in the neonatal and adult rat, *J. Comp. Neurol.*, 166 (1976) 163–172.
23 Sabel, B.A., Anatomical mechanisms whereby ganglioside treatment induces brain repair: what do we really know? In D.G. Stein and B.A. Sabel (Eds.), *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury,* Plenum, New York, 1988, pp. 167–194.
24 Sabel, B.A., Dunbar, G.L. and Stein, D.G., Gangliosides minimize behavioral deficits and enhance structural repair after brain injury, *J. Neurosci. Res.*, 12 (1984) 429–443.
25 Sabel, B.A., Dunbar, G., Fass, B. and Stein, D.G., Gangliosides, neuroplasticity and behavioral recovery after brain damage. In B.E. Will, P. Schmidt and J.C. Delrymple-Alford (Eds.), *Brain Plasticity, Learning and Memory,* Plenum, New York, 1985, pp. 481–493.
26 Sabel, B.A., Dunbar, G.L., Butler, W.M. and Stein, D.G., GM_1 ganglioside stimulates neuronal reorganization and reduces rotational asymmetry after hemitransections of the nigrostriatal pathway, *Exp. Brain Res.*, 60 (1985) 27–37.
27 Sabel, B.A., DelMastro, R., Dunbar, G.L. and Stein, D.G., Reduction of anterograde degeneration in brain damaged rats by GM_1-gangliosides, *Neurosci. Lett.*, 77 (1987) 360–366.
28 Sabel, B.A. and Schneider, G.E., Enhanced sprouting of retinotectal fibers after early superior colliculus lesions in hamsters treated with gangliosides, *Exp. Brain Res.*, in press.
29 Sabel, B.A., Slavin, M.D. and Stein, D.G., GM_1 ganglioside treatment facilitates behavioral recovery from bilateral brain damage, *Science,* 225 (1984) 340–342.
30 Schneider, G.E., Mechanisms of functional recovery following lesions of visual cortex or superior colliculus in neonate and adult hamsters, *Brain Behav. Evol.*, 3 (1970) 295–323.
31 Schneider, G., Early lesions of the superior colliculus: factors affecting the formation of abnormal retinal projections, *Brain Behav. Evol.*, 8 (1973) 73–109.
32 Schneider, G.E., Early lesions and abnormal neuronal connections, *Trends Neurosci.*, 4 (1981) 187–192.
33 Sofroniew, M.V., Pearson, R.C.A., Cuello, A.C., Tagari, P.C. and Stephens, P.H., Parenterally administered GM_1 ganglioside prevents retrograde degeneration of cholinergic cells of the rat basal forebrain, *Brain Research,* 398 (1986) 393–396.
34 Toffano, G., Savioni, G., Moroni, F., Lombardi, G., Calza, L. and Agnati, F., GM_1 ganglioside stimulates the regeneration of dopaminergic neurons in the central nervous system, *Brain Research,* 261 (1983) 163–166.
35 Toffano, G., Savoini, G., Moroni, F., Lombardi, G., Calza, L. and Agnati, L.F., Chronic GM_1 ganglioside treatment reduces dopamine cell body degeneration in the substantia nigra after unilateral hemitransection in the rat, *Brain Research,* 296 (1984) 233–239.
36 Vanier, M., Holm. M., Ohman, R. and Svennerholm, L., Developmental profiles in human and rat brain, *J. Neurochem.*, 18 (1971) 581–592.
37 Will, B., Hefti, F., Pallage, V. and Toniolo, G., Nerve growth factor: effects on CNS neurons and on behavioral recovery from brain damage. In D.G. Stein and B.A. Sabel (Eds.), *Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury,* Plenum, New York, pp. 339–360.
38 Willinger, M. and Schachner, M., GM_1 ganglioside as a marker for neuronal differentiation in mouse cerebellum, *Dev. Biol.*, 74 (1980) 101–117.
39 Wójcik, M., Ulas, J. and Oderfeld-Nowak, B., The stimulating effect of ganglioside injections on the recovery of choline acetyltransferase and acetylcholinesterase activities in the hippocampus of the rat after septal lesions, *Neuroscience,* 7 (1982) 495–499.