

# Behavioral Effects of Gangliosides: Anatomical Considerations

Dwaine F. Emerich

*Cellular Transplants, Inc.,  
Four Richmond Square, Providence, RI 02906*

## ABSTRACT

Gangliosides are endogenous sialic acid containing glycosphingolipids which are highly concentrated in the central nervous system. Although they were first characterized over 40 years ago, the function(s) played by this unique class of lipids remain largely unknown. Gangliosides have been suggested to play a prominent role in both normal and abnormal developmental processes. In addition, several lines of convergent evidence have indicated that gangliosides exert pronounced trophic effects following damage to peripheral and central nerves. Gangliosides have been shown to (1) enhance cell survival and outgrowth in cultured and developing neurons; (2) promote the regeneration of damaged peripheral and central nerves, and (3) facilitate behavioral recovery by altering the pattern, extent and persistence of the biochemical, morphological and behavioral changes induced by neural trauma. Little is known, however, concerning the neurobiological mechanisms which subserve the behavioral protection afforded by ganglioside treatment. This review focuses on the evidence suggesting that gangliosides mediate functional recovery by minimizing primary or secondary cell loss or promoting the regeneration or sprouting of damaged central nerves subsequent to injury. An understanding of the mechanisms by which gangliosides produce their effects may lead to

**the development of more efficacious and rational primary or adjunct pharmacological treatments for central nervous system disorders.**

## KEY WORDS

gangliosides, recovery of function, sprouting, trophic factors, regeneration.

## INTRODUCTION

One of the major goals of neuroscience is to understand and treat brain disorders. Despite technical and conceptual advances in the neurosciences we still face fundamental questions in neurology. Among the most problematic neurological disorders are those associated with the degeneration of brain neurons. Neurodegenerative diseases, strokes and penetrating wounds all produce unique constellations of behavioral symptoms that result from the destruction of specific neuronal populations. Although research is beginning to reveal the pathophysiology and molecular biology of these disorders there remains a wide gap between our understanding of the substrates of these diseases and our ability to prevent or treat them.

Traditional approaches to the treatment of acute and more chronic neurological disorders typically produce very limited functional recovery and may even produce deleterious side effects. Even in cases where some degree of functional recovery occurs, residual deficits typically continue to compromise the individuals' quality of life /51/. Based on the limited efficacy of current treatment modalities it is imperative to develop treatments which limit the extent and

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Reprint address:

Dr. Dwaine F. Emerich  
Cellular Transplants, Inc.  
Four Richmond Square  
Providence, RI 02906  
USA

persistence of neural trauma and promote functional recovery.

Gangliosides are sialic acid containing glycosphingolipids which are highly concentrated in neuronal membranes /40,45,65,66/. These large, complex lipids are typically associated with the outer surface of the neuronal membrane and consist of a hydrophobic sphingosine and steric acid complex inserted into the neuronal membrane and a hydrophilic component containing sialic acid residues and a variety of carbohydrates which protrude towards the extracellular fluid. Although over 70 different forms of gangliosides /96/ have been identified, most investigators have focused on the monosialoganglioside GM1 because of its demonstrated *in vivo* and *in vitro* efficacy as a trophic agent.

The location of gangliosides on the outer portion of the neuronal membrane has led to the speculation that these molecules play a role in cell surface events such as cell to cell recognition, synaptic transmission and receptor-ligand functions. Morphological, developmental, biochemical and behavioral studies have shown that gangliosides participate in a variety of neurobiological functions related to the maturation and repair of neural tissue. For example, there are pronounced changes in ganglioside concentrations and distribution during synaptogenesis and central nervous system (CNS) maturation /29,59/. In addition, developmental disorders which result from impairments in the metabolism of gangliosides (e.g., Tay Sachs disease) are characterized by anomalous patterns of neuronal differentiation and synaptogenesis as well as progressive cognitive deterioration /85,86/. Moreover, administration of antibodies to GM1 ganglioside to developing animals produces permanent alterations in the extent of synaptogenesis and myelination of nervous tissue together with deficits in learning performance /59/. In contrast, exogenously administered gangliosides increase cell survival and enhance neurite outgrowth when added to cultures of developing neurons /12,26,28,38,73/, promote regeneration of damaged central and peripheral nerves /4,43-45/, and facilitate behavioral recovery by altering the pattern, extent and

persistence of the biochemical, morphological and behavioral changes induced by neural trauma /16,17,20,30,32,34,35,37,82,83,89,92-97, 115,120/. Based upon these observations it has been proposed that gangliosides may serve as useful pharmacological agents for augmenting the plasticity of the CNS and promoting functional recovery. Results such as those described above, coupled with the practical considerations that gangliosides are capable of crossing the blood-brain barrier /64,81,111/ and produce no overt toxic side effects /52/, make gangliosides particularly attractive neurotrophic agents for the treatment of CNS injury. Although several studies have documented the efficacy of gangliosides in a variety of animal models and human conditions, the mechanism by which gangliosides exert their beneficial effects has remained elusive (see Table 1). The purpose of this article is to review the possible anatomical mechanisms by which gangliosides might produce behavioral recovery in various models of CNS injury.

#### EFFECTS OF GANGLIOSIDES ON DEVELOPMENT AND PERIPHERAL NERVOUS SYSTEM REGENERATION

The expression of gangliosides correlates with the development of the CNS and has led to the suggestion that gangliosides play a role in normal neuronal growth. Willinger /117/ reported that the expression of ganglioside GM1 was markedly increased on the neuronal membrane of differentiating cells. Moreover, Rosner /91/ demonstrated that increases in the concentrations of a variety of endogenous gangliosides paralleled cell migration and neuronal differentiation in the optic lobes of the chicken. The differential alterations in ganglioside concentrations during development do not provide direct evidence for a causative role in specific developmental phenomena. However, the abnormal neuronal development associated with disorders of ganglioside metabolism does suggest a direct modulatory role for gangliosides in neuronal growth. GM1 gangliosidosis results from a deficiency of the enzyme  $\beta$ -galactosidase which is responsible for the degradation of GM1. As a

TABLE 1

EFFECTS OF GANGLIOSIDES ON BEHAVIORAL AND NEUROBIOLOGICAL RECOVERY  
FOLLOWING CNS LESIONS

Species/ Lesion	Ganglioside Treatment	Anatomical/ Neurochemical Results	Behavioral Results	Ref
Rat peroneal nerve	Mix of GM1, GD1a, GD1b, and GT1 (50 mg/kg, IP)		Enhanced neuromuscular control	13
Rat sciatic nerve	Mix of GM1, GD1a, GD1b, and GT1 (5 mg/kg, IP)	Sprouting of moto- neurons		44
Rat fourth lumbar root	Mix from bovine brain (50 mg/kg, IP)		Enhanced isometric tension of plantaris muscle	102
Rat sciatic nerve; alloxan- induced diabetes	Mix of GM1, GD1a, GD1b and GT1b (10 mg/kg, IP)		Ameliorated loss of axonal transport	70
Rat sciatic nerve; diabetic peripheral neuro- pathy	Mix of GM1, GD1a, GD1b, and GT1b (20 mg/kg, SC)	Improvement of nerve conduction		108
Rat; vinblastine- induced sym- pathectomy	GM1 and/or NGF (30 and 1 mg/kg, SC)	Reduced NE loss		117
Rat ventro- medial septal nucleus	Mix of GM1, GD1a, GD1b, and GT1 (50 mg/kg, IP)	Promoted recovery of ChAT and AChE		124
Rat septal nucleus	GM1 (30 mg/kg, IP)		Decreased emotionality	84
Rat dorsal hippocampus	GM1 (30 mg/kg, IM)	Enhanced recovery of AChE, ChAT and 5-HT		46
Rat dorsal hippocampus	GM1 (30 mg/kg, IP)	Enhanced 5-HT uptake		61
Rat hippo- campus/ neocortex	GM1 (30 mg/kg, IP)	Enhanced ChAT activity		104
Rat entorhinal cortex	Mix from bovine brain (50 mg/kg, IM)		Promoted recovery of T- maze alternation	57

cont.

Table 1 (cont.)

Species/ Lesion	Ganglioside Treatment	Anatomical/ Neurochemical Results	Behavioral Results	Ref
Rat entorhinal cortex	Mix of GM1, GD1a, GD1b, and GT1 (30 mg/kg, IM)	Decreased sprouting	Reduced hyperactivity	36
Rat dentate gyrus	GM1 (30 mg/kg, IP)	No reduction of cell loss	Promoted recovery of hyperactivity and pharm- acological sensitivity	120
Rat dentate gyrus	GM1 (30 mg/kg, IP)	No reduction of cell loss	Increased ODC activity on contralateral side	119
Rat dentate gyrus	GM1 or AGF2 (15 and 10 mg/kg IP)	No reduction of cell loss	Induced recovery of T- maze performance	32
Rat hippo- campus	GM1 (15 mg/kg, IP)	Prevent loss of ChAT and HACHU		27
Rat nucleus basalis	GM1 (30 mg/kg, IP)	Prevent cholinergic cell loss		20
Rat nucleus basalis	GM1 (5 mg/kg, ICV)	Prevent decrease in ChAT	Promoted recovery of passive avoidance and water maze performance	31
Rat septal nucleus	AGF2 (10 mg/kg, IP)	Induce recovery of ChAT	Partial recovery of radial arm maze task	34
Rat septal nucleus	AGF2 (10 mg/kg, IP)	Prevent loss of ChAT and HACHU	Prevented hyperactivity and cognitive deficits	35
Rat nucleus basalis	GM1 (10 mg/kg, IM)	Reduced loss of AChE and ChAT	Reduced mortality	69
Rat striatum	GM1 (10, 30, or 50 mg/kg, IP)	Reduced loss of ChAT and GAD		68
Rat striatum	GM1 (30 mg/kg, IP)	Reduced anterograde cell loss		97
Rat striatum	GM1 or AGF2 (30 mg/kg IP)	Regeneration of nigro- striatal DA system		4
Rat substantia nigra	GM1 (30 mg/kg, IP)	Reduced retrograde cell loss		116
Rat nigrostriatal pathway	GM1 (30 mg/kg, IP)		Reduced rotational beh- avior	94
Rat nigrostriatal pathway	GM1 or AGF2 (30 mg/kg, IP)	Induced recovery of TH	Reduced rotational beh- avior	115
Rat striatum	GM1 (30 mg/kg, IP)		Promoted recovery of cognitive behavior	93

cont.

Table 1 (cont.)

Species/ Lesion	Ganglioside Treatment	Anatomical/ Neurochemical Results	Behavioral Results	Ref
Rat cerebral cortex	GM1 (30 mg/kg, IP)	Induced regrowth of NE terminals		62
Mouse sciatic nerve; diabetic peripheral neuro- pathy	Mix of GM1, GD1a, GD1b, GT1 (1 or 10 mg/kg, IP)	Promoted recovery of axonal flow		76
Mouse periph- eral neuropathy	Mix of GM1, GD1a, GD1b, GT1 (20 mg/kg, SC)	Induced fiber sprouting		109
Mouse sub- stantia nigra (MPTP)	GM1 or AGF2 (30 or 10 mg/kg, IP)	Produced recovery of DA DOPAC		47
Mouse sub- stantia nigra (MPTP)	GM1 (30 mg/kg, IP)		Eliminated haloperidol induced sensorimotor effects	121
Hamster sup- erior colliculus	GM1 (30 mg/kg, IP)	Enhanced sprouting of retinotectal fibers		98
Human stroke	GM1 (20 mg, IM)		Produced neurological improvement	7
Human cerebro- vascular disease	GM1 (40 mg, IM)		Enhanced neurophysio- logical and clinical recovery	8
Human diabetic neuropathy	Mix of GM1, GD1a, GD1b, GT1b (40 mg, IM)		Produced recovery of sen- sory perception of lower extremities	53
Human idio- pathic facial paralysis	Mix of GM1, GD1a, GD1b, GT1b (20 mg, IM)		Complete recovery	54
Human Alzheimer's disease	GM1 (100 mg, IM)		No improvement on cog- nitive or psychosocial indices	3
Human retinitis pigmentosa	Mix of GM1, GD1a, GD1b, GT1b (40 mg, IM)		Improved visual field area and electroretino- graphic responses	72

NOTE: AChE = acetylcholine esterase; ChAT = choline acetyltransferase; DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; GAD = glutamic acid decarboxylase; HACHU = high affinity choline uptake; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, NE = norepinephrine; NGF = nerve growth factor; ODC = ornithine decarboxylase; IP = intraperitoneal; SC = subcutaneous; IM = intramuscular; ICV = intraventricular.

result of this deficiency, meganeurites, characterized by multiple neuritic processes, neurites, and spines develop throughout the nervous system /85,86/. Since this pattern of cell growth is typically observed only during early stages of development, these results indicate that an abnormal accumulation of gangliosides has a direct influence on the subsequent growth of neurons.

Numerous *in vitro* experiments have demonstrated that exogenous gangliosides alter the morphological characteristics of cultured neurons /12,26,28,38,73,125/. Gangliosides promote neuritogenesis in a variety of cell lines, with certain cells responding only to specific species of gangliosides. For instance, Carine *et al.* /15/ reported that GM1, but not GD1a, enhanced neuritogenesis in S20Y murine neuroblastoma cells. Similarly, the selectivity of afferent connections in tetrodotoxin-treated spinal cord explants is dependent on the species of gangliosides used /6/. Mixed bovine ganglioside treatments produced a selective afferent connectivity in these culture preparations while GM1 alone produced a nonselective connectivity. These studies indicated that the exogenous addition of gangliosides enhances the outgrowth of cultured neurons but that this outgrowth is dependent on the molecular form of the ganglioside used. Since the distribution of gangliosides in the nervous system is variable, it is conceivable that gangliosides normally play a role in mediating specific forms of neuronal connectivity and that the extent of functional recovery afforded by gangliosides is a function of precise and subtle anatomical changes.

Ceccarelli and colleagues /18/ initially reported that the exogenous administration of gangliosides promoted sprouting in the peripheral nervous system (PNS). Following a pre- and postganglionic anastomosis of the superior cervical ganglion in cats, daily administration of gangliosides resulted in a complete recovery from paralysis together with normal pupillary and nictitating membrane function. Since animals typically exhibit a partial recovery from this surgery as a result of regenerating axons reinnervating the cervical ganglion it was concluded that the gangliosides

had promoted the regeneration of the axotomized fibers. Support for the assumption of regeneration came from visualization of increased catecholaminergic histofluorescence in the cervical ganglion following the anastomosis. Since these initial studies, several laboratories have confirmed that gangliosides promote the regeneration and sprouting of damaged peripheral nerves and muscle /13,44,45,56,60/.

## GANGLIOSIDE-INDUCED REGENERATION AND SPROUTING FOLLOWING NEURAL INJURY

### Septohippocampal cholinergic system

Given that gangliosides could markedly potentiate the regenerative response of peripheral nerves, Oderfeld-Nowak and colleagues /77-80/ investigated the effects of ganglioside treatment on the sprouting response which results from partial denervation of the cholinergic input to the hippocampus (HPC). Following damage to the septum, there is an initial decrease in the enzymes acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) in the HPC which is then followed by a progressive recovery of enzymatic activity over the next several months /75,79/. The recovery of neurochemical activity correlates with the appearance of newly formed synaptic contacts in the HPC and is considered to be an accurate marker of sprouting of surviving hippocampal afferents. Following electrocoagulation lesions, a mixture of gangliosides accelerated the recovery of AChE and ChAT activity in the HPC /77,79,80/. While the initial decline in enzymatic activity was equivalent regardless of whether the animals received gangliosides or not, gangliosides produced a significant increase in AChE and ChAT activity compared to controls at both 18 and 50 days post-surgery. These same investigators reported that ganglioside GM1 promoted sprouting following ablations of the entorhinal cortex /78/. At 3 weeks following surgery, GM1 enhanced the recovery of AChE and ChAT activity in the HPC indicating an enhancement of the sprouting response of the septohippocampal pathway.

Given that gangliosides appeared to enhance the plasticity of the septohippocampal cholinergic pathway, investigators examined the behavioral consequences of this accelerated response. Karpiak /57/ examined the performance of rats on a spatial alternation task following unilateral lesions of the entorhinal cortex. Animals receiving daily injections of GM1 made significantly fewer errors on the first postoperative day compared to lesioned animals not receiving ganglioside treatment. Because this reduction in the lesion-induced behavioral deficits preceded the period of time considered necessary for reinnervation to begin, these results were interpreted as indicating that GM1 exerted a protective rather than regenerative effect. To evaluate this possibility Fass and Ramirez /36/ bilaterally lesioned the entorhinal cortex producing increases in open-field activity which typically recover over a two week period. Animals treated with GM1 were less active than lesioned animals receiving saline treatment as early as two days post-surgery suggesting that the behavioral protection was independent of alterations in the sprouting response. Furthermore, a *reduction* in the density of AChE staining was observed in the dentate gyrus of animals treated with gangliosides indicating that GM1 might have actually suppressed the sprouting response. The interpretation of these results was further complicated by a comparison between the behavioral and histological analysis which revealed that there was no obvious relationship between the recovery of the behavioral impairments and the alterations in AChE staining. Poplawsky and Isaacson /84/ further demonstrated that ganglioside treatment decreased the hyper-emotionality following septal lesions. Again, these changes were observed within two days following surgery, making it unlikely that structural changes, such as sprouting, accounted for the observed behavioral effects.

More recently, Emerich and Walsh /34/ examined the effects of ganglioside AGF2 (the internal ester of GM1) on the behavioral and neurochemical consequences of bilateral injections of ethylcholine mustard aziridinium ion (AF64A) into the lateral ventricles of rats.

AF64A produced an impairment on both a standard radial arm maze (RAM) task and when a 1 hour delay was imposed between the fourth and fifth arm choices. Although the initial impairment was equivalent regardless of whether the AF64A-treated animals received AGF2 or saline, the AGF2-treated rats recovered on the standard RAM task within 4 weeks but were permanently impaired on the delay version of the task. Neurochemical analysis revealed that AGF2 enhanced ChAT activity in the hippocampus at 20, but not 2 or 11 weeks following surgery. Cell counts of cholinergic neurons in the medial septum revealed that AGF2 did not reduce the extent of neuronal loss. As in the study by Fass and Ramirez /36/, these data indicated that AGF2 might have enhanced the sprouting of cholinergic terminals following the initial insult, but that the behavioral recovery exhibited by these animals was incomplete and unrelated to that sprouting.

It appears, then, that there is no clear evidence which directly links ganglioside-induced sprouting to behavioral recovery following damage to central cholinergic systems. Gangliosides have been shown to influence behavioral recovery at times too early to be accounted for by structural changes or at times removed from the occurrence of such changes /34,36,57,84/. The interpretation of the relationship of sprouting to behavioral recovery is also complicated by the methods used to assess sprouting. Changes in enzyme levels do not necessarily reflect an effect of ganglioside on sprouting. Rather gangliosides could be modulating a lesion-induced up-regulation of transmitter biosynthesis and release. Gangliosides could also directly stimulate the activity of the enzymes involved in the synthesis of acetylcholine (ACh). Cuello and colleagues /20/ found that GM1 significantly increased ChAT activity in the nucleus basalis in sham-operated rats. In addition, Hefti *et al.* /49/ reported that a ganglioside mixture directly increased ChAT activity in cultures of dissociated fetal septal neurons without altering fiber outgrowth of those cholinergic neurons. Accordingly, gangliosides might enhance ChAT activity following septal lesions by increasing the activity of this

enzyme in surviving neurons. However, enhanced sprouting and direct stimulation of ChAT activity need not be mutually exclusive events and might act in a coordinated manner to create a more conducive neural environment for the initiation of structural repair processes which promote functional recovery.

### **Nigrostriatal dopamine system**

The nigrostriatal dopamine system also lends itself to an analysis of the correlation between behavioral recovery and regeneration/reinnervation processes. Transections of the nigrostriatal tract result in a marked reduction in tyrosine hydroxylase (TH) and homovanillic acid (HVA) content in the denervated caudate. Behaviorally, rats with unilateral lesions exhibit a stereotyped rotational behavior when challenged with dopaminergic agonists such as apomorphine or amphetamine. Toffano and colleagues /113-115/ reported that gangliosides reduced the number of apomorphine-induced rotations at 14 and 30, but not 8 days, following a unilateral hemitransection of the nigrostriatal pathway. It was also reported that TH levels in saline-treated controls were 50% of normal while animals receiving gangliosides exhibited only a 20% decrease in TH. Increased levels of HVA and TH-immunofluorescence substantiated the ganglioside related increase in dopaminergic activity at the later, but not earlier, time points. Since this time course coincided with that expected for terminal reinnervation of the striatum, it was concluded that gangliosides enhanced behavioral recovery through increased sprouting of dopaminergic fibers. However, the interpretation of these studies are also subject to the possibility that gangliosides simply increased HVA and TH activity through a compensatory increase in neurotransmitter synthesis and release. Following damage, surviving dopaminergic neurons increase their rate of synthesis and metabolic activity. Ganglioside treatments might act to accelerate this process, thereby affording the animal some degree of behavioral recovery.

To address this issue more carefully, Sabel *et al.* /92,94/ injected HRP into the striatum

following transection of the nigrostriatal pathway. It was assumed that if GM1 ganglioside was promoting sprouting then an increased number of HRP-positive cells should be visualized within the substantia nigra. At post-operative day 3 there were no differences in the number of labeled cells between the saline and GM1-treated animals. However, at day 15 there were significantly more labeled neurons in those animals treated with GM1. While these results strongly suggested that GM1 stimulated reorganizational processes, these same processes do not necessarily underlie behavioral recovery. In fact, a clear dissociation of behavioral and morphological recovery was observed in these studies. While ganglioside-treated animals exhibited decreases in rotational behavior as early as two days following surgery, there were no apparent differences in HRP-labeled cells in the substantia nigra. Although these two indices may correlate at later time points, the proposed sprouting of dopaminergic fibers cannot account for this early behavioral recovery.

Other studies have failed to find a clear correlation between anatomical/neurochemical and behavioral recovery following ganglioside treatment. Sabel *et al.* /93/ reported that gangliosides ameliorated the deficits in spatial alternation behavior following bilateral electrolytic lesions of the caudate nucleus. Upon histological evaluation, it was revealed that there were no differences in the number of neurons or glia adjacent to the lesion site or in the substantia nigra between those lesioned animals receiving gangliosides and those receiving vehicle control injections. While a protective effect of gangliosides was not likely in these studies, the role of sprouting remains a possible contributor to the observed functional recovery. Dunbar *et al.* /30/ examined the effects of gangliosides on rotational behavior, aphagia and adipsia following complete transections of the nigrostriatal pathway. Following total lesions, gangliosides were ineffective in reducing amphetamine-induced rotational behavior /30/. However, these same ganglioside-treated animals demonstrated a significant attenuation of the initial decrease in body weight. Together with previous neurochemical studies, these data

suggested that there may be a dissociation between functional and neurobiological recovery in ganglioside-treated animals.

### Other systems

The ability of gangliosides to promote sprouting has been evaluated in a variety of other neural regions. Kojima *et al.* /62/ infused 6-hydroxydopamine (6-OHDA) into the cerebral cortex via an osmotic minipump. Fluorescent histochemistry and neurochemical analysis revealed a significant loss of fluorescent nerve terminals and norepinephrine (NE) levels in the cortex within 3-7 days. Within 5 weeks there was a reappearance of nerve terminals within the damaged region. Treatment with ganglioside GM1 significantly elevated NE levels at 7 and 14 days following 6-OHDA with no effect on the initial loss of NE terminals.

Jonsson *et al.* /55/ reported that GM1 restored serotonin (5-HT) levels in the cortex following systemic injections of the serotonergic neurotoxin 5,7-DHT. Again, GM1 had no effect on the initial loss of 5-HT terminals, rather the effects were observed to occur following the initial result.

Fujito *et al.* /41,42/ conducted a series of experiments which suggested that gangliosides promoted sprouting in the red nucleus of kittens. Following neocortical damage, the probability of producing excitatory postsynaptic potentials (EPSPs) in the red nucleus decreased to approximately 20%. Gradually, EPSPs reappeared, indicating a sprouting response of the previously degenerating cortical afferents. Placement of a sponge containing a crude ganglioside extract into the wound cavity resulted in an increased probability of inducing EPSPs in the red nucleus at 3 weeks post-lesion. These data indicated that gangliosides promoted a sprouting response and that this response had a functional consequence (i.e., production of EPSPs).

Other investigators have suggested that gangliosides promote sprouting in the spinal cord following transections /10,19/ and in the hamster visual system following lesions of the superior colliculus /98/.

### GANGLIOSIDE-INDUCED PREVENTION OF NEURONAL DEGENERATION

The foregoing discussion strongly suggests that ganglioside-induced enhancement of sprouting does not necessarily account for the promotion of functional recovery. Given that behavioral recovery processes have been frequently reported to occur relatively soon following injury (i.e., at times prior to the initiation of sprouting), a more parsimonious explanation for these effects might be that they limit the extent of neuronal damage produced immediately following surgery. The following section reviews the evidence for this possibility and discusses possible biological mechanisms underlying this prophylactic effect.

### Nucleus basalis-cortical cholinergic system

Following lesions of the magnocellular nuclei, high affinity choline uptake (HACHU) and ChAT activity is decreased by approximately 50% in the frontal cortex. Treatment with GM1 prevented the decreases in HACHU in the frontal and parietal cortex as early as 4 days following surgery /82/. Because of this early time course of recovery, the sparing of HACHU cannot be accounted for by collateral sprouting. Following unilateral cortical lesions, GM1 also prevented the retrograde cell atrophy in the nucleus basalis /20/. Accompanying this attenuation was a significant protection against the loss of ChAT activity in this same region. Unfortunately, a time course analysis was not performed to determine whether GM1 actually prevented or reversed the cell shrinkage.

Casamenti and colleagues /17/ confirmed that ganglioside treatment not only prevents the loss of cortical cholinergic parameters following damage but also improves the rate of acquisition of an active avoidance task. These behavioral effects were observed during the initial stages of behavioral testing (4-7 days post-surgery) and ruled out the possibility that collateral sprouting underlay the observed alterations in cholinergic function. Rather, these results may be interpreted as indicating that GM1 either

prevented terminal degeneration or produced compensatory increases in cholinergic enzymatic activity at time points shortly following injury. The contention that exogenous ganglioside administration attenuates the initial consequences of damage to the magnocellular nuclei is supported by a recent study by Cuello and colleagues /20/. These investigators reported that GM1 prevented the retrograde atrophy and loss of cholinergic neurons in the nucleus basalis following cortical damage. Importantly, this effect was observed both following systemic administration of GM1 and following the placement of microencapsulated GM1 onto the damaged cortical surface /71/. Elliott and colleagues /31/ examined the effects of GM1 on the neurochemical and behavioral changes produced by unilateral devascularizing lesions of the neocortex. Following surgery, animals exhibited a series of behavioral alterations, including hyperactivity, motor uncoordination, impaired passive avoidance retention and reacquisition, and decreased retention of a Morris water maze task. Infusion of GM1 directly into the lateral ventricles via minipumps prevented the retrograde degeneration of cholinergic nuclei in the nucleus basalis and enhanced the reacquisition of both the passive avoidance and water maze task but had no effect on the hyperactivity or motor coordination tasks.

### **Septohippocampal cholinergic system**

Ganglioside-induced prevention of cholinergic cell loss has also been reported following damage to the septohippocampal cholinergic system. DiParte *et al.* /27/ reported that the intraventricular injection of vincristine decreased ChAT and HACHU in the HPC and that these decreases were prevented by prior treatment with ganglioside GM1. Sofroniew *et al.* /104/ further demonstrated that gangliosides prevented the retrograde cell loss in the medial septum and vertical limb of the diagonal band following hippocampal ablation.

To evaluate the ability of gangliosides to prevent damage to the septohippocampal cholinergic system and the associated behavioral consequences, a series of studies using the

neurotoxin colchicine were conducted /33,35/. Intraventricular administration of colchicine produced marked decreases in hippocampal ChAT activity and HACHU levels without damage to hippocampal granule cells which are susceptible to direct administration of colchicine. Behaviorally, colchicine-treated animals exhibited a pronounced hyperactivity and marked cognitive deficits which were prevented by AGF2 administration. While previous studies had indicated that gangliosides produced a task dependent behavioral recovery /17,34/ the recovery in these studies was complete. Lesioned animals receiving AGF2 were able to perform a radial arm maze task as well as controls, even when a delay as long as four hours was imposed between the fourth and fifth arm choices. Neurochemical and anatomical analyses indicated that AGF2 prevented the decreases in hippocampal ChAT and HACHU as well as the loss of cholinergic neurons in the medial septum. These data clearly indicated that gangliosides are capable of preventing the biochemical, anatomical and behavioral consequences of neural damage.

Interestingly, ganglioside treatment appeared to exert a different profile of effects following intradentate injections of colchicine. Following intrahippocampal administration, colchicine produced extensive decreases in the thickness of both the superior and inferior blades of the dentate gyrus and an associated retrograde degeneration of cholinergic neurons in the medial septum /32,33/. GM1 administration facilitated the recovery of normal locomotor activity, attenuated the impaired retention of a passive avoidance task and reduced the alterations in sensitivity to the motor stimulating effects of apomorphine and the analgesic effects of morphine /118/. Furthermore, both GM1 and AGF2 facilitated recovery of working memory in a multiple component T-maze task /32/. However, quantitative analysis revealed that, despite the facilitative effects of ganglioside treatment on behavior, there was no obvious reduction in the extent of colchicine-induced granule cells destruction or the retrograde degeneration of cholinergic neurons in the medial septum.

In contrast to the failure to find any anatomical correlate to the ganglioside-induced behavioral recovery following intrahippocampal colchicine, biochemical evidence suggesting a possible protective effect of GM1 has been obtained. Following intrahippocampal injections of colchicine, Walsh *et al.* /119/ examined the effects of GM1 on the expression of ornithine decarboxylase (ODC) activity in the HPC. Ornithine decarboxylase is the rate-limiting enzyme in the synthesis of the polyamines putrescine, spermine, and spermidine and has been suggested to provide a reflection of the extent of neural damage present. Following unilateral injections of colchicine into the dentate gyrus, ODC activity increased 8-fold in the HPC. Prior treatment with GM1 ganglioside significantly reduced this response on the ipsilateral side but enhanced the ODC response on the contralateral side. These ganglioside-induced changes were independent of any protective action of GM1 on hippocampal morphology. Since changes in ODC activity are correlated with the magnitude of neural damage, it appeared that GM1 may have altered some of the secondary consequences of hippocampal damage.

The mechanism by which gangliosides exert their protective effects on cholinergic systems is unclear but may relate to the interaction of gangliosides with endogenous neurotrophic factors at or near the site of injury. Several lines of evidence indicate that gangliosides augment endogenous biological responses that are initiated by injury and subserve neuroplasticity. It is well established that levels of endogenous trophic factors increase following neural damage /74,75/. Furthermore, the early administration of trophic agents, including nerve growth factor (NGF) and gangliosides, are effective in reducing both cell death and the behavioral deficits associated with neural injury /21,22, 27,31,35,50,63,107,122/. The typical increases in injury mediated trophic factors may not be sufficient to prevent the lesion-induced cell loss which occurs immediately following injury, or these increases may occur at a time subsequent to the majority of cell loss. The administration of compounds capable of interacting with

endogenous trophic factors or altering the response of neurons to the levels of endogenous trophic factors may represent one method for minimizing injury related cell loss. Gangliosides have been suggested to exert their trophic effects by interacting with endogenous NGF. This suggestion is supported by several convergent lines of evidence: (1) gangliosides and NGF promote cell survival and increase ChAT activity in the nucleus basalis in a synergistic manner following cortical damage /20/; (2) GM1 potentiates the ability of NGF to prevent the decreases in norepinephrine levels in the heart, spleen and kidneys following systemic administration of vincristine /117/; (3) GM1 initiates neurite outgrowth in fetal chick dorsal root ganglionic cells and PC12 cells in a NGF-dependent manner /28,103/, and (4) the ability of GM1 to prevent the loss of cholinergic neurons in the nucleus basalis diminishes as the age of the animal increases and the normal and lesion-induced levels of trophic factors decrease /107/.

Although the exact mechanism by which gangliosides potentiate the actions of NGF remains unknown, it may relate to a modification of membrane surface properties subsequent to the incorporation of gangliosides into the membrane. Unfortunately Ferrari /39/ failed to find increased binding of NGF to PC12 cells following ganglioside treatment. However, gangliosides could still conceivably modulate a variety of neuronal processes including surface transduction or specific NGF related post-translational events (see /22/ and /101/ for reviews).

Gangliosides may also alter axonal transport and in turn increase the availability of NGF or other trophic factors to deprived neurons. Gangliosides promote the formation of microfilament networks in cultured neuroblastoma cells /106/ as well as stimulating tubulin gene expression following transections of the nigrostriatal pathway /126/. By accelerating or promoting the organization of cytoskeletal elements, gangliosides may enhance the transport and subsequent availability of trophic factors such as NGF under normal conditions and following injury. The ability of gangliosides to modulate the regeneration of damaged nerves

by altering the axoplasmic transport of macromolecules has been investigated following nerve crush of the optic nerve in goldfish. Following nerve crush, the optic nerve regenerates over a 2-4 week period in parallel with increased visual acuity /23,24/. Sbaschnig-Agler *et al.* /100/ reported an 8-fold increase in the axonal transport of gangliosides following crush of the optic nerve. This increase was evident within 8 days following surgery and persisted for up to 25 days. In addition, intraocular injections of antibodies to gangliosides inhibited the regeneration of the optic nerve following nerve crush /105/. Daily injections of antiserum beginning 5 days prior to surgery resulted in a 40% decrease in the distance of axonal outgrowth measured 10 days after the crush. These data suggested that gangliosides may play a role in neural outgrowth and regeneration by modulating the transport of various macromolecules such as NGF.

### Nigrostriatal dopamine system

As already mentioned, the evidence supporting a role for gangliosides in mediating behavioral recovery via sprouting of surviving dopaminergic afferents is questionable. Several lines of evidence indicate that behavioral recovery occurs at time points too early to be accounted for by structural changes. On the other hand, there is evidence to suggest that gangliosides may act to reduce the initial consequences of damage to this system.

Within 2 weeks following a partial hemitransection of the nigrostriatal pathway, animals treated with gangliosides exhibit an attenuated decrease in TH activity. Toffano *et al.* /116/ reported that this amelioration was associated with a ganglioside-induced reduction of the loss of dopamine neurons. Both the TH and immunocytochemical indices necessarily use dopamine to evaluate cell number and these results could also be interpreted as indicating that gangliosides are simply increasing transmitter synthesis.

In an attempt to evaluate more carefully the possible protective effects of gangliosides, Sabel

*et al.* /97/ used the Fink-Heimer technique to quantify the extent of anterograde degeneration in the substantia nigra following hemitransections of the nigrostriatal pathway in the left hemisphere and an electrolytic caudate lesion in the right hemisphere. Seven days following the lesion, the animals treated with GM1 had significantly smaller areas of neuronal degeneration on the side with the caudate lesion than those animals treated with saline. In contrast, there was no effect of ganglioside treatment on the side with the hemitransection. While GM1 had no effect following the hemitransection in these studies, Raiteri and colleagues /88/ reported that AGF2 decreased the reduction of [<sup>3</sup>H]dopamine uptake into striatal synaptosomes five days following surgery. Although not conclusive, the combined anatomical and neurochemical data suggest that gangliosides exert some protective effect following damage to the nigrostriatal system.

The contention that gangliosides produce behavioral recovery by minimizing cell damage is supported by a study by Li *et al.* /67/. Two days following unilateral nigrostriatal transections, amphetamine-induced rotational behavior was reduced by ganglioside treatment. The ganglioside treatment also prevented the loss of striatal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity typically observed following damage. Two days following transection, the level of Na<sup>+</sup>,K<sup>+</sup>-ATPase was decreased 36% in lesioned animals but only 7% in lesioned animals receiving gangliosides. Because increased Na<sup>+</sup>,K<sup>+</sup>-ATPase activity had been previously shown to correlate with the extent of edema, it was concluded that the gangliosides had minimized the extent of edema and in turn promoted functional recovery.

### Ischemia

Karpiak and colleagues have used a model of transient cerebral ischemia to investigate the effects of gangliosides on behavioral recovery /58/. In particular, this model has been employed to evaluate the suggestion that gangliosides produce their effects through the induction of discrete metabolic events immediately after damage. The most studied metabolic alterations

concern the ability of gangliosides to reduce the decrease in membrane  $\text{Na}^+, \text{K}^+$ -ATPase which occurs following cell damage. Gangliosides produced a 33% reduction in edema 48 hours following cortical damage in rats /58/. This reduction was accompanied by a significant attenuation of lesion-induced reduction in plasma  $\text{Na}^+, \text{K}^+$ -ATPase levels and paralleled enhanced cerebral blood flow, reduced cell damage, electrocorticographic alterations and neurological deficits /14/.

There have been a limited number of clinical studies examining the effects of gangliosides in human stroke patients. Bassi /7/ reported that patients receiving gangliosides had a higher level of neurological functioning than a similar group of individuals treated with placebo. Likewise, Battistin /8/ demonstrated that ganglioside treatment resulted in improved neurophysiological ratings in patients with cerebrovascular disease. Importantly, the ganglioside treatment in both these studies was effective even though it began at least 10 days following the initial lesion. Therefore, it may not be absolutely necessary to administer gangliosides during the acute phase of such an injury. Such a treatment may still be effective during the subacute phase and might actually exert a more powerful effect if given earlier.

### CONCLUSIONS

Gangliosides appear to be capable of producing potent behavioral recovery following a variety of CNS perturbations /16,17,20,30,32,34, 35,82,89,92-97,113,115,120/. Gangliosides have been shown to reduce motor and cognitive deficits in laboratory animals as well as the clinical signs of neurological functioning in humans. In contrast, only a small number of studies has failed to demonstrate a beneficial effect of ganglioside treatment /11,17,30,34,84/. Importantly, in those studies failing to show any significant improvement following ganglioside treatment in humans, there have not been any demonstrated deleterious effects. For instance, chronic administration (up to 12 months) of GM1 ganglioside to Alzheimer's patients did not

result in any neurological improvement but had no toxic side effects either, demonstrating the long-term safety of chronic ganglioside treatment /3/. Unfortunately, these investigators were not able to determine if the GM1 treatment significantly slowed the progression of the disease. Recent studies have suggested that antibodies to GM1 are present in high levels in individuals afflicted with Guillain Barre syndrome, suggesting that chronic administration of gangliosides could contribute to the development of this disease /87,127/. However, a recent study failed to reveal antibody formation following parenteral injection of gangliosides in Alzheimer patients /110/. Nevertheless, the possibility of untoward side effects from ganglioside treatment deserves serious attention prior to large scale clinical trials. The possibility of side effects further underscores the need to determine the mechanism underlying ganglioside-induced recovery.

Several lines of evidence suggest that gangliosides promote sprouting of CNS neurons and that this sprouting response mediates behavioral recovery /92,93,113-115/. However, there are also numerous studies which seriously question this conclusion /34,36,57,84/. Most of the evidence of ganglioside-induced sprouting is based on observations of increased transmitter levels or their metabolites. Additionally, increased retrograde cell labeling has been provided as evidence of enhanced sprouting. These indices do not, however, provide direct evidence of enhanced sprouting and could simply reflect increased transmitter levels, enzyme activity, axonal transport processes or a unique combination of these events. Future studies using electron microscopy, tract tracing techniques, autoradiography, electrophysiology and *in vitro* techniques are needed to substantiate ganglioside-induced sprouting.

Regardless of whether gangliosides do actually promote sprouting, it appears that this is not always the basis of behavioral recovery. Functional recovery has been frequently reported prior to, or independent of, measures indicative of sprouting /34,36,84/. Such a dissociation suggests that events which precede sprouting may be the basis for some observed

recovery. Although few studies have actually demonstrated neuronal sparing following ganglioside treatment, some have provided direct evidence of a protective effect [20,35]. While either an enhancement of sprouting or a prophylactic effect of gangliosides is appealing, neither can account for all observed behavioral recovery. It appears then that a variety of factors, including the type, location, and extent of the lesion, may affect the ability of gangliosides to modulate behavioral recovery. Further complicating our understanding of recovery are questions concerning the effective species of gangliosides for eliciting recovery from various lesion types, as well as practical considerations, including the doses, timing and route of administration and which portion of ganglioside molecule is responsible for an observed effect. These considerations withholding, gangliosides appear to be useful for treating a variety of CNS injuries. Although not a panacea, answers to the above questions may accelerate our development of an efficacious and rational approach for using gangliosides or other pharmacological treatments as primary or adjunct therapies in disorders of the CNS.

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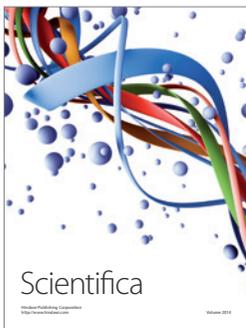
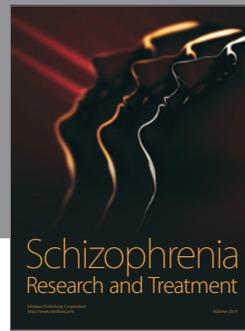
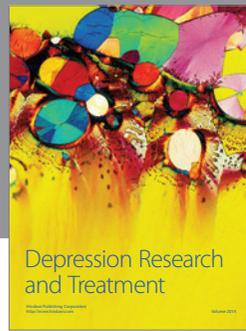
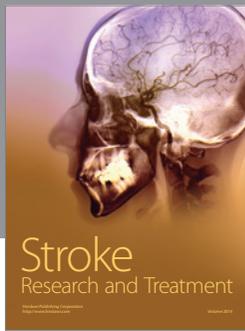
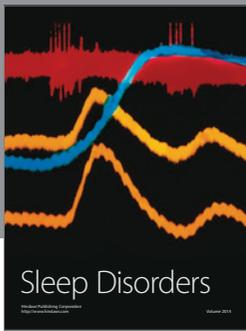
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