TOWARD A UNIFIED THEORY OF GANGLIOSIDE-MEDIATED FUNCTIONAL RESTORATION AFTER BRAIN INJURY: LESION SIZE, NOT LESION SITE, IS THE PRIMARY FACTOR DETERMINING EFFICACY

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Abstract. Pharmacological intervention is receiving increasing attention as a strategy to promote recovery of function after central nervous system injury. In this review we describe numerous studies indicating that gangliosides have been particularly successful in promoting CNS repair across a wide spectrum of brain areas and behavioral paradigms. A detailed comparison of these studies reveals common features among those studies where ganglioside treatment was found to be beneficial and those where no effect was observed. Specifically, treatment efficacy was only seen in those paradigms where recovery occurs naturally after the injury and not in those where either the behavioral deficit was too small ("ceiling effect") or too extensive for spontaneous recovery to occur ("floor effect"). Thus, ganglioside treatment is only effective in cases of moderate (partial) injury and/or when behavioral recovery occurs naturally. The effect is independent of lesion site or lesion type. On the basis of these observations, we propose a model based on the relationships of neural aggregates (cell groups) which have either a pri-

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mary or modulatory role in brain function. Our model may provide a heuristic basis for future research efforts aimed at elucidating the mechanism(s) whereby gangliosides enhance brain repair.

INTRODUCTION

Within the last twenty years, the conceptualization of the central nervous system's (CNS) ability to respond to injury has undergone a dramatic revision. Previously, the CNS was considered to be hard-wired, incapable of actively responding to insult in other than a degenerative fashion (ref. 17, for a review). Ramon y Cajal's (40) dictum that the CNS "is fixed and immutable; everything may die, nothing may regenerate" was the essence of the doctrine believed throughout most of this century and it is still frequently invoked in contemporary neurology. Although it was recognized that behavioral recovery from CNS damage was possible, many of the models accounting for recovery reflected this doctrine (24) and, for the most part, little progress was made towards the amelioration of impairments associated with CNS injury.

In 1969, Geoffrey Raisman published a seminal report (35) in which he significantly challenged this "immutability" doctrine. Raisman demonstrated in an electron microscopic investigation that removal of the septum's hypothalamic or hippocampal afferents resulted in significant sprouting by the remaining surviving inputs. In the early 1970's, however, it was still unknown whether this kind of CNS plasticity was the exception rather than the rule (30), but a flurry of reports subsequently confirmed that lesion-induced synaptogenesis was indeed a ubiquitous CNS phenomenon (7, 8, 51, for a review 53).

We now know that the CNS actively responds to injury in a multiplicity of ways — e.g. reactive synaptogenesis, altered regulation of neurotransmitter synthesis, denervation supersensitivity, unmasking of silent synapses, for example (reviewed in 17). Because of the recognition that the CNS is a dynamic participant in recovery of function, neuroscientists have recently become involved in a multidisciplinary research effort to identify and manipulate factors which may contribute to recovery from CNS injury.

Of the therapeutic strategies, we propose that the pharmacological approach may in the long run be the most practical and useful. In fact, in recent years we have witnessed a great deal of activity in the use of pharmacological agents to ameliorate the behavioral impairments after CNS injury. Drug therapies have effectively reduced behavioral deficits after damage to entorhinal cortex, striatum, the nigro-striatal pathway, and the nucleus basalis of Meynert, to name but a few (see 54, for a re-
view). Since many of these drugs can be given peripherally, the potential complications associated with surgical interventions (e.g. brain grafts) are circumvented.

Although a variety of pharmacological manipulations have met with limited success in ameliorating impairments associated with brain and spinal cord injury (54), we will focus our attention in this review on gangliosides, a family of sialic acid-containing glycosphingolipid molecules (25). Of the pharmacological agents used to treat CNS injury, gangliosides combine a number of features making them potentially therapeutically important: (1) they have reliably been shown to exert powerful ameliorative effects in numerous animal model preparations and on a variety of behavioral tasks; (2) they are easily administered (i.e. intraperitoneally or intramuscularly); and (3) there are presently no known toxic side effects associated with their administration (20).

The objectives of this article are twofold: A) to briefly review the studies of the behavioral effects of ganglioside administration to animals with brain injury; and B) to identify common features among studies that successfully employed gangliosides to facilitate recovery in order to serve as a heuristic for future research efforts. Unfortunately, despite the fact that there is now a substantial body of evidence demonstrating that gangliosides effectively facilitate recovery from brain injury, little is known about the mechanism(s) of ganglioside action.

GANGLIOSIDES: A HISTORICAL PERSPECTIVE

Two studies in the mid-1970's were pivotal in the identification of gangliosides as potential neuritogenic/neuromotrophic agents and subsequently sparked a series of biochemical, anatomical, physiological, and behavioral experiments which continue into the present time. The first systematic study (6) of gangliosides' neuritogenic properties was initiated after Ceccarelli and his colleagues had earlier serendipitously discovered that gangliosides could promote sprouting of peripheral nerves (cited in 42). Ceccarelli and his colleagues (6) examined the effects of phospholipid administration in cats that sustained pre- and post-ganglionic transection and subsequent anastomosis of the cervical sympathetic nerves innervating the nictitating membrane (NM). In this experiment, they used mixed ganglioside administration (50 mg/kg; i.p.) as a control injection (Ceccarelli, personal communication: cited in 42) injection and subsequently reported the unexpected result; while untreated cats showed no evidence of recovery of NM response to nerve stimulation 45 days after the transections, the NM response of ganglioside-treated animals recovered to near normal levels. Ceccarelli et al.
also demonstrated a recovery of catecholaminergic fluorescence in the NM inferior smooth muscle. They concluded that both the electrophysiological and anatomical evidence indicated that ganglioside administration had enhanced peripheral nerve regeneration.

In 1977, Purpura and Baker (34) examined mature cortical neurons in a cat suffering from GM₁-ganglioside storage disease. Feline GM₁-gangliosidosis involves a deficiency of B-galactosidase resulting in an accumulation of GM₁ in the brain. One of the most striking features of cortical neurons in the B-galactosidase deficient cat was the presence of enormous neurites between the perikaryon and axon hillock as well as many fine neurites projecting from these meganeurites. This finding led Purpura and Baker to speculate that the abnormally high concentrations of GM₁ in these neurons may have produced the anomalous neurites. Indeed, subsequent research has confirmed that GM₁ exhibits neuritogenic properties in cell cultures (e.g. 9, 11, 41).

GANGLIOSIDE FACILITATES BEHAVIORAL PERFORMANCE AFTER BRAIN INJURY

To date, researchers have examined the behavioral effects of ganglioside administration in a number of different model preparations of brain injury which will now be discussed. Unless stated otherwise, mixed ganglosides (GM₁, GD₁α, GD₁β, GT₁ or purified GM₁) at concentrations of 30 to 50 mg/kg were injected daily throughout the length of these studies (see also review by Dunbar et al. 13).

Recovery from subcortical lesions

Caudate nucleus. Among the first demonstrations that gangliosides reduce learning deficits resulting from brain injury was that by Sabel et al. (48). In this experiment, rats were given bilateral lesions of the caudate nucleus by electrocoagulation, and the animals were subsequently tested in a two-armed learning maze. The rats had to learn to avoid or escape footshock by running through one of the doors, and, upon reaching criterion, the door was reversed. Rats which had sustained bilateral lesions of the caudate nucleus were unable to undergo such spatial reversals and thus obtained low learning scores. However, when treated daily with intraperitoneal ganglioside injections, the brain-injured rats learned the task quite readily. This group difference was seen as early as the first day of testing (postoperative day 9), suggesting that the treatment had reduced the behavioral deficit (sparing) rather than enhanced “recovery” of function. In fact, despite significant brain injury, these treated animals were indistinguishable from sham operated counterparts on a number of behavioral parameters.
**Nigrostriatal pathway.** The dopaminergic nigro-striatal system has several features which makes it an attractive model to study brain-behavior relationship (61). Unilateral lesions can be created in the nigrostriatal system by injections of the neurotoxin 6-hydroxydopamine (6-OHDA) into the substantia nigra pars compacta or by transection of the dopaminergic axon bundle with a surgical knife. Rats with such lesions show asymmetries in their body posture and a characteristic rotational behavior (turning in circles) due to the unilateral loss of dopamine. This rotational behavior is conveniently measured with an automated testing apparatus and the impairment is even more obvious when dopaminergic agonists (such as amphetamine or apomorphine) are given to amplify the asymmetry in dopaminergic neurotransmission between the two hemispheres.

Several investigators have created this type of lesion and studied the behavioral and anatomical consequences of GM1 treatment. Agnati et al. (1) observed, for example, that rotational behavior following injections of apomorphine was less pronounced in the GM1 group. The improvement was evident at postoperative day 8. Sabel et al. (44, 46) have found similar effects of GM1 and have shown, in addition, that amphetamine-injections, which stimulate the release of dopamine from surviving fibers, also lead to less pronounced rotational behavior in GM1-treated animals. It is interesting to note that the reduction of behavioral deficits was noted as early as 2 days postlesion. An anatomical analysis of neuronal connectivity was performed, using intrastriatal HRP-injections to retrogradely trace the nigro-striatal neurons after lesion. It was found that ganglioside treated animals had significantly more labeled neurons. Li et al. (27) confirmed these behavioral results and by varying the onset of treatment (beginning at 0, 2, 4, 8, or 12 h. after the lesion) they showed that when started 4 h or longer postsurgery, treatment was no longer effective.

Intracerebral, unilateral injections of (6-OHDA) have widely been used to simulate Parkinson’s disease in rats. Most recent reports have presented evidence indicating that ganglioside therapy is beneficial even in this animal model (56, intraventricular infusion of gangliosides) and also improves outcome after peripheral MPTP-administration in mice (19, 52).

**Nucleus basalis magnocellularis.** Because of the evidence implicating the nucleus basalis of Meynert in Alzheimer’s disease (see 2, for a review), several investigators have explored the role of the cholinergic nucleus basalis magnocellularis (NBM; the rat homologue of the nucleus basalis of Meynert) in learning and memory. Nucleus basalis lesions in rats disrupt the acquisition of active and passive avoidance (18, 28),
radial arm maze performance (3) as well as serial spatial discrimination reversal performance (26). Thus, the NBM preparation may serve as a good model for the diseased nucleus basalis of Meynert observed in Alzheimer's patients.

Accordingly, Casamenti et al. (5) examined the possibility that gangliosides might reduce the severity of behavioral and neural impairments after unilateral electrolytic lesions of the NBM. Rats with NBM lesions were treated with GM\(_1\) or saline and tested for acquisition of active and passive avoidance. The NBM lesions seriously disrupted the acquisition of avoidance behavior; however, the GM\(_1\)-treated group successfully avoided the shock more frequently than the saline-treated animals. In contrast, passive avoidance was equally impaired in both treatment conditions. Casamenti et al. also measured ChAT activity in cortex after NBM damage and observed significant declines in the cortex ipsilateral to the lesion of saline-treated rats. Although ChAT activity was reduced in the frontoparietal cortex ipsilateral to the NBM lesion in GM\(_1\)-treated animals, the parieto-occipital areas of the cortex in the intact hemisphere had significantly elevated levels of ChAT activity. The increases were evident in those brain regions with the greatest residual innervation. Casamenti et al. propose that this elevation may signify either: (1) an increase in cholinergic activity of residual cholinergic cortical inputs; or (2) a sprouting response by remaining intact cholinergic projections. Of the two hypotheses, sprouting is an unlikely candidate to account for these results since NBM innervation of cortex is primarily ipsilateral. Thus, if sprouting had occurred in the hemisphere contralateral to the lesion, it would have occurred in a fully innervated structure in adult animals. Nevertheless, GM\(_1\) ganglioside administration facilitated recovery of active avoidance behavior in NBM-lesioned rats and restored cholinergic activity to a significant portion of denervated cortex. Cholinergic deficits can also be induced by intracerebral vin-cristine injections. Here, ganglioside therapy has again been found to be beneficial (10).

**Septum.** In a recent investigation, Poplawsky and Isaacson (32) demonstrated that GM\(_1\) ganglioside promotes behavioral recovery from bilateral damage to the medial and lateral septal nuclei of rats. These lesions typically result in hyperemotionality, decreased rearing behavior in an open-field, and facilitation of avoidance behaviors (see 21, for a review). Poplawsky and Isaacson studied these behaviors in septal-lesioned rats, and GM\(_1\) treatment significantly reduced hyperemotionality within two days of surgery and accelerated recovery to control levels of emotionality. Although GM\(_1\) did not affect the number of avoid-
ance responses, the GM\(_1\)-treated rats made fewer intertrial crossings than the saline-treated animals. However, as Poplawsky and Isaacson pointed out, GM\(_1\) exerted ameliorative effects only on those behaviors which are normally transient after septal damage (i.e. emotionality and intertrial crossings).

**Vestibular system.** Unilateral labyrinthectomies in guinea pigs severely disrupt postural and ocular movements from which the animals recover several weeks after the lesions. Reactive synaptogenesis occurring in the denervated vestibular nuclei after labyrinthectomy is generally considered to contribute to vestibular recovery (31).

Insofar as gangliosides appear to have neuritogenic properties (see above) and vestibular recovery may rely on sprouting, Petrosini (31) examined the possibility that GM\(_1\) ganglioside would promote functional recovery from vestibular impairments. Guinea pigs with unilateral labyrinthectomies were treated with GM\(_1\) ganglioside and were tested on a battery of postural and ocular behavior tests. Although GM\(_1\) treatments had somewhat limited facilitative effects after the labyrinthectomies, the recovery of two behaviors was particularly accelerated: head posture in the horizontal plane and ocular stability.

**Recovery from cortical lesions**

**Entorhinal cortex.** By the early 1980's evidence consistent with the possibility that exogenous gangliosides were capable of promoting neuritogenesis in vitro (9, 11, 41), in the peripheral nervous system (6), and in the CNS (60) had begun mounting.

Consequently, it became of interest to determine whether ganglioside administration could enhance recovery from CNS injury in a model preparation wherein recovery was thought to be dependent on sprouting (23). Previous research (29) had shown that recovery from learned alternation deficits after unilateral entorhinal cortex lesions in rats was correlated with the reinnervation of the dentate gyrus by one of its surviving afferents (i.e. the crossed temporodentate projection). Karpiak (23) therefore treated rats that had sustained unilateral entorhinal cortex lesions with gangliosides or saline the day before surgery and every day thereafter. He tested the rats for retention of a learned alternation task and observed that rats treated with gangliosides: (1) committed fewer errors immediately upon retesting and (2) recovered to preoperative levels of performance significantly faster than their saline-treated counterparts. Unfortunately, crossed temporodentate sprouting was never assessed after the entorhinal lesions so it is unknown whether the gan-
the enhanced recovery was consistent with the possibility that gangliosides had increased the rate of crossed temporodentate sprouting, the rapidity with which the recovery occurred (i.e. within 2 days postlesion) makes it more likely that gangliosides had affected some more acute lesion-related processes. Using a short-term treatment regimen, we have since corroborated Karpiak's findings, and have also found that ganglioside-detreated rats with entorhinal lesions exhibit significantly less perseverative behavior than saline-treated animals (38).

Following Karpiak's initial experiment, Fass and Ramirez (16) reexamined the possibility that gangliosides might effect their ameliorative actions via sprouting in a study of locomotor activity after bilateral entorhinal cortex lesions. Bilateral entorhinal lesions produce dramatic increases in locomotor activity in an open-field chamber from which the animals recover in about 8-10 days postlesion (55). Since the time-course for recovery parallels that of dentate gyrus reinnervation by the septodentate and commissural/associational pathways (see 7, 8, for a review), Steward et al. suggested that the recovery may be related to the lesion-induced sprouting. Consequently, Fass and Ramirez (16) treated rats that sustained bilateral entorhinal lesions with ganglioside or saline and tested the animals in an open-field apparatus. The ganglioside-treated rats exhibited a level of hyperactivity intermediate to that of saline-treated brain-damaged rats or the ganglioside-treated sham operates. Ramirez et al. (36) later confirmed these findings.

In order to evaluate the effects of gangliosides on sprouting by the septodentate pathway, Fass and Ramirez prepared animals with unilateral entorhinal lesions and treated them with mixed gangliosides. Analyses of tissue histochemically stained for acetylcholinesterase (a sensitive marked for sprouting by the septodentate pathway) indicated that there was a reduction in the sprouting response of the septodentate pathway. Because previous research demonstrated a strong correlation between sprouting in the dentate gyrus and recovery from locomotor hyperactivity in rats during the first two weeks postlesion, it is unlikely that gangliosides exerted their facilitative effects by suppressing sprouting. Rather, as Karpiak (23) had suggested earlier, gangliosides might stabilize membranes of neurons adjacent to lesions. As an extension of this proposal, we postulated that gangliosides may also stabilize membranes of denervated target cells (e.g. granule cells), thereby resulting in two outcomes: (1) a reduced behavioral deficit and (2) a reduction in denervation-induced changes in the target which may normally be involved in eliciting a sprouting response from its afferents (16).

Insofar as these studies indicate the possibility that gangliosides enhance recovery independent of sprouting, Ramirez et al. (39) next exa-
mined the effects of ganglioside treatments in a model preparation where-in sprouting is thought to make only a minor contribution to recovery from CNS injury. Recovery of alternation performance after bilateral entorhinal lesions usually occurs after approximately 30 days of post-operative training on an alternation task with a 0 sec. intertrial interval (38). Sprouting is not believed to play an important role in this paradigm because: (1) the recovery does not occur during the time-course within which sprouting occurs, but rather appears to be training-dependent (38); and (2) whereas the recovery of alternation performance relies on sprouting by homologous afferents, the sprouting occurring after bilateral lesions is heterologous. Rats with bilateral entorhinal lesions were tested for the retention of a preoperatively learned alternation task and were administered either gangliosides or saline for six consecutive days, beginning the day before surgery and every other day thereafter. Ganglioside–treated rats committed significantly fewer errors and perseverative errors, and recovered to preoperative performance levels sooner than the saline–treated rats. Therefore, gangliosides effectively improved the performance of brain–damaged rats even in the absence of sprouting by homologous afferents.

Hippocampus. Two groups of investigators (15, 57) have injected a neurotoxin, colchicine, into the dentate gyrus of the hippocampal formation to evaluate the efficacy of ganglioside therapy. Tilson et al. (57) injected 2.5 µl of the neurotoxin bilaterally in the dentate gyrus and assessed hypermotility by recording the number of interruptions of a photo-beam in a recording chamber. It was found that the colchicine lesion did not induce hypermotility in rats that were handled regularly after the lesion, despite marked loss of granule cells in dentate gyrus. The lack of a behavioral deficit was paralleled by the absence of any ganglioside effect ("ceiling effect", see discussion below). In contrast, when rats were not handled during the postoperative period, a significant hypermotility was observed. Unfortunately, the authors did not assess the effects of gangliosides in the latter, more interesting, condition.

Using the same paradigm, Emerich and Walsh (15) studied reference and working memory impairments using a T-maze. As in the experiment by Tilson et al. (57), Emerich and Walsh did not find that ganglioside therapy had any beneficial effects on the neurotoxin-induced morphological damage, neither did the treatment improve reference memory. It should be noted, however, that the reference memory deficit was very small and the treatment effect was therefore probably masked by the "ceiling effect". When assessing working memory, in contrast, a significant functional loss was noted, followed by spontaneous recovery. Here, ganglioside therapy (or treatment with the compound
AGF2, a ganglioside derivative) significantly improved the recovery process.

Frontal cortex. Since GM₁ gangliosides had effectively reduced the severity of bilateral caudate nucleus lesions (discussed above), Sabel et al. (46) wanted to determine whether these facilitative GM₁ effects could be observed in other preparations involving spatial deficits. More importantly, they examined the possibility that the type and locus of lesion as well as type of reinforcement (water reward) used in training would be important variables in obtaining a GM₁ effect. Therefore, they subjected rats to bilateral 

 aspiration lesions of the mediofrontal cortex which seriously disrupt spatial alteration performance in a manner similar to that of caudate lesions. Despite extensive mediofrontal damage, rats treated with GM₁ performed on the spatial task at a level intermediate to both the sham operates and the untreated brain-damaged group. GM₁-treated rats committed fewer perseverative errors and required fewer trials to obtain criterion performance than the saline-treated group.

Visual cortex. Butler et al. (4) have recently shown that impairments in visual discrimination after bilateral ablation of the visual cortex in rats are not ameliorated after treatments with gangliosides. Saline-treated and GM₁-treated rats were equally impaired in pattern discrimination throughout the two weeks of testing. Moreover, although visual cortex damage usually produces transient deficits in brightness discrimination, Butler et al. observed that GM₁ treatment mildly exacerbated the effects of the aspiration lesions. Butler and his colleagues suggested either that visual activity may be necessary for an ameliorative GM₁ effect or that the cortical lesions may have severely disrupted the rats’ circadian rhythm which resulted in these findings. In fact, Toffano et al. (58) had demonstrated earlier that recovery of tyrosine hydroxylase activity after unilateral nigrostriatal damage and GM₁ treatment was disrupted when rats were on a constant dark-cycle. This interpretation is also consistent with previous studies suggesting that the circadian rhythm may determine whether behavioral impairments or recovery are manifested (37, 49).

PIECING THE PUZZLE TOGETHER

Despite a plethora of proposals, the mechanisms whereby gangliosides promote behavioral recovery essentially remain enigmatic. Exogenous gangliosides participate in a wide assortment of cellular processes (reviewed by Sabel, ref. 42), so it is conceivable that gangliosides contribute to recovery in multiple ways (e.g., by reducing degeneration or enhancing axonal sprouting). In any event, we believe that a careful
consideration of the current behavioral literature might provide us with additional clues as to the circumstances under which ganglioside treatment can be expected to improve behavior and under which circumstances it might not.

In the following sections we therefore first identify common features among those studies which reported a ganglioside-induced amelioration of behavioral deficits after CNS injury as well as ascertain potentially important factors in model preparations wherein gangliosides failed to facilitate recovery. Thereafter, we will present a model which may help us to better conceptualize the postlesion response to injury and of the conditions necessary for ganglioside treatment to improve behavioral outcome after injury.

Ganglioside injection regimen. The most common treatment regimen used in the studies discussed above involved daily administration of gangliosides throughout the testing periods. Short-term treatment (i.e. one week or less), however, was effective in reducing impairments after septal damage (32, 33), entorhinal cortex damage (39), and nigro-striatal transection (27).

Perhaps one of the most critical factors to be considered when administering gangliosides is the time at which treatment is begun. Evidently, treatment should be initiated as close as possible to the time of injury. As Li et al. (27) demonstrated, the later the therapy is initiated, the less likely the treatment will be effective: treatments begun 4 h after the nigro-striatal damage failed to reduce rotational behavior. In addition, it appeared that pretreatment alone was not sufficient to improve behavior.

Lesion parameters: site, size and type

Lesion site. To varying degrees and with the exception of the visual cortex, gangliosides have induced behavioral improvement after lesions in most cortical and subcortical areas examined so far. They included regions involved in sensory and motor processing (1, 4, 12, 27, 44, 56, 59), emotion (16, 32, 36), learning and memory (5, 15, 23, 37, 39, 46, 48). In addition, the ganglioside-effect does not appear to be restricted to particular transmitter systems, for primarily monoaminergic (1, 44, 59), cholinergic (5, 16, 36), and glutaminergic (37) systems have all responded to the ganglioside treatment.

Because of the nearly ubiquitous nature of these facilitative effects, it would be reasonable to argue that gangliosides affect fundamental cellular processes common to neurons across the CNS. By way of contrast, nerve growth factor is known to exert its neurotrophic effects primarily on cholinergic neurons in the CNS (62).
Lesion size. A factor which appears to be the most critical determinant whether gangliosides improve behavior is the size of the injury. For example, when the nigrostriatal pathway is partially damaged, GM₁ reduces behavioral deficits (44). However, if lesions are total, the drug-induced rotational deficit does not recover spontaneously and gangliosides fail to promote recovery (12, 59). In addition, Stein and co-workers (4) point out the possibility that GM₁ ganglioside was without effect in their visual cortex preparation because the lesions were very large. Conversely, they suggest that GM₁ facilitated recovery in their mediofrontal preparation because more cortical tissue remained after the lesions. Thus, an exceedingly large lesion may ultimately preclude the possibility of recovery, regardless of any pharmacological treatment, because the substrate for recovery is absent.

The only major exception to the rule that total lesions may not be amenable to treatment is a study using the entorhinal cortex preparation. Despite the fact that lesions of the entorhinal cortex are complete, gangliosides have reliably accelerated the rate of behavioral recovery on a variety of tasks (e.g., open field activity and learned alternation). This point will be discussed in greater detail below and a model is proposed which may account for these observations.

Lesion type. Efficacy of treatment is not dependent on the type of injury in the CNS. Ganglioside treatment is not only effective in various mechanical lesion paradigms such as electrocoagulation, transection, or aspiration but also after lesions using neurotoxins which are administered to destroy specific cell groups of the brain (for references, see discussion above).

“Ceiling effect” and “floor effect”. As presented above, Emerich and Walsh (15) reported a therapeutic activity of ganglioside treatment when working memory was assessed. In contrast, using the same lesion paradigm, Tilson et al. (57) could not document efficacy when hypermotility was studied. While the colchicine injections done by Tilson’s group lead only to minor behavioral deficits (hypermotility) compared to CSF-injections (ref. 57), Emerich and Walsh observed a severe deficit in working memory from which untreated animals gradually recovered. Here, ganglioside treatment significantly improved the animals’ performance. The discrepancy in these two experiments can in part be explained by differences in the extent of granule cell damage. Alternatively, these two behavioral paradigms may respond differentially to ganglioside treatment.

It is interesting to note that a “ceiling effect” found by Tilson et al. was also observed by Emerich and Walsh when they assessed reference memory (i.e. small behavioral deficit, no ganglioside effect).
<table>
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<td>Butler et al. (4)</td>
<td>visual cx</td>
<td>tot</td>
<td>pattern discr.</td>
<td>(minor deficit)</td>
<td>0</td>
</tr>
<tr>
<td>Dunbar et al. (12)</td>
<td>nigro-striatal</td>
<td>tot</td>
<td>rotation</td>
<td>no</td>
<td>0</td>
</tr>
<tr>
<td>Poplawsky and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isaacs (32)</td>
<td>septum</td>
<td>tot</td>
<td>avoidance learn.</td>
<td>no</td>
<td>0</td>
</tr>
<tr>
<td>Toffano et al. (59)</td>
<td>nigro-striatal</td>
<td>tot</td>
<td>rotation</td>
<td>no</td>
<td>0</td>
</tr>
<tr>
<td>Butler et al. (4)</td>
<td>visual cx</td>
<td>tot</td>
<td>brightness discr.</td>
<td>no</td>
<td>0/-</td>
</tr>
</tbody>
</table>

The table displays the reference, the site of the lesion, an estimation of whether lesions were partial (part) or total (tot), and the behavioral paradigm. The table also displays if a given behavioral deficit was followed by spontaneous recovery in untreated animals and whether ganglioside treatment influenced behavioral outcome; +, effective; (+), marginally effective; 0, no change; —, detrimental. In some experiments the time course of behavioral changes was not published and it is not possible to judge whether spontaneous recovery was observed in animals with lesions and no treatment (n.a.*) **Within 30 days postoperatively, the animals grew into a deficit, with no apparent recovery. Whether recovery occurs at a later time cannot be judged from the data presented. ***The behavioral assessment of this study revealed no spontaneous recovery, but a later replication did demonstrate spontaneous recovery when animals were tested at a different time of the day. The table shows that ganglioside efficacy was always associated with spontaneous recovery.
As with the colchicine neurotoxicity, the detrimental effects of intracerebral injection of 6-hydroxydopamine (6-OHDA) can either be reversed (56) by intraventricular infusion of gangliosides or not reversed (60) by treatment. Again, the outcome depends largely on the size of the lesion.

**Persistence of behavioral deficits.** One potentially important characteristic of model preparations wherein gangliosides have facilitative effects is whether behavioral recovery occurs spontaneously in these preparations, that is in the absence of any treatment. Obviously, this is closely related to the size of the lesion (see discussion above). For instance, Poplawsky and Isaacson (32) state that “...gangliosides have their greatest effects on transient changes in behavior...” (p. 156) following brain injury. Recovery of function is known to spontaneously occur after partial unilateral nigro-striatal damage (41), unilateral and bilateral entorhinal cortex lesions (29, 38), septal lesions (32), unilateral labyrinthectomy (31), and lesions of the granule cells of the dentate gyrus (15). As displayed in Table I, gangliosides effectively accelerated recovery in each of these preparations. By contrast, gangliosides failed to enhance recovery in preparations wherein the deficits are particularly persistent. This was the case after complete nigro-striatal lesions which induce stable rotational behavior (12, 59), septal lesions which facilitate avoidance behavior and decreased rearing in an open field (discussed in 32), and posterior neocortex lesions which result in stable loss of pattern discrimination. Likewise, when the behavioral deficit is too small, GM₁ is also without effect. This was noted in the hippocampal lesion paradigm when reference memory was assessed (15), in the posterior neocortex lesion paradigm when brightness discrimination was measured (4) and after incomplete transection of the nigro-striatal pathway (12), resulting in only very few rotations per day. Figure 1 displays the behavioral results of a number of experiments done in various laboratories after conversion of the score to allow for direct comparison. These graphs clearly show the close relationship between the ability of animals to recover spontaneously and efficacy of GM₁-treatment in all studies.

**When does ganglioside therapy improve behavioral outcome?**

Based on the research reports on the efficacy of ganglioside administration discussed above, we can draw some general conclusions:

**Conclusion 1.** Gangliosides are only effective in cases of moderate (partial) injury and/or when behavioral recovery occurs naturally. The lesion size has to be within a certain range of tissue loss: If the lesion is too large, gangliosides have too little substrate upon which to exert an effect (“floor effect”), unless the damage involves a modulatory nu-
Fig. 1. It is generally difficult to directly compare the various studies on recovery of function. In order to demonstrate the apparent similarities among the various lesion paradigms, we have therefore converted the behavioral scores (such as number of perseverative errors, number of rotations, % correct responses, etc.) taken from several publication into values displaying "% function", i.e. full (intact) function would be represented by the value of 100, whereas 0% would represent full deficit. We do not claim that our conversion from the absolute to relative values is indisputably correct in each case. Nevertheless, the graphs clearly show the spontaneous recovery seen in untreated animals as compared to subjects treated with GM1 (Part A, upper 3 panels). Note that gangliosides did not improve function when the behavioral deficit was either severe ("floor effect", Fig. 1B) or very mild ("ceiling effect", Fig. 1C). C, control; L, lesion; LG, lesion plus gangliosides.
cleus (see discussion below). If the lesion and/or the behavioral deficit is too small, gangliosides’ facilitative effect would be difficult to detect (“ceiling effect”).

**Conclusion 2.** Ganglioside treatment is effective independent of lesion site or lesion type. As the above discussion shows, a large variety of brain systems and transmitter pathways can be successfully treated with gangliosides following moderate injury. This suggests that exogenous ganglioside treatment affects some fundamental cellular process in the partially damaged structure and the “mechanism of action” likely involves fundamental molecular events. It also appears that it does not matter what type of lesion is present.

**Conclusion 3.** Behavioral effects of ganglioside therapy are consistent with alterations on the anatomical-morphological level. The initial proposal that gangliosides enhance behavioral recovery by accelerating neuronal regeneration and/or sprouting in the damaged brain (23, 44, 46, 50, 58) is probably not sufficient to account for all the instances of recovery described above. Indeed, at least in one case (16), the facilitation of behavioral recovery was correlated with reduced neuronal sprouting. Several investigators have recently suggested that the major mechanism of ganglioside action is that of rescuing neurons in a partially injured structure from further deterioration and cell death (for review see 42, but also 1, 16, 22, 23, 37, 43, 46, 48). Despite all of the research available today, no single causal relationship between physiological/anatomical and behavioral effects of ganglioside administration could be established.

**Conclusion 4.** In order to elucidate the mechanism of ganglioside action, a brain-injury model is needed to produce lesions of graded severity in a highly predictable fashion. Our retrospective analysis of the literature supports the notion that degree (size) of injury is the primary determinant of whether spontaneous recovery occurs and whether ganglioside therapy is beneficial. Therefore it is essential that a brain injury model is available in which the degree of injury can be controlled with great reliability and predictable behavioral outcome. We have now developed a reproducible method to create a graded crush the rat optic nerve, and the electrophysiological and behavioral outcome has been described elsewhere (14). Using this new injury model, we are now underway to evaluate the efficacy of ganglioside therapy under controlled traumatic conditions, and we believe that such a model will help us to better elucidate the action of ganglioside therapy.
Toward a unified theory

Taking into consideration the experiments discussed above, we now propose a theoretical brain-injury model that synthesizes the available information regarding gangliosides' ameliorative effects into a coherent framework. This model may help to explain why brain systems recover to different degrees and why ganglioside administration does not produce uniform improvements across all lesion paradigms. Although we recognize that this model is only a simplified representation of a number of complex events and features involved in the post-lesion response to injury, we hope to bring the different behavioral paradigms into a coherent heuristic framework. It is hoped that this would allow us to make specific predictions of behavioral outcome in future lesion experiments. Although we acknowledge the importance of identifying the salient cellular mechanism(s) which may underlie ganglioside's ameliorative effects, the model we are proposing below emphasizes the role of functional relationships among various CNS structures affected by injury.

The model

The model assumes that a lesion may affect three principal locations in the brain: primary aggregates, modulating aggregates, and/or their interconnections (Fig: 2). Within each of these locations, the lesion may be either total (i.e. destroying the majority of the structure or pathway) or it may be partial (either a unilateral lesion or destroying a fraction of the structure or pathway uni- or bilaterally).

Primary aggregate. A primary aggregate is a brain structure, or a component of a neural system, which is most salient for the expression of a certain behavior. Thus, total destruction of this region is associated with a severe behavioral deficit from which the animal does not recover to any appreciable extent. In contrast, a partial lesion of the primary aggregate will also disrupt function, but only temporarily, and a variety of compensatory mechanisms are available (e.g. collateral sprouting, denervation supersensitivity) whereby the remaining tissue can compensate for the loss. Examples of primary aggregates include the superior colliculus for control of visually elicited head orientation or the primary visual cortex for visual perception.

Modulating aggregate. A modulating aggregate influences function of a primary circuit but is not itself a major element of a neuronal system contributing to a given behavior. When the modulating aggregate is damaged, the function can recover due to the continued presence of the primary circuit with its outputs to and inputs from other modulating aggregates. Because the primary aggregate or its afferent fibers may
Fig. 2. Illustration of a theoretical model describing the behavioral consequences of different types of brain lesions. Part A is the representation of a “primary” aggregate (i.e. neuronal cell group or nucleus) without damage (upper panel), with partial damage (middle panel) or with complete damage (lower panel). I, input to the primary nucleus; O, functional output (i.e. behavioral recovery) from this simplified circuit. According to our brain injury model, a partial lesion of a primary aggregate (R1) will lead initially to behavioral impairment which can be compensated for by the surviving part of the aggregate. Thus, the “spared” portion of the nucleus provides the physiological basis for behavioral recovery, providing sufficient input to nucleus R2 to function “normally”. In case of a complete lesion of a primary aggregate, the animal will display a rather complete deficit, from which it will not recover. Part B. A modulatory aggregate is a group of cells which is not part of the primary neuronal circuitry, but which normally fulfills the function to modulate the primary aggregate (upper panel). Here, a partial lesion may not be noticeable to the observer, and only a complete lesion will lead to a significant functional loss. In contrast to a total lesion of a primary aggregate, a total lesion of a modulating aggregate will be compensated for (probably by some element of the primary circuit), leading to significant recovery of function.

compensate for the loss of the modulating input, the behavior may be recovered, even after total destruction of the modulating aggregate. For example, the entorhinal cortex may be considered a modulating aggregate for the hippocampal formation for locomotor activity. Even total lesions of the entorhinal area will not produce permanent behavioral deficits, because the hippocampal formation itself remains intact.
Disconnection of aggregates. Disconnection implies the disruption of axon bundles and pathways linking modulating and/or primary aggregates. The severity of the disconnection-induced deficit (and thus the ability to recover from it) depends on whether the pathway emerges from a primary or a modulating aggregate.

The distinction of primary and modulating aggregates is not meant to imply that an aggregate is exclusively committed to a single behavioral function. Rather, we assume that a given area may be differentially and hierarchically-integrated into a variety of neural circuits subserving multiple functions. The structure could be simultaneously involved in processing different types of information and may thus be considered either a modulating or a primary aggregate, depending on the function in question. For example, the septum can be considered a primary aggregate in avoidance behavior (21) since this behavior is permanently disrupted after septal lesions. In contrast, the septum could be classified as a modulatory aggregate in emotionality because normal emotionality recovers after 1-2 weeks following septal damage (21).

In terms of rate and final outcome of recovery from brain injury, we propose the following specific events, depending on whether the lesion affects a primary aggregate or a modulating aggregate (see Figure 2): Total lesions of primary aggregate induce permanent behavioral deficits and little recovery. In contrast, a total lesion of a modulating aggregate or a partial lesion of a primary aggregate will fail to persistently disrupt behavior and recovery of function can be readily observed. Of course, the recovery will depend on the relative degree of injury within that system as well as the level of task difficulty (38). The least behavioral impairments, if any, would be expected after partial lesions of a modulating area.

Gangliosides and the model. How might this model be used to explain and predict ganglioside effects after brain injury? We propose that three lesion conditions determine the effectiveness of ganglioside-induced brain repair: (1) Total lesions of primary aggregates: gangliosides will not facilitate brain repair in systems where behavioral recovery does not occur spontaneously (see Table I). Thus, they will be least effective, if at all, in preparations involving total lesions of primary structures of their outputs ("floor effect"). (2) Total lesions of modulating aggregates: gangliosides will be effective in preparations wherein modulating aggregates or their projections are totally destroyed. (3) Partial lesions of primary aggregates: gangliosides will promote recovery/sparing in partial lesion preparations involving primary aggregates or their efferent projections. (4) Since partial lesions of the modulating aggregates do not normally produce significant behavioral deficits, the gan-
Glioside treatment would not be expected to improve behavior dramatically ("ceiling effect").

In summary, our brain-injury model predicts that ganglioside treatment improves behavior most effectively after total lesions of a modulating aggregate or partial lesions of a primary aggregate where recovery occurs spontaneously (see examples in Table I). A partial lesion of a modulating aggregate, in contrast, might not be accompanied by sufficient functional deficits and a demonstration of an improvement due to treatment may be hampered by the "ceiling effect". Therefore, we propose that gangliosides will be most effective in cases of partial CNS injury, and may thus be particularly useful for the treatment of trauma such as moderate head injury in humans.

REFERENCES


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