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G_{M1} Ganglioside Treatment Facilitates Behavioral Recovery from Bilateral Brain Damage

Abstract. *Adult rats with bilateral lesions of the caudate nucleus were treated with G_{M1} ganglioside. Although animals injected with a control solution were severely impaired in their ability to learn a complex spatial task, those treated with ganglioside were able to learn spatial reversals.*

Until recently the central nervous system was believed to lack the capacity for repair, and damage to the brain was believed to result in permanent loss of critical mental and motor functions. As a result of this pessimistic view, virtually no effort had been made to develop effective treatments to restore function lost as a result of traumatic brain injuries. However, this view is now gradually changing (*1*).

Within the last few years, a number of neurotrophic factors known to play an important role in the stimulation and guidance of regrowing axons after damage in the peripheral and the central nervous system have been isolated from mammalian brain tissue (*2*).

Gangliosides, glycolipid molecules located in the outer leaflet of neuronal membranes (*3*), are among these neurotrophic factors now being examined for their potential capacity to restore function of damaged neuronal tissue. When applied to neuronal cell cultures gangliosides stimulate neurite outgrowth (*4*), and when injected systemically into animals with peripheral nerve damage (*5*) they promote sprouting into the denervated target area. Nevertheless, the question of whether gangliosides facilitate central sprouting after brain injury (*6, 7*) or enhance recovery from resulting behavioral deficits is just beginning to be addressed (*7, 8*).

We now report that ganglioside injected after bilateral injury to the caudate nucleus significantly reduces behavioral deficits in spatial learning ability.

Prior to surgery, male albino rats (Sprague-Dawley, 320 to 420 g, 90 to 95 days old) were handled daily for 1 week and then tested for 2 days on a two-choice footshock discrimination-learning maze (*9*). In the preoperative phase, the rats were given ten daily trials in which they could escape from or avoid footshock by running into one of two safe goal areas (*10*). On the first day of training we evaluated the animal's choice preference. The side to which the animal escaped or avoided the footshock more than 50 percent of the time was considered its preferred side. On the next day, the rats were trained to run straight to their nonpreferred side. Those rats that did not run eight out of ten trials were eliminated from the study. Although this training procedure was too short for the rats to acquire spatial reversal habits, it did permit us to eliminate animals that refused to run at all in the test situation. Approximately 20 percent of the animals were thus eliminated from the study.

The remaining animals were randomly assigned to one of three surgical groups: the control group (group C) (n = 8) underwent sham surgery, and the lesion group (group L) (n = 8) were given radio-frequency–induced bilateral lesions of the caudate nucleus (*9*). Both groups received daily intraperitoneal injections of Ringer solution for 14 days. The lesion-ganglioside group (group LG) (n = 7) received, in addition to the same bilateral caudate lesions, daily intraperitoneal injections of G_{M1} ganglioside for 14 days (*11*).

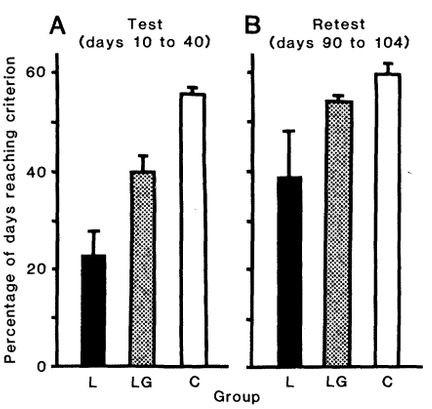

Fig. 1. Mean percentage of days (with standard errors) on which animals reached criterion (nine out of ten responses correct). The groups are L, bilateral caudate nucleus lesion with control injections; LG, bilateral caudate nucleus lesion with injections of G_{M1} ganglioside; C, no lesion with control injections.

According to our previous procedures (*9*), postoperative behavioral testing began after a 9-day recovery period and continued to the nonpreferred side until the rats met a criterion of avoiding or escaping shock correctly on every trial for two consecutive days. Thereafter, the animals were trained to the opposite side of the goal area with the same criterion. In this manner, animals underwent a continuous series of spatial habit reversals for 30 days of testing (with a total of 300 trials). Starting on postoperative day 90, all animals were retested on the same task for 14 days (140 trials).

Each trial was scored for the animals' response to shock (escape or avoidance) and perseverative errors (response to the wrong side after reversal of the correct side). The behavioral data were analyzed separately for the first 30-day testing session and for the 14-day retest period.

For the first testing period, a one-way analysis of variance revealed differences among the three groups for the following measures: (i) number of failures to reach the goal area per reversal [$F(2, 20) = 8.05$, $P < 0.01$], (ii) number of days to reach a criterion after the first reversal [$F(2, 20) = 16.0$, $P < 0.01$], and (iii) the percentage of days on which a criterion of nine correct responses out of ten trials (9/10) was attained [$F(2, 20) = 19.7$, $P < 0.01$].

Subsequent a priori comparisons with Dunnett's test (based on one-tailed probabilities) revealed that animals with lesions but no treatment (group L) were significantly impaired on the behavioral task when compared with animals without brain damage (group C). In contrast, brain-damaged animals treated with G_{M1} showed little impairment, differing significantly in only the percentage of days on which criterion was reached from controls (*12*) (Fig. 1A).

When compared with their untreated, brain-damaged counterparts (group L), animals given G_{M1} ganglioside reached the goal area significantly more often per reversal ($t = 2.86$, $P < 0.01$), took fewer days to reach criterion after the first reversal ($t = 4.46$, $P < 0.01$), and reached criterion (9/10) more often ($t = 3.09$, $P < 0.01$) (Table 1). With respect to the ganglioside-induced im-

provements in learning, the significant group differences became apparent within the first 10 days of training and remained throughout the 30-day test period.

The results of the retest indicate that behavioral performance of ganglioside-treated animals did not deteriorate (Fig. 1B). Both groups with lesions retained what they had learned and even showed significant improvement in all measures (*13*). Although ganglioside-treated animals no longer differed significantly from controls except in percentage of days reaching criterion, untreated brain-damaged animals still performed significantly less well than controls in all three measures (*14*).

When behavioral testing was completed, all animals were killed and prepared for histological verification of the lesion (*15*). With respect to extent of brain damage, groups L and LG did not significantly differ [$F(1, 12) < 1.0$]. In all cases, the center of the head of the caudate nucleus was destroyed (Fig. 2). An examination of neuron and glia populations in remaining caudate tissue and in the substantia nigra pars compacta revealed no statistically significant differences between groups in cell death or reactive gliosis (*16*).

As a result of bilateral caudate nucleus damage, a predictable pattern of behavioral deficits occurs in experimental animals. Rats with such lesions show, for example, an increase in perseverative behavior (*17, 18*), an impaired learning of spatial reversal tasks (*18*), deficits in active avoidance learning (*19*), and impaired ability to escape footshock successfully (*9*). Our findings indicate that these impairments are significantly reduced by repeated intraperitoneal injections of G_{M1} ganglioside.

Our experiment extends previous findings showing that gangliosides reduce behavioral deficits after unilateral brain lesions in adult laboratory rats (*7, 8*). For example, Toffano *et al.* observed a ganglioside-induced reduction of behavioral asymmetries after a unilateral transection of the nigro-striatal pathway (*7*); Karpiak noted that, in animals injected with ganglioside before and after unilateral lesions of the entorhinal cortex were made, behavioral deficits were less severe in a spatial alternation learning task (*8*). In these preparations, however, the contralateral, homologous structure remained intact, resulting in only transient behavioral deficits.

We found posttraumatic ganglioside treatment to be effective in reducing behavioral deficits even after massive, bilateral lesions of the caudate nucleus, which typically produce severe and long-lasting impairments.

Table 1. Means ± standard error of the mean of postoperative behavioral measures. Testing occurred on days 10 to 40 and retesting on days 90 to 104.

Group	Behavioral measure					
	Escape failures per reversal (No.)		Time to criterion after first reversal (days)		Days criterion reached (%)	
	Test	Retest	Test	Retest	Test	Retest
Control	5.1 ± 2.1	1.5 ± 0.7	4.6 ± 0.5	3.5 ± 0.4	56 ± 1.4	60 ± 2.6
Lesion-G_{M1}	33.9 ± 7.6	3.7 ± 1.2	9.9 ± 2.3	5.3 ± 0.5	40 ± 3.5	54 ± 1.5
Lesion-Ringer	130.4 ± 38	35.5 ± 19.1	23.8 ± 3.6	6.6 ± 1.8	23 ± 5.3	39 ± 9.8

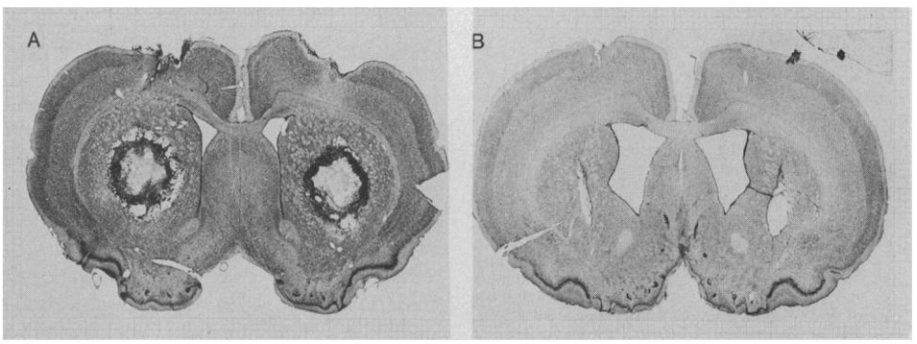

Fig. 2. Photomicrographs of brain sections from animals with bilateral damage to the caudate nucleus that survived 7 days (A) or 4 months (B) after surgery.

Although peripherally injected gangliosides cross the blood-brain barrier in small amounts (*20*), they seem to be without any biochemical or behavioral effects in animals without injury to the nervous system (*7, 8*), which suggests that gangliosides may be active only in the presence of brain lesions. In the damaged brain, however, ganglioside administration may influence several molecular and neuroanatomical events that could, in turn, account for the enhancement of behavioral recovery.

After lesions of the nigro-striatal pathway, for example, systemic injections of ganglioside increase homovanillic acid and tyrosine hydroxylase activity in the denervated striatum (*7*). These changes have been taken as evidence for enhanced collateral sprouting of remaining fibers into the denervated target area. In addition, gangliosides modify properties of postsynaptic membranes and receptors, reducing denervation supersensitivity and number of receptor sites (*21, 22*). While both of these mechanisms may contribute to behavioral recovery, gangliosides may also prevent tissue deterioration secondary to brain trauma, such as the atrophy and death of neurons that lose their target area (*22*). Thus, in the damaged adult brain, gangliosides may exert simultaneous, multimodal actions in preventing spared tissue from secondary destruction and in influencing compensatory mechanisms such as collateral sprouting and denervation supersensitivity.

In the treatment of brain injury with other neurotrophic substances such as nerve growth factor, gangliosides have a distinct advantage because they can cross the blood-brain barrier (*20*) and thus can be administered by systemic injections. In addition, no toxic effects have been observed in doses that facilitate the rate of recovery from brain injury (*23*). If results of future studies resemble ours, ganglioside administration may become a useful chemotherapy for the treatment of brain injury and degenerative disorders in humans.

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10. A trial was begun by placing the animal in the start box. Five seconds later the guillotine door was raised, activating the discriminative cues (light and tone) for 5 seconds. At the termination of the cues, a 0.2-mA footshock was delivered through a scrambler into the grid floor for 10 seconds. Animals that escaped or avoided the shock successfully were permitted to remain for 20 seconds in the safe compartment (goal area).
11. G_{M1} ganglioside (purification 99+ percent, molecular weight 1546.9) was dissolved in Ringer solution at a concentration of 30 mg/ml. Animals received daily injections of 30 mg per kilogram of body weight starting on the day of surgery.
12. Behavioral results of the first testing period (the direction of difference is indicated for each comparison by the symbol <; $\alpha = 0.05$): (i) number of escape failures per reversal: C < L [$t(14) = 3.85$, $P < 0.01$], C < LG [$t(13) = 0.86$, NS (not significant)]; (ii) number of days to reach criterion after the first reversal: C < L [$t(14) = 6.15$, $P < 0.01$], C < LG [$t(13) = 1.47$, NS]; (iii) percentage of days criterion (9/10) was reached: C > L [$t(14) = 6.23$, $P < 0.01$], C > LG [$t(13) = 2.9$, $P < 0.01$].
13. A one-way analysis of variance for repeated measures was used for the statistical comparison of test-retest behavioral performance based on one-tailed probabilities. Behavioral improvement was apparent in every one of the following measures: (i) number of escape failures per reversal for group L [$F(1, 6) = 5.49$, $P < 0.05$], group LG [$F(1, 6) = 19.5$, $P < 0.01$], and group C [$F(1, 7) = 4.65$, $P < 0.05$]; (ii) number of days to reach criterion after the first reversal: group L [$F(1, 6) = 23.1$, $P < 0.01$], group LG [$F(1, 6) = 5.36$, $P < 0.05$], and group C [$F(1, 7) = 2.03$, NS]; (iii) percentage of days when criterion (9/10) was reached: group L [$F(1, 6) = 3.98$, $P < 0.05$], group LG [$F(1, 6) = 8.9$, $P < 0.01$], and group C [$F(1, 7) = 2.04$, NS].
14. To account for heterogeneous variances, Jonckeere-Terpstra's distribution-free test for ordered alternatives (one-tailed, $\alpha = 0.05$) [R. P. Runyon and H. Haber, *Fundamentals of Behavioral Statistics* (Addison-Wesley, Reading, Mass., 1971)] was used to analyze the number of escape failures per reversal: C < L ($U' = 43.5$, $P < 0.05$), C < LG ($U' = 35.5$, NS), L > LG ($U' = 36.0$, NS). In the analyses of the other measures, Dunnett's test was used ($\alpha = 0.05$). The results were as follows: number of days to criterion after the first reversal: C < L [$t(14) = 1.93$, $P < 0.05$], C < LG [$t(13) = 1.12$, NS], L > LG [$t(13) = 0.79$, NS); percentage of days on which criterion was reached: C > L [$t(14) = 2.63$, $P < 0.05$], C > LG [$t(13) = 0.75$, NS], L < LG [$t(13) = 1.81$, $P < 0.05$].
15. After the rats had been perfused transcardially with 0.9 percent saline followed by 10 percent Formalin in saline, the brains were cut coronally at 40 μm on a freezing microtome, and every sixth section was mounted on microscope slides and stained with cresyl-echt violet. To measure lesion size, the perimeter of the lesion extent was traced from successive, coronal sections with an overhead microprojector, and the lesion volume was determined by means of a Graphic tablet-menu on an Apple II plus computer.
16. Mean neuron-to-glia ratios and standard errors in caudate areas medial and adjacent to the lesion (L, 0.61 ± 0.18; LG, 0.64 ± 0.14; NS) and in substantia nigra pars compacta (L, 0.29 ± 0.04; LG, 0.23 ± 0.04; NS) were evaluated in both lesion groups ($n = 7$) [B. A. Sabel and D. G. Stein, *Exp. Neurol.* **73**, 507 (1981)].
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Monoclonal Antibody to a Human Germ Cell Membrane Glycoprotein That Inhibits Fertilization

Abstract. *A monoclonal antibody to an antigen in the human germ cell membrane did not agglutinate or immobilize sperm but inhibited binding and penetration of zona-free hamster ova by human sperm and blocked murine fertilization in vitro. The antibody, of the 2a subclass of immunoglobulin G, was germ cell–specific but not species-specific. It recognized a single antigen of 23 kilodaltons that has been isolated from human germ cells. This fertilization antigen, located on the postacrosome, midpiece, and tail of human sperm, is a glycoprotein of testicular origin associated with some types of human involuntary immunoinfertility.*

Immunization of male or female animals of different species with homogenates of mature sperm, testis, or their extracts results in infertility (*1*). Antigenicity of sperm has also been implicated in involuntary infertility in human beings (*2*) and is the cause of continued infertility after vasovasostomy (*3*). However, use of whole sperm and testis homogenates is not appropriate for immunization since (i) many antigens of the germ cells are likely to be shared with other somatic tissues (*4, 5*) and (ii) immunization could result in undesirable immune complexes. Other germ cell–specific antigens do cause a reduction, although not a complete block, of fertility. Lactate dehydrogenase C_4 reduced fertility in mice, rabbits, and baboons (*6*), and antiserum to a rabbit sperm 13-kilodalton (kD) autoantigen inhibited fertility in female rabbits (*7*).The advent of hybridoma technology has made it possible to identify and purify sperm-specific antigens (*8, 9*) that are involved in fertility and infertility.

In our study, BALB/c mice were immunized intraperitoneally with 10^6 sperm (washed ejaculated or epididymal human sperm) in Freund's complete adjuvant. Hybridomas were prepared by fusion of the mouse myeloma line P3-NS1/1-Ag4-1 (*10*) with spleen cells from mice with high antibody titers to sperm. The hybridomas secreting these antibodies were selected and cloned. The preliminary screening was performed by an indirect enzyme-linked immunosorbent assay (ELISA) with whole washed human sperm or a membrane preparation solubilized with LIS (0.3*M* lithium diiodosalicylate) (*11*). Positive clones (reading higher than 2 standard deviations above the mean for controls) were selected and recloned, and 10^7 hybrid cells were injected into BALB/c mice previously treated with pristane to generate ascites fluid.

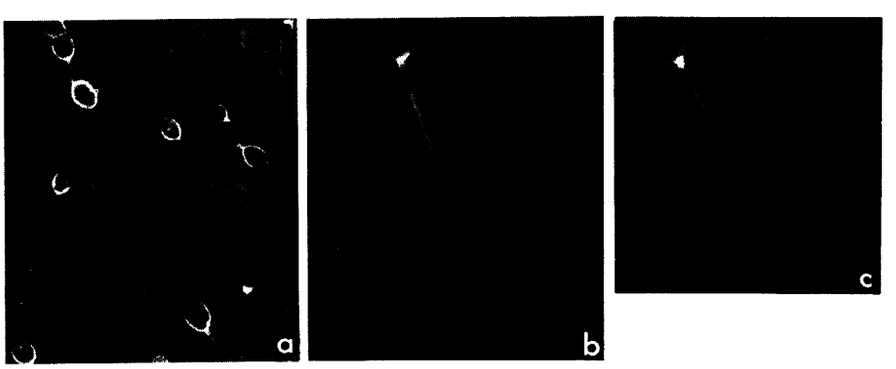

Fig. 1. Location of the FA-1 antigen by indirect immunofluorescence on (a) human sperm that have been reduced (swollen), (b) a methanol-fixed spermatozoon, and (c) a capacitated spermatozoon. The MA-24 clone reacted with the postacrosomal, tail, and (to a limited extent) midpiece regions of the sperm (×1100).