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GM1 Ganglioside Reduces Cognitive Dysfunction After Focal Cortical Ischemia

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ORTIZ, A., J. S. MACDONALL, C. G. WAKADE AND S. E. KARPIAK. *GM1 ganglioside reduces cognitive dysfunction after focal cortical ischemia*. PHARMACOL BIOCHEM BEHAV **37**(4) 679–684, 1990.—The functional consequences of cortical focal ischemia and the effect of monosialoganglioside (GM1) treatment on learning/performance of a spatial reversal task were investigated. Cortical focal ischemia was induced by a permanent occlusion of the left common carotid artery and the ipsilateral middle cerebral artery, with a 1-h clamping of the contralateral carotid artery. Twenty-six rats were randomly assigned to three groups: sham controls, a saline-treated ischemic group, and a GM1 ganglioside-treated ischemic group (10 mg/kg/day: IM). Fifteen days after surgery rats were trained on a spatial reversal task in a two-lever operant chamber where food reward was contingent on lever pressing. Training continued from day 15 to day 21 after surgery. Cortical focal ischemia resulted in learning/performance deficits that were reduced by GM1 ganglioside treatment. The cognitive deficits were characterized by a significantly higher number of nonperseverative errors and number of responses to criterion. There was a significant difference between left and right lever performance in the saline-treated ischemic group, which was absent in shams and GM1-treated ischemic rats. On all measures GM1-treated rats were not different from sham controls.

Ganglioside GM1 Ischemia Learning Perseveration Parietal Memory Stroke Cortical stroke

BRAIN stroke is the third leading cause of death, and one of the major factors contributing to neurological and functional disability in the United States (10,12). The majority of central nervous system (CNS) strokes are classified as ischemic, a localized tissue anemia due to the obstruction of the arterial blood inflow thereby resulting in oxygen and glucose loss (26). Cerebral ischemia triggers a spectrum of physiological and biochemical sequelae (14) that ultimately result in neuronal plasma membrane failure and cell death (8). This irreversible CNS damage is manifested in behavioral dysfunction (7, 18, 23). If CNS cell damage can be minimized by interventions that prevent membrane failure, subsequent behavioral and neurological deficits may also be reduced.

Studies of the effectiveness of monosialoganglioside (GM1) treatment in various animal models of CNS ischemia suggest that these glycosphingolipids protect plasma membrane structure and function (5, 13, 14, 17). This membrane protection is evident by the reduction of Na^+,K^+-ATPase losses in hippocampal and cortical plasma membranes in gerbils after global ischemia (13) and in rat after cortical focal ischemia (24). In transient global ischemia, produced by a 60-minute occlusion of the common carotid arteries (CCAs) in rats, GM1 ganglioside therapy limited the extent of the edematous reaction, K^+ efflux, and accumulation of Ca^{2+} ions (5). Similar results were observed after cortical focal ischemia in rats produced by permanent occlusions of the left middle cerebral artery (MCA) and ipsilateral CCA, with 1-h clamping of the contralateral CCA (25). The ability of GM1 ganglioside to reduce membrane pathology in CNS ischemic tissue should also be manifested in improved behavioral function. This therapeutic effect of monosialoganglioside and/or its inner ester (AGF2) have been observed in the reduction of neurological dysfunction after CNS ischemia in monkeys (4) and after ischemic stroke in humans (1). In addition, it has been shown that GM1 ganglioside reduces levels of hyperactivity and sensorimotor performance deficits in rats after cortical focal ischemia (2).

Cognitive impairments after CNS ischemia are thought to be the primary factor contributing to long-term disability in humans (11,22). Therefore, one goal of this study was to initiate a series of experiments designed to better define the cognitive dysfunctions associated with cortical focal ischemia. An earlier study found that GM1-treated rats with transient global ischemia did not show any retention deficits as measured on a passive avoidance paradigm (one-trial learning) (5). A more complex testing procedure would provide a basis for a more systematic and detailed analysis of which aspects of learning/performance are disrupted by focal cortical ischemia. Hence, the present study assessed the effect of ischemia and GM1 treatment on learning and performance parameters of a complex learning task using a rat model of cortical focal ischemia.

The rat model of cortical focal ischemia used in this labora-

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tory consists of the permanent ligation of the left CCA followed by a permanent occlusion of the ipsilateral middle cerebral artery (MCA), combined with a 60-min clamping of the contralateral CCA (MCAo+CCAo). This model, originally described by Chen et al. (6), results in a highly reproducible, large and consistent focal infarct that is localized to the parietal cortex and does not involve subcortical tissue damage (6). Because parietal cortex is primarily affected by this cortical focal ischemia, a spatial reversal task was selected for cognitive testing. Cortical parietal injury has been associated with functional deficits in spatial learning tasks (3, 15, 16, 19), particularly in tasks that involve the reversal of a previously learned discrimination (15,16). Therefore, it was expected that animals with an MCAo+CCAo-induced lesion would show poor learning/performance on the spatial reversal task. The following experiment assessed which aspects of this learning paradigm are affected by cortical parietal ischemic injury, and determined whether GM1 ganglioside treatment ameliorated the associated cognitive deficits.

METHOD

Subjects

Twenty-six male Sprague Dawley rats (Hilltop Lab Animals, Scottsdale, PA, 220–260 grams) were used. Rats were housed in groups of three–four and were maintained on a 12-hour light/12-hour dark schedule, with ad lib food and water. Rats were randomly assigned to three groups. One group (n = 10) was exposed to focal ischemia (MCAo+CCAo) and received daily IM injections of 10 mg/kg GM1 ganglioside (FIDIA Research Labs.) in saline, beginning immediately after surgery and then daily for 20 days. The second group (n = 8) was also exposed to focal ischemia (MCAo+CCAo) but received saline injections for the same duration. The third group (n = 8) was a sham-operated control group with saline injections. Sham rats were exposed to the same surgical procedures, excluding the temporary or permanent occlusion of the MCA and CCA arteries. Beginning four days after surgery, rats received ad lib food one hour daily for the duration of the experiment.

Apparatus

Behavior was assessed in a two-lever operant chamber, 10½″ × 9⅛″ × 9½″ long (Gerbrand Corp.). The light above each lever and the house light were "on" continuously. The operant chamber was in a sound- and light-attenuating enclosure, equipped with fan and white noise source. A Walter/Palya ECbasic Experiment Controller controlled the contingencies, reinforcer delivery, and data collection. Reinforcers consisted of 45 mg food pellets (Noyes Co.).

Surgical equipment included a Nikon SMZ-2t Type 102 stereomicroscope with fiber optic bifurcated illumination and ring/lens illumination, a dental drill, microscissors, bipolar radiofrequency forceps (Tieman No. 160-1841) and a Birtcher Hyfrecator (model 733, Solid State Electro-surgery). A nontraumatic microaneurysm clip (BRI-34-3550) were used for the temporary contralateral CCA occlusion.

Focal Cortical Ischemia

Rats were anesthetized (IM) with a mixture of Vetalar (87.5 mg/kg) and Rompun (7.5 mg/kg). After the left CCA was permanently ligated, the ipsilateral MCA was permanently occluded. The MCA was coagulated with bipolar radiofrequency forceps at two sites: 1 mm below the point where the MCA crosses the rhinal fissure and 4 mm above this point at the bifurcation of the MCA. After coagulation, the MCA was cut with microscissors at the two occluded sites. A nontraumatic microaneurysm clip was applied for one hour to the contralateral CCA. Immediately after the removal of the clip, rats were injected (IM) with either GM1 ganglioside (10 mg/kg) or saline.

Reversal Learning

Behavioral procedures began seven days after MCAo+CCAo or sham surgery. Rats were handled daily, for seven days, for one minute and placed in the operant chamber for five minutes. Fourteen days after surgery rats were magazine trained (i.e., trained to approach the food cup at the sound of food delivery). Then, using the method of successive approximations, the behavior of each rat was shaped to *press the left lever*. After shaping, the first 10 left lever presses were reinforced; right lever presses were not reinforced. Following these 10 reinforcements, the lever producing the reinforcer was switched three times as follows: the first two right lever presses were reinforced, the next two left lever presses were reinforced, and finally, the next two right lever presses were reinforced.

Spatial reversal learning began on the next day (day 15 after surgery). At the beginning of each session only left-lever presses were reinforced. Right-lever presses were not reinforced and were recorded as errors. After eight consecutive left lever presses (correct responses), the right lever became the correct lever, delivering a reinforcer for each response, and the left lever became the incorrect lever (a reversal). At this time any response on the left lever was classified as an error. After eight consecutive correct right-lever presses, the location of the correct and incorrect levers was reversed again. A session ended after 10 reversals.

In summary, all rats were exposed to daily sessions of reversal training from day 15 to day 21 postsurgery (MCAo+CCAo). At the start of each session, only responses on the left lever were reinforced. During each session the location of the correct lever (left or right) was reversed ten times. Reversals always occurred after eight consecutive correct responses.

Rats' performance was measured by the number of errors and the number of responses emitted to reach the criterion for each reversal. Errors were classified as either nonperserverative or perseverative. A nonperseverative error was the first error occurring after a reinforced response. Any subsequent consecutive responses on the unreinforced lever were classified as perseverative errors.

RESULTS

Figure 1 presents the cumulative average nonperseverative errors (days 15–21). The ischemic-saline group made significantly more errors than the ischemic-GM1 group ($F = 3.96$, $p < 0.03$; $t = 2.73$, $p < 0.05$). The inset panel in Fig. 1 presents the daily average frequency of nonperseverative errors for all groups. On each day of testing the ischemic-saline group made the greatest number of errors. There was no interaction effect of group condition by days of testing ($F = 0.60$).

The cumulative average perseverative errors (days 15–21) for each group are presented in Fig. 2; the inset panel shows the average frequency of perseverative errors for each group for each day. For all three groups, the frequency of perseverative errors decreased across days ($F = 74.8$, $p < 0.001$). There were no differences between groups on this measure ($F = 0.79$) and no interaction effect of group condition by day of testing ($F = 0.90$).

The responses to criterion measure is the sum of the errors (perseverative and nonperseverative) and correct responses made until the criterion of 8 consecutive correct responses was reached. Figure 3 presents the average responses to criterion for all days; the inset panel presents the mean responses to criterion as a func-

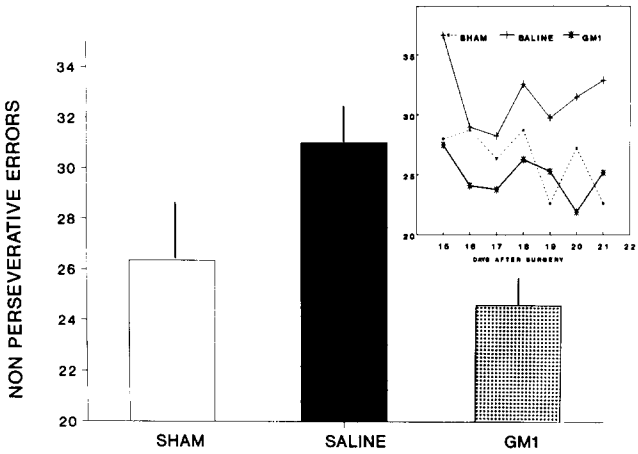

FIG. 1. Cumulative average nonperseverative errors (days 15–21) for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats. Inset panel shows the daily average frequency of nonperseverative errors for each group.

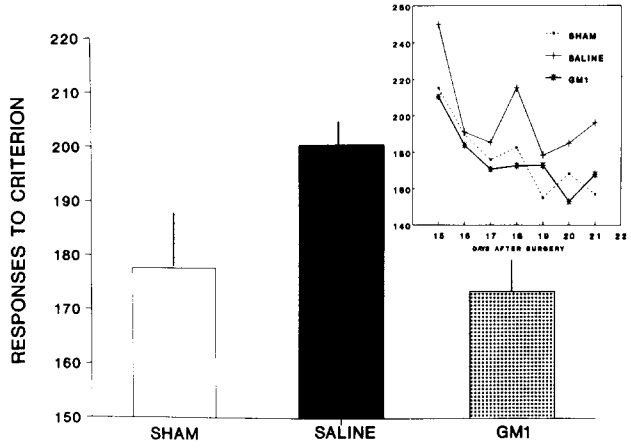

FIG. 3. Cumulative average responses to criterion (days 15–21) for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats. Inset panel shows the daily average frequency of responses to criterion for each group.

tion of testing day for each group. The ischemic-saline rats made significantly more responses than either the sham control ($t = 2.14$) or the ischemic-GM1 group ($t = 2.67$) for the entire experiment ($F = 3.92$, $p < 0.03$). The inset shows that during each session the ischemic-saline group made more responses to reach criterion than the sham-control or ischemic-GM1 groups. The responses to criterion for all groups decreased from days 15 to 21 ($F = 7.92$). There was no interaction effect between group condition by days of testing ($F = 0.60$). Also, there was no significant difference between groups in the number of food pellets ingested ($p < 0.05$).

To further analyze the performance deficits seen for the measures of nonperseverative errors and responses to criterion, data were categorized in terms of left or right lever responses. In this left/right analysis the frequency of nonperseverative errors and responses to criterion were analyzed on the basis of which lever was "correct" during testing. Figure 4 presents the cumulative average of left/right nonperseverative errors for each group. The ischemic-saline group made significantly more nonperseverative errors than the GM1 or saline group when the right lever was the correct lever ($F = 5.19$, $p < 0.01$). Only the saline-ischemic group showed significant differences on the right/left lever analysis ($t = 2.48$, $p < 0.05$). This same asymmetric response pattern was seen when the average number of responses to criterion were analyzed on the basis of the left/right lever analysis (Fig. 5). Comparison between groups showed a similar number of lever presses to reach criterion when the left lever was the "correct" choice ($F = 0.19$, N.S.) and a significant difference between groups when the right lever was correct ($F = 6.68$, $p < 0.005$). In this analysis (right lever correct) saline-treated ischemic rats had more responses to reach criterion than GM1-treated rats ($t = 3.5$, $p < 0.01$) or sham controls ($t = 2.77$, $p < 0.05$). This asymmetric response pattern was observed for all groups at day 16 (Fig. 6). However, after repeated exposure to the task, GM1-treated and sham control groups showed a consistent decrease in this left/right lever response asymmetry. Saline-treated rats did not reduce this asymmetric response pattern from day 16 thru 21 (Fig. 6).

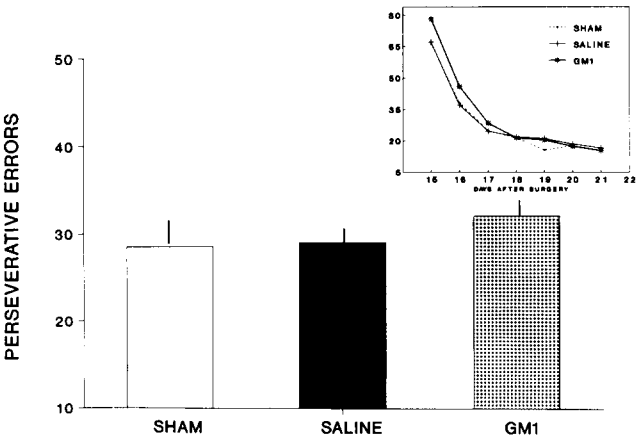

FIG. 2. Cumulative average perseverative errors (days 15–21) for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats. Inset panel shows the daily average frequency of perseverative errors for each group.

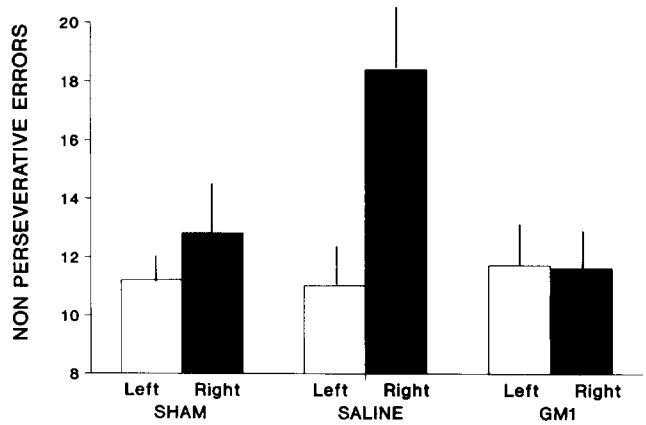

FIG. 4. Cumulative average nonperseverative errors (days 15–21) when the left or the right levers were correct for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats.

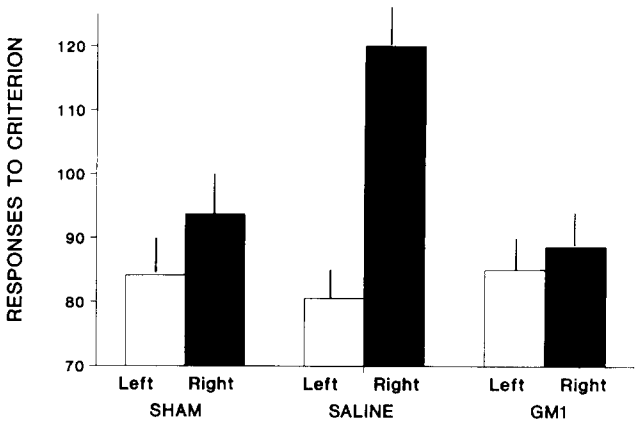

FIG. 5. Cumulative average responses to criterion when the left or right levers were correct for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats.

DISCUSSION

Performance on a spatial reversal learning task was significantly disrupted in rats with unilateral parietal cortical ischemia (MCAo+CCAo). These deficits were seen (Figs. 1 and 3) when testing on a reversal task began at 15 days after the initial ischemic injury and persisted throughout the testing period (15–21 days post-MCAo+CCAo). Although all groups (shams, ischemic-saline and ischemic-GM1) were not different in their acquisition of the reversal learning task (first reversal: day 15), subsequent task performance (days 16–21) indicated that ischemic-saline rats showed only marginal improvement in their performance of the task, whereas both sham controls and ischemic-GM1 treated rats significantly improved their performance. From the first day of testing (day 15), the GM1-treated rats did not differ from sham controls in their performance (Figs. 1 and 3).

The mean number of perseverative errors did not differ among the three groups (Fig. 2). This lack of difference is noteworthy since it illustrates that GM1 administration does not affect "normal" functioning, but does affect those functions that have been altered by a pathology (in this case, cortical focal ischemia). Also, this lack of difference (on perseverative errors) among the groups allows us to conclude that the poor performance of the ischemic-saline group is not the result of generalized learning impairments, motor weakness or motivational changes. The ischemic injury produced specific functional effects (deficits), and only those deficits are affected by GM1 treatment.

Analyses of nonperseverative errors indicated that this cognitive performance measure was markedly affected by the ischemic injury (Figs. 1 and 4). This measure, sensitive to the ischemic pathology (unlike the perseverative error measure), was significantly reduced in GM1-treated rats so that their performance was not different from sham controls. Analysis of the animal behavior in this task suggests that these differences induced by the ischemia might be attributed to a memory (possibly working memory) or spatial performance dysfunction. Typically a nonperseverative error is committed when the rat fails to press the lever that in the

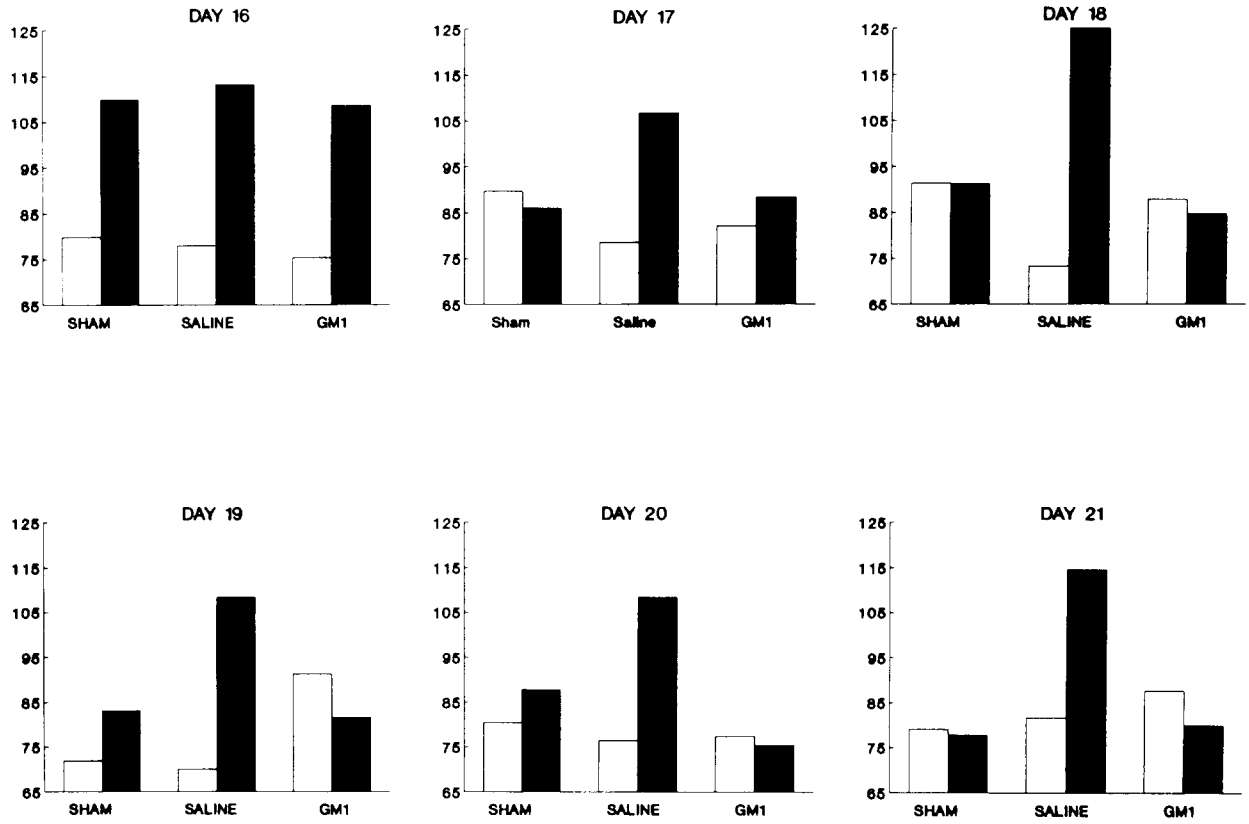

FIG. 6. Daily average responses to criterion (days 16–21) when the left (open bar) or right levers were correct for GM1-treated ischemic rats, saline-treated ischemic rats, and sham-operated rats.

previous response resulted in food delivery. In this task, the food cup is located at the same distance from each lever. After eating the food pellet the rat must return to the lever that was producing the reinforcement. It is at this point that working memory dysfunction or spatial difficulties can result in an incorrect choice. Similar working memory dysfunctions after ischemia have been reported in studies using different rat models (21,23). However, specifically defining the types of learning dysfunctions observed is only speculative at this time until more studies are completed.

A memory or spatial dysfunction is also supported by data collected at the termination of this experiment. Starting at day 22 after ischemia a light signal was introduced in the procedure and all rats were exposed to four additional testing days. The light signal identified the lever that was associated with the reinforcement on each reversal (correct lever). The introduction of the signal immediately resulted (day 22) in a decrease of nonperseverative errors for all groups and eliminated the previously observed group differences. In this case the light signal (a visual stimulus) might be accessing another modality of the brain, thereby compensating for any memory deficit or spatial discrimination dysfunction. This further confirms our conclusion that the differences observed were not the result of sensorimotor deficits.

Cognitive dysfunction was also evident in the measure of number of responses to criterion (Figs. 3 and 5). In saline-treated rats it is apparent that this measure was also affected (adversely) by the ischemic injury, and that little improvement occurred on this measure after the second day of testing (Fig. 6). Again, GM1-treated rats were significantly improved on this measure. In fact, even during the early stages of the testing paradigm (days 15–18), the GM1-treated rats had the lowest average of responses to criterion and were not different from sham controls (Fig. 3).

The extent of the cognitive dysfunction resulting from the ischemic injury is best illustrated by data where the responses of the rat are parcelled into a left/right lever analysis. Since all rats were trained (day 14) to initially press the left lever for food reinforcement it is not unexpected that there would be an asymmetric (bias) response pattern. The greatest number of nonperseverative errors and number of responses occurred when the rat was required to respond to the right lever (the "nonpreferred" lever). This is evident in all three experimental groups at the beginning of the testing period (Fig. 6). After 7 days of testing there is no longer any significant difference between performance on either lever in the sham controls. Quite simply, learning occurred. GM1-treated rats showed this identical pattern of improvement (Figs. 4 and 5). The saline-treated rats do not demonstrate this learning pattern. After 7 days of testing, the saline rats continued to manifest an enormous discrepancy (asymmetry) on left/right lever performance (Fig. 6).

This study extends previous reports that GM1 ganglioside treatment of CNS injury results in reduced CNS dysfunction as measured by morphological, biochemical and behavioral analysis (2, 4, 5, 13, 17, 25). In studies which have focused on the efficacy of GM1 treatment for CNS ischemia, investigators have shown that sensory/motor behavioral dysfunction is ameliorated (2,4). This study provides initial evidence that monosialoganglioside treatment can also improve cognitive deficits that results from CNS ischemic injury. The mechanism(s) by which this glycosphingolipid (GM1) is effective are being actively explored (9,17). However, the ability of this compound to improve both neurological (sensory/motor) as well as cognitive performance after CNS ischemia is significant. Clinical studies of the effectiveness of monosialoganglioside therapy for focal ischemia reflect this promise (1).

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