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Differential Effects of Amphetamine and Haloperidol on Recovery After Global Forebrain Ischemia

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WISHART, T. B., S. IJAZ AND A. SHUAIB. *Differential effects of amphetamine and haloperidol on recovery after global forebrain ischemia.* PHARMACOL BIOCHEM BEHAV 47(4) 963–968, 1994.—Gerbils subjected to sham surgery or to bilateral occlusion of the carotid arteries were given an injection, during the recovery period, of saline, *d*-amphetamine, or haloperidol. The animals were subsequently tested once daily for 50 days in an eight-arm radial maze. Global forebrain ischemia had no effect on learning to avoid unbaited arms (reference memory), but greatly increased the number of times animals reentered previously visited arms (working memory errors). Gerbils made ischemic and treated with amphetamine reduced working memory errors more rapidly than did saline-treated ischemic gerbils; conversely, animals made ischemic and treated with haloperidol made more working memory errors than the ischemic controls. Although all ischemic animals were hyperactive, the differential radial maze behaviors of the ischemic groups cannot be explained on the basis of increased activity.

Global ischemia Amphetamine Haloperidol Recovery of function Radial arm maze

BILATERAL occlusion of the carotid arteries in the animal model of forebrain ischemia produces widespread neuronal damage and particularly severe cell loss in the pyramidal cell layers of the hippocampus. Behavioral observations of rats or gerbils after forebrain ischemia indicate that CA1 cell loss is accompanied by impairments in spatial learning and in working memory (4,23).

Ischemia-induced CA1 damage does not necessarily preclude learning a spatial memory task, but learning may proceed at a slower rate in the period immediately after ischemia (25,26). When a lengthy recovery period is permitted after the ischemic procedure, animals may not show spatial memory impairments (1,3), indicating that brain regions other than the CA1 cell area may be involved in spatial learning or may be involved in the recovery process following ischemia.

Behavioral effects after brain damage may be exacerbated or alleviated by drug treatment. For example, it has been known for some time that amphetamine facilitates the recovery of simple motor patterns after motor cortex lesions (19,20). More recently, Feeney and Hovda (9) have shown that a single dose of amphetamine or apomorphine administered 24 h after motor cortex lesions facilitates recovery of beam walking. Conversely, a single injection of haloperidol delays recovery in the same paradigm although these effects appear to be dependent upon task-specific postlesion practice (15). Schallert, Hernandez, and Barth (24) reported that daily treatment with diazepam for 3 weeks after unilateral electrolytic lesions of anteromedial cortex indefinitely delayed recovery of sensorimotor asymmetry. Phenobarbital may also have a similar effect (24).

Facilitation of recovery after brain damage by amphetamine is not limited to simple motor behaviors. Amphetamine has been reported to reinstate binocular depth perception after bilateral visual cortex lesions in cats (9,17), to promote the recovery of a previously learned visual discrimination habit in rats, and even to diminish a spatial learning deficit after striatal lesions in rats (6). It is interesting, but not unexpected given their antagonistic actions, that haloperidol has been reported to block the recovery-facilitating effect of amphetamine in at least one paradigm (16). There is preliminary evi-

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dence from clinical studies that amphetamine may enhance recovery after stroke, and other drugs (haloperidol, diazepam, dilantin) may slow recovery after stroke (14).

Previous experiments investigating the effects of amphetamine or haloperidol on recovery of function have typically induced focal brain insults, or used simple sensorimotor tasks to measure recovery. We attempted to determine whether single-dose amphetamine or haloperidol, administered in the period after global forebrain ischemia, but prior to training, could influence the rate of acquisition of a complex spatial memory.

METHOD

Subjects

Twenty-eight 3-month-old male gerbils obtained locally and housed in clear plastic cages were used in the study. The animals were randomly divided into four groups with one animal from each group placed in each of seven cages. Water was available ad lib. One week after their arrival in the laboratory the gerbils were placed on a food restriction schedule designed to lower their body weights to 80–85% of normal. Food, in the form of pellets, was placed directly into each cage for a 2-h period daily.

Apparatus

An eight-arm radial maze with 40-cm long arms was constructed of clear Plexiglas. The arms of the maze had Plexiglas walls measuring 15 cm high, and the octagonal, central, open area from which the arms radiated measured 20 cm in diameter. A small (0.25 cm), circular food well was drilled in the floor of each arm 2.5 cm from the end wall and equidistant from the side walls. The maze was contained in a 3.0 × 2.5 m, well-lit room with a large variety of external visual room cues.

Locomotor activity was measured in an open field measuring 40 × 40 cm with walls 30 cm high. The open field was placed in a small, dimly lit room. Movement was monitored using a digitizing camera and a computerized software program (Columbus Instruments, Columbus, OH).

Surgical and Histological Procedures

Once body weights had been lowered to 80%, the gerbils were subjected to a sham operation (exposure of the carotid arteries), or to a single 5-min period of ischemia. Anaesthesia was achieved with 3% halothane and a mixture of nitrous oxide and oxygen. Rectal and scalp temperatures were monitored in all animals and body temperature was maintained with a heating pad. Following a midline incision and dissection, the carotid arteries were occluded with aneurysm clips under visual inspection. The halothane anaesthesia was terminated immediately after occlusion of the carotid arteries, but the nitrous oxide was maintained. After 5 min the aneurysm clips were removed, restoration of blood flow visually verified, and the skin lesion closed with suture. Nitrous oxide was then discontinued and the animals were allowed to recover.

Following the completion of behavioral testing, the animals were sacrificed with an overdose of pentobarbital and perfused through the heart initially with normal saline, then by a phosphate buffer containing 10% formalin. One hour later, the brains were removed and placed in 30% sucrose buffer for cryoprotection and later stained with a modification of Gallyas' silver impregnation method (11,12). The sections were washed with water, pretreated with alkaline ammonium nitrate, impregnated with 0.32% silver nitrate in alkaline ammonium nitrate, treated with ethanolic sodium carbonate/ammonium nitrate solution, and finally developed in Nauta reducer. Representative sections were cut through the cortex, hippocampus, striatum, thalamus, medial geniculate nucleus, and the substantia nigra pars reticulata. The degree of neural damage was assessed by examination of the silver-stained sections. Ratings of regional brain damage used a scoring system similar to that of Pulsinelli et al. (23) on a scale of 0–4 (where 0 = no detectable damage, 1 = less than 25% damage, 2 = 25–75% damage, 3 = more than 75% damage, and 4 = a completed infarction) and were performed by an investigator blind to the treatment of each animal.

Drug Treatment

Twenty-four hours after surgery, each animal was injected once IP with physiological saline, *d*-amphetamine sulphate (3 mg/kg) dissolved in saline, or haloperidol (3 mg/kg).

Behavioral Testing

During the 3 days immediately prior to surgery, the animals in each cage were placed together in the maze and allowed to explore for a 15-min period. Pellets (45 mg, P. J. Noyes Co., Lancaster, NH) were strewn throughout the maze.

Beginning 72 h after surgery, animals were tested once daily for 50 days in the eight-arm radial maze. Five of the eight arms were baited by placing a 45-mg pellet in the food well. The identical five arms were baited for all animals on all days.

Each animal was given one trial per day. The trial was initiated by placing the animal in the central, open area of the maze. To eliminate the possibility of the development of specific movement patterns, the direction the animal faced at the start of the trial was varied systematically for all animals across trials. Entries into each arm were recorded, and the trial terminated when all five pellets were eaten or 10 min elapsed.

Locomotor activity was measured once weekly beginning 1 week before surgery. Tests were conducted 2 h after the daily 2-h feeding schedule. The gerbil was placed in the center portion of the open field and the VideoMex system was activated. The gerbil was allowed 2 min to explore the field while exploratory/locomotor activity was recorded.

RESULTS

The effects of the single, 5-min ischemic procedure on regional brain damage scores are presented in Table 1. The 5-min ischemic procedure produced no detectable brain damage in the thalamus, striatum, substantia nigra, or medial geniculate nucleus; consequently, the data from these regions are omitted. Brain damage ratings were generally higher in the cortex and all areas of the hippocampus in the three experimental groups compared to the sham-operated controls. Statistically, brain damage was significantly greater in the CA1 region in the amphetamine and haloperidol groups than in controls ($p < 0.05$; Mann-Whitney *U*-test) and in the CA4 region in the haloperidol group compared to controls ($p < 0.05$).

Locomotor activity scores are presented in Fig. 1. Although prior to surgery all groups were equivalent in terms of

TABLE 1
REGIONAL BRAIN DAMAGE RATINGS

Group	Region			
	CTX	CA1	CA3	CA4
Sham operates	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Ischemia-saline	0.3 ± 0.3	0.6 ± 0.4	0.3 ± 0.3	0.3 ± 0.3
Ischemia-amphetamine	0.9 ± 0.3	2.6 ± 0.4*	0.4 ± 0.4	0.9 ± 0.4
Ischemia-Haloperidol	0.6 ± 0.4	1.9 ± 0.6*	0.1 ± 0.1	1.6 ± 0.6*

Values are mean ± SE.
*Significantly different from sham-ops ($p < 0.05$; Mann-Whitney U test).

locomotor activity, the three groups that experienced ischemic insult became hyperactive postsurgically, moving about the open field approximately 1.5 times as much as controls, on average. A repeated measures ANOVA on the postsurgery scores revealed group, $F(3, 24) = 5.04$, $p < 0.01$, and trials, $F(6, 144) = 13.47$, $p < 0.001$, main effects but no interaction. Subsequent analysis using the Tukey test indicated that, overall, the sham-operated group was significantly less active than all other groups ($p < 0.05$), the amphetamine-treated group was significantly more active than all other groups ($p < 0.05$), but that the saline- and haloperidol-treated groups did not differ. The Spearman rank-order correlation between the CA1 brain damage score and locomotor activity was 0.34 ($p < 0.05$); correlations between activity and other brain damage scores were all insignificant.

Performance in the radial maze was subdivided into reference memory errors (first-time entries into unbaited arms) and working memory errors (reentries into previously visited baited or unbaited arms). For ease of illustration and analysis, trials were grouped into 10 blocks of five trials each (trials 1-5, 6-10, etc.).

Figure 2 provides the average number of reference memory errors made by the animals in the four groups. These results were analyzed with a repeated measures ANOVA. A significant effect was obtained for trials, $F(3, 216) = 43.5$, $p < 0.001$, but there was no significant group effect, $F(3, 24) = 1.6$, $p > 0.10$, nor was there a group by trials interaction, $F(27, 216) = 1.3$, $p > 0.10$. Examination of Fig. 2 shows that initially the gerbils indiscriminately entered all arms, but that the animals in all four groups learned at the same rate to avoid the unbaited arms. By the end of 50 trials, the animals in all groups averaged approximately one reference error/trial.

Figure 3 illustrates the number of working errors made by the animals in each group. These data were analyzed using a repeated measures ANOVA with subsequent post hoc (Tukey) tests where appropriate. There was a significant main effect for group, $F(3, 24) = 3.3$, $p < 0.01$, trials, $F(3, 216) = 76.3$, $p < 0.001$, and a group by trials interaction, $F(27, 216) = 4.7$, $p < 0.001$.

On the first block of five trials the sham-operated animals committed an average of 5.1 working memory errors/trial, but quickly reduced these errors to about 1/trial. These animals gradually improved their performance over the 50 trials such that they made only 0.4 working memory errors per trial, on average, during the last 10 trials.

Examination of Fig. 3 and post hoc statistical analyses revealed that the three groups of animals subjected to the ischemic procedure made significantly more working memory errors than did controls ($p < 0.05$) during the first four

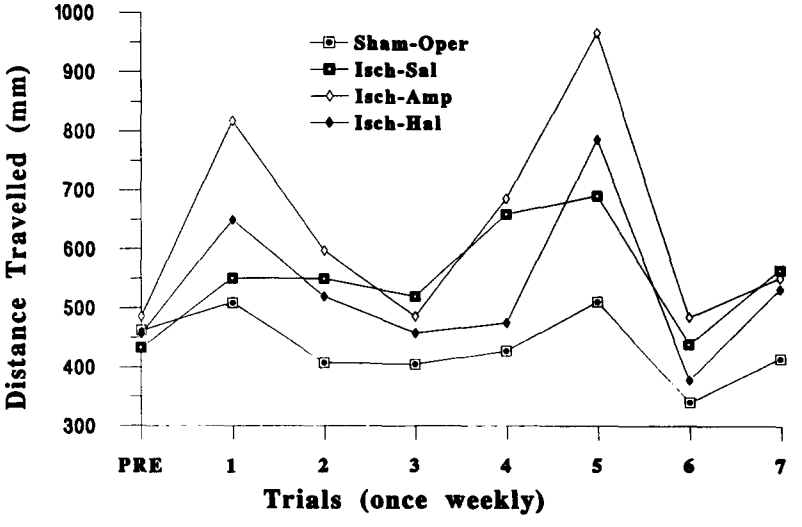

FIG. 1. Locomotor activity scores pre- and post ischemia.

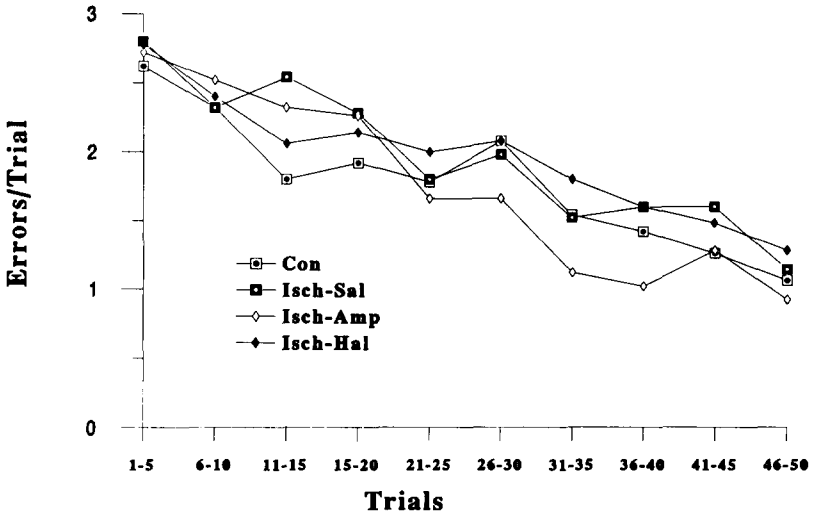

FIG. 2. Average number of reference memory errors following sham operations or ischemia.

blocks of five trials, but there were no differences between the ischemic groups during these initial 20 trials. Subsequently, the animals in these three groups reduced their working memory errors at different rates.

Animals made ischemic and treated with a single dose of saline continued to make significantly more working memory errors than did control animals on trials 21–25, and again on trials 41–45 ($p < 0.05$). Though not statistically different from controls on trials 26–40 or 46–50, the saline-treated animals consistently made more working memory errors than did controls on all trials.

The amphetamine-treated animals reduced their working memory errors to control levels in the fifth block of five trials; thereafter, the performance of this group was indistinguishable from that of the sham-operated group.

The haloperidol-treated group made significantly more working memory errors than did controls on all trials ($p < 0.05$).

Statistical comparison of the three ischemic groups revealed that the amphetamine-treated group made significantly fewer working memory errors than did the haloperidol-treated group on trials 26–50, and fewer than the saline-treated group on trials 41–45. The saline-treated group differed from the haloperidol group on trials 26–40 and 46–50.

Spearman rank-order correlations between regional brain damage scores and various measures of working and reference memory performance were determined. All correlations between brain damage scores and reference memory errors were insignificant. All but two correlations between brain damage scores and working memory errors were also insignificant; however, the correlation between CA1 damage and the total number of working memory errors made in the first 25 trials

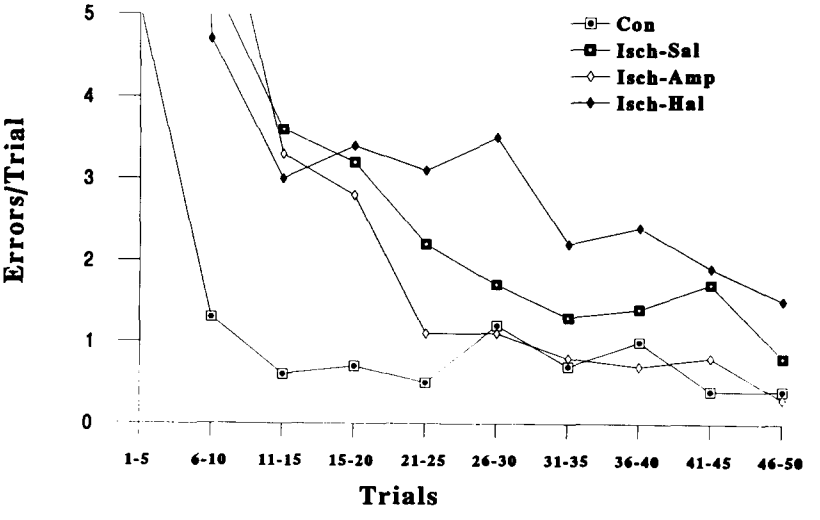

FIG. 3. Average number of working memory errors following sham operations or ischemia.

was 0.37 ($p < 0.05$), and that between total brain damage (obtained by summing all regional brain damage scores) and total working memory errors in trials 1–25 was 0.39 ($p < 0.05$).

DISCUSSION

A single, 5-min ischemic insult compromised radial arm maze performance in this experiment. The ischemic procedure did not impair the ability to learn to avoid unbaited arms, a finding reminiscent of that by Davis et al. (5). However, gerbils that received only saline after bilateral carotid artery occlusion made many more working memory errors (defined as reentries to previously visited arms) than did controls in the first 20 trials and in two of the remaining six blocks of five trials. Even though there were no statistical differences on the remaining four blocks, saline-treated ischemic animals never achieved the level of performance of sham-operated controls. These results are similar to those of others who have tested ischemic gerbils (18) or rats (5) in the radial arm maze. Because the hippocampal subfields were the brain structures most damaged by the ischemic procedure, and because a significantly positive correlation was obtained between CA1 cell damage and working memory errors, these findings provide additional support for the hypothesis that the hippocampus is involved in labile or working memory (22), and/or in spatial perception (21).

A single injection of amphetamine, administered 24 h after ischemia, was found to have prolonged effects on spatial memory by reducing the total number of working memory errors in the maze in comparison to ischemic animals given saline only. Further, after the initial trials when they repeatedly reentered already visited arms, the maze performance of amphetamine-treated gerbils became indistinguishable from that of sham-operated controls These results confirm those of Dunbar et al. (6), who found a facilitation of spatial memory in animals administered amphetamine after striatal damage. Conversely, a single injection of haloperidol administered 24 h after ischemia was associated with poorer working memory as measured by repeated reentry into already visited arms in the radial arm maze.

Colbourne and Corbett (2) recently reported that amphetamine had no recovery-enhancing effect in an open field test following forebrain ischemia in the gerbil. Contending that open field behavior following ischemia is related more to spatial mapping than to simple motor hyperactivity (25), they suggest that amphetamine may have beneficial restoration effects related to sensorimotor abilities, but not to cognitive functions such as memory. This conclusion directly contradicts the findings reported in this experiment. The discrepant findings may be related to the different tasks employed in our studies (open field vs. radial arm maze), or to some other procedural variable. Additional experiments need to be carried out to resolve these apparently contradictory findings.

The mechanism by which amphetamine might promote recovery in the radial maze task is unclear, particularly when the one-time drug administration was so temporally removed from the behavioral tests. The drug is certainly not neuroprotective because the amphetamine-treated gerbils experienced significant CA1 and CA4 cell damage. Colbourne and Corbett (2) reported similar findings. The effect may be mediated by one or more of the specific effects this drug has on monoaminergic systems, as has been argued for the recovery-facilitating effects of amphetamine in the case of motor disturbances after sensorimotor cortex lesions (15). The observation that a haloperidol injection after ischemia was associated with consistently poorer working memory performance in the gerbil is consistent with this presumption, because amphetamine and haloperidol are considered to have antagonistic neuropharmacological actions. The possibility exists, however, that the poor working memory performance of the haloperidol-treated animals may have been due, in part, to the greater extent of damage in the CA4 region in this group.

Alternatively, the effects of amphetamine and haloperidol on working memory, although clearly diametrical, may be the result of more general, nonspecific influences exerted by these drugs in the aftermath of the ischemic insult. Amphetamine has well- known stimulatory properties reflected in locomotor activity/stereotypy; conversely, haloperidol inhibits motor activity and antagonizes amphetamine-induced activity and stereotypy (10). It is most unlikely that hyperactivity can account for the improved performance of the amphetamine-treated animals because, contrary to the actual observations on reference memory, one would expect hyperactivity to be associated with nonselectively increased arm entries. Furthermore, even the haloperidol-treated ischemic animals were relatively hyperactive (compared to controls), yet these latter animals had markedly poorer working memories.

All gerbils subjected to the ischemic procedure became hyperactive in the weeks after surgery. Similar observations, including the correlation of the degree of hippocampal damage to the magnitude of the increase in activity, have been previously reported (13). Wang and Corbett (25) have provided evidence that ischemia-induced hyperactivity may be related to the inability to form spatial maps, which may account for the positively correlated relationships obtained in this study between CA1 damage and locomotor activity and between CA1 damage and working memory errors.

Although most earlier studies have concentrated on simple motor behaviors or sensory abilities and have induced focal brain damage, the results of this experiment provide clear support to the growing number of studies demonstrating that psychoactive agents administered in the period immediately following an ischemic episode may have profound behavioral consequences (6,9,17). Such medications are frequently used in patients after acute stroke or head injury and, because Goldstein (14) has collected some preliminary clinical data indicating that these drugs may affect recovery of function after acute stroke, more work is needed to better understand the mechanisms involved.

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