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Striatal Dopamine-Mediated Motor Behavior Is Altered Following Occlusion of the Middle Cerebral Artery

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BORLONGAN, C. V., R. MARTINEZ, R. D. SHYTLE, T. B. FREEMAN, D. W. CAHILL AND P. R. SANBERG. *Striatal dopamine-mediated motor behavior is altered following occlusion of the middle cerebral artery.* PHARMACOL BIOCHEM BEHAV **52**(1) 225–229, 1995. – Cerebral infarct (stroke) causes striatal damage with subsequent deterioration of sensorimotor and cognitive functions that may be mediated by the dopamine receptor system. In the present study, transient, focal ischemia was induced in Sprague–Dawley rats by middle cerebral artery occlusion. Ischemic animals exhibited significantly less dopamine antagonist (haloperidol)-induced catalepsy and more dopamine agonist (amphetamine)-induced hyperactivity than sham-operated animals. Younger ischemic animals showed more profound behavioral alteration but also displayed greater recovery over time than older ischemic animals. Histologic data revealed a lateral striatal lesion in all ischemic animals. These results place the striatal dopaminergic system as a possible strategic venue for the treatment of cerebral ischemia. In addition, aging is found to be a risk factor for stroke as noted in humans.

Stroke Middle cerebral artery Striatum Dopamine Haloperidol Amphetamine Catalepsy Locomotor behavior Aging

CEREBRAL infarction or stroke is usually secondary to cerebrovascular diseases, which often lead to irreversible neuropathologic damage (i.e., ischemic necrosis) and subsequent deterioration of motor, sensory, and cognitive functions (8, 24). Investigations into the etiology and possible therapies for cerebral ischemia have used animal models. Various strategies have been explored to establish a physiologic, biochemical, and histologic similarity between animal models and the human disease. The extracranial, transient, focal occlusion of the middle cerebral artery (MCAo) seems best to resemble the clinical cerebral ischemia (10,16). The MCAo technique uses an embolus to occlude blood supply that flows through the MCA to the brain without extensive surgery or craniectomy. Experimental studies have repeatedly shown that MCAo results in a localized degeneration of the basal ganglia, in particular the striatum (1,9,15). Rats with MCAo-induced ischemia demonstrate impairments in passive avoidance learning and memory (16), water-maze learning (9), spontaneous locomotor activity (26), and neurologic functioning (11,12). In the present study, we investigated the role of the nigrostriatal dopaminergic system in ischemic-induced behavioral deficits.

Sanberg and colleagues (21,19) reported that dopamine receptors localized postsynaptically on striatal neurons mediate cataleptic effects of dopamine antagonist haloperidol, a central dopamine receptor blocker. Considerable haloperidol-induced catalepsy was observed in normal animals with intact striatum, but an almost complete absence of cataleptic behavior was noted in animals with striatal lesions (19). Because striatal damage follows MCAo, we thought that the same striatal dopamine-mediated mechanism may be responsible for ischemic-induced behavioral effects. We hypothesized that ischemic animals would display minimal cataleptic response following haloperidol injection. To further confirm this dopamine-mediated mechanism, we also investigated the locomotor activity of ischemic animals in response to the dopamine agonist *d*-amphetamine. Previously, hyperactivity was ob-

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served in animals with excitotoxic striatal lesions following injection of *d*-amphetamine (13,14,18,20,22). Our hypothesis was that ischemic animals would exhibit an exaggerated motor response to *d*-amphetamine injection. Finally, we explored the possible age effects that have long been identified as a risk factor in stroke (6). Two age groups of Sprague-Dawley rats were used; one group consisted of 5-mo-old rats ($n = 12$) and had a mean weight of 510 ± 42 g, whereas the other group consisted of 8-week-old rats ($n = 12$) with a mean weight of 320 ± 24 g. We hypothesized that older animals would exhibit longer-lasting behavioral deficits than younger animals in response to haloperidol and amphetamine.

METHOD

Surgery

Animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were kept under a 12 L : 12 D cycle and allowed free access to food and water before and after surgery. We used the MCAo procedure, with a slight modification, originally described by Koizumi et al. (10) and Nishino et al. (16). Twelve animals, six from each age group, were randomly selected as experimental animals. Each animal was anesthetized with 0.60 ml/kg sodium pentobarbitol and shaved on the ventral portion of the neck. A V-shaped incision was made beginning at the caudal end of the sternomastoid and sternothyroid muscles and cutting toward the ears. After dissection, the right common carotid artery was exposed and carefully separated from the vagus nerve. The external carotid artery was then tied off tightly with two polypropylane sutures. The internal carotid artery was tied loosely and a small clamp was placed distal to the tie. Upon clamping off the common carotid artery, a small cut was made in the external carotid artery just above the bifurcation. The embolus was then introduced through the external carotid artery and guided into the internal carotid artery until resistance was felt. The embolus, which was made by coating a 30-mm piece of silk suture with silicone mixed with hardener, was used to block the origin of the right MCA; it was left in place for 1 h and then removed. The embolus point of entry was then cauterized and the skin sutured. The animal was then placed in a heating pad to keep its body temperature at normal limits. The animal's condition was monitored constantly 24 h following surgery or until recovery. The remaining 12 animals, which served as sham-operated subjects, underwent the same surgical procedures except that the embolus was not inserted.

Behavioral Tests

Haloperidol-induced catalepsy test. The procedure for the catalepsy test followed the procedures by Sanberg et al. (21) and was performed 1 mo following surgery. Animals were injected with 1.0 mg/kg haloperidol. This dosage has been demonstrated to induce catalepsy in normal animals for about 6–12 h (3,7,19). At 2 and 4 h postinjection of haloperidol, animals were tested for catalepsy. The bar test was performed using two bar heights of 8 and 12 cm for the two-weight group of animals, <350 and >450 g, respectively. The rats were gently picked up from under the forelimbs and dragged toward the bar, with the hindpaws allowed to remain in contact with the surface top. Timing began as soon as the investigator's hand was removed from the animal and ended as soon as the animal's right and left paws touched the table. Two separate latency scores (one for each paw) were obtained for each rat.

d-*Amphetamine-induced locomotor activity.* One week following the haloperidol-induced catalepsy test, the *d*-amphetamine-induced locomotor activity test was carried out. The Digiscan Locomotor Activity System (Omnitech Electronic, Columbus, OH) was used to quantify 13 different locomotor variables. Detailed descriptions of the Digiscan system is reported elsewhere (23). Initially, the animals were placed in the Digiscan and baseline locomotor activity was recorded during a 1-h period in three 20-min intervals. Immediately following this baseline recording, animals received 1.0 mg/kg, IP, of *d*-amphetamine (Sigma Chemical, St. Louis, MO) dissolved in deionized water (pH 7.4). *d*-Amphetamine-induced locomotor activity was subsequently measured by the Digiscan over a 2-h period in six 20-min intervals.

Histology

At the completion of all behavioral tests, all animals were decapitated and their brains studied for histology via cresyl violet and acetylcholinesterase (AChE) stains. Initially, animals were injected with 50.0 mg/kg of pentobarbital. Intracardial perfusions by 0.9% saline then followed by 4% paraformaldehyde in phosphate-buffered saline were carried out. Brain sections were cut at 40 μm using the vibroslice (World Precision Instruments, Sarasota, FL). Qualitative comparisons between ischemic and sham-operated brain sections were then performed in a blind-randomized manner.

Statistical Analysis

Group differences in cataleptic response to haloperidol were determined using Student's *t*-test. Repeated measures of ANOVA were used to analyze differences in *d*-amphetamine-induced locomotor activity. Posthoc *t*-tests were also carried out.

RESULTS

Histopathology

Brain damage in ischemic animals was fairly localized in the lateral aspect of the striatum ipsilateral to the occluded MCA (Fig. 1). In most ischemic brains, extrastriatal damage extended to the underlying cortex (olfactory nuclei) and the lateral horns of the corpus callosum. Gliosis and cell loss—in particular, disappearance of AChE-positive neurons—were widespread in the damaged areas of the striatum. There was no damage to the contralateral hemisphere. Differences between old and young ischemic brains were not visible at our level of gross macroscopic analysis. No corresponding CNS damage was noted in the sham-operated animals.

Behavioral Tests

Catalepsy test. At 2 and 4 h postinjection of haloperidol, ischemic rats exhibited a cataleptic response to haloperidol less than that of sham-operated animals ($t = 12.62$ and 5.28, $p < 0.01$, $df = 22$ for 2 and 4 h, respectively) (Fig. 2). When data were analysed across age, at 2 h postinjection of haloperidol, both age groups of ischemic rats spent significantly less time on the bar than sham-operated animals, with the younger ischemic rats spending the shortest time on the bar. At 4 h postinjection, the younger ischemic rats exhibited a cataleptic response that did not differ from that of the sham-operated animals ($t = 0.86$, $p > 0.05$, $df = 22$), but the older ischemic rats were still spending significantly less time on the bar than younger ischemic and sham-operated animals (each $t =$

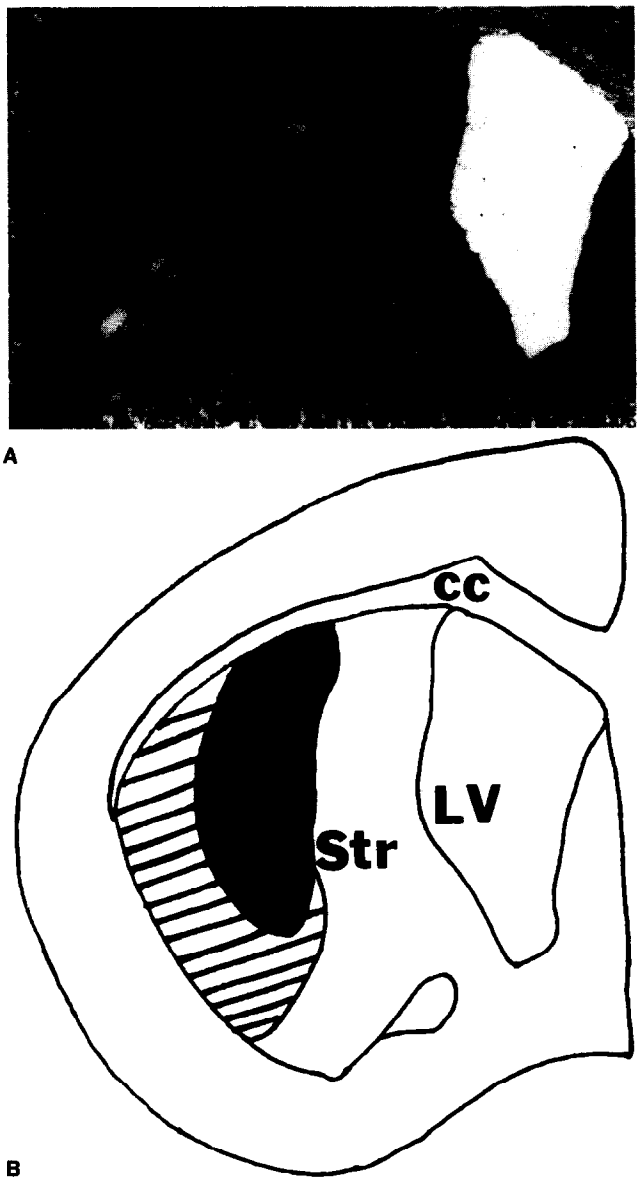

FIG. 1. Representative brain section from an ischemic rat. (A) An example of striatal infarct observed in animals that sustained right MCAo. The lesion was limited to the lateral aspect of the striatum ipsilateral to the MCAo. Gliosis and cell loss, particularly a decrease in AChE-positive neurons, were noted in the lesioned area. (B) Shaded areas correspond to severe infarction; striped areas are regions of moderate striatal degeneration. Ventricular dilation is also seen. LV, Lateral ventricle; Str, striatum; CC, corpus callosum.

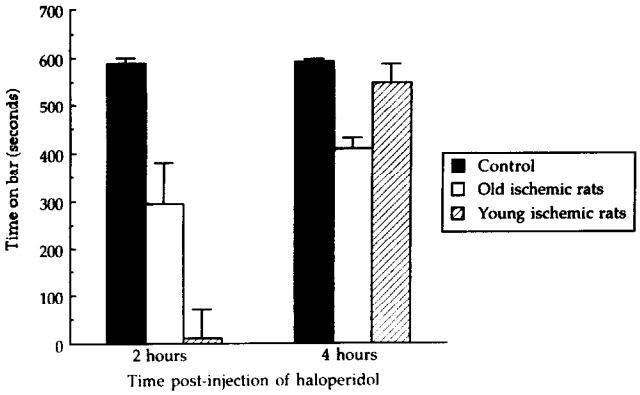

FIG. 2. Haloperidol-induced catalepsy. Data from both age groups of sham-operated animals were combined because no age difference was found. Values are group means ± SEM. At 2 h postinjection of haloperidol, both age groups of ischemic rats spent significantly less time on the bar than sham-operated animals, with the younger ischemic rats spending the shortest time on the bar. However, at 4 h postinjection of haloperidol, younger ischemic rats exhibited a cataleptic response that did not differ significantly from that of the sham-operated animals. In contrast, older ischemic rats continued to display a cataleptic response with a duration that was still significantly less than that of the sham-operated animals.

7.26 and 8.10, $p < 0.01$, each $df = 10$ and 16 vs. younger ischemic and sham-operated rats, respectively). No age difference was found in sham-operated animals across test sessions.

d-Amphetamine-induced locomotor activity. ANOVA revealed a significant treatment effect (preamphetamine vs. postamphetamine test sessions) [$F(1, 20) = 60.02$, $p < 0.0001$] (Fig. 3). In contrast, no significant age effect was noted [$F(1, 20) = 0.46$, $p > 0.05$]. Also, there was no significant treatment × age interaction [$F(1, 20) = 0.701$, $p > 0.05$]. Posthoc t-tests revealed that ischemic animals were significantly more hyperactive than sham-operated animals in all locomotor variables across the post-*d*-amphetamine injection sessions (each t and $p < 0.001$, $df = 22$). A significant time effect was also found [$F(7, 140) = 53.46$, $p < 0.0001$], as well as a treatment × time interaction [$F(7, 140) = 23.33$, $p < 0.0001$], age × time interaction [$F(7, 140) = 6.82$, $p <$

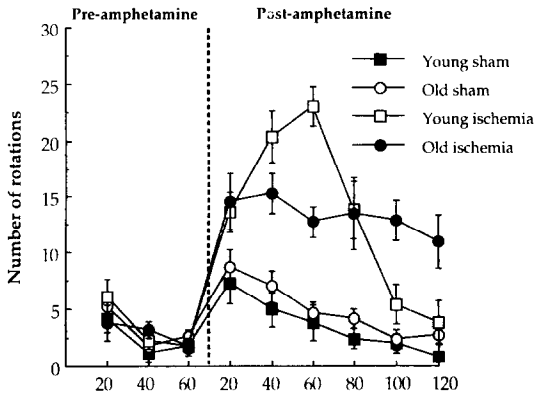

FIG. 3. *d*-Amphetamine-induced locomotor activity. Counterclockwise rotation (contralateral to the ischemic-damaged side) was chosen to represent the general locomotor activity of ischemic animals across all locomotor variables sampled at pre- and postinjection of amphetamine. Across the three 20-min pre-*d*-amphetamine sessions, no significant group difference was observed. In contrast, there was significant group difference across the six 20-min sessions of post-*d*-amphetamine injection. Both age groups of ischemic animals were significantly more hyperactive than sham-operated animals across the post-*d*-amphetamine injection sessions. Younger ischemic rats were significantly more hyperactive than older ischemic rats at 40 and 60 min post-*d*-amphetamine injection sessions, whereas the older ischemic rats were significantly more hyperactive than younger ischemic rats at 100 and 120 min post-*d*-amphetamine injection sessions.

0.0001], and treatment × age × time interaction [$F(7, 140) = 7.04$, $p < 0.0001$]. Posthoc tests showed that younger ischemic rats were significantly more hyperactive than older ischemic rats at 40 and 60 min post-*d*-amphetamine injection sessions [$t = 18.36$ and 20.35, $p < 0.001$, $df = 10$]. However, at 100 and 120 min post-*d*-amphetamine injection sessions, older ischemic animals were significantly more hyperactive than younger ischemic animals ($t = 7.61$ and 12.04, $p < 0.001$, $df = 10$). At 120 min post-*d*-amphetamine injection session, the younger ischemic animals were not significantly different from sham-operated animals ($t = 0.16$, $p > 0.05$, $df = 16$).

DISCUSSION

The present study demonstrated that ischemic animals were impaired in their locomotor functions. Specifically, ischemic rats showed a marked reduction in cataleptic response to haloperidol and a potentiation of the locomotor response to *d*-amphetamine. Age effects were also found to play a significant role in these behavioral changes. The possible mechanism underlying the behavioral alterations following ischemia may be the neuropathologic damage to the striatal dopaminergic system.

The present histologic data supported findings that brain damage following MCA-induced ischemia is concentrated in the lateral aspect of the striatum (9,16). Previous studies have also reported that striatal lesions may result in disruption of the dopamine system (2,25). The loss of striatonigral projections, which inhibit the activity of mesolimbic dopaminergic neurons, has been demonstrated to facilitate locomotor activity of the rat (3,13,14,17,21]. Within the striatum, haloperidol and other neuroleptics may act at dopamine receptor sites (4,5). In ischemic animals, postsynaptic striatal dopamine receptors may have been destroyed, which would result in a reduction of haloperidol-induced catalepsy. Accordingly, a damaged dorsolateral striatum could lead to a hyperresponsive mesolimbic dopamine system (17,21) thereby enhancing locomotor behavior following *d*-amphetamine administration, as was observed in the present study.

An age effect was noted in both behavioral tests, with the younger ischemic animals being less cataleptic and more hyperactive than the older ischemic ones in response to haloperidol and amphetamine, respectively. However, the younger ischemic animals showed recovery over time compared to the older ischemic animals, which displayed maintained hyperactivity. In humans, age has been reported as a risk factor of stroke (6). Although they suggest that older age may result in long-term ischemic-induced behavioral deficits, our present findings also provide evidence that younger age may be vulnerable to a more severe motor dysfunction. Further investigations are warranted to explore age effects on stroke.

The present study supports a striatal dopamine receptor-mediated mechanism for MCAo-induced ischemia. Further studies should determine direct changes in dopamine receptor number and function.

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