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Neonatal 6-Hydroxydopamine-Induced Dopamine Depletions: Motor Activity and Performance in Maze Learning

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ARCHER, T., W. DANYSZ, A. FREDRIKSSON, G. JONSSON, J. LUTHMAN, E. SUNDSTRÖM AND A. TEILING. *Neonatal 6-hydroxydopamine-induced dopamine depletions: Motor activity and performance in maze learning.* PHARMACOL BIOCHEM BEHAV 31(2) 357–364, 1988.—Three experiments were performed to study the effect of dopamine (DA) depletions, induced by neonatal intracerebroventricular (ICV) treatment with 6-hydroxydopamine (6-OHDA), upon measures of spontaneous motor activity. Instrumental learning for food reward in an Olton radial arm maze and escape learning from a large, circular water maze were studied also. Motor activity was measured by direct observation of rats in a modified radial arm maze and by use of automated test cages equipped with photocell devices. 6-OHDA-treated rats demonstrated considerable and long-lasting locomotor (ambulation) activity and total activity increases. 6-OHDA-treated rats showed notably less rearing activity than the vehicle-treated rats during the initial 20 min of each 60-min test period. However, over the second half of these 60-min test periods, the 6-OHDA-treated rats demonstrated significantly more rearing activity than the vehicle-treated rats. In the acquisition of the running response, to obtain the 8 food pellets placed in each of the 8 arms of the radial arm maze, 6-OHDA rats showed a retarded acquisition, as measured by the latency and number of arms visited to acquire all eight pellets. 6-OHDA-treated rats failed completely to acquire the Morris-type swim maze task by which they were required to locate a platform just under the water surface in a circular water tank. The neurochemical assays indicated severe DA depletion in several forebrain regions. The present findings add to existing indications of the potential of this DA depletion condition as an animal model of the minimal brain dysfunction syndrome.

Neonatal 6-hydroxydopamine Intracerebroventricular Ambulation Rearing Total activity
Test cages Acquisition Radial-arm maze Swim-maze Dopamine

THE destruction of dopamine (DA) neurones in the central nervous system as a result of intracerebral neonatal microinjections of 6-hydroxydopamine (6-OHDA) causes alterations in the functioning of both the juvenile and adult rats (5, 10–13). The behavioural alterations following DA depletion after neonatal 6-OHDA appear to consist of 1) a marked degree of hyperactivity which may (2,9) or may not (19) persist well into adulthood, 2) deficits in the performance of shock-avoidance tasks (18,20), and 3) a retarded acquisition of lever pressing responses on a fixed-ratio schedule of water reinforcement (4). It has been suggested (19) that this model bears a strong similarity to the disorder characterized by a constant involuntary hyperactivity accompanied by a range of cognitive and perceptual problems (22,23). Surprisingly, the functional aspects of neonatal DA depletion have not been investigated in an instrumental maze learning procedure. Thus, the radial-arm Olton maze task, a procedure that is sensitive to deficits in learning performance (11–13), was adopted to evaluate both cognitive and motor functions of neonatally DA-depleted rats. In a second experiment, the spontaneous motor activity of 6-OHDA- and vehicle-treated rats was measured in automated test cages during 60-min periods over several days. Finally, in a third experiment, spatial learning acquisition was tested again in a Morris-type swim maze.

METHOD

Male Sprague-Dawley rats aged 70–80 days, weighing 240–280 g at the time of behavioural testing, were used in all three experiments to study the effects of neonatal 6-OHDA intracerebroventricular (ICV) treatment on later behaviour. They were housed in groups of three or four animals under laboratory conditions with a 12 hr on/12 hr off lighting schedule in a thermostatically-controlled room (21±1°C).

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Behavioural testing was carried out during the hours of daylight between 10.00 and 16.00 hours.

Apparatus

An automated device (1) consisting of rat cages (40×25×15 cm) placed within two series of infrared beams (low level and high level) was used to measure spontaneous behaviour (Rat-O-matic, ADEA elektronik AB, Uppsala, Sweden). Each rat was alone in its test cage. The following parameters were measured.

Locomotion was registered by the low level grid of invisible infrared beams. Counts occurred only when the rats moved horizontally, showing predominantly locomotor behaviour.

Rearing was registered when rats raised their front legs and/or rested on their haunches, with the upper part of the body breaking the high level infrared beams. Counts (at a rate of 4 per sec) occurred as long as a single high level beam remained interrupted, i.e., the number of rearing counts obtained was proportional to the number of times the animal reared or to the time the rat interrupted the beam (i.e., the time spent rearing).

Total activity was registered by a pick-up (mounted on a lever with a counterweight) with which the test cage was in contact. The pick-up registered all types of vibrations within the test cage, e.g., those caused by particular rat movements when standing in one place, like tremors, head-shakes, or grooming behaviour.

Olton radial-arm maze. The radial arm maze task, a procedure sensitive to deficits in learning performance (11), was adapted both to evaluate spontaneous motor activity and cognitive function. Each of the 8 arms (each 54 cm long and 10 cm wide) of the Olton maze was "marked" off (divided) into three units (each 18 cm long and 10 cm wide) which gave a total of 25 units (counting the central hub, 25 by 25 cm) within walls that were 20 cm high. The radial arm maze was always placed on the floor, and a video-camera positioned on the ceiling filmed each rat. Behaviour was monitored on a TV screen placed in an adjoining room. The food cups, on the floor of the maze at the extremity of each arm, were not accessible during motor activity testing due to the presence of an extra, removable back wall. For the learning tests the "false" back walls were removed and each food cup was exposed. Standard food pellets (45 mg) were obtained from Campden Instruments Ltd. (London).

Morris-type swim maze. Five trials were presented to each rat after the platform had been placed at a particular position within the circular pool. For each trial, each rat was placed at the same point in the pool and allowed to swim around to find the platform and escape from the water onto the platform which was located 1 cm below the water level. Five trials were presented to each rat on each of two days. On reaching the platform each rat was allowed to remain upon it for 30 sec before being placed in the water again for the next trial. If a rat failed to locate the platform within 65 sec it was removed from the water and placed on the platform for 30 sec.

Treatment

Female rats (Sprague-Dawley, ALAB, Sollentuna, Sweden) were obtained on the fourteenth day of pregnancy and were housed individually in a room maintained at 21±1°C with illumination provided from 0600 to 1800 hr. Food and water were continuously available. Male rat pups were randomly assigned to litters of 4 at birth and were housed with a lactating dam in plastic cages. The male rat pups were weaned at 23 days of age and were housed thereafter in groups of 2 animals. At 3 days of age, the male rat pups were given subcutaneous injections of desmethylimipramine-HCl (DMI, 20 mg/kg, Ciba-Geigy, Basel). Thirty minutes later, the pups were anaesthetized by cooling and were given an ICV injection of 6-hydroxydopamine hydrobromide (6-OHDA, 100 μg/5 μl vehicle) or the vehicle solution (0.9% saline containing 0.1% ascorbic acid). The skull was exposed with a midline incision and a 10 μl Hamilton syringe equipped with a 27-gauge, 2.5 mm long needle was held perpendicular to the skull while the needle was inserted through the skull at a site located 1.0 mm lateral to the sagittal suture at the bregma. Five μl of solution were then delivered into the right lateral ventricle over a period of 30 sec. The incisions were closed with adhesive sutures and the pups were returned to the lactating dams. On day 6 after birth, each rat pup received the same DMI + 6-OHDA or DMI + vehicle treatment as on Day 3, except that 6-OHDA or vehicle was injected into the left lateral ventricle.

Statistical Analysis

Ambulation and rearing data from the spontaneous motor activity tests in Experiment 1 as well as the latency and number of arms visited, and latency to locate the platform data from the learning tests in Experiments 1 and 3 were all subjected to two-way ANOVA (21). Locomotion, rearing and total activity data from Experiment 2 were subjected to Split-plot ANOVA (8). Pairwise testing between groups was performed with the Tukey HSD test (8). The 1% level of significance was maintained throughout unless where otherwise stated.

Neurochemical Analysis

At the end of the behavioural tests in each experiment the rats were sacrificed by decapitation. The brains were rapidly removed and dissected out as described by Jonsson and Sachs (6). For Experiment 1, catecholamine concentrations in several regions of the CNS of adult rats treated ICV with 6-OHDA neonatally were measured. Endogenous catecholamine concentrations were determined using high pressure liquid chromatography with electrochemical detection (l.c.e.c.) [Keller *et al.* (7) as modified by Jonsson *et al.* (5)]. For Experiments 2 and 3, endogeneous levels of monoamines were assayed by reversed phase high pressure liquid chromatography with electrochemical detection as modified by Hallman *et al.* (3).

EXPERIMENT 1

Procedure

Following birth, on Day 3 rat pups from each dam were assigned to either the 6-OHDA or vehicle treatments following DMI (20 mg/kg SC) injections to each pup. Microinjections of 6-OHDA (100 μg/5 μl, ICV) or vehicle were administered to each pup on Day 3 and Day 6 after birth. Following weaning at 23 days of age, the 6-OHDA and Vehicle pups were housed in pairs until spontaneous motor activity was measured in the modified Olton radial arm maze on Days 30, 45, 52 and 60 after birth. In each group, 6-OHDA and vehicle, n=8. Each rat's behavior was monitored on the video TV apparatus placed in an adjoining room. Ambulation and rearing were measured over 10-min

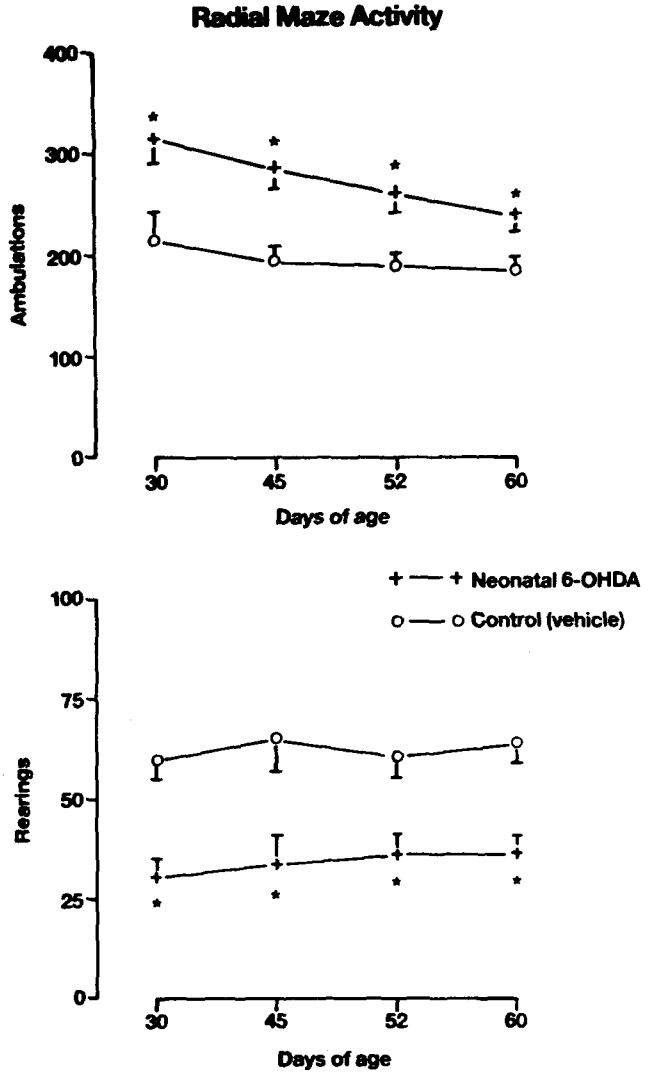

FIG. 1. Mean ambulation and rearing counts by 6-hydroxydopamine- and vehicle-treated rats in a modified radial arm maze on Days 30, 45, 52 and 60 after birth. *Ambulation* was defined as the passage of a rat's body from one unit to another. *Rearing*, when each rat raised itself onto its hindlegs, was measured also. Spontaneous activity was measured over 10-min periods on each test occasion. 6-Hydroxydopamine rats showed more ambulatory but less rearing behaviour than the vehicle-treated controls (Tukey HSD test, $p<0.01$). Values are expressed as mean±s.e.m.

periods on each test occasion. Ambulation is defined as the passage of a rat's body from one unit to another, and rearing was scored each time a rat raised itself onto its hindlegs. After each animal was tested the maze was wiped carefully with a sponge rinsed in water containing liquid soap. Following testing on the final test day, all the rats were placed on a total deprivation for 48 hours. Acquisition performance in the radial arm maze was tested at ages 64 and 65 days (Tests 1 and 2). For the learning tests, the "false" back walls were removed exposing the 8 food cups (one at the extremity of each arm) into each of which a 45 mg food pellet was placed. At the start of each test, each rat was placed in the central hub and then monitored for its performance in acquiring all the pellets. Body weights at the onset of testing were: 6-OHDA=216±18, vehicle=249±11. Immediately after the second test each rat was given free access to food and one week later all the rats were sacrificed and several brain regions were dissected out and analyzed for catecholamine concentrations.

Results

The rats that had been treated neonatally with 6-OHDA (2×100 μg/5 μl, ICV) showed long-lasting increases in ambulatory activity during the 10-min test period but a decrease in rearing activity during each test. Two-way ANOVA indicated significant Groups effects both for the Ambulation data, $F(1,60)=34.7$ and for the Rearing data, $F(1,60)=50.2$. Pairwise testing between groups over all four motor activity tests with Tukey HSD tests indicated that the 6-OHDA rats made significantly more ambulations and significantly fewer rearings during each test occasion. Figure 1 presents the mean number of ambulations and rearings by the 6-OHDA- and vehicle-treated rats on Days 30, 45, 52 and 60 after birth.

In the test of acquisitive performance in the radial arm maze, the 6-OHDA-treated rats demonstrated a retardation of acquisition in comparison with the vehicle-treated control rats. Two-way ANOVA indicated a significant Groups × Tests interaction for the latency data, $F(1,30)=7.0$ and a significant Groups effect, $F(1,30)=12.7$ and Tests effect, $F(1,30)=12.5$, for the number-of-arms-visited data. Tukey HSD tests indicated that the 6-OHDA-treated rats required significantly more time to acquire all eight pellets (latency) than the vehicle-treated rats during Test 1; no significant differences between the groups were observed during Test 2. 6-OHDA-treated rats also visited significantly more arms in acquiring all eight pellets during both tests. All the rats tested reduced latencies and the number of arms visited from Test 1 to Test 2 (i.e., pairwise testing between Test 1 and Test 2 over both groups indicated significantly shorter latencies and fewer arms visited in taking all 8 pellets) which indicates some acquisition performance whether 6-OHDA- or vehicle-treated. To control for differences in motivation a simple food intake test was performed after the rats had been food-deprived. No difference in latency to consume a single 4 g pellet was obtained.

Figure 2 presents the mean latency to take all eight pellets and the mean number of arms visited by 6-OHDA-treated and vehicle-treated rats. Table 1 presents the catecholamine concentrations of brain regions from the 6-OHDA and vehicle groups tested in Experiment 1. The neurochemical assays indicate marked DA depletions (4% to 24% of control values) in the frontal cortex, nucleus accumbens, septum and striatum, and lesser but quite noteworthy DA depletions in the amygdala and A9 regions (34% and 36% of control values, respectively). Noradrenaline (NA) concentrations were largely unchanged, except for a small but significant decrease in the amygdal (77% control values).

EXPERIMENT 2

Procedure

On Days 3 and 6 following birth rat pups from each dam were assigned to either the 6-OHDA or vehicle treatment groups and administered either 6-OHDA (2×100 μg/5 μl, ICV) or vehicle. In order to test more specifically the alterations in spontaneous motor activity incurred by neonatal 6-OHDA treatment (200 μg/5 μl, ICV), rats were tested in

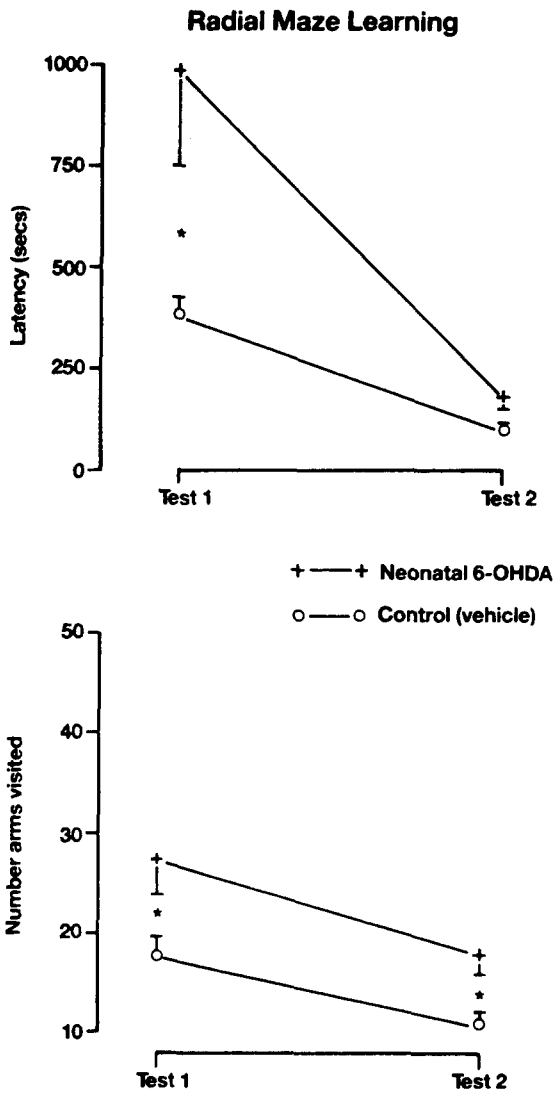

FIG. 2. Mean latency to take all eight food pellets and mean number of arms visited in taking all eight food pellets by 6-hydroxydopamine- and vehicle-treated rats in a radial-arm maze on Days 64 and 65 after birth. 6-Hydroxydopamine rats showed longer latencies and visited more of the arms in taking all eight pellets (Tukey HSD tests, $p<0.01$). Values are expressed as mean±s.e.m.

automated activity test cages over five consecutive days, Days 1 to 5 of testing (Days 61 to 65 after birth), for a 60-min period on each test day. On each test occasion each rat, 6-OHDA and vehicle, was removed from its housing group cage and placed, singly, in the automated test cage. 6-OHDA and vehicle groups were allowed to remain in the test cages over the five 12-min (total 60 min) test periods during each of the five consecutive days of testing. Locomotion, rearing and total activity were measured over this period.

Results

The 6-OHDA rats demonstrated considerable hyperactivity as measured by the locomotion and total activity parameters of motor activity, but a clear hypoactivity during the initial part of the test periods as measured by the rearing parameter. During the latter part of each test period, 6-OHDA rats showed clear increases in rearing activity over the vehicle rats. Split-plot ANOVA indicated significant Treatment × Time period × Days interactions, $F(16,224)=34.9$ and, $F(16,224)=37.6$, for the locomotion and rearing data, respectively, and significant Treatment × Time periods interaction, $F(4,56)=31.7$ and Treatment × Days interaction, $F(4,56)=50.3$, for the total activity data. Figure 3 presents the locomotion, rearing and total activity data of 6-OHDA and vehicle rats during each 12-min time period over each of the five days of testing (Days 61 to 65 after birth). Each motor activity parameter will be described in turn:

Locomotion. Tukey HSD tests indicated significantly more locomotion counts by the 6-OHDA group during each 12-min period over all five test days.

Rearing. Tukey HSD tests indicated significantly fewer rearing counts by the 6-OHDA group during the first two 12-min periods on test days 1, 3 and 4; and fewer counts during the first 12-min period on Days 2 and 5. The 6-OHDA group made more rearing counts than the vehicle group during the final three 12-min periods on test days 1, 2, 3 and 4, whereas more rearing counts were made during the final four 12-min periods on Day 5.

Total activity. Tukey HSD tests indicated significantly more total activity counts by the 6-OHDA group over the whole 60-min test period over all five test days. Pairwise testing within groups over periods and days indicated that the 6-OHDA group made more locomotion and total activity counts during the final two 12-min periods on Day 5 as compared with Day 1, i.e., 6-OHDA rats tended to show more hyperactivity over successive time periods and over the five test days.

In order to confirm the DA depletions caused by neonatal 6-OHDA treatment, the frontal cortex and striatum regions were dissected out and assayed for catecholamine concentrations. In the frontal cortex DA concentration was 29 percent of control values and NA concentration was 109 percent of control values. In the striatum DA concentration was 4% of control values and NA levels were not detectable. Thus the DA depletions obtained from the rats tested in Experiment 2 compared closely with those of Experiment 1, whereas NA does not seem to be affected.

EXPERIMENT 3

On days 3 and 6 following birth rat pups from each dam were assigned to either the 6-OHDA or vehicle treatment groups and administered either 6-OHDA (2×100 μg/5 μl, ICV) or vehicle. In order to provide another test of spatial learning to complement the Olton maze test of Experiment 1 this third batch of 8 6-OHDA and 8 vehicle rats were tested in the swim maze on each of two consecutive days.

Results

The neonatal 6-OHDA groups were drastically impaired in the swim maze task compared with the vehicle group. Mean latencies to reach the platform were: Day 1: 6-OHDA=62.4±1.0 sec, Vehicle=41.5±1.5 sec; Day 2: 6-OHDA=63.6±0.5 sec, Vehicle=13.6±0.8. Two-way ANOVA indicated a significant Groups effect, $F(1,30)=16.8$, as a result of the significant increase in latency to locate the platform by the 6-OHDA rats, as compared with the vehicle rats. Neurochemical analysis of the striatum regions of the 8

TABLE 1

REGIONAL CHANGES IN NORADRENALINE AND DOPAMINE CONCENTRATIONS IN THE CNS OF ADULT RATS TREATED INTRAVENTRICULARLY WITH 6-OHDA NEONATALLY

Region	Noradrenaline			Dopamine		
	Control	6-OHDA	%	Control	6-OHDA	%
Frontal Cortex	388 ± 16	338 ± 74	−13	37 ± 3	9 ± 2†	−76
Nucleus Accumbens	404 ± 198	249 ± 60	−39	10063 ± 914	1580 ± 419†	−84
Septum	829 ± 44	1005 ± 818	+21	1148 ± 221	131 ± 39*	−89
Striatum	not detectable	not detectable		12726 ± 384	542 ± 226†	−96
Amygdala	871 ± 35	667 ± 32*	−23	398 ± 31	137 ± 32†	−66
Hypothalamus	2547 ± 208	2174 ± 264	−15	488 ± 46	671 ± 89	+37
A9	223 ± 22	259 ± 36	+16	632 ± 43	227 ± 24†	−64

6-OHDA (100 μl/5 μl) was administered at 3 and 6 days in the right and left ventricle, respectively, and the animals were killed in the adult stage (2½ months old).

The data are expressed as ng/g; mean ± SEM of 6–9 determinations, male animals only. %=percent change compared to control. Statistical comparison by Student's *t*-test (6).

*$0.01>p>0.001$; †$p<0.001$.

Catecholamine assays were performed as described previously (5).

6-OHDA and 8 vehicle rats tested above confirmed the severe DA depletions (6-OHDA=2% of vehicle values).

DISCUSSION

The present findings may be summarized as follows: 1) 6-OHDA-treated (neonatally, ICV) rats showed significant and long-lasting increases in locomotor (ambulatory) activity over the vehicle-treated rats when the animals were tested in a modified version of the Olton radial arm maze; whereas at the same time the 6-OHDA rats showed decreases in rearing activity. Note that activity testing in the modified Olton maze was over 10 min only on each occasion. Spontaneous activity measurements performed in automated test cages confirmed these results as the 6-OHDA rats showed hyperactivity for the locomotion parameter throughout the 60-min test periods but hypoactivity for the rearing parameter during the first 10–25 min of test, i.e., more locomotion and total activity than the vehicle rats over 60 min but less rearing over the initial 10–25 min. During the final 24 minutes of the 60-min tests the 6-OHDA rats demonstrated a marked increase in rearing activity over the vehicle group. For the total activity parameter, the 6-OHDA rats showed clear hyperactivity over the total 60-min throughout the five consecutive testing periods. 2) 6-OHDA-treated rats showed a retarded acquisition of running responses to acquire all the eight available food pellets, one of which was placed at the extremity of each arm of the radial arm maze, as measured both by latency and by the number of arms visited in acquiring all eight pellets. 3) 6-OHDA-treated rats failed completely to learn to locate a platform hidden 1 cm under the water surface in a Morris-type swim-maze. 4) The catecholamine assays of the rats tested in the first experiment indicated severe or notable DA depletions in the frontal cortex, nucleus accumbens, septum, striatum, amygdala and A9 regions of the 6-OHDA-treated rats. Noradrenaline (NA) was largely unchanged, except for a small, but significant decrease of NA concentrations in the amygdala (77% of control value). Severe DA depletions in the striatum and frontal cortex were confirmed for the rats tested in Experiments 2 and 3.

The present findings generally confirm the results from previous investigations of the DA depletions caused by neonatal ICV 6-OHDA (bilateral) treatment since the 6-OHDA-treated rats demonstrated consistent and considerable hyperactivity. New evidence regarding the effects of neonatal DA depletions and cognitive performance is presented here since the 6-OHDA rats indicated a retarded acquisition of an Olton maze learning task and showed a total failure to acquisition of the platform-location in the circular pool, even though each rat was placed upon the platform after each 65-sec trial. The neonatal 6-OHDA rats had severe DA depletions in several forebrain regions. However, these results extend the documented findings in some other respects also: Both the direct observation of rats and the use of automated test cages indicated that the increases in ambulation/locomotion (horizontal activity) were accompanied by decreases in rearing (vertical activity); note, however, that the decrease in rearing behavior lasted up to 24 min of the test period after which 6-OHDA rats indicated notable increases in rearing. Thus, these results may be reconciled also with other pre- or neonatal manipulations of the DA system where increases in ambulation were accompanied by increases in rearing (10). The 6-OHDA-treated rats were found to be clearly retarded in acquiring the radial arm maze instrumental task after having shown a clear decrease in rearing behavior during all the previous four exposures to the maze; but these rats did show some notable degree of acquisition already on the second trial which was performed 24 hours after the first (see Fig. 2). It may be interesting to note that the vehicle treated rats seemed to have reached an asymptotic performance by the second trial.

Earlier studies concerning this type of neonatal DA depletion model have utilised operant conditioning tasks in a skinner-box (4) and shock-avoidance tasks (18,20); clear performance deficits were obtained in each case. It should be noted that the radial arm maze task was chosen in order to provide a measure of instrumental learning different to that investigated previously and not to generate learning curves. Although this task is generally interpreted to assess spatial memory or working memory (11) the purpose of the experi-

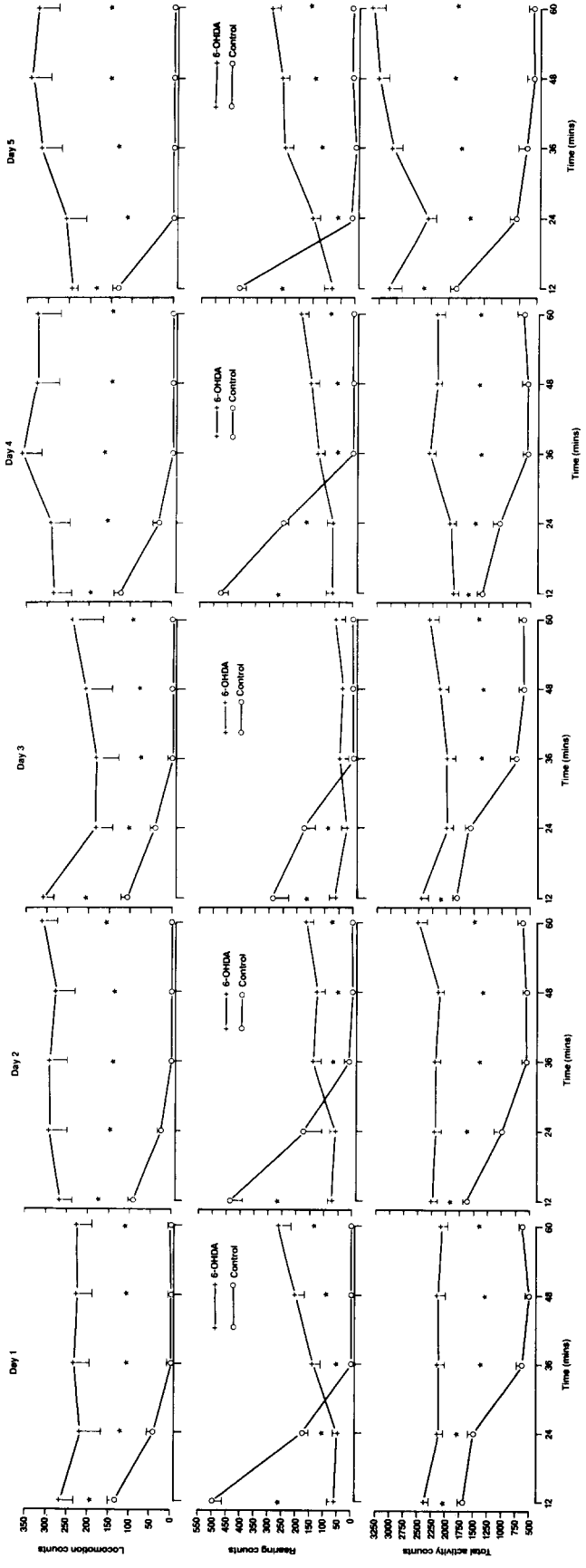

FIG. 3. Mean locomotion, rearing and total activity counts by 6-hydroxydopamine- and vehicle-treated rats in the automated (ADEA) test cages during 60-min periods on Days 61 to 65 after birth (Days 1, 2, 3, 4 and 5).

ment was not primarily to test memory but rather performance. It is possible, however, that the 6-OHDA rats are hampered by some alteration of working memory. At least the results of the swim maze task where the 6-OHDA rats were so severly impaired offer additional evidence in favor of a spatial memory dysfunction. Present research is directed towards separating the effects of DA depletion upon working and reference memory in maze learning tasks.

The motor activity test performed in previous studies utilized stabilimeter cages (2,9) or chambers measuring movement (14), presumably mechanically, but in neither case does there appear to be any distinction made between rearing and ambulation. Shaywitz *et al.* (17) measured several parameters including slight activity (sniffing, grooming, scratching) and total activity (ambulation, climbing, eating, drinking, rearing and slight activity). The total activity parameter, registered in Experiment 2 measured mechanically using a "pick-up" device in the automated ADEA boxes (Experiment 2), appears to be similar to that same parameter of the Shaywitz study; in both cases a drastic and very long-lasting state of hyperactivity is obtained in 6-OHDA-treated rats. A consistent decrease in rearing activity compared to the vehicle rats was observed and it is important to observe that this decrease was in each case limited to the initial testing periods when both rearing and locomotor/ambulatory activity are at their peak. There are some indications that rearing activity, for example in an open-field, may be a more selective measure of "exploratory" behavior than locomotion [e.g., (16)]. One may suggest that the 6-OHDA rats fail to show sufficient "exploration," possibly as a result of the massively increased ambulatory/locomotor activity. This explanation may be further developed to suggest that the performance deficit in the Olton maze may be due, indirectly, to the hyperactivity of these animals rather than any specific effect of the lesions on learning ability or spatial memory per se, i.e., the hyperactivity of 6-OHDA rats may retard maze performance by disrupting the normal "exploratory/search" behavior during the prelearning activity test exposures when the rats were allowed to investigate the maze on Days 30, 45, 52 and 60. In the swim maze task where 6-OHDA rats failed completely to learn to locate the platform, it was observed that, without exception, these rats swam rapidly round in circles that kept them on a path just outside or just inside the position of the platform. In this task it is probable that the disruptive effects of hyperactivity were potentiated by stress effects aggravated by the repeated failure to locate the platform.

The suitability of the neonatal intracerebroventricular 6-OHDA dopamine depletion treatment as an animal model for the minimal brain dysfunction (MBD) syndrome in hyperactive children has been argued (2, 14, 17) and clearly there seems much evidence supporting the utility of this model. Certainly the consistency of the present results derived from both a direct observation technique and from automated test cages must be an indication also of the reliability of the model. However, one conclusion suggested from the present findings could be to emphasize that much additional functional and neurochemical analysis is required not only to obtain some explanation for the learning deficit but also to realize the full potential of this animal model for the eventual purpose of development of therapeutic agents.

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