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Neonatal Brain Dopamine Depletion and the Cortical and Behavioral Consequences of Enriched Postweaning Environment

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PAPPAS, B. A., S. J. E. MURTHA, G. A. S. PARK, K. T. CONDON, R. M. SZIRTES, S. I. LAVENTURE AND A. ALLY. *Neonatal brain dopamine depletion and the cortical and behavioral consequences of enriched postweaning environment.* PHARMACOL BIOCHEM BEHAV 42(4) 741-748, 1992.—This study investigated the effects of neonatal intraventricular administration of 6-hydroxydopamine (6-OHDA, 15 μg total with and without desmethylimipramine pretreatment) on the cortical thickening and behavioral effects of 35 days of enriched postweaning housing (ENR) in the rat. The 6-OHDA treatment depleted cortical dopamine (DA) to about 40% of control. It did not affect the thickness of the cerebral cortex nor did it affect the capacity for the cortex to be thickened by ENR. In addition, it did not alter the superior performance on two spatial water maze tasks that was caused by ENR. Thus, the potential for neurobehavioral plasticity was not changed by neonatal DA depletion. ENR eliminated the spatial learning/memory deficits that were caused by neonatal DA depletion and that were manifested when the rat was raised in standard (impoverished) laboratory conditions. Hence, environmental factors can modulate the cognitive effects of neonatal DA depletion. ENR did not attenuate the hyperactivity of the neonatal DA-depleted rat. This may reflect the subcortical mediation of this behavioral abnormality.

Cortical thickness Spatial learning Neonatal 6-Hydroxydopamine Plasticity

CATECHOLAMINE (CA) neurons differentiate relatively early in the prenatal development of the rat brain and CA axons are evident in the cerebral cortex at about embryonic day 17 (2,25,35). Furthermore, a disproportionately high number of synapses in the newborn rat cortex have a CA uptake/storage mechanism (3,22). These findings have led to the suggestion that CA neurons may have a special role in cortical maturation (3,25,30,35). Accordingly, it would be expected that early destruction of these neurons would affect cortical development and cognitive function. Several laboratories have examined the effects of perinatal destruction of norepinephrine (NE) axons on cortical development. The results have been inconsistent, however (7,19,20,30,40).

In an interesting elaboration of this theme of special relevance of NE for cortical maturation, Kasamatsu and Pettigrew (16) proposed that intact forebrain NE afferents from the locus coeruleus were required for the environmental modification of the kitten cortex during a critical period of development. Thus, these NE afferents were suggested to have an essential role in cortical "plasticity." This subsequently controversial (8) hypothesis stimulated our own and other laboratories to assess the role of forebrain NE for the environmental modification of the rat behavior and cortex. These experiments have examined whether neonatal depletion of forebrain NE affected the consequences of enriched postweaning housing, namely, enhanced performance on spatial mazes and thickening of the cerebral cortex (5,9,33). While there have been indications that such consequences are eliminated by forebrain NE depletion (21,27,29), there have also been reports of no effect (4,42).

The current experiment determined the relevance of forebrain dopamine (DA) to the consequences of enrichment for the rat. The rationale for doing so was twofold. First, it is important to have a "positive" control against which the outcome of the NE depletion experiments could be compared, that is, would a similar outcome occur after damage to other types of diffuse forebrain innervation, suggesting that differing forms of perinatal forebrain damage can share similar consequences regarding the modification of the brain by enrichment? If so, then there is no specific relevance of forebrain NE to the plasticity that is reflected by the enrichment phenomenon. Second, DA cell differentiation and DA innervation of the forebrain also occurs relatively early in brain development and may precede the maturation of NE innervation

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(2,39). Perinatal depletion of forebrain DA by intracranial injection of 6-hydroxydopamine (6-OHDA) has profound behavioral effects, including enduring hyperactivity and deficits in the acquisition of a variety of behavioral tasks (11,32,37). Thus, the evidence to suggest DA's special role in forebrain maturation is as compelling as that for NE. Accordingly, the following experiment determined if neonatal intraventricular injections of 6-OHDA would alter rats' responsiveness to enriched postweaning housing as assessed by measures of cortical thickness and, behaviorally, by locomotion on an elevated plus-maze and on two spatial water maze tasks. The 6-OHDA injections were preceded in about half the rats by systemic injections of desmethylimipramine (DMI) so as to cause damage to only DA terminals (37). The dose of 6-OHDA administered was selected to cause a moderately severe, selective DA depletion approximately equivalent to that created by Kalsbeek and coworkers (14,15), who reported that neonatal thermal lesions of the ventral tegmental area of the 1-day-old pup reduced the total length of the basal dendrites of layer 5 pyramidal cells in the prefrontal cortex, suggesting a trophic role for DA in the development of the cortex.

METHOD

Subjects

One-hundred and seventy-four male hooded rats of the Long-Evans strain were used in this study. They were born and bred in our own colony from breeding stock (nonprimiparous females) obtained from Charles River Laboratories (Montreal, Quebec). All litters were cross-fostered within 12 h of birth, and culled to six to nine male pups per dam.

Drug Treatment

On postnatal day 0 (within 24 h of birth), pups received a 0.05-ml SC injection in the nape of the neck of either saline (SAL) or 25 mg/kg DMI to protect NE neurons from the toxic effect of 6-OHDA. After 25 min, animals were immobilized by being placed in crushed ice for 5 min. The SAL and DMI groups were then given bilateral intraventricular injections of either 0.2 mg/ml ascorbic acid in saline (groups SAL-VEH, DMI-VEH, respectively), or 11.1 μg (7.5 μg free base each ventricle) of the neurotoxin 6-OHDA hydrobromide (Sigma Chemical Co., St. Louis, MO) in the ascorbic acid solution (SAL-6-OHDA, DMI-6-OHDA). Injections of 1.0 μl per ventricle were delivered through a 30-ga stainless steel cannula by a microdrive pump over a 1-min period. A further 30 s were allowed for diffusion of the drug after it was administered. A modified version (the needle insert into the foramen magnun was eliminated) of the neonatal stereotaxic described by Heller and colleagues (13) was used. The injection coordinates were 0.55 mm anterior to bregma, ± 1.4 mm lateral to the midsagittal suture, and 3.5 mm ventral from the scalp surface.

Enriched/Impoverished Postweaning Environment

Neonates were born and raised under a reverse light cycle (lights on at 20:00 and off at 08:00 h). All dams and their litters were housed in polypropylene cages (45 × 23 × 15 cm) until weaning at 25 days of age. The four treatment groups were then randomly divided in two, half being placed in an impoverished environment (IMP) and the other half going into an enriched one (ENR) (33,34). This resulted in eight groups: ENR SAL-VEH, ENR DMI-VEH, ENR SAL-6-OHDA, ENR DMI-6-OHDA, and the matching four IMP groups.

The enriched condition consisted of 8–11 rats per large wire mesh cage (72 × 72 × 45 cm), each cage containing many different kinds of toys (wheels, ladders, tunnels, etc.). They were moved to a new clean cage that contained a different selection of toys every third day. As well, they received intermittent auditory stimulation from a radio in the room.

The impoverished condition consisted of pairs of rats housed in wire mesh hanging cages (20 × 24 × 18 cm). Every third day, they were put in a weighing pan and then placed back into their cages. This was done to eliminate the potential differences caused by the handling of enriched animals during cage changes. It also served to accustom animals to the handling that occurred during behavioral testing. All animals were maintained with ad lib access to Purina Rat Chow and water.

At 60 days of age, all ENR and IMP rats were weighed and placed singly into hanging cages. Testing began on day 61, when rats were assigned to measures of cortical thickness or one of the three behavioral tests.

Cortical Thickness

After completion of ENR/IMP housing at 60 days of age, 8–10 rats per group were perfused with 10% formalin on days 62 and 63 after an overdose of pentobarbitol. Brains were stored in formalin and later sectioned on a microtome at 40 μm. Following the procedures of Diamond et al. (4,5), sections were taken at the decussation of the anterior commissure (AC) and the thickness of the dorsal, lateral, and ventral regions of the cortex was subsequently recorded. These measures were taken using commercially available software (Imaging Research, St. Catherines, Ontario) to capture and digitize an image of the most intact section closest to the decussation of the AC. The digitized image was projected on a monitor and templates adapted from Diamond et al. were laid over the image to standardize the measurement procedure. Five to seven measures of cortical thickness were taken at each region and these were averaged to yield a single value for each region.

Elevated Plus-Maze

The elevated plus-maze was adapted from the design of Pellow et al. (31). It consisted of two open arms (50 × 10 cm) opposite one another and two opposing, enclosed (50 × 10 × 40 cm) arms that were perpendicular to the open ones. The arms were elevated 50 cm above the floor. The maze was constructed of opaque white Plexiglas. A videocamera was hung above the maze to record animals' behavior. The maze was situated in the center of an illuminated room.

For testing, each rat was placed in the center "neutral zone" of the maze and allowed to freely ambulate for 5 min. The videotapes of the rat's behavior was scored for total arm entries, a measure of activity, and for open and closed arm entries. Rats treated with anxiolytic drugs typically show a higher ratio of open to closed arm entries than do undrugged rats (31).

Morris Water Maze

The Morris water task was adapted from Morris et al. (23) and was a circular aluminum, white pool 135 cm diameter by 50 cm high filled with tapwater (21 ± 1°C) to a height of 40 cm. The pool was placed in the center of a room that contained various objects (desk, cabinet, etc.) that served as distal cues for the rat. A videocamera was mounted above the pool to record the rats' paths. One of two platforms served as an

escape for rats. A white platform could be placed with its upper surface 1.0 cm below the surface of the water. A black platform could be placed to protrude 1.0 cm above the water's surface. Its top was concave so that rats would be standing in approximately the same amount of water as when standing on the white platform.

The procedure used was a variation of the one described by Morris et al. (23). Powdered milk was added to the water to make it opaque, thus making the white underwater platform invisible. For the first 4 days of training, the white platform was positioned in the middle of the southwest quadrant of the pool. Each animal was randomly placed into the pool facing either the north, south, east, or west wall and their latency to find the platform was recorded (120 s max). They were removed from the platform after 20 s. If a rat did not find the platform within 20 s, it was placed onto the platform for 20 s. This procedure was repeated four times such that each rat began at each starting location once.

On day 5, a no-platform test was conducted. The platform was removed and animals allowed to swim for 60 s. Their paths were videotaped for subsequent determination of the time spent in the quadrant that had previously contained the platform and the remaining three quadrants.

On days 6 and 7, rats were tested for cued platform finding. The black visible platform was placed in the northeast quadrant of the pool (opposite from where the white platform was).

At the end of each battery of four trials, each animal was toweled off and placed under heat lamps to dry for 5 min before being returned to its cage. All behavioral testing was conducted blind such that the experimenter did not know which drug or rearing condition to which the animal belonged.

Concentric Ring Maze

An annular ring 101 cm diameter and fabricated of white sheet vinyl could be inserted into the Morris maze described earlier. This created a circular channel around the outer perimeter of the pool that was 17.5 cm in width. An opening was cut in the ring and by rotating the entire ring this opening could be placed at 90° left or right from the rat's starting position. The opening was 10 cm wide and extended from 10 cm above water level to 15 cm below it. A white Plexiglas barrier slightly less than 17.5 cm wide and extending from the top of the ring to the maze bottom was used to occlude the left or right channel at 135° from the start point. A black 6-cm diameter circular platform made of roughened Plexiglas was situated at the center of the pool. Its surface was about 1 cm above water level.

Testing consisted of two stages: The platform-only (cued) stage required that rats swim to and mount the above-water platform. The one-ring test consisted of the annular ring arrangement and required that the rat learn to criterion a left–right discrimination. For half the rats in each group, the opening was on the right and vice versa for the other half.

On the platform-only day of testing, animals were placed into the pool with only the black platform placed in the center. The latency to find the platform was recorded for four trials to a maximum of 2 min per trial. If the rat did not reach and mount the platform in 10 s or less, an additional fifth trial was run. If the rat failed to mount the platform within 10 s, it was given an additional four trials the next day (by which time all rats had reached criterion). Four equally spaced points around the perimeter served as the four start points for each rat.

On the one-ring test day, the rat was placed facing the inner ring at a constant perimeter location that was within the outer channel and 90° from the entry door to the center of the pool. The rat's latency to reach and mount the platform was recorded. If the rat did not reach the platform within 2 min it was picked up and placed on the platform. Two types of errors were also recorded and summed: errors where the rat swam the wrong way in the outer section until it encountered the barrier and errors where the animal reentered the outer section after having been in the central area but not having found the platform. Rats were required to perform three consecutive errorless trials to reach criterion. Rats that did not achieve criterion in 10 trials were retested for up to 10 trials the following day and, if necessary, again the day following that. All animals had reached criterion by the third day.

CA Analysis

Brain catecholamine levels were assayed for rats that had received neurochemical lesions but had not undergone behavioral testing. Rats were decapitated at 70 days of age and their brains dissected over ice into whole cortex, caudate, and hippocampus. The tissue was subsequently stored at −70°C until assayed using a reverse-phase, ion pairing high-performance liquid chromatography (HPLC) method slightly modified from Ally et al. (1). The solvent system used was 25 mM citric acid, 25 mM disodium phosphate (dibasic), 1 mM sodium octane sulphonic acid, 5 μm EDTA, pH 3.5, with phosphoric acid. All buffer solutions were made in distilled, HPLC-grade water. These were passed through 0.22-μm filters to eliminate any contaminants.

Analysis was conducted on an LKB-2150 isocratic HPLC pump. A μ-bondapak (Waters Associates, Toronto, Ontario) C-18, 30-cm column with an internal diameter of 3.9 μm was used. Electrochemical detection, using a BAS model LC-4B amperometric detector with 0.5-s filter, at 20 nA sensitivity, was set to a potential of 0.7 V. Data was collected using a linear chart recorder.

Samples were extracted in 0.4 M perchloric acid with 1.0 mM EDTA and 1.0 mM sodium metabisulphite. One μM 3,4-dihydroxybenzylamine HBr was used in the extraction medium as an internal standard. Tissues were weighed and extracted with the extraction mixture in the ratio of 10 ml/g tissue. The extraction was carried out on ice with the tissue being homogenized for about 15 s using a Polytron (Brinkmann, Rexdale, Ontario) homogenizer. The sample was returned to the ice and allowed to cool for 10 s, then rehomogenized for a further 15 s. It was then transferred to 1.5-ml Ependorff (Brinkmann, Rexdale, Ontario) tubes and centrifuged for 3 min at high speed. The supernatant was then passed through a 0.22-μm filter. The pH of the extract was adjusted to 4–5 and 25 μl extracted sample was injected for analysis.

RESULTS

Body Weight

6-OHDA-injected rats did not exhibit aphagia or akinesia. A 2 × 2 × 2 analysis of variance (ANOVA) was performed on the postenvironmental body weights. There was a significant drug effect, $F(1, 69) = 6.33$, $p < 0.05$, with 6-OHDA animals weighing less (321.7 ± 3.9 g) than VEH-treated ones (342.6 ± 4.3 g). There was no significant effect of environment or subcutaneous DMI injection. Another ANOVA con-

ducted on the weight of rats prior to sacrifice again indicated a significant drug effect, $F(1, 69) = 5.55$, $p < 0.05$, VEH animals weighing more (437.5 ± 5.4 g) than 6-OHDA-injected rats (411.6 ± 5.4 g). There were no other significant main effects or interactions.

CA Assays

The assay results are shown in Table 1. Neither NE nor DA levels were affected by postweaning environment. DMI by itself also had no enduring effect on catecholamine levels. Pooled across environmental condition, 6-OHDA injections without DMI pretreatment depleted hippocampal and cortical NE to 26 and 25% of vehicle control levels, respectively. DMI pretreatment completely prevented NE depletions. 6-OHDA injections reduced caudate and cortical DA to 26 and 41% of vehicle control levels, respectively. The levels of 6-OHDA-injected DMI-pretreated rats were 28 and 38% of controls, respectively.

Cortical Thickness

ANOVAs showed that the left and right cortices did not differ in thickness. Accordingly, they were averaged for subsequent analyses. Similarly, DMI pretreatment had no effect on cortical thickness for either saline- or 6-OHDA-injected rats. Accordingly, the results for VEH- and 6-OHDA-injected rats were averaged across the DMI/SAL condition.

A 2 × 2 × 3 ANOVA was computed on cortical thickness. Between-group variables were neonatal treatment (VEH vs. 6-OHDA) and environment (ENR vs. IMP) and the single within-subject variable was plane of section (dorsal, lateral, and ventral). The ANOVA showed a significant main effect only for environment, $F(1, 36) = 15.64$, $p < 0.001$. Neither the 6-OHDA main effect nor any of the interactions involving neonatal treatment or postweaning environment approached significance. The results are shown in Fig. 1, where it can be clearly seen that 6-OHDA treatment neither affected cortical thickness nor modified the effect of environment on it. Separate t-tests comparing ENR and IMP groups combined over the neonatal treatment variable showed that enrichment increased cortical thickness at all three planes [dorsal, $t(36) = 5.35$, $p < 0.001$; lateral, $t(36) = 3.26$, $p < 0.01$; ventral, $t(36) = 2.90$, $p < 0.01$].

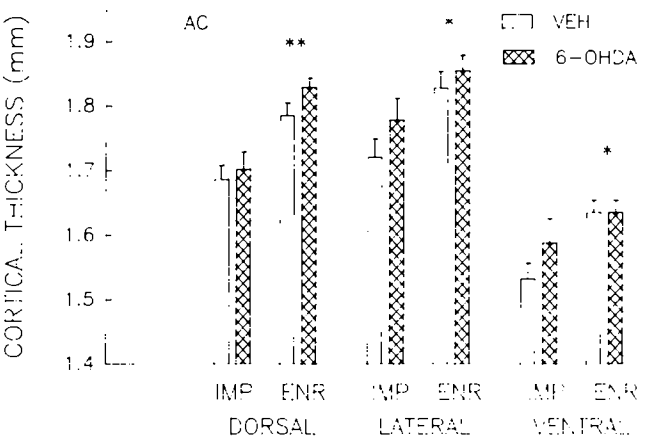

FIG. 1. Mean (± SEM) thickness of the cerebral cortex at the decussation of the anterior commissure (AC, upper panel) or at the crossing of the posterior commissure (PC, bottom panel). The data are indicated for the dorsal, lateral, and ventral planes.

Behavioral Tests

The DMI and SAL control groups did not differ on any of the behavioral tests and therefore their data were pooled for simplification of presentation. Similarly, SAL- and DMI-pretreated 6-OHDA groups did not differ and their data were pooled as well.

Elevated Plus-Maze

Figure 2 shows the data for the maze expressed as total arm entries (upper panel), the ratio of open to total arm entries (middle panel), and the proportion of the total test time during which the rat was in the open arms.

TABLE 1

MEAN (± SEM) NE AND DA CONTENTS (nmol/g TISSUE) AFTER NEONATAL INTRAVENTRICULAR 6-OHDA OR VEHICLE

	NE		DA	
	Hippocampus	Cortex	Caudate	Cortex
Vehicle				
ENR	2.54 ± 0.12	2.51 ± 0.25	76.9 ± 8.7	4.36 ± 0.49
IMP	2.31 ± 0.23	2.26 ± 0.11	81.9 ± 8.3	4.91 ± 0.42
6-OHDA				
ENR	0.70 ± 0.33	0.41 ± 0.22	20.5 ± 3.9	2.10 ± 0.23
IMP	0.58 ± 0.23	0.77 ± 0.25	20.3 ± 6.4	1.72 ± 0.33
Vehicle & DMI				
ENR	2.66 ± 0.21	2.39 ± 0.32	83.6 ± 7.5	4.62 ± 0.49
IMP	2.24 ± 0.21	2.39 ± 0.29	86.9 ± 8.9	4.39 ± 0.30
6-OHDA & DMI				
ENR	2.39 ± 0.19	2.31 ± 0.31	21.8 ± 7.0	1.40 ± 0.14
IMP	2.39 ± 0.22	2.39 ± 0.20	23.4 ± 3.9	2.09 ± 0.40

The data are shown for groups raised in enriched (ENR) or impoverished (IMP) postweaning environments.

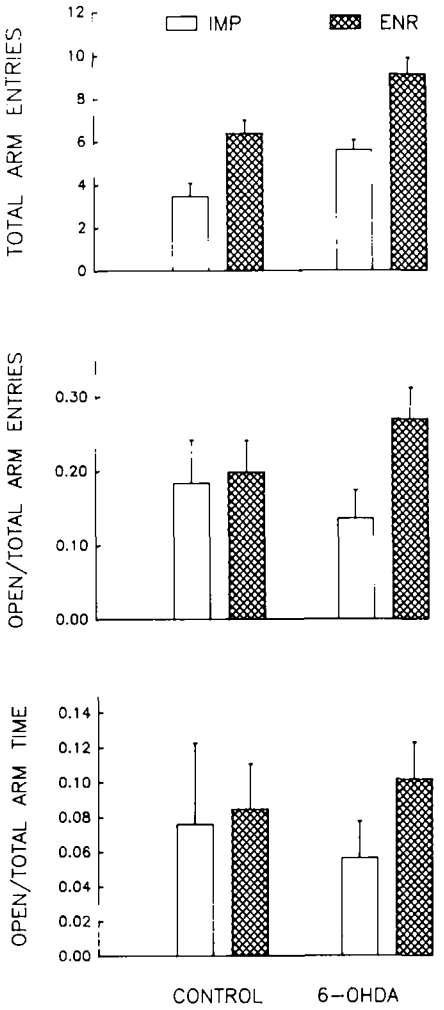

FIG. 2. Elevated maze performance. The upper panel shows the (mean ± SEM) total arm entries, the middle panel shows the ratios of open to total arm entries, and the lower panel shows the ratios of the time spent on the open arms to total time on the maze.

As the upper panel shows, ENR rats irrespective of their neonatal treatment entered more arms, $F(1, 78) = 15.11$, $p < 0.001$. As well, 6-OHDA-treated rats entered more arms than did their VEH counterparts, $F(1, 78) = 26.29$, $p < 0.001$.

There were no significant main or interaction effects for either the open to total arm entry ratios or the proportion of total test duration that was spent on the open arms (all $ps > 0.10$, ANOVA). As the middle and lower panels of Fig. 2 show, all groups entered fewer open than closed arms and spent less time on the open than on the closed arms.

Morris Maze

Figure 3 shows the latencies to find the submerged platform for IMP- (left panel) and ENR (right panel)-reared rats. Comparison of these upper and lower panels shows that ENR-reared rats, both normal and DA depleted, had shorter escape latencies, a result confirmed by ANOVA, which indicated a significant environment × trials interaction, $F(15, 59) = 2.20$, $p < 0.05$, but no environment × drug interaction.

While there was no significant overall neonatal 6-OHDA effect for escape latency over the 16 trials for either ENR- or IMP-housed rats, inspection of the data shown in Fig. 3 suggested 6-OHDA-injected, IMP-reared rats were slower to find the platform on the first trial of days 2 and 3. This impression was confirmed by ANOVAs carried out only on the first daily trial (trials 1, 5, 9, and 12) latencies and on the latencies for all other trials. The ANOVA of the first trial latencies of IMP-reared rats indicated a significant interaction for 6-OHDA injection × trials, $F(3, 71) = 2.73$, $p < 0.05$. ANOVA of the comparable data for ENR rats did not show a similarly significant interaction. ANOVAS performed on the latencies for trials two through four of each day failed to show any effects.

A 2 × 2 ANOVA of the no-platform test data indicated a significant main effect for environment, $F(1, 73) = 11.52$, $p < 0.001$, and 6-OHDA injection, $F(1, 73) = 7.12$, $p < 0.01$. ENR rats, both normal and DA depleted, spent more time in the platformless training quadrant than did IMP animals (21.5 ± .9 vs. 17.2 ± 1.0 s). Similarly, VEH-treated rats spent more time in the training quadrant than did DA-depleted animals (21.1 ± 1.0 vs. 17.9 ± .9 s). Planned comparisons of VEH- and 6-OHDA-injected rats showed that only the 6-OHDA rats that were raised in impoverishment spent significantly less time in the training quadrant than did their vehicle controls [$t(34) = 2.01$, $p < 0.05$, one tailed].

Cued navigation. ANOVA showed that all groups performed equally regardless of drug group or environment.

Concentric Ring Maze

IMP rats were generally inferior to their ENR counterparts in first platform-only phase of testing, which required that rats locate the black platform. This difference reached statistical significance for total time to criterion, $F(1, 86) = 16.7$, $p < 0.001$, and for the total time to locate the platform for the first four trials, $F(1, 86) = 18.91$, $p < 0.001$. There were no significant differences between VEH and the 6-OHDA groups for any of the three measures.

On the one-ring test (see Fig. 4), 6-OHDA-injected rats required more trials to reach criterion, $F(1, 85) = 5.46$, $p < 0.03$, more total time to reach criterion, $F(1, 85) = 5.03$, $p < 0.03$, and committed more errors before reaching criterion, $F(1, 85) = 6.34$, $p < 0.02$. As shown in Fig. 4, these effects were significant only for IMP rats. Post-hoc analyses (Tukey's test) showed that for each of these variables IMP-housed, 6-OHDA rats were impaired in comparison to IMP-housed controls ($ps < 0.05$). ENR-housed 6-OHDA rats did not differ from ENR-reared controls.

There were also significant overall main effects due to post-weaning environment. Overall, IMP rats required more total time to reach criterion, $F(1, 85) = 5.02$, $p < 0.03$, and committed more errors, $F(1, 85) = 6.34$, $p < 0.02$, than did ENR rats.

DISCUSSION

The main findings of this experiment were as follows. First, neonatal 6-OHDA-induced depletion of brain DA had no enduring effect on cortical thickness. Furthermore, it did not affect the capacity for the cerebral cortex to be thickened by ENR. Behaviorally, DA-depleted rats were hyperactive but there was no evidence that they were abnormally anxious. They were impaired at the learning and retention of spatial maze habits. This impairment was eliminated by ENR, however.

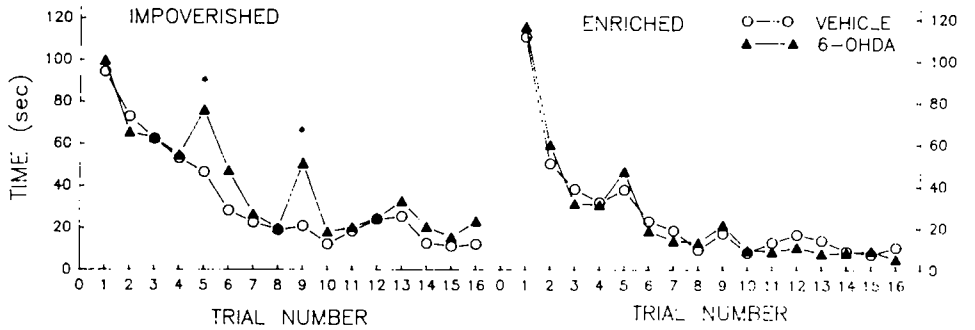

FIG. 3. The left panel shows the mean group latencies across trials to find the submerged platform in the Morris water maze for impoverished-housed 6-OHDA- or vehicle-treated rats. The right panel shows comparable data for enriched-housed rats. Four trials were administered per day.

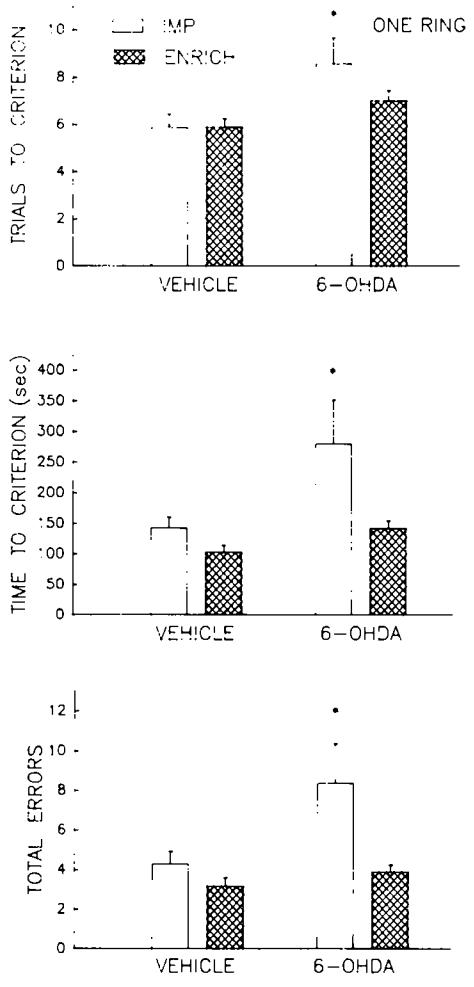

FIG. 4. Results of the one-ring version of the water maze. The upper and middle panels show the mean (± SEM) trials and times to criterion, respectively. The lower panels show the total errors committed.

Because of their relatively early prenatal differentiation (2,25,35) and their disproportionately high synaptic density in the newborn rat cortex (3,22), CA neurons have been posited to have a special role in cortical development. Onteniente et al. (26) administered neonatal injections of 100 μg 6-OHDA (free base) into the fourth ventricle, observing only a slight, nonsignificant increase in thickness of the temporal cortex at 16 days of age. There was, however, a significant loss of cells there, with the loss being greatest in the upper layers, but the staining technique did not differentiate the loss to decreased glia or neurons. In that study, 6-OHDA injections were not preceded by DMI pretreatment and, as a result, rats showed a substantial depletion of both NE and DA. Accordingly, the cell loss could have been due to perinatal depletion of either NE or DA or both. The evidence for effects of perinatal NE depletion only upon the development of cortical cellular architecture or cortical thickness is at variance, however (7, 17,18,20,40), while the effects of neonatal depletion of only DA on the cortex has received scant attention. Neonatal thermal lesions of the ventral tegmental area of the 1-day-old pup have been effected and the results on cortical thickness are conflicting. In one instance, this lesion was found to cause an approximate 6% reduction in cortical thickness (14) and in another to have no effect on cortical thickness (15). In this latter experiment, however, lesioned rats showed a reduction in the total length of the basal dendrites of layer 5 pyramidal cells in the prefrontal cortex. It was suggested that the results of these two experiments supported a trophic role for DA in the development of the prefrontal cortex. It should be noted, however, that the effects of these thermal lesions were not restricted to the DA cells of the ventral tegmentum.

The extent of the cortical DA depletions caused by the aforementioned neonatal thermal lesions varied from 27-69% depending upon the side of the brain assayed. In the present experiment, cortical DA depletions after neonatal intraventricular 6-OHDA ranged from 51-70%. It seems, therefore, that neonatal DA depletions of moderately severe magnitude do not affect cortical thickness, despite their having clear behavioral effects. It does seem to affect cortical cell number (26) and dendritic morphology (15) and this merits further exploration. It would be of particular interest to examine the consequences of more extensive selective depletions of DA. Preliminary observations in this laboratory indicate that substantially higher doses (100 μg with DMI pretreatment) cause

severe ventricular hypertrophy, suggesting that this dose of 6-OHDA could cause nonspecific tissue damage, although it remains possible that this is directly attributable to very severe DA depletions.

The present experiment indicated that not only does moderately severe forebrain DA depletion in the neonatal rat have no effect on cortical thickness but it also fails to alter the capacity for enriched postweaning housing to thicken the cortex. A similar absence of effect has been observed after neonatal NE depletion (24). Thus, at least as reflected by this enrichment model, forebrain catecholamines do not seem to have a special role in cortical plasticity.

Hyperactivity is a hallmark of neonatal DA depletion (6,28,36) and was observed in this experiment—DA-depleted rats entered more arms on the elevated plus-maze. Unlike the impairment on the two water maze tasks, this behavioral abnormality was not eliminated by enrichment. It is not clear what aspects or consequences or neonatal 6-OHDA-induced DA terminal degeneration cause the enduring hyperactivity displayed by these rats. One possibility is that the hyperproliferation of serotonin terminals that occurs in the striatum (38) could mediate this. This is supported by the demonstration that the paradoxical activity-reducing effects of amphetamine and other stimulants in neonatal DA-depleted rats *may* be mediated by their augmentation of serotonergic transmission (11). On the other hand, neonatal electrolytic lesions of the medial ventral tegmental but not the nigrostriatal DA pathways cause hyperactivity (10), which indicates that the critical site for damage may not be the neostriatum (and the serotonergic hyperproliferation there) but the frontocortical and/or limbic terminations of the ventral tegmental DA neurons. If so, then it is not surprising that enriched postweaning housing did not ameliorate the hyperactivity since the neural effects of such housing are primarily on dendritic branching and synapses in neocortex (9,33).

This experiment showed that the likelihood of observing impaired acquisition of spatial tasks after neonatal DA depletion depends upon the postweaning environment. If they had been raised in an impoverished environment, DA-depleted rats were found to be impaired at 24-h retention of platform location in the Morris maze, showed significantly less swim time in the previous platform-containing quadrant in the no-platform test, and were impaired at the acquisition of the one-ring version of the concentric ring water maze. DA-depleted rats raised in an enriched environment did not show these. Previous studies that have documented impaired acquisition in neonatal DA-depleted rats have all involved "standard," impoverished postweaning housing (12,32,37,41). Thus, the present experiment is testament to the powerful effect of postweaning environment on the cognitive function of neonatal DA-lesioned rats. It shows that impoverished postweaning environment can precipitate cognitive impairment in neonatal DA-lesioned rats. Arguably, the enriched environment used in the present experiment probably only begins to simulate the complexity of the rat's natural environment (34) while the "standard" impoverished environment represents an aberration unlikely to occur in this animal's natural habitat. Additional research is necessary to determine if particular aspects of enrichment (e.g., group housing, cage size, objects, etc.) ameliorate cognitive deficits in neonatal DA-depleted rats. In addition, it would be instructive to determine the spectrum of neonatal DA depletion-induced abnormalities that are/are not eliminated by enrichment. This could clarify the interpretation of the psychological effects of the permanent loss of DA terminals early in life.

DA-depleted rats showed the same proportion of open to total arm entries on the elevated plus-maze as did controls. This proportion is increased by clinically useful anxiolytic drugs and decreased by drugs with anxiogenic properties (31) and it may reflect the rat's state of anxiety. Accordingly, neonatal DA-depleted rats seem not to be more anxious than their controls and therefore excessive anxiety seems not to be the reason why they were impaired on the maze tasks (after impoverished postweaning housing).

In conclusion, moderately severe neonatal DA depletion affects neither the thickness of the rat cerebral cortex nor the capacity for the cortex to be thickened by enriched postweaning housing. In addition, it does not alter the cognitive enhancement from enriched housing as reflected by spatial water maze tasks. Thus, neither the gross development of the cortex nor the potential for neurobehavioral plasticity is changed by this degree of neonatal DA depletion. Postweaning enrichment eliminates spatial learning/memory deficits that are caused by this neonatal DA depletion and manifested when the rat is raised in standard (impoverished) conditions. Thus, spatial learning and memory deficits are not an immutable consequence of neonatal DA depletion. Rather, they are at least in part precipitated by the impoverishment of standard laboratory housing. Postweaning enrichment does not attenuate the hyperactivity of the neonatal DA-depleted rat. This may reflect the subcortical locus of this behavioral abnormality.

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