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Effects of Neonatal and Adult 6-Hydroxydopamine Treatment on Random-Interval Behavior[1,2]

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LEVINE, T. E., L. ERINOFF, D. P. DREGITS AND L. S. SEIDEN. *Effects of neonatal and adult 6-hydroxydopamine treatment on random-interval behavior.* PHARMAC. BIOCHEM. BEHAV. **12**(2) 281–285, 1980.—Rats were given intraventricular injections of 6-hydroxydopamine (6-HDA) or saline-ascorbate vehicle as neonates (3-days old) and as adults (49 and 51 days old). At 73 days of age, they were trained on a random interval 90-sec schedule of water reinforcement. The rats treated with 6-HDA as adults stabilized at response rates approximately twice those of vehicle-treated rats, while rats treated with 6-HDA as neonates showed response rates which were not significantly different from vehicle-treated rats. Both L-Dopa and apomorphine decreased response rates at all doses tested. There were no differences among the groups with respect to the effect of these drugs. Adult-treated rats showed greater response rate decreases following peripheral decarboxylase inhibition with Ro 4-4602. Catecholamine analyses revealed the rats treated with 6-HDA as neonates had greater depletions in the striatum and the remainder of telencephalon than adult-treated rats but an increase in brainstem norepinephrine. These findings suggest that age of treatment is an important determinant of the biochemical and behavioral effects of treatment with 6-HDA.

6-Hydroxydopamine Operant behavior Development Apomorphine L-DOPA Catecholamines

THE ontogeny of central catecholamine (CA) systems has been studied in order to further understand the development of brain function and behavior [3, 9, 12]. One method for studying the interaction between the ontogeny of CA's and behavior involves interfering with immature brain CA systems, and comparing the behavioral effects of this treatment with those produced by treatment which destroys mature CA systems. The neurotoxin 6-hydroxydopamine (6-HDA), can be used to selectively destroy catecholaminergic neurons in both neonate and adult rats and is therefore a useful tool for assessing the effects of destruction of this transmitter system in the neonate in comparison to the adult rat.

Studies on the effects of 6-HDA on operant behavior in adult rats have by and large failed to show long-term effects [5, 10, 16, 17]. However, following treatment of adult rats with 6-HDA, Peterson and Sparber [14] found increased responding on a fixed ratio 30 (FR-30) and Schoenfeld and Uretsky [15] found rate increases in rats performing on a variable interval 1.5 min (VI-1.5 min). In the latter study, the increases in rate were 3–4 times that of control rats and occurred in rats treated with 6-HDA irrespective of whether the rats were trained before or after lesioning.

The large increase in VI response rates following 6-HDA administration provides a good basis for comparison of the effects of neonatal and adult treatment with 6-HDA. Therefore, the study reported here evaluated the effects of neonatal and adult treatment with 6-HDA on a similar schedule. Dosages of 6-HDA for neonates and adults were chosen in an attempt to produce equivalent effects on DA and NE in these groups. Schoenfeld and Zigmond [17] postulated that the increased response rates of 6-HDA treated animals is due to depletion of dopamine (DA). Therefore the effects of two DA agonists, apomorphine and L-DOPA were also determined in these animals.

The results of the present study indicate that neonatal treatment with 6-HDA does not produce as large a rate increase in RI performance seen with adult treatment. However, the effects of L-DOPA and apomorphine were similar

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[2]Portions of this study were presented at the Annual Neurosciences Meetings in St. Louis, MO, November 5–9, 1978.
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TABLE 1
OUTLINE OF TREATMENT PROCEDURE

Treatment group	Treatment as neonate	Treatment as adult
Neonatal	6-HDA (100 μg)	Saline-ascorbate
Adult	Saline-ascorbate	6-HDA (2 < 250 μg)
Vehicle control	Saline-ascorbate	Saline-ascorbate

in both neonatal and adult lesioned groups; there was little evidence that either lesion affected the behavioral response to these drugs.

METHOD

Subjects

Pregnant Sprague-Dawley rats were obtained from Holtzman Company (Madison, WI) at 14–15 days gestation. At 3 days of age, 30 male pups were anesthetized with ether. Ten were treated with 6-HDA intraventricularly (50 μg/μl in each lateral ventricle), and 20 male pups were treated similarly with an equivalent volume of vehicle (0.9% saline and 0.1% ascorbate). Four to five hours after treatment, pups of like treatment were assigned to lactating dams, 10 pups/dam. When the rats reached 49 days of age, they were again anesthetized with ether and treated. Ten of the rats which had been treated with vehicle at 3 days of age were given 6-HDA (250 μg/20 μl) into the right lateral ventricle; at 51 days of age, this procedure was repeated with the exception that the 6-HDA (250 μg/20 μl) was injected into the left lateral ventricle. The other 20 rats were anesthetized with ether and injected with 20 μl of vehicle into the right and left lateral ventricles at 49 and 51 days of age, respectively (Table 1). During the two weeks following treatment, rats were given access to food and a .01% solution of tetracycline. They were then placed on water deprivation with 15 min access to water each day.

Apparatus

Twelve modified Gerbrands rat chambers (Model C), 20.5 cm long × 23 cm wide × 20 cm high served as experimental enclosures. The front and rear walls were Plexiglas; the left and right walls and top of the chamber were stainless steel. The left wall contained a houselight which remained on throughout the session. A Lehigh Valley lever was mounted on the right wall, 3.0 cm from the front wall, 2.5 cm from the floor and 6.5 cm from the rear wall and 1.0 cm from the floor. A force of 20–30 g was required to operate the lever. A solenoid-operated dipper provided 0.1 ml of water reinforcement. The opening for the dipper was 4.5 cm wide × 4.5 cm high and was located 2.5 cm from the rear wall and 1.0 cm from the floor. Each chamber was enclosed in a modified Coleman camping cooler equipped with ventilating fan.

All the chamber devices were interfaced to a PDP/8e computer. The computer was programmed to control the reinforcement contingencies and store the data [18]. The data were recorded as interresponse times (IRTs). An IRT is defined as the interval between two successive microswitch closures. Each IRT was recorded with a resolution of 0.1 sec. The data were analyzed off-line by a PDP/8a computer.

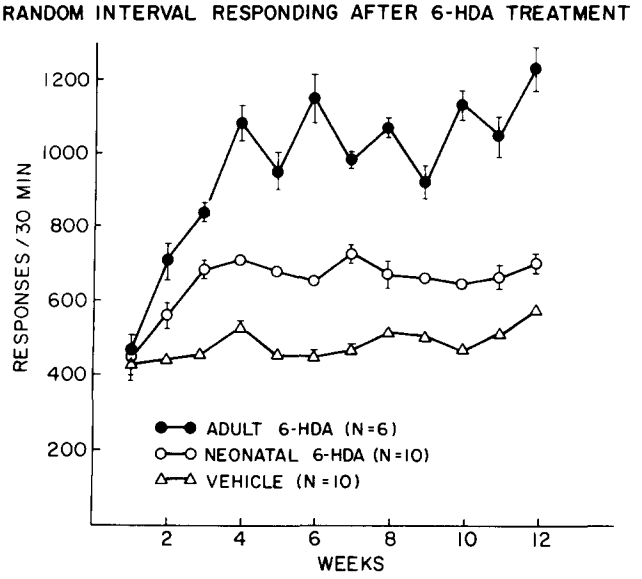

FIG. 1. Weekly mean responses rates (±SE) for rats treated with vehicle or 6-HDA as adults or neonates. Each point is the mean of daily group means, usually for 5 days. Four of the adult treatment rats died prior to initiation of training, one in infancy and three following 6-HDA treatment.

Procedure

At 73 days of age, training was begun. The rats were placed in the chambers and allowed to acquire the lever press response. The session duration was one hour and all responses were reinforced. Four days later, when all rats were emitting at least 100 responses/session, training was initiated on a random interval 90-sec schedule of reinforcement and the session length was shortened to 30 min. On this schedule, the interval between reinforced responses is determined randomly with an average interval of 90-sec by generating a 0.033 probability of reinforcement every 3 sec. Rats were run Monday–Friday at the same time each day (1–4 p.m.). Drug testing began after 3 months exposure to the schedule. Drugs were given Tuesday and Friday with Thursday serving as a control day for both drug days. Drug testing was done over a two-month period during which control rates were the same as at the end of predrug training (mean ± SE): V=622 ± 88; N=736 ± 122; A=1228 ± 175). All rats received the same drug treatment on a given day.

At the termination of the study, the rats were decapitated and the brains dissected into four parts: striatum, telencephalon without striatum, diencephalon, and brainstem and cerebellum removed. Dopamine and norepinephrine (NE) content were determined by the method of Anton and Sayre [1].

Drugs

The 6-HDA HBr was purchased from Regis Chemical Company (Chicago, IL). Apomorphine HCl was purchased from Merck and Company, Inc. (Rahway, NJ) and was injected subcutaneously immediately before the session. L-Dopa was purchased from Regis Chemical Company (Chicago, IL). L-Dopa was dissolved in hot 0.9% saline acidifed with 0.001 N HCl (final pH=5.0). L-Dopa was in-

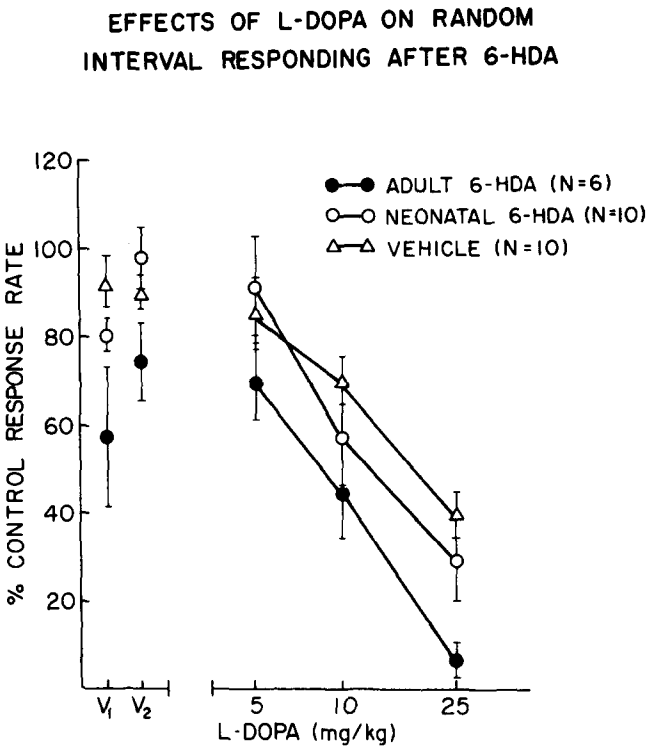

FIG. 2. Dose-response curves for the effects of L-Dopa on random interval responding after adult or neonatal treatment with 6-HDA. V_1 and V_2 refer to two treatments in which Ro 4-4602 was followed by L-Dopa vehicle only. Each point is the mean of one determination in each animal (±SEM). Absolute control response rates were as follows: Vehicle=607 (±83); Neonatal=785 (±131); Adult=1288 (±179).

jected IP 15 min before the daily session. A peripheral decarboxylase inhibitor, Ro 4-4602, was administered before the L-Dopa in order to ensure that the effects of L-Dopa were due to the central action of the drug. Ro 4-4602 was kindly supplied by Hoffmann-LaRoche (Nutley, NJ). It was dissolved in 0.9% saline and injected IP 75 min before the daily session.

RESULTS

Neonatally lesioned rats stabilized at response rates that were similar to those of vehicle-treated rats whereas rats lesioned as adults stabilized at much higher response rates (Fig. 1). Although the initial response rates were very similar in the three groups, response rates in the adult lesioned rats began to increase relative to the vehicle-treated rats by the second week and remained fairly stable after four weeks. There was more variability in the adult-lesioned group due to a smaller N and higher rates of responding; however, there was no trend upward or downward across time; the response rates tended to oscillate around 1100 responses/30 min. The vehicle-treated rats showed the lowest terminal rate (400–500 responses/30 min), adult-treated rats had rates which were 200–250% greater than the vehicles (900–1250 responses/30 min), and the neonatally-treated rats had rates which were slightly higher than vehicle (600–700 responses/30 min). A one-way analysis of variance was done on day 58, one week prior to the initiation of drug treatment. Analysis of variance

TABLE 2

EFFECTS OF L-DOPA AS PERCENT OF VEHICLE CONTROL

	L-DOPA (mg/kg) 5	10	12
Control	92.7 ± 6.8%	79.5 ± 8.6%	42.2 ± 4.9%
Neonate	103 ± 12.0%	64.6 ± 11.9%	30.4 ± 8.6%
Adult	90.3 ± 12.9%	67.5 ± 26.0%	10.7 ± 7.4%

revealed a significant treatment effect, ($F2,23=5.59$; $p<0.05$). Scheffes' tests indicated that this was due to a significant increase in the adult-treated group; the neonatal treatment group did not differ significantly from vehicle.

L-Dopa did not affect behavior differentially in the treatment groups (Fig. 2). The dose-response curve of the adult-treated group appears to be shifted to the left in that 10 mg/kg of L-Dopa decreased response rates as much as 25 mg/kg in the other two groups. In addition, the 25 mg/kg of L-Dopa decreased rates of adult-treated rats to 6% of the predrug rates. At this dose, the rates of vehicle and neonatally-treated animals were only decreased to 40% and 30% of control responding, respectively. However, there was a small rate decrease following administration of Ro 4-4602 plus vehicle which was most pronounced in the adult-treated group (% predrug rate: vehicle-treated 92.5 ± 5.9, 90.2 ± 5.9; neonatally-treated 80.6 ± 3.8, 98.4 ± 6.5; adult-treated 57.6 ± 16.0, 74.4 ± 9.1). Therefore, the increased sensitivity of the adult group to L-Dopa is questionable when L-Dopa effects are calculated as a percent of Ro 4-4602 plus vehicle. Table 2 shows this data. Apomorphine caused a dose-related decrease in response rate in all three groups but there were no differences among the groups (Fig. 3).

Group analysis of IRTs also indicated little difference among the three treatment groups in response to L-Dopa or apomorphine. IRTs were divided into three categories: short IRTs (<3 sec), pauses (>3 sec) not including post-reinforcement pauses (PRPs), and PRPs. Adult 6-HDA treatment shortened all three categories of IRTs. In all three categories, neonate and control rats were similar with the largest change in the adult group occurring in the PRP category ($F2,23=3.79$; $p<0.05$). IRT data for saline and no treatment days are shown in Table 3.

The neonatal treatment groups showed greater depletions of striatal dopamine and telencephalic norepinephrine than the adult treatment groups. There were equivalent depletions of norepinephrine in diencephalon (Table 4). Brainstem norepinephrine was increased in the neonatal treatment group while levels in the adult treated group were unchanged. The response rates of individual animals did not correlate with degree of CA changes in any region.

DISCUSSION

The results of this study show a large increase in random interval responding in rats treated with 6-HDA as adults but not in rats treated with 6-HDA as neonates. The lack of a rate increase in the neonatally-treated rats suggests that removal of catecholamine innervation early in development leads to different behavioral sequelae than removal of catecholamine innervation in the adult animal.

The large rate increase in the adult-treated rats confirms

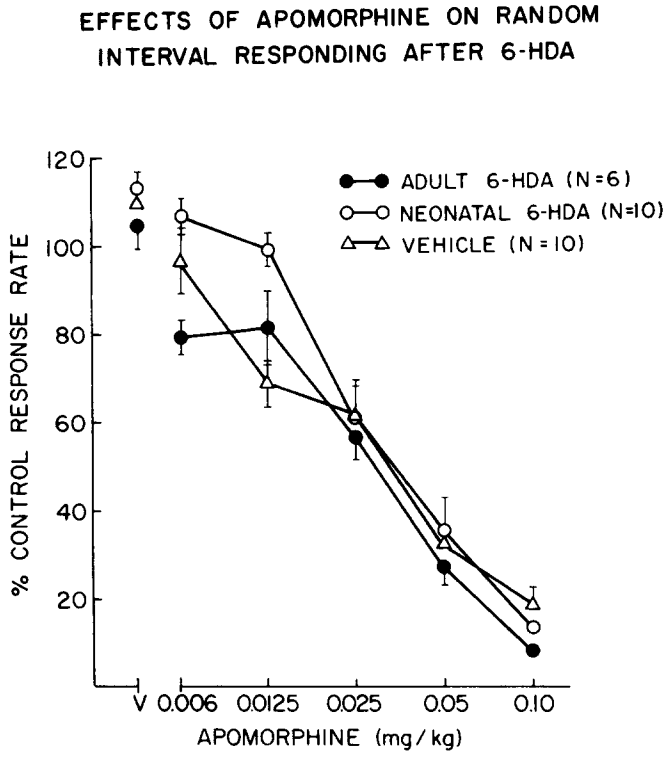

FIG. 3. Dose-response curves for the effects of apomorphine on random interval responding after adult or neonatal treatment with 6-HDA. Each point is the mean of one determination in each animal (± SE). Absolute control response rates were as follows: vehicle = 628 (± 94); Neonatal = 410 (± 124); Adult = 1286 (± 212).

the finding of Schoenfeld and Uretsky [15] using a similar schedule of reinforcement, the VI 1.5-min schedule. In both the variable interval and random interval schedules, response rate and reinforcement frequency are independent of each other, although extremely low rates of responding do decrease reinforcement frequency. The loose relationship between response rate and reinforcement frequency may contribute to the sensitivity of these schedules to the changes produced by 6-HDA treatment since more rigorous contingencies such as those of fixed interval, fixed ratio, and DRL schedules produce no change or variable results following 6-HDA treatment in adult rats [14, 16, 17] (Levine, McGuire, Heffner and Seiden, in press).

Peterson and Laverty [13] failed to find an increase in VI 60-sec responding following neonatal treatment with 6-HDA and attributed this result to the failure of the subcutaneous injection technique to deplete dopamine in their animals. It had previously been suggested by Schoenfeld and Zigmond [17] that the selective depletion of DA, but not NE, was important in producing the rate-increase in VI performance in the adult rat. Although the dosage used for neonates and adults were chosen to produce equivalent depletions, the neonatally-treated rats showed greater dopamine depletions than the adult-treated rats. Despite the larger dopamine depletions, they failed to show the behavioral effects seen following adult treatment.

Two possible explanations of the failure of neonatally-treated rats to show rate increases are: (1) the age at which the animal is treated with 6-HDA is an important determinant of the behavioral effects of destruction of brain transmitter systems. Recently, Erinoff *et al.* have reported that age of treatment is an important factor in determining the locomotor effects of treatment with 6-HDA [9]. The differences between the behavioral effects of neonatal and adult treatment in the present report demonstrate the importance of this experimental design to understanding the role of catecholamines in behavior; (2) the increase in brainstem NE in the neonatally-treated rats, absent in the adult-treated group, is responsible for the absence of a significant rate increase in the neonatally-treated group. This increase is thought to be due to an increased outgrowth of NE terminals and increased intraneuronal NE concentration [11].

TABLE 3

EFFECTS OF 6-HDA TREATMENT ON THREE CLASSES OF IRT

	Untreated	Saline
Control		
IRTs≤3 sec	0.95 ± 0.01	0.94 ± 0.01
IRTs>3 sec	7.24 ± 0.11	7.50 ± 0.11
PRPs	9.84 ± 0.29	9.71 ± 0.28
Neonate		
IRTs≤3 sec	0.86 ± 0.01	0.86 ± 0.01
IRTs>3 sec	7.07 ± 0.11	6.77 ± 0.09
PRPs	9.40 ± 0.32	9.13 ± 0.27
Adult		
IRTs≤3 sec	0.76 ± 0.01	0.77 ± 0.01
IRTs>3 sec	5.84 ± 0.11	6.12 ± 0.13
PRPs	5.51 ± 0.20	5.13 ± 0.18

The data from the administration of apomorphine and L-Dopa indicate that supersensitivity was not present in rats treated with 6-HDA either as adults or neonates. Supersensitivity to these drugs following neonatal 6-HDA treatment has been reported by Creese and Iverson with respect to locomotor activity and stereotypy [6]. However, Creese and Iverson obtained a very large depletion of DA (98%) and it may be that depletions of this magnitude are necessary for supersensitivity to occur. The types of behavior measured in the present study and that of Creese and Iverson are quite different and may result in differing drug effects. For example, both L-Dopa and apomorphine increase the frequency and/or intensity of locomotor activity and stereotypy whereas apomorphine has been shown to depress operant behavior [2,7]. Little work has been done with L-Dopa and appetitive operant tasks, but in the present study, it also depressed operant behavior.

There are additional factors which may account for the apparent lack of supersensitivity in the operant paradigm used in this study. One possibility is that the drugs may decrease operant behavior by interacting with receptors (possibly supersensitive) which mediate incompatible locomotor activity and/or stereotypy. Furthermore, recent evidence suggests that low doses of apomorphine and L-Dopa may act on the presynaptic rather than the postsynaptic DA receptor [4,8]. The doses of L-Dopa and apomorphine used in this study were low and activation of presynaptic receptors cannot be ruled out.

In summary, this study showed that neonatal 6-HDA treatment may yield different behavioral effects than adult treatment with 6-HDA. Although rats treated with 6-HDA as

TABLE 4

Treatment group	Catecholamine levels in 6-HDA-treated animals: Striatum DA	Telencephalon NE	Diencephalon NE	Brainstem NE
Vehicle	4.88 ± 0.60	0.43 ± 0.01	0.88 ± 0.03	0.51 ± 0.02
Neonatal	0.89 ± 0.12 (18%)*†	0.03 ± 0.003 (7%)†	0.47 ± 0.04 (53%)†	0.85 ± 0.05 (167%)†
Adult	2.62 ± 0.60 (54%)‡§	0.07 ± 0.01 (16%)†	0.38 ± 0.04 (43%)†	0.40 ± 0.17 (78%)§

*% vehicle-treated.
†Significantly different from vehicle, $p<0.001$.
‡Significantly different from vehicle, $p<0.01$.
§Significantly different from neonatal, $p<0.05$.

neonates had greater catecholamine depletions in the striatum and the remainder of telencephalon, they did not show the high rates on the random interval seen in the adult 6-HDA-treated rats. These results demonstrate that alterations in the development of CA systems produced by neonatal 6-HDA treatment produce rats with similar or more severe forebrain CA depletions than that of adult treatment but with behavior which is similar to vehicle-treated rats. The mechanism for these behavioral differences remains to be elucidated.

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