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Impaired Acquisition and Operant Responding After Neonatal Dopamine Depletion in Rats

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MOY, S. S. *Impaired acquisition and operant responding after neonatal dopamine depletion in rats.* PHARMACOL BIOCHEM BEHAV **52**(2) 433-441, 1995.—The effect of neonatal dopamine depletion in rats was examined using operant conditioning. Rat pups were given 6-hydroxydopamine (6-OHDA) or sham lesions at 3 days of age. When tested as adults, 6-OHDA treated subjects were impaired in the acquisition of lever pressing for reward and displayed stereotyped sniffing patterns not observed in the control subjects. In addition, significantly lower rates of responding were measured for the lesion group during continuous reinforcement (CRF), dilution of reinforcer efficacy, and with progressively increasing fixed ratio requirements. Alpha-methyl-*p*-tyrosine (AMPT), given before one CRF session, attenuated responding in over half the lesion animals and in none of the controls. Dopamine content in caudate nucleus was found to significantly correlate with number of trials to acquisition and rates of responding following AMPT in the lesion group, but not in the control group. Overall, the results of this experiment showed that neonatal dopamine depletion does not lead to severe motor impairment or the inability to learn, but does disrupt the normal patterns of behavior associated with operant conditioning.

Dopamine 6-Hydroxydopamine Alpha-Methyl-p-tyrosine Motor behavior Instrumental learning Neonatal rat

DEPLETION of dopamine in adult animals, via intraventricular 6-hydroxydopamine (6-OHDA) injection, leads to severe, acute aphagia (7,24) and movement deficits, including akinesia, excessive rigidity, and festination (24,35). In contrast, neonatal rats given 6-OHDA injections show few overt behavioral symptoms when tested at maturity, even when dopamine levels are 99% depleted (5). It, therefore, appears that the plasticity of the neonatal rat brain allows the development of compensatory mechanisms that attenuate, to some degree, the debilitating effects of dopamine depletion. At the same time, studies using avoidance procedures and maze learning have suggested that there may be limits to such compensation, because adult animals receiving lesions as neonates showed deficits in performing these tasks (1,18,22,34).

Few studies have examined operant conditioning in animals given neonatal 6-OHDA treatment. In operant conditioning, the likelihood that a selected behavior (such as a lever press) will occur is increased through the use of reinforcement. Levine et al. (13) used 6-OHDA to produce catecholamine depletions in neonatal animals and in adults. The subjects were then tested in adulthood using a random interval 90-s schedule of water reinforcement. The rates of responding found for subjects given 6-OHDA treatment as neonates were slightly higher than the rates shown by the control animals, while the animals given 6-OHDA treatment as adults responded at a much higher rate than did the control subjects. Impairment in acquisition of the operant response was not reported for any of the groups.

In contrast, research using an autoshaping procedure showed that early, selective dopamine lesions resulted in clear impairments in the acquisition of a lever-pressing response (11). Overall, almost one-fourth of the dopamine depleted subjects failed to acquire the operant response. However, the apparent absence of deficits in rates of responding for the neonatally treated animals that did acquire the performance corresponds to previous findings (13). Motoric dysfunction was not observed, and the 6-OHDA-treated animals that did learn to lever press were able to maintain rates comparable to control rates.

Other studies using operant conditioning procedures have focused on the effect of neonatal dopamine lesions on intracranial self-stimulation (25,27,30). Although neonatal 6-OHDA lesions do not block the rewarding impact of stimulation, lower rates of responding in the lesion groups were observed (25,27). In other work, the dopamine depleted subjects did not show any impairment in response rates or acquisition (30). Therefore, adaptations following neonatal dopamine lesions still allow sparing of intracranial stimulation

reward, but some reports suggest that compensation may not be complete when measured in terms of acquisition of an operant response and/or rates of operant responding.

Considerable evidence demonstrates that neuroleptics disrupt operant responding for a variety of reinforcers [e.g., (9,33)]. However, animals given neonatal dopamine lesions are subsensitive to many dopamine antagonists (4,6,8,32). For example, studies involving stimulation of the lateral hypothalamus found that neonatally treated rats are subsensitive to the rate-decreasing effects of the neuroleptic pimozide (25). These findings suggest that the sparing observed with intracranial stimulation, therefore, cannot be mediated by residual dopaminergic neurons, because disruption of operant responding did not occur in the lesion group at doses of pimozide that virtually halted lever pressing in control animals.

In contrast, other work has shown that animals sustaining lesions as neonates are supersensitive to alpha-methyl-*p*-tyrosine (AMPT), a catecholamine synthesis inhibitor (17,19). Both of these studies measured the effect of AMPT on food intake and found that doses of the drug that had little effect on the control animals almost completely blocked eating in the 6-OHDA treated group.

In the present experiment, subjects given neonatal dopamine lesions were trained using an operant conditioning paradigm. Behavioral observations were recorded during the initial shaping phase, because the deficits in acquisition observed in previous studies might have been secondary to differential profiles of behavior in the operant chamber. Rates of lever pressing were then measured under several different conditions: continuous reinforcement (CRF), dilution of reinforcer efficacy, and progressively increasing fixed-ratio requirements. These procedures were used to determine if the 6-OHDA-treated animals were different from the controls in their sensitivity to changes in reward value or motoric requirements for reinforcement.

One issue not addressed in previous studies involving neonatal lesions was whether other behavioral measures besides food intake could be disrupted by the administration of AMPT. Therefore, the effect of a single dose of AMPT on the rate of responding under a CRF schedule was determined.

METHOD

Subjects

Four Sprague-Dawley dams, with all-male litters of 11 pups each, were obtained from Charles River Laboratories, Inc. (Raleigh, NC). An unknown number of the pups were siblings. Pups were 2 days old at the time of arrival. For the first 26 days after arrival, each mother and her litter were housed in a clear plastic tub with water and food (Purina Rat Chow). Two litters received 6-OHDA treatment and two litters received sham injections (see below) at 3 days of age. After weaning at 28 days of age, the pups were individually housed in standard hanging stainless steel wire cages. The colony room was maintained at 75°F (±2°) with a 0700-1900 h light/dark cycle. Water was available ad lib. Rats were fed from 10 to 25 g of food daily (Purina rat chow), and weights at maturity were maintained between 350 g and 400 g.

Twenty animals served in the present experiment. These subjects had been previously tested in an activity pattern monitor (16). Subjects were approximately 4½ months old at the start of the experiment. One of the control animals broke off a top tooth and so was no longer able to eat the standard rat pellets; this animal was dropped from the study, resulting in nine control animals. Ten lesion animals (i.e., 6-OHDA-treated animals) served as subjects during the shaping phase of this experiment. Seven of the 10 lesion animals acquired the operant response and continued through the remaining experimental phases; the three animals that did not consistently lever press were discontinued from the study.

Neonatal Injections

On day 3 after birth, each rat pup was treated with a SC injection of 20 mg/kg desipramine. One hour later, neonates were anesthetized with ether and injected intracisternally (IC) with 100 μg (free base) 6-OHDA in 10 μl saline (0.5% ascorbic acid) (3). Control animals were given IC injections of the saline vehicle. Pups were then returned to their dams.

Determination of Dopamine Levels in Caudate Nucleus

Following completion of the experiments, animals were anesthetized by ether and then decapitated. The whole brain was removed, placed on an ice-cold glass plate, and the left hemisphere was removed and saved for a different study. The caudate nucleus was then dissected from the right hemisphere. This tissue was weighed and placed on dry ice subsequent to storage at −70°C. Levels of dopamine, serotonin, and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were quantified for the caudate nuclei of 8 control animals and 10 lesion subjects using the method of reversed-phase high performance liquid chromatography with electrochemical detection (12). The caudate nucleus of a ninth control animal was lost due to experimenter error.

Apparatus

The operant conditioning procedure utilized four rat chambers (30 × 30 × 24 cm, Coulburn instruments), equipped with two rodent levers (Model E21-03) positioned at one end of the box, 5 cm on either side of the midline and at a height of 4 cm from the floor rods. Between the two levers was positioned a liquid feeder magazine (Model E140-05 with a 0.2 cc cup). An incandescent lamp (#1829-voltage reduced), centered 5 cm over the liquid magazine, provided dim illumination within the chamber. When the reinforcer was available, this house light was extinguished and a light in the magazine was turned on for 5 s. The rat chambers were individually enclosed in sound-attenuating boxes. In addition, white noise provided sound masking. The reinforcer consisted of sweetened condensed milk (Borden Eagle Brand), diluted 2 : 1 with tap water. Operant responses were automatically controlled and recorded by computer with a precision of 0.01 s (31).

Procedure

Acquisition. Subjects were introduced to the reinforcer by the presentation of sweetened condensed milk in their home cages. All animals were observed drinking on the first day of presentation. Next, the animals were shaped in operant conditioning chambers to press a lever for the milk reinforcer. Each animal was given one 15-min session per day. A set of keyboard codes was devised so that the experimenter could obtain a continuous computer record of the animals' behavior during the shaping sessions. This record consisted of the temporal duration of 13 mutually exclusive responses: active locomotion, rearing, climbing sides of chamber, scratching, grooming, biting lever, biting magazine, sniffing/pawing lever or magazine, drinking from magazine (dipper up), head in

magazine (dipper down), sniffing at cage or in air, inactive (no overt movement, eyes open), and sleeping.

CRF schedule. Seven lesion animals and nine controls were able to meet a criterion level of acquisition, defined by 50 rewarded presses in one 15-min session. These 16 animals were then placed on a continuous reinforcement (CRF) schedule, in which every response on the lever was followed by a reward. One hundred reinforcers were available during each session, and the subjects were given approximately one-half hour to earn all of the rewards. Occasionally, longer sessions were conducted for the lesion animals. One dopamine-depleted subject did not show consistent lever pressing until the amount of time in which the reinforcer was available was changed from 5 s to 10 s following each press.

Reinforcer dilution. The next phase of the experiment assessed the effect on response rates of varying the reinforcer dilution. The original reinforcer, sweetened condensed milk diluted 2 : 1 with tap water, was given the value of 100%. Further dilution was achieved by adding varying amounts of tap water, resulting in six different dilution values: 50%, 25%, 12%, 6%, 3%, and 0% (plain tap water) of the original reinforcer. Each dilution value was tested in two to four sessions using a CRF schedule.

Fixed-ratio schedule. The next phase of the experiment involved performance under a fixed-ratio (FR) schedule. Responding was observed with a progressive increase in the response requirement to earn reinforcement across days (rather than within a session). Some FR requirements were repeated across two sessions to allow a more accurate assessment of average response rates. The sequence of FR requirements used was: FR 2 (every second response reinforced), FR 3, FR 4, FR 5, FR 7, FR 9, FR 11, FR 14, FR 17, FR 20, and FR 24.

*Effect of alpha-methyl-*p*-tyrosine.* For the final phase, animals were returned to a CRF schedule at 100% initial milk solution and given four sessions with 100 reinforcers available and then five sessions with 200 reinforcers available. After these nine sessions, response rates had returned to previous levels (data not shown). Next, two sessions were given with a pretreatment of sterile water, the vehicle for AMPT (Regis Chemical Company, Morton Grove, IL). The IP injections were given 1 h before the beginning of the session. The effects of AMPT, 40 mg/kg, were tested the day following the second vehicle session. The drug was suspended in sterile water and administered to all animals 1 h before placement in the operant chambers. Subjects responded on a CRF schedule, with 200 reinforcers available.

Data Analysis

Results from the postmortem brain assays and acquisition phase were analyzed using a two-tailed Student's *t*-test. Response rates were tested with a repeated measures ANOVA with one between-group factor (control or lesion) and one within-group factor (dependent on experimental phase). Linear regression analyses were used to test correlations between caudate dopamine levels and performance during operant conditioning. Significance level was set at $p < 0.05$.

RESULTS

Postmortem Analysis

As shown in Table 1, 6-OHDA treatment resulted in significantly low levels of dopamine (ng/mg protein) in the caudate nucleus of the lesion animals, $t(16) = 6.20$, $p < 0.0001$. DOPAC, a metabolite of dopamine, was also depleted in the lesion subjects in comparison to the controls, $t(16) = 7.25$, $p < 0.0001$. In line with previous findings (2,4,29), the administration of 6-OHDA led to higher levels of both serotonin, $t(16) = 2.23$, $p < 0.05$, and 5-HIAA, $t(16) = 6.19$, $p < 0.0001$, in the caudate nucleus. Levels of HVA were below the measurement threshold of the HPLC procedure; therefore, these data were not analyzed.

TABLE 1

EFFECT OF 6-OHDA TREATMENT ON DOPAMINE, SEROTONIN, AND THEIR METABOLITES (ng/mg PROTEIN) IN THE CAUDATE NUCLEUS

	Experimental Group	
Monoamine/Metabolite	Control	Lesion
Dopamine	219.5 ± 38.9	5.1 ± 1.8*
DOPAC	42.9 ± 2.6	4.4 ± 2.6*
Serotonin	9.3 ± 0.9	12.8 ± 1.2†
5-HIAA	11.4 ± 0.5	17.7 ± 0.8*

Values given are means ± SEM. For control animals, $n = 8$. For lesion animals, $n = 10$. *$p < 0.0001$, lesion vs. control. †$p < 0.05$, lesion vs. control.

Acquisition

The dopamine-depleted animals were impaired in their ability to acquire an operant response. Although all of the control animals learned to lever press, three animals from the lesion group did not learn the operant response within 12 sessions and were not given further training. Seven lesion animals learned to consistently press the lever in an average of eight sessions, while the nine control animals needed only an average of three sessions to acquire the operant response, $t(14) = -3.88$, $p < 0.0017$. Figure 1 shows the percentage of animals meeting criterion during the acquisition phase. Criterion was defined as one session with 50 rewarded responses within 15 min.

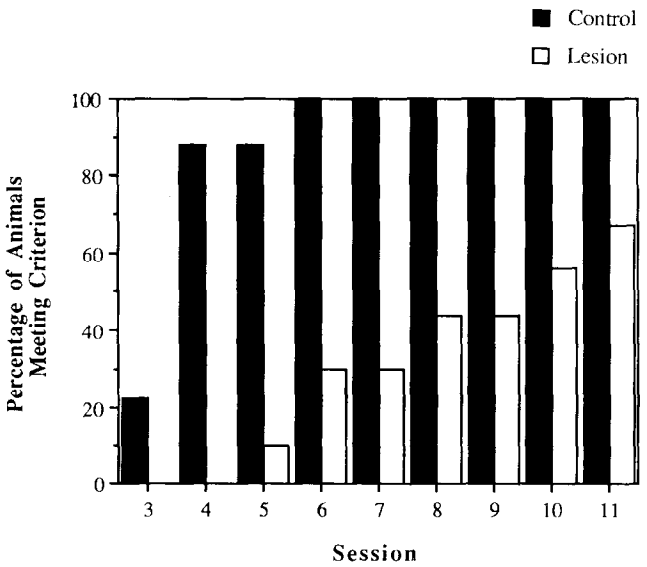

FIG. 1. Percentage of control ($n = 9$) and lesion ($n = 10$) animals meeting criterion during the acquisition phase. Criterion was defined as one session with 50 rewarded responses within 15 min.

mals from each group that met criterion during a given shaping session, starting on day 3.

The results of the behavioral observations taken during the shaping sessions are presented in Fig. 2. Records were taken from 9 control animals and 10 lesion animals. The data from this phase included the temporal duration of each response; overall, the recorded behavioral observations encompassed 91.4% of the total time for the control group and 91.6% of the total time for the lesion group during the first 3 days of shaping.

The upper panel of Fig. 2 shows the percentage of total time the animals spent on two activities: sniffing or pawing at the magazine or right lever, and drinking from the magazine. Together, these activities accounted for approximately 60% of the temporal observations. During the first 3 days of shaping, both the lesion group and the control group demonstrated virtually identical rates of sniffing around the magazine and right lever, suggesting that the dopamine-depleted subjects associated these areas of the chamber with reinforcement in a manner comparable to normal animals. At the same time, the lesion animals were less likely to place their heads in the magazine opening during the time when milk was available and, therefore, were less likely to drink the reinforcer, $t(17) = 3.13, p < 0.007$.

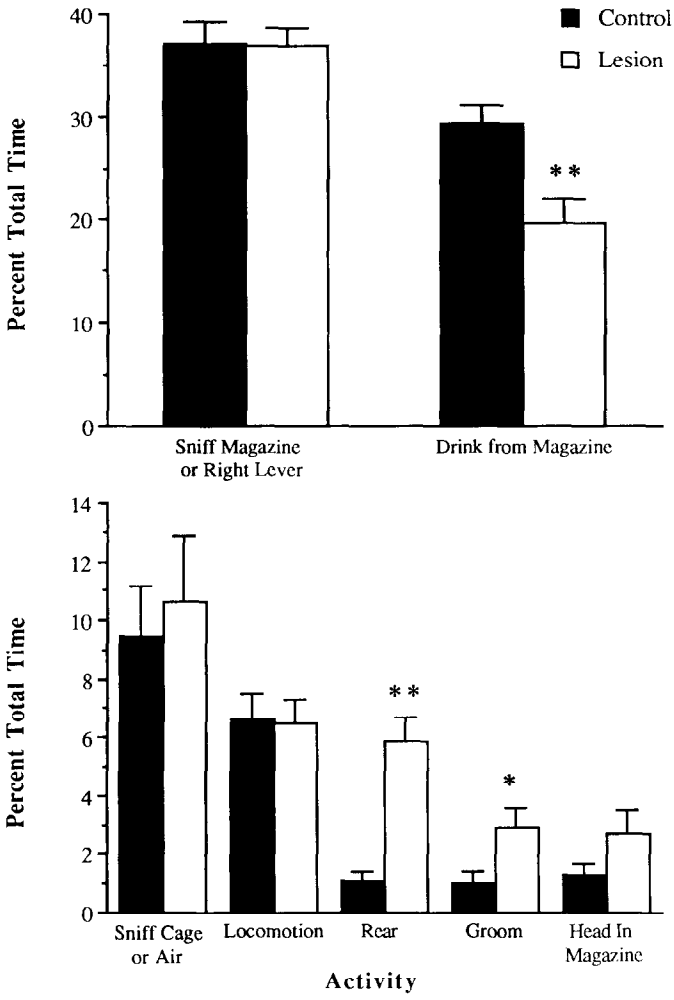

FIG. 2. Mean (±SEM) percentage of total time for high-rate activities (upper panel) and low-rate activities (lower panel) for the first three acquisition sessions. Records were taken from 9 control subjects and 10 lesion animals. *$p < 0.05$, **$p < 0.01$; lesion vs. control.

The lower panel of Fig. 2 depicts the percentage of total time associated with several activities performed at relatively low rates. The lesion group was comparable to the control group in the percentage of time spent actively locomoting, sniffing in the air or at other areas of the operant chamber apart from the lever, and in percentage of time spent with head in magazine (dipper not present). The lesion animals did spend more time rearing, $t(17) = -5.57, p < 0.0001$, and grooming, $t(17) = -2.47, p < 0.024$, than did the controls. However, these behaviors occurred at quite low rates in comparison to the substantial portion of time invested in drinking or sniffing at the magazine and right lever. A similar behavioral profile emerged from data derived for the lesion group across all 11 days of shaping (data not shown).

Additional observations were recorded in session notes that further characterize the significantly lower levels of drinking seen in the 6-OHDA-treated group. Five of the 10 lesion animals consistently showed a distinctive type of behavior that involved a stereotyped sniffing response when the magazine apparatus was activated. Although the normal animals immediately approached the magazine when reinforcement was available, the lesion subjects consistently engaged in a particular pattern, different for each individual, of sniffing around the lever, on the floor, or above and below the magazine. These responses not only introduced a delay between presentation of the reinforcer and consumption of the milk solution, but persisted despite the fact that the reinforcer was available for only 5 s. The word ritualistic seemed to best describe these patterns. One animal, which learned to press the lever consistently for milk, did not maintain responding on a CRF schedule because the persistent insertion of these conditioned stereotypes prevented the rat from getting to the magazine before the dipper was removed. Steady rates of responding were established for this subject by doubling (from 5 to 10 s) the amount of time the reinforcer was available after each lever press.

Another behavior that distinguished the lesion animals from the normal controls was a sudden, abrupt jumping response. This jumping was primarily observed at the beginning of a shaping session, immediately after the animal was placed in the chamber. Across the shaping sessions, jumping behavior was recorded for 6 of the 10 lesion subjects and in only 1 out of 9 control subjects. Overall, the dopamine depleted group demonstrated a total of 101 jumps, while only 2 jumps (emitted one after the other) were observed for the single control animal.

Responding on a CRF Schedule

Figure 3 presents the results from 17 sessions of responding on a CRF schedule after the shaping phase was completed. The 6-OHDA-treated group had significantly lower rates of responding in comparison to the controls, $F(1, 14) = 21.32, p < 0.0004$. Although there was a significant main effect for sessions, $F(16, 224) = 2.29, p < 0.004$, a significant interaction between group and sessions was not found, $F(16, 224) = 0.996, p > 0.45$, indicating that the lesion group and the control group changed comparably across time.

Dilution of Reinforcer

Figure 4 presents the results from the reinforcer dilution phase of the experiment. Overall, the lesion animals continued

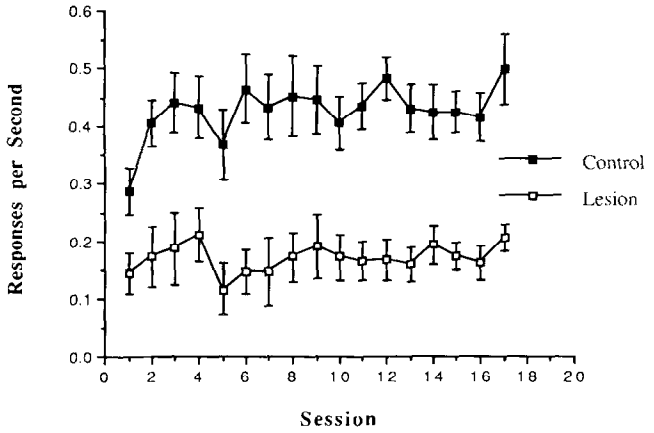

FIG. 3. Mean (±SEM) response rates with a CRF schedule for control (n = 9) and lesion (n = 7) subjects. Sessions were limited to 30 min, with 100 reinforcers available per session.

the trend evident under the CRF schedule and responded at significantly lower rates than the controls (significant main effect of group, $F(1, 14) = 22.38$, $p < 0.0003$). The rates of responding tended to decrease as the solution became more dilute [significant main effect of dilution, $F(6, 84) = 41.27$, $p < 0.0001$]. A significant group by dilution interaction was found, $F(6, 84) = 6.38$, $p < 0.0001$, indicating that the changes in responding observed in the two groups occurred to different degrees, although a similar pattern emerged in each group. The 6-OHDA-treated animals did decrease responding for the diluted reinforcers, but their low rates of responding were more resistant to the rate-decreasing effects of dilution of reward in comparison to the controls.

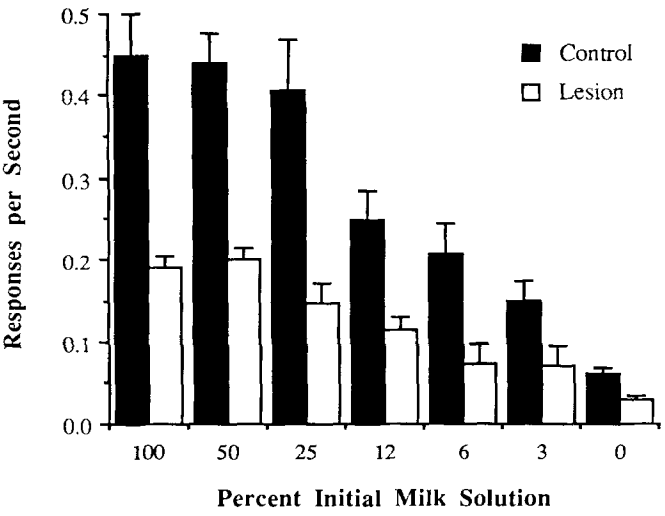

FIG. 4. Mean (±SEM) response rates with reinforcer dilution for control (n = 9) and lesion (n = 7) subjects. Sessions were limited to 30 min, with 100 reinforcers available per session under a CRF schedule. Dilution values were derived by assigning the original reinforcing solution the value of 100%.

Progressive Increase in FR Requirement

Figure 5 presents the results from the progressive increase in FR requirement. As depicted in the upper panel, the lesion animals responded at a significantly lower rate than the controls, $F(1, 14) = 15.9$, $p < 0.0013$. The increase in the number of responses necessary for reinforcement had a significant, although variable, effect on rate of lever pressing, as seen in the significant main effect of FR requirement, $F(11, 154) = 5.5$, $p < 0.0001$. A significant interaction was found between group and FR requirement, $F(11, 154) = 4.1$, $p < 0.0001$, indicating that a different pattern of change in rates emerged in the lesion group in comparison to the controls. It is notable that the rates seen for the lesion subjects increased to levels comparable to the baseline CRF performance for the control subjects. Thus, the lesion animals are shown to be capable of maintaining higher response rates than was apparent in the previous phases.

As the FR requirement increased beyond seven responses

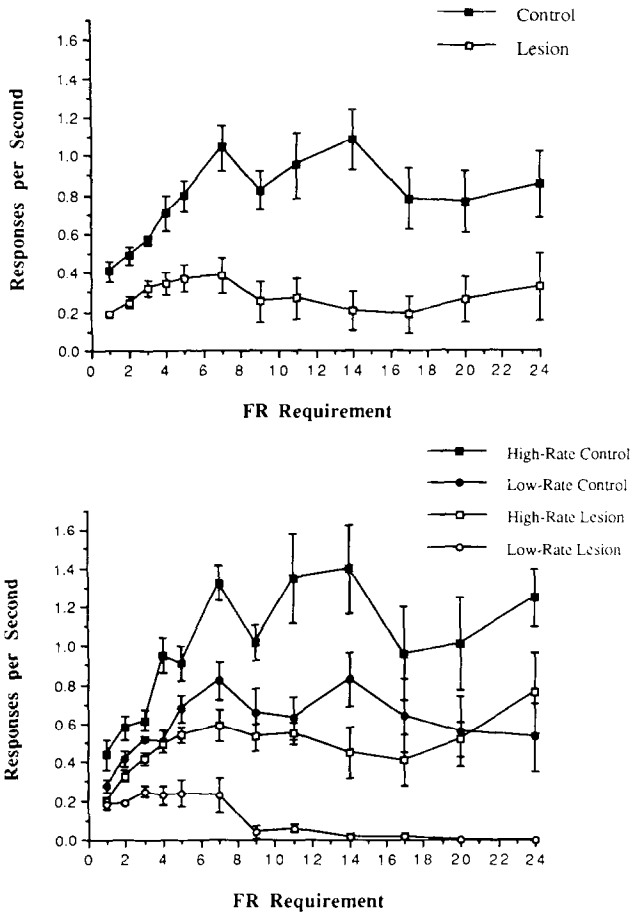

FIG. 5. Mean (±SEM) response rates obtained with a progressive FR schedule. FR requirements were increased between sessions and not within sessions. Sessions were limited to 30 min, with 100 reinforcers available per session. Upper panel shows overall group results for control (n = 9) and lesion (n = 7) subjects. Lower panel depicts mean response rates for high-rate control (n = 4), low-rate control (n = 5), high-rate lesion (n = 3), and low-rate lesion (n = 4) subjects.

per reinforcer, the rates of responding in the lesion group started to decline. At FR 9, two of the lesion animals stopped responding. By FR 17, four of the seven lesion animals had virtually ceased responding. The separate results for these four low-rate lesion animals and the three remaining high-rate lesion animals are shown in the lower panel of Fig. 5. Similarly, the control group has been divided into five low-rate subjects and four high-rate subjects, based on performance under the progressive FR schedule. By the last FR session, the three high-rate lesion animals had an average response rate of 0.76 responses per second (SEM = 0.21), a value that was actually higher than that observed in the control low-rate group: 0.53 responses per second (SEM = 0.18). In all, then, over half of the dopamine depleted subjects were unable to maintain responding beyond the initial FR requirements, while the three remaining members of the lesion group showed normal performance.

*Effect of Alpha-Methyl-*p*-Tyrosine*

The final phase of the present experiment determined the effect of a single dose of AMPT on response rates. The upper panel of Fig. 6 presents the overall rates of responding for this phase. An ANOVA performed on these data found a significant main effect of group, $F(1, 14) = 11.51$, $p < 0.005$, but the effect of experimental condition was not significant, $F(1, 14) = 3.22$, $p < 0.095$, although the 6-OHDA-treated group did demonstrate a notable decrease on the day of drug administration. This rate-decreasing effect was most evident for four subjects, the same four subjects that had been most sensitive to the changes in FR requirement and reinforcer dilution.

The lower panel of Fig. 6 shows the separate averages for the same low-rate and high-rate groups described during the progressive FR schedule phase. Administration of AMPT had a profound effect on the lesion animals that had previously demonstrated low rates of responding at the higher FR requirements. Although the responding of the high-rate control animals and high-rate lesion subjects was not affected, the low-rate control animals did show some reduced responding following AMPT. However, only the low-rate lesion animals exhibited an almost complete disruption of lever-pressing activity.

Correlation Between Caudate Dopamine Levels and Behavior

Table 2 presents the Pearson product-moment correlation coefficients between levels of dopamine in the caudate nucleus and several behavioral measures for the control and lesion groups. Overall, the r values for the control group were quite low, indicating that behavioral performance was a poor predictor of caudate dopamine levels in these subjects. On the other hand, simple regression analyses demonstrated that caudate dopamine in the lesion group was significantly correlated with both number of trials to acquisition, $F(1, 8) = 5.95$, $p < 0.05$, and responding after the AMPT pretreatment, $F(1, 5) = 21.32$, $p < 0.006$. The animals with the most extensive lesions tended to require more trials to learn the operant task and showed greater disruption of lever pressing following AMPT. In addition, the correlation between caudate dopamine levels and responding by the lesion animals after vehicle injections approached significance, $F(1, 5) = 6.01$, $p < 0.058$. No significant correlations were found between levels of serotonin or the metabolite 5-HIAA and any of the behavioral measures tested.

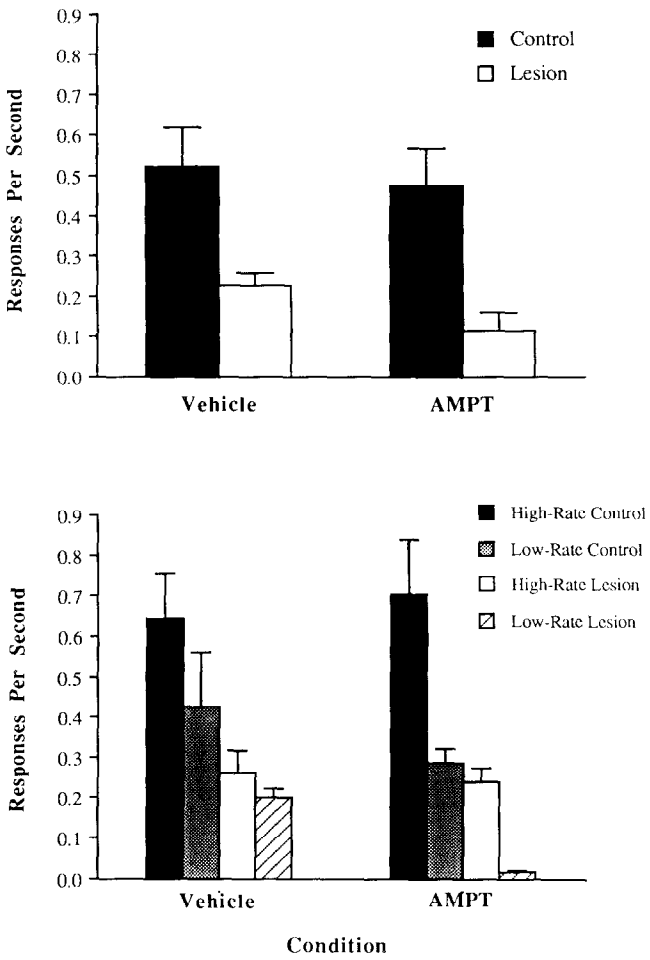

FIG. 6. Mean (±SEM) response rates following the administration of either vehicle (sterile water) or 40 mg/kg AMPT 1 h before testing. Sessions were limited to 30 min, with 200 reinforcers available per session under a CRF schedule. Upper panel shows overall group results for control ($n = 9$) and lesion ($n = 7$) subjects. Lower panel depicts mean response rates for high-rate control ($n = 4$), low-rate control ($n = 5$), high-rate lesion ($n = 3$), and low-rate lesion ($n = 4$) subjects.

DISCUSSION

The present study demonstrated significant and consistent differences between lesion animals and controls under several different experimental conditions. The dopamine depleted subjects showed impairments in learning an operant response and responding under an FR schedule. At the same time, the performance of the lesion subjects was not more severely disrupted by reductions in reinforcer efficacy in comparison to the controls. A single dose of AMPT was found to suppress responding only for the four lesion subjects that had previously shown very low rates of responding under a progressive FR schedule.

The analysis of behavioral records taken during the acquisition trials suggests that the lesion and control groups differed in their initial responses to the operant chamber and to the shaping procedure. When these same subjects were tested in an activity pattern monitor (16), the lesion group showed

TABLE 2

CORRELATIONS BETWEEN DOPAMINE CONTENT IN CAUDATE NUCLEUS AND BEHAVIORAL MEASURES FOR CONTROL AND 6-OHDA TREATED RATS

	Control		Lesion	
Operant Schedule	Mean ± SEM	r	Mean ± SEM	r
Trials to acquisition	3.0 ± 0.29	−0.07	8.2 ± 0.95	−0.65*
CRF, 100 rf	0.50 ± 0.06	0.37	0.21 ± 0.02	0.52
FR7	1.04 ± 0.11	−0.01	0.39 ± 0.09	0.36
VEH/CRF, 200 rf	0.52 ± 0.08	−0.05	0.23 ± 0.03	0.74
AMPT/CRF, 200 rf	0.47 ± 0.09	0.08	0.11 ± 0.05	0.90†

Behavioral measures are number of trials or responses per second. For the control group, $n = 8$. For the lesion group, $n = 10$ for trials to acquisition and $n = 7$ for other behavioral measures.

*$p < 0.05$.

†$p < 0.01$.

aberrant patterns of behavior and decreased locomotion during habituation. Stoof et al. (26) have found differential behavioral profiles and enhanced locomotion in lesion animals placed in a novel environment. The present experiment did not reveal any significant changes in locomotor activity. Rather than a difference in overall activity levels, the 6-OHDA-treated animals showed a different sort of activity.

Drinking from the magazine was found to be significantly reduced in the lesion animals. This decrease was attributed to two factors: first, the lesion animals sometimes failed to approach and consume all of the given reinforcers; activation of the dipper mechanism did not interrupt on-going behavior in the lesion subjects as consistently as it did for the normal animals. Second, one-half of the dopamine-depleted subjects developed ritualistic sniffing responses that were performed after the dipper mechanism was activated. These conditioned stereotypes continued to be produced even when they resulted in the loss of reinforcement.

Additional behavioral differences were found to exist between the experimental group and the control group during acquisition. Although all of the normal animals readily learned to press a lever for reward, almost one-third of the lesion subjects failed to acquire this operant response. Those dopamine-depleted animals that did learn to lever press required, on the average, more than twice as many shaping sessions as the controls. Similar impairments in acquisition have been previously reported (11), although other studies using lever press as an operant response have reported no differences in acquisition for animals given neonatal 6-OHDA treatment (27,30). Dopamine blockade via the administration of neuroleptics has also been demonstrated to retard acquisition (28,33). In the present study, a significant correlation was found between number of trials to acquisition and dopamine content in the caudate nucleus for the lesion animals, but not for the control subjects.

One final behavioral difference noted during shaping was a distinctive jumping response observed primarily in the 6-OHDA-treated group (only two jumps were ever recorded in the control group). This jumping behavior appeared to be an aberrant response to the stress of handling, because most jumps occurred soon after animals were placed in the operant chamber. The failure to habituate to handling has been previously reported for adult animals given 6-OHDA as neonates (21,34).

Despite these differences, behavioral records showed that the lesion animals were, in many ways, similar to the controls. The rates for responses such as locomotion and sniffing were comparable for the two groups. The measures recorded for the lesion subjects clearly suggested that all of the animals were motivated to obtain the reinforcer of sweetened milk and that they learned to associate nosing or pawing the area of the lever with the reward. Therefore, the deficit in learning to lever press did not appear to arise from a lack of motivation or an inability to associate the reinforcer with the sound and light cues, but rather an inability of the reinforcer to modify the initial conditioned responses.

The lesion animals also demonstrated significantly lower levels of responding under a CRF schedule. Some previous research has shown reduced responding by lesion animals when compared to controls [(25,27); see also (23)], although other studies reported normal rates of responding after neonatal 6-OHDA treatment (11,30). It is possible that the reduced rates observed for the lesion subjects of the present study were due to a motoric impairment stemming from the neonatal exposure to 6-OHDA. Yet, the comparison of response rates taken during different experimental phases shows that the lesion subjects, as a group, were able to increase their rate of lever pressing in response to changes in the FR requirement. For example, in the progressive FR phase, the lesion animals had an overall average response rate of 0.387 responses per second (SEM = 0.093) at FR 7, which was actually higher than the performance of control animals at FR 1: 0.353 responses per second (SEM = 0.048). A similar best score analysis has previously been used to dissociate changes in reward processes from motor impairment in the neuroleptic-induced reduction of operant responding (14). In the present study, the simple inability to respond at high rates, thus, does not explain the consistently low levels of responding, because the animals were capable of producing higher rates.

One question asked by the present experiment was whether the performance of the rats given neonatal 6-OHDA treatment would be more easily disrupted than that of sham lesion controls. The anhedonia hypothesis (33) predicts that dopamine-depleted animals would be especially sensitive to reductions in reinforcer efficacy. In particular, dopamine blockade by neuroleptics has been found to decrease resistance to extinction, so that treated animals cease responding more quickly than control subjects under conditions of nonreward [e.g., (10,33)]. The results from the reinforcer dilution procedure demonstrated that the performance of the lesion group was actually less disrupted by changes in dilution than the controls. At the same time, the lesion group did show some rate reduction in response to the lower dilution values. Therefore, the dopamine depleted animals did respond to changes in the reinforcer efficacy, but did not show the augmented disruption of operant responding predicted by the anhedonia hypothesis (33).

Another prediction based on the anhedonia hypothesis is that disruption of dopaminergic activity would suppress responding maintained with schedules of low reinforcement frequency more than responding maintained with schedules of high reinforcement frequency (15). In line with this prediction, the response rates of four dopamine depleted rats were greatly disrupted by the increasing FR requirements. However, three other lesion subjects did maintain rates of responding within control levels. Several researchers have demonstrated that early 6-OHDA treatment can lead to some deficits in motor performance involving the forelimbs (21,25,34). In the present study, motor impairment could explain this reduced capacity

for high-rate operant responding in the lesion animals; however, it would not provide an account for the cessation of responding at a low FR requirement.

The final phase of the study evaluated the effect of a single dose of AMPT on operant responding. Only the four lesion animals that had previously shown a deficit in responding during the FR schedule were profoundly disrupted by the catecholamine synthesis inhibitor. This supersensitivity to the rate-suppressing effects of AMPT found for the four lesion animals replicated research involving the reduction of food intake for neonatally treated rats (17,19), and provided evidence that residual dopamine activity retained an important role in operant conditioning. It is notable that caudate levels of dopamine were significantly correlated with responding after AMPT only in the lesion subjects. Similarly, Wishaw et al. (34) found significant correlations between levels of dopamine and several behavioral measures, including performance of a water maze task, for animals given neonatal 6-OHDA treatment.

Salamone (20), in a discussion of the role of striatal dopamine, hypothesizes that the processes governing conditioned reinforcement are mediated by dopamine. At the same time, he points out that instrumental learning is a complex cognitive process that subsumes motivation, reward, and motor ability, so impairments in learning following interference with dopaminergic activity cannot be simply attributed either to anhedonia or to motor dysfunction. Overall, the present experiment demonstrates that reduced motor capacity cannot explain all of the impairments following neonatal dopamine depletion. A number of different interpretations may be offered for the results, including changes in motivation and cognitive functioning in the 6-OHDA-treated animals. However, the complexity of operant conditioning prevents the attribution of the observed deficits to impairment in any one process.

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REFERENCES

1. Archer, T.; Danysz, W.; Fredriksson, A.; Jonsson, G.; Luthman, J.; Sundstrom, E.; Teiling, A. Neonatal 6-hydroxydopamine-induced dopamine depletions: Motor activity and performance in maze learning. Pharmacol. Biochem. Behav. 31:357–364; 1988.
2. Breese, G. R.; Baumeister, A. A.; McCown, T. J.; Emerick, S. G.; Frye, G. D.; Crotty, K.; Mueller, R. A. Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: Relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. J. Pharmacol. Exp. Ther. 231(2):343–354; 1984.
3. Breese, G. R.; Traylor, T. D. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. Br. J. Pharmacol. 44(2):210–222; 1972.
4. Bruno, J. P.; Jackson, D.; Zigmond, M. J.; Stricker, E. M. Effect of dopamine-depleting brain lesions in rat pups: Role of striatal serotonergic neurons in behavior. Behav. Neurosci. 101(6):806–811; 1987.
5. Bruno, J. P.; Snyder, A. M.; Stricker, E. M. Effect of dopamine-depleting brain lesions on suckling and weaning in rats. Behav. Neurosci. 98(1):156–161; 1984.
6. Bruno, J. P.; Stricker, E. M.; Zigmond, M. J. Rats given dopamine-depleting brain lesions as neonates are subsensitive to dopaminergic antagonists as adults. Behav. Neurosci. 99(4):771–775; 1985.
7. Cooper, B. R.; Howard, J. L.; Grant, L. D.; Smith, R. D.; Breese, G. R. Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. Pharmacol. Biochem. Behav. 2:639–649; 1974.
8. Duncan, G. E.; Criswell, H. E.; McCown, T. J.; Paul, I. A.; Mueller, R. A.; Breese, G. R. Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. J. Pharmacol. Exp. Ther. 243(3):1027–1034; 1987.
9. Ettenberg, A. Dopamine, neuroleptics and reinforced behavior. Neurosci. Biobehav. Rev. 13:105–111; 1989.
10. Feldon, J.; Katz, Y.; Weiner, I. The effects of haloperidol on the partial reinforcement extinction effect (PREE): Implications for neuroleptic drug action on reinforcement and nonreinforcement. Psychopharmacology (Berlin) 95:528–533; 1988.
11. Heffner, T. G.; Seiden, L. S. Impaired acquisition of an operant response in young rats depleted of brain dopamine in neonatal life. Psychopharmacology (Berlin) 79:115–119; 1983.
12. Kilts, C. D.; Breese, G. R.; Mailman, R. B. Simultaneous quantification of dopamine 5-hydroxytryptamine and four metabolically related compounds by means of reversed-phase high-performance liquid chromatography with electrochemical detection. J. Chromatogr. 225:347–357; 1981.
13. Levine, T. E.; Erinoff, L.; Dregits, D. P.; Seiden, L. S. Effects of neonatal and adult 6-hydroxydopamine treatment on random-interval behavior. Pharmacol. Biochem. Behav. 12:281–285; 1980.
14. McDougall, S. A.; Nonneman, A. J.; Crawford, C. A. Effects of SCH 23390 and sulpiride on the reinforced responding of the young rat. Behav. Neurosci. 105(5):744–754; 1991.
15. Morley, M. J.; Bradshaw, C. M.; Szabadi, E. The effect of pimozide on variable-interval performance: A test of the "anhedonia" hypothesis of the mode of action of neuroleptics. Psychopharmacology (Berlin) 84:531–536; 1984.
16. Moy, S. S.; Eckerman, D. A.; Breese, G. Altered activity patterns following neonatal 6-hydroxydopamine lesions to dopaminergic neurons: Effect of SKF-38393. Brain Res. 645:49–60; 1994.
17. Potter, B. M.; Bruno, J. P. Food intake of rats depleted of dopamine as neonates is impaired by inhibition of catecholamine biosynthesis. Neurosci. Lett. 107:295–300; 1989.
18. Raskin, L. A.; Shaywitz, B. A.; Anderson, G. M.; Cohen, D. J.; Teicher, M. H.; Linakis, J. Differential effects of selective dopamine, norepinephrine or catecholamine depletion on activity and learning in the developing rat. Pharmacol. Biochem. Behav. 19:743–749; 1983.
19. Rogers, D. C.; Dunnett, S. B. Hypersensitivity to α-methyl-*p*-tyrosine suggests that behavioural recovery of rats receiving neonatal 6-OHDA lesions is mediated by residual catecholamine neurones. Neurosci. Lett. 102:108–113; 1989.
20. Salamone, J. D. Complex motor and sensorimotor functions of striatal and accumbens dopamine: Involvement in instrumental behavior processes. Psychopharmacology (Berlin) 107:160–174; 1992.
21. Schallert, T.; Petrie, B. F.; Whishaw, I. Q. Neonatal dopamine depletion: Spared and unspared sensorimotor and attentional disorders and effects of further depletion in adulthood. Psychobiology 17:386–396; 1989.
22. Shaywitz, B. A.; Pearson, D. A. Effects of phenobarbital on activity and learning in 6-hydroxydopamine treated rat pups. Pharmacol. Biochem. Behav. 9:173–179; 1978.
23. Sidhu, K. S.; Stellar, J. R.; Garity, D.; Bruno, J. P. 6-OHDA-treated weanling rats show normal neuroleptic sensitivity as adults on LHSS. Pharmacol. Biochem. Behav. 44:901–905; 1993.
24. Snyder, A. M.; Stricker, E. M.; Zigmond, M. J. Stress-induced neurological impairments in an animal model of parkinsonism. Ann. Neurol. 18(5):544–551; 1985.

25. Stellar, J. R.; Waraczynski, M.; Bruno, J. P. Neonatal dopamine depletions spare lateral hypothalamic stimulation reward in adult rats. Pharmacol. Biochem. Behav. 30:365–370; 1988.
26. Stoof, J. C.; Dijkstra, H.; Hillegers, J. P. M. Changes in the behavioral response to a novel environment following lesioning of the central dopaminergic system in rat pups. Psychopharmacology (Berlin) 57:163–166; 1978.
27. Takeichi, T.; Kurumiya, S.; Umemoto, M.; Olds, M. E. Roles of catecholamine terminals and intrinsic neurons of the ventral tegmentum in self-stimulation investigated in neonatally dopamine-depleted rats. Pharmacol. Biochem. Behav. 24:1101–1109; 1986.
28. Tombaugh, T. N.; Tombaugh, J.; Anisman, H. Effects of dopamine receptor blockade on alimentary behaviors: Home cage food consumption, magazine training, operant acquisition, and performance. Psychopharmacology (Berlin) 66:219–225; 1979.
29. Towle, A. C.; Criswell, H. E.; Maynard, E. H.; Lauder, J. M.; Joh, T. H.; Mueller, R. A.; Breese, G. R. Serotonergic innervation of the rat caudate following a neonatal 6-hydroxydopamine lesion: An anatomical, biochemical and pharmacological study. Pharmacol. Biochem. Behav. 34:367–374; 1989.
30. Umemoto, M.; Takeichi, T.; Kurumiya, S.; Olds, M. E. Selective neonatal depletion of dopamine has no effect on medial prefrontal cortex self-stimulation in the rat. Neurosci. Res. 1:295–307; 1984.
31. Walter, D. E.; Palya, W. L. An inexpensive experiment controller for stand-alone applications or distributed processing networks. Behav. Res. Methods Instrum. Comput. 16(2):125–134; 1984.
32. Weihmuller, F. B.; Bruno, J. P. Drinking behavior and motor function in rat pups depleted of brain dopamine during development. Dev. Psychobiol. 22(2):101–113; 1989.
33. Wise, R. A. Neuroleptics and operant behavior: The anhedonia hypothesis. Behav. Brain Sci. 5:39–87; 1982.
34. Wishaw, I. Q.; Funk, D. R.; Hawryluk, S. J.; Karbashewski, E. D. Absence of sparing of spatial navigation, skilled forelimb and tongue use and limb posture in the rat after neonatal dopamine depletion. Physiol. Behav. 40:247–253; 1987.
35. Zigmond, M. J.; Stricker, E. M. Parkinson's disease: Studies with an animal model. Life Sci. 35(1):5–18; 1984.