*Pharmacology Biochemistry & Behavior*, Vol. 36, pp. 321–325. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

# Effect of Forebrain Dopamine Depletion on Novelty-Induced Place Preference Behavior in Rats

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Received 17 October 1989

PIERCE, R. C., C. A. CRAWFORD, A. J. NONNEMAN, B. A. MATTINGLY AND M. T. BARDO. *Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats.* PHARMACOL BIOCHEM BEHAV **36**(2) 321–325, 1990.— Novelty-induced place preference behavior of rats was studied in two experiments. In the first experiment, separate groups of animals were habituated to a distinct environment 30 min daily for either zero, one, two, four or eight days. On the day following the last habituation day, animals were allowed 15 min free access to both the habituated (familiar) and a distinct novel environment. The results revealed a significant novelty preference in the two-, four- and eight-day habituation groups. In these same animals, the rate of horizontal and vertical activity was lower in the novel environment relative to the familiar environment. The influence of forebrain dopamine (DA) projections on novelty preference behavior was studied in the second experiment. Animals were given an injection of 6-hydroxydopamine (6-OHDA) into the nucleus accumbens or were given sham surgery, and then they were given four habituation days to one environment. Novelty-induced place preference was blocked in the lesioned animals, as the amount of time spent in the novel and familiar environments was not significantly different. Lesioned animals also failed to show a difference in locomotor activity between the novel and familiar environments. Subsequent assay data revealed that the 6-OHDA lesion reduced DA levels in the nucleus accumbens, anterior striatum and olfactory tubercles by over 65% as compared to sham surgery. These results suggest that novelty preference behavior may be mediated by a central DA pathway similar to that involved in other types of reinforcing stimuli, such as food, water and drugs of abuse.

Place preference Novelty Dopamine 6-Hydroxydopamine Reinforcement

WHEN allowed free choice access to both a novel and familiar stimulus environment simultaneously, rats and mice prefer the novel environment. This place preference is evident as an increase in duration spent in the novel environment relative to the familiar environment (3, 8, 12). During the free choice test, animals also show a lower rate of locomotor activity in the novel environment relative to the familiar environment (1,9). These behavioral effects are blocked by systemic administration of dopamine (DA) antagonists (1,10), suggesting that exposure to novel stimuli activates a DA system within the brain similar to some drugs of abuse (2,17).

Lesion studies also support the hypothesis that DA systems mediate some behavioral responses to novelty. Fink and Smith (5–7) microinjected the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) into the anterolateral region of the hypothalamus of rats, which caused extensive destruction of catecholamine terminals in the neocortical, mesocortical, mesolimbic, hippocampal and nigrostriatal projection fields. The lesioned animals locomoted and reared less in a novel open field, and they investigated an introduced novel object less than controls. These behavioral deficits presumably reflect destruction of DA terminals, as the animals that received 6-OHDA lesions were pretreated with the noradrenergic reuptake blocker desmethylimipramine. In addition, the behavioral deficit was shown to be reversed by the DA agonist apomorphine.

Despite this evidence, however, it is not known whether the depletion of DA disrupts the animals' *preference* for novelty in a free choice test. The place preference test has been used extensively to assess the reinforcing efficacy of various drugs of abuse (16). The present investigation used the place preference test to assess the reinforcing effects of novelty following forebrain 6-OHDA lesions in rats. A preliminary experiment was first performed with nonlesioned animals in order to determine how much stimulus habituation is required in order to observe reliable novelty preference behavior (Experiment 1). In Experiment 2, a separate group of animals received either microinjections of 6-OHDA into the mesolimbic terminal fields or was given sham surgery, and novelty preference behavior was subsequently assessed. Horizontal and vertical activity rates were also monitored

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within the novel and familiar stimulus environments in both experiments.

## EXPERIMENT 1

Previous studies have shown that animals show novelty preference behavior when habituated to one of two different stimulus environments (1, 3, 8–10). In each of these studies, habituation consisted of either one prolonged (24 hr) exposure or 6–8 brief (30 min) exposures to one environment. It is unclear from these studies, however, whether such extensive habituation is necessary in order to obtain novelty preference behavior. In the first experiment, therefore, we varied the number of 30-min habituation exposures in nonlesioned rats in order to determine how many habituation sessions might be optimal for assessing the effect of a 6-OHDA lesion on novelty preference behavior.

### METHOD

#### *Animals*

The animals were 50 male Sprague-Dawley albino rats (Harlan Industries, Indianapolis, IN) weighing 300–400 g. The rats were housed individually in wire mesh hanging cages. The colony room was maintained at a constant temperature and humidity with a 12-hour light/dark cycle (lights on at 0700). Behavioral testing was conducted during the light phase. Food and water were provided ad lib in the home cage. Prior to conditioning, animals were handled approximately 2 min per day for two days.

#### *Apparatus*

The apparatus consisted of a wooden, rectangular chamber with three compartments separated by two removable wooden partitions. The two end compartments measured $24 \times 30 \times 45$ cm (length, width and height, respectively), while the middle compartment measured $24 \times 10 \times 45$ cm. One end compartment had white walls, a wire-mesh floor and pine bedding below the floor, while the other end compartment had black walls, a metal-grid floor and cedar bedding below the floor. The smaller middle compartment was gray with a solid wood floor. The partitions separating the three compartments were either solid to confine an animal in one end chamber or were constructed with a $10 \times 10$ cm opening centered at floor level to allow the animal access to the entire apparatus. The apparatus was located in a laboratory room which contained an 8 ohm speaker driven by a white noise generator (70 dB, ambient) as well as a video camera suspended over the apparatus. The video camera was used to record behaviors on the test day.

#### *Procedure*

Animals were assigned randomly to one of five treatment groups (n = 10 per group). Four of the groups were habituated to either the black or white compartment (counterbalanced within groups) for 30 min daily on either one, two, four or eight consecutive days. The fifth group received no habituation to either compartment. On the day following the last habituation day, the solid partitions were replaced with those containing openings. The animal was placed in the center gray compartment and allowed access to the entire apparatus for fifteen minutes. Observers who were blind to the rats' group assignment viewed the animals on a video monitor and recorded duration in each compartment (operationally defined as both front paws in a compartment), as well as horizontal activity (operationally defined as the number of times that both front paws crossed a line which bisected the compartment parallel to the opening into the compartment) and vertical activity (operationally defined as the number of times that both front paws left the floor). Horizontal and vertical activity data were collected only when the animal was in one of the two end compartments.

TABLE 1

MEAN (± SEM) BASELINE VALUES OBTAINED FROM NONHABITUATED CONTROL ANIMALS (N = 10) IN EXPERIMENT 1

| Dependent Variable | Compartment | |
|---|---|---|
| | White | Black |
| Duration (sec) | 247.0 (±10.7) | 291.7 (±14.8) |
| Number of Entries | 14.2 (± 0.8) | 15.0 (± 1.0) |
| Horizontal Activity (Line Crosses per sec) | 0.11 (± 0.008) | 0.11 (± 0.006) |
| Vertical Activity (Rears per sec) | 0.05 (± 0.008) | 0.05 (± 0.004) |

#### *Statistics*

A randomized block analysis of variance (ANOVA) was performed on the duration and activity data from the nonhabituated control animals. Activity rates were calculated as the total number of behaviors (horizontal line-crosses or vertical rears) observed within each compartment divided by the total duration spent within each compartment.

A split-plot ANOVA was performed on the duration and activity data from the four habituation groups. The between-subjects measure was number of habituation days, while the within-subjects measure was the compartment entered (novel or familiar).

### RESULTS

#### *Nonhabituated Controls*

Table 1 summarizes the duration, entry and activity rate data for the nonhabituated control animals in Experiment 1. The statistical analysis revealed no significant differences between the white and black compartments in any dependent variable measured. These data show that there was no baseline preference or activity difference inherent in the two choice compartments of our apparatus.

#### *Novelty Preference*

Figure 1 summarizes the duration data for the four habituation groups in Experiment 1. The overall ANOVA revealed a significant length of habituation by compartment interaction, $F(3,36) = 3.22$, $p<0.05$. Subsequent analyses revealed that the 2-, 4- and 8-day habituation groups displayed a novelty preference, as they spent significantly more time in the novel compartment than the familiar compartment. There were no significant differences among the 2-, 4- and 8-day habituation groups as assessed by Tukey's HSD test. The 1-day habituation group did not show a novelty preference, as the time spent in the novel compartment did not differ significantly from the time spent in the familiar compartment.

#### *Activity*

Regardless of the number of habituation sessions (1, 2, 4 or 8), animals displayed a significantly lower rate of horizontal, $F(3,36) =$

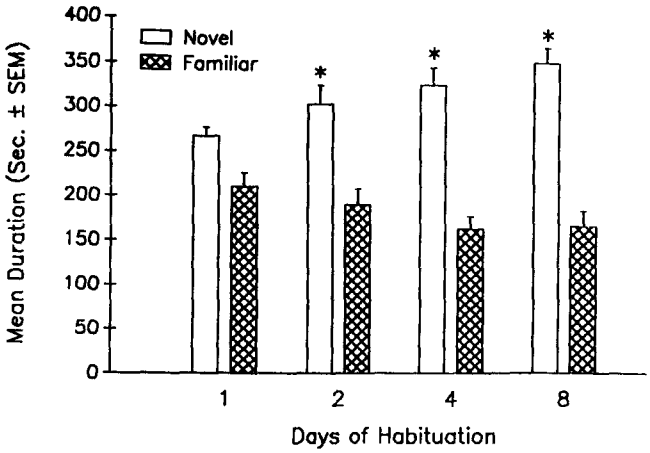


FIG. 1. Results from Experiment 1 showing that the 2-, 4-, and 8-habituation day groups' mean duration in the novel compartment was significantly greater than the duration in the familiar compartment. *Represents a significant within-group difference from the familiar environment, $p<0.05$.

21.84, $p<0.05$, and vertical, $F(3,36)=6.96$, $p<0.05$, activity in the novel compartment relative to the familiar compartment (data not shown). Animals also made significantly more entries into the novel compartment than into the familiar compartment, $F(3,36)=18.57$, $p<0.05$. There were no significant interaction effects between the length of habituation and compartment (novel vs. familiar) factors for these dependent measures.

## EXPERIMENT 2

The results from Experiment 1 showed that two, four and eight 30-min exposures to a previously novel environment all produced novelty preference behavior in rats given free choice access to both the habituated and a distinct novel environment. The two-day habituation group, while producing a distinct novelty preference, displayed a somewhat weaker effect which may not be as reliable as that obtained following 4 or more habituation days. Therefore, four habituation days were used in Experiment 2 to assess the effects of forebrain 6-OHDA lesions on novelty preference behavior.

### METHOD

*Animals and Apparatus*

Twenty-three male Sprague-Dawley albino rats (300–400 g) were obtained and housed as described in Experiment 1. The apparatus was also the same as described in Experiment 1.

*Procedures*

Randomly chosen animals received either lesion (n = 13) or sham (n = 10) surgery prior to habituation. Three lesioned animals died following surgery, thus only 10 lesioned and 10 sham animals received habituation sessions. The habituation and test procedures were the same as those described in Experiment 1, except that all animals were habituated for four days beginning on postoperative day 15.

*Surgery*

The animals were anesthetized with chloral hydrate (100 mg/ml, 3.0 ml/kg) and administered the monoamine oxidase inhibitor pargyline (50 mg/ml, 1.0 ml/kg) and the norepinephrine uptake blocker desipramine (25 mg/ml, 1.0 ml/kg) approximately 30 min prior to surgery. The animals in the lesion group were placed into a stereotaxic instrument and received bilateral injections of 6-OHDA (8.0 μg/2.0 μl/side) at a rate of 0.5 μl/min. Stereotaxic coordinates were: A +2.0 mm, L ±1.5 mm, V −7.0 mm, relative to Bregma (11). The needle was left in place for five minutes following the injection. The sham operates received the same treatment, but no injection of 6-OHDA was administered. All animals received a 0.25-ml intramuscular injection of penicillin (Combiotic, Pfizer) immediately following surgery.

*Drugs*

The chloral hydrate, pargyline HCl and desipramine HCl (Sigma) were mixed in 0.9% NaCl and injected IP. The 6-OHDA HBr (Sigma) was mixed fresh in ice-cold 0.9% NaCl containing 0.2 mg/ml ascorbic acid. All dosages were based on the salt form of the drug.

*Assay for Dopamine*

Ten days after behavioral testing concluded, all animals were sacrificed by rapid decapitation. The brain was removed, placed on an ice-cold dissecting plate, and then was dissected into the nucleus accumbens, striatum (anterior to optic chiasm), olfactory tubercles and medial prefrontal cortex. All samples were frozen at −70°C between dissection and assay.

For assay, each sample was thawed on ice, sonicated in 15 volumes of 0.1 N $HClO_4$ and then centrifuged at 40,000 × g for 10 min at 4°C. Twenty microliters of supernatant were assayed for dopamine using a high-pressure liquid chromatograph with an electrochemical detector (Bioanalytical Systems, LC-304T) and a temperature-controlled (25°C) 5 μm column (Bioanalytical Systems, PN 6207). The mobile phase was prepared as described previously (13), and external standards for DA (Sigma) were assayed daily.

*Statistics*

A split-plot ANOVA was performed on the duration data. The between-subjects factor was treatment (sham or lesion) while the within-subjects factor was compartment (novel or familiar). Planned randomized block ANOVAs were performed on the duration data comparing the mean duration spent in the novel and familiar compartment for each of the treatment groups.

A split-plot ANOVA was also performed on the activity data. Activity rates were calculated as the total number of behaviors (horizontal line-crosses or vertical rears) observed within each compartment divided by the total duration spent within each compartment. Significant effects were subsequently analyzed with Bonferroni's test of significance, $p<0.05$.

A Student's *t*-test was performed on the assay data comparing the DA concentrations from each brain region in sham and lesioned animals, $p<0.05$. A dopamine concentration in the nucleus accumbens greater than two standard deviations from the mean of the lesion group was operationally defined as a misplaced lesion. Any animals meeting this criterion were excluded from subsequent analyses.

### RESULTS

*Assay for Dopamine*

Table 2 summarizes the mean DA levels in the nucleus accum-

TABLE 2

ASSAY RESULTS FROM EXPERIMENT 2 REFLECTING REGIONAL CONCENTRATIONS OF DA IN BOTH THE SHAM (N = 10) AND LESION GROUPS (N = 7) AS WELL AS PERCENT DEPLETION IN THE LESION RELATIVE TO SHAM GROUP

| | Dopamine Concentration (Mean μg/g tissue ± S.E.M.) | | |
|---|---|---|---|
| Brain Region | Sham | Lesion | Depl. |
| Nucleus Accumbens | 9.66 (±0.87) | 3.21 (±0.42) | 67* |
| Olfactory Tubercles | 10.41 (±0.39) | 3.30 (±0.74) | 68* |
| Anterior Striatum | 11.07 (±1.34) | 2.80 (±0.61) | 75* |
| Medial Prefrontal Cortex | 0.16 (±0.01) | 0.14 (±0.01) | 12 |

*Represents a significant difference between sham and lesion treatments, $p<0.05$.

bens, olfactory tubercles, anterior striatum and medial prefrontal cortex for both the sham and lesion treatments. Three animals in the lesion group met the criterion of a misplaced lesion and were excluded from all analyses. The assay results from the remaining animals reflect significantly lower levels of DA in the lesioned animals relative to the sham animals for the nucleus accumbens, $t(15)=5.82$, $p<0.05$, anterior striatum, $t(15)=4.89$, $p<0.05$, and olfactory tubercles, $t(15)=9.21$, $p<0.05$, but not prefrontal cortex.

*Lesion Effects on Novelty Preference*

Figure 2 summarizes the duration data for both sham and lesion treatments. The overall ANOVA revealed that the interaction effect did not reach statistical significance, $F(1,15)=2.24$, $p<0.15$, perhaps due to larger variability observed in the lesioned group relative to the sham group. Nonetheless, a randomized block analysis of variance did reveal that the sham group displayed a novelty preference, as their mean duration in the novel compartment was significantly greater than their mean duration in the familiar compartment, $F(1,9)=36.01$, $p<0.05$. This novelty preference in the sham group was similar to that of the 4-day habituation group in Experiment 1. In contrast, lesioned animals' novelty preference was disrupted, as their mean duration in the novel and familiar compartments did not differ statistically.

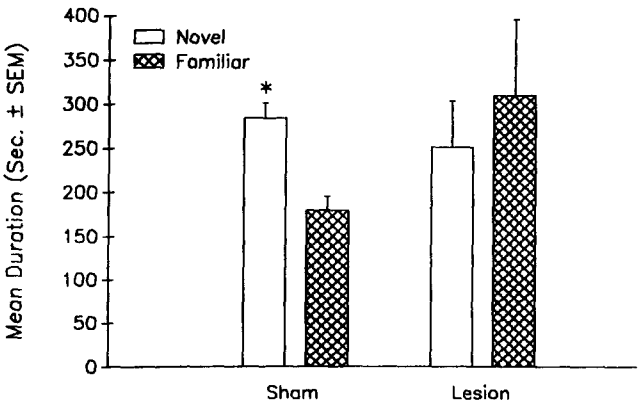


FIG. 2. Results from Experiment 2 showing novelty preference in the sham group, as their mean duration in the novel compartment was significantly different from their mean duration in the familiar compartment. Novelty preference was disrupted in the 6-OHDA-lesioned animals, as the mean duration in the novel and familiar compartments were not significantly different. *Represents a significant within-group difference from the familiar environment, $p<0.05$.

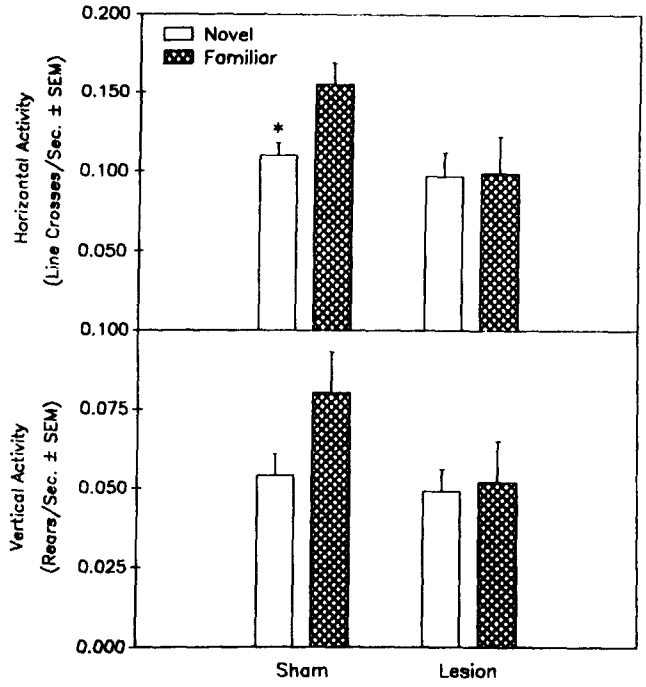


FIG. 3. Results of activity data from Experiment 2. Top panel: Results showing a significant difference in horizontal activity rates between the familiar and novel environments in the sham but not the lesion group. Bottom panel: Results which revealed no significant difference in vertical activity rates between familiar and novel environments for either the sham or lesion group. *Represents a significant within-group difference from the familiar environment, $p<0.05$.

*Lesion Effects on Horizontal and Vertical Activity*

The results from the activity data revealed a significant interaction effect for horizontal activity rate, $F(1,15)=12.45$, $p<0.05$ (see Fig. 3, top), but not vertical activity rate (see Fig. 3, bottom). Subsequent analysis of the sham group's horizontal activity data showed a significantly lower line crossing rate in the novel compartment relative to the familiar compartment. The lesion group's horizontal activity in their novel and familiar compartments did not differ statistically.

## GENERAL DISCUSSION

In previous studies, novelty preference behavior was obtained following either a single prolonged (24 hr) exposure to one environment (8–10) or 6–8 brief (30 min) repeated exposures to one environment (1,3). In the current report, novelty preference behavior was clearly shown to be affected by the number of habituation exposures used. That is, following one 30-min exposure to one of two distinct environments, animals did not show a novelty preference, as the time spent in the novel and familiar compartments did not differ statistically. However, following two, four or eight exposures to a previously novel compartment, separate groups of animals displayed a distinct novelty preference, as they spent significantly more time in the novel than familiar compartment. The effect of novelty was more robust following 4

exposures than following 2 exposures however. These results show that extensive habituation is not necessary to produce novelty preference in rats.

The current results also provide evidence that the novelty preference behavior observed here involves a forebrain DA mechanism. A 6-OHDA lesion of forebrain DA terminal fields disrupted subsequent novelty preference behavior. In addition, the lesion eliminated the differential rate of activity in the novel and familiar environments observed in sham animals. These findings are consistent with earlier reports in which lesions of the forebrain DA system produced a deficit in novelty-elicited behaviors (5,7). Whereas Fink and Smith (5–7) observed the behavior of animals confined to a novel open field and approach behaviors to a novel object introduced into the open field, the current report extends these previous studies by assessing choice behavior between a habituated and a distinct novel environment. Further, unlike the lesion produced by Fink and Smith with 19.5 μg 6-OHDA into the anterolateral hypothalamus, we found that the lesion produced with 8 μg 6-OHDA into the nucleus accumbens did not invade the mesocortical dopamine system. This suggests that a mesolimbic and/or nigrostriatal DA system may be more critical than the mesocortical DA system in novelty-seeking behavior.

The current results are consistent with the hypothesis that a 6-OHDA lesion of forebrain DA terminals blocked the reinforcing effect of novelty. A growing body of evidence now implicates the mesolimbic DA system in the reinforcing effects of various stimuli, including food, water, electrical brain stimulation and drugs of abuse (2,17). Perhaps exposure to novel stimuli, like exposure to other rewarding stimuli, activates the mesolimbic DA pathway. Consistent with this notion, it has been shown that novel visual stimuli can activate neurons within the ventral tegmental area of monkeys (4).

An alternative interpretation of these results is that forebrain DA depletions interfere with stimulus habituation, and thus lesioned animals may treat both environments as "novel" on the test day. Consistent with this interpretation, we found that lesioned animals displayed a level of activity in both the novel and familiar environments which was comparable to the level of activity displayed by sham control animals within the novel environment (see Fig. 3). Despite this, however, other reports mitigate against the possibility that forebrain DA depletions interfere with stimulus habituation. In particular, Fink and Smith (5) showed that rats given forebrain 6-OHDA lesions are able to discriminate between novel and familiar stimuli because they spontaneously alternate normally, approach novel objects in some situations, and show a normal rate of locomotion during an open-field trial. Thus, the deficit in novelty preference behavior observed in the present report may reflect a relatively selective diminution in the incentive value of novel stimuli.

Finally, the present results may have some implications for studies involving drug-conditioned place preference. Similar to the preference for novelty, rats show a place preference for stimuli previously associated with various drugs, including opiates, stimulants and sedative-hypnotics (16). Interestingly, a 6-OHDA lesion similar to that produced in the present report is also known to eliminate the conditioned place preference produced by amphetamine (15) and diazepam (14). Although these latter findings likely reflect a diminution in the reinforcing efficacy of amphetamine and diazepam, an alternative interpretation is that the 6-OHDA lesion may have disrupted the habituation process, thus producing a "novel" test environment which obscured the normal preference for drug-associated stimuli. Further research may be needed to rule out this possibility.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of a number of students who helped in scoring the behavioral measures and in preparing surgery, including M. Castle, K. Harrod, R. Middleton, T. Nodurft, B. Pierce, K. Placier and D. Schneider. This research was supported by United States Public Health Service grant DA05312.

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