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6-Hydroxydopamine Reduces Preference for Conspecific But Not Other Familiar Odors in Rat Pups

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MARASCO, E. M., C. A. CORNWELL-JONES AND S. K. SOBRIAN. *6-Hydroxydopamine reduces preference for conspecific but not other familiar odors in rat pups.* PHARMAC. BIOCHEM. BEHAV. 10(3) 319–323, 1979.—Rat pups injected subcutaneously with 6-hydroxydopamine (6-OHDA) showed reduced preference for a familiar combination of conspecific and botanical odors when subsequently tested in a two-choice situation. However, drug treatment did not influence preference for the simple botanical odor. 6-OHDA also reduced norepinephrine (NE) concentrations in the forebrain and the olfactory bulbs. The data implicate NE in the ontogeny of acquired responses to conspecific odors.

Forebrain Imprinting Norepinephrine Olfactory bulb Pheromone 6-Hydroxydopamine Olfaction

ODORS of animal origin attract neonates of many mammalian species [2, 5, 7, 9, 10, 13, 18, 23, 25, 26]. These odors may subserve important ethological functions by helping to ensure that infants remain near their mothers and by facilitating suckling [3, 11, 12, 30]. In the rodents studied to date, attraction to conspecific odors is not innate. For example, cross-fostered *Acomys* and *Mus* mouse pups prefer bedding odors from females of their foster species to odors produced by females of their own species [23]. Similarly, conspecific odors deposited on bedding of a familiar but not an unfamiliar scent attract hamster pups [4], and fecal odors of lactating female conspecifics fed a familiar but not an unfamiliar diet attract rat pups [16]. In each case, unfamiliar odors produced by conspecific females are neutral for pups, indicating that preferences for such odors are not genetically preprogrammed in rodent neonates, but are acquired during exposure in the nest.

Systemic injection of the neurotoxin 6-hydroxydopamine (6-OHDA) impairs attraction of rat pups reared in pine shavings to conspecific odors from the nest [28]. However, treated pups show normal avoidance of novel botanical odors, suggesting that olfactory sensitivity is not impaired. In addition to its behavioral effects on neonates, 6-OHDA treatment permanently reduces levels of the neurotransmitter norepinephrine (NE) peripherally in the sympathetic system [1,14]. Centrally, the treatment reduces NE levels in widespread forebrain regions including some areas receiving olfactory innervation [15, 21, 24, 29]. Brainstem NE levels increase following neonatal 6-OHDA injection, but regional brain dopamine (DA) levels are not significantly altered [15,20].

In combination, the behavioral and neurochemical data imply that NE may help mediate attraction of rat pups to familiar odors. The present experiments were designed to determine if the observed 6-OHDA-induced deficits in attraction to conspecific odors [28] indicated a general learning deficit, a general impairment of approach responses, or a specific odor-guided response deficit restricted to conspecific scents.

EXPERIMENT 1

Exposing rats to botanical odors during development induces attraction [17]. If 6-OHDA treatment impairs appraoch responses or produces a general learning deficit, we predicted that drug-treated animals would not demonstrate a preference for a familiar botanical odor. On the other hand, if 6-OHDA injection selectively impairs responses to conspecific odors, the treatment would not affect attraction to familiar botanical odors. We tested these predictions by rearing pups in a normally aversive botanical odor [28] and then determining if drug treatment prevented the demonstration of a preference for the odor.

Animals

Thirteen litters of Sprague-Dawley rats born in the Princeton animal colony were used in this experiment. Prospective mothers were housed with males for 20 days and then placed in individual cages containing pine shavings. Dams were checked for births at 0800 and 1600 hr daily. The day a litter was found was designated Day 0. Within four hr of the discovery of birth, half of the pups received sub-

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TABLE 1
TESTING SCHEDULE

Odor Choices	Rearing	Litter	Treatment N 6-OHDA	Treatment N NaCl	Days Tested 5	6	7	8	10	12	14
		1	3	5	x	x	x	x	x	x	x
Nat. Cedar	Cedar	2	4	3	x	x	x	x	x	x	x
		3	5	4	x	x	x	x	x	x	x
vs.		4	3	2	x	x	x	x	x	x	x
		5	3	2	x	x	x	x	x	x	x
Nat. Pine		6	5	5	x			x			x
		7	4	5	x			x			x
		1	3	5	x	x	x	x	x	x	x
	Pine	2	3	3	x	x	x	x	x	x	x
		3	4	3	x	x	x	x	x	x	x
		4	3	2	x	x	x	x	x	x	x
		5	4	4	x			x			x
		6	2	4	x			x			x
Cedar Nest		1	5	5	x	x	x	x	x	x	x
vs.	Cedar	2	4	5	x	x	x	x	x	x	x
Nat.		3	3	5	x	x	x	x	x	x	x
Cedar		4	4	4	x	x	x	x	x	x	x
		5	4	4	x	x	x	x	x	x	x

N: Pups surviving to last test day. Five pups per litter were assigned to each treatment group on Day 0.

cutaneous injections of 50 μg/g of 6-OHDA hydrobromide (Regis Chemical) dissolved in 0.9% saline vehicle with ascorbic acid (1 mg/ml). Remaining pups served as controls and received vehicle injections only.

After injection, pups were transferred with dams to rearing cages 36×30×16 cm and maintained on a 12 hr reversed light/dark cycle. Cages contained 4 liters (by volume) of one of two kinds of shavings. Seven litters were placed in cages containing cedar shavings, the other 6 litters in Ponderosa pine shavings (Rosedale Mills, Princeton, NJ). Within each rearing group, 1/2 the pups had been injected with 6-OHDA, the other 1/2 with vehicle. Injections were repeated on each of the next three days (Days 1, 2 and 3) while pups were temporarily removed from their nests. Thus, each pup received a total of 4 injections of either 6-OHDA or vehicle.

Animals were reared in a special filtered air system which provided each litter with an isolated olfactory environment. A top with an inlet for air was clamped over each rearing cage. A container of charcoal was placed in the inlet to filter incoming air. The air passed through each rearing cage to a screened outlet leading to a common exhaust line. The air was thence vented to a duct leading outside the building. This system prevented the exchange of odors between cages.

Procedure

Odor preferences of pups were measured on an apparatus 20×26×8 cm having a Plexiglas frame and a screen floor which was directly on top of two compartments, each filled with 0.5 liters of wood shavings. This arrangement allowed odors but not tactile or gustatory cues to pass from the shavings to the animals tested. One compartment contained fresh natural pine shavings, the other fresh natural cedar. The time a pup spent over cedar odor was monitored with a stopwatch. Pups five days old were tested for 2 min, pups 6–14 days old for 3 min.

Five of the cedar-reared and 4 of the pine-reared litters were given a single trial on pine vs. cedar on Days 5, 6, 7, 8, 10, 12 and 14. In addition, to control for the effects of repeated testing, two additional litters from each rearing group were tested on Days 5, 8 and 14 (Table 1). These spot-tested pups were marked with India ink under the skin of their paws for individual identification.

Data Analysis

Analysis of variance for repeated measures was used to evaluate the effects of age and drug treatment on scores of spot-tested pups within each rearing group. The effects of rearing odor differences were evaluated by t tests applied to combined scores of spot- and repeatedly-tested pups.

RESULTS

Data from spot and frequently tested pups were statistically similar in both rearing and treatment groups. On Days 5–12, pups reared in cedar spent significantly more test time over natural cedar odor than did analagously tested pine-reared pups (Fig. 1, t tests for the significance of the difference between two means [8]). Cedar- but not pine-reared pups generally preferred cedar odor, indicating that exposure to cedar not only eliminated aversion, but induced attraction to this odor.

Drug treatment did not influence attraction to natural cedar odor in exposed pups, $F(1,17)=0.2604$, $p>0.05$ (spot-tested cedar-reared pups) nor avoidance of the odor in unex-

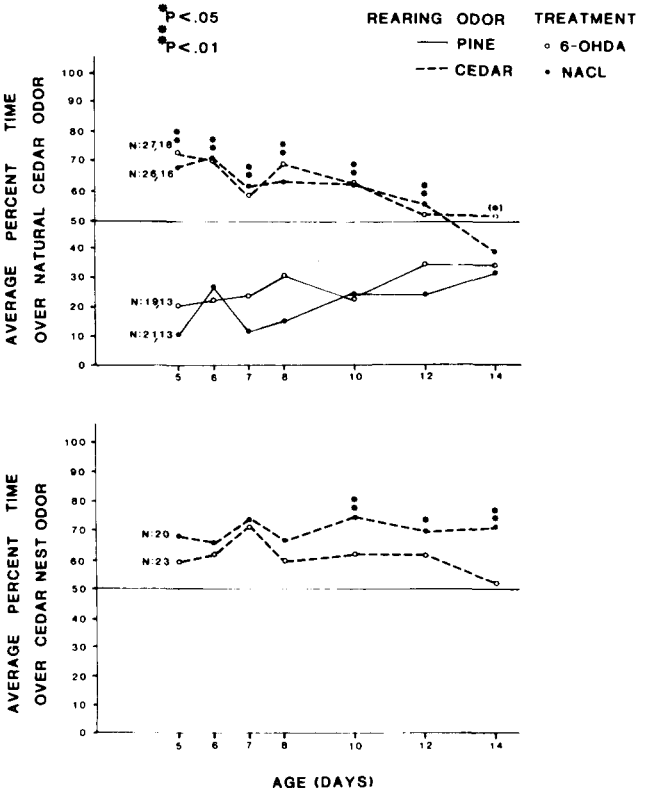

FIG. 1. Effects of 6-OHDA and olfactory environment on responses to animal and nonanimal odors. Upper graph: Average percent of test time drug- and vehicle-injected rats in both rearing groups spent over cedar odor. Numbers = maximum and minimum number of pups tested on each day. Asterisks refer to rearing differences. Lower graph: Average percent of test time cedar-reared animals spent over cedar nest odor as a function of neonatal treatment. Asterisks refer to treatment differences.

posed pups, $F(1,12)=0.01439$, $p>0.05$ (spot-tested pine-reared pups, Fig. 1). There was no significant effect of age, $F(2,24)=3.0146$, $p>0.05$, nor was there a significant age by treatment interaction, $F(2,24)=3.375$, $p>0.05$, for averages of spot-tested pine-reared pups. For spot-tested cedar-reared pups there was a significant age by treatment interaction, $F(2,34)=22.11$, $p>0.001$, and a significant age effect, $F(2,34)=19.21$, $p<0.001$. The age effect and interaction were attributable to a decline in preference for natural cedar odor by saline-treated pups; spot-tested animals in this group spent significantly less time over natural cedar odor on Day 14 than on Day 5, $t(9)=4.45$, $p<0.002$, two-tailed. Age did not significantly influence scores of drug-treated cedar-reared pups.

Rat pups reared in cedar shavings acquired a preference for cedar odor in spite of 6-OHDA treatment. Therefore, the drug induced neither a general learning deficit, nor a motor inability to approach a familiar odor. Moreover, drug treatment did not influence aversion to cedar odor by pups reared in pine, suggesting that 6-OHDA did not impair general olfactory sensitivity.

EXPERIMENT 2

This experiment was designed to determine if conspecific animal odors deposited on nest shavings attract cedar-reared pups, and if 6-OHDA treatment reduces this preference.

Method

Animals. Pups from five litters reared in cedar shavings, injected with drug or vehicle, and maintained as in Experiment 1, were used.

Procedure. All pups were individually marked and were tested repeatedly (Table 1) as in Experiment 1, except that fresh natural cedar shavings were in one odor compartment, and soiled cedar shavings taken from pups' nests were in the other odor compartment. The time spent over the nest shavings were recorded for each pup. No spot-tested animals were used because data from the first experiment indicated that testing frequency did not influence scores. Analysis of variance for repeated measures was applied to scores of all pups to evaluate age and treatment effects.

Results

As seen in Fig. 1, there were significant effects of treatment, $F(1,141)=52.4672$, $p<0.001$, and age, $F(6,246)=11.229$, $p<0.001$, and there was a significant age by treatment interaction, $F(6,246)=14.263$, $p<0.001$, on responses to cedar nest vs. fresh cedar odor. The interaction was attributable to a decline in time spent over cedar nest odor by drug- but not saline-treated pups. Cedar nest odor attracted drug-treated pups on Day 7 significantly more than on Day 14, $t(22)=2.50$, $p<0.05$, two-tailed. Age did not affect scores of saline-treated pups. As a result, on Days 5–8, saline- and drug-treated pups were similarly attracted by cedar nest odor, while on Days 10–14, the odor attracted drug-treated pups significantly less than controls, $t(41.0)=2.40$, $p<0.01$; $t(42.0)=1.70$, $p<0.05$; $t(35.6)=3.39$, $p<0.005$, one-tailed, Days 10, 12 and 14 respectively.

In spite of the preference reduction, drug treatment did not eliminate attraction to cedar nest odor. For example, 6-OHDA-treated pups spent significantly more than 50% of test time over cedar nest odor on Day 10, $t(22)=2.89$, $p<0.005$. In the first experiment, natural cedar odor also attracted drug-treated cedar-reared pups on Day 10, $t(17)=2.59$, $p<0.01$. Thus, 6-OHDA treatment did not eliminate the motor ability to approach either familiar odor on Day 10, but selectively diminished attraction to conspecific odors.

EXPERIMENT 3

The object of this experiment was to verify that 6-OHDA treatment depletes forebrain NE concentrations in cedar-reared rats, and to see if the degree of depletion changes with post-injection time.

Method

Animals. Pups from 5 litters were reared in cedar shavings, injected with drug or vehicle on Days 0–3, and maintained as in Experiment 1.

Procedure. Twenty-one pups from 3 litters and 12 pups from 2 litters were sacrificed on Days 8 and 14 respectively. Pups' brains were dissected on an ice-cooled, saline-rinsed glass plate. The skull was opened and the olfactory bulbs removed. A coronal cut was made anterior to the superior colliculus and through the mammilary bodies. Tissue posterior to the cut was discarded. The olfactory bulbs and the entire forebrain between the cut and the frontal pole were separately frozen in liquid nitrogen.

Tissue samples were subsequently assayed by the

fluorometric method of Richardson, Cowan, Hartman and Jacobowitz [24] for endogenous levels of the catecholamines (CA) NE and dopamine (DA). Brain areas were weighed, slightly acidified with 0.01 N HCl and homogenized in 5 ml chilled 1-butanol. The homogenate was centrifuged for 10 min at 3000 rpm. CA present in the butanol supernatant was extracted into a phosphate buffer and aliquots of the buffer were oxidized. Fluorescence intensities of the oxidized aliquots were read on a spectrophotofluorometer, and CA concentration was calculated.

Results

For the DA assays, fluorometric readings were less than twice the reading for the respective tissue blanks, indicating that tissue DA concentrations were below assay sensitivity levels. For NE assays, fluorometric readings were always at least threefold higher than the appropriate tissue blank. On Day 8, forebrain NE recovery levels were 64.93%. Therefore, tissue sample values were corrected for that assay. NE recovery levels for all other assays ranged from 77.5% to 78.7% and sample values were uncorrected.

As shown in Fig. 2, NE concentrations (±SEM) in the olfactory bulbs of drug-treated pups were significantly below those of saline controls on both Days 8, $t(14.91)=7.2088$, $p<0.001$, two-tailed, and 14, $t(9.95)=12.88$, $p<0.001$, two-tailed. Mean olfactory bulb NE concentrations did not change significantly with age in either drug- or vehicle-treated animals.

Figure 2 indicates that NE concentrations in the forebrains of drug-treated pups were similar to those of saline controls on Day 8, $t(14.14)=1.851$, $p>0.05$, but significantly lower on Day 14, $t(11.37)=3.1523$, $p<0.01$. Forebrain NE concentrations of saline-treated pups increased significantly between Days 8 (Mean=0.074 ± 0.004 μg/g) and Day 14, Mean=0.088 ± 0.005 μg/g, $t(11.51)=2.305$, $p<0.05$. This NE increase in the forebrain is similar to one reported previously using a different assay method [19]. NE forebrain concentrations in drug-treated animals did not increase significantly between Days 8, Mean=0.056 ± 0.009 μg/g, and 14, Mean=0.068 ± 0.004 μg/g, $t(14.73)=1.22$, $p>0.05$.

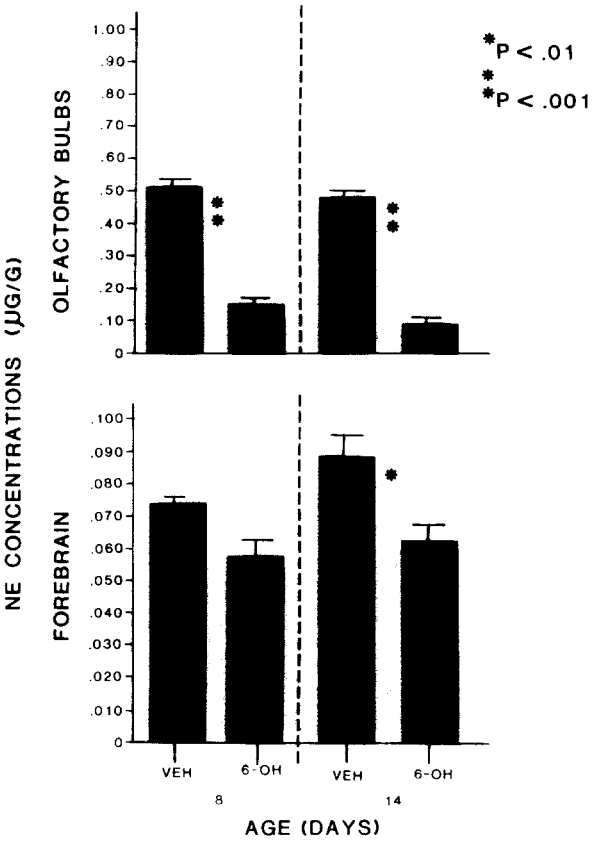

FIG. 2. Effects of age and 6-OHDA on olfactory bulb and forebrain NE levels. Each histogram represents the averaged concentrations of 6–12 brains.

DISCUSSION

Rearing rat pups in cedar shavings induced a preference for natural cedar odor which was not altered by injections of 6-OHDA on Days 0–3. These data indicate the drug treatment did not induce a general olfactory learning deficit, nor a general impairment of approach responses. In contrast to its lack of influence on responses to the botanical odor, drug treatment reduced preferences for conspecific odors beginning on Day 10. The fact that the deficits in response to conspecific odors were first observed 7 days after the final injection suggests that the change was not due to some acute traumatic effect of drug treatment.

A previous study demonstrated that neonatal 6-OHDA injections reduce preferences of pine-reared rat pups for conspecific odors [28]. Responses to unfamiliar botanical odors were not impaired in that study, regardless of odorant concentration. While the spectrum of odors tested in these studies is limited, the combined data suggest that 6-OHDA treatment influences responses to certain odors, but not others.

Forebrain and olfactory bulb NE levels in drug-treated pups were below normal on Day 14, when attraction to conspecific odors was reduced. The coincidence of deficits in brain and behavior on Day 14 suggests that the depletion of forebrain NE might have been critical in reducing preference for cedar nest odor. However, systemic 6-OHDA reduces peripheral as well as central NE levels: the treatment causes peripheral sympathectomy in neonate rats [1,14]. Although cervical sympathectomy improves rather than impairs odor discrimination ability in adult female rats [22] the behavioral deficits observed in the present study using neonates may represent selective changes in peripheral or central neural substrates.

Forebrain NE levels in rat pups normally increase during the second postnatal week as the neurotransmitter is transported rostrally from cell bodies in the brainstem to their axon terminals [6,19]. In the present study, NE concentrations increased between Days 8 and 14 in vehicle- but not drug-injected pups. The data imply that 6-OHDA disrupts rostral transport of NE, preventing the usual increases in forebrain NE concentrations from occurring between Days 8 and 14 [20].

The level of forebrain NE depletion in 6-OHDA treated pups 14 days old reported here, is small compared to reductions reported in rats 40 days old treated neonatally [29]. However, forebrain NE levels in normal rats increase fourfold between the second postnatal week and adulthood [19]. The degree of forebrain NE depletion caused by neonatal 6-OHDA treatment would be expected to rise with age as neurotransmitter levels rose in vehicle- but not drug-treated animals.

In summary, our findings imply that neonatal subcutaneous injections of 6-OHDA preferentially impair responses to familiar conspecific odors by rat pups. The selective behavioral deficits observed following NE depletion in the forebrain and the olfactory bulbs, suggest that this neurotransmitter may be involved in the normal ontogeny of responses to conspecific odors.

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