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Impaired Hoarding and Olfactory Learning in DSP-4-Treated Rats and Control Cagemates

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CORNWELL-JONES, C., T. PALFAI, T. YOUNG, J. DESAI, D. KRASENBAUM AND J. MORRISON. *Impaired hoarding and olfactory learning in DSP-4-treated rats and control cagemates.* PHARMACOL BIOCHEM BEHAV **36**(4) 707–711, 1990.—The possibility that variables affecting rats' home-cage odor preferences also influence hoarding behavior was examined. Neonatal male rats were injected SC with the noradrenergic neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), or with vehicle. At weaning, rats were assigned to control-only, DSP-4-only, or mixed groups of DSP-4 and control rats. For the next 10 days, half the rats in each social condition were housed in cedar shavings, and remaining rats were housed in pine. Exposure to cedar significantly increased preference for the odor in control-only groups, but not in DSP-4-only or mixed treatment groups. Control-only groups also hoarded significantly more pellets per animal than rats in the other two social conditions. The results suggest that both olfactory adaptation and hoarding can be impaired by either neonatal NE depletion or an abnormal social environment.

Olfaction DSP-4 Hippocampus Hoarding Monoamines

RATS are one of many species which, in the wild, establish food hoards at weaning (13). A hoard provides a food source upon which animals may rely in times of food shortage. Miller and Viek (10) have shown that olfactory cues play an important role in this form of behavior. When given a choice, rats prefer to hoard in cages with familiar rather than novel odors, and show reduced hoarding when placed in a cage with an unfamiliar odor (10).

In recent studies, Cornwell-Jones and her colleagues have shown that several experimental manipulations can impair responses to home-cage odors in recently weaned rats. For example, placing normal weanling rats in a new bedding odor for 10 days increases preference for the odor (2). However, this is not the case when the animals' forebrain norepinephrine (NE) is depleted neonatally with either one of three neurotoxins: 6-hydroxydopamine, 6-hydroxydopa, or N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) (3,4). These results implicate a possible role of NE in this type of odor-guided behavior.

An unexpected finding resulting from these experiments was that control animals that were housed with their DSP-4-treated littermates, also showed impaired olfactory adaptation to the novel odor. Since control animals housed with rats treated with the other two neurotoxins did not show this effect, it appeared that the social environment created by mixing DSP-4-treated animals and their littermates was responsible for this unusual phenomenon.

The experimental results above provided the rationale for investigating whether impaired olfactory adaptation to the home cage environment transfers to other odor-guided behaviors, such as hoarding. Therefore, in the present experiment, we examined the effects of neonatal DSP-4 treatment and three different housing conditions on olfactory learning and hoarding behavior in juvenile rats.

METHOD

Animals and Testing

Seventy-five male Sprague-Dawley rats from 13 litters (Charles River stock) born in the laboratory were used in the experiment. Breeder males and females were housed together for 16 days, and then females were placed in clear plastic maternity cages measuring 47 × 36 × 19 cm containing Ponderosa pine shavings. Females were checked daily for births.

On the day a litter was found (Day 0), it was culled to no more than 10 pups, including at least two females, so that the males used in the experiment would be reared in mixed-sex groups. Half the males in each litter received 50 μg/g subcutaneous injections of DSP-4. Control littermates received water vehicle. Litters were placed in an 18-cage Laminar Flo clean room enclosure with six shelves. Each shelf had a separate air supply so that animals could

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smell odors from cages on their own shelf, but not the other shelves. Animals were maintained on a 12/12-hr reversed light cycle. Purina Rat chow and water were provided ad lib from containers in the cage lids.

Litters were weaned on postnatal Day 25. Female siblings were housed separately from males in maternity cages containing pine shavings. Each litter of males was assigned to either a mixed social housing condition in which control and DSP-4-treated juveniles were housed together, or a separate condition in which the treatment groups were housed in different cages. Half the groups in each social housing condition were placed in maternity cages containing 4 liters (by volume) of cedar shavings, the other animals remained in pine. Cages of pine- and cedar-housed animals were placed on different shelves of the clean air enclosure so that the rats could only smell odors similar to those in their home cages.

Ten days after weaning, animals were given olfactory preference tests. Animals were tested individually under red light to minimize visual cues. The testing apparatus measured 53 × 48 × 25 cm and consisted of a Plexiglas frame with a screen floor placed over two odor compartments containing differently scented shavings. Each compartment was divided into 2 right-angle triangles with a common hypotenuse. The two inner triangles formed a V at the apparatus midline and were empty. One of the two outer sections contained 1 liter (vol.) of fresh pine shavings. The other outer section contained either 1 liter of soiled pine nest shavings from the female littermates of the males being tested or fresh cedar shavings. Each rat was given two odor preference tests: pine vs. pine nest, and pine vs. cedar. The rat was placed in the middle of the screen floor, its behavior was observed for 3 min, and the time spent over each of the two odors was recorded.

Four days after the preference tests were given, group hoarding behavior was measured following the procedures of (14). Rabbit pellets rather than rat chow were used because the smaller pellets permitted more precise measurement of the amount of food hoarded. Fifteen grams of pellets per rat in the cage were weighed and placed on a plastic tray. The tray was left in the cage for an hour, at which time the animals and the tray were removed. The location of any hoards were drawn on a diagram of the cage, and the number of pellets in the hoards were counted. A hoard was defined as at least three pellets lying in contact with each other and not with the tray (i.e., pellets that had obviously spilled from the tray were not included). The number of pellets hoarded was divided by the number of rats in the cage to yield the hoarding score.

Biochemical Analysis

To verify the neurotoxic effects of DSP-4, within a week of testing, animals were sacrificed by decapitation. The hippocampus was dissected on an ice-cooled, saline-rinsed glass petri dish, using a method modified from the procedure of (6). The tissue was frozen on dry ice and stored at −70°C until it was assayed for monoamines and their metabolites. High pressure liquid chromatography was used to measure concentrations of norepinephrine (NE), serotonin (5-HT), dopamine (DA), and the serotonergic metabolite 5-hydroxyindole acetic acid (5-HIAA), following procedures modified from (8), as described in Cornwell-Jones *et al.* (5).

Statistical Analysis

For the preference tests, a difference score was computed by subtracting the time spent over cedar or pine nest from the time spent over fresh pine. An odor preference was inferred if mean difference scores were significantly higher or lower than zero, the score expected if a group had no preference between odors. Preference scores and neurochemical data were evaluated for each housing condition by analysis of variance (ANOVA) followed by 2-tailed comparisons using the Newman-Keuls test. Student *t*-tests for the significance of the difference between an observed and hypothetical mean were used to determine if the mean difference scores of individual groups were statistically different from zero. Hoarding scores were analyzed using ANOVA followed by planned comparisons of control-only group means with the means of the other two housing groups [Dunnett test, (9)]. The comparisons were one-tailed since we predicted that animals housed in control-only groups should hoard more than rats in the other two housing conditions.

RESULTS

Biochemistry

Neonatal DSP-4 treatment greatly depleted concentrations of hippocampal NE, moderately depleted 5-HT, and 5-HIAA, but did not significantly affect DA concentrations (mean DA values = 9.9–17.8 μg/g wet weight tissue). Drug treatment depleted NE concentrations, $F(1,28)=96.363$, $p<0.001$, to between 9% and 15% of control values (Fig. 1A). Treatment and housing conditions interacted to influence hippocampal concentrations of 5-HT, $F(1,28)=5.699$, $p<0.025$: DSP-4 significantly lowered 5-HT concentrations for animals housed in the mixed condition (to 68% of their controls' value), but not the separate condition (Fig. 1B). Control 5-HT values in the two housing conditions were statistically similar. Finally, treatment significantly influenced 5-HIAA concentrations, $F(1,28)=15.183$, $p<0.001$. Drug treatment tended to lower 5-HIAA concentrations for animals in both housing conditions, but the effect was only significant for those in the separate condition (53% of control values, Fig. 1C).

Behavior

Preferences for pine vs. cedar odor were significantly influenced by the home cage odor, $F(1,67)=25.25$, $p<0.001$. The effect resulted from the fact that animals tended to spend more time over their current housing odor than the other odor, but the tendency was not consistently statistically significant. In agreement with previous findings (3), olfactory experience significantly influenced preferences of controls living with similarly treated cagemates, but not preferences of other groups. For control-only groups, cedar odor was aversive to pine-housed rats, $t(10)=4.64$, $p<0.001$, but neutral to cedar-housed rats (Fig. 2A). As a result of these preference patterns, exposure to cedar significantly increased preference for the odor in control animals housed in separate treatment groups ($p<0.01$, Newman-Keuls, Fig. 2A).

Rats housed in DSP-4-only groups showed qualitatively similar behavior. Cedar odor was aversive to pine-housed drug-treated animals, $t(10)=3.78$, $p<0.01$, and neutral to cedar-housed rats, but preference differences were not large enough to be statistically significant ($p>0.05$, Newman-Keuls, Fig. 2A). Therefore, neonatal DSP-4 treatment attenuated the effects of olfactory experience, as seen in an earlier study (3).

None of the groups of rats housed in the mixed treatment condition showed statistically significant preferences (Fig. 2A). Olfactory experience did not significantly influence preferences of either control or drug-treated rats living in this condition. Therefore, as demonstrated previously, housing controls with DSP-4-treated littermates prevented normal experience-dependent changes in odor preferences.

Current housing odor also significantly influenced preferences

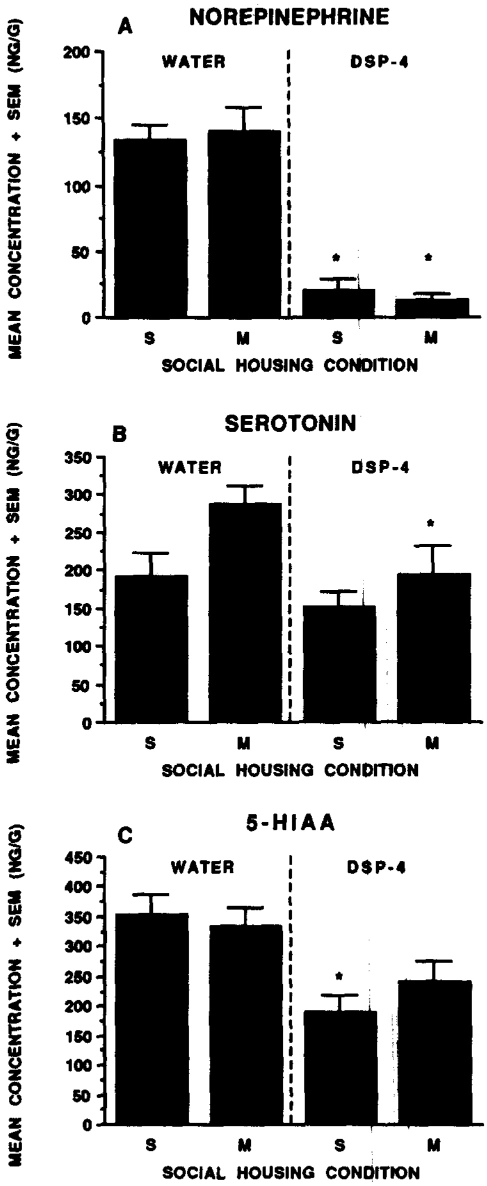

FIG. 1. Effects of neonatal DSP-4 treatment and social housing conditions on hippocampal (A) NE, (B) 5-HT and (C) 5-HIAA. S's on the abscissa indicate means for animals housed in separate water-only and DSP-4-only groups, M's indicate means for animals housed in mixed water/DSP-4 groups. Asterisks indicate means significantly different from similarly housed controls, $p<0.01$, Newman-Keuls test. N = 8 tissue pieces per histogram.

for pine nest vs. pine odor, F(1,67) = 19.72, $p<0.001$. Animals housed in cedar spent significantly more time over pine nest than fresh pine odor, while pine-reared rats showed no preference between the odors (Fig. 2B). However, preference differences between pine and cedar-housed rats were statistically significant only for DSP-4-treated animals in the separate housing condition ($p<0.01$, Newman-Keuls, Fig. 2B). In summary, DSP-4 treatment and mixed housing impaired experience-dependent preferences for home-cage bedding odors. The deficit was selective: neither variable impaired experience-dependent preferences for bedding odors from cages of female conspecifics.

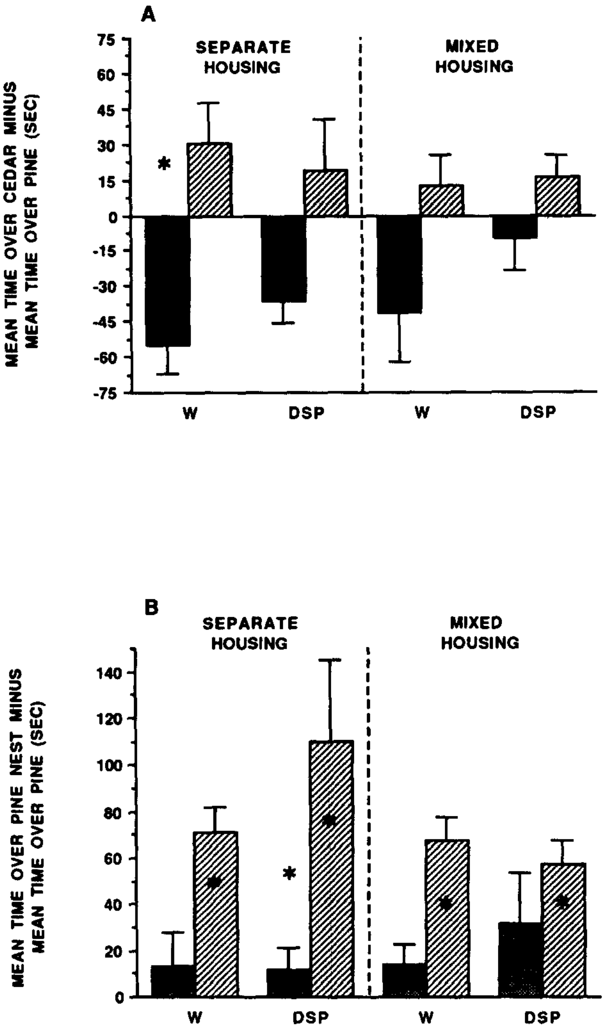

FIG. 2. Effects of olfactory experience, neonatal NE depletion and social housing conditions on preferences for (A) cedar vs. pine odor (B) pine nest vs. pine odor. W's indicate means of rats treated neonatally with water, DSP's means for neonatally drug-treated rats. Histograms indicate mean preference scores ± SEM. Asterisks inside histograms indicate odor preferences, i.e., means are significantly different from zero, $p<0.01$, t-test for the significance of the difference between an observed and hypothetical mean. Asterisk outside histograms indicates an effect of olfactory experience, i.e., differences between adjacent means are statistically significant, $p<0.01$, Newman-Keuls. N = 7–11 animals per histogram. Days in cedar: dark bars: 0; hatched bars: 10.

Hoarding scores were not significantly affected by odor, F(1,12) = 1.002, $p>0.05$, so the data for the two rearing odors were pooled for further analysis. The effect of housing on the number of pellets hoarded approached, but did not reach significance, F(2,15) = 3.162, $0.10>p>0.05$. However, the planned comparisons indicated that animals in the control-only groups hoarded significantly more pellets per rat than either DSP-4-only or mixed groups, $p<0.05$, Dunnett test, Fig. 3. Therefore, neonatal DSP-4 treatment disrupted hoarding by drug-treated animals. Moreover, scores of DSP-4-only and mixed groups were virtually identical, suggesting that the presence of DSP-4-treated cagemates interfered with hoarding by controls. In summary, hoarding deficits paralleled deficits in adaptation to the home-cage olfactory environment.

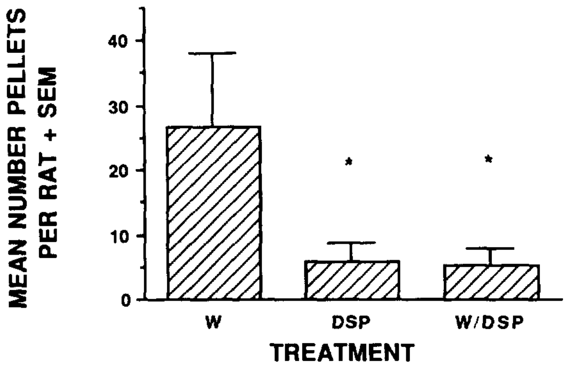

FIG. 3. Effects of neonatal DSP-4 treatment and social housing conditions on hoarding behavior. Each histogram indicates mean+SEM for 6 cages of animals. Asterisks indicate means are significantly different from control-only group, $p<0.05$, Dunnett test.

DISCUSSION

As seen in an earlier study (3), housing juvenile rats in a new bedding odor after weaning increased preference for the odor if the animals were living in control-only groups. However, significant preference increases did not result if the animals had been neonatally treated with DSP-4, or were living in mixed groups of control and drug-treated animals. The control-only groups also hoarded more pellets of chow than animals in the other two social conditions. The data support the idea that manipulations that disrupt olfactory learning about the home environment, also decrease hoarding behavior.

The attenuated olfactory learning shown by DSP-4-treated rats is consistent with previous data suggesting that neonatal NE depletion impairs the ability to adapt to a new olfactory environment after weaning (3,4). The finding that mixed housing impairs experience-dependent responses by controls to home-cage odors also agrees with earlier results (3). Evidently, housing the two groups together creates a social environment that is aversive to controls, and the negative effects of this situation generalize to home-cage odors.

Data from the pine-nest vs. pine test indicates that deficits in olfactory learning did not reflect anosmia or an inability to respond to odors in the testing situation. Cedar-housed animals in all treatment and housing groups showed statistically significant preferences for pine bedding soiled by odors from their female siblings. The finding that these conspecific odors were more attractive to animals housed in cedar than in pine is similar to data obtained earlier in an experiment using 6-hydroxydopamine (4), although it was not seen in another study, conducted in a different laboratory, using 6-hydroxydopa and DSP-4 (3).

We think that the explanation for the difference in results is that animals providing the shavings in the latter study were housed in metal cages while donors in the present study and the 6-hydroxydopamine experiment were housed in plastic cages similar to those of the animals tested. A comparison of preference scores from the three experiments indicated that pine bedding from the metal cages was less attractive than pine bedding from the plastic cages to cedar-housed rats, implying that the metal imparted an odor to the bedding. The data from the two experiments using plastic donor cages support the idea that pine-reared animals associated pine-nest odor with their postweaning environment, without the dam, and that cedar housed animals associated the odor with the preweaning environment (4).

The hoarding data are consistent with findings by Bindra (1) that normal rats which have never been food deprived will hoard under some circumstances. The failure to hoard by DSP-4-treated rats could reflect a direct effect of monamine depletion, and/or an indirect, socially mediated influence. A previous study which depleted NE centrally in food-deprived adult rats found no substantial effects on hoarding behavior (7). However, this does not eliminate the possibility that systemic neonatal NE depletion might influence hoarding by satiated juvenile rats. This question should be pursued by examining the effects of other NE neurotoxins on hoarding behavior.

In addition, neonatal DSP-4 treatment might alter social behavior or other cues which influence hoarding. The finding that mixed housing impaired hoarding as much as DSP-4 treatment alone supports this hypothesis. One explanation of this result is that DSP-4-treated rats may have actively interfered with hoarding by control cagemates. Animals were not observed during hoarding, so we do not know whether this was the case. Future experiments should videotape the animals so that this ambiguity can be resolved.

Another possibility is that DSP-4-treated rats produced abnormal social cues which impaired hoarding by themselves and their cagemates. For example, experiments in progress in our laboratory indicate that normal naive rats prefer the odor of bedding from water-treated rats to bedding from DSP-4-treated rats. These results imply that DSP-4 treatment changes the odors the rats produce and that these changes can be detected by conspecifics. Additional experiments should determine whether exposing controls to the bedding odor of DSP-4-treated rats impairs hoarding.

Our data compliment evidence from other studies indicating that the social environment influences feeding behavior during development. For example, Pfister *et al.* (12) have demonstrated that suckling by juvenile rats can be prolonged by housing them with a dam who is still nursing younger pups. The juveniles usually suckled only after pups had attached to the dam. In addition, social factors can influence hoarding by the hamster, which is generally a solitary species in the wild (11). Turpin *et al.* (14) have reported that housing weanling hamsters in an environment which allows them to hear, see and smell, but not touch conspecifics, reduced hoarding behavior. The present data suggest that exposure to abnormal conspecifics can impair hoarding in a colonial species. The specific social factors which induce this deficit need to be investigated in future studies.

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REFERENCES

1. Bindra, D. The nature of motivation for hoarding food. J. Comp. Physiol. Psychol. 41:211–218; 1948.
2. Cornwell-Jones, C. A. Olfactory sensitive periods in albino rats and golden hamsters. J. Comp. Physiol. Psychol. 93:668–676; 1979.
3. Cornwell-Jones, C. A.; Decker, M. W.; Gianulli, T.; Wright, E. L.; McGaugh, J. L. Neonatal DSP4 reduces environmental influences on brain neurochemistry and olfactory learning in developing rats. Soc. Neurosci. Abstr. 15:466; 1989.
4. Cornwell-Jones, C. A.; Stephens, S. E.; Dunston, G. A. Early odor preferences of rats are preserved by neonatal 6-hydroxydopamine. Behav. Neural Biol. 35:217–230; 1982.
5. Cornwell-Jones, C. A.; Velasquez, P.; Wright, E. L.; McGaugh, J. L. Early experience influences adult retention of aversively motivated tasks in normal, but not DSP4-treated rats. Dev. Psychobiol. 21:

177–185; 1988.

6. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brain: I. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J. Neurochem. 13:655–669; 1966.
7. Kelley, A. E.; Stinus, L. Disappearance of hoarding behavior after 6-hydroxydopamine lesions of the mesolimbic dopamine neurons and its reinstatement with L-dopa. Behav. Neurosci. 99:531–545; 1985.
8. Kim, C.; Campanelli, C.; Khanna, J. M. Determination of picogram levels of brain catecholamines and indoles by a simplified chromatographic electrochemical detection method. J. Chromatogr. 282:151–159; 1983.
9. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks/Cole; 1968.
10. Miller, G. A.; Viek, P. An analysis of the rat's response to unfamiliar aspects of the hoarding situation. J. Comp. Psychol. 37:221–231; 1944.
11. Murphy, M. R. Natural history of the Syrian golden hamster—A reconnaissance expedition. Am. Zool. 11:632; 1971.
12. Pfister, J. F.; Cramer, C. P.; Blass, E. M. Suckling in rats extended by continuous living with dams and their preweanling litters. Anim. Behav. 34:415–420; 1986.
13. Sherry, D. F. Food storage by birds and mammals. Adv. Stud. Behav. 15:153–188; 1985.
14. Turpin, B.; Johnston, T. D.; Fulk, K. R. Sibling inhibition of hoarding in postweaning hamster pups (*Mesocricetus auratus*). Dev. Psychobiol. 21:467–476; 1988.