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Postnatal Pup Brain Dopamine Depletion Inhibits Maternal Behavior

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WILKINS, A. S., M. LOGAN AND P. KEHOE. *Postnatal pup brain dopamine depletion inhibits maternal behavior.* PHARMACOL BIOCHEM BEHAV **58**(4) 867–873, 1997.—The interactions between dams and their pups and among siblings were investigated in litters with (a) all pups depleted of striatal dopamine by 6-hydroxydopamine (6-OHDA on PND3), (b) all pups treated with vehicle, or (c) half of the pups depleted of dopamine and the other half treated with vehicle. On PND10, two sets of four pups from each litter were videotaped in a novel environment with the dam; pup and maternal behaviors were later scored by blind observers. We observed a 70% decrease in striatal dopamine in 6-OHDA–treated pups but found no effect of treatment on pup weight gain. Dams with some or all DA-depleted pups (a) were slower to retrieve a pup and establish a nest, (b) retrieved pups less frequently, and (c) spent less time huddling with pups than dams with only vehicle-treated pups. When compared with DA-depleted pups in homogeneous litters, DA-depleted pups in mixed litters were less hyperactive and spent more time huddling with other pups than in isolation. These results suggest that DA-depleted pups receive compromised maternal care but can benefit from interactions with normal siblings. © 1997 Elsevier Science Inc.

Maternal behavior 6-Hydroxydopamine Pup growth Licking Retrieval behavior HPLC Dopamine Ultrasonic vocalizations Sprague–Dawley rats

THE mother–infant relationship is typically regarded as symbiotic (12,13,19,35). In the rodent, the nursing behavior that occurs between the dam and her newborn pups involves participation of both members (37,38). When separated from the dam, pups signal the dam to retrieve them through olfactory (34,36), visual (36), and auditory cues (1,3,23). After the dam retrieves her pups into a nest, she then hovers over and licks them (37). This licking typically involves anogenital licking of the pup, which is beneficial for both the dam and the pup (12,13). Pups are active participants in the licking interaction, but males are licked more than females, perhaps because they respond more rapidly to maternal stimulation than do females, or because they have a different odor than do females (20).

The neonatal dopaminergic system may play an important role in mediating pup behavior and dam–pup interactions. While one study (29) demonstrated that 6-OHDA treatment of pups did not disrupt maternal behavior (nursing, nest building, retrieval, and locomotor activity), neonatal 6-OHDA treatment has been shown to impair pup body growth (9,26,27,29), and learning (2,10,22,30,32,33,41), while increasing locomotor activity (2,11,30–33,40). These results have varied across laboratories, however, as others have found no changes in body growth (6,7,30,40), decreases in locomotor activity (12,30), and no changes in behavior (25).

The environment in which pups develop has a significant impact on their development. For example, separation of a pup from its dam leads to a variety of immediate behavioral and physiological consequences, including increased motor activity (39), ultrasonic vocalizations (15,18), loss of body temperature (24), and a decrease in heart and respiratory rate (14). Hyperactivity (2,11,30,31,32,40) and impairments in learning (2,10,22,30,32,33,41) caused by treatment with 6-OHDA can, to some extent, be overcome when the environment in which pups are reared is changed. One experiment found that 6-OHDA–treated pups reared artificially (not with their dams) did not, unlike treated pups reared with their dams, display hyperactivity during the first month of life (11). Moreover, 6-OHDA–treated pups reared with control siblings displayed less hyperactivity (27,39) and improved learning in a shuttle box aversive learning paradigm (29) compared to 6-OHDA–treated pups reared only with other 6-OHDA–treated siblings. Another group enriched the environment of 6-OHDA–treated pups on PND0 from PND25 (after weaning) to PND60 (26). They found that placing DA-depleted an-

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imals in an enriched environment (i.e., large cage, new toys in cage every 3 days, intermittent exposure to music) eliminated spatial and memory deficits, as measured in the Morris water maze, that were caused by neonatal DA-depletion (hippocampal NE was reduced to 26% of vehicle control levels).

The goal of the present study was to answer two primary questions. First, what role does the neonatal dopaminergic system have in influencing or modulating the dam–pup relationship? To address this question, we analyzed the dam's ability, in homogeneous litters containing all 6-OHDA–treated pups or all control pups, to demonstrate maternal behavior by retrieving her pups, establishing a nest, and licking her pups. We also studied the pups' level of activity and ability to stimulate effectively the dam to provide these maternal behaviors. Second, what impact does a novel environment have on the dam–pup relationship for DA-depleted vs. control pups? Dams were challenged in a novel environment to determine the impact on maternal behavior and dam–pup interactions. Dam–pup interactions in heterogeneous litters containing both 6-OHDA–treated and control pups were compared with homogeneous litters containing all 6-OHDA–treated pups or all control pups to investigate the role sibling interactions might have on pup development and the dam–pup relationship.

METHOD

Subjects

Sprague–Dawley female rats (200–300 g) were mated in the Psychobiology Laboratory at Trinity College, Hartford, CT. Mothers and their litters were housed in plastic tubs with stainless steel lids providing Purina Lab Chow and water ad lib. Lights were maintained on a 12-h light:12-h dark cycle (lights on at 0700 h), and the colony temperature ranged from 23 to 25°C. The day on which newborn litters were found was designated as postnatal day 0 (PND0), and litters were culled to 12, with an equal number of males and females in each litter, on PND1. Twenty-five litters were used in the study.

Drug Treatment on PND3

Pups were removed from the dam, numbered with nontoxic markers [1–12], weighed, and huddled in a plastic bin with bedding at constant temperature (32°C) under a heat lamp. Litters were labeled as homogeneous control litters (Sal Homo), homogeneous treatment litters (6-OHDA Homo), or heterogeneous treatment litters (Hetero). All pups received intraperitoneal (IP) injections of either DMI (desmethylimipramine) or saline 30 min prior to receiving bilateral, 10 μl intracerebroventricular (ICV) injections. The dose was divided into two equal 5-μl portions and administered slowly (about 4 s each side) into each lateral ventricle (the injection volume and length are consistent with that of the following studies: (5–8,40). Unpublished experiments in our laboratory with cresyl violet dye indicated that the dye is distributed primarily and consistently into the lateral ventricles (17) and that the success rate of the procedure is 0.90. The experimental design is represented in Fig. 1.

ICV injections were performed with a hand-held 10 μl syringe equipped with a 27-gauge needle adjusted to a length of 3.2 mm. The researcher with one hand gently grasped the back of a pup's head to expose bregma as visible through the skin. The other hand was used to insert the needle according to the following coordinates: 0.5 mm anterior to bregma, 1.5 mm lateral to the saggital suture, and 3.2 mm ventral to the skull surface.

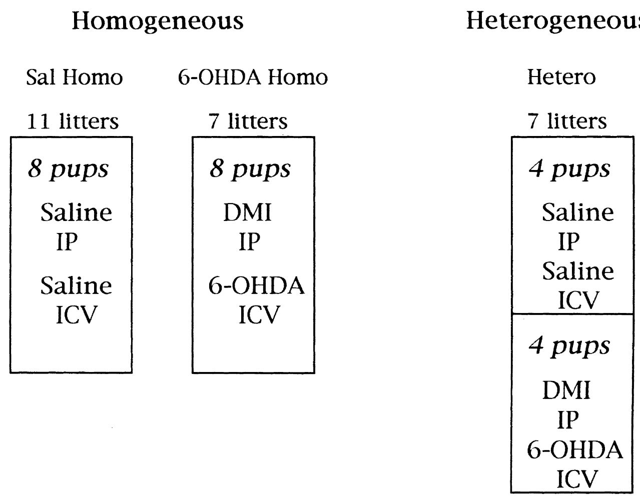

FIG. 1. In the experimental design, pups on PND3 were separated into a Sal Homo, 6-OHDA Homo, or Hetero treatment group. All pups in the Sal Homo group received saline (IP) prior to treatment with saline (ICV). All pups in 6-OHDA Homo group received desmethylimipramine (DMI, IP) prior to treatment with 6-hydroxydopamine (6-OHDA, ICV). Half of the pups in the each heterogeneous litter received saline (IP) prior to treatment with saline (ICV); the other half of the pups received DMI (IP) prior to treatment with 6-OHDA (ICV).

All control pups received 0.05 ml saline IP injections and saline ICV injections. In 6-OHDA Homo litters, all pups received DMI (25 mg/kg) IP injections prior to 300 mg of 6-hydroxydopamine (6-OHDA) in 10 μl ICV injections (150 mg/5 μl into each ventricle). In the Hetero litters, six pups received DMI IP injections and 6-OHDA ICV injections. The other six received saline IP injections and saline ICV injections. All pups were returned to the dam 1 h following ICV injections. We did not include a DMI + saline treatment group because we have previously observed (unpublished) no differences in maternal or pup behavior or pup weight when compared with the saline + saline treatment group.

Dam–pup Interactive Testing on PND10

Clean bedding was added to a transparent aquarium (50 × 26 × 30 cm), and a mirror (46 × 30 cm) was placed against the wall of the aquarium farthest away from the video camera (Panasonic) to prevent any behaviors of the dam or the pups from being hidden.

The dam was placed alone in the aquarium and allowed to habituate for 20 min. During this time, pups were weighed and divided into three groups. Because maternal dams interact differently with their male and female pups (21) both males and females were always included in the experimental design (two male:two female, one male:three female, or three male:one female). Mixed litters always consisted of two 6-OHDA–treated and two vehicle-treated pups.

Pups 1–4 were videotaped during the first session with the dam, pups 5–8 were videotaped during the second session. Pups to be videotaped were colored with nontoxic magic markers (e.g., red, blue, yellow, or green) to allow for pup differentiation during videotape scoring. After pups were weighed and colored, they were huddled in a plastic bin with home bedding in a controlled chamber at 30°C.

After the dam's 20-min habituation period, pups 1–4 were retrieved from the huddle, placed in the aquarium with the dam, and videotaped for 20 min. At the beginning of the recording session, each of the four pups was placed in a separate corner of the aquarium. A videotaped session was repeated for pups 5–8 after pups 1–4 were returned to the huddle.

Within half an hour of recording, pups 1–8 were sacrificed and the striatum of each pup removed and placed in a weighed Eppendorf tube on dry ice [see (5–8,40)]. All Eppendorf tubes were reweighed and placed in a freezer at −70°C. Throughout videotaping and during dissections, nonvideotaped pups 9–12 were maintained in the pup huddle on home bedding to prevent any of pups 1–8 from spending time in isolation.

Videotape Scoring Criteria

Videotape scorers were blind to pup treatments. Each 20-min session was viewed five times: the first viewing allowed for scoring of the dam's behavior, and the other four viewings allowed for scoring of behavior of each of the pups in the videotaped group.

Dam-initiated Behaviors

Latency to first retrieval. The time elapsed before the dam picked up a pup in her mouth and moved it to another location in the aquarium.

Latency to one nest. The time elapsed before all four pups were in physical contact with each other in a huddle.

Pup retrieval. Any time in which a pup was picked up by the dam and moved to another location in the aquarium (not a rearrangement of a pup within the nest).

Pup licking. Any period in which the dam was licking a pup on any part of the pup's body.

Dam isolation. A maternal isolation period began when the dam ceased physical contact with any of her pups and ended as soon as physical contact was resumed with any pup.

Burrowing. Any episode during which the dam was pushing the aquarium bedding with her snout or paws.

Wall climbing. Any attempts to climb out of the aquarium, or any period in which one or both front paws were placed on the wall of the aquarium. A wall climbing incident ceased when the dam returned all four limbs to the bedding of the aquarium.

Self-grooming. Any period during which the dam was licking or stroking herself.

Pup-dependent behaviors. These behaviors are interdependent. For example, a pup cannot be in isolation and in a dam huddle at the same time.

Pup movement. Any time during which a pup was using all four limbs to locomote itself from one location to another.

Pup isolation. Any time during which a pup was not touching the dam or any other pups.

Dam huddle. Any time during which a pup was in physical contact with the dam, except her tail. The pup may or may not have been in contact with other pups.

Pup huddle. Any time during which a pup was in physical contact with one or more pups, but not the dam.

By pausing the videotape at appropriate times, the scorer was able to note the time at the beginning and end of each behavior. This allowed for the determination of the total time spent on each behavior. Pup retrievals were scored by simply noting the total number of times a pup was retrieved. Overlap of behaviors was possible so that, for example, a pup could be licked by a dam while in a dam huddle.

Neurochemical Analyses

Catecholamines were extracted from striatal tissue by sonicating tissue in 250 ml of perchloric acid and 250 ml of DHBA for 20 s. The homogenate was then centrifuged (Fischer micro-centrifuge, Model 235B) at top speed for 15 min, and the supernatant was placed in a separate Eppendorf and frozen at −70°C until ready for chemical analysis.

Catecholamine concentrations of the supernatant were determined by using high-performance liquid chromatography (HPLC) with electrochemical detection (EG&G, Princeton Applied Research, Model 400). A Gilson pump 307 delivered a mobile phase (pH 3.7) containing 275 mg of sodium octyl sulfate, 100 mg of ethylene-diaminetetraacetic acid (EDTA), 55.2 g of NaH_2PO_4, and 80 ml of acetone, per 2 liters. External standards (10^{-6}) of DHBA, NE, DOPAC, and DA were run approximately every six samples.

The data were stored by a Gateway 2000 computer, and analysis was performed using a Gilson 712 HPLC program. The chromatograms provided catecholamine height measurements, from which picograms of dopamine and norepinephrine were calculated. To obtain percentage dopamine depletion consequent to 6-OHDA treatment, the picograms of dopamine of each treated pup were compared to the average picograms of dopamine of all control pups.

Statistical Analyses

One-way analyses of variance were performed to determine main effects of treatment group (Sal Homo vs. 6-OHDA Homo vs. Hetero) on various dam and pup behaviors. Post hoc statistical comparisons (Newman–Keuls) were performed to dissociate significance between treatment groups. The following data were collapsed because no differences were found: 1) behavioral data between the first and second testing sessions with each dam, 2) data between males and females [except for offspring weight and licking duration, in which two-way ANOVAs (treatment × sex) were performed], and 3) data between DA-depleted and control pups in mixed litters.

RESULTS

Catecholamine Levels

Brain striatal DA levels in animals injected on PND3 with 6-OHDA following pretreatment of DMI were reduced on P10 to 30% of control levels, $F(1, 66) = 10.79$, $p < 0.002$. Brain NE levels of 6-OHDA–treated animals on P10 were not significantly reduced from levels of control animals (treated mean = 0.687 pg; control mean = 0.581 pg). There were no significant differences in DA levels between Homo and Hetero 6-OHDA–treated pups or between Homo and Hetero control pups.

Offspring Weight Gain

A two-way ANOVA (treatment × sex) indicated that there was no significant difference in percentage weight gain between PND3 and PND10 for 6-OHDA–treated and control animals. Separate two-way ANOVAs (treatment × sex) indicated no differences in weight on either day across treatment groups. However, there was a main effect of sex on weight on PND10, $F(1, 41) = 6.78$, $p < 0.01$.

Dam-initiated Behaviors

Burrowing. A one-way ANOVA revealed a significant main effect of treatment group on maternal burrowing duration, $F(2, 41) = 3.22$, $p < 0.05$. 6-OHDA dams spent significantly more time burrowing in each interactive session than Sal Homo or Hetero dams (Newman–Keuls, $p < 0.05$; Table 1). There were no significant effects of treatment group on maternal wall climbing or self-grooming.

Dam isolation. There was a significant effect of treatment group on dam isolation, $F(2, 41) = 12.53$, $p < 0.0001$. Both 6-OHDA (Newman–Keuls, $p < 0.05$) and Hetero (Newman–Keuls, $p < 0.01$) dams spent more time in isolation (i.e., not in contact with any pups) than Sal dams (Table 1).

Retrieval Behavior

An ANOVA revealed a significant main effect of treatment group on dam latency to retrieve one pup, $F(2, 41) = 4.12$, $p < 0.03$; Table 1. 6-OHDA (Newman–Keuls, $p < 0.05$) and Hetero (Newman–Keuls, $p < 0.05$) dams took significantly more time to retrieve one pup than Sal dams. After retrieving one pup, dams then established a nest by retrieving all four pups into one location. There was a main effect of nest latency across groups, $F(2, 41) = 3.76$, $p < 0.04$; Table 1; on average, 6-OHDA dams took 5 min longer and Hetero dams 3 min longer to establish a nest than Sal dams.

In addition to retrieving one pup and establishing a nest more quickly than 6-OHDA and Hetero dams, Sal dams retrieved their pups more frequently in each testing session than 6-OHDA (Newman–Keuls, $p < 0.05$) and Hetero (Newman–Keuls, $p < 0.05$) dams, $F(2, 24) = 3.48$, $p < 0.05$; Fig. 2. There were no differences in nest latency, retrieval latency, or number of retrievals between 6-OHDA or Hetero dams.

Pup Licking

A two-way ANOVA (treatment × sex) revealed a main effect of pup sex on maternal licking duration, $F(1, 41) = 4.81$, $p < 0.03$; (Fig. 3). Males were licked for a longer amount of time than females in both the Sal and 6-OHDA groups. In the Hetero group, however, there was no significant difference in the amount of time licked between males and females. Hetero males were licked less than both Sal (Newman–Keuls, $p < 0.05$) and 6-OHDA (Newman–Keuls, $p < 0.05$) males. 6-OHDA and Hetero females were licked less than Sal pups (Newman–Keuls, $p < 0.05$). There was no difference in licking duration between 6-OHDA and Sal pups in the Hetero group.

TABLE 1

DAM-INITIATED BEHAVIORS

Behavior	Sal Homo	6-OHDA Homo	Hetero
Dam burrowing	27 ± 6	82 ± 34*	32 ± 12
Dam isolation	211 ± 18	312 ± 58*	448 ± 44†
Dam huddle	804 ± 22	615 ± 50†	573 ± 23†‡
Retrieval latency	90 ± 23	285 ± 87†	325 ± 113
Nest latency	225 ± 54	512 ± 121*	415 ± 82

Duration each behavior was performed during 20-min testing sessions with the dam and her pups in a novel environment.

Means ± SEM.

Note: these behaviors are interdependent.

*$p < 0.05$ when compared with Sal Homo group.

†$p < 0.01$ when compared with Sal Homo group.

‡$p < 0.01$ when compared with 6-OHDA Homo group.

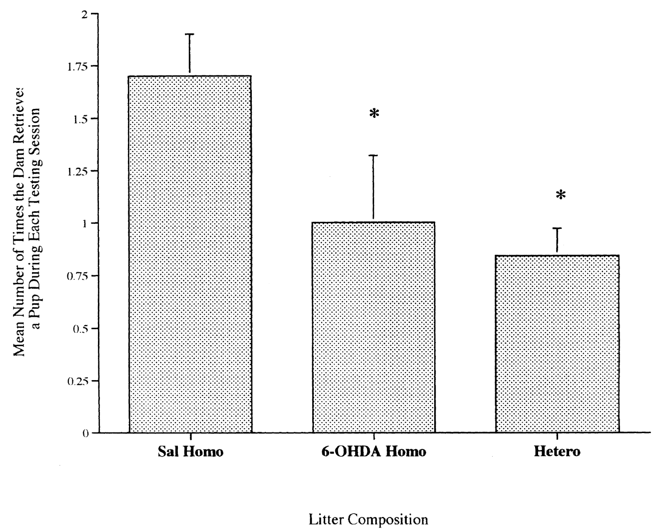

FIG. 2. The mean number of times dams retrieved each pup during each testing session. *$p < 0.05$ when compared with the Sal Homo group. Error bars indicate SEMs.

Pup-dependent Behaviors

Huddling vs. isolation. Tables 1 and 2 represent a balance of time in which pups were either huddling with the dam (dam huddle), huddling with one or more siblings but not the dam (pup huddle), or in isolation (pup isolation). These three measures are interdependent; as a pup spends more time with the dam, the amount of time that it can spend in a pup huddle or in isolation must decrease (dam huddle time + pup huddle time + pup isolation time = total testing time).

A one-way ANOVA revealed a main effect of pup time spent with the dam, $F(2, 24) = 8.92$, $p < 0.002$ (Table 1). 6-OHDA and Hetero pups spent less time with the dam than did Sal pups (Newman–Keuls, $p < 0.05$). During each 20-min

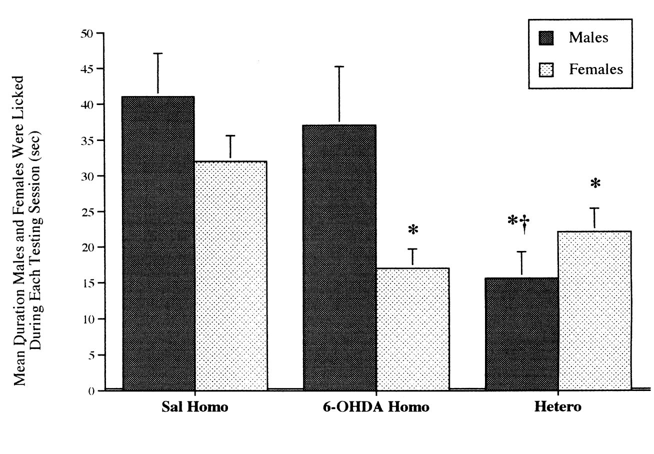

FIG. 3. The mean amount of time dams spent licking males and females during each testing session. *$p < 0.05$ when compared with pups of the same sex within the Sal Homo group. †$p < 0.05$ when compared with 6-OHDA Homo group. Error bars indicate SEMs.

TABLE 2
PUP-DEPENDENT BEHAVIORS

Behavior	Sal Homo	6-OHDA Homo	Hetero
Pup huddle	249 ± 15	400 ± 50†	543 ± 21†
Pup isolation	70 ± 15	125 ± 18*	88 ± 19
Pup movement	8.5 ± 0.9	26 ± 5.1†	12 ± 0.9

Duration each behavior was performed during 20 minute testing sessions with the dam and her pups in a novel environment.
Means ± SEM.
Note: these behaviors are interdependent.
*$p < 0.05$ when compared with Sal Homo group.
†$p < 0.01$ when compared with Sal Homo group.

testing session, 6-OHDA pups on average spent 3 min, and Hetero pups 4 min less time huddling with the dam than Sal pups. Conversely, 6-OHDA and Hetero pups spent more time huddling with siblings than Sal pups, $F(2, 24) = 8.25$, $p < 0.003$ (Table 2). Yet as Hetero pups spent more time huddling with siblings than 6-OHDA pups (Newman–Keuls, $p < 0.05$), 6-OHDA pups spent more time in isolation than Hetero or Sal (Newman–Keuls, $p < 0.05$) pups. There were no differences between 6-OHDA and saline-treated pups in the Hetero group.

Pup Activity

A one-way ANOVA revealed a main effect of treatment group on pup activity level, $F(2, 25) = 4.30$, $p < 0.03$ (Table 2). 6-OHDA pups spent more time locomoting in each testing session than Sal or Hetero pups (Newman–Keuls, $p < 0.05$). There was no significant difference between 6-OHDA and saline-treated pups in the Hetero group, and the combined Hetero mean did not differ from the mean for Sal pups.

DISCUSSION

6-OHDA Treatment and Pup Weight

We observed no difference in percentage weight gain from PND3 to PND10 between control and DA-depleted pups, regardless of litter composition. Likewise, there were no differences between control and DA-depleted pups in heterogeneous litters. While several groups have found decreases in neonatal weight consequent to 6-OHDA treatment (9,26, 27,29), others have found no effect of 6-OHDA on weight (6,7,27,28,30,40). The varying effects of 6-OHDA treatment on pup weight may be attributed to a number of factors, which may include a) strain differences, b) the age at which pups were treated with 6-OHDA, c) the age at which pups were later weighed, and d) the dose of 6-OHDA used.

In the present experiment, the lack of a significant weight difference between 6-OHDA–treated and control pups on PND10 may initially seem incompatible with the behavioral differences found between experimental groups. Indeed, it may be the case that a deficit in pup growth would have emerged after PND10 (6) and/or the neurotoxin treatment was not effective in creating a large enough deficit in pup growth. Although either or both of these may be true, the fact remains that behavioral differences exist independent of a decrement in pup weight.

The testing situation is not representative of what happens in the home cage with a full litter present. It seems likely, given our behavioral findings independent of a 6-OHDA–induced deficit in pup growth, that the challenging nature of the testing environment (i.e., dam separation from pups prior to videotaping, testing chamber different from home cage, short testing sessions, partial litter during testing) is necessary to evoke the effects of pup DA-depletion on maternal behavior. Specifically, the relatively short 20-min habituation sessions [compare with 4 h: (38)] and 20-min testing sessions [compare with 1 h: (29,30) and 30 min: (38)] emphasize not only the dams' adaptability to their surroundings but also the dams' ability to demonstrate maternal behavior in their surroundings.

The Initiation of Dam–Pup Interactive Sessions

Dams with some or all DA-depleted pups were delayed in initiating interactive sessions with their pups. Because there was no difference in retrieval latency for pups tested immediately following dam habituation or 20 min after the first testing session, retrievals were probably dam initiated. Pups that were tested second were not treated differentially from pups tested first. Dams with any DA-depleted pups were slower to retrieve a pup to build a nest and slower to establish a complete nest of four pups. In addition, throughout testing sessions, these dams retrieved pups less frequently than dams with control pups. Although the dams with DA-depleted pups eventually retrieve them all, they seem to be less efficient at the task.

We suspect that the increased latency and reduced frequency to retrieve DA-depleted pups stems, at least partially, from these pups' impaired ability to notify the dam of their presence via ultrasonic vocalizations, which are known to contribute to the rapidity with which dams locate and retrieve displaced pups (1,4,16,23,34,37,38). In our laboratory, we have observed that 6-OHDA–treated pups in isolation vocalize less than untreated pups (unpublished observations) (17). In this experiment, perhaps the 6-OHDA treatment disrupted the development of brain neurochemical systems that influence pup vocalizations, which would have impaired these pups' ability to vocalize when in isolation (i.e., not in contact with the dam). However, because the retrieval delay of dams with DA-depleted pups in this experiment is greater than that of dams with anesthetized pups incapable of vocalizing (38), another dam-pup interactive deficiency may be additionally affecting the rate at which dams retrieve their pups. This deficiency is probably dependent upon the challenging nature of the testing paradigm, but it is possible that the dam–pup deficiency may exist in the dam–litter interaction history prior to the test.

Dam–Pup Interactive Behaviors

After pups are retrieved to a nest, the dam nuzzles, hovers over, and licks her pups (38). In this experiment, males were licked more than females, which is consistent with previous findings (20,21). Across treatment groups, pups in heterogeneous litters but not DA-depleted pups in homogeneous litters were licked less than controls. Yet while all pups in heterogeneous litters were licked less, they did not 1) spend less time huddling with the dam than pups in homogeneous litters, or 2) demonstrate decreased weight gain between PND3 and PND10. These results suggest that there may be significant behavioral differences among the pups in the heterogeneous litters, presumably between DA-depleted and control pups, that may interrupt the dam's licking of her pups in the novel environment.

Dams with all DA-depleted pups spent more time burrowing during the testing sessions than did dams with some DA-depleted pups, which might indicate a greater level of arousal in dams with all DA-depleted pups because laboratory rodents burrow when they are excited or stimulated, and dams may be excited by the behavior of the DA-depleted pups. This heightened level of arousal may have affected dam–pup interactions, because dams with all DA-depleted pups spent less time with their pups than dams with control pups (i.e., in a dam huddle; Table 1). The novel environment in which dams were tested may have contributed to increased burrowing and decreased huddling. Although allowed to habituate to the novel environment for 20 min, dams with all DA-depleted pups (vs. dams with some DA-depleted pups) may have demonstrated a heightened stress response to the novel environment.

Dams with all or some DA-depleted pups spent less time huddling with their pups than did dams with only control pups (Table 2). This is not surprising, because dams with DA-depleted pups spent more time in isolation (Table 2) than dams with only control pups. Moreover, isolation and huddling are interdependent measures (i.e., the dam cannot be doing both at the same time). Although all dams did eventually establish a nest before the end of each testing session, dams with DA-depleted pups may be less effective in responding to the challenge of displaying maternal behavior (huddling) in the novel environment. Because there was no difference in pup percentage weight gain across groups from PND3 to PND10, dams with DA-depleted pups in the home cage may demonstrate maternal behavior comparable to that of dams with control pups. Moreover, the effect that the novel environment had on pup's behavior is unknown (i.e., were the pups challenged in the novel environment?), even though the bedding, the dam, and siblings were all familiar to the pups during testing.

Impact of the Environment on the Dam–Pup Relationship

Litter composition (DA-depleted vs. control pups) had a significant impact on the dam–pup relationship. When not with the dam, DA-depleted pups in homogeneous litters spent more time in isolation than huddling with siblings; pups in heterogeneous litters, conversely, spent more time huddling with siblings (and not the dam) than in isolation. DA-depleted pups in homogeneous litters were also more hyperactive than pups in heterogeneous litters. One hypothesis that might account for these differences involves the "normal" behavior of the control pups in the mixed litters. One group observed that 6-OHDA treated pups tested on PND15, 19, or 22 tended to be "the more vigorous initiators of activity, poking and prodding their littermates into continuing activity" (39). In our experiment we observed a similar effect. In the litters with all DA-depleted pups, pups showed increased movement and upon contact, successfully prodded one another into initiating movement. Conversely, in the heterogeneous litters, when the DA-depleted pups prodded the control pups, the control pups were not prodded into movement, and DA-depleted pups usually ceased their movement. In this respect, the DA-depleted pups in heterogeneous litters did not display increased movement over controls, which is consistent with previous findings (39).

The testing environment also affected the dam–pup relationship. The discrepancy between the current findings and those of a similar study (29), which found no effect of 6-OHDA pup treatment on maternal behavior, may be explained by several differences in experimental design between paradigms. In general, dams in this experiment were perhaps more challenged by the testing environment than dams in the previous study. Specifically, 1) pups in the previous experiment were placed together in the middle of the testing chamber (at the onset of testing) instead of in the corners of the testing chamber, so that dams did not have to "work" to retrieve pups or establish nests; 2) dams were not isolated from their pups prior to testing (i.e., no habituation period); 3) mothering activity was recorded for 1-h sequences rather than 20-min sessions; and 4) eight pups were videotaped at a time with the dam instead of four pups. Another laboratory demonstrated why the number of pups used in testing may affect dam–pup interactions: they found that dams with four pups (vs. eight pups) had increased activity and self-grooming and somewhat less time crouching during 30-min testing sessions (38).

CONCLUSIONS

Dams with some or all DA-depleted pups displayed a delayed onset of maternal behavior, increased isolation from their pups, decreased licking of a subset of their pups, and fewer retrievals of their pups. Dams with heterogeneous litters treated their pups as a unit, as though all were DA-depleted, even though these dams spent significantly more time burrowing than dams in other groups. The presence of control pups did not serve to "improve" the maternal care that DA-depleted pups in mixed litters received: there were no specific differences in maternal care received between DA-depleted and control pups in heterogeneous litters, and DA-depleted pups with control siblings received comparable care to DA-depleted pups in homogeneous litters.

Despite the fact that maternal care to DA-depleted pups was not improved in the heterogeneous litters, the control siblings in mixed litters may somehow compensate for the behavior deficits of their DA-depleted siblings. It has been shown that DA-depleted pups reared in heterogeneous litters (vs. DA-depleted pups in homogeneous litters) were improved in avoidance learning on PND27 (27). In addition, we found that pups in mixed litters were less hyperactive than DA-depleted pups in homogeneous litters (27). We similarly found that there were no differences in activity levels between DA-depleted and control pups in mixed litters. This parallels the finding of an insignificant 1.5% decrease in 6-OHDA–treated pup activity in mixed litters (39).

In the present experiment we have extended these findings: we observed that pups in mixed litters spent more time huddling and DA-depleted pups in homogeneous litters spent more time in isolation. Taken together, these results suggest that sibling interactions and not maternal care transiently improve the developing pups' environment. The extent to which sibling interactions affect long-term maturation remains to be determined.

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