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Interaction With Pups Enhances Dopamine Release in the Ventral Striatum of Maternal Rats: A Microdialysis Study

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HANSEN, S., A H. BERGVALL AND S. NYIREDI. *Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: A microdialysis study.* PHARMACOL BIOCHEM BEHAV **45**(3) 673–676, 1993.—A growing body of evidence suggests that an interference with dopamine (DA) transmission disrupts maternal behavior in the rat. The present brain microdialysis study was therefore conducted to investigate whether infants can modulate ventral striatal DA release in mother rats. There was a significant rise in the extracellular concentrations DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in the ventral striatum when mothers were reunited with their litters following separation overnight. Nursing was the predominant behavior during this phase of the experiment. More active behaviors were elicited by soiling pups with flowerpot earth, and this was accompanied by further increases in DA, DOPAC, HVA, and 5-HIAA. It is suggested that pup-induced stimulation of ventral striatal DA release facilitates parental responses such as pup retrieval.

Dopamine Mesolimbic dopamine system Maternal behavior Microdialysis Nucleus accumbens
DOPAC HVA 5-HIAA Ventral striatum

RECENT studies show that the activity of the mesolimbic dopamine (DA) system in rodents modulated by social cues from influential conspecifics. For example, during separation from the mother DA turnover in the septum increases in infant guinea pigs (27). High-ranking male rats can enhance DA release in the nucleus accumbens of submissive animals (16), and the presence of a sexually active female increases ventral striatal DA release in male rats (2,3,5,17,20,21). Considering the growing number of studies demonstrating a link between maternal behavior and central DA mechanisms in the rat (6–8,10,11,18,24,26), the present in vivo microdialysis (29) experiment was undertaken to determine whether infant rats can alter DA transmission in their mothers. Lesion studies suggest that DA neurons innervating the nucleus accumbens may be particularly important for certain aspects of maternal behavior (10,11). Therefore, neurochemical measurements were made from dialysates collected from the ventral striatum of maternal females interacting with pups after a period of separation.

METHOD

Subjects were 11 primiparous Wistar rats (Möllegaard Breeding Laboratories, Denmark), nursing litters of 10 pups. They were maintained in a temperature-controlled colony room in which the lights were off between 10:00 a.m. and 10:00 p.m., and they had free access to water and food (Ewos, Sweden). Sometime between days 3–10 postpartum, each mother was anesthetized with Brietal (sodium methohexital, Lilly; 80 mg/kg), trepanated 1.6 mm anterior to bregma and 1.5 mm lateral to midline, and a guide cannula (3 mm) was fixed to the skull by acrylic dental cement (Svedia, Enköping, Sweden). A microdialysis probe (CMA10 with a dialysis polycarbonate membrane length of 4 mm; molecular cut off below 20,000 Da) was then inserted through the cannula such that it protruded approximately 8 mm below the skull (19). The animal was then placed into a circular transparent cage (40 cm in diameter; CMA120), the floor being covered with wood chips, food pellets, and nesting material, and connected to a balancing arm with a liquid swivel. The inlet tubing of the probe was connected to a CMA 100 perfusion pump and the outlet tube ending secured to an Eppendorf vial holder on the swivel.

On the following day, 15–18 h after surgery, the probe was perfused with a Ringer solution (Pharmacia) at a rate of 2 μl/min and samples were collected at 20-min intervals. The perfusion fluid (pH = 6, osmolality: 290 mOsm) contained 300 mg KaCl, 330 mg CaCl, and 8.6 g NaCl per 1 l sterile water. Three to five baseline samples were collected until stable values were attained. Pups, which had been housed in an incubator (32°C) overnight, were then reunited with their

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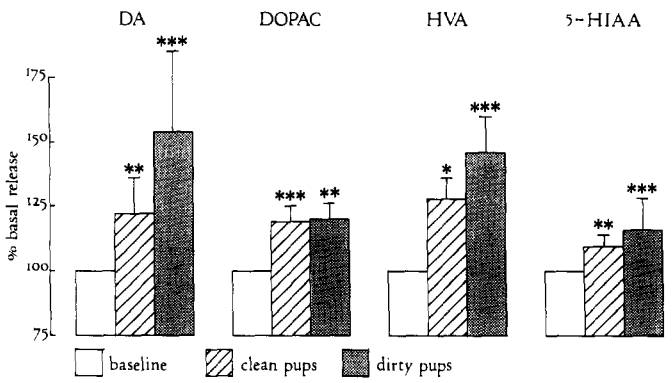

FIG. 1. Changes in the extracellular concentrations of dopamine (DA),3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in maternal rats interacting with their litter after a period of separation. *$p < 0.05$, **$p < 0.02, p < 0.01$ (Wilcoxon matched-pairs signed-ranks test).

mother for 1 h while sampling continued. In preliminary experiments, it was noted that nursing was the predominant maternal response during this time. To elicit more active maternal behaviors, pups were subsequently smeared with flowerpot soil mixed with water, whereupon two additional samples were collected. The mother was finally given an IP injection of *d*-amphetamine sulfate (0.5 mg/kg; Sigma Chemical Co., St. Louis, MO) to ensure that our system was sensitive enough to pick up changes in DA and its metabolites, an important control if no significant alterations in DA release would be detected as a consequence of pup exposure. In eight cases, mothers' behavior toward pups was videotaped using a Panasonic MS95 camera. The cumulative time spent *rearing, grooming, feeding, drinking, digging, nest building, pup sniffing, pup licking*, and *nursing*, together with the frequency of *wet-dog shaking* and *pup retrieval*, was later determined by examining the videorecordings. Following completion of the observations, animals were given a lethal dose of Brietal and brains were stored in 10% formalin. Brains were subsequently cut with a microtome through the relevant segment. Following staining with cresyl violet and microscopic examination of the probe tracks, it was verified that the probes had been situated in the ventral striatum. However, because the dialysis membrane lengths were 4 mm long it is likely that neurochemicals from the dorsal striatum also diffused into the perfusion fluid.

Immediately after collection of the samples, the concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were determined. A BAS 460 HPLC system with electrochemical detection (Bioanalytical Systems, West Lafayette, IN) was used together with a BAS Phase II ODS column. The mobile phase consisted of a 0.1 M NaH_2PO_4 buffer (pH = 4.0) to which 6.0 g acetic acid, 112 mg Na_2 EDTA, 302 mg Na-octylsulfonate, 52.3 g acetonitrile, and 11.4 g tetrahydrofuran (Sigma) had been added. The flow rate was 0.5 ml/min and the working electrode was set at 0.7 V.

The values are expressed as percent of the last baseline value. The median concentrations of DA, DOPAC, HVA, and 5-HIAA during the first hour of pup reunion (i.e., three samples) was compared with the last baseline sample by the Wilcoxon test (23). Similarly, the average neurochemical concentrations detected in dialysates collected while the mother was interacting with dirty pups (two samples) were compared with the last clean pup sample. Finally, the values determined 20–40 min after *d*-amphetamine injection were contrasted to those seen in the last dirty pup sample. Because of technical difficulties, the DA values for two rats could not be determined.

RESULTS

Figure 1 shows that in the ventral striatum there were significant increases in the extracellular levels of DA ($T = 3$, $n = 9$, $p < 0.02$), DOPAC ($T = 0$, $n = 11$, $p < 0.01$), HVA ($T = 11$, $n = 11$, $p < 0.05$), and 5-HIAA ($T = 0$, $n = 11$, $p < 0.002$) following pup reunion. Table 1 shows that mothers spent more than 50% of the time nursing their litters during this period. Further increases in the concentration of these neurochemicals were seen when pups were soiled with earth (Fig. 1). Thus, the levels of DA ($T = 2$, $n = 9$, $p < 0.01$), DOPAC ($T = 7$, $n = 11$, $p < 0.02$), HVA ($T = 0$, $n = 11$, $p < 0.01$), and 5-HIAA ($T = 0$, $n = 11$, $p < 0.01$) were significantly higher when dirty rather than clean pups were present in the cage. As seen in Table 1, in which the values from both collection periods are collapsed, the introduction of dirty pups significantly stimulated active maternal behaviors such as nest building, as well as other activities (grooming, wet-dog shakes, digging). During the first 20 min of this phase, pup retrieval was also significantly enhanced ($p < 0.05$ vs. the previous phase) whereas the time spent nursing decreased (16%, $p < 0.01$ vs. the previous phase).

As expected from many previous studies (28), *d*-amphetamine (0.5 mg/kg) increased the extracellular concentrations of DA (273%, $T = 0, n = 9, p < 0.01$) and 5-HIAA (135%, $T = 8, n = 11, p < 0.05$) relative to baseline levels whereas DOPAC levels decreased (74%, $T = 0, n = 11, p < 0.01$).

DISCUSSION

The present results demonstrated that the extracellular concentrations of DA, DOPAC, and HVA increase significantly in the striatum of maternal rats reunited with their pups after several hours of separation. During the first hour of reunion, at which time mothers mostly nursed infants, the DA levels increased by an average of 123% from baseline (100%). A

TABLE 1

BEHAVIOR OF MOTHER RATS FOLLOWING REUNION WITH THEIR PUPS DURING MICRODIALYSIS SAMPLING

	Clean Pups	Dirty Pups
Rearing	4.8 ± 3.8	8.0 ± 7.2
Grooming	47.8 ± 20.4	80.8 ± 24.7*
Wet-dog shaking	1.4 ± 0.8	5.5 ± 2.1*
Feeding/drinking	0.0 ± 3.3	54.3 ± 54.9
Digging	37.8 ± 14.9	113.5 ± 21.3†
Nest building	18.3 ± 19.5	83.5 ± 27.1*
Pup retrieval	2.5 ± 0.5	4.0 ± 3.2
Pup licking/sniffing	207.8 ± 71.1	98.0 ± 67.5*
Nursing	621.3 ± 77.7	453.8 ± 208.3

The data are derived from the median values for three (clean pups) or two (dirty pups) 20-min-periods. Values for wet-dog shaking and pup retrieval are frequency measures; the remaining ones are durations (s/20 min). Values are medians ± semiinterquartile ranges.

*$p < 0.05$, †$p < 0.01$ (Wilcoxon test).

further increase (154% from baseline) was observed when pups were made dirty by coating them with flowerpot soil; this manoeuvre decreased nursing behavior and stimulated more active maternal and nonmaternal responses. Comparable observations of offspring modulation of DA release in the mother were reported by Kendrick, Keverne, and their coworkers in their pioneering brain dialysis studies of postpartum sheep. In this species, DA release is enhanced in the substantia nigra and olfactory bulb during suckling and attenuated following lamb separation (12).

Our results indicate that the mesolimbic DA system of maternal rats is activated by cues from newborns. Infants in acute demand of active parental care—by being dirty, outside the nest, perhaps also cold and vocalizing due to the smearing procedure—appeared particularly effective in this regard. It is not unlikely that this pup-induced DA activation in the mother is behaviorally important. For example, systemic administration of DA receptor antagonists disrupts most forms of active parental behaviors in the rat (6,8,10,26), and mesolimbic DA depletions impair the pup retrieval response (10,11). Thus, pups may promote their own welfare by stimulating ventral striatal DA release in the mother, a neurochemical event that might facilitate parental responses such as pup retrieval.

At present, one can only speculate as to the mechanism whereby pup exposure enhances DA release in the ventral striatum of maternal rats. Because the ventral striatum is afferented by olfactory-related brain areas (9), it is possible that pup odors are responsible. That conspecific olfactory cues indeed regulate mesolimbic DA transmission is illustrated by the demonstration that the odor of an estrous female activates DA release in the ventral striatum of male rats (5). Another possibility is that snout contact with the young in connection with sniffing, licking, and retrieval activities plays a role. Tactile information from the perioral region is crucial for maternal behavior in rats (14,25) and stimulates DA release in the striatum (1). Neuroendocrine mechanisms associated with suckling may also be involved. For example, there is evidence that prolactin, the levels of which are enhanced in the cerebrospinal fluid of nursing females (22), stimulates striatal DA release in the rat (15). Finally, one cannot exclude that nonspecific activation, enhanced activity, and/or the novel odor of the flowerpot soil brought about the increased DA levels, but it should be noted that neither forced locomotion nor exposure to a novel environment increase ventral striatal DA release appreciably in the rat (5).

Along with the increase of DA and its metabolites, there was a parallel increase in 5-HIAA levels. This appears to be in contrast to the situation in sheep, in which no reliable changes in 5-HIAA in relation to maternal behavior was detected (12,13). Our data indicate that serotonin neurons are also activated in maternal females when in contact with young. In view of the dramatic disruption of maternal behavior that accompanies depletion of central serotonin levels (4), it is possible that this activation contributes to the maintenance of maternal responsiveness in the rat.

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