*Pharmacology Biochemistry & Behavior*, Vol. 13, pp. 391–395. Printed in the U.S.A.

# Effects of 6-Hydroxydopamine and Amphetamine on Rat Mothering Behavior and Offspring Development

M. PICCIRILLO, J. E. ALPERT, D. J. COHEN[1] AND B. A. SHAYWITZ

*Child Study Center and Departments of Pediatrics, Psychiatry, and Neurology*
*Yale University School of Medicine, New Haven, CT 06510*

Received 15 October 1979

PICCIRILLO, M., J. E. ALPERT, D. J. COHEN AND B. A. SHAYWITZ. *Effects of 6-hydroxydopamine and amphetamine on rat mothering behavior and offspring development.* PHARMAC. BIOCHEM. BEHAV. 13(3) 391–395, 1980.—Selective CNS dopamine depletion was produced in neonatal female rats by administration of 6-hydroxydopamine and desmethylimipramine. These rats were subsequently tested as adults for maternal behavior toward their own offspring and received d-amphetamine prior to half of these sessions. The effects of early, selective CNS dopamine depletion and its related developmental disruptions on later care of offspring and response to amphetamine were thereby assessed. Pup retrieval, nest building, crouching, and locomotor behaviors were studied in 6-OHDA + DMI treated mothers while offspring were examined with respect to weight, density of fur, age of eye opening, and shuttlebox avoidance learning. Depletion of brain dopamine to 30% of control concentrations was associated with increased crouching time but with no other differences in caregiving, offspring development, or response to amphetamine. Although profound neonatal depletion of dopamine leads to a variety of disturbances in early life, later maternal behavior is adequate and apparently enhanced. The possible role of compensatory brain mechanisms is discussed.

Maternal behavior 6-Hydroxydopamine Amphetamine Dopamine Developmental disturbances Rat behavior

INVESTIGATIONS of physiological processes underlying the expression of maternal behavior have traditionally emphasized lesioning or ablation of neural structures or manipulation of hormonal parameters [5]. Within the last five years, attention has been directed to the study of discrete brain neurochemical systems which may be involved. In particular, several studies have implicated the integrity of central catecholamine and indolamine systems in the initiation and regulation of mothering. While acute depletion of brain serotonin with para-chloro-D,L-phenylalanine has been associated with unusual maternal aggression toward pups [1], depletion of norepinephrine with 6-hydroxydopamine (6-OHDA) 2 days preceding parturition has been reported to interfere with nursing and nest building behaviors, but not with pup retrieval [9]. Pervasive deficits in maternal responsiveness which encompass nursing, nest building, and pup retrieval have been described in dams sustaining depletion of hippocampal norepinephrine accomplished during the first week of gestation either with bilateral electrolytic lesions of the dorsal noradrenergic bundle or with surgical transection of the fornix [18]. Less acute and specific treatment, involving concomitant reduction of brain norepinephrine and dopamine concentrations with 6-OHDA at approximately 8 weeks prior to testing, was found to produce increased maternal protectiveness near the nest, reflected in defensive attack of an unfamiliar male and decreased latency of pup retrieval [17]. It has also been observed that tail pinch stimulation accelerates the onset of maternal behavior in virgin rats to whom newborn pups have been proferred. This facilitatory effect may be the result of activation of the nigrostriatal dopamine system [19].

The relationship between dopamine and maternal behavior has been studied with indirect (tail pinch stimulation) and nonspecific (6-OHDA depletion of both norepinephrine and dopamine) methods. The purpose of the present study was to examine the effects of selective and enduring brain dopamine depletion on rat mothers and the development of depleted dams' offspring. To avoid confounding the effects of dopamine depletion with the acute neurologic insult associated with adult administration of a neurotoxin, 6-hydroxydopamine and desmethylimipramine treatment leading to dopamine depletion was performed during the neonatal period.

Selective central dopamine depletion in the first postpartum week has been associated during the subsequent month with disturbances of habituation, conditioned avoidance learning, and the normal ontogeny of motor activity [10,14]. During the same period, these animals show a distinctive response to phenobarbital, methylphenidate and amphetamine [11, 12, 13]. Neonatally, dopamine depleted

[1]Send reprint requests to: Dr. Donald J. Cohen, Yale Child Study Center, 333 Cedar Street, New Haven, CT 06510.

pups have also been shown to be more promptly retrieved by their dams than controls [8]. Less is known about the behavioral and pharmacologic status of these animals as adults. The study presented in this paper examines the question of whether or not neonatally depleted dams continue to exhibit behavioral deviance and altered responsiveness to catecholaminergic stimulation as adults. Maternal behaviors and response to amphetamine during lactation are evaluated.

## METHOD

### *Animals*

Eight primiparous female Sprague-Dawley rats with one day old litters of ten female pups each were obtained from the Charles River Breeding Laboratories (Wilmington, MA) and housed individually on a 12 hr light-12 hr dark cycle (lights on at 7:30 a.m.) in 40×50×21 cm high opaque plastic bins with wire lids continuously supplied with Purina Rat Chow and water. Pups received experimental (selective brain dopamine depletion) and sham treatments at age 5 days, were weaned at age 25 days, and were housed as experimental-sham pairs. Randomly selected females were mated when they reached between 100 and 200 days of age and immediately thereafter were housed individually for the pre- and post-partum periods. Four treatment groups were formed from those dams successfully mated during the specified age range: of the dopamine depleted dams, five received 0.05 mg/kg d-amphetamine sulfate and five received 0.25 mg/kg; of the sham-treated dams, eight received 0.05 mg/kg d-amphetamine sulfate and eight received 0.25 mg/kg. All dams received saline on alternate days. No offspring received treatment.

### *Treatment*

Experimental and sham treatment of pups took place at age 5 days. Experimental treatment leading to selective depletion of dopamine consisted of intraperitoneal injection of 0.4 mg desmethylimipramine HCL and 0.2 mg L-ascorbic acid in 0.2 ml saline followed one hour later by intracisternal injection of 150 μg 6-hydroxydopamine and 10 μg L-ascorbic acid in 25 μl saline. Shammed animals received 10 μg L-ascorbic acid in 25 μl saline intracisternally. As mothers, each of these dams received intraperitoneal injections of either zero, 0.05, or 0.25 mg/kg of d-amphetamine sulfate (Sigma Chemical Co.) in 0.2 ml saline when her pups were 3, 4, 10, and 11 days of age. In each of the four subject groups, approximately half the dams received a given dose of amphetamine when their pups were 3 and 11 days of age and saline on Days 4 and 10 while the remaining dams received amphetamine on Days 4 and 10 and saline on Days 3 and 11. This design permitted each animal to serve as her own control for amphetamine treatment.

### *Materials*

One hour videotaped sessions of continuous maternal behavior were conducted in a lacquered plywood enclosure (1 $m^2$×30 cm) and were monitored and recorded with equipment employed previously [8]. Pup weights were determined with a Mettler PR 700 and adult weights, with a Dial-O-Gram scale (cap 1600 g, Ohaus Scale Corp.). Pup escape-avoidance learning was studied with a single unit, enclosed shuttle box consisting of two compartments, each 20×18×15 cm high and separated by an 18×8 cm high hurdle. Two mA shocks were delivered by a shock generator/scrambler (BRS/LVE Model SG-004 Tech Serve, Inc.), time intervals were set with Sigma electric timers (Sigma Instruments, Model B8RC01A), and escape and avoidance latencies were determined as the animals triggered a photo-interrupter switch mounted above the hurdle.

### *Procedure*

Litter size of adult dopamine depleted and sham depleted dams was noted, and litters were reduced to eight pups at age 1 day. One hour squences of dams' mothering behavior were video-recorded on Days 3, 4, 10 and 11 between the hours of 9:00 a.m. and 1:30 p.m. A modification of the procedure previously reported [8] involved allowing each mother to remain in the experimental mothering enclosure in the absence of her pups for 30 min after saline or amphetamine injection to allow for the onset of drug action. After 30 min, the mother was briefly transferred to a holding bin while her pups were placed together in the center of the mothering enclosure. The session began immediately thereafter as the dam was placed near one wall. Following a brief initial period of exploratory locomotion during which the mothers repeatedly initiated physical contact with their pups, the dams typically retrieved them in rapid succession from the center of the mothering enclosure to its corners. Mothers then spent the duration of the hour engaging in locomotion, crouching over pups, and nest-building using paper strips folded over the enclosure walls (10 strips per wall).

Dams' weights were recorded on Day 3 or 4 and 10 or 11 depending on which day amphetamine was administered. Several indices of pup maturation were recorded. Pups were weighed on Days 3, 10 and 32, and on Day 10 the density of pup fur was scored as sparse, normal, or dense. The age of eye opening was recorded as the first day on which half the pups in each litter had both eyes open. Pups' escape-avoidance learning was studied at age 32 days between 1:00 and 6:00 p.m. (before weights were obtained) in a one-way, active avoidance shuttle box task with 5 sec avoidance interval and 30 sec inter-trial interval. Rats were allowed a maximum of 25 sec of shock before manual prompting over the hurdle into the "safe" compartment. Performance was evaluated on the basis of 20 consecutive trials per pup.

After completion of the studies, dams were decapitated and their brains were removed and immediately stored at −60°C. Fluorometric and high pressure liquid chromatographic-amperometric norepinephrine and dopamine assays were run eight weeks later to insure that selective depletion of brain dopamine was achieved [4,7].

### *Behavioral Measures*

*Maternal measures.* Measures of maternal behavior are similar to those described previously [8]. The interval between the beginning of the session and retrieval of the first pup from the center of the enclosure to a corner was scored as retrieval latency. The number of pups retrieved and the number of corners to which they were retrieved were also noted. Inter-retrieval interval (IRI) was defined as the average time elapsed between successive pup retrievals:

IRI=time of last retrieval−retrieval latency/number of pups retrieved

Nest building activity was quantified as the amount of time spent nest building and as the number of paper strips detached from their positions by the dam with her teeth. The time spent crouching over one or more pups was scored as

an index of nursing activity. The time the dam's hind legs remained in motion was recorded as a measure of locomotor activity, and the number of squares (grid superimposed on videomonitor screen) the dam entered constituted a measure of distance traveled in one hour. Rate of locomotion (RL) was defined as:

$$RL = \text{squares entered/time in motion}$$

*Pup measures.* Shuttle box escape-avoidance learning was measured by the number of avoidance responses per litter and by the sum of escape and avoidance times over 20 consecutive trials.

### *Data Analysis*

Measures of maternal behavior was subjected to a four-factor analysis of variance for a repeated measures design [20]. The factors were: Depletion (sham; 6-OHDA + DMI), Dose (0.05 mg/kg dams on and off amphetamine; 0.25 mg/kg dams on and off amphetamine), Treatment (saline; amphetamine) and Age (Days 3–4; Days 10–11). Measures which yielded significant Age effects were examined with separate analyses of variance at each age for three factors: Depletion, Dose and Treatment. A three-factor analysis of variance was also performed on mother and pup weights with Depletion, Dose and Age as factors. Pup measures that were not repeated across days were analyzed with a two-factor (Depletion and Dose) analysis of variance.

## RESULTS

The effects of 6-OHDA + DMI treatment and amphetamine are reported in two parts. First, the impact of 6-OHDA + DMI on brain catecholamines, maternal behavior, and offspring development are presented. Second, the response of dams to intraperitoneal amphetamine injection is described.

### *Effects of 6-OHDA + DMI Treatment*

*Brain catecholamines.* The impact of experimental treatment at age 5 days on adult brain catecholamine concentrations is presented in Table 1. Brain dopamine concentrations of 6-OHDA + DMI treated dams were reduced to a mean of 30.2% (SE= ± 7.6%) of control values whereas norepinephrine concentrations were not significantly affected.

*Mothering behavior.* Despite the known developmental disturbances and profound neurotoxic disruption of central dopaminergic mechanisms associated with neonatal 6-OHDA + DMI treatment, the pattern of maternal behavior of treated dams was virtually identical to that of controls. Offspring of dopamine depleted dams were neither neglected, injured, nor cannibalized and there were no differences between depleted and control dams on measures of contact initiated with pups, retrieval behavior, and nest building activity. While the time spent in motion by both groups of mothers decreased as offspring matured and dams gained exposure to the experimental enclosure, $F(1,22)=5.04$, $p=0.04$, there was a trend for both groups toward increased time crouching (Table 2). An apparent behavioral distinction, however, is that 6-OHDA + DMI treated mothers spent more time crouching over their pups than did control dams, $F(1,22)=7.03$, $p=0.02$ (Fig. 1), suggesting an enhancement of maternal attentiveness. In addition, 6-OHDA + DMI treated dams spent less time in motion on Days 3–4 than controls, $F(1,22)=4.81$, $p=0.04$, but this

TABLE 1

BRAIN CATECHOLAMINE CONCENTRATIONS (ng/g)

| | Sham treated | 6-OHDA + DMI treated |
|---|---|---|
| DA | 706.7 ± 34.5 | 212.9 ± 54.1* |
| NE | 411.0 ± 43.5 | 393.7 ± 24.4* |

*$p<0.001$.

TABLE 2

BEHAVIORAL EFFECTS OF 6-OHDA + DMI TREATMENT*

| | Day | Sham treated | 6-OHDA + DMI treated |
|---|---|---|---|
| Time crouching (sec) | 3–4 | 1886 ± 325 | 2588 ± 255† |
| | 10–11 | 2149 ± 261 | 2681 ± 187† |
| Time in motion (sec) | 3–4 | 250 ± 43 | 174 ± 38† |
| | 10–11 | 206 ± 47 | 150 ± 29 |

*There were no significant interactions between 6-OHDA + DMI treatment and amphetamine response for these measures. Thus, means for saline and amphetamine trials were combined.

†$p<0.05$.

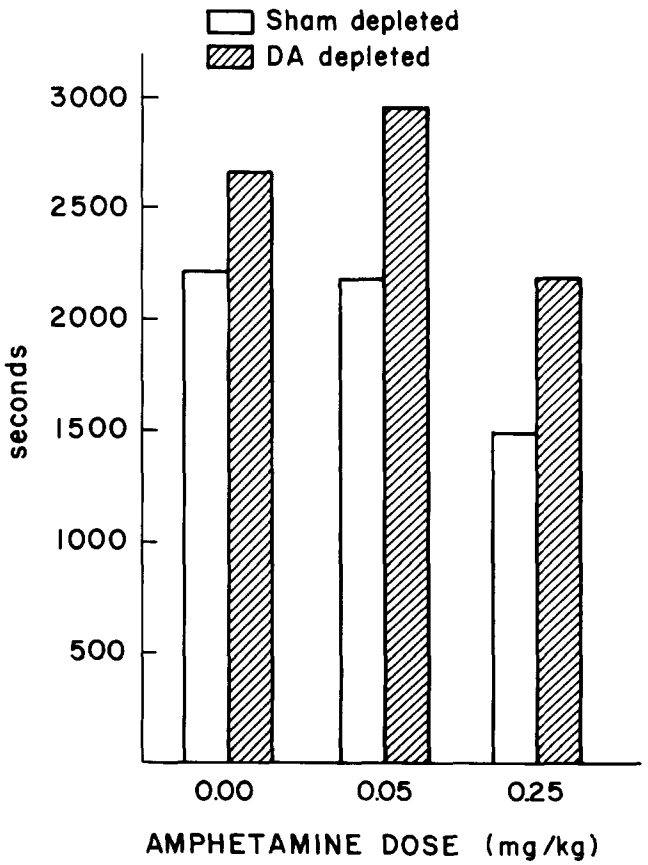


FIG. 1. Crouching behaviour of dopamine depleted and sham depleted mothers at 0.00, 0.05 and 0.25 mg/kg amphetamine. Values are combined for all pup ages tested.

TABLE 3
MEASURES OF OFFSPRING DEVELOPMENT

| Measure | Sham treated dams' offspring | 6-OHDA + DMI treated dams' offspring |
|---|---|---|
| Age of eye opening (days) | 14.3 ± 0.3 | 14.2 ± 0.2 |
| Weight at age 32 days (g) | 106.0 ± 1.3 | 106.7 ± 1.3 |
| Number of shuttle box avoidance responses per litter | 39.6 ± 4.7 | 45.1 ± 4.0 |

difference failed to reach significance on Days 10–11. No associated differences emerged in the number of squares entered and rate of locomotion.

*Pup development.* 6-OHDA + DMI treatment of dams was associated with no disruption of offspring maturation and no discrepancy in number of pups per newborn litter compared to sham treated controls. There was no disparity in pup weight at 3, 10 and 32 days of age, nor was there a difference in the age of eye opening and the density of fur at age 10 days. 6-OHDA + DMI treatment was not associated with an effect on offspring shuttlebox learning at age 32 days: there was no difference between pups of treated dams and controls in the sum of escape and avoidance times and in the number of avoidance responses (Table 3).

*Effects of Amphetamine*

There were no differences between 6-OHDA + DMI and control dams in their response to low dose (0.05 and 0.25 mg/kg) intraperitoneal amphetamine administration. Effects of amphetamine (6-OHDA + DMI and sham treated dams considered together on both doses) emerged for crouching time and time nest building, $F(1,22)=6.71$, $p=0.02$ and $F(1,22)=5.48$, $p=0.03$, respectively, with dams spending less time crouching and nest building on the drug. In addition, a Dose×Treatment interaction was observed for crouching time, $F(1,22)=12.18$, $p=0.03$, reflecting a 22% decrement in mean values (6-OHDA + DMI and control dams considered together) produced by 0.25 mg/kg and a 3% increase produced by 0.05 mg/kg. A more complete description of dose dependent amphetamine effects in primaparous maternal dams has been submitted (M. Piccirillo, J. E. Alpert, D. J. Cohen and B. A. Shaywitz).

## DISCUSSION

During the first month of life, rats treated neonatally with 6-OHDA and DMI have been distinguished from controls with respect to rate of habituation to novel environments, conditioned avoidance learning, ontogeny of behavioral arousal, protective maternal care received, and behavioral response to catecholamine agonists and phenobarbitol [8, 10–14]. Recent studies of neurotransmitter systems and maternal behavior have suggested that catecholamine manipulations, particularly if acute, may influence the pattern and adequacy of maternal caregiving in the rat [9, 17–19]. The major finding of this study is that despite the well documented developmental aberrance of neonatally 6-OHDA + DMI treated dams, and despite dopamine depletion to a mean of 30% of control concentrations, neonatal 6-OHDA + DMI treatment had no pervasive influence on later maternal care. Measures of pup retrieval, nest building, and offspring maturation revealed no differences between 6-OHDA + DMI and sham treated dams. Similarly, an analysis of trends in maternal behavior related to pup age and the use of low doses of amphetamine as a pharmacologic probe failed to discriminate between these two groups. Nevertheless, 6-OHDA + DMI treated dams were distinguishable from controls: they spent significantly more time crouching over their offspring, and at a young pup age (3–4 days) spent less time in motion away from the nest.

Both hyperactivity and hypoactivity have been reported in adult rats following neonatal depletion of CNS dopamine [3,16], but in the present study, no difference in the rate of locomotion and number of squares entered was found between 6-OHDA + DMI and sham treated rats. The difference in time in motion emerged only at the younger pup age when the discrepancy in nursing time between 6-OHDA + DMI and sham treated rats was greatest. Since decreased time in motion was observed only at the younger pup age (Days 3 and 4), and since no other measures of locomotion were affected at both pup ages, it seems unlikely that hypoactivity provides an explanation for the greater crouching time of 6-OHDA + DMI dams observed across Days 3, 4, 10 and 11.

Much work has been devoted to effects of adult maternal undernutrition on caregiving activities [2], and two groups of investigators have found evidence for increased time spent with pups in the nest by undernourished mothers [6,15]. Although selective dopamine depletion in the neonatal period is known to result in growth retardation during the first month of life [8], weights of 6-OHDA + DMI and sham treated dams as adults were not significantly different in this study during offspring development. Since little is known about the impact on adult maternal behavior of transient lags in growth during early development, the extent to which these lags influence behavior 6-OHDA + DMI treated rats is difficult to estimate.

The pharmacologic intactness of dopamine depleted dams may best be accounted for by long term compensatory processes following neonatal destruction of dopaminergic terminals. These include increased turnover of dopamine in residual neurons escaping neurotoxic damage, postsynaptic receptor supersensitivity, and, perhaps, synaptic regeneration [21]. The predominant behavioral intactness of depleted dams may be explained by these same mechanisms or by the possibility that dopaminergic systems do not play a central role in neuroendocrine processes mediating maternal behavior in the rat. If the results of acute administration of 6-OHDA + DMI to gestating dams are compatible with our findings, it should be possible to distinguish the role of dopamine in maternal rat behavior from the effects of aberrant development, the action of chronic compensatory mechanisms, and the consequences of acute trauma to the central nervous system.

## ACKNOWLEDGEMENTS

We gratefully acknowledge technical assistance from David

Blatt, Karen Cone, Clifford Felig and Perry Zigler. Special thanks to Margrethe G. Cone for editorial contributions and to John P. Kootz and Hedevig L. Rusch for assistance with data analyis.

The research was supported in part by Public Health Service Grant HD-03008, Children's Clinical Research Center Grant RR-00125, the William T. Grant Foundation, Mr. Leonard Berger, the Jean T. Schall Trust, Mental Health Clinical Research Center Grant No. P50 MH 30929, and The Solomon R. and Rebecca D. Baker Foundation, Inc.

## REFERENCES

1. Copenhaver, J. H., R. L. Schalock and M. J. Carver. para-Chloro-D,L-phenylalanine induced filicidal behavior in the female rat. *Pharmac. Biochem. Behav.* **8:** 263–270, 1978.
2. Crnic, L. S. Maternal behavior in the undernourished rat *(Rattus norvegicus). Physiol. Behav.* **16:** 677–680, 1976.
3. Ernioff, L., R. C. Macphail, A. Heller and L. S. Seiden. Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. *Brain Res.* **164:** 195–205, 1979.
4. Keller, R., A. Oke, I. Mifford and R. W. Adams. Liquid chromatographic analysis of catecholamines routine assay for regional brain mapping. *Life Sci.* **19:** 995–1004, 1976.
5. Lamb, M. E. Physiological mechanisms in the control of maternal behavior in rats: A review. *Psychol. Bull.* **82:** 104–119, 1975.
6. Massaro, T. F., D. A. Levitsky and R. H. Barnes. Protein malnutrition in the rat: Its effects on maternal behavior and pup development. *Devl Psychobiol.* **7:** 551–561, 1974.
7. Nagatsu, T. *Biochemistry of Catecholamines: The Biochemical Method.* Baltimore: University Park Press, 1973, p. 217.
8. Piccirillo, M., D. J. Cohen, B. A. Shaywitz, J. E. Alpert and D. Marinelli. Maternal care received by rat pups treated with 6-hydroxydopamine. *Physiol. Behav.* **22:** 69–75, 1979.
9. Rosenberg, P., A. Halaris and H. Moltz. Effects of central norepinephrine depletion on the initiation and maintenance of maternal behavior in the rat. *Pharmac. Biochem. Behav.* **6:** 21–24, 1977.
10. Shaywitz, B. A., J. W. Gordon, J. H. Klopper and D. A. Zelterman. The effect of 6-hydroxydopamine on habituation of activity in the developing rat pup. *Pharmac. Biochem. Behav.* **6:** 391–396, 1977.
11. Shaywitz, B. A., J. H. Klopper and J. W. Gordon. Methylphenidate in 6-hydroxydopamine-treated developing rat pups. *Archs Neurol.* **35:** 463–469, 1978.
12. Shaywitz, B. A., J. H. Klopper, R. D. Yager and J. W. Gordon. Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. *Nature* **261:** 153–155, 1976.
13. Shaywitz, B. A. and D. A. Pearson. Effects of phenobarbital on activity and learning in 6-hydroxydopamine treated rat pups. *Pharmac. Biochem. Behav.* **9:** 173–179, 1978.
14. Shaywitz, B. A., R. D. Yager and J. H. Klopper. Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science* **191:** 305–308, 1976.
15. Smart, J. L. and J. Preece. Maternal behaviour of undernourished mother rats. *Anim. Behav.* **21:** 613–619, 1973.
16. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185:** 609–619, 1973.
17. Sorenson, C. A. and M. Gordon. Effects of 6-hydroxydopamine on shock-elicited aggression, emotionality and maternal behavior in female rats. *Pharmac. Biochem. Behav.* **3:** 331–335, 1975.
18. Steele, M. K., D. Rowland and H. Moltz. Initiation of maternal behavior in the rat: Possible involvement of limbic norepinephrine. *Pharmac. Biochem. Behav.* **11:** 123–130, 1979.
19. Szechtman, H., H. I. Siegel, J. S. Rosenblatt and B. R. Komisaruk. Tail-pinch facilitates onset of maternal behavior in rats. *Physiol. Behav.* **19:** 807–809, 1977.
20. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.
21. Zigmond, M. J. and E. Stricker. Behavioral and neurochemical effects of central catecholamine depletion: A possible model for "subclinical" brain damage. In: *Animal Models in Psychiatry and Neurology,* edited by I. Hanin and E. Usdin. New York: Pergamon Press, 1977, pp. 415–429.