Pharmacology Biochemistry & Behavior, Vol. 18, pp. 725–729, 1983. © Ankho International. Printed in the U.S.A.

Sexual Dimorphism in Rats with Respect to Locomotor Activity and Circling Behavior

JAMES F. HYDE AND THOMAS P. JERUSSI

Department of Pharmacology and Toxicology, College of Pharmacy, Rutgers University
P.O. Box 789, Busch Campus, Piscataway, NJ 08854

Received 2 July 1982

HYDE, J. F. AND T. P. JERUSSI. *Sexual dimorphism in rats with respect to locomotor activity and circling behavior.* PHARMACOL BIOCHEM BEHAV 18(5) 725–729, 1983.—Male and female rats were tested for locomotor activity and spontaneous circling (rotation) at 4, 6, 8, 11, 13 and 15 weeks of age. Locomotor activity of females increased with age, and significant intersex differences which became apparent by 8 weeks of age were attributed to the greater perseverative tendency of the females. Spontaneous rotation, on the other hand, did not change with age and significant intersex differences were not evident. Moreover, locomotor activity and rotation were not correlated at any age. In contrast to spontaneous rotation, amphetamine induced significantly more rotation in older (18 week) than in younger (5 week) females and males of both ages. Apomorphine, on the other hand, also elicited more rotation in older than in younger females, but not in males. In addition, intersex differences were not evident in younger animals tested with either drug. These data suggested that the greater perseverative tendency and lateralization of females compared to males may be related to bilateral functional imbalances in nigrostriatal activity.

Laterality Rotation Circling behavior Locomotor activity Sexual dimorphism Ontogenesis
Habituation Perseveration Amphetamine Apomorphine

THE relationship of sex and age to open field behavior in rats has been extensively investigated. Females have been reported to locomote more than males [2, 5, 24, 25, 28, 30, 34], and older animals show equal or less general activity than younger rats of either sex [4, 9, 29, 30, 32]. Developmentally, sex differences in locomotor activity appear near the onset of sexual maturity [30] and various manipulations of gonadal steroids appear to confirm these observations [1,25].

Differences between the sexes have also been reported in drug-induced circling behavior (rotation) in normal rats [3,22]. It appears that normal surgically intact female rats rotate more than their male counterparts when challenged with amphetamine. Moreover, this behavior is also subject to modification by gonadal hormones [8,12]. Normal rats will also show significant rotation without any drug when tested for extended periods of time, especially during the dark phase of their diurnal cycle [10,13]. And similar to the rotation induced by drugs, both the direction and magnitude of spontaneous nocturnal rotation are consistent between test sessions. However, in contrast to the studies on locomotor activity, ontogenetic differences between the sexes with respect to circling behavior have not yet been established.

Although locomotor activity appears to be neurochemically mediated, in part, by dopamine [7], most agree that circling behavior is primarily an expression of dopaminergic function. A bilateral imbalance in nigrostriatal dopaminergic activity biases the animal to rotate or turn in a dominant or "preferred" direction [6, 14, 22, 26, 27]. However, some suggest that the dopamine imbalance between the left and right corpora striata accounts for only the asymmetry of posture, whereas the general level of activity produces the "drive" necessary to induce circling in the biased direction [15,20]. Because of the apparent relationship among locomotor activity, circling behavior and dopaminergic-hormonal function, the ontogenesis of these behaviors in both male and female rats was investigated.

METHOD

Ten male and ten female four week old Sprague-Dawley rats (Blue Spruce Farms, Inc., Altamont, NY) weighing between 60–100 g were separated by sex and maintained five per cage on a 12 hr diurnal cycle (1830–0630 hr for the dark phase). Food and water were available ad lib except during testing.

Locomotor activity and circling behavior (rotation) were measured at 4, 6, 8, 11, 13 and 15 weeks of age. Males and females were transported from their home cages to the testing area in separate holding cages approximately 30 minutes prior to testing. Animals were placed in the center of an activity meter (33×43 cm, Model "ECO," Columbus Instruments, Columbus, OH; with a cover, 26×30.5×43 cm) at the beginning of each test session. Locomotor activity counts were obtained in the dark, each minute, for 14 minutes between 1700–1800 hr. Between each test session the apparatus was cleaned with distilled water until it appeared odor free. Males and females were alternately tested and the order of testing was the same each week. Immediately following the locomotor activity test, the animals were tested for spontaneous circling behavior in a rotometer previously described [13]. Following a short period (15–20 min) of ac-

climation to the apparatus, rotations were automatically recorded between 1830–0630 hr, during the dark phase of the animals' diurnal cycle.

At 18 and 19 weeks of age, all rats (except 3 males which had died) were tested for drug-induced rotation between 1500–1800 hr with doses of d-amphetamine sulphate (1.0 mg/kg) and apomorphine hydrochloride (10.0 mg/kg), respectively, which have been shown to induce optimal rotation in normal rats [14]. Fifteen minutes after the animals had been placed in the rotometers they were injected intraperitoneally (IP) with drug. Rotations were then recorded for one hour. Subsequent to these experiments, another group of normal rats (10 male, 10 female) were tested for drug-induced rotation under similar conditions at 5 and 6 weeks of age with d-amphetamine (1.0 mg/kg) and apomorphine (10.0 mg/kg), respectively. For each test of rotation, full rotations (360 degrees) to the right and left were separately totalled and then net rotations (i.e., rotations to the left minus rotations to the right) were determined.

Rotations and locomotor activity counts were compared by linear regression analysis and independently analyzed by analyses of variance followed by multiple comparisons, using the Newman-Keuls procedure for tests on means [33].

RESULTS

Figure 1 illustrates the development of locomotor activity in males and females from 4 to 15 weeks of age. Three-way analysis of variance (sex×age×time) with repeated measures over age and time (i.e., the 14 minutes of testing) indicated significant main effects of sex, $F(1,18)=4.84$, $p<0.05$, age, $F(5,90)=11.19$, $p<0.01$, and time, $F(13,234)=33.03$, $p<0.01$. There were also significant sex×age, $F(5,90)=4.23$, $p<0.05$, and sex×time, $F(13,234)=2.23$, $p<0.05$, interactions. Multiple comparisons indicated that the locomotor activity of the males did not differ significantly over the six weeks of testing. In contrast, the locomotor activity of the females significantly increased at week 11 and remained elevated thereafter. Moreover, beginning at 8 and continuing to 15 weeks of age, significant differences in locomotor activity were apparent between the sexes (see Fig. 1).

For both sexes, locomotor activity declined significantly within each test session. Multiple comparisons indicated that the decline occurred primarily during the first seven minutes of the test session and locomotor activity between the sexes was not significantly different during this time interval (see Fig. 2). By contrast, multiple comparisons indicated that the significant sex×time interaction could be attributed to the latter half (8–14 min) of the test session (see Fig. 3). Although significant sex differences were apparent only during the latter half of the test session, generally age-related changes, illustrated in Figs. 2 and 3, were not markedly different *within* a test session. A significant change in locomotor activity during the first but not the second half of the test session was evident in males only at 13 weeks of age, but from 11–15 weeks postnatally females showed similar significant age-related increases for both halves of the test session.

Figure 4 illustrates rotational behavior over the ages tested. Although female rats appear to show greater net rotations than males, two-way analysis of variance failed to indicate any significant effect of sex, $F(1,18)=1.48$, $p>0.25$, age, $F(5,90)=2.04$, $p>0.10$, or a sex×age interaction, $F(5,90)=1.37$, $p>0.25$. Similarly, significant differences were not found for total rotations (data not shown). In addition, total and net nocturnal rotations for each sex were not

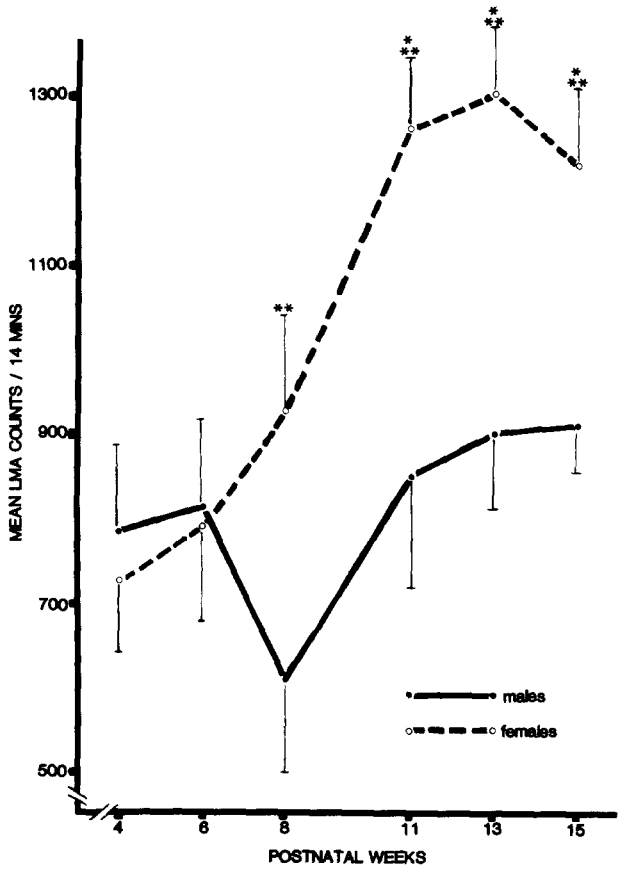

FIG. 1. Mean (±SE) locomotor activity (LMA) counts during the 14 minute test sessions for males and females 4–15 weeks of age. *Significantly greater than postnatal week 4 ($p<0.01$; Newman-Keuls). **Females significantly greater than males ($p<0.01$; Newman-Keuls) of the same age.

significantly correlated ($r=-0.53246$ to 0.53588) with locomotor activity at any age.

A three-way analysis of variance (sex×age×drug) of the data in Table 1 indicated that there were significant main effects of sex, $F(1,18)=6.70$, $p<0.01$, and age, $F(1,18)=30.16$, $p<0.01$, and a significant sex×age interaction, $F(1,18)=10.64$, $p<0.01$. The effect of drug was not significant, $F(1,18)=0.04$, $p>0.25$. Multiple comparisons showed that older females tested at 18 weeks with amphetamine rotated significantly more than their male counterparts. This was not the case when they were retested at 19 weeks of age with apomorphine. In contrast, younger rats tested at 5 and 6 weeks with amphetamine and apomorphine, respectively, did not show any significant intersex differences in rotation. However, the rotation induced by either amphetamine or apomorphine in older females was significantly greater than the drug-elicited rotation in the younger female rats.

DISCUSSION

From the results it can be seen that the female rats locomote more than the males. Although this phenomenon has been observed by others [2, 5, 24, 25, 28, 30, 34], in this report significant increases in locomotor activity became evident as the females grew older. Males, on the other hand,

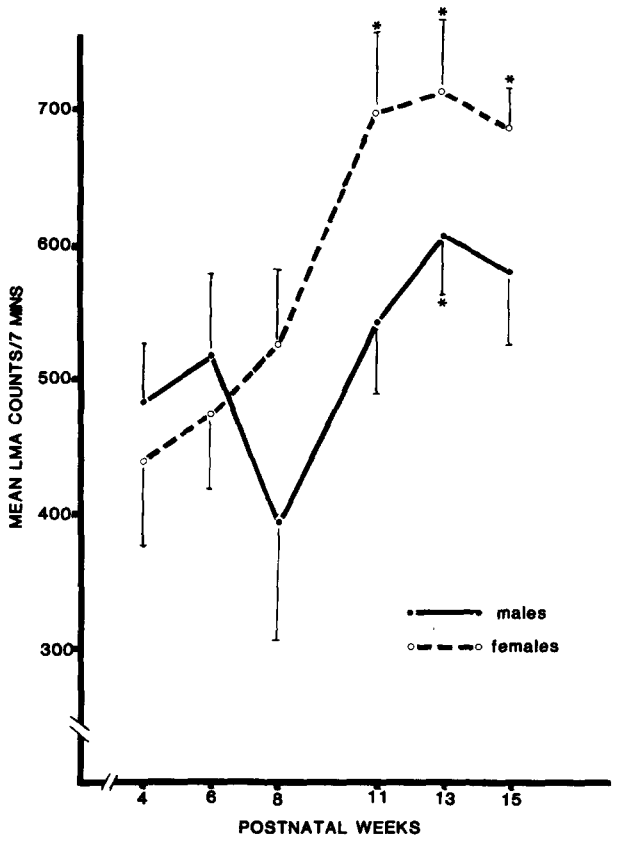

FIG. 2. Mean (±SE) locomotor activity (LMA) counts during minutes 1–7 of the 14 minute test sessions for males and females 4–15 weeks of age. *Significantly greater than postnatal week 4; females ($p<0.01$; Newman-Keuls); males ($p<0.05$; Newman-Keuls). Differences between males and females of the same age were not statistically significant.

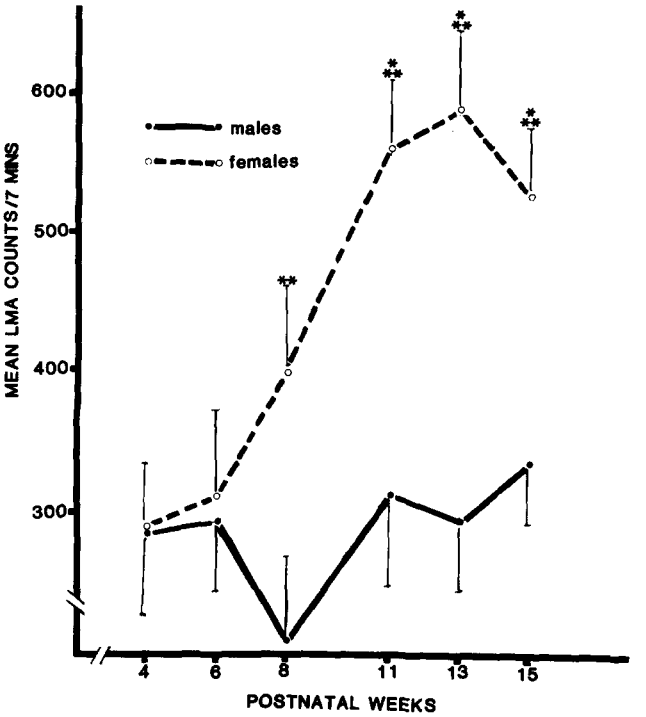

FIG. 3. (Mean (±SE) locomotor activity (LMA) counts during minutes 8–14 of the 14 minute test sessions for males and females 4–15 weeks of age. *Significantly greater than postnatal week 4 ($p<0.01$; Newman-Keuls). **Females significantly greater than males ($p<0.01$; Newman-Keuls) of the same age.

did not show similar age-related changes. These findings are in contrast to reports [9, 29, 32] demonstrating a significant decrease in older rats. Yet other studies [1, 2, 5] indicate that aging rats show increases in locomotor activity. This discrepancy arises because the terms "young" and "old" are not used consistently to refer to rats of specific age groups. A review of the literature indicates that male rats show little if any change in locomotor activity up to about 13 weeks of age, after which their locomotor activity begins to decrease [4, 9, 29, 32]. The locomotor activity of females, on the other hand, increases significantly [1, 2, 5] until about the 16th week postnatally; then it too declines. Eventually, no intersex difference or only a small one is observed [4,32].

In this present study, significant differences between the sexes were apparent *only* during the last seven minutes of the test session. Frequently, rats are tested for shorter periods of time, ranging from 2 to 5 minutes [1, 2, 4, 5, 9, 30–32], and therefore, the way the two sexes differentially adapt to the test situation is not readily apparent. The present data indicate that males habituate more rapidly than females with respect to locomotor activity. As a result, significant intersex differences are evident during the latter half of the test session.

The greater locomotor activity of female rats compared to males has been proposed as a way to increase the frequency

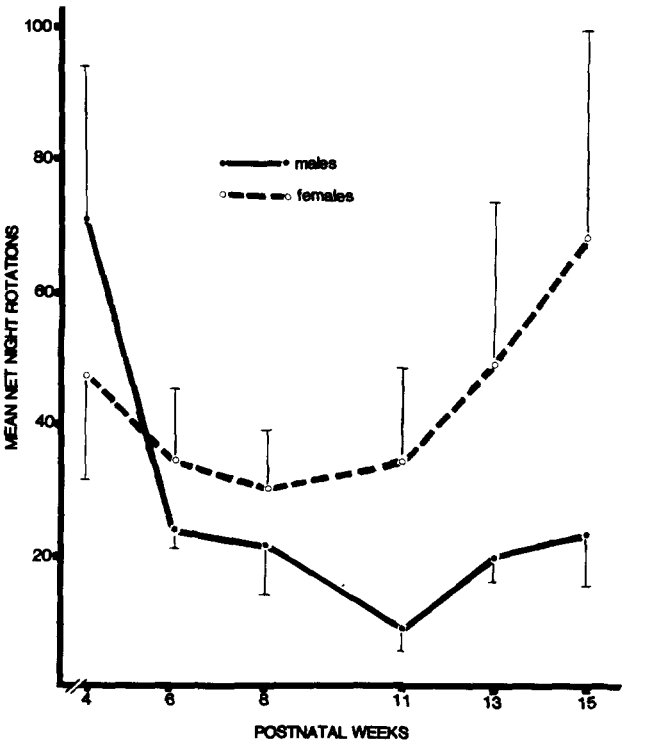

FIG. 4. Mean (±SE) net night rotations for males and females 4–15 weeks of age. Differences between males and females of the same age were not statistically significant.

TABLE 1
EFFECTS OF SEX AND AGE ON DRUG-INDUCED CIRCLING

	Mean Net Rotations/Hour ± SD			
	Older Rats		Younger Rats	
	Males (N=7)	Females (N=10)	Males (N=10)	Females (N=10)
d-Amphetamine	21.0 ± 22.7	102.4 ± 77.5*	12.0 ± 18.6	10.6 ± 14.5
Apomorphine	36.3 ± 25.6	54.4 ± 47.0†	28.2 ± 28.1	21.4 ± 16.3

*Significantly greater than older amphetamine-treated males and younger amphetamine-treated males and females ($p<0.01$; Newman-Keuls).
†Significantly greater than younger apomorphine-treated females ($p<0.01$; Newman-Keuls).

of finding a mate [23]. Moreover, because the onset of puberty in females occurs weeks before that of males [21], we propose that the greater activity of the females coupled with their tendency to perseverate at this behavior increases the probability of the female leaving the nest or home territory and mating with sexually mature males from other litters. As a result, inbreeding between littermates is reduced.

Although females were significantly more active than males with respect to locomotor activity, this was not the case for spontaneous circling behavior. Females generally displayed more net and total rotations than males across ages, but these apparent differences were not statistically significant possibly due to the large variability of the females' circling behavior. In addition, for each sex at all ages investigated, significant correlations were not observed between locomotor activity and circling behavior. Therefore, although a certain amount of locomotion is necessary to produce circling in the dominant direction, the lack of any significant correlation indicates that these behaviors are independent of one another and that the magnitude of rotation cannot be attributed solely to an animal's tendency to spontaneously ambulate.

Younger rats tested for rotation with either amphetamine or apomorphine did not show any intersex differences. In contrast, differences between the sexes were apparent in the older animals. Older females rotated significantly more with amphetamine than younger females and males of both ages. Therefore, these data indicate that younger male and female rats are equally insensitive to the rotation-augmenting effects of the two drugs. As the animals get older, however, the females become significantly more sensitive to amphetamine than the males. Since amphetamine- or apomorphine-induced rotation involves predominantly pre- or postsynaptic elements, respectively, the differential sensitivity between the sexes could occur if older females were more lateralized than males with respect to the presynaptic concentration of striatal dopamine, as some neurochemical data appear to indicate [22]. Others have shown that by 4 weeks of age, ^{3}H-haloperidol binding to striatal dopamine "receptors" has reached peak values [18]. Whereas even at 6 to 8 weeks of age the biosynthetic enzymes for dopamine are only 75 to 80 percent of their adult activities, and dopamine concentration in septum-caudate is not quite 70 percent of maximum [19]. Therefore with age, presynaptic dopamine concentrations increase into adulthood.

From the results of this present study there is also some evidence of an increase in the postsynaptic sensitivity of the older *females*, in contrast to a recent report finding Sprague-Dawley males more sensitive than females to the rotation-stimulating effects of apomorphine [3]. However, in agreement with others [22], our data indicates that the *primary* difference between males and females with respect to circling behavior appears to be presynaptic and not postsynaptic. Spontaneous rotation presumably reflects the combined and possibly equivalent effects of pre- and postsynaptic nigrostriatal activity. Therefore, the presynaptic intersex difference in striatal dopamine is not fully expressed during spontaneous rotation. However, this threshold or "latent" tendency of the females to rotate more than males may be unmasked when the animals are tested with amphetamine.

Although previous work demonstrated that amphetamine was not asymmetrically distributed between the two halves of the brain [14], others report that brain concentrations of the drug are higher in female than in male rats [11,17]. However, cerebral drug levels, per se, cannot account for the amphetamine-induced intersex differences in rotation because it has been shown that these differences still persist even when males are given a much larger dose than their female counterparts [3].

Amphetamine increases arousal and perseveration [7,16]. Locomotor activity and rotation involve nigrostriatal function and are repetitive, stereotyped, and perseverative behaviors which are also augmented by amphetamine. Therefore, we propose that the greater nigrostriatal lateralization of the females is expressed behaviorally as a reduced capacity to habituate. It is this greater perseverative tendency of females that is unmasked by amphetamine and causes them to rotate more than males.

ACKNOWLEDGEMENTS

This research was assisted by Biomedical Research Support Grant PHS RR 07058-17, Rutgers University Research Council Award 2-02214, and NIMH Grant 37488-01.

REFERENCES

1. Blizard, D. A., H. R. Lippman and J. J. Chen. Sex differences in open-field behavior in the rat: The inductive and activational role of gonadal hormones. *Physiol Behav* **14:** 601–608, 1975.
2. Bond, N. and E. DiGiusto. Open-field behavior as a function of age, sex and repeated trials. *Psychol Rep* **41:** 571–574, 1977.
3. Brass, C. A. and S. D. Glick. Sex differences in drug-induced rotation in two strains of rats. *Brain Res* **223:** 229–234, 1981.
4. Broadhurst, P. L. Determinants of emotionality in the rat II. Antecedent factors. *Br J Psychol* **49:** 12–20, 1958.
5. Bronstein, P. M., H. Neiman, F. D. Wolkoff and M. J. Levine. The development of habituation in the rat. *Anim Learn Behav* **2:** 92–96, 1974.
6. Christie, J. E. and T. J. Crow. Turning behavior as an index of the action of amphetamines and ephedrines on central dopamine-containing neurons. *Br J Pharmacol* **43:** 658–667, 1971.
7. Cole, S. O. Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy: a review. *Neurosci Biobehav Rev* **2:** 89–100, 1978.
8. Euvrard, C., C. Oberlander and J. C. Boissier. Antidopaminergic effect of estrogens at the striatal level. *J Pharmacol Exp Ther* **214:** 179–185, 1980.
9. Furchtgott, E., S. Wechkin and J. W. Dees. Open-field exploration as a function of age. *J Comp Physiol Psychol* **54:** 386–388, 1961.
10. Glick, S. D. and R. D. Cox. Nocturnal rotation in normal rats: correlation with amphetamine-induced rotation and effects of nigrostriatal lesions. *Brain Res* **150:** 149–161, 1978.
11. Groppetti, A. and E. Costa. Factors affecting the rate of disappearance of amphetamine in rats. *Int J Neuropharmacol* **8:** 209–215, 1969.
12. Hruska, R. E. and E. K. Silbergeld. Estrogen treatment enhances dopamine receptor sensitivity in the rat striatum. *Eur J Pharmacol* **61:** 397–400, 1980.
13. Jerussi, T. P. A simple, inexpensive rotometer for automatically recording the dynamics of circling behavior. *Pharmacol Biochem Behav* **16:** 353–357, 1982.
14. Jerussi, T. P. and S. D. Glick. Drug-induced rotation in rats without lesions: behavioral and neurochemical indices of a normal asymmetry in nigro-striatal function. *Psychopharmacology (Berlin)* **47:** 249–260, 1976.
15. Kelly, P. H. and K. E. Moore. Mesolimbic dopaminergic neurones in the rotational model of nigrostriatal function. *Nature* **263:** 695–696, 1976.
16. Kokkinidis, L., M. D. Walsh, R. Lahue and H. Anisman. Tolerance to d-amphetamine: behavioral specificity. *Life Sci* **18:** 913–918, 1976.
17. Meyer, E. M. and L. D. Lytle. Sex related differences in the physiological disposition of amphetamine and its metabolites in the rat. *Proc West Pharmacol Soc* **21:** 313–316, 1978.
18. Pardo, J. V., I. Creese, D. R. Burt and S. Snyder. Ontogenesis of dopamine receptor binding in the corpus striatum of the rat. *Brain Res* **125:** 376–382, 1977.
19. Porcher, W. and A. Heller. Regional development of catecholamine biosynthesis in the rat brain. *J Neurochem* **19:** 1917–1930, 1972.
20. Pycock, C. J. and C. D. Marsden. The rotating rodent: a two component system? *Eur J Pharmacol* **47:** 167–175, 1978.
21. Ramirez, V. D. Endocrinology of puberty. In: *Handbook of Physiology*, section 7, *Endocrinology*, vol 2, *Female Reproductive System*, Part 1, edited by S. R. Geiger. Washington, DC: American Physiological Society, 1973, pp. 1–28.
22. Robinson, T. E., J. B. Becker and V. D. Ramirez. Sex differences in amphetamine-elicited rotational behavior and the lateralization of striatal dopamine in rats. *Brain Res Bull* **5:** 539–545, 1980.
23. Russell, P. A. Sex differences in rats' stationary exploration as a function of stimulus and environmental novelty. *Anim Learn Behav* **5:** 297–302, 1977.
24. Russell, R. L. and R. O. Pihl. Determinants of amphetamine-produced stereotyped behavior in the rat. *Psychol Rep* **43:** 575–579, 1978.
25. Savageau, M. M. and W. W. Beatty. Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine in rats. *Pharmacol Biochem Behav* **14:** 17–21, 1981.
26. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behavior. *Acta Physiol Scand* Suppl. **367:** 49–68, 1971.
27. Ungerstedt, U. and G. W. Arbuthnott. Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigro-striatal dopamine system. *Brain Res* **24:** 485–493, 1970.
28. Valle, F. P. Effects of strain, sex, and illumination on open-field behavior of rats. *Am J Psychol* **83:** 103–111, 1970.
29. Valle, F. P. Rats' performance on repeated tests in the open field as a function of age. *Psychon Sci* **23:** 333–335, 1971.
30. Valle, F. P. and R. J. Bols. Age factors in sex differences in open-field activity of rats. *Anim Learn Behav* **4:** 457–460, 1976.
31. Walsh, R. N. and R. A. Cummins. The open-field test: a critical review. *Psychol Bull* **83:** 482–504, 1976.
32. Werboff, J. and J. Havlena. Effects of aging on open field behavior. *Psychol Rep* **10:** 395–398, 1962.
33. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Book Company, 1962.
34. Wong, P. T. A behavioral field approach to general activity: sex differences and food deprivation in the rat. *Anim Learn Behav* **7:** 111–118, 1979.