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Strain and Sex Differences in Amphetamine-Induced Rotation

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STORRIE-BAKER, H. J., M. F. NOVOTNY AND D. P. CROWNE. *Strain and sex differences in amphetamine-induced rotation.* PHARMACOL BIOCHEM BEHAV **43**(3) 795–797, 1992.—Studies of rotational behavior in female rats have investigated Fischer, Sprague-Dawley, Madison, WI, and Holtzman strains. The present study of amphetamine-induced rotational preference looked at the most widely used of the pigmented strains, Long-Evans hooded rats, examining rotation in females and comparing rotational magnitude and direction to males of the same strain. We corroborate in Long-Evans animals the greater rotation of females, but our findings oppose the right-sided female and left-sided male rotational preferences reported in earlier studies. Only female rats in this experiment had a significant directional bias, and it was to the left. This result strongly points to the importance of strain in the lateralization expressed by rotation.

Rotation   Strain differences   Sex differences   Amphetamine

BOTH unilateral lesions of ascending nigrostriatal fibers (2) and the application of dopamine (DA) directly into the striatum (33) result in motor asymmetries such as circling. Injections of amphetamine (AMPH) exaggerate the imbalance that unilateral lesions produce by releasing DA from nigrostriatal nerve endings on the intact side (13). Unilateral lesions of the nigrostriatal system are not necessary, however, to produce rotational behavior (17). AMPH produces a dominant direction of rotation in intact rats that is reliable over several weeks (16,17,23).

The consistency with which differences have been reported across many different measures of behavioral and brain asymmetries in nonhuman animals suggests that sex is a very important variable in determining the pattern of cerebral lateralization (11,29). Indeed, studies suggest that sex differences exist in the mechanisms underlying rotational behavior (32).

Intact female Fischer (8), Sprague-Dawley (8,22), and Holtzman (6,10,30) rats show more vigorous AMPH-induced rotational behavior than males. Greater nigrostriatal activity in females is demonstrated by stronger perseveration (25) and a reduced capacity to habituate, both generated by AMPH and resulting in increased rotation (22).

A right-sided population bias has been demonstrated in females among both Sprague-Dawley (20) and Holtzman (28) strains. Following injection of AMPH, right rotators are significantly more active and have stronger side preferences than left rotators. Male Holtzman rats exhibit a leftward rotational bias (10), are less active, and, therefore, are thought to be less lateralized than females. In fact, in all cases where the strength or magnitude of postural/motor asymmetries have been estimated females appear to be more strongly lateralized. Females exhibit stronger paw preferences, increased locomotion, and more consistent asymmetries in neonatal tail posture and side preferences in the open field (7).

We examined AMPH-induced rotational preference in Long-Evans hooded rats, a widely used but previously unstudied strain, exploring gender differences in lateralization and rotational behavior.

METHOD

Subjects

Forty-eight adult female and 48 adult male Long-Evans hooded rats were obtained from either a University of Waterloo breeding colony originally stocked by or directly from Harlan Sprague-Dawley. They were housed individually in standard lab cages and maintained on a 12 L : 12 D cycle with free access to lab chow and tapwater. They were handled daily for 1 week prior to testing. At the onset of the study, animals were 105 days old, females weighed approximately 245 g, and males weighed between 250–350 g.

Materials

To determine direction of rotational preference, animals were observed in a cylindrical glass container, 24.9 cm high with a diameter of 23.9 cm. To reduce environmental distractions, the container was surrounded with cardboard (18), which increased the height to 30.5 cm.

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Design and Procedure

Estrus determination. To control for the significant variation in amphetamine-induced rotational behavior exhibited across the five stages of the estrous cycle (6,10,28), female animals were observed during estrus. Female Holtzman, Madison, and WI rats (5) tend to complete more net rotations during the estrus phase of the cycle than in any other (17). Failures to find sex differences in rotational behavior may result from fluctuations in estrous cycle (6).

Because examination of vaginal cell distributions has proven to be a reliable method of determining the stages of estrous (3,4,26), samples were obtained each morning for 15 days to chart at least two full cycles for each animal. Using a method refined by Wang (34), distilled water was introduced into the vagina through a glass Pasteur pipette and immediately retracted. Fluids were then transferred to slides and examined microscopically for the presence of leukocytes, cornified, and nucleated epithelial cells under 10/0.25× magnification with reduced lighting (31).

The onset of estrus is indicated by a smear containing 75% nucleated, 25% unclustered cornified epithelia, and a complete absence of leukocytes (9,12).

Amphetamine-Induced Rotational Behavior. Preferred direction of rotation was determined using an amphetamine-induced rotational procedure. Following a 10-min habituation period in the observation container, each animal received an AMPH injection (1.0 mg/kg, IP) (14). This dose has proven sufficient to induce optimal rotation in normal rats (24).

After a 20-min interval for maximum drug effect (18,19), the number and direction of 360° rotations were recorded during a 20-min observation session. Only full 360° turns completed within 5 s were considered true rotations (35). This procedure was repeated 1 week later for males and for females on the day of estrus during the subsequent estrous cycle to determine the consistency of both strength and direction of rotation.

The hemisphere contralateral to the animal's preferred direction of rotational behavior was nominated the dominant side for rotational behavior and the other as nondominant (19,27).

RESULTS AND DISCUSSION

A spatial dominance index (SDI) was calculated to reflect both the magnitude and direction of spatial dominance (21). Two SDI measures were calculated, one for each AMPH-induced rotation session, using the following formula:

$$\text{SDI} = \frac{\#\text{ of turns to right} - \#\text{ of turns to left)}}{\#\text{ of turns to right} + \#\text{ of turns to left)}} \times 100.$$

The SDI ranged from −100, representing a complete leftward rotational bias and right hemisphere dominance, to +100, indicating a complete rightward bias and left hemisphere dominance. To determine both the effectiveness of our estrous determination procedure and the stability of the SDI measure, the two scores were correlated for females. The resulting correlation of 0.93 was higher than the values of 0.52 and 0.71 obtained by Adelstein and Crowne (1) from two cohorts of Sprague-Dawley females.

By one-way analysis of variance, female rats showed a significantly greater magnitude of circling behavior (M = 70.81 net rotations/h) than males (M = 33.41 net rotations/h), $F(1, 92) = 30.0$, $p < 0.001$. Using the criteria of Willar and Crowne (35) and Zimmerberg et al. (36), animals were classified as lateralized (more than 20 net rotations/h) or nonlateralized (fewer than 20 net rotations/h). A larger percentage of the male sample did not meet the criteria for rotation (34.04%) as compared to 6.38% of the females characterized as nonrotating. This greater lateralized AMPH-induced rotational behavior in Long-Evans females conforms to results previously demonstrated in Fischer (8), Holtzman (6,10,30), and Sprague-Dawley (22) strains.

To examine direction of rotation, χ^2 contingency analyses were performed. They demonstrated the dependence of rotational direction upon gender, $\chi^2(1, N = 94) = 9.86$, $p < 0.01$, and in addition a significant leftward bias for females, $\chi^2(1, N = 47) = 11.26$, $p < 0.01$, whereas males tended toward more rightward rotation. Although this finding corroborates evidence of an opposite directional bias between the sexes, it is inconsistent with the established view of female right-sided and male left-sided bias (15,20) and may represent a strain difference.

Taken together, the greater magnitude of rotation and the significant directional bias provide compelling evidence for stronger female lateralization.

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