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# Sex Differences and Asymmetries of Catecholamines: Relation to Turning Preferences[1]

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DARK, K. A., G. ELLMAN, H. V. S. PEEKE, D. GALIN AND V. I. REUS. *Sex differences and asymmetries of catecholamines:Relation to turning preferences.* PHARMACOL BIOCHEM BEHAV 20(3) 327–330, 1984.—Male and female Sprague-Dawley rats were tested for turning preferences in a multiple alley maze. The left and right caudate-putamen were dissected and assayed for norepinephrine and dopamine. Dopamine was not found to be lateralized contralateral to turning preference for females as a group. However, dopamine was significantly lateralized contralateral to the females turning preference if a strong turning bias was present. No relationship between dopamine asymmetry and turning preference was evident for males. Females were found to have norepinephrine significantly lateralized to the left caudate-putamen; in males greater striatal norepinephrine levels were equally distributed between left and right sides. This sexual dimorphism in norepinephrine lateralization was not related to turning preference.

Lateralization Sex differences Dopamine Norepinephrine

FEMALE rats have a natural turning bias and an asymmetry of dopamine in the caudate nucleus which is correlated with this turning preference; rats turn contralateral to the caudate nucleus with the higher concentration of dopamine [4]. This behavioral predisposition is accentuated by the administration of d-amphetamine, a drug which affects dopaminergic activity [4].

Only recently has attention been directed to sex differences in asymmetrical patterns of rotational behavior or neurotransmitter distribution [5]. Unlike female rats, there is no correlation between the behavioral preference of males and their neurotransmitter distribution [9].

The majority of studies which investigated rotational behavior have used a bowl-shaped device for recording the amount of turning an animal exhibits as well as the dominant direction of rotation [4]. Because we believe this is a somewhat restrictive situation for an animal, we have investigated turning preference in a Dashiell maze. In this maze the animal has access to an open area which is divided into a number of alleys; thus it allows for determining turning preferences during exploration in a less restrictive situation.

In this study we examined the extent to which a turning preference in the Dashiell maze was predictive of the direction of dopamine asymmetry in the caudate-putamen. We also investigated sex differences in both directional preferences and catecholamine asymmetry.

## METHOD

### *Animals*

Thirty-eight adult Sprague-Dawley rats, 19 males and 19 females were used in this study. Animals were housed in pairs. Food and water were available ad lib. The animal colony was maintained on a 12:12 light-dark cycle.

### *Behavioral Testing*

Testing was carried out in a modified Dashiell maze, 121×121 cm square, within which were 16 plywood boxes (17×17×29 cm) arranged in four equally spaced rows of four (Fig. 1).

Each rat was tested individually under dim red illumination. After a 15 sec adaptation period in the start box the sliding door was raised and the animal was allowed to ex-

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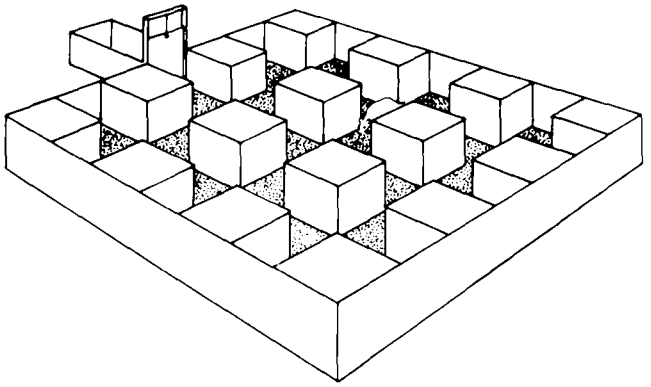

FIG. 1. The Dashiell Maze.

plore the maze for 6 min. During this time the path of the animal was traced by the experimenter on a map of the maze from which the number of left and right turns were determined. The % turning preference was calculated by dividing the side with the greater number of turns by the total number of turns for each animal. Only animals whose activity reached a minimum of 10 turns in the 6 min of observation were included in the statistical analysis. In addition, any animal who did not exhibit a behavioral preference was not included in the analysis (i.e., equal number of right and left turns).

We operationally defined a significant turning preference as 60% or more turns in one direction; such a value would occur <5% of the time in random samples of 100 turns ($\chi^2$, goodness of fit, $p<0.05$).

*Assay Procedure*

All animals were sacrificed by rapid decapitation on the day following testing and their brains were dissected over ice, preserving for assay the left and right caudate-putamen (CP). The tissue samples were individually wrapped in aluminum foil and frozen at −80°C until the time of assay. The neurochemical assay used was a modification of one by Jacobowitz and Richardson [6] which improves the reproducibility of the assays: the coefficient of reliability (SD/mean) decreased from 18% to 4% (Ellman *et al.*, manuscript in preparation). Each tissue sample was weighed and homogenized in 0.01 N HCl to which 0.2% EDTA was added. Then 90% TCA (0.15 ml/100 mg tissue) was added to precipitate the protein. The mixture was centrifuged at 2000 × *g* for 20 min after which aliquots of the supernatant were oxidized into trihydroxyindole fluorophores. Fluorescence readings were taken at the wavelengths appropriate for each neurotransmitter and converted into concentration values in μg/g of tissue. Fluorescence readings for the dopamine-norepinephrine assays were made on an Aminco-Keirs Spectrophosphorimeter connected to a digital readout.

## RESULTS

The concentrations of dopamine and norepinephrine in the caudate are shown in Tables 1 and 2. The mean values for females and males were calculated in two ways; left vs. right caudate, and contra- vs. ipsilateral. The latter consisted of classifying the transmitter concentration in the hemisphere opposite from that of the turning preference as contralateral and that of the hemisphere on the same side as the turning preference as ipsilateral.

TABLE 1

CAUDATE DOPAMINE CONCENTRATIONS (μg/g)

| | Left | Right | Contra | Ipsi |
|---|---|---|---|---|
| Females with significant turning preference (n = 7) | | | | |
| Mean | 5.55 | 5.32 | 5.76* | 5.11 |
| St. Dev. | 0.94 | 1.33 | 1.05 | 1.15 |
| Females with no turning preference (n=8) | | | | |
| Mean | 5.85 | 6.02 | 5.70 | 6.17 |
| St. Dev. | 0.44 | 0.56 | 0.46 | 0.44 |
| Males with significant turning preference (n = 2) | | | | |
| Mean | 4.93 | 5.76 | 5.76 | 4.93 |
| St. Dev. | 0.66 | 0.28 | 0.28 | 0.66 |
| Males with no turning preference (n = 12) | | | | |
| Mean | 4.76 | 4.42 | 4.56 | 4.62 |
| St. Dev. | 0.84 | 1.00 | 1.07 | 0.79 |

*Significant at $p<0.05$, calculated for correlated samples.
The standard deviations given are those of the groups indicated.

TABLE 2

CAUDATE NOREPINEPHRINE CONCENTRATIONS (μg/g)

| | Left | Right | Contra | Ipsi |
|---|---|---|---|---|
| Females with significant turning preference (n = 7) | | | | |
| Mean | 0.26 | 0.19 | 0.21 | 0.24 |
| St. Dev. | 0.05 | 0.06 | 0.07 | 0.05 |
| Females with no turning preference (n=8) | | | | |
| Mean | 0.26 | 0.22 | 0.24 | 0.23 |
| St. Dev. | 0.06 | 0.08 | 0.06 | 0.08 |
| Males with significant turning preference (n = 2) | | | | |
| Mean | 0.25 | 0.25 | 0.25 | 0.25 |
| St. Dev. | 0.09 | 0.05 | 0.05 | 0.09 |
| Males with no turning preference (n = 12) | | | | |
| Mean | 0.22 | 0.22 | 0.24 | 0.21 |
| St. Dev. | 0.06 | 0.08 | 0.08 | 0.06 |

Of the 19 females tested, 3 were eliminated from analysis because they made less than 10 turns, and 1 because it showed no turning preference. The remaining 15 females had a mean preference score of 59%, and thus failed to show a significant turning preference as a group. In addition, no group relationship between dopamine lateralization and turning preference was immediately apparent; 8 animals had higher dopamine content lateralized ipsilaterally and 7 contralaterally to their turning preference.

Eight of the 15 females had preference scores less than 60% and 7 had preference scores greater than 60%. In

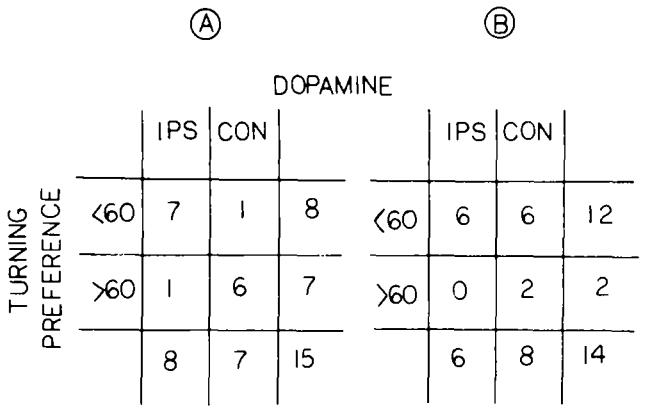


FIG. 2. (A) Distribution of strength of preference in females as a function of dopamine content. (B) Distribution of strength of preference in males as a function of dopamine content.

females which had preferences greater than 60%, higher dopamine concentration was significantly lateralized to the contralateral striatum ($t=3.14$, $p<0.05$; Fig. 2A). Females with turning preferences less than 60% showed an opposite trend; 7 of the 8 had higher dopamine concentration ipsilateral to their preference (Fig. 2A). To further illustrate this relationship a correlation between % behavioral preference for all of the females as a group with their % dopamine concentration contralateral to their preference (i.e., contralateral dopamine concentration/total dopamine concentration) yielded a significant correlation coefficient of 0.78 ($p<0.05$).

Of 19 males tested, 5 were eliminated from analyses because they made less than 10 turns during the testing procedure. The remaining 14 males had a mean score of 57% and failed to show a significant turning preference as a group. Only 2 of these 14 males had preferences greater than 60% and both of these had higher dopamine concentration contralateral to their preferred side. Twelve males had preference scores less than 60% and of these 6 had higher dopamine concentration contralateral and 6 had higher dopamine ipsilateral to their preferred side (Fig. 2B). No significant differences were found between the contralateral and ipsilateral dopamine concentrations for the males as a group, or in groups with preferences either greater or less than 60%. A correlation between the % behavioral preference for all males and the % dopamine concentration contralateral to their turning preference was not significant ($r=0.43$, $p>0.05$).

Although no significant correlations were found between norepinephrine values and behavioral preferences, an unexpected finding was that 16 of the 19 females tested had higher norepinephrine concentrations in the left CP, whereas only 2 had higher concentrations of norepinephrine on the right and one animal had equal norepinephrine values in the left and right CP. This asymmetry was not found in the males. Eight males had higher norepinephrine concentrations on the left, 10 had higher concentrations on the right and 1 male had equal values on the left and right. Testing with a $\chi^2$ found the left-right distribution of norepinephrine in males non-significant, but a significant proportion of females showed higher concentrations of norepinephrine in the left CP than in the right (males: $\chi^2=0.220$; $p>0.05$; females: $\chi^2=10.889$; $p<0.01$).

## DISCUSSION

The finding that approximately 84% of the females had higher norepinephrine concentrations in the left CP while males were as likely to have higher norepinephrine values in the left as the right CP is a new and robust neurochemical asymmetry. However we have not, as yet, been able to find a correlation between our norepinephrine results and any of the behavioral measures used (e.g., turning preference, activity level), either for groups as a whole or for any of the subgroups. Several other behaviors which have been shown to be related to striatal asymmetries were not investigated in this study. Oke and colleagues [7,8] have shown norepinephrine asymmetries both in the rat and the human. However, the asymmetries they observed in the thalamus were made up of localized mosaics of high and low concentrations as opposed to the overall left vs. right differences we found with the CP norepinephrine concentration.

These data indicate that female rats may show different degrees of directional asymmetries. In addition, these differences may be characterized by different patterns of dopamine asymmetry, those animals with strong preferences having higher dopamine contralateral to their preferred side and those with weak preferences having higher dopamine content ipsilateral to their preferred side. The data for males cannot be looked at in this way due to the small number of males showing a strong preference. This may be important in itself, however, as an indicator that males may be only weakly lateralized behaviorally. It is a possibility that these results are due to the nature of our testing procedure which we believe to be a less restrictive measure of turning biases in animals than a spherical apparatus which could force stereotyped movements.

Our findings are further evidence that male/female differences in behavioral patterns may reflect underlying differences in the organization of the extrapyramidal system. These findings are relevant in light of the growing body of clinical research demonstrating sexual dimorphism in the incidence of extrapyramidal disorders in humans, as well as possible sex differences in the response to drugs used in the treatment of these disorders [1, 2, 3, 10].

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