

## NEUROCHEMICAL ASYMMETRIES IN THE ALBINO RAT'S CORTEX, STRIATUM, AND NUCLEUS ACCUMBENS

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(Received in final form January 13, 1984)

### Summary

The concentrations of dopamine (DA), norepinephrine (NE), serotonin (5-HT), dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the right and left cortex, striatum, and nucleus accumbens of adult Purdue-Wistar rats. There was more DA in the right cortex and accumbens and a greater concentration of NE in the left striatum. There is more 5-HT in the left striatum and right accumbens, more 5-HIAA in the left cortex, as well as a greater 5-HT turnover in the left accumbens. These results are considered in the light of previous findings concerning the relationship of neurochemical asymmetries and behavioral lateralization.

Circling behavior in rodents is related to nigrostriatal dopaminergic asymmetry: animals circle contralateral to the side of higher dopamine concentration. This occurs whether they circle spontaneously or after injection of high doses of amphetamine (1,2). Consistent with this is the finding that injections of amphetamine following unilateral lesions of the nigrostriatal system induce ipsiversive circling in rats (1). Glick *et al.* (3) found an intrinsic asymmetry in striatal dopamine of approximately 10-15% favoring the hemisphere contralateral to the rotational bias, while injection of amphetamine resulted in a difference between the contralateral and ipsilateral striata of 25%.

The relationship of lateralized behaviors and neurochemistry extends to other neuroanatomical areas as well (4). Following bilateral lesions of the frontal cortex, animals previously found to be left rotators showed a marked increase in the degree of asymmetry, while preoperative right-turners exhibited slightly less asymmetry (4). This difference following the bilateral lesion presumably reflects an intrinsic dopamine asymmetry in the frontal cortex. In addition, recent work (5) has demonstrated a dopaminergic asymmetry favoring the left frontal cortex of Wistar derived rats (WEzob).

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Dopaminergic asymmetries have also been reported in the nucleus accumbens of Holzman Sprague-Dawley rats with more dopamine in the right hemisphere of males handled in infancy (6). In addition, Camp *et al.* (6) found behavioral asymmetries in the open field: handled males and nonhandled females both turned to the left. These results replicate previously published reports from our laboratory with the Purdue-Wistar rat (7,8). Since Holzman Sprague-Dawley and Purdue-Wistar rats are nearly identical in terms of behavioral asymmetries, this suggests that the neurochemical asymmetries might be similar as well. The following study was designed to test this hypothesis and, in addition, to search for neurochemical asymmetries in other areas of the brain of the Purdue-Wistar rat.

#### Materials and Methods

**Subjects.** Subjects were 40 adult Purdue-Wistar rats (20 males and 20 females) selected at random from a population of animals tested for tail posture in infancy. Testing involved removing the pups from their mother, placing them prone on a flat wooden surface and recording the deviation of the tail from midline. The tail posture was recorded daily for 3 minutes with an observation every ten seconds for the first four days of life. These animals were tested in adulthood for rotation in a water-filled apparatus and for activity in the open field. The results of these experiments are reported elsewhere (9,10).

**Dissection.** The animals were sacrificed by decapitation at approximately seven months of age. The brain was quickly removed, placed ventral side up onto a glass microscope slide and immediately frozen on dry ice. The brain was then wrapped loosely in aluminum foil and packed in dry ice. Four days later, the brains were transferred to a -70°C freezer where they remained until dissection three months later when the brain was placed, ventral side up, on a micrometer controlled slicing device. Using the notch of the optic chiasm as an external landmark, five coronal cuts were made to separate each brain into four "slices" that were used for subsequent dissection on ice/dry ice. Slice 1 extended from 3.0-0.5 mm rostral to the chiasm; Slice 2 from 0.5 rostral to 2.5 mm caudal to the chiasm; Slice 3 from 2.5 caudal to 4.0 mm caudal to the chiasm; and Slice 4 extended from 4.0 to 8.0 mm caudal to the chiasm. Slice 3 was discarded.

Sections of dorsolateral cortex were dissected from Slices 1, 2, and 4. Sections of nucleus accumbens were dissected from Slice 1 and anterior striatum from Slice 2. For each of these structures, samples were divided into right and left halves, weighed, and put immediately into dry ice and stored at -70°C for up to five weeks before assay.

**Assay of Monoamines and Metabolites.** Monoamines and metabolites were assayed by high performance liquid chromatography with electrochemical detection (HPLC/EC). On the day of assay, individual tissue samples were homogenized by sonication (Biosonik Sonicator, Bronwill Scientific Co., Rochester, NY) in 0.7 ml of an "homogenization solution" containing 0.05 N perchloric acid, 1.0mM disodium EDTA and 0.4 mM sodium metabisulfite as antioxidants, and 35 ng epinephrine (E, Sigma Chemical Co., St. Louis, MO) as an internal standard. (The amount of added E far exceeded the trace amounts detectable in tissue samples). The catecholamines norepinephrine (NE), dopamine (DA), and E in 0.4 ml of supernatant homogenate were extracted (by adsorption and desorption on alumina) and chromatographed according to modifications of Sperk *et al.* (11) of the methods of Felice *et al.* (12). A 100 ul aliquot of homogenate was injected directly into a second HPLC/EC system to resolve dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) following the method of Sperk (13).

Three standard solutions, containing 1, 5, and 10 ng/100 ul, respectively, of authentic NE and DA (Sigma) in "homogenization solution" (containing 5 ng/100 ul

of internal standard (E)), were subjected to adsorption/elution on alumina and were chromatographed along with each batch of tissue samples. Three standard solutions containing 1, 5, and 10 ng/100  $\mu$ l of DOPAC, 5-HT, and 5-HIAA (Sigma) respectively, were also chromatographed. Using these data, standard lines for NE and DA were plotted through the origin, with the ratio of peak height of standard to that of the internal standard (E) on the ordinate. Using peak height alone, standard lines were also plotted for DOPAC, 5-HT, and 5-HIAA. Concentration of monoamines and metabolites in tissue samples were calculated by mathematical interpolation on these lines and expressed as ng/mg wet weight tissue. The ratios of DOPAC/DA and 5-HIAA/5-HT were computed as estimates of DA and 5-HT turnover rates, respectively.

### Results

The data were analyzed using a repeated measures analysis of variance with Sex as the independent measure and Hemisphere as the repeated measure. This is equivalent to a correlated t-test. There were no main effects of Sex nor were there any interactions with Sex, so the male and female data were pooled.

The mean levels of monoamines and metabolites in the right and left cortex, striatum, and accumbens are summarized in Table I. There was significantly more DA in the right cortex ( $F = 5.28$ ,  $df = 1,34$ ,  $p < 0.05$ ) and right accumbens ( $F = 4.46$ ,  $df = 1,38$ ,  $p < 0.05$ ). In addition, there was more NE in the left striatum ( $F = 12.00$ ,  $df = 1,38$ ,  $p < 0.01$ ). There was an increased concentration of 5-HIAA in the left cortex ( $F = 4.89$ ,  $df = 1,34$ ,  $p < 0.05$ ), more 5-HT in the left striatum ( $F = 8.51$ ,  $df = 1,38$ ,  $p < 0.01$ ) and right accumbens ( $F = 10.29$ ,  $df = 1,38$ ,  $p < 0.01$ ), and more 5-HT turnover in the left accumbens ( $F = 8.29$ ,  $df = 1,38$ ,  $p < 0.01$ ).

### Discussion

The hypothesis under investigation was that the Purdue-Wistar rat would have dopaminergic asymmetries favoring the right hemisphere. This hypothesis was generalized from findings obtained by Camp *et al.* (6) that there is more dopamine in the right nucleus accumbens of Sprague-Dawley rats handled in infancy. This hypothesis was confirmed in our strain: there were higher levels of dopamine in the right accumbens and, additionally, the right cortex. While our animals were not handled in the traditional sense, the act of measuring tail posture during the first four days of life might act as a significant early experience (14). The manipulations took about 3 minutes--the same amount of time as involved in handling. Indeed, we found a leftward turning bias in these animals in the open field (9)--results that are typical of handled males (7). The dopaminergic asymmetries in the cortex are in the opposite direction from those reported by Slopeema *et al.* (5), although they assayed tissue punches from the frontal cortex as compared to our assay of large portions of dorsolateral neocortex. In addition, strain differences may also account for this discrepancy.

Neuroanatomical connections between the nucleus accumbens and the cortex via the median forebrain bundle are well documented (e.g. 15). These results suggest an asymmetric neurochemical link between the cortex and accumbens as well. Although neither we nor Camp *et al.* (6) find any evidence of a population asymmetry in striatal DA, there is evidence to suggest that the nucleus accumbens is strongly linked to striatal control of circling behavior. Indeed, the striatal dopamine system primarily controls postural motor movements while the accumbens dopaminergic system may be responsible for gross motor movements (16, 17). Circling activity, then, is the result of asymmetrical distribution of DA in the striatum as well as a neurochemically active nucleus accumbens (18). Our results, considered with those of Camp *et al.* (6), suggest that the dopaminergic asymmetry in the nucleus accumbens might play an important role in the

TABLE I

Mean (+ S.E.M.) Levels of Neurochemicals (ng/mg wet weight) in the Right and Left Striatum, Cortex, and Nucleus Accumbens in the Purdue-Wistar Rat

| NEUROCHEMICALS <sup>1</sup> | STRIATUM      |                 |    |
|-----------------------------|---------------|-----------------|----|
|                             | Right         | Left            | N  |
| NE                          | 0.245 ± 0.020 | 0.309 ± 0.024** | 40 |
| DA                          | 8.442 ± 0.301 | 8.119 ± 0.276   | 40 |
| DOPAC                       | 1.375 ± 0.077 | 1.430 ± 0.071   | 39 |
| 5-HT                        | 0.589 ± 0.021 | 0.619 ± 0.024** | 40 |
| 5-HIAA                      | 0.588 ± 0.019 | 0.609 ± 0.020   | 40 |
| DA TURNOVER                 | 0.169 ± 0.010 | 0.182 ± 0.010   | 39 |
| 5-HT TURNOVER               | 1.022 ± 0.032 | 1.011 ± 0.031   | 40 |

  

|               | CORTEX <sup>2</sup> |                |    |
|---------------|---------------------|----------------|----|
|               | Right               | Left           | N  |
| NE            | 0.264 ± 0.008       | 0.273 ± 0.008  | 36 |
| DA            | 0.063 ± 0.008*      | 0.046 ± 0.007  | 36 |
| DOPAC         | ---                 | ---            | -- |
| 5-HT          | 0.259 ± 0.008       | 0.264 ± 0.009  | 36 |
| 5-HIAA        | 0.190 ± 0.006       | 0.214 ± 0.012* | 36 |
| DA TURNOVER   | ---                 | ---            | -- |
| 5-HT TURNOVER | 0.742 ± 0.020       | 0.841 ± 0.064  | 36 |

  

|               | ACCUMBENS       |                 |    |
|---------------|-----------------|-----------------|----|
|               | Right           | Left            | N  |
| NE            | 0.260 ± 0.008   | 0.263 ± 0.008   | 40 |
| DA            | 3.697 ± 0.183*  | 3.427 ± 0.208   | 40 |
| DOPAC         | 1.084 ± 0.050   | 1.023 ± 0.050   | 40 |
| 5-HT          | 0.801 ± 0.040** | 0.748 ± 0.043   | 40 |
| 5-HIAA        | 0.514 ± 0.020   | 0.517 ± 0.026   | 40 |
| DA TURNOVER   | 0.306 ± 0.013   | 0.322 ± 0.015   | 40 |
| 5-HT TURNOVER | 0.667 ± 0.023   | 0.728 ± 0.026** | 40 |

<sup>1</sup>Abbreviations: NE = norepinephrine; DA = dopamine; DOPAC = dihydroxyphenylacetic acid; 5-HT = 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindoleacetic acid; DA TURNOVER = DOPAC/DA; 5-HT TURNOVER = 5-HIAA/5-HT.

<sup>2</sup>DOPAC was not present in measurable quantities in cortex.

\*significantly greater concentration when compared to opposite hemisphere;  $p < 0.05$ .

\*\*significantly greater concentration when compared to opposite hemisphere;  $p < 0.01$ .

asymmetry of this behavior. Additionally, the asymmetry in cortical DA leads to the speculation that the cortex might mediate this asymmetric system, as suggested by Ross and Glick (4).

The asymmetry seen in the serotonergic system in the three areas is intriguing. The left striatum has more 5-HT than the right, and there is a greater concentration of 5-HIAA in the left cortex. However, it is the right accumbens

that has a greater amount of 5-HT. This could be the result of faster metabolism of 5-HT in the left accumbens which may account for the finding of more 5-HT in the right accumbens. These results have possible implications for circling behavior since it has been demonstrated that increased serotonin concentrations inhibit ipsiversive circling (see 19).

The results from the catecholaminergic assays are consistent with previous research. Since work from Glick's laboratory has shown a link between striatal dopamine asymmetry and adult rotation, and since we have demonstrated a link between contralateral striatal DA and postural asymmetries in infancy (10), it would be interesting to explore the relation of asymmetries in the nucleus accumbens to neonatal and adult asymmetrical behaviors. More generally, the relationship between dopaminergic and serotonergic asymmetries in the accumbens and cortex to lateralized behaviors may prove to be of further interest.

#### Acknowledgements

This research was supported, in part, by a Dissertation Fellowship from the Research Foundation of the University of Connecticut to GDR.

This work was done during the tenure of a Scientist Award to SF from the American Heart Association and with funds contributed, in part, by its Massachusetts affiliate.

Also partially supported by USPHS (NIMH) Research Grant MH-31154 to McLean Hospital and an award from the Anderson Foundation.

The authors gratefully acknowledge Loraine Karol for superb secretarial assistance.

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