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Sexually dimorphic brain and behavioral asymmetries in the neonatal rat

(cerebral laterality/spatial preference/postnatal development/deoxyglucose)

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ABSTRACT **The 2-deoxy-D-glucose method was used to study asymmetries in cerebral metabolic activity in neonatal rats. Left–right asymmetries in 2-deoxy-D-glucose uptake were observed in hippocampus, diencephalon, cortex, and medulla–pons: 2-deoxy-D-glucose incorporation was greater in right hippocampus, right diencephalon, left cortex, and left medulla–pons. These asymmetries occurred only in females. We also observed neonatal asymmetries in tail position that, in both sexes, were predictive of adult turning preferences; females had right-sided biases in both neonatal and adult characteristics. Collectively these data indicate that cerebral lateralization is sexually dimorphic and is present at birth.**

Cerebral lateral asymmetry was once believed to be a unique attribute of humans (1) but can no longer be considered as such. An ever-increasing body of knowledge clearly indicates that the brains of normal animals are lateralized (2) and that this lateralization is oftentimes manifest behaviorally (e.g., refs. 3–5). Thus, it has been shown in this laboratory that spontaneous side preferences and nocturnal (6) and amphetamine-induced (7) rotation are related to an intrinsic asymmetry in dopamine content (8), metabolism (9), and dopamine-stimulated adenylate cyclase activity (9) in the corpus striatum of rats. In addition, it has been demonstrated that rats have asymmetries in self-stimulation thresholds that are also related to rotation (10). Moreover, we have recently shown that there exist asymmetries in 2-deoxy-D-glucose (dGlc) incorporation in several regions of the adult (11) and neonatal (12) rat brain.

It is known that the human brain is characterized by an asymmetry in temporal lobe gross anatomy and cytoarchitectonics and that this asymmetry can be observed prenatally (13). Other studies have revealed asymmetries in head posture in human neonates during the first 24 hr of life (14). With these findings in mind, coupled with those that suggest that asymmetry in the rat is in some way reflective of that in the human, we thought it of considerable interest to determine whether the rat brain is lateralized at birth and if this lateralization is in any way related to behavioral phenomena. To study these questions we observed the behavior of neonatal rats during their first week of life and measured dGlc incorporation in various bilaterally dissected brain regions. The present study details behavioral and biochemical findings that, collectively, suggest that cerebral asymmetry is present at birth, is related to an asymmetry in tail posture, and is sexually dimorphic.

MATERIALS AND METHODS

Subjects. Male and female Sprague–Dawley-derived albino rats were obtained from Perfection Breeders, Douglassville, PA. One week was allowed for acclimatization prior to mating. Breeding was accomplished by placing one male with three females in large breeding cages [10 × 18 × 8 inches (1 inch = 2.5 cm)] for 1 week; then the males were removed and the pregnant females were placed into separate breeding cages until parturition, which generally occurred 3 weeks later. On the day of birth the litters were culled to five males and five females. A 12:12 light/dark cycle (lights on at 7:00 a.m.) was maintained and food and water were available ad lib.

dGlc Procedure. dGlc (2-deoxy-D-[1,2-^{3}H]glucose; New England Nuclear; 40.0 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) was dissolved in physiological saline and administered intraperitoneally to rats at a dose of 2 μCi/g of body weight in 50 μl. Three males and three females were used on each day from birth (day 0) through day 7. The rats were removed from their mothers, weighed, quickly injected with isotope, and maintained at 37°C by using a rheostat-controlled heat lamp. Thirty minutes after injection, the animals were sacrificed by decapitation, and their brains were removed and dissected bilaterally into 12 structures (Fig. 1). Each region was weighed and homogenized in 1.0 ml of distilled water. Three aliquots, each 100 μl, were removed and their radioactivities were measured in a Beckman LS 9000 liquid scintillation spectrometer, using New England Nuclear 963 liquid scintillation cocktail. To preclude litter effects, no more than one male and one female per litter were used on any given day. Variability due to prandial condition was minimized by not allowing the pups access to their mothers' teats during the experiments and by performing the experiments at the same time each day (9:00 a.m.).

Behavioral Studies. Behavioral testing was performed on the day of birth (day 0), day 1, or day 2. The mother was removed from her pups for approximately 3 min, during which time the pups were removed from their cage and placed in an open field maintained at 37°C. Testing consisted of placing the pups in a head-to-tail symmetrical posture for 5 seconds and then allowing them to assume their preferred posture, which was invariably an asymmetric one (Fig. 2). A score of left or right was determined by the position of the tail, the sex was then determined by the anogenital distance, and the rat was placed back into its cage. Tail posture was chosen as our index of lateralization because it was robust and easy to score in these young neonates, which rarely locomote at such an early age. We also observed head movements and body posture, but we found that these characteristics were more subtle and difficult to document in an equally objective manner. It should be emphasized that tail scores were determined prior to the determination of sex to preclude observer bias. The pups (n = 231) tested for tail bias were not used for dGlc studies. Randomly selected animals

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Abbreviation: dGlc, 2-deoxy-D-glucose.

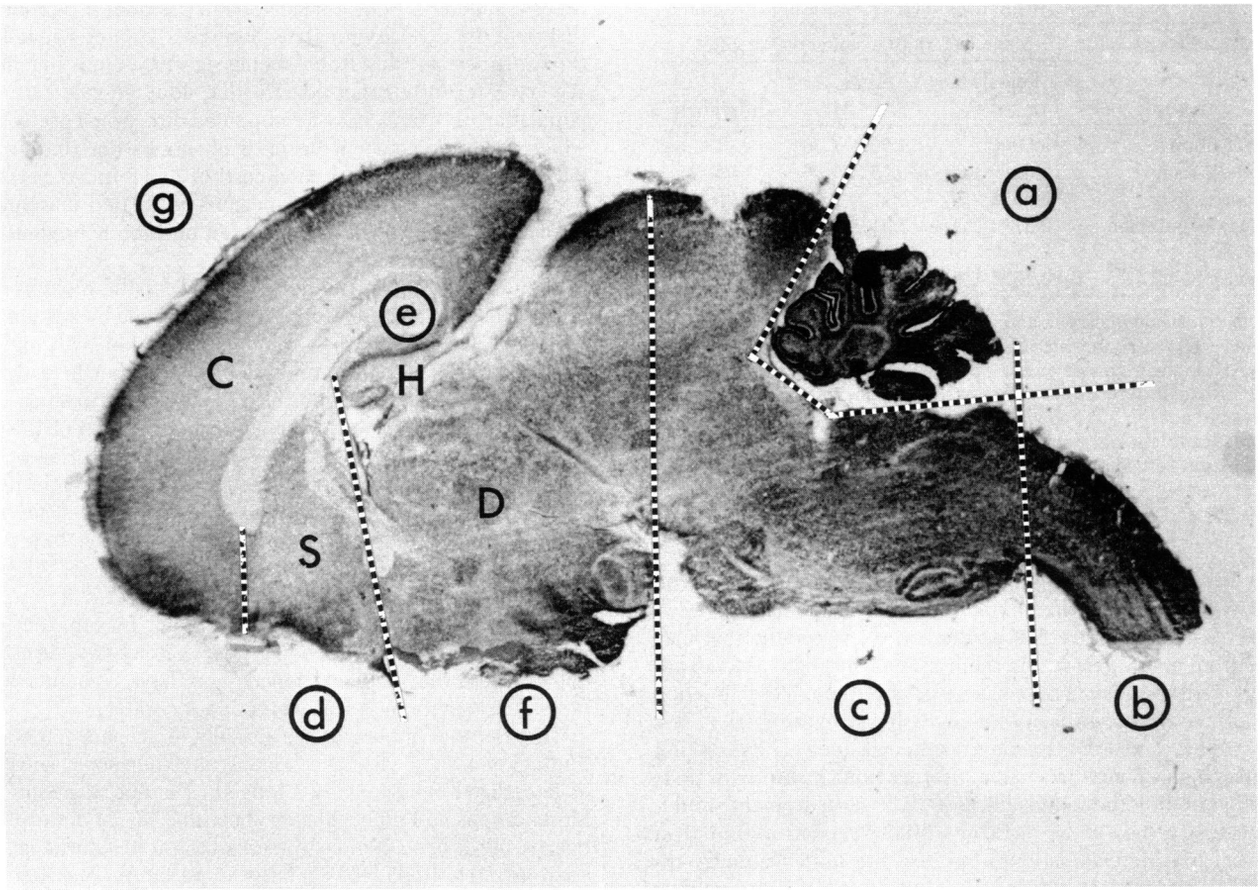

FIG. 1. Sagittal section of neonatal rat brain, illustrating dissection procedure: (*a*) Cerebellum is removed from brainstem. (*b*) Lower medulla/spinal cord is removed and discarded. (*c*) Medulla–pons is removed from diencephalon (note that this includes midbrain). (*d*) Caudoputamen is removed lateral to septum. (*e*) Hippocampus is removed in its entirety lateral to the diencephalon. (*f*) Diencephalon is removed from cortex. (*g*) Entire cortex is removed. H, hippocampus; S, septum; C, cortex; D, diencephalon.

(n = 33) were saved for testing as adults. The latter rats were weaned at day 21 and caged together until day 40, when the males (n = 16) and females (n = 17) were separated and caged in pairs with food and water ad lib. On day 85 these animals were placed in rotometers, administered *d*-amphetamine sulfate (1.0 mg/kg, intraperitoneally), and tested for rotation for 1 hr as described (6).

RESULTS

Neonates Have Asymmetries in dGlc Incorporation. A two-way analysis of variance on the dGlc uptake data was performed separately for each sex. The two factors were side (left–right) and structure. Although the main effect of structure was significant ($P < 0.001$) in both sexes, the interaction between side and structure was significant ($P < 0.02$) only in females. Week 1 mean left–right asymmetries for each structure are summarized in Table 1. A two-way analysis of variance on these asymmetry data showed a significant ($P < 0.02$) interaction between sex and structure. Specific differences between sides and sexes were subsequently demonstrated in Newman–Keuls multiple-range tests (indicated in Table 1). Significant left–right asymmetries in dGlc incorporation were seen only in females and included the medulla–pons, hippocampus, cortex, and diencephalon: dGlc concentration was significantly higher in left medulla–pons, right hippocampus, left cortex, and right diencephalon. Sex differences were significant in all structures.

Asymmetries in Tail Posture Are Sexually Dimorphic. As with the asymmetries in dGlc incorporation, a significant asymmetry in tail posture was noted only in female pups. Frequencies for both sexes are shown in Table 2, which indicates that the tails of females are directed preferentially to the right. Although the tails of the males were biased towards the left, the preference was not significant. However, the difference between the sexes was highly significant. Furthermore, there was a significant ($P < 0.05$, t test) negative correlation ($r = -0.50$,

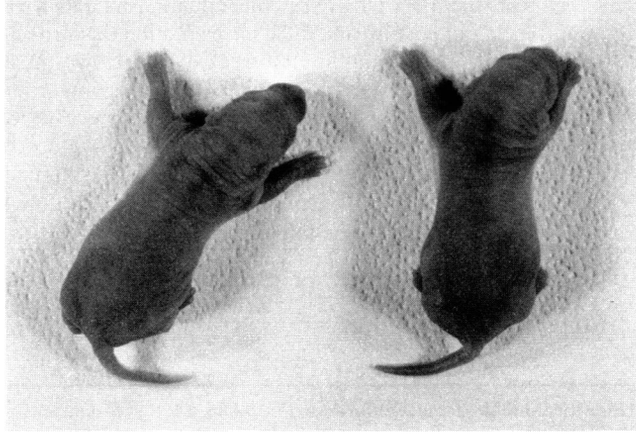

FIG. 2. Day 1 rat pups exhibiting tail bias.

Table 1. Regional left–right asymmetries in brain dGlc uptake

Structure	Mean left–right asymmetry* ± SEM: Female (n = 24)	Male (n = 24)	Differences
Cerebellum	−3.59 ± 2.72	1.60 ± 1.30	†
Medulla–pons	2.82 ± 1.08	−0.48 ± 1.36	‡
Hippocampus	−5.56 ± 2.18	0.22 ± 2.10	‡
Caudate–putamen	0.27 ± 2.30	3.91 ± 4.00	†
Cortex	2.77 ± 1.51	−2.12 ± 1.45	‡
Diencephalon	−2.95 ± 1.32	0.65 ± 1.01	‡

* Left–right asymmetry is defined as [(dpm/g high side)/(dpm/g low side) − 1)] × 100, in which the difference is designated positive when the left is the high side and negative when the right is the high side.
† Significant difference between sexes ($P < 0.05$, Newman–Keuls test) but not between left and right sides.
‡ Significant left–right asymmetry ($P < 0.05$, Newman–Keuls test on dpm/g for left and right structures) and significant difference between sexes ($P < 0.05$, Newman–Keuls test on the above asymmetry ratios).

n = 19) between the percentage of females in each litter with right-sided tails and the number of males in the litter.

The tail asymmetry is a population characteristic that may vary from test to test in the same animal. In pilot work, 15 animals were tested on 2 consecutive days. Although the direction of tail bias was significantly consistent in a one-tailed χ^2 test ($\chi^2 = 3.27$; $P < 0.05$), the degree of consistency (73%) did not appear high enough to enable a direct relationship with dGlc incorporation to be found, because dGlc measurement would, of necessity, have to be made at a different point in time than the tail bias determinations. Because the tail asymmetry diminishes and is no longer apparent by day 4, it is not surprising that the consistency of this measure is less than 100%.

Neonatal Tail Posture Is Predictive of Adult Rotational Preference. In view of the above, with regard to the consistency of tail posture, we felt it important to ascertain whether the tail position was in any way related to or reflective of the adult directional bias measured in a rotometer. We compared the neonatal tail direction with the direction of *d*-amphetamine-induced rotation at 85 days of age: these directions were the same in 28 of the 33 animals (15/16 males, 13/17 females). This association was significant for the whole group (χ^2 test, $P < 0.001$) as well as for each sex separately (χ^2 tests, $P < 0.001$ and 0.03, respectively, for males and females).

DISCUSSION

The results of this study clearly establish that normal rats have asymmetries in tail posture and dGlc incorporation that are observable at birth and during the first week of life. However, it should be noted that the behavioral and metabolic asymmetries we report are left–right asymmetries and are not necessarily related to each other in a contralateral–ipsilateral fashion. Although, as noted above, it was not feasible to make concomitant determinations of dGlc uptake and tail position, it is of interest that both the dGlc asymmetries and the asymmetry in tail posture were sexually dimorphic, being significant only in females. We have recently reported (15) that adult female rats, as a population (n = 602), have a right-sided directional preference, which was predicted on the basis of our earlier finding of a left–right asymmetry in frontal cortical dGlc uptake (11). The neonatal and adult findings appear to be related inasmuch as we have now shown that neonatal tail position is predictive of the adult directional preference.

Table 2. Frequencies of tail bias in neonatal rats

Sex*	Direction†: Left	Right
Male (n = 114)	65	49
Female (n = 117)	45	72

* Significant male–female difference ($P < 0.005$, 2×2 χ^2 test on entire table).
† Significant left–right difference in females only ($P < 0.02$ in females and $P > 0.1$ in males, one-sample χ^2 test on data for each sex).

We did not employ the dGlc method for the measurement of local cerebral glucose utilization as described by Sokoloff and coworkers (16) due to the problems associated with multiple blood sampling in neonatal rats. However, other investigators have employed modifications of this procedure to study cerebral development (17–20). Using our modification of this procedure (21), we were able to discern lateralized differences in dGlc uptake in various structures. These side-to-side differences in dGlc incorporation were observed within individual animals and are, therefore, most likely attributable to intrinsic lateralization in glucose uptake and not reflective of other factors, such as plasma glucose, that would affect dGlc incorporation equally on the two sides of the brain. Inasmuch as dGlc incorporation reflects functional neuronal activity (16), these data suggest that neonatal female rats have asymmetries in metabolic activity in at least three brain regions.

That these asymmetries are sexually dimorphic extends the findings of others, who have demonstrated hormonal sensitivity of lateralized behaviors (22). Indeed, it is not surprising that hippocampus and diencephalon show this sexual dimorphism, because it has been demonstrated that these structures can concentrate [^{3}H]estradiol (23), and the hippocampus manifests sexual dimorphism in sprouting after experimental deafferentation (24). Although a mechanism for the sex difference in medulla–pons is not obvious, there are many anatomical connections between this structure, hippocampus, cortex, and diencephalon that may serve to modulate or drive the medulla–pons asymmetry. Oke *et al.* (25, 26) have shown that the concentration of norepinephrine in thalamus is lateralized, and our diencephalic dGlc asymmetry may, in some way, be related to this—especially when one considers that dGlc uptake is thought to be primarily into nerve terminals (27). The asymmetry in neonatal cortical dGlc uptake is in the same direction as we observe in the adult rat (11), suggesting that it may be present during the entire life span of the female.

Our finding of a tail asymmetry may have some relevance to the findings of others, who have demonstrated a relationship between tail pinch and dopaminergic nigrostriatal function (28). At the very least, this suggests that tail sensory input is in some way related to neuronal systems that are known to be lateralized (8, 9). The reason why this tail asymmetry is sexually dimorphic is at this time elusive. However, it has recently been reported that female mice developing between male fetuses *in utero* have significantly higher concentrations of testosterone in their blood and amniotic fluid and that later in life these females differ from their littermates in various sexually related characteristics (29). The probability of female mice having this *in utero* position should increase with the number of males in the litter. Considering these data, and our observation of sexual dimorphism in tail posture, we performed a regression analysis of the strength of the female tail bias versus the number of males in the litter. The fact that we found a significant inverse correlation between the percentage of females in each litter with right-biased tails and the number of males in the litter suggests that hormonal status prior to birth may modulate asymmetries observed at a later time. These findings are likely to

have important implications for developmental theories of sexual differences in cognitive function and brain lateralization (30).

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