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# Effects of Intrauterine Position on the Metabolic Capacity of the Hypothalamus of Female Gerbils[1]

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JONES, D., F. GONZALEZ-LIMA, D. CREWS, B. G. GALEF, JR. AND M. M. CLARK. *Effects of intrauterine position on the metabolic capacity of the hypothalamus of female gerbils.* PHYSIOL BEHAV **61**(4) 513–519, 1997.—The intrauterine position that a rodent fetus occupies relative to members of the same or opposite gender affects both its reproductive physiology and behavior when adult. Cytochrome oxidase histochemistry was used to assess regional differences in the oxidative metabolic capacity of the hypothalamus of female Mongolian gerbils that developed in utero between 2 female fetuses ($n = 15$) or between 2 male fetuses ($n = 14$). Cytochrome oxidase reactivity was measured densitometrically by experimenters unaware of subject intrauterine position. Gray-to-white matter ratios of optical density in 11 brain regions were used as a normalized index of metabolic capacity. Significant group differences in the metabolic capacity of the medial and the posterior parts of the anterior hypothalamus were revealed. Females that developed in utero between 2 male fetuses showed significant increases (19–22%) in cytochrome oxidase reactivity in these brain regions compared to that in females that developed between 2 female fetuses. The medial part of the anterior hypothalamus contributes to copulatory behavior, whereas the posterior part of the anterior hypothalamus may be involved in the control of pituitary gonadotropin secretion. Both these functions are influenced by intrauterine position during fetal life. To our knowledge, this is the first demonstration of metabolic changes in hypothalamic areas of the adult related to the differences in intrauterine position. © 1997 Elsevier Science Inc.

Intrauterine position  Cytochrome oxidase  Functional capacity  Brain metabolism  Androgenization

THE intrauterine position that a rodent fetus occupies relative to fetuses of the same or opposite gender affects its prenatal level of exposure to testosterone (3,33,35,36). Androgens secreted by male rodent fetuses during the last week of gestation diffuse through amniotic fluid and cross fetal membranes to adjacent fetuses (12,37). Consequently, late in gestation, fetuses located between 2 males (2M males or 2M females) are exposed to higher levels of exogenous testosterone than are fetuses located between 2 females (2F males or 2F females) (3,35,36). In Mongolian gerbils (*Meriones unguiculatus*), the subject species in the present study, as in house mice (35), 2M female fetuses have higher circulating levels of testosterone late in gestation than do 2F female fetuses (3).

Such naturally occurring variation in prenatal exposure to androgens, correlated with the intrauterine position that a fetal female rodent occupies, appears to affect its reproductive behavior when adult. Gerbil females that develop in 2F intrauterine positions exhibit vaginal introitus at an earlier age than do their 2M sisters (4), and such early maturing females reproduce for the first time when younger, have more litters and a greater percentage of females per litter than do late-maturing female gerbils (5,7). Late-maturing female gerbils tend to attack, but their early maturing sisters tend to mate, with strange males introduced into their home cages (8).

The marked differences in the reproductive physiology and behavior exhibited by early and late-maturing female gerbils, taken together with observed correlations between intrauterine position and fetal exposure to gonadal hormones, suggest the hypothesis that intrauterine position affects the probability of occurrence of hormonal events that modulate the functional capacity of the nervous system of female gerbils. However, to date, there is little direct evidence that the naturally occurring variation in prenatal exposure to gonadal hormones that occurs in Mongolian gerbils and other litter-bearing rodent species, as a consequence of fetal intrauterine position, actually affects the functional capacity of the nervous system. Faber and Hughes (14) reported a significant correlation between neonatal anogenital distance (a correlate of intrauterine position) and the size of the sexually dimorphic nucleus (SDN) of female rats. However, they failed to consider the possibility that both anogenital distance and size of SDN might covary with body weight. Consequently, Faber and Hughes (14) may have shown only that larger female

[1] Results of this study have previously been published in abstract form (Jones, D.; Gonzalez-Lima, F.; Crews, D.; Galef, B. G., Jr.; Clark, M. M. Effects of intrauterine position on hypothalamic activity of female gerbils: A cytochrome oxidase histochemical study. Soc. Neurosci. Abstr. 20(3): 1739; 1994 (26).

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rats have both relatively long anogenital distances and relatively large brains. Tobet et al. (34) did not find an effect of intrauterine position on aromatase activity in homogenates of the preoptic area of rat hypothalamus.

The differences in reproductive biology exhibited by early and late-maturing gerbils suggest, however, that the naturally occurring variation in exposure to hormones experienced by female gerbils from different intrauterine positions might affect the structure or function of areas in gerbil hypothalamus that are involved in reproduction and modifiable by exposure to gonadal steroids in infancy or adulthood (9,33, for review, see 21).

The objective of the present study was to utilize cytochrome oxidase (C.O.) histochemistry (18–20,40–42) to assess hypothalamic metabolic capacity in relation to intrauterine position in female Mongolian gerbils. Histochemistry of C.O. reactivity has become the tool of choice for displaying visually the regional oxidative metabolic capacity of the nervous system (40–42). Due to its rate-limiting role in oxidative metabolism, C.O. is an excellent marker of cellular oxidative metabolic capacity: sustained oxidative metabolism of nerve cells in a brain region leads to increased C.O. in their mitochondria (41). The functional capacity of nerve cells is mainly determined by their ability to use ATP for the high-energy-requiring membrane potentials involved in synaptic communication (17). Because C.O. is the rate-limiting enzyme for the oxidative energy metabolism of nerve cells coupled to ATP production, the level of C.O. reactivity assessed postmortem is an endogenous marker of the level of nervous tissue metabolic capacity (41). Using this technique, brain metabolic capacity was compared in female gerbils that developed in utero between 2 female fetuses or between 2 male fetuses. The C.O. histochemical technique served to map relative differences in regional neural metabolic capacity between 2M and 2F female gerbils (18–20).

## MATERIALS AND METHODS

### *Subjects*

We used 30 female Mongolian gerbils (*Meriones unguiculatus*) aged 12–15 months and weighing 66–115 g at the time of sacrifice. Gerbils were obtained from a colony maintained at McMaster University (Hamilton, Ontario). Subjects used for the study were divided into 2 groups based on their intrauterine position. 2F female gerbils were those that developed in utero between 2 female fetuses and 2M females developed between 2 male fetuses (3). Intrauterine position was determined during surgical removal of subjects from the uterus; for details, see (8). After weaning at 30 days of age, subjects were maintained in groups of 3–4 same-gender littermates, and mated in pairs starting at 60 days of age. Each subject was killed 1 week after the birth of her seventh litter, between 1 and 3 PM in the daylight portion of a 12:12 light:dark cycle (light onset at 5 am). Subjects were lightly anesthetized with ether, killed by decapitation, and their unfixed brains were quickly wrapped in aluminum foil and dropped in liquid nitrogen. The frozen brains from all subjects were coded and shipped to the University of Texas (Austin), where they were analyzed by experimenters unaware of subject condition (2M or 2F). Initially, the brains of 15 subjects were analyzed using C.O. histochemistry, resulting in evidence of group differences. The first study was replicated using 15 additional subjects to confirm the initial results and conclusions. One 2M subject was excluded due to artifacts during brain removal, leaving 15 2F and 14 2M subjects.

### *Tissue Processing*

Brains were sectioned in a Reichert-Jung cryostat at −20°C in the flat skull plane of Loskota et al. (27). The 40 $\mu$m sections were picked up on clean slides and stored at −40°C in an ultra freezer. To aid in region identification and correlate the C.O. labeling with tissue morphology, adjacent sections were processed for Nissl or C.O. stains. One series of sections was delipidized, buffered, stained in a 0.01% Cresyl Violet solution, differentiated for 20 min, dehydrated, cleared, and cover-slipped with Permount, for Nissl staining. The adjacent series of frozen sections from each brain was processed by the modified C.O. histochemical procedure described in Gonzalez-Lima and Jones (20). This C.O. staining procedure involved metal intensification through cobalt preincubation, as well as oxygenation and heating of the reaction medium. Briefly, the following solutions were prepared: 1. phosphate buffer (0.1 M, pH 7.6); 2. preincubation solution of 0.05 M Tris buffer (pH 7.6), mixed with 0.0275% cobalt chloride, 10% sucrose, and 1% dimethylsulfoxide (DMSO); 3. an incubation solution similar to Silverman and Tootell's (32), as modified from (40), containing 0.05% diaminobenzidine (DAB), 0.0075% cytochrome c, 5% sucrose, 0.002% catalase, 0.25% DMSO (v/v), and phosphate buffer added to make 700 ml. Fresh preincubation and incubation solutions were prepared for each individual rack of slides stained. Sections were kept frozen on the slides until they were processed for C.O. The following sequence of baths and times were used: 1. 0.5% glutaraldehyde in 10% sucrose phosphate buffer for 5 min.; 2. phosphate buffer, 4 changes for 5 min each; 3. preincubation in Tris buffer mixture for 10 min; 4. rinse in phosphate buffer; 5. incubation in DAB solution (oxygenated for 5 min before introduction of sections) at 37°C with continuous stirring in a dark oven for 1 h; 6. 10% buffered formalin with 10% sucrose for 30 min; 7. dehydration baths of 30, 50, 70, 90, 95 (2 changes), and 100% (3 changes) ethanol for 5 min each; 8. 3 changes of xylene for 5 min each; and, finally, 9. cover-slipping with Permount. All procedures were performed at room temperature with the exception of the incubation bath (37°C).

### *Delineation of Brain Regions and Image Analysis*

Using the adjacent sections stained for Nissl substance, representative sections of the 11 regions of interest from each brain were chosen. A stereotaxic atlas of the gerbil brain (27) was used to select gerbil brain sections that approximately matched the levels of sections between bregma 0.0 and −1.3 mm. These bregma coordinates were used for reference points and do not indicate actual coordinates in the gerbil brain. The levels chosen in the gerbil brain are shown in Fig. 1A–H. They included the preoptic area: medial (MPO) and lateral (LPO) parts; anterior hypothalamic area: medial (AHy-M), lateral (AHy-L), and posterior (AHy-P) parts; bed nucleus of stria terminalis (BST); suprachiasmatic nucleus (SCN); paraventricular nucleus (PV); lateral hypothalamic area: anterior (LH-A) and posterior (LH-P) parts; and ventromedial hypothalamic nucleus (VM).

For image analysis, the MPO was defined as that area just lateral to the third ventricle, bounded dorsally by the anterior commissure and ventrally by the optic chiasm (corresponding to a level of bregma 0.0 mm) (27). This area was differentiated from the LPO by its higher cell body density as visualized with Nissl stains (Fig. 1A, B). The AHy was defined as a loose collection of cells lateral from the third ventricle, but not extending past the lateral edge of the fornix. Rostrally (bregma −0.4 mm) the AHy is bounded by the BST dorsally and by the SCN ventrally. The BST and the medial (AHy-M) and lateral (AHy-L) parts of the AHy were measured at the level of the SCN. The SCN is a distinct area of densely packed somata close to the midline and just dorsal to the optic chiasm. The BST was defined as the cell bodies just ventral to the fiber bundle of the fornix

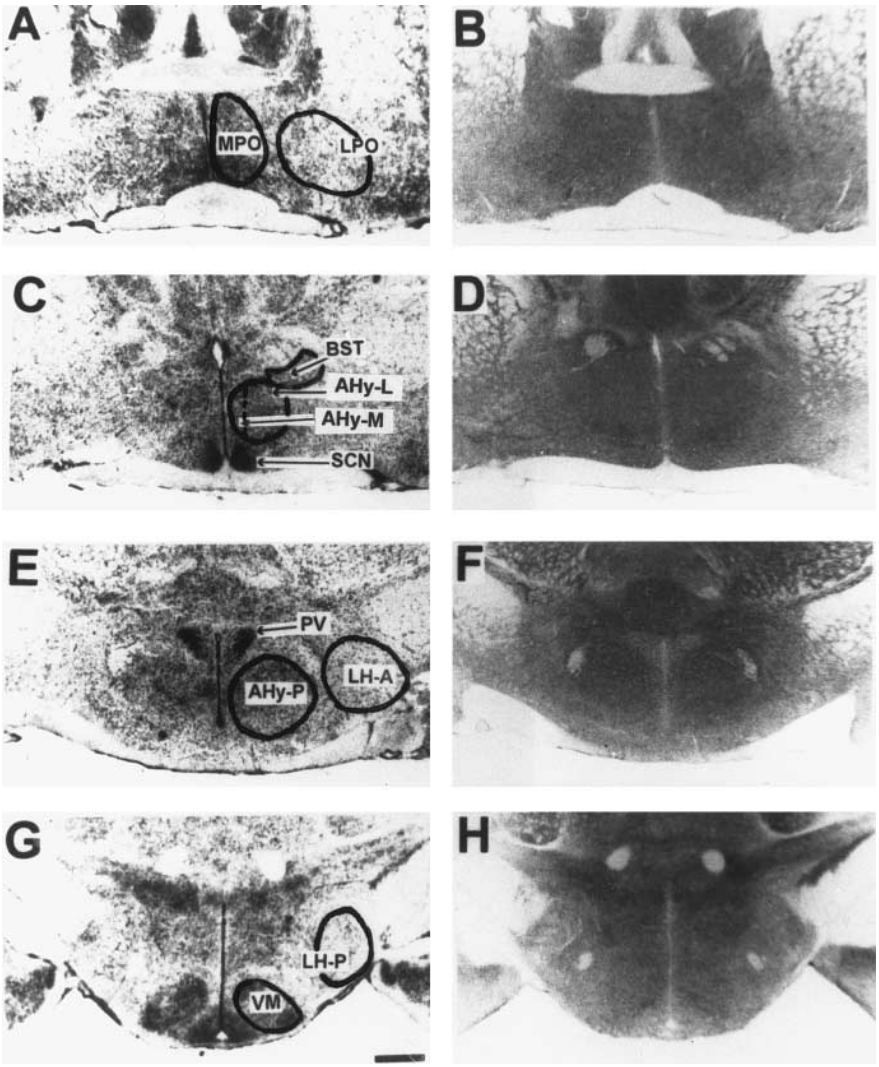


FIG. 1. Photomicrographs of transverse sections of the hypothalamus processed with Nissl (left column) and cytochrome oxidase (right column) stains showing the regions analyzed. Each row corresponds to pairs of adjacent sections of a 2F female gerbil, ordered from rostral (A, B) to caudal (G, H) levels. Sections correspond to bregma levels 0.0 (A, B), −0.4 (C, D), −0.8 (E, F), and −1.3 (G, H) (27). The labels for the regions correspond to the abbreviations listed in Table 1. Scale bar = 0.7 mm.

and dorsal to the AHy-L. Measurements for the AHy-M were taken just lateral to the third ventricle and medial to the AHy-L (Fig. 1C, D). The posterior portion of the AHy (AHy-P) was measured at the level of the PV (bregma −0.8 mm). The PV is a distinct area of high cell body packing just lateral to the third ventricle and ventral to the zona incerta (Fig. 1E, F). The most caudal region analyzed was the VM (Bregma -1.3 mm). The VM was easily identified as an oval cluster of cell bodies lateral to the third ventricle and dorsal to the arcuate hypothalamic nucleus (Fig 1 G,H).

Also delineated for measurement was the LH. This was defined as a relatively homogenous area extending laterally from the vertical boundary delineated by the fornix, but not extending past the optic tract. Measurements for the anterior portion (LH-A) were taken at the level of AHy-P and measurements for the posterior portion (LH-P) were taken at the level of the VM. Measurements for both the anterior and posterior LH were taken dorsal to the supraoptic decussation, along the horizontal plane of the anterior or basolateral amygdaloid areas, respectively.

In addition to the gray matter regions of interest, reference white matter readings were taken from each animal to normalize its data for the comparison between subjects. The white matter of the sensory root of the trigeminal nerve was used to formulate a relative measure of optical density in the form of a gray-to-white matter ratio for each brain region in each subject. In this manner, nonspecific staining of the white matter within a brain was weighted less heavily than C.O. staining that was specific to cell metabolic capacity. There were no significant staining differences between groups in the white matter used as reference in the ratios ($t = -0.73$, $p > 0.5$). Therefore, optical density values for the regions of interest were normalized using gray-to-white matter ratios in each subject, and compared across groups using 2-tailed Student's *t*-tests.

TABLE 1

CYTOCHROME OXIDASE REACTIVITY IN THE BRAIN OF 2M AND 2F FEMALE GERBILS

| Area | 2M | 2F |
|---|---|---|
| Medial preoptic area (MPO) | 3.26 ± 0.12 | 3.31 ± 0.18 |
| Lateral preoptic area (LPO) | 3.34 ± 0.12 | 3.31 ± 0.17 |
| Bed nucleus of stria terminalis (BST) | 3.20 ± 0.11 | 3.21 ± 0.12 |
| Anterior hypothalamic area, medial (AHy-M) | 4.18 ± 0.32 | 3.48 ± 0.08* |
| Anterior hypothalamic area, lateral (AHy-L) | 3.47 ± 0.10 | 3.46 ± 0.14 |
| Suprachiasmatic nucleus (SCN) | 4.18 ± 0.48 | 3.67 ± 0.18 |
| Paraventricular hypothalamic nucleus (PV) | 3.24 ± 0.09 | 3.08 ± 0.08 |
| Lateral hypothalamic area, anterior (LH-A) | 3.69 ± 0.17 | 3.33 ± 0.08 |
| Anterior hypothalamic area, posterior (AHy-P) | 4.75 ± 0.37 | 3.86 ± 0.11* |
| Ventromedial hypothalamic nucleus (VM) | 4.55 ± 0.49 | 4.13 ± 0.27 |
| Lateral hypothalamic area, posterior (LH-P) | 3.76 ± 0.24 | 3.82 ± 0.21 |

Values are mean ± SEM of gray-to-white matter ratios of optical density in the sections. * Significant difference ($p < 0.05$), 2-tailed independent Student's t test.

The optical density in the C.O.-stained sections was analyzed as previously described (20), using an image-processing system consisting of a high-gain camera, a Targa-M8 image capture board, a 486 computer, Sony color monitor, DC-powered illuminator, and JAVA software (Jandel Scientific, San Rafael, CA, U.S.A.). The system was calibrated using an optical density step tablet (Kodak Calibration Tablet No. 2) and the histochemical reaction product (chromatic indicator of C.O.) was measured in optical density (O.D.) units. In each region measured, 4 readings were taken per section with 3 sections per brain (i.e., 12 readings per region per brain). Multiple readings were taken from both hemispheres of the brain to avoid artifacts. For each region measured, the size of the measuring window of the densitometer was set to approximately 1 quarter the size of the whole region, and 4 adjacent sites were measured to cover the region. The size of this window was held constant across subjects. For example, in the LH, a window of 13 × 13 pixels was used. Thus, for all animals, in each of 3 sections, 4 separate sites of 13 × 13 pixels were measured in such a way that the majority of the LH was measured. Thus, the area measured was constant across all animals, and fell within the borders of the anatomical region of interest.

## RESULTS

### *General C.O. Staining Characteristics*

The histochemical reaction product appeared fairly evenly distributed throughout regions of the hypothalamus, with distinct boundaries seen between regions in the SCN, PV, and VM nuclei. The lateral boundaries of both the SCN and VM nuclei could be observed by visual inspection as slightly darker arcs corresponding to the lateral edges of these nuclei, as seen in Nissl stains of adjacent sections. Conversely, the PV was easily identified by its light C.O. staining. In C.O.-stained sections, the PV appeared as a well-defined area of low (median of 0.037 O.D. units) reactivity surrounded by a homogenous area of higher reactivity. However, other regions of the hypothalamus could only be clearly differentiated with the aid of quantitative densitometry. For example, the boundary between the AHy-L and the BST was discernible as a small shift from moderate (median of 0.041 O.D. units) to low (median of 0.038 O.D. units) reactivity.

### *Group Comparison of C.O. Reactivity*

The 12 readings taken per subject per area were averaged to obtain a single score per subject per area. The mean scores from subjects from each group were compared statistically with independent *t*-tests (2-tailed). Two of the regions of interest showed a significantly ($p < 0.05$) greater C.O. reactivity in 2M females than in 2F females (Table 1). These 2 areas were the posterior portion of the anterior hypothalamic area ($t = 2.49$, $p < 0.05$), with a 22.1% increase, and the medial portion of the anterior hypothalamic area ($t = 2.24$, $p < 0.05$), with a 19.1% increase, in 2M over 2F females. The patterns of reactivity in the AHy-M and the AHy-P are illustrated in Fig. 2A–D. It is possible to inspect the photomicrographs and notice some darker labeling of AHy-M and AHy-P in 2M gerbils. However, such simple in-

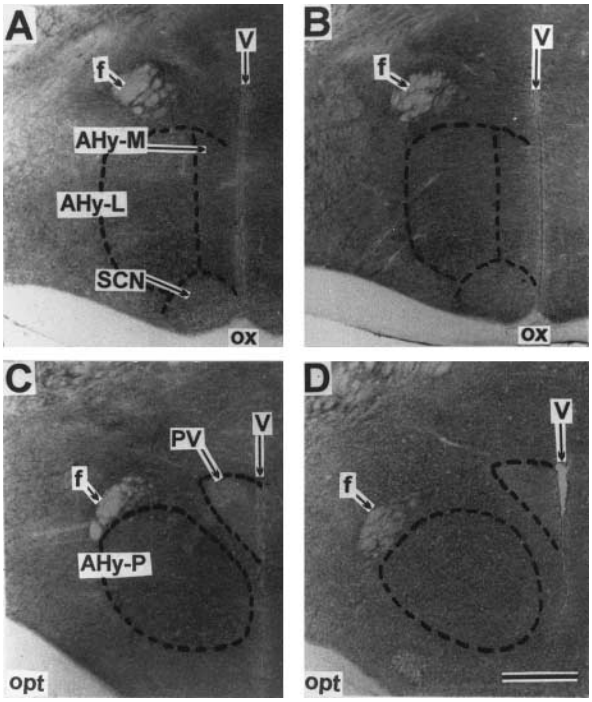


FIG. 2. Photomicrographs of the left hypothalamus of 2M (A, C) and 2F (B, D) female gerbils. (A) and (B) show a comparison at the level of the medial (AHy-M) and lateral (AHy-L) parts of the anterior hypothalamus. A vertical dashed line from the center of the suprachiasmatic nucleus (SCN) delineates the area of measurement for the AHy-M, located parallel to the third ventricle (V). Immediately lateral to the AHy-M was the AHy-L, and its dorsolateral boundary was delineated using the fornix (f) as a landmark. (C) and (D) show a comparison at the level of the posterior part of the anterior hypothalamus (AHy-P) and the paraventricular nucleus (PV), also delineated by dashed lines, in 2M (C) and 2F (D) gerbils. The AHy-P is bounded dorsolaterally by the fornix (f), and medially by the third ventricle (V). Also labeled are the optic chiasm (ox) and the optic tract (opt) used as landmarks to identify these levels. Top is dorsal, left is lateral. Scale bar = 0.5 mm.

spection of photomicrographs is inadequate to evaluate the data because photomicrographs may show contrast differences due to developing and processing factors and other merely incidental differences. The effects reported here were based on reproducible differences determined by direct densitometric analysis of C.O.-stained sections from each structure in every subject and found to be statistically reliable for the group (Table 1).

## DISCUSSION

The major point of this study is that there are functional changes in the adult brain as a result of intrauterine position. To our knowledge, this is the first direct evidence of metabolic changes in areas of the adult brain related to differences in the individual's intrauterine position during fetal life. We demonstrated that 2 of the 11 regions measured exhibited significant differences between females from different intrauterine positions. Increases in C.O. reactivity in these regions were seen in female gerbils from 2M compared to 2F intrauterine position. In many of the regions of interest, 2M females had higher mean ratios of C.O. reactivity than the 2F females, though this was not found in every case and many of these differences were not significant (Table 1). Thus, it was not simply the case that 2M females had a global increase in metabolic capacity or that differences seen were equal across all brain regions.

### *Functional Implications of Brain Changes in C.O. Reactivity*

Due to the essential role C.O. plays in cellular respiration, this mitochondrial enzyme has been used as an endogenous marker of regional tissue metabolic capacity (41). C.O. catalyzes the electron transfer necessary for ATP synthesis (39). The major energy-consuming mechanism using ATP in neurons is the $Na^+/K^+$ pump that preserves the ion balance necessary to maintain the resting membrane potential. Increased neuronal electrical activity leads to increased need for ATP and, thus, an increase in cellular aerobic respiration to supply the necessary energy (25). Based on this coupling between C.O. activity and energy-demanding electrical activity, there is a coupling of neuronal C.O. activity with neuronal level of functional activity (40). The more active neurons in a brain region have increased C.O. content in their mitochondria (25). Hence, C.O. staining utilizing the chromagen diaminobenzidine has been used as a mitochondrial stain to provide a visual display of changes in the metabolic capacity of nervous tissue (41). Recent modifications of the C.O. method using internal standards and quantitative densitometry have facilitated the measurement of differences in C.O. activity in histochemically stained brain slices (18–20,28).

Previously, C.O. techniques have been used to visualize functional changes in brain regions as an effect of manipulations such as long-term visual deprivation (42) or electroconvulsive treatment (28). Because both behavioral and brain morphological differences have been reported as a result of intrauterine position and hormonal differences (6,9), we applied the C.O. functional measure to reveal neural metabolic correlates of known effects of intrauterine position on behavior in the female Mongolian gerbil. The present findings suggest that quantitative C.O. histochemical methods may be useful to study hormonally mediated effects on brain metabolic capacity.

One way to explain the energy metabolic processes represented by C.O. histochemistry is to compare C.O. with 2-deoxyglucose (2-DG) uptake (17). C.O. histochemistry and 2-DG autoradiography reveal energy processes analogous to potential and kinetic energy, respectively (18). C.O. reactivity visualizes a longer-lasting level of brain metabolic capacity. In contrast, 2-DG and related glucose utilization techniques visualize a shorter-term expression of metabolic energy use by the brain during the period of tracer uptake. 2-DG is better suited to assess short-term changes in metabolic activity evoked by a stimulus situation during the postinjection survival period than is C.O.; C.O. is better suited to assess long-term levels in enzymatic content that develop slowly. This means that the goal of C.O.-mapping experiments cannot be to assess acute changes in metabolic activity over a period of min in response to a stimulus as in the case of 2-DG studies (18). The C.O. technique is suited for assessing the baseline potential capacity for metabolism of brain regions, as done in this study. Therefore, the C.O. approach is suitable to assess the effects of chronic treatments over days or longer periods (28,40-42). This would reveal alterations in metabolic capacity linked to the sustained metabolic demands on brain regions affected by the intrauterine environment.

Although hypothalamic areas have been shown to be morphologically different between male and female rats (22) and gerbils (9,11,43) or after adult testosterone treatment (9), the present study is the first to reveal a difference in C.O. reactivity and, hence, functional tissue metabolic capacity. Although this metabolic capacity was not quantified in terms of absolute biochemical enzyme activity units, the gray-to-white matter O.D. ratios found correspond to a level of C.O. reactivity similar to that found in sections of whole rat brain homogenates (20). The gray-to-white O.D. ratios we found were consistent with findings that hypothalamic nuclei have lower metabolic capacity than other brain regions such as auditory nuclei (2,18,20,24). Hypothalamic regions had C.O. reactivities ranging between 3 to 5 times that of the reference white matter; brain nuclei with sustained high metabolic capacity in the gerbil have been shown to have C.O. reactivity levels 6 to 10 times that of reference white matter (20). In addition, all the hypothalamic regions, as well as the BST, exhibited C.O. staining characteristic of neuropil. In the regions examined, few neurons were observed with higher concentrations of C.O. reaction product in cell bodies than were found in neuropil. This pattern of higher neuropil C.O. reactivity is similar to that reported in forebrain thalamic and cortical regions, and it is the opposite of the pattern of higher C.O. reactivity found in cell bodies of medullary and pontine sensory nuclei (20). This pattern suggests that the cellular distribution of C.O. reactivity in the hypothalamus and the BST is similar to that of other forebrain regions.

### *Relationship to Sexually Dimorphic Areas and Rodent Reproductive Behavior*

Adult testosterone levels influence the morphology of a sexually dimorphic area (SDA) in the caudal preoptic area-anterior hypothalamus of the gerbil, as defined by Commins and Yahr (9). This region appears to be homologous to the rat's anterior hypothalamic nucleus (1), which includes the sexually dimorphic nucleus of the medial preoptic area (22). Studies have shown that the rat sexually dimorphic nucleus, and its presumed homologue in the gerbil, are involved in regulation of sexually differentiated endocrine and behavioral reproductive functions (23,30,38,43,44). In rats, neural substrates, that facilitate lordosis (23) tonically inhibit lordosis performance (30) and initiate the required surge of luteinizing hormone secretion for ovulation (38), seem to involve the sexually dimorphic nucleus of the preoptic area (1).

The AHy-M, as defined here, appeared to overlap with portions of the region previously defined as the medial SDA in gerbils (9,11,43). However, a direct comparison of the AHy-M with the SDA is difficult because Yahr and collaborators (9,11,43) used a plane of sectioning different from the flat skull plane (27)

used in this study. In the Yahr et al. studies, the medial SDA lies between the anterior commissure and the SCN (9,11,43). In our plane of sectioning, the anterior commissure cannot be seen at the level of the AHy-M and the SCN, only in more rostral sections. Because the AHy-M lies dorsal to the SCN, serial Nissl-stained sections indicated that the AHy-M may overlap only with ventrocaudal portions of the medial SDA in gerbils (9,11,43). Similarly, our AHy-L appeared to overlap with ventrocaudal portions of the region defined as the lateral part of the SDA in the gerbil (9,11,43).

The SDA contains sex steroid hormone-concentrating cells (10,29), and it is essential for copulation in male gerbils (43,44). The role of the SDA on gender-differentiated behavior is consistent with the observed intrauterine-position effect on the functional capacity of the AHy-M. The lack of an observed difference between 2M and 2F females in the AHy-L, which includes some of the lateral SDA, may be due to the fact that the region measured encompassed a larger area. Another possibility is that there is no functional difference in the lateral SDA, or the AHy-L as measured here, between 2M and 2F female gerbils.

The functional changes seen in metabolic capacity were not limited to areas possibly related to the SDA. The AHy-P showed increased metabolic capacity in a region that has not been described as morphologically dimorphic in gerbils. A population of gonadotropin-releasing hormone (GnRH)-containing neurons has been found in the AHy-P in all vertebrates studied to date (31); these send their axons to the median eminence, where they terminate in the hypothalamo-hypophyseal portal system. It is possible, therefore, that, in the gerbil, the AHy-P contains neurons that secrete GnRH that, in turn, controls the release of luteinizing hormone (LH) from the pituitary. In the female, the pattern of LH release is cyclic, leading to ovulation, whereas, in the male, the pattern of LH release is relatively constant. Because this GnRH-LH system is necessary for puberty and fertility (13), the observation that 2M female gerbils reach puberty later (4) and produce fewer young as adults (5,7) may reflect the observed differences in metabolic capacity in the AHy-P.

Our findings may serve as a basis for investigating the functional contributions of the identified brain regions in the differences in reproductive behavior exhibited by androgenized females (15,16). The present C.O. method may also be used to detect changes in brain activity reflecting different behavioral experiences (17). For example, allowing 1 set of females to engage in reproductive behaviors, examining C.O. activity, and then comparing that to animals for whom reproductive experiences had been prevented. These are future questions, for which this report might provide a starting point.

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