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Sex and Intrauterine Position Influence the Size of the Gerbil Hippocampus

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SHERRY, D. F., B. G. GALEF, JR. AND M. M. CLARK. *Sex and intrauterine position influence the size of the gerbil hippocampus.* PHYSIOL BEHAV **60**(6) 1491–1494 1996.—Sex differences in home range size and spatial ability are predictive of sex differences in the relative size of the hippocampus in rodents. Such differences in behavior and hippocampal volume are presumed to be, in part, the result of differences in perinatal exposure to hormones. We predicted from differences in the size of home ranges of male and female Mongolian gerbils (*Meriones unguiculatus*) in the wild that the hippocampus of male gerbils would be relatively larger than that of females. We examined the effect of prenatal hormonal influences on hippocampal size by comparing hippocampal volume of males and females from 2F and 2M intrauterine positions to that of randomly selected males and females. We found that, as predicted, randomly selected males had a significantly larger hippocampus, relative to telencephalon, than did randomly selected females. However, males and females from 2F and 2M intrauterine positions did not differ in relative hippocampal size. Possible explanations for the absence of a sex difference in hippocampal size in male and female gerbils from 2F and 2M intrauterine positions are discussed. *Copyright © 1996 Elsevier Science Inc.*

Hippocampus Sex difference Intrauterine position Spatial ability Gerbil

IN A number of rodent species in which males have larger home ranges than do females, males tend to have a larger hippocampus and to perform better than females on laboratory tests of spatial ability (6,8–10,15,16). For example, male meadow voles (*Microtus pennsylvanicus*) have larger home ranges during the breeding season than do female meadow voles, show superior performance in mazes, and have a larger hippocampus relative to brain size (8–10,15). Male bannertail and Merriam's kangaroo rats (*Dipodomys spectabilis* and *D. merriami*) mate polygamously and have larger home ranges than do females of these species. In both, the hippocampus is relatively larger in males than in females (16). Mongolian gerbils (*Meriones unguiculatus*), the subjects of the present study, also exhibit a sex difference in home range size in the wild. The home ranges of adult males can be 2 to 3 times larger than those of adult females (1,11).

In many litter-bearing rodents, such as the Mongolian gerbil, house mouse (*Mus musculus*) and Norway rat (*Rattus norvegicus*), the intrauterine position occupied by a fetus, relative to fetuses of the same or opposite sex, can profoundly influence the hormonal milieu in which development occurs (2). Male fetuses that occupy an intrauterine position between two males (2M males) have greater blood concentrations of testosterone than do their brothers in an intrauterine position between two females (2F males). Female fetuses located between two males (2M females) have higher testosterone titers than do their sisters located between females (2F females). This difference in testosterone levels observed in rodent fetuses from different intrauterine positions is the result of androgens, secreted by the testes of male fetuses during the last week of gestation, diffusing through the amniotic fluid and across fetal membranes to adjacent fetuses (5,22).

Perinatal exposure of rodent fetuses to androgens produces differences between the sexes in use of space in natural environments (14,24), in performance on laboratory tests of spatial ability (7,23), and in hippocampal anatomy (18,19). Female house mice gestated in intrauterine positions between two male fetuses (2M females) and released as adults into a natural habitat had home ranges that were significantly larger than those of females gestated between two females (2F females) (24). Female voles (*Clethrionomys rufocanus*) from litters with a high proportion of males, and therefore likely to have been adjacent to males in utero, dispersed further than females from less male-biased litters (14). Female rats from litters with a high proportion of males performed more accurately in a 12-arm radial maze than did females from litters with a low proportion of males (23). Administration of testosterone propionate to neonatal female rats improved their performance as adults in the water maze and caused the sexually dimorphic granule cell layer of the hippocampus to more closely resemble that of males (18,19).

If differences in the relative hippocampal size of male and female rodents that differ in home range size in nature result from a difference in their perinatal exposure to testosterone, we would predict that male Mongolian gerbils would have a larger hippo-

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campus than do females. We would also predict that intrauterine position and consequent exposure to different levels of testosterone during the perinatal period would affect the relationship between sex and hippocampal size.

METHOD

Subjects

Twenty-six male and 26 female Mongolian gerbils born and reared in the vivarium of the McMaster University Department of Psychology served as subjects. All were second or third generation descendants of breeding stock acquired from Tumblebrook Farms (Brookfield, MA). Seventeen male and 16 female subjects were delivered by cesarian section. The remaining 19 subjects (9 males and 10 females), referred to below as group R, were randomly selected from among males and females used as breeding stock in the colony. Less than 10% of these animals would be expected to come from 2F or 2M intrauterine positions (Clark and Galef, unpublished observations). All animals were maintained on a 12L:12D photoperiod with light onset at 0500 h.

Procedures

Cesarian delivery. Each subject delivered by cesarian section and its littermates were removed from their deeply anesthetized dam on the last day of gestation, and were foster reared by a dam that had delivered a litter vaginally on the same day. Detailed descriptions of surgery and foster rearing can be found in (3,4). As fetuses, 10 male and 6 female subjects delivered by cesarian section had developed in 2M intrauterine positions and 7 male and 10 female subjects had developed in 2F intrauterine positions. All 33 of these subjects were sacrificed for histological examination at 60 days of age. Group R subjects were sacrificed at a mean age of 74.2 days.

Histology. Under pentobarbitol anesthesia (40 mg/kg body weight), each subject was perfused transcardially with physiological saline and 10% formalin buffered with saline. Perfused brains were embedded in 10% gelatin and postfixed in 37% formalin before sectioning. Frozen sections were cut in the coronal plane at 40 μm intervals and all sections were mounted and stained with Cresyl Violet.

Morphometry. The areas of the left and right hippocampus (including dentate gyrus, fimbria, and subiculum) and left and right telencephalon (excluding hippocampus) were determined at 160 μm intervals (every 4th 40 μm section) using a Leitz Dialux microscope (Leitz, Wetzlar, Germany), a Panasonic WV-1500 video camera (Secaucus, NJ), and Jandel JAVA software (Jandel, San Rafael, CA). Area measurements were combined using the formula for a truncated cone to obtain volume estimates (20). Hippocampus measurements were taken from a mean of 21.6 sections per brain (range 14–27) and telencephalon measurements from a mean of 55.7 sections per brain (range 44–76). All measurements were performed blind to sex and intrauterine position by coded labelling of slides.

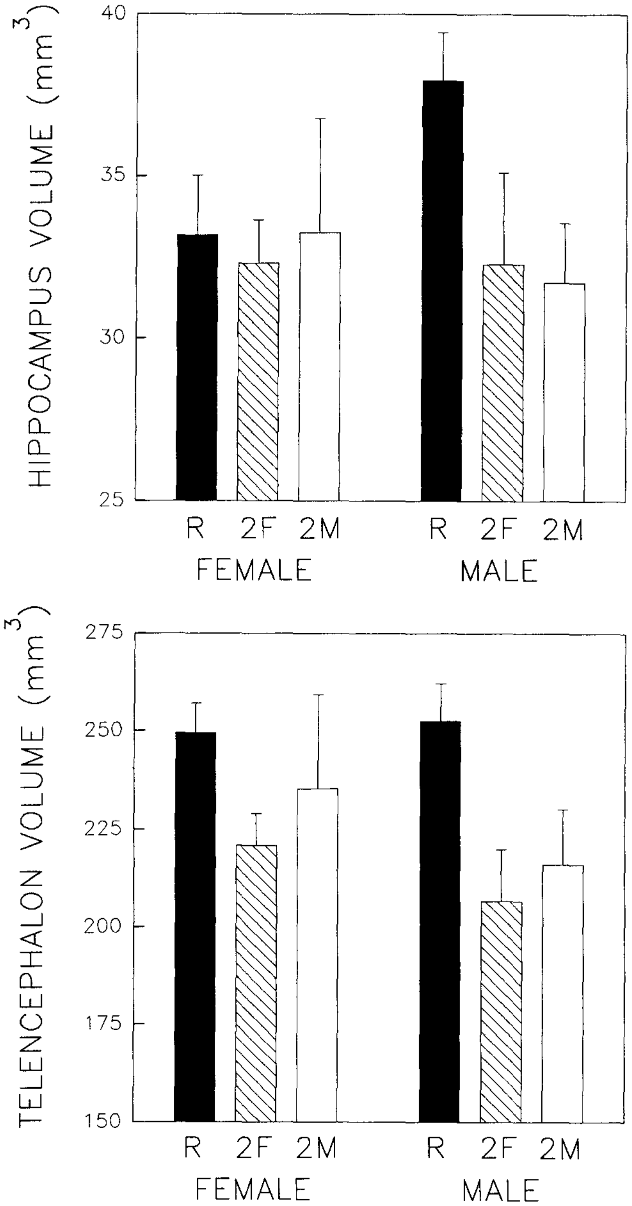

FIG. 1. Mean volume of the hippocampus (upper) and telencephalon (lower) for each sex and intrauterine position. R, randomly selected males and females; 2F, intrauterine position between two females; 2M, intrauterine position between two males. Error bars indicate one standard error of the mean.

RESULTS

Volume of the hippocampus and telencephalon for all males and females is shown in Fig. 1. Analysis of covariance was used to examine the effects of sex and intrauterine position on the size of the hippocampus, using size of the telencephalon as a covariate. Homogeneity of the regression of hippocampal volume on telencephalon volume was confirmed for both sexes and for the three intrauterine conditions (2M, 2F, and R) prior to analysis of covariance. Mean residuals from this regression are shown in Fig. 2.

Planned comparisons (21) showed that the only significant difference between groups in relative hippocampal size occurred between R males and R females [$F(1,45) = 4.70$, $p < 0.05$]. Omnibus analysis of covariance of hippocampal volume with telencephalic volume as a covariate showed no overall significant effect of sex [$F(1,45) = 3.53$, $p < 0.06$], intrauterine position [$F(2,45) = 0.44$, $p < 0.65$], nor any interaction between sex and intrauterine position [$F(2,45) = 0.86$, $p < 0.43$]. Because there were a priori reasons to expect a sex difference in at least some groups, and because the effect of sex was near the criterion

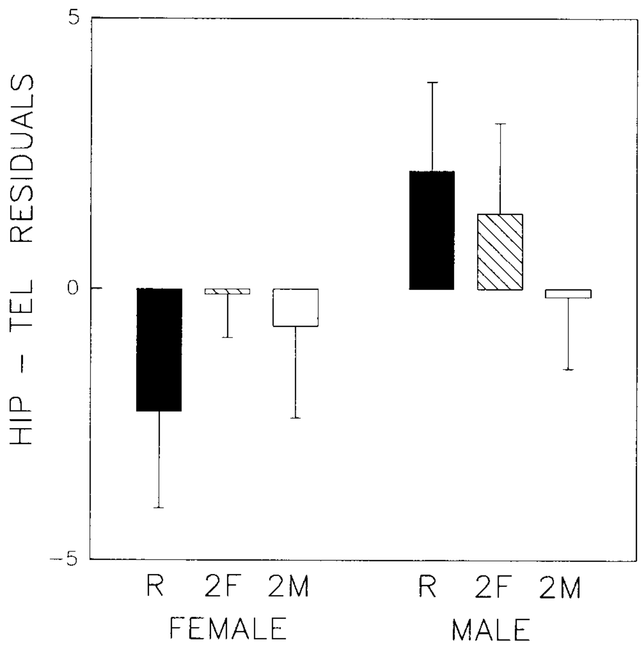

FIG. 2. Mean residuals from the regression of hippocampus volume on telencephalon volume for each sex and intrauterine position. Error bars indicate one standard error of the mean.

for statistical significance, tests of simple main effects were performed despite the nonsignificant interaction between sex and intrauterine position. Tests of simple main effects showed a significant effect of sex in the R group [$F(1,45) = 4.73, p < 0.05$], confirming the results of the planned comparison reported above, but no significant effect of sex was found in either the 2F or 2M groups [$F(1,45) = 0.36, p < 0.55$; $F(1,45) = 0.03, p < 0.86$, respectively]. Tests of simple main effects showed no significant effect of intrauterine position within either sex [$F2,45) = 0.48, p < 0.62$, $F(2,45) = 0.75, p < 0.48$ for males and females, respectively]. Analysis of variance of telencephalon volume showed no significant effect of sex [$F(1,46) = 0.82$ $p < 0.37$], a significant effect of intrauterine position [$F(2,46) = 4.91, p < 0.05$], and no significant interaction between sex and intrauterine position [$F(2,46) = 0.46, p < 0.64$]. Post hoc tests showed that, compared to R animals, the telencephalon was smaller in both 2F ($t(35) = 3.05, p < 0.01$) and 2M animals ($t(34) = 2.03, p < 0.05$)].

Analysis of covariance revealed no left-right asymmetry in the hippocampus and no interaction between hippocampal asymmetry and either sex or intrauterine position. Analysis of variance showed no left-right asymmetry in the size of the telencephalon and no interaction between telencephalic asymmetry and either sex or intrauterine position.

DISCUSSION

The results provide evidence for a sex difference in relative hippocampal size in a random selection of Mongolian gerbils in the direction expected on the basis of sex differences in home range size in the wild. Like male meadow voles and kangaroo rats (15,16), male gerbils have a larger home range and a relatively larger hippocampus than do females.

As can be seen most clearly in Fig. 2, gestation in either 2F or 2M intrauterine positions eliminated the sex difference found in randomly selected male and female gerbils. It is perhaps not surprising that androgenization of 2M females and reduced exposure to androgens in 2F males might reduce the magnitude of the sex difference observed between randomly selected males and females. Reduction of relative hippocampal size in 2M males is somewhat unexpected. It has been previously shown, however, that increased exposure to androgens does not necessarily have further masculinizing effects on spatial ability and the hippocampus of males. Perinatal administration of exogenous testosterone to gonadally intact rats impaired water maze performance by males (but improved performance by females) and, although it increased the thickness of the dentate gyrus granule cell layer in females, it had no effect in males (18,19).

In addition, Hampson and Moffat (12) concluded, from a review of research on the effects of androgens on cognitive ability in humans, that intermediate levels of exposure to androgens may have a facilitating effect on spatial performance, but exposure to higher or lower levels of androgens may produce a decline in spatial performance. For example, boys with congenital adrenal hyperplasia (CAH), which causes exposure to high levels of androgens during fetal development, performed worse on tests of spatial ability than did their unaffected male siblings. Girls with CAH performed better than did their unaffected female siblings (13).

The expected masculinizing effect of androgens on 2M females was not observed in a statistical comparison of R and 2M females in the present experiment, but it is clear from Fig. 2 that the magnitude of the sex difference observed between R males and R females is reduced when comparing R males with 2M females.

2F and 2M animals differ from R animals not only in their intrauterine environment but also in being cesarian-delivered. Is it possible that the size of the hippocampus in 2F and 2M animals is unrelated to their intrauterine hormonal environment and is, instead, a result of cesarian delivery? Cesarian delivery could be stressful to neonates, with consequent adrenal steroid-mediated effects on the hippocampus (17). Intrauterine position was found to have a significant effect on telencephalon size, and both Fig. 1 and statistical analyses reported above show a smaller telencephalon in cesarian-delivered 2F and 2M animals, compared to R animals. An overall reduction in hippocampal size as a result of cesarian delivery would tend to preserve sex differences, however, by reducing hippocampal size in both sexes. Similarly, an overall reduction in telencephalon size without effects on hippocampal size would tend to increase relative hippocampal size in both sexes, leaving sex differences intact. For cesarian delivery to be the sole explanation for the absence of sex differences among 2F and 2M animals (see Fig. 2), it would be necessary that cesarian delivery had the effect of increasing relative hippocampal size in female gerbils and decreasing relative hippocampal size in males. It seems unlikely, therefore, that cesarian delivery provides a full explanation for the pattern of results observed. A more complex hypothesis in which the effect of cesarian delivery interacts with sex might be entertained, but the present experiment was not designed to test this.

The results show that in Mongolian gerbils, a sex difference in home range size in the wild is correlated with a sex difference in the relative size of the hippocampus. The data also show that the size of the hippocampus in adults may be affected by intrauterine position. Exposure to either 2F or 2M intrauterine environments reduced or eliminated the sex difference in relative hippocampal size observed in randomly selected animals. We suggest that these effects on relative hippocampal size in 2F and 2M male and female Mongolian gerbils are the result of perinatal exposure to higher or lower than normal levels of androgens.

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