Intrauterine Position Effects on Sexually Dimorphic Asymmetries of Mongolian Gerbils: Testosterone, Eye Opening, and Paw Preference

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A series of experiments was undertaken to both discover and explore the causes of sexually dimorphic phenotypic asymmetries in infant and adult Mongolian gerbils. We found that (1) the order in which the eyes of individual gerbil pups opened varied with their gender; right eyes of female pups opened before left eyes, while left eyes of male pups opened before right eyes, and (2) the paw that adult gerbils held in the air while maintaining a species-typical tripedal stance varied with gender; female gerbils held their right paws in the air significantly more often than did male gerbils. Both order of eye opening and laterality of paw use while in a tripedal stance varied significantly as a function of the intrauterine position which subjects had occupied as fetuses. These data implicate exposure to testosterone as a mediator of the development of asymmetries exhibited by Mongolian gerbils.

In the course of raising large number of Mongolian gerbils (Meriones unguiculatus) for use in behavioral studies, we noticed a potentially interesting feature of development in gerbil pups that we rarely observed in young Norway rats (Rattus norvegicus), the other rodent species bred in substantial numbers in our laboratory. Although we often found infant gerbils with only one of their two eyes open, we infrequently found rat pups in the same state. The eyes of rat pups do not, of course, open at exactly the same moment, but in young rats the interval between

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the opening of one eye and the opening of the other is quite brief. In contrast, it is not unusual for one eye of a gerbil pup to open 1 or 2 days before the other. Consequently, the probability of seeing a rat pup with only one eye open is noticeably less than the probability of seeing a gerbil pup in the same state.

The greater apparent asynchrony in the eye opening of gerbil pups than of rat pups is not the result of young gerbils simply using only one eye at a time after both have opened spontaneously. The closed eye of those gerbils with only one eye open is usually sealed shut; it can only be unsealed and opened by applying considerable pressure (Clark, unpublished observation).

Search of the literature revealed that, although others had commented on the substantial asynchrony in the age at which the two eyes of some gerbil pups open (De Ghet, 1972; McManus, 1971), there had been no further discussion of the phenomenon.

Our own curiosity concerning asynchronous eye opening in gerbils was aroused because informal observation suggested that there was a strong correlation between the gender of a gerbil pup and the order in which its eyes opened: The left eyes of male gerbils seemed to open before their right eyes, while the right eyes of female gerbils appear to open before their left eyes.

The present studies were undertaken: (1) to formalize the observation of gender differences in order of eye opening exhibited by Mongolian gerbil pups, (2) to examine the role of intrauterine position, and consequent differential prenatal exposure to testosterone, in determining the order in which the eyes of gerbils opened and, (3) to determine whether asymmetry in order of eye opening was correlated with other phenotypic asymmetries of Mongolian gerbils.

**Experiment 1**

Experiment 1 was designed to determine whether there were, in fact, gender differences in the order of eye opening exhibited by young Mongolian gerbils.

**Method**

**Subjects**

Subjects were 210 male and 210 female Mongolian gerbils from 105 litters born in the vivarium of the McMaster University Psychology Department. Each member of each litter was permanently marked for individual identification on the day of its birth.

The 105 litters that participated in the present study also served as subjects in other experiments (Clark, Bone, & Galef, 1990; Clark & Galef, 1991) and, for purposes relevant to those experiments (and irrelevant to the present research), 35 of the 105 litters had been culled on the day of their birth to 2 male and 2 female pups, 35 to 4 male pups, and 35 to 4 female pups.

All 420 subjects were housed together with their respective dams in polypropylene shoe-box cages (35 × 30 × 15 cm). The entire breeding colony was maintained on ad lib Purina Rodent Laboratory Chow #5001 and water in a tempera-
ture- and humidity-controlled colony room that was illuminated on a 12:12 hr light/dark cycle.

Procedure

To determine the order in which pups' eyes opened, we examined each pup in each litter three times/day from the time that a pup was 14 days of age until both its eyes had opened spontaneously. Examinations of pups were carried out at approximately 3-hr intervals during the light portion of the light/dark cycle. The experimenter recorded both the sex of each pup and the order in which its eyes opened.

Results and Discussion

The results of Experiment 1 are presented in Figure 1 which shows the percentage of both male and female gerbil pups whose right eyes opened before their left eyes. Figure 1 presents data from only 210 of the 410 pups examined because both eyes of the remaining 200 pups opened during the night, and they were seen only when both their eyes were still sealed shut and after both their eyes had already opened.

As is clear from examination of Figure 1, and as statistical tests confirmed, primacy of right eye opening was observed significantly more often in female than in male gerbil pups, $\chi^2 = 23.3, p < .0001$. Furthermore, both the left eye of male gerbils, $\chi^2 = 12.8, p < .001$, and the right eye of female gerbils, $\chi^2 = 10.5, p < .01$, opened before the contralateral eye significantly more often than one would expect by chance.

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Fig. 1. Percentage of male and female gerbil pups whose right eyes were observed open before their left eyes.
These data clearly confirm our informal observation of a gender-correlated asymmetry in eye opening in Mongolian gerbil pups.

**Experiment 2**

Rosen and colleagues (1983) have shown that the greater left bias in the tail posture of 1-day-old female than of 1-day-old male rat pups immediately following release from restraint in a symmetric posture is modifiable by prenatal exposure to testosterone.

We have shown previously (Clark, Crews, & Galef, 1991) that the levels of plasma testosterone to which fetal gerbils are exposed in utero are correlated with the intrauterine positions which they occupy; both male and female fetuses that occupy an intrauterine position between two male fetuses (2M males and 2M females) have higher circulating levels of testosterone than do their littermates occupying intrauterine positions between two female fetuses (2F males and 2F females; Clark et al., 1991). If the gender-correlated asymmetry in eye opening exhibited by Mongolian gerbils (like the gender-correlated postural asymmetry exhibited by neonatal rat pups studied by Rosen et al., 1983) is mediated by prenatal exposure to testosterone, then one would expect to find effects of intrauterine position on the order in which the eyes of Mongolian gerbil pups open: 2M gerbils should exhibit a higher frequency of the male pattern of asymmetry in eye opening (left eye open before right eye) than their 2F siblings.

**Method**

**Subjects**

Fifty male and fifty female, Caesarian-delivered Mongolian gerbil pups served as subjects in the present experiment. Twenty-five males and 25 females had developed in intrauterine positions between two male fetuses (2M males and 2M females) and 25 males and 25 females had developed in intrauterine positions between two female fetuses (2F males and 2F females). These 100 pups were the first 25 2M males, 25 2F males, 25 2M females, and 25 2F females (in a group of more than 200 Caesarian-delivered young raised for use in other experiments) whose order of eye opening we succeeded in observing. All 100 pups were reared by foster dams in mixed-sex litters of five.

Each dam and her foster young were maintained throughout the experiment in a polypropylene shoe-box cage as in Experiment 1, and were held in the same colony room in which Experiment 1 was conducted.

**Breeding, Caesarian Delivery, and Maintenance**

At 90 to 100 days of age, virgin female Mongolian gerbils were each weighed and then placed individually with a proven male. The date on which each breeding pair first mated was determined by observation, and pair members were separated 2 weeks later, when females were conspicuously pregnant.

Twenty-four days after observed copulation (i.e., 1 day before anticipated vaginal delivery), each female that had gained weight at a rate consistent with her
impregnation on the day of observed copulation was anesthetized by ether inhalation, her abdomen opened, her uterus externalized and her fetuses removed one by one. The gender of each fetus was determined on the basis of its anogenital distance (Clark & Galef, 1990) and its position was recorded relative to that of other fetuses found in the same horn of its dam's uterus (Clark, vom Saal, & Galef, 1992). After all fetuses had been removed from a dam, she was euthanized by anesthetic overdose.

Each infant was toe-clipped at delivery for permanent identification and then, using the procedures of Clark and Galef (1988), fostered to a female that had delivered a litter vaginally on the day of delivery of her foster pups.

Testing

Each pup in each litter was examined three times/day from the time it was 14 days of age until both of its eyes were open to determine the date on which each of its eyes opened.

Results and Discussion

The results of Experiment 2 are presented in Figure 2 which shows the percentage of male and female gerbil pups from 2M and 2F intrauterine positions whose right eyes opened before their left eyes. As is clear from examination of Figure 2, we replicated the main effect reported in Experiment 1 of a sexually dimorphic asymmetry in eye opening. Primacy of right eye opening was significantly more frequent in female than in male gerbils, \( \chi^2 = 5.84, p < .01 \).

Further, we found a significant effect of intrauterine position on order of eye opening. Those gerbil pups from 2M intrauterine positions exhibited primacy of left eye opening (the male pattern) significantly more frequently than did those

![Figure 2](image_url)

Fig. 2. Percentage of male and female Caesarian-delivered gerbil pups from 2M and 2F intrauterine positions whose right eyes opened before their left eyes.
gerbil pups from 2F intrauterine positions, $\chi^2 = 4.05$, $p < .02$. This result is consistent with the hypothesis that differential exposure to testosterone while in utero causes the gender-correlated asymmetry in eye opening reported in Experiment 1. 2F males exhibited primacy of right eye opening significantly more frequently than did 2M males, $\chi^2 = 3.31$, $p < .03$; 2F females did not exhibit primacy of right eye opening significantly more frequently than did 2M females, $\chi^2 = 1.30$, ns.

**Experiment 3**

We undertook a series of studies to find other sexually dimorphic asymmetries in the behavior of adult Mongolian gerbils that might be correlated both with the asymmetry in eye opening described in Experimenters 1 and 2 and the intrauterine position that male and female gerbils occupied as fetuses.

Our attempts to find in Mongolian gerbils either a sex difference in neonatal postural asymmetry like that reported in 1-day-old rats by both Ross and colleagues, 1981 and Rosen and colleagues, 1983), or a sex difference in the rotational direction selected by adults like that observed in adult rats by Glick and Ross (1981) were not successful. Similarly, we were not able to find sex differences in the paw preferences of adult gerbils in the reaching task Collins (1975) developed for use with mice.

However, in the course of these unsuccessful studies we noticed an apparent difference between male and female adult gerbils in their use of left and right paws while standing in a species-typical tripodal posture (De Ghett, 1972; see Fig. 3). While in the tripodal stance a gerbil remains still, resting on both hind feet and one forepaw, and holding the other forepaw in the air thus maintaining, albeit briefly, a pose reminiscent of that exhibited by a hunting dog while on point (De Ghett, 1972).

Experiment 3 was undertaken both to formalize our observation of possible gender differences in use of left and right forepaws by adult gerbils while in the tripodal stance and to examine effects of intrauterine position on this postural asymmetry.

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**Fig. 3.** Drawing, from a photograph, of a young adult Mongolian gerbil in the tripodal stance with its left forepaw in the air.
Method

Subjects

Fourteen male and 10 female Caesarian-delivered gerbils from 2M intrauterine positions and 14 female and 10 male Caesarian-delivered gerbils from 2F intrauterine positions served as subjects. Each pup was foster reared in a mixed-sex litter of four or five under the conditions described in Method of Experiment 2.

After weaning at 21 days of age, subjects were maintained in same-sex groups of 2 or 3 littermates in the colony room described in Experiment 1.

Testing

Eye Opening

From Days 14–21 postpartum, each subject was examined thrice daily to determine which of its eyes opened first. Those 20 pups that were seen with only one eye opened were used in the correlational analysis, results of which are presented in Figure 5.

Paw Preference

At 60 days of age, each pup was examined on 5 consecutive days to determine whether it exhibited an asymmetry in paw use while in the tripod stance.

On each day, a subject pup was introduced into a small Plexiglas box (9.5 x 9.5 x 18 cm) and watched as it walked about the enclosure. Whenever a subject was not in contact with one of the walls of the enclosure, stopped moving, and assumed a tripod stance, an observer who was unaware of either the sex of the subject or the intrauterine position in which it had developed recorded which of its forepaws it held in the air while standing on three feet. Each subject was watched each day until it had assumed a tripod stance on 10 occasions, and at the end of the 5th day, each subject was awarded a score indicating the percentage of the 50 occasions when it was observed in a tripod stance when it held its right paw in the air.

Results and Discussion

The main results of Experiment 3 are presented in Figure 4 which shows the mean percentage of trials on which male and female, 2M and 2F gerbils held their right paws in the air when assuming a tripod stance. A 2 x 2 ANOVA performed on arcsine-transformed percentage scores revealed significant effects both of subject gender, $F(1,44) = 4.12, p < .05$, and of subject intrauterine position, $F(1,44) = 7.61, p < .01$, on paw preference and no significant interaction, $F(1,44) = 0.06, ns$.

Figure 5 shows the relationship between primacy in eye opening and handedness in the tripod stance for those 20 gerbils whose order of eye opening we had been successful in observing. As is obvious from examination of Figure 5, there
was a significant difference between gerbils whose right and left eyes opened first in which paw they held in the air when in a tripodel stance, \( U = 10, \ p < .002 \). All 10 gerbils whose left eyes had opened first held their left paws in the air on a majority of the 50 occasions when they were observed to assume a tripodel stance, while 8 of the 10 gerbils whose right eyes opened first held their right paws in the air on a majority of the 50 occasions when they were observed in a tripodel stance.

The results of the present experiment demonstrate both gender-correlated and intrauterine-position-correlated behavioral asymmetries in adult Mongolian ger-
bils and a positive correlation between primacy in eye opening and asymmetry in paw use by gerbils while in the tripod stance. These results are again consistent with the view that prenatal exposure to androgen, as the result either of gender or of intrauterine position, causes asymmetries in phenotypic development.

**General Discussion**

We are not in the first either to find evidence of sexually dimorphic phenotypic asymmetries in rodents or to provide evidence consistent with the view that such asymmetries are affected by exposure to testosterone early in life. As discussed in the introduction to Experiment 2, Ross and colleagues (1981) have reported a sex difference in the position in which newborn rats hold their tails after they have been held and released; Glick and Ross (1981) have reported a positive correlation between such bias in tail orientation in infants and their preferred direction of rotation in adulthood, while Rosen and colleagues (1983) and Glick (1983) have provided evidence implicating prenatal exposure to testosterone in production of such behavioral asymmetries. However, both the magnitude and spontaneous nature of the sexually dimorphic asymmetries described here are unusual as is our identification of a normally occurring developmental variable (intrauterine position) that can account for some of the within-sex variance in the direction and extent of observed phenotypic asymmetries.

The present findings, like those discussed above, have possible theoretical significance because they may be relevant to Geschwind and Galaburda's (1984) hypothesis that, in humans, prenatal exposure to high levels of testosterone differentially affects neuronal growth in the two cerebral hemispheres, resulting in development of sexually dimorphic behavioral asymmetries. Our results are consistent with such a hypothesis in that they suggest that naturally occurring differences in the levels of testosterone to which Mongolian gerbils are exposed early in life are correlated with both the direction and extent of phenotypic asymmetries which gerbils exhibit both in infancy and in adulthood. The present evidence suggests that two characteristics of Mongolian gerbils, order of eye opening and paw use in the tripod stance, may provide useful model systems for exploring the development of phenotypic asymmetries in mammals.

**Notes**

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