Behavioral Neuroscience
1998, Vol. 112, No. 4, 1026–1029

0735-7044/98/$3.00

Effects of Gonadectomy in Infancy and Adulthood on Handedness in Male and Female Mongolian Gerbils

Mertice M. Clark, Belinda T. Santamaria, Rohan K. Robertson, and Bennett G. Galef, Jr.
McMaster University

When assuming a species-typical tripodal posture, female Mongolian gerbils most often rest on their left forepaws and hold their right forelimbs aloft; male gerbils most often do the reverse. This experiment examined effects of gonadectomy, both in infancy and in adulthood, on the sexually dimorphic asymmetry in forelimb use by Mongolian gerbils when maintaining a tripodal stance. In adulthood, both male and female gerbils that had been gonadectomized at birth reversed their forelimb use while in a tripodal stance: Gonadectomized males used their forelimbs as did sham-operated females, and gonadectomized females used their forelimbs as did sham-operated males. Gonadectomy in adulthood abolished the sexual dimorphism in forelimb use seen in sham-operated subjects. It was concluded that gonadal hormones have organizational as well as possible activational effects on adult patterns of forelimb use by gerbils.

When male and female Mongolian gerbils (*Meriones unguiculatus*) assume a species-typical tripodal posture, resting on both hindfeet and on one forepaw while holding the other forepaw in the air (De Ghett, 1972), they use their right and left forelimbs differently: Males most often stand on their right forepaw and hold their left forelimb aloft; females most often do the reverse (Clark, Robertson, & Galef, 1993, 1996). Unlike rats and mice (Collins, 1975; Ross, Glick, & Meilbach, 1981; Rosen, Berrebi, Yutzey, & Denenberg, 1983), infant gerbils do not show sex differences in postural asymmetries, and adult gerbils do not exhibit sex differences in either rotational direction or paw use in reaching tasks (Clark et al., 1993).

There is evidence consistent with the view that gerbils' tendency to use either the right or the left forepaw for support while in a tripodal stance is affected by exposure to testosterone (T): (a) male Mongolian gerbils have higher circulating levels of T than do females of their species (Clark, vom Saal, & Galef, 1992; Clark, Crews, & Galef, 1991), and as noted previously, male and female gerbils hold opposite forelimbs aloft while standing on three legs; (b) gerbils of each sex gestated in intrauterine positions between males have higher circulating levels of T throughout life than do gerbils gestated between females (Clark et al., 1991), and adult gerbils of each sex gestated between males are more likely to use their forelimbs in the male-typical way (i.e., to stand on their right forelimbs and to hold their left forelimbs off the ground) than are adult gerbils of each sex gestated between females (Clark et al., 1993); and (c) use of forepaws by both adult male and adult female gerbils while in a tripodal stance can be altered by injecting them with testosterone propionate (TP) shortly after birth (Clark et al., 1996). Although the direction of such exogenously induced changes in handedness are not those expected on the simple hypothesis that exposure to T masculinizes behavior (in adulthood, male gerbils given a single 200-mg injection of TP at 4 days of age showed an increase in the frequency with which they displayed the female-typical use of forepaws when in a tripodal stance, and 50-, but not 100-mg, injections of TP delivered on Day 6 postpartum masculinized forepaw use by females when adult), the results do clearly implicate T in modulating forelimb use by Mongolian gerbils (Clark et al., 1996).

Explorations of hormonal effects on lateral asymmetries in behavior are potentially important because sex differences in behavioral asymmetry may reflect sex differences in neuroanatomical lateralization that, like sex-specific behavioral asymmetries, are modifiable by exposure to gonadal hormones in infancy (for reviews, see Denenberg & Yutzey, 1985; Diamond, 1984, 1991).

In the present experiment, we examined effects of gonadectomy, both on the day of birth and in adulthood, on forepaw use by adult male and female Mongolian gerbils when in a tripodal stance. Our goals were (a) to provide additional evidence of hormonal mediation of lateral asymmetries in behavior (Geschwind & Galaburda, 1987), and (b) to begin to determine whether hormonal effects on use of forelimbs by gerbils are organizational or activational in nature (Phoenix, Goy, Gerall, & Young, 1959).

Method

Subjects

We used as subjects 40 male and 40 female Mongolian gerbils randomly selected from 50 litters born to descendants of breeding

Mertice M. Clark, Belinda T. Santamaria, Rohan K. Robertson, and Bennett G. Galef, Jr., Department of Psychology, McMaster University, Hamilton, Ontario, Canada. Rohan K. Robertson is now at the University of Toronto, Faculty of Medicine, Toronto, Ontario, Canada.

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. We thank Paul Ramos, Elaine Whiskin, and Jessica Stevens for technical assistance.

Correspondence concerning this article should be addressed to Mertice M. Clark, Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1. Electronic mail may be sent to MClark@McMaster.CA.

stock purchased from Tumblebrook Farm (Brookfield, MA) and reared in the vivarium of the McMaster University Psychology Department.

Maintenance

After weaning at 30 days of age, we maintained subjects in groups of 2 or 3 juveniles of the same sex in opaque, polypropylene, shoe box cages (35 × 30 × 15 cm) housed in a temperature- and humidity-controlled colony room illuminated each day from 5 a.m. to 5 p.m.

We left subjects undisturbed from the time that they were weaned until they were 70 days of age except for biweekly cage cleaning and surgical treatment of 40 subjects when they were 50 days old. All subjects had *ad libitum* access to water and Purina Rodent Laboratory Chow 5001 (Ralston-Purina Canada, Woodstock, Ontario) throughout the experiment.

Procedure

Surgery performed on infants. On the day of their birth, we bilaterally gonadectomized 10 male and 10 female subjects while under cryoanesthesia. After gonadectomy, we sealed incisions with New-Skin (Medtech Inc., Jackson, WY). We then warmed subjects and returned them to their respective dams. We treated 10 males and 10 females that we had randomly assigned to a sham-operated, control group exactly as we treated gonadectomized subjects except that, after incision, we did not remove the gonads of control subjects.

Surgery performed on adults. We bilaterally gonadectomized 10 male and 10 female subjects when they were 50 days old. We anesthetized subjects by intraperitoneal injection with sodium pentobarbitol (30 mg/kg) supplemented, when necessary, with inhalation of Metofane (Janssen Pharmaceutica, North York, Ontario, Canada). We treated a further 10 male and 10 female subjects randomly assigned to a sham-operated, control group exactly as we treated gonadectomized subjects except that, after incision, we did not remove the gonads of control subjects.

Verifying surgical success. We killed each of the 40 gonadectomized subjects at 80 days of age and verified their gonadal status by visual inspection. We discarded data from 2 female subjects that we had ovariectomized in infancy when we discovered ovarian tissue in each, during necropsy.

Determining handedness. When each of the 80 subjects reached 70 days of age, we determined their use of left and right forelimbs while standing in a tripodal posture. On each of 10 consecutive test days, we introduced subjects individually into a 5-gallon aquarium (20 × 40 × 20 cm); an observer who was unaware of the individual identity, sex, or gonadal status of subjects scored their use of left and right forelimbs while in a tripodal stance.

Whenever a subject was not in contact with a wall of the aquarium, had stopped moving, and was standing on three legs, the observer recorded which forelimb the subject held aloft. Each subject was observed each day until its use of forelimbs in a tripodal stance had been observed on 10 occasions.

At the end of the 10 days of testing, we awarded each subject a score equal to the number of times out of 100 that it held its right forelimb in the air while in a tripodal stance.

Statistical analyses. We analyzed differences among groups in the number of times their members held their right forelimbs aloft using a 2 × 2 × 2 analysis of variance (ANOVA; Age at Surgery × Sex × Gonadal Status), 2 × 2 ANOVAs, and post hoc Student's *t* tests.

Results

Results of the 2 × 2 × 2 ANOVA

The main results of the experiment are presented in the two panels of Figure 1, which show the number of times that male and female subjects gonadectomized in infancy (upper panel) and in adulthood (lower panel), and their respective sham-operated controls, held their right forelimbs aloft on the 100 occasions when each was observed in a tripodal stance.

Main effects. Not unexpectedly, the overall analysis revealed no significant main effects on forelimb use of age at surgery, $F(1, 70) = 0.22$, *ns*, gonadal status, $F(1, 70) = 0.01$, *ns*, and sex, $F(1, 70) = 3.38$, *ns*.

Interactions. More important, because it permitted post hoc analyses, there was a significant three-way interaction of effects on forepaw use of sex, age at surgery, and gonadal status of subjects, $F(1, 70) = 3.80, p < .05$.

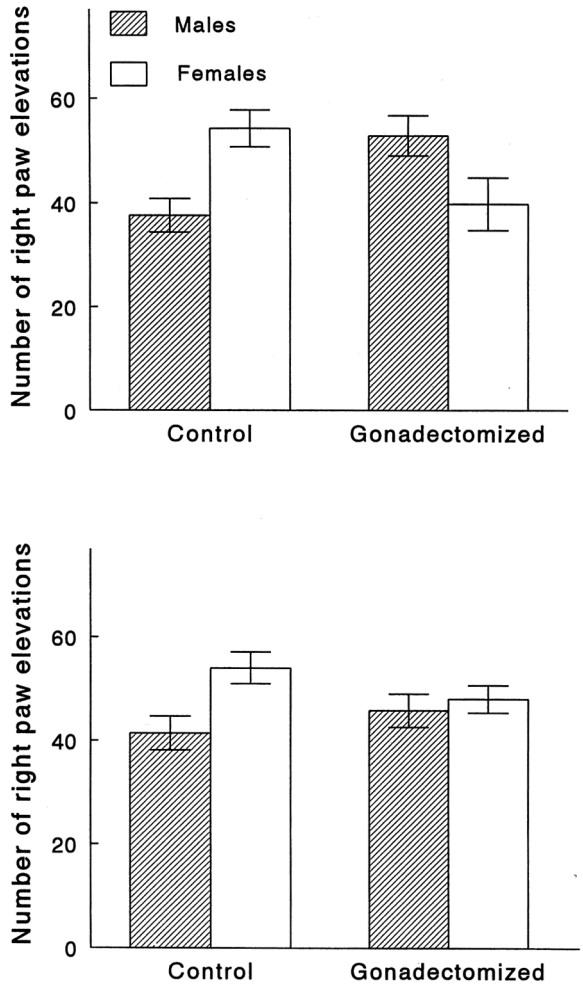

Figure 1. Mean (±*SEM*) number of times out of 100 that male and female gerbils that were gonadectomized either on their day of birth (upper panel) or when adults (lower panel; and their respective sham-operated controls) held their right forepaws aloft while in a species-typical tripodal stance.

Results of Post Hoc Tests

Sex differences in forelimb use by sham-operated gerbils. As can be seen in both the upper and lower panels of Figure 1, females assigned to each of the two sham-operated groups held their right forelimbs aloft while in a tripodal stance significantly more frequently than did males assigned to sham-operated groups (both $ts > 2.69$, both $ps < .02$). This sexually dimorphic asymmetry in forelimb use observed in sham-operated subjects repeats findings reported by Clark et al. (1993, 1996) of a sex difference in forelimb use by unmanipulated gerbils.

Age at sham operation did not affect forelimb use in adulthood by either male or female subjects (both $ts < .79$, both *ns*).

Effects on forelimb use of gonadectomy in infancy. As can be seen in the upper panel of Figure 1, gonadectomy in infancy reversed the normal pattern of forelimb use by both male and female gerbils. This reversal in forepaw use was due to two factors, the first of which is feminization of the tripodal stance of gonadectomized males; males gonadectomized in infancy held their right forepaws aloft significantly more frequently than did control males that received sham operations in infancy, $t(18) = 3.05$, $p < .01$, and with the same frequency as did sham-operated females, $t(18) = 0.27$, *ns*. The second factor is masculinization of forepaw use in gonadectomized females; females gonadectomized in infancy held their right forepaws aloft significantly less frequently than did females that had received sham operations in infancy, $t(16) = 2.37$, $p < .03$, and with the same frequency as did sham-operated males, $t(16) = 0.36$, *ns*.

Finally, adult females gonadectomized on their day of birth held their right forepaws aloft marginally less frequently than did adult males gonadectomized on the day of their birth, $t(16) = 2.07$, $p < .06$.

Effects of gonadectomy in adulthood on forelimb use. A 2×2 ANOVA revealed a significant main effect of sex on forelimb use, $F(1, 36) = 5.60$, $p < .03$, no effect of treatment, $F(1, 36) = 0.07$, *ns*, and a marginal interaction of sex and treatment, $F(1, 36) = 2.77$, $p = .10$. Examination of the lower panel of Figure 1 shows that the sex difference in handedness seen in sham-operated subjects and reported by Clark et al. (1993, 1996) was not present in gerbils gonadectomized in adulthood, $t(18) = 0.53$, *ns*. Post hoc *t* tests revealed that forepaw use by both male and female subjects gonadectomized in adulthood was marginally different from that of their respective sham-operated, control subjects: males, $t(28) = 1.56$, $p < .07$; females, $t(28) = 1.64$, $p < .06$, both *ts* one-tailed.

Relative effects of age at gonadectomy on forepaw use. As might be expected, given the relatively large effect on use of forelimbs of gonadectomy in infancy and the relatively small effect on use of forelimbs of gonadectomy in adulthood, there was a significant interaction between effects on forelimb use of age at surgery and sex of subjects (2×2 ANOVA), $F(1, 34) = 4.27$, $p < .05$.

Discussion

Gonadectomy in infancy feminized forelimb use by adult male gerbils and masculinized forelimb use by adult female gerbils. Gonadectomy in adulthood abolished, but did not reverse, the statistically significant sex difference in forelimb use seen repeatedly in intact and sham-operated adult gerbils. The significantly greater impact of gonadectomy in infancy than in adulthood suggests that there exists a period early in life during which the development of sex differences in forelimb use by gerbils is particularly sensitive to hormonal influences. It is possibly relevant to note that concentrations of T in newborn male gerbils are as high as those seen in adulthood, drop to low levels within a week, and remain reduced until a prepubertal surge in T occurs at 2 to 3 months of age (Probst, 1987).

The finding that gonadectomy in infancy both masculinized the behavior of females and feminized the behavior of males is difficult to interpret. Hormonal effects on handedness, whether in gerbils (Clark et al., 1993, 1996), cats (Tan, Kara, & Kutlu, 1991), rhesus monkeys (Drea, Wallen, Akinbami, & Mann, 1995), or humans (Moffat & Hampson, 1996; Nass et al., 1987) do not reflect a straightforward masculinization of behavior by exposure to T, and we know nothing of the effects, if any, of exposure to other gonadal hormones on asymmetries in forepaw use.

We speculate that differences either in production or transformation of adrenal steroids by gonadectomized male and female Mongolian gerbils (Dittmann, 1992) may underlie the complex effects we observed of gonadectomy on gerbils' use of forelimbs while in a tripodal stance. Adrenal glands of gerbils are large (relative to body weight) for a rodent (Cullen, Pare, & Money, 1971) and show unusual structural and secretory characteristics (Kadioglu & Harrison, 1975; Oliver & Peron, 1964; Nickerson, 1971), some of which become exaggerated after gonadectomy (Nickerson, 1975). As Fenske (1986, p. 22) stated, "the adrenal and testicular physiology of the gerbil adrenal gland differs in several aspects from that of other laboratory animals species [sic]." The relationship, if any, between the physiology of gerbils' adrenal glands and effects of gonadectomy on gerbils' use of forepaw when standing tripodally remains to be determined.

The present finding—that depriving infant gerbils of normal exposure to gonadal hormones affected handedness—is consistent with Geschwind and Galaburda's (1987) hypothesis that the hormonal milieu in which vertebrates mature organizes lateral asymmetries in their behavior. However, abolition by gonadectomy in adulthood of sex differences in use of forelimbs seen in intact gerbils suggests that, in addition to whatever organizational effect gonadal hormones have on development of asymmetries in forepaw use by gerbils, circulating levels of such hormones in adults affect expression of sex differences in forepaw use. The present data thus provide strong evidence of organizational effects and weaker evidence of activational effects on lateral asymmetries in forelimb use by Mongolian gerbils.

References

Clark, M. M., Crews, D., & Galef, B. G., Jr. (1991). Sex-steroid levels of pregnant and fetal Mongolian gerbils. *Physiology & Behavior, 49,* 239–243.

Clark, M. M., Robertson, R. K., & Galef, B. G., Jr. (1993). Intrauterine position effects on sexually dimorphic asymmetries in Mongolian gerbils: Testosterone, eye-opening and handedness. *Developmental Psychobiology, 26,* 185–194.

Clark, M. M., Robertson, R. K., & Galef, B. G., Jr. (1996). Effects of perinatal testosterone on handedness of gerbils: Support for part of the Geschwind-Galaburda hypothesis. *Behavioral Neuroscience, 110,* 413–417.

Clark, M. M., vom Saal, F. S., & Galef, B. G., Jr. (1992). Fetal intrauterine position correlates with endogenous testosterone levels in adult Mongolian gerbils. *Physiology & Behavior, 51,* 957–960.

Collins, R. L. (1975). When left-handed mice live in right-handed worlds. *Science, 187,* 181–184.

Cullen, J. W., Pare, W. P., & Money, A. L. (1971). Adrenal weight to body weight ratios in the Mongolian gerbil (*Meriones unguiculatus*). *Growth, 35,* 169–176.

De Ghett, V. J. (1972). The behavioral and morphological development of the Mongolian gerbil (*Meriones unguiculatus*) from birth until 30 days of age. *Dissertation Abstracts International, 33,* 5536B. (University Microfilms No 73-12, 022)

Denenberg, V. H., & Yutzey, D. A. (1985). Hemispheric laterality, behavioral asymmetry, and the effects of early experience in rats. In S. D. Glick (Ed.), *Cerebral lateralization in nonhuman species* (pp. 110–131). New York: Academic Press.

Diamond, M. C. (1984). Age, sex, and environmental influences. In N. Geschwind & A. M. Galaburda (Eds.), *Cerebral dominance: The biological foundations* (pp. 134–146). Cambridge, MA: Harvard University Press.

Diamond, M. C. (1991). Hormonal effects on the development of cerebral lateralization. *Psychoneuroendocrinology, 16,* 121–129.

Dittmann, R. W. (1992). Body position and movement patterns in female patients with congenital adrenal hyperplasia. *Hormones and Behavior, 26,* 441–456.

Drea, C. M., Wallen, K., Akinbami, M. A., & Mann, D. R. (1995). Neonatal testosterone and handedness in yearling rhesus monkeys (*Macaca mulatta*). *Physiology & Behavior, 58,* 1257–1262.

Fenske, M. (1986). Adrenal function in the Mongolian gerbil (*Meriones unguiculatus*): Influence of confinement stress upon glucocorticosteroid, progesterone, dehydroepiandosterone, testosterone and androstenedione plasma levels, adrenal content and in vitro secretion. *Experimental and Clinical Endocrinology, 87,* 15–25.

Geschwind, N., & Galaburda, A. M. (1987). *Cerebral lateralization: Biological mechanisms, associations and pathology.* Cambridge, MA: MIT Press.

Kadioglu, D., & Harrison, R. G. (1975). The ultrastructure of the adrenal cortex of the Mongolian gerbil (*M. unguiculatus*). *Journal of Anatomy, 20,* 179–189.

Moffat, S. C., & Hampson, E. (1996). Salivary testosterone levels in left- and right-handed adults. *Neuropsychologia, 34,* 225–233.

Nass, R., Baker, S., Speiser, P., Virdis, R., Balsamo, A., Cacciari, E., Loche, A., Dumic, M., & New, M. (1987). Hormones and handedness: Left-handed bias in female congenital adrenal hyperplasia patients. *Neurology, 37,* 711–715.

Nickerson, P. A. (1971). Fine structure of the Mongolian gerbil adrenal cortex. *Anatomical Record, 171,* 443–456.

Nickerson, P. A. (1975). Stimulation of the adrenal gland of the Mongolian gerbil after ovariectomy. *Cell and Tissue Research, 165,* 135–139.

Oliver, J. T., & Peron, F. G. (1964). 19-hydroxy-11-deoxycortisol, a major steroid secreted by the adrenal gland of the Mongolian gerbil. *Steroids, 4,* 351–363.

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology, 65,* 369–382.

Probst, B. (1987). Developmental changes in the pituitary-gonadal-axis in male Mongolian gerbils from birth to adulthood. *Experimental and Clinical Endocrinology, 90,* 157–166.

Rosen, G. D., Berrebi, A. S., Yutzey, D. A., & Denenberg, V. H. (1983). Prenatal testosterone causes a shift of asymmetry in neonatal tail posture of the rat. *Developmental Brain Research, 9,* 99–101.

Ross, D. A., Glick, S. D., & Meilbach, R. C. (1981). Sexually dimorphic brain and behavioral asymmetries in the neonatal rat. *Proceedings of the National Academy of Sciences, 78,* 1958–1961.

Tan, U., Kara, I., & Kutlu, N. (1991). The effects of testosterone on paw preference in adult cats. *International Journal of Neuroscience, 56,* 187–191.

Received September 17, 1997
Revision received December 30, 1997
Accepted January 15, 1998 ■