Behavioral Neuroscience
1996, Vol. 110, No. 2, 413-417

0735-7044/96/$3.00

Effects of Perinatal Testosterone on Handedness of Gerbils: Support for Part of the Geschwind–Galaburda Hypothesis

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

When assuming their species-typical tripodal stance, male Mongolian gerbils (*Meriones unguiculatus*) use their right forepaw for support more frequently than do females. This experiment determined whether, as N. Geschwind and A. M. Galaburda (1987) have proposed, the direction of such sexually dimorphic lateral asymmetry of forelimb use is affected by perinatal exposure to testosterone (TP). It was found that male gerbils injected withTP when 4 days old were significantly less likely to rest on their right forepaw when in a tripodal stance than were their oil-injected siblings. Female gerbils injected with TP when 6 days old were more likely than oil-injected controls to use their right forepaws for support. The findings demonstrate effects of perinatal exposure to TP on handedness in gerbils and suggest that the relationship between TP exposure and asymmetrical forelimb use is not always as direct as Geschwind and Galaburda's model suggests.

Male and female Mongolian gerbils (*Meriones unguiculatus*) use their left and right forepaws differently when they assume a species-typical tripodal stance in which they support themselves on both hind feet and one forepaw while holding the other forepaw in the air (De Ghett, 1972). When in the tripodal stance, male Mongolian gerbils stand on their right forepaws and hold their left forepaws aloft significantly more frequently than do female Mongolian gerbils (Clark, Robertson, & Galef, 1993).

Also relevant to the experiments described below is evidence that, regardless of sex, adult gerbils that were gestated in intrauterine positions between two males are more likely to stand on their right forepaws while in the tripodal stance than are adult gerbils that were gestated between two females (Clark et al., 1993).

Because male gerbils have higher circulating levels of testosterone than do females (Clark, vom Saal, & Galef, 1992; Clark, Crews, & Galef, 1991) and because gerbils that as fetuses resided in intrauterine positions between males have higher circulating levels of testosterone throughout life than do gerbils that were gestated between females (Clark et al., 1991), there is reason to suppose that the tendency to use the right forepaw for support while in a tripodal stance is increased by exposure to testosterone during development.

Such a supposition is consistent with Geschwind and Galaburda's (1987) hypothesis that in humans, exposure to high levels of testosterone both prenatally and in infancy affects neuronal growth in the two cerebral hemispheres differentially and results in sexually dimorphic behavioral asymmetries, handedness among them.

Although our previous observations of both sex differences in and intrauterine-position effects on forepaw use by Mongolian gerbils (Clark et al., 1993) are in accord with Geschwind and Galaburda's hypothesis that early hormonal exposure plays an important role in producing sexually dimorphic behavioral asymmetries, evidence of effects of testosterone on handedness in gerbils (Clark et al., 1993), like most other data in the literature concerning hormonal effects on lateral asymmetries, is entirely correlational (for reviews, see Bradshaw & Rogers, 1993; James, 1988; Rogers, 1989).

Our laboratory has been engaged in studies of effects of perinatal exposure to testosterone on the potency and fertility of male Mongolian gerbils. These experiments made available male gerbils that had been exposed to exogenous testosterone in infancy and, thus, provided us with an opportunity to examine directly the effects, if any, of neonatal androgenization on development of handedness in male Mongolian gerbils. The outcome of our experiment with male gerbils led us to a further experiment designed expressly to examine effects of early exposure to testosterone on handedness in females of the species.

On both the Geschwind and Galaburda hypothesis and our previous data describing both sex and intrauterine-position effects on forepaw use by Mongolian gerbils, one would predict that animals with a history of early exposure to artificially elevated levels of testosterone would be more likely than would control animals lacking such exposure to exhibit the male pattern of forepaw use, that is, to stand on their right forepaw and to hold their left forepaw in the air while in a tripodal stance.

Experiment 1

Experiment 1 was undertaken to explore the effects of early exposure to testosterone propionate (TP) on use by male

Mertice M. Clark, Rohan K. Robertson, and Bennett G. Galef, Jr., Department of Psychology, McMaster University, Hamilton, Ontario, Canada.

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. We thank Jennifer Vonk and Elaine E. Whiskin for technical assistance. Some of the data reported here formed a portion of a thesis submitted to the Faculty of Science of McMaster University by Rohan K. Robertson in partial fulfillment of the requirements for the M.Sc. degree.

Correspondence concerning this article should be addressed to Mertice M. Clark, Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada.

Mongolian gerbils of right and left forelimbs while in the tripodal posture.

Method

Subjects

Subjects were 1 female and 2 male Mongolian gerbils randomly selected from within each of 18 litters born in the vivarium of the McMaster University Psychology Department to descendants of gerbils originally acquired from Tumblebrook Farm (Brookfield, MA). Each litter selected for use in the experiment contained at least 2 male and 2 female pups at birth.

There were deaths among experimental subjects in 4 of the 18 litters, leaving 14 litters in which we could use within-litter comparisons to control for litter effects in our study of paw use by gerbils while in the tripodal stance.

Procedure

Maintenance. Subjects were housed throughout the experiment in opaque, polypropylene shoebox cages in a single temperature- and humidity-controlled colony room illuminated on a 12-hr light–dark cycle. Dams and their young were housed together until pups were 35 days of age, when each pair of siblings of the same sex was removed to a separate cage where it remained until the experiment ended 35 days later.

On the day of birth (Day 1), litter members were toe-clipped for permanent identification. Care was taken to clip toes on the right and left feet of equal numbers of subjects assigned to each condition.

Administration of testosterone. On Day 4 postpartum, 1 male in each litter was assigned to the experimental condition and injected subcutaneously with 200 μg of testosterone propionate (TP; Sigma Chemical, St. Louis, MO) dissolved in .02 ml peanut oil. Date of injection was selected on the basis of Turner's (1984) data demonstrating a sensitive period between days 3 and 6 postpartum for effects of neonatally administered TP on scent marking by adult male gerbils.

It was our intention to mimic in experimental subjects some of the increased level of exposure to testosterone normally experienced, both pre- and postnatally, by male gerbils that mature in intrauterine positions between male rather than between female fetuses (Clark et al. 1991, 1992).

The second male subject in each litter was assigned to the control group and injected with .02 ml of unadulterated peanut oil, as was each female subject. All injection sites were sealed with collodion (Fischer Scientific, Toronto, Ontario, Canada) immediately following injection.

Testing for paw preference. To determine whether subjects exhibited asymmetry in paw use while in the tripodal stance, each subject was examined for 10 successive days at each of two ages, first 2 to 5 days after both its eyes had opened (at 15 to 20 days of age) and second at 60 days of age, when it was sexually mature.

On each test day, a subject was introduced into a 5-gallon aquarium (20 × 40 × 24 cm) and then watched by an experimenter who was unaware of the sex or experimental condition to which the subject she was observing had been assigned. Whenever a subject was not in contact with an aquarium wall, had stopped moving, and had assumed a tripodal stance, the observer recorded which of its forepaws it used for support. Each subject was observed each day until it had assumed the tripodal stance 10 times.

At the end of each of the two 10-day testing periods, a subject was awarded a score indicating the proportion of the 100 occasions when it was observed in a tripodal stance that it rested on its right forepaw.

Results

The main results of Experiment 1 are presented in Figure 1, which shows the mean proportion of times that subjects in each of the three groups in Experiment 1 used their right forepaws for support while in the tripodal stance. As can be seen in Figure 1, for both juvenile and adult subjects, there were significant effects of treatment on use of the right forepaw while in the tripodal stance; one-way, repeated measures analyses of variance (ANOVAs): juveniles, $F(2, 26) = 7.41$, $p < .004$; adults, $F(2, 26) = 6.55$, $p < .006$. Post hoc tests revealed that (a) untreated females were significantly less likely to stand on their right forepaw while in the tripodal stance than were untreated males (least significant difference [LSD] tests for both juveniles and adults, $p < .05$), a result consistent with Clark et al.'s (1993) finding of a sex difference in forepaw use by male and female Mongolian gerbils while in the tripodal stance, and (b) TP-treated males were significantly less likely than were oil-treated males to use their right forepaws for support while in the tripodal stance (LSD tests for both juveniles and adults, $p < .01$).

Figure 2 provides data describing the stability of paw preferences in subjects from weaning age to early adulthood. Pearson's correlation coefficients revealed that, across all three groups, there was a highly significant correlation between an individual's tendency to stand on its right forepaw when juvenile and its tendency to do so in early adulthood (Pearson's $r = .63, p < .001$). Similar correlational analyses of stability of paw preference within each of the three treatment groups in Experiment 1 revealed a significant tendency for male subjects in both control and experimental groups to continue to use the same forepaw for support while in a tripodal stance (both Pearson's rs $> .53$, both ps $< .05$). Female subjects also exhibited a positive correlation between forepaw use for support when juvenile and when adult (Pearson's $r = .38$), but that relationship was not statistically reliable.

Experiment 2

The results of Experiment 1 suggested that paw use by male gerbils when resting in a tripodal stance was affected by perinatal exposure to testosterone, but that the direction of the effect of testosterone on behavior was not what one might predict on the simple model that increased exposure to testosterone masculinizes behavior. In the present experiment, we extended our investigations of effects of TP on asymmetrical paw use to female gerbils, which normally are more likely than are males to stand on their left forepaws when in a tripodal posture (Clark et al., 1993).

Method

Subjects

Subjects were 1 male and 3 female Mongolian gerbils randomly selected from within each of 17 litters born in the vivarium of

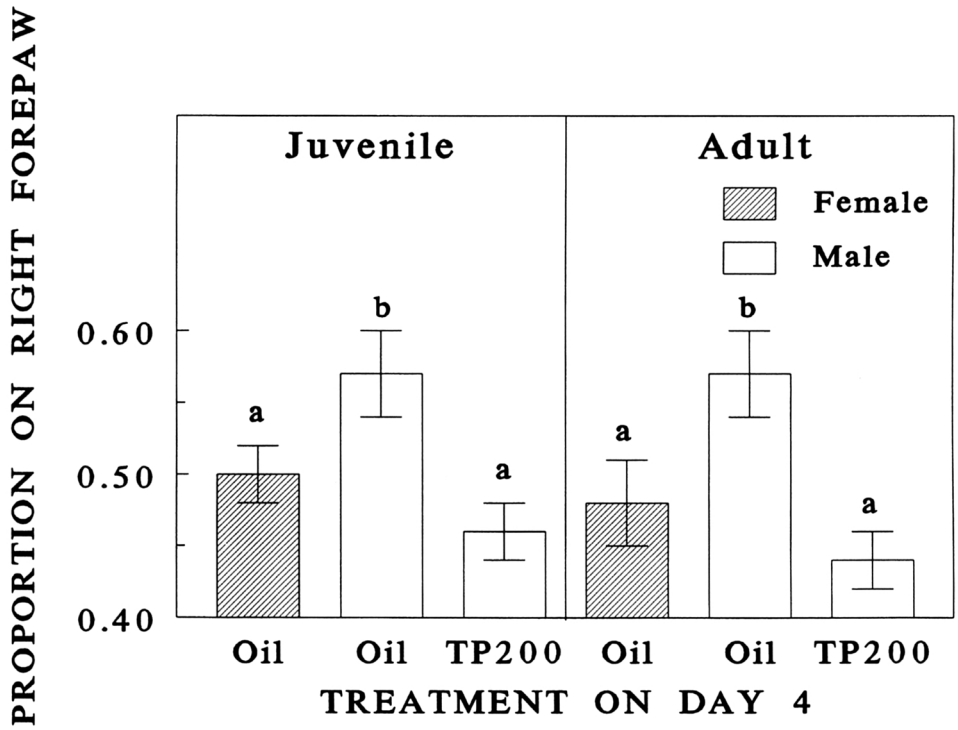

Figure 1. Mean proportion of instances in which juvenile and adult subjects used their right forepaws for support while in a tripodal stance. Error bars indicate ± 1 SEM. Bars within a panel with different letters above them differed significantly. TP200 = subjects injected with 200 μg testosterone propionate.

the McMaster University Psychology Department. Each litter chosen for use contained at least 2 male and 3 female pups at birth. There were deaths among experimental subjects in 3 of the 17 litters, leaving 14 litters in which we could make comparisons among subjects while controlling for litter effects. The male and female subjects injected with oil (see *Procedure*) in 4 of the 14 litters used in Experiment 2 had also served as vehicle-injected control subjects in Experiment 1.

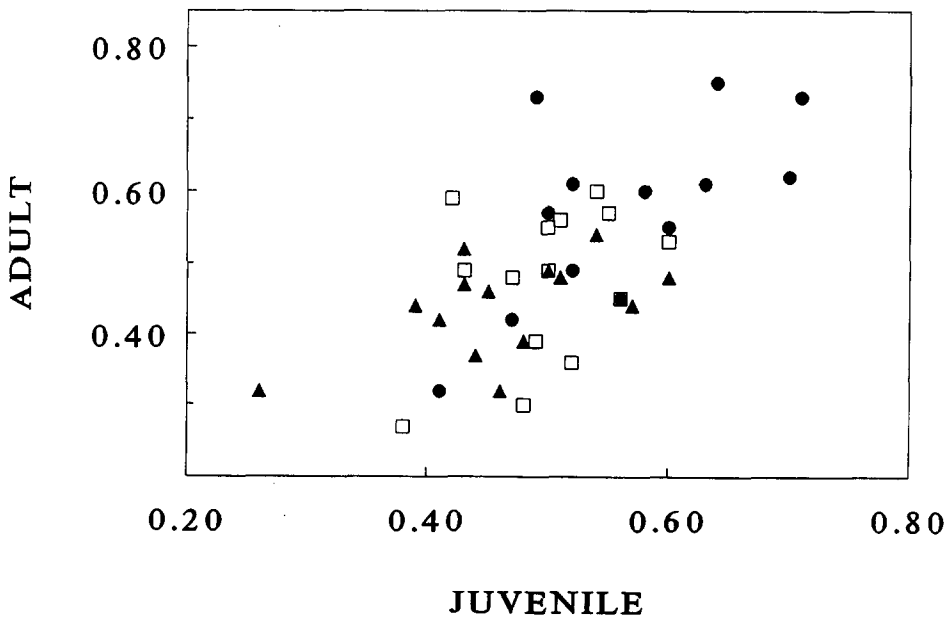

Figure 2. Proportion of instances in which individual subjects, as juveniles and as adults, used their right forepaws for support while in the tripodal stance. Circles indicate males injected with oil; triangles indicate males injected with testosterone propionate; squares indicate females injected with oil.

Procedure

The procedure of Experiment 2 was identical to that of Experiment 1, except that, in Experiment 2, when they reached 6 days of age, 1 male and 1 female subject in each litter were injected with .02 ml of unadulterated peanut oil, 1 female in each litter was injected with 50 μg of TP dissolved in .02 ml peanut oil, and another female in each litter was injected with 100 μg TP dissolved in .02 ml peanut oil. As in experiment 1, the date and amount of TP injected were selected on the basis of Turner's (1984) study of effects of single perinatal injections with TP on frequency of scent marking.

As in Experiment 1, all subjects were tested for paw preference shortly after eye opening and again when sexually mature.

Results

The main results of Experiment 2 are presented in Figure 3, which shows the mean proportion of times that each subject in each of the four groups in Experiment 2 used its right forepaw for support while maintaining a tripodal stance. As can be seen in Figure 3, when tested both as juveniles and adults, subjects exhibited significant effects of treatment on forepaw use in the tripodal stance; one-way, repeated measures ANOVA: when juvenile, $F(3, 39) = 3.38, p < .03$; when mature, $F(3, 39) = 2.99, p < .05$. Post hoc tests revealed that, as in Experiment 1, males injected with oil were more likely to stand on their right forepaws than were females injected with oil (LSD tests for both juveniles and adults, $p < .05$). Post hoc tests also showed that (a) juvenile females that had been injected with 50 μg of TP were significantly more likely to stand on their right forepaws than were either oil-treated females or females that had been injected with 100 μg TP (LSD tests, both $ps < .05$), and (b) females tested when adult and injected as infants with 50 μg TP were significantly more likely to stand on their right forepaws than were their adult sisters that had been treated with oil when infants (LSD test, $p < .05$).

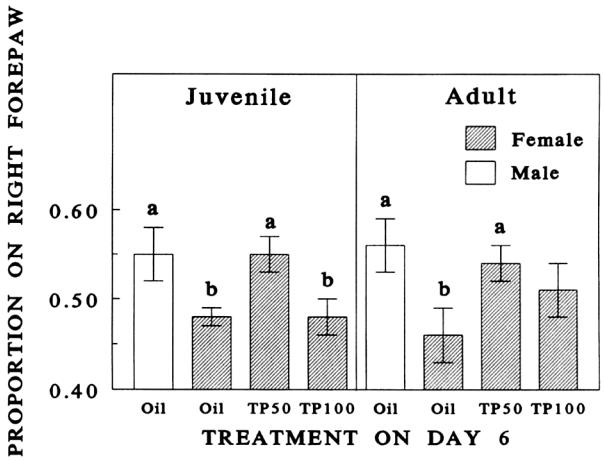

Figure 3. Mean proportion of instances in which juvenile and mature subjects used their right forepaws for support while in a tripodal stance. Bars within a panel that have different letters above them differed significantly (least significant difference tests). Error bars indicate ± 1 SEM. TP50 = subjects injected with 50 μg testosterone propionate. TP100 = subjects injected with 100 μg testosterone propionate.

It is relevant to note that in Turner's (1984) study, the frequency of scent marking (a masculine behavior) seen in adult female gerbils that had received a single injection of 100 μg of testosterone on Day 5 postpartum was significantly elevated relative to that of females injected at the same age with either 50 μg of TP or vehicle.

Again, as in Experiment 1, there was a highly significant correlation between an individual's tendency to use its right forepaw for support when a juvenile and its tendency to do so in early adulthood (Pearson's $r = .47$, $p < .001$). The probability that an individual would stand on its right forepaw when tested as a juvenile and as an adult was positively correlated within each of the four groups in Experiment 2, but that correlation was statistically significant only in the case of oil-treated males (Pearson's $r = .62, p < .02$) that also had the greatest within-group variability in probability of using the right paw for support both when tested as juveniles and when tested in adulthood.

After we combined data across Experiments 1 and 2, to achieve a large enough number of subjects to make statistically reliable outcomes likely, binomial tests revealed that juvenile males in control groups were significantly more likely to stand on their right forepaws than on their left ($x = 6, p < .01$). By similar testing, neither juvenile nor adult females in control groups approached significant deviation from chance in their choice of a forepaw on which to stand (both $xs > 12$), whereas adult males in control groups approached, but did not reach statistical significance in their tendency to stand on their right forepaws (binomial test, $x = 9, p = .09$, two-tailed).

General Discussion

Geschwind and Galaburda (1987, p. 10) began their discussion of the causes of lateralization with the assertion that, although genes contribute to lateralization patterns, the hormonal milieu in which an organism matures also has significant effects on its lateral asymmetries in both morphology and behavior. The results of the present studies provide experimental evidence consistent with that view: (a) adult, male Mongolian gerbils that as neonates had received a single administration of TP used their left and right forepaws for support differently than did their brothers that lacked early exposure to exogenous TP, and (b) both juvenile and adult female gerbils that as infants had been injected with 50 μg of TP were more likely to rest on their right forepaws while in a tripodal stance than were their oil-injected sisters. In Mongolian gerbils, preferential use of left and right forelimbs is affected by early exposure to hormones, and such hormonal effects are relatively stable from the juvenile period into early adulthood.

The present experiments, like an earlier report of effects of hormonal manipulations on tail posture in female (but not in male) rats (Rosen, Berrebi, Yutzey, & Denenberg, 1983), demonstrated effects of perinatal administration of testosterone on lateralization of behavior; this is consistent with a larger body of correlational evidence (for reviews, see Bradshaw & Rogers, 1993; James, 1988; Rogers, 1989) implicating testosterone in the development of lateral asymmetries, as Geschwind and Galaburda (1987) indicated.

On the other hand, our expectations as to the direction of

effects of exposure to testosterone on use of left and right forepaws were not realized. We had assumed, on the basis of both Geschwind and Galaburda's (1987) hypothesis and the results of our earlier study of effects of sex and intrauterine position on forelimb use by gerbils (Clark et al., 1993), that exposing infant gerbils to exogenous testosterone would increase their tendency to exhibit the male pattern of forepaw use in adulthood. We found, to the contrary, that neonatal exposure to TP significantly increased the frequency of expression of the female pattern of forepaw use in male gerbils and, depending on the dosage administered, either masculinized or left unaffected forepaw use by female gerbils. Clearly, the relationship between exposure to testosterone and masculinization of behavioral lateralization is not as straightforward as Geschwind and Galaburda suggested (and we had hoped) that it might be.

Perhaps, if we had exposed our subjects to different amounts of testosterone or had androgenized them at different points in ontogeny, we would have seen the straightforward relationship between exposure to testosterone and lateralized use of forelimbs that we had expected. We do not know. What is clear from the present results is that the simple notion that increased exposure to testosterone in infancy results in increased expression of the male pattern of lateralized forelimb use by gerbils is inadequate.

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Received April 10, 1995
Revision received June 13, 1995
Accepted August 7, 1995 ■