NEONATAL TESTOSTERONE AFFECTS GERBILS' PAW ELEVATION

NEONATAL TESTOSTERONE TREATMENT AFFECTS THE PAW ELEVATION OF THE MONGOLIAN GERBIL (MERIONES UNGUIGULATUS)

By

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Abstract

The present thesis reports an experiment that investigated the phenomenon of asymmetric paw elevation in the tripedal stance of the Mongolian gerbil, and tested the Geschwind-Galaburda extra-genetic theory of human handedness. Neonate gerbils of both sexes were injected with testosterone propionate during the "critical" period of brain development. They were then assessed for asymmetry in eye opening, for anogenital distance, and for paw elevation and scent marking before and after puberty. Eye opening asymmetry was not affected by treatment. Paw elevation was affected by treatment, with treated gerbils of both sexes displaying more right elevations before and after puberty than untreated gerbils. Control females displayed systematic patterns in paw elevation before and after puberty. Adult gerbils in all conditions displayed more consistency in paw elevation than young gerbils. Anogenital distance was increased with treatment, but only in the females. Adult scent marking behavior was marginally reduced with treatment, but only in the males. Results are interpreted within the Geschwind-Galaburda theory of handedness, and the hormonal basis of paw elevation is discussed.

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Third, I thank Ian Begg for his support, his statistical knowledge, and his patience during the weepings, the wailings, and the gnashings of teeth. My parents, too, deserve some acknowledgment for the latter. Thanks to Miney Ham and Elaine Whiskin for their friendship in lab and out (truly they co-rule!), to Caroline Woods for her empathy, and to Jennifer Vonk for listening to my multitudinous complaints. To James Taylor, I accredit the very clever "anogenital proportion", which managed to withstand Dr. Daly's skepticism.

This thesis is dedicated to all the little gerbils who gave their lives for my (comparatively) inane dilettantism.

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Many creatures exhibit structural and functional asymmetries, but asymmetric appendage use was long thought to be a uniquely human characteristic (Rogers, 1989). Comparative studies, however, have demonstrated that individuals of many species, from parrots to rodents, preferentially use left or right appendages to perform various tasks (see Rogers, 1989, for review). The finding that non-human species display asymmetry in appendage use enabled theorists to use animal models in the formulation and testing of hypotheses about the ontogeny of human handedness.

What factors influence the development of hand use? Collins (1969, 1985; Collins, Sargent & Neumann, 1991) believed that asymmetries in hand use were encoded in the genome. Using the mouse as his model, Collins performed selective breeding studies with the hope of producing one strain that reached for food with the left paw, and one strain that reached for food with the right paw. Although he was not able to achieve the desired result, he was able to produce two strains that differed in the degree to which one paw or the other was used. Collins' findings, and findings from human studies (Bryden, 1987, Bryden & Steenhuis, 1991) led genetic theorists to conclude that human hand use involves two distinct components, degree and direction, and, although genetic factors could explain the development of the first, they could not explain the development of the second (Hellige, 1993). The ontogeny of hand use must be governed by factors other than simply genes.

An Extra-Genetic Theory of Hand Use

Geschwind and Galaburda (1985a, 1985b, 1985c, 1987) proposed that the chemistry of the intrauterine environment shapes many human structural and functional

asymmetries by affecting the development of the central nervous system. Although fetal genes make an important contribution to intrauterine chemistry, Geschwind and Galaburda believed that factors lying outside the fetal gene pool make important contributions as well.

Testosterone is one intrauterine chemical that is not wholly governed by the fetal genome; it may enter the uterus from the outside, for example, from maternal hormone therapy or maternal adrenal gland production, or it may be produced internally, for example, from the testes of a twin brother. In humans, the left cerebral hemisphere develops more slowly than the right, and, when fully developed, is generally larger than the right (Geschwind & Galaburda, 1985a, 1987). Geschwind and Galaburda (1985a, 1987) proposed that testosterone has the effect of further slowing the growth and development of some or all of the left hemisphere. The right hemisphere, or portions of it, will consequently enjoy more growth and development than it would have in the absence of testosterone. The net effect of retarding left and enhancing right is a brain in which the hemispheres are structurally symmetrical in many or all areas.

According to Geschwind and Galaburda (1985a, 1987, and Rosen, Sherman & Galaburda, 1991), the physical size of a hemispheric region reflects its capacity for function. Ordinarily, fine motor function resides predominantly in the (larger) left hemisphere for the contralateral production of movement; most people will therefore tend to use their right hands for tasks such as writing. In the more symmetrical testosterone-altered brain, however, fine motor function has a roughly equal chance of residing in either hemisphere. Thus, people for whom fine motor function is controlled by the right hemisphere will tend to use their left hands for tasks such as writing.

Predictions of the Theory

The Geschwind-Galaburda theory predicts that exposure of an individual to high levels of prenatal testosterone will result in left hand use. There is empirical support for this prediction in the human literature. For example:

1. The human population supports about three times as many left-handed males as females (e.g. Porac & Coren, 1981), a statistic that is consistent with the fetal contribution to the intrauterine testosterone pool;
2. There is a high rate of left-handedness in the female member of opposite-sex twins (Koch, 1966, in Miller, 1994), a finding that is consistent with the male twin's contribution to the intrauterine testosterone pool;
3. The brains of males are more structurally symmetrical than the brains of females (Habib, Touze & Galaburda, 1990);
4. A high incidence of left-handedness has been reported among adults who had been prenatally exposed to diethylstilbestrol, an estrogen metabolite of testosterone (Geschwind & Galaburda, 1987);
5. An elevated rate of left-handedness among homosexual males has been reported (Becker, Bass, Dew, Kingsley, Selnes & Sheridan, 1992), a phenomenon that may be attributable to rapid rise and fall of intrauterine testosterone levels (e.g. Geschwind & Galaburda, 1987; Ward & Weisz, 1980).

Although testosterone may well be a factor in the development of asymmetrical hand use, some studies have not upheld the Geschwind-Galaburda theory's prediction that the direction of hand use will be leftward. Grimshaw, Niccols and Finegan (1990) found no relationship between fetal testosterone levels and the direction of hand use of 7-year olds. Subsequently, Grimshaw (1992), using more reliable measures of hand use, found that prenatal testosterone levels were positively correlated with right-handedness, but only in girls.

Only one non-human study has related prenatal testosterone to asymmetries in appendage use: Clark, Robertson and Galef (1993) reported that exposure to high levels of fetal testosterone was correlated with left forepaw elevation during the tripedal stance in adult Mongolian gerbils (Meriones unguiculatus) of both sexes, a finding that is consistent with the Geschwind-Galaburda theory. Prenatal testosterone has been implicated as a factor in other structural and functional asymmetries, such as tail posture (Rosen, Berrebi, Yutzey, & Denenberg, 1983) and rotation (see Bradshaw & Rogers, 1993, for a review) in rats. In both cases, males were found to be less strongly lateralized than females, a result that is consistent with the Geschwind-Galaburda theory's prediction that the brains of males will be more symmetrical than the brains of females.

Like prenatal testosterone, neonatal testosterone has been shown to produce structural and functional asymmetries in non-humans, including asymmetries in the spatial abilities of rats (Roof, 1993; see Williams & Meck, 1991, for review), in the visual discrimination of male chickens (Zappia & Rogers, 1987), and in the vocalization of Mongolian gerbils (Holman & Hutchison, 1991). The lateralization of human brain function is present from birth, but the lateralization of rodent brain function is not complete until about postnatal Day 10 (Rosen et al., 1991). The neonatal rodent brain is therefore developmentally equivalent to the prenatal human brain; hence, the neonatal manipulation of rodent brain development by exogenous testosterone, with the subsequent measurement of asymmetry in appendage use, may be used to test the Geschwind-Galaburda theory's prediction that exposure of the fetus to high levels of testosterone is a factor in the development of left hand use.

The Present Experiment

The present experiment sought to determine whether neonatal testosterone treatment could directly affect paw elevation in the Mongolian gerbil (Meriones unguiculatus), and, if so, whether this direction was indeed leftward.

Gerbil pups were injected with a single dose of testosterone propionate (TP) shortly after birth. Females were chosen as the chief experimental subjects because it was thought that they would be more sensitive to the effects of testosterone than males. Female gerbils display more consistency in adult paw elevations (Clark et al., 1993), female mice show a greater degree of paw preference (Collins, 1977), and female rats show a greater bias in tail posture (Rosen et al., 1983) and in the direction of rotation (Glick, 1983), than do their male counterparts. Presumably, testosterone is one factor responsible for these sex differences. To assess the effect of dose on the degree of paw elevation, females were placed into two dosage groups.

Males also served as experimental subjects, because there is some evidence that exposure to high levels of perinatal testosterone is associated with the incomplete masculinization of brain and behavior (Ward & Weisz, 1980; Stewart & Kolb, 1988). However, only one very high dose of testosterone was used with male subjects, because male behaviors were suspected to be at a "testosterone ceiling", and it was thought that the changes in their magnitude that would generated by lower doses of hormone would not be detectable. For example, detection of change in the size of the scent gland (which is highly correlated with territorial marking frequency) in response to testosterone treatment is not possible beyond a certain dose (Theissen & Yahr, 1977).

The single injection of TP was administered on Day 4 in males (where date of birth is Day 1) and on Day 6 in females, because the territorial marking behavior of the adult Mongolian gerbil shows a maximal response to injections at this time (Turner, 1984). Neonatal rats of both sexes experience an elevation of free plasma testosterone

around Day 2 (vom Saal, Montano & Wang, 1992). Because the ontogenies of the gerbil and the rat are very similar (vom Saal et al., 1993), gerbils would also experience this surge, and, because gerbils lag behind rats in development by several days, an injection at Day 4 to 6 should enhance the naturally-occurring phenomenon.

The experimental males received a higher dose of TP (200 μg) than the high dose females (100 μg), because it was thought that the male-appropriate dose would sicken the females, and 100 μg had been documented as a completely androgenizing dose for other dependent measures in female gerbils (eg. Turner, 1984; Holman & Hutchinson, 1991) and in female rats (Raible & Gorzalka, 1987).

To assess the lifespan plasticity of paw use, paw elevation was measured at two stages of the animals' lives. The times were chosen to bracket puberty; thus, any changes in paw elevation from juvenile to adult would coincide with a change in plasma hormone levels.

The first eye to open after birth was recorded. Eye development is asynchronous in several rodent species: In Mongolian gerbils, one eye generally opens before the other (Clark et al., 1993), and in rabbits, the optic nerve of the first eye to open myelinates at a faster rate than that of the other eye (Narang, 1977). In mice, eye opening is affected by both uterine environment and genotype (Nosten & Roubertoux, 1988). The eye is ontogenetically part of the brain, and, if the net effect of testosterone is to produce hemispheres of equal size (retarding left with subsequent enhancement of the right), then more left eye openings in response to TP treatment should be observed.

Territorial marking behavior before and after puberty, and anogenital distance as a function of body length, were measured. In the body, testosterone is metabolized to dihydrotestosterone (DHT, an androgen) and estradiol (E, an estrogen); this conversion occurs in parallel fashion, and is irreversible (Breedlove, 1993). The effects of testosterone on sexually differentiated behaviors are well-established and result from

direct binding to testosterone receptors, from the binding of either metabolite to its proper receptors, or from a combination of these effects (Yahr, 1988; vom Saal, Montano & Wang, 1992). Dihydrotestosterone appears to be impeded in its access to the brain by peripheral tissues (Yahr, 1979), but testosterone and E have been shown to have large effects on brain development (vom Saal et al., 1992). Anogenital distance is affected by DHT (e.g. Breedlove, 1993), and territorial marking behavior is mediated by unmetabolized testosterone (e.g. Yahr, 1981). The relative magnitudes of these measures may therefore serve as indices of the path of metabolization taken by TP, and a clue as to which hormone mediates asymmetrical paw elevation.

Method

Subjects were 28 male and 47 female Mongolian gerbils from 15 litters born in the vivarium of the McMaster University Psychology Department. Most animals were delivered through natural birth, but a few were delivered by caesarean section on the day preceding the expected date of birth. Each member of a litter was permanently marked for individual identification via metacarpal amputations ("toeclips") on the day of birth. To eliminate the possible interaction between toeclip and paw elevation, an attempt was made to assign equal numbers of left and right clipped animals to each condition.

There were no constraints on the sex ratio of the litters except that there be at least two males and four females in each. All treatments were thus represented within each litter, thereby controlling for litter effects. During the course of the experiment, some of the subjects died or were born malformed; it was therefore not possible to have equal numbers of animals in each condition.

The day of birth was deemed Day 1 for natural deliveries, and Day 0 for caesarean deliveries. Males in each litter were randomly assigned to one of two conditions and were injected on Day 4. Females in each litter were randomly assigned

to one of four conditions and were injected on Day 6. Males received either 200 μg of TP (Sigma) in .02 mL of peanut oil, or .02 mL of peanut oil alone, while females received either 100 μg or 50 μg of TP in .02 mL of peanut oil, .02 mL of peanut oil alone, or no treatment at all.

All injections were performed between 09:30 and 11:30, using a 1 mL syringe fitted with a 30 gauge, 1/2 inch needle[1]. The needle was inserted under the skin at the right side of the neck, and was threaded to the hilt towards the posterior of the animal[2]. Upon injection, the solution formed a small bubble. On some occasions, the needle punctured the skin at a site other than the site of entry, causing the solution to leak. These occasions were duly recorded, and, if leakage was judged to be large, the bubble was popped, the site was flushed and blotted dry, and the injection was readministered on the left side of the animal. The puncture wounds of the first four litters were sealed with Vaseline; thereafter, the wounds were sealed with collodion (Fischer Scientific).

Subjects were housed in polyurethane shoebox cages in a room that was maintained at a constant temperature and light cycle (0400 - 1600). Subjects lived with their mother (or, in the case of the caesarean subjects, their foster mother) until about Day 40, at which time they were weaned. Thereafter, they were housed in same-sex groups of 2 to 6.

Eye Opening

The experimenter, who was blind to sex and condition, examined subjects for eye opening 3 times a day starting on about Day 12. When eyes began to open (approximately Day 17), animals were checked every 2-3 hours, between 07:00 and 17:00 each day. The first eye to open and the time of opening were recorded. The same

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1 Smaller syringes were not available.

2 It was realized too late that side should have been counterbalanced for. However, side was held constant across all conditions.

was done for the second eye.

Mass and Anogenital Distance

The experimenter, who was blind to sex and condition, measured mass and anogenital distance on Day 10. To obtain anogenital distance, each animal was inverted, and its tail and one hind leg were gently stretched to a maximum in opposite directions. The distance between the center of the anal opening and the tip of the genital ridge was measured using a Mitutoyo digimatic caliper (CD-6"B). Because within-subject measurements were so variable, the mean of five successive trials is reported. The mass of each animal was assessed using a Mettler scale (BB300).

Paw Elevation

The paw elevated by the gerbil is defined as the forepaw that was elevated during the tripedal stance (Clark et al., 1993). Because gerbils do not perform the tripedal stance until after eye-opening (De Ghett, 1972), and because subjects required several days to become habituated to the testing environment, before-puberty testing of paw elevation was begun anywhere from the second to the fifth day after both eyes had opened. On each of 10 consecutive days, a subject was placed into a 5 gallon aquarium and was watched as it wandered about the enclosure. Whenever the subject assumed the tripedal stance in the center of the enclosure, the experimenter recorded which of the forepaws it held in the air while it balanced upon three feet. Each subject was watched each day until it had assumed the tripedal stance on 10 occasions. At the end of the 10 day testing period, each subject was awarded a score, P(R), that indicated the proportion of the 100 occasions upon which it was observed in the tripedal stance with its right paw elevated.

After-puberty testing of paw elevation was begun on Day 60 and was performed in the same fashion as it was before puberty. The experimenter was blind to both sex and condition during prepubertal testing, and to condition during postpubertal testing.

Scent Marking

Subjects were tested for scent-marking behavior using a modified procedure of Thiessen (1968). The arena consisted of a 90 x 90 cm Plexiglas floor with 60 cm high wooden walls. A painted grid divided the floor into sixteenths, and a roughened Plexiglas peg (2.6 x 1.2 x 0.7 cm) was located at each of the 9 nodes. Subjects were introduced into the arena for a 5 min session on each of 4 consecutive days. The number of times that the animal marked any of the pegs was recorded.

Females ordinarily mark more when they are in estrus. Because of the difficulty of judging estrus cycle, particularly in the treated females, all subjects were tested at fixed ages, once at Day 45 and again at Day 70. The experimenter was blind to condition during both testing periods.

Results and Discussion

Data from the 50 μg and intact females were not analyzed because of a high rate of birth defects in the former animals, and because there were so few of the latter animals. Thus, throughout the analyses, three orthogonal comparisons are made, namely, between experimental and control males, between experimental (100 μg) and control (oil injected) females, and between control males and control females. Two independent-variable ANOVAs, i.e. treatment and sex, were also performed, but it was realized that statements about sex differences in response to treatment could not be made

because sex and dose were confounded. However, statements about treated vs. untreated gerbils in general (the main effect of treatment) are made.

Paw Elevation

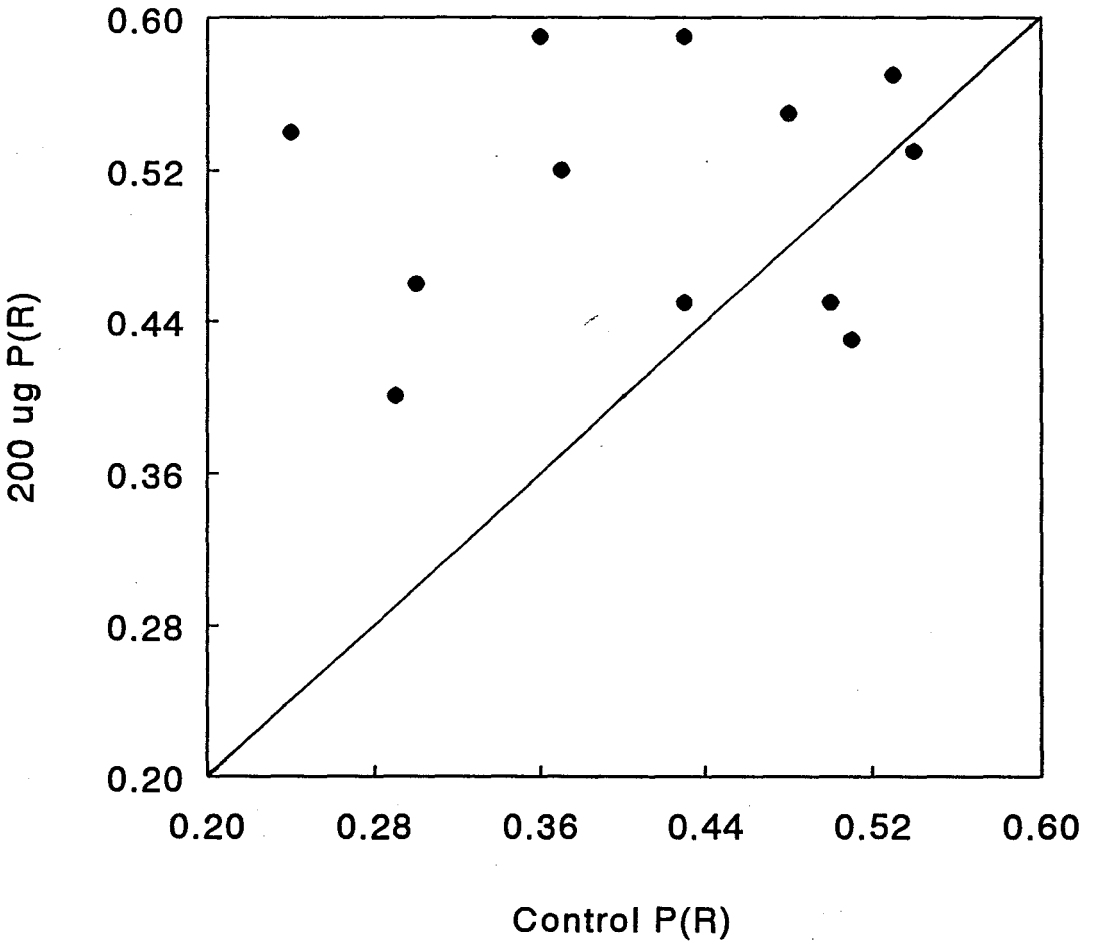
Table 1 depicts P(R) means and standard errors for the four groups before and after puberty; a litter's data was included in the calculations only if it contained animals from all conditions. Although paw elevation scores were rather variable, the Wilcoxon signed ranks test produced essentially the same results as the repeated measures ANOVA (in which litters were treated as subjects, and each littermate was treated as an observation). Thus, only the parametric statistic is reported unless homogeneity of variance is suspect, in which case both statistics are reported. The technique of toe clipping the animals for identification did not interfere with paw elevation either before or after puberty, as determined by a series of two-way ANOVAs (treatment x toe).

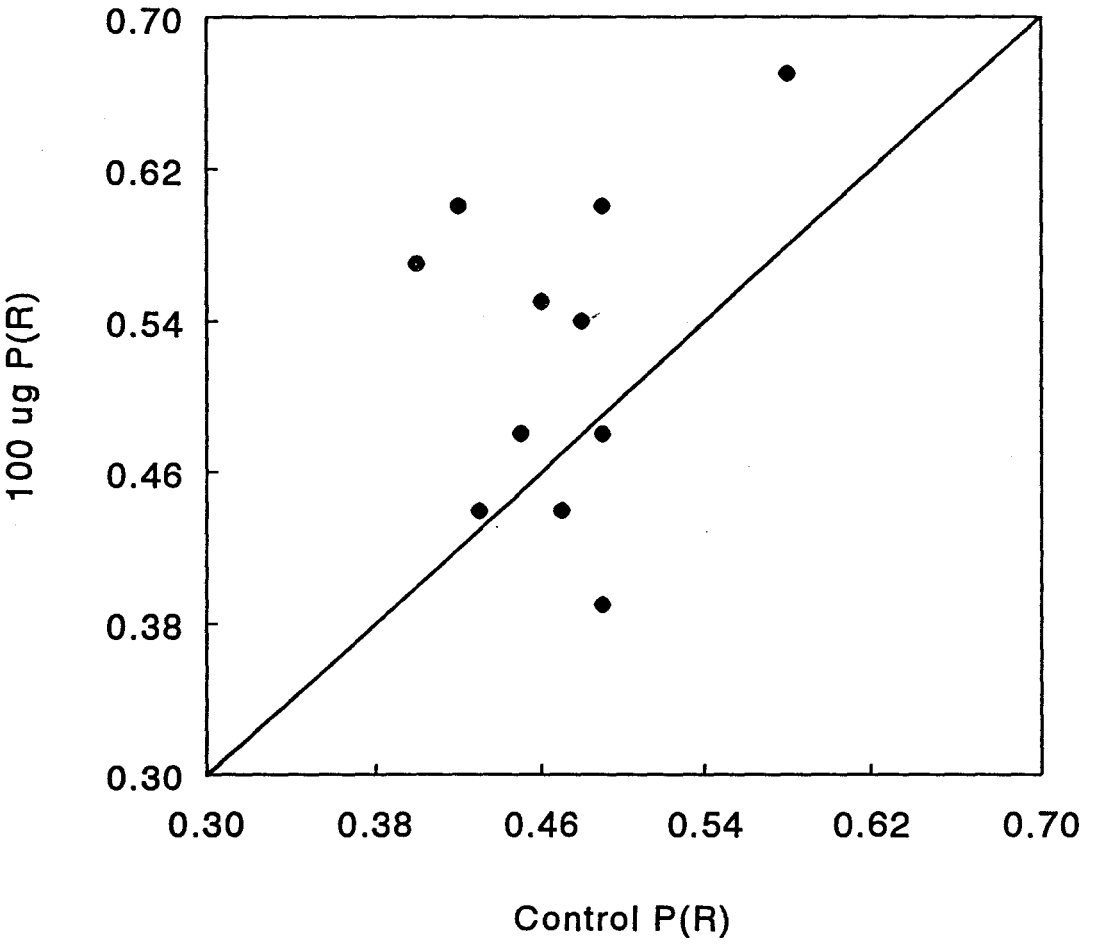
Prepubertal paw elevation. Prepubertal paw elevation was first analyzed by a two-way ANOVA of sex x treatment, which revealed a main effect of treatment, $F(1,10) = 9.625$, $p < .05$, with treated animals being significantly more right-pawed than untreated animals. A separate one-way ANOVA within the males revealed that treated males were significantly more right-pawed than control males, $F(1,11) = 7.715$, $p < .05$. Figure 1 shows the distribution of male P(R)s; in nine of the twelve litters, the treated male was more right-pawed than his control brother, a result that is marginally significant by the sign test ($p = .073$).

A separate one-way ANOVA within the females revealed that treated females were marginally more right-pawed than the control females, $F(1,10) = 4.516$, $p = .0575$. Figure 2 shows the distribution of female P(R)s; in eight of the eleven litters, the treated female was more right-pawed than her control sister, a result that is not significant by the sign test ($p = .113$).

Table 1. Proportion Of Right Paw Elevations Before and After Puberty.

	Before			After		
	N	$\overline{X}$	SE_m	N	$\overline{X}$	SE_m
Male						
Oil	11	0.4136	0.0322	9	0.4467	0.0552
200 μg	11	0.4991	0.0190	9	0.5322	0.0331
Female						
Oil	11	0.4691	0.0144	9	0.4433	0.0208
100 μg	11	0.5236	0.0256	9	0.4933	0.0361


0.60
0.52
0.44
0.36
0.28
0.20
200 ug P(R)
0.20
0.28
0.36
0.44
0.52
0.60
Control P(R)

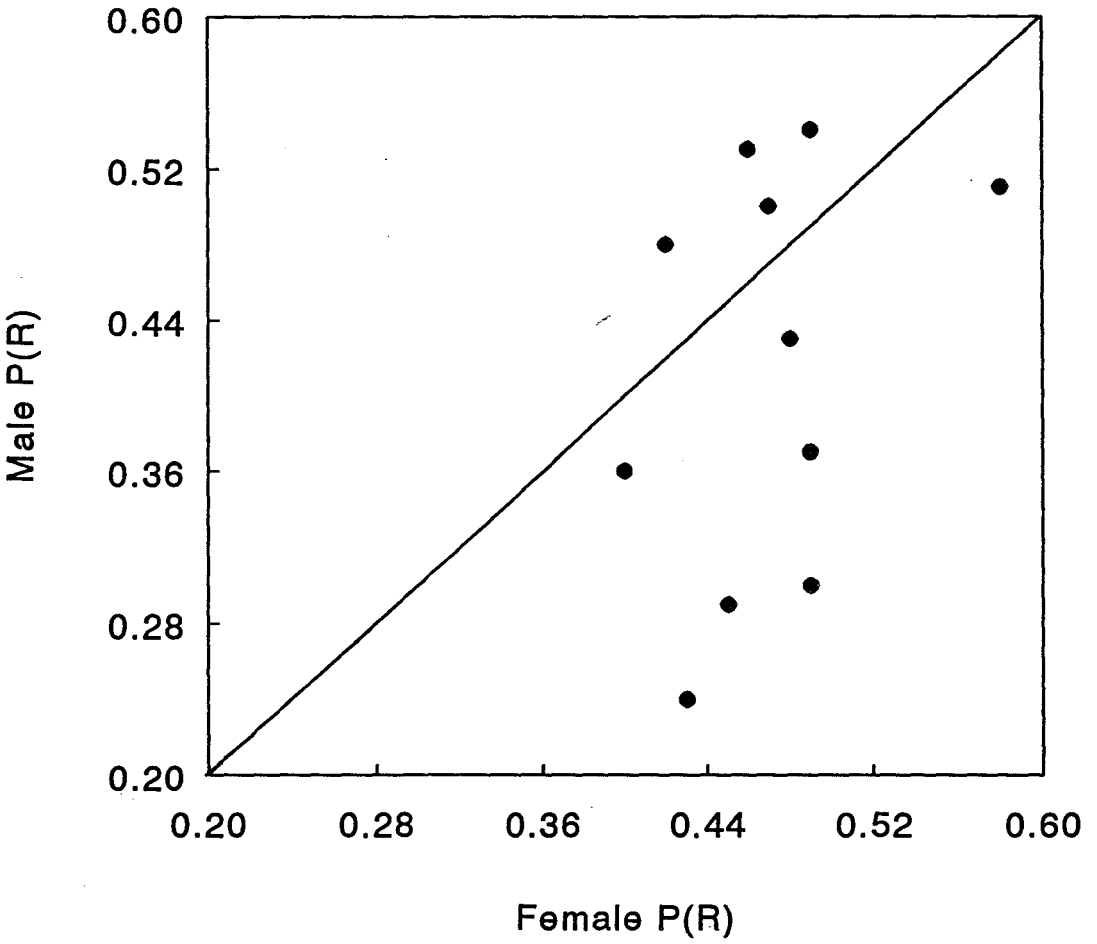

0.70
0.62
0.54
0.46
0.38
0.30
100 ug P(R)
0.30
0.38
0.46
0.54
0.62
0.70
Control P(R)

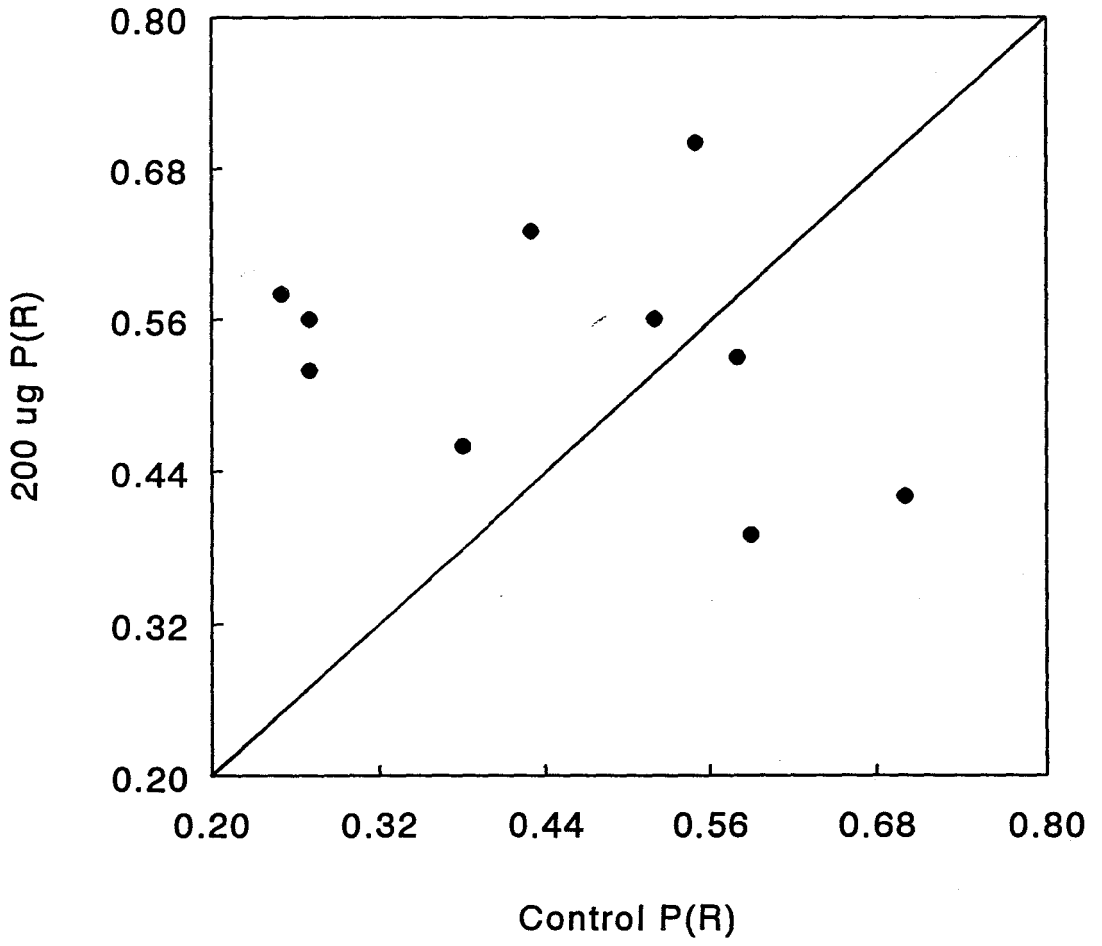
A separate one-way ANOVA within the controls revealed that control females were marginally more right-pawed than control males, $F(1,11) = 4.475$, $p = .0560$; because of nonhomogeneity of variance, the analysis was repeated using the Wilcoxon test, which failed to reveal a significant difference ($T = 16$, $n = 11$, $p > .10$). Figure 3 shows the distribution of control P(R)s; in seven of the eleven litters, the female was more right-pawed than her brother, a result that is not significant by the sign test ($p = .274$).

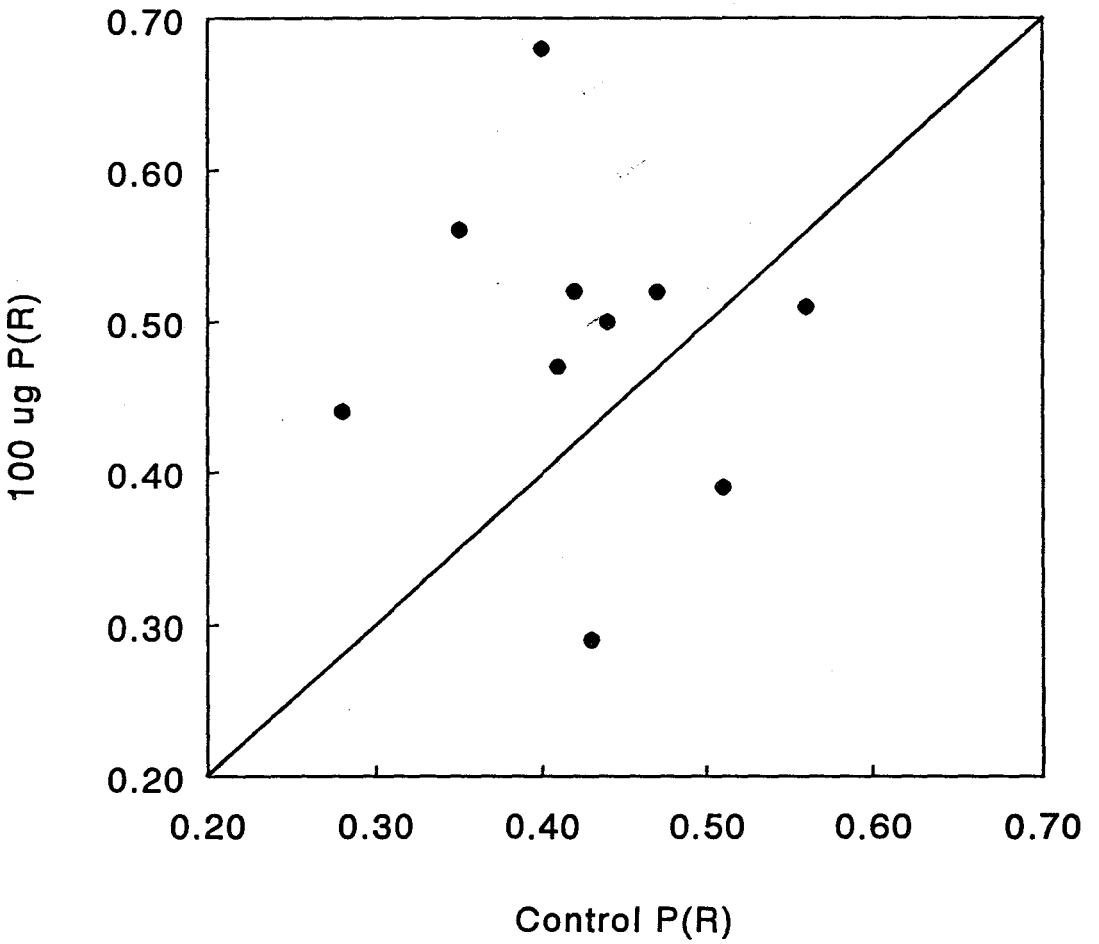
Postpubertal paw elevation. Postpubertal paw elevation was first analyzed by a two-way ANOVA of sex x treatment, which revealed a main effect of treatment, with treated animals being significantly more right-pawed than untreated animals, $F(1,8) = 6.085$, $p < .05$. A one-way ANOVA within the males revealed no significant difference in paw elevation between the treated males and the control males, $F(1,9) = 1.549$, $p > .10$. Figure 4 shows the distribution of male P(R)s; in seven of the ten litters, the treated male was more right-pawed than his control brother, a result that is not significant by the sign test ($p = .172$).

A one-way ANOVA within the females revealed no significant difference in paw elevation between the treated females and the control females, $F(1,9) = 2.004$, $p > .10$. Figure 5 shows the distribution of female P(R)s; in seven of the ten litters, the treated female was more right-pawed than her control sister, a result that is not significant by the sign test ($p = .172$).

A one-way ANOVA within the controls revealed no significant difference in paw elevation between the males and the females, $F(1,9) < 1$; because of nonhomogeneity of variance, the analysis was repeated using the Wilcoxon test, which failed to reveal a significant difference ($T = 16$, $n = 11$, $p > .10$). Figure 6 shows the distribution of


0.60
0.52
0.44
0.36
0.28
0.20
Male P(R)
0.20
0.28
0.36
0.44
0.52
0.60
Female P(R)


0.80
0.68
0.56
0.44
0.32
0.20
200 ug P(R)
0.20
0.32
0.44
0.56
0.68
0.80
Control P(R)


0.70
0.60
0.50
0.40
0.30
0.20
100 ug P(R)
0.20
0.30
0.40
0.50
0.60
0.70
Control P(R)

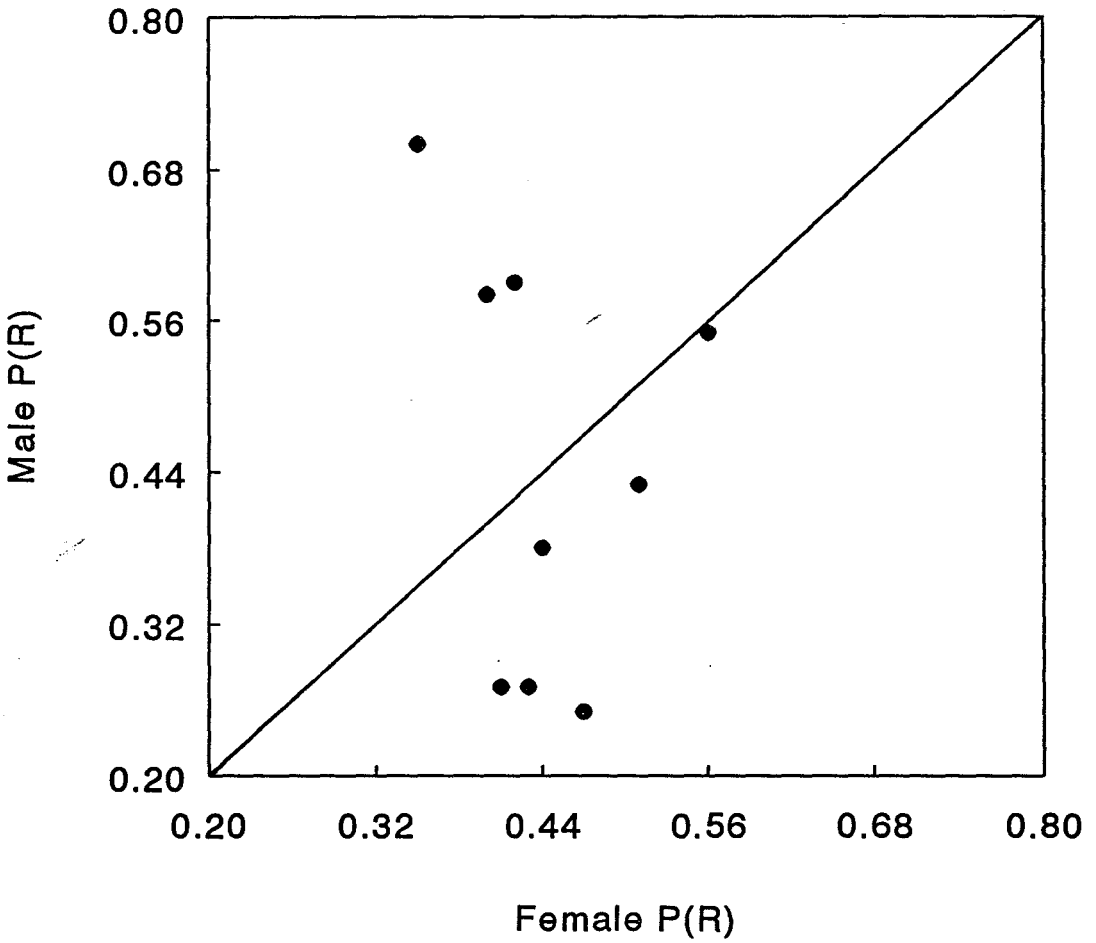
control P(R)s; in six of the nine litters, the female was more right-pawed than her brother, a result that is not significant by the sign test ($p = .254$).

Prepubertal paw elevation across days. The scores for the ten days on which prepubertal paw elevation was measured were parsed into two blocks of five days in order to assess the reliability of the measure. Pearson r correlations between blocks revealed low consistency in prepubertal paw elevation across days in all four groups. None of the correlations were significantly greater than zero, ranging from $r = .0659$, $n = 12$, $p > .10$ for the experimental males, to $r = -.4232$, $n = 11$, $p > .10$ for the control females.

Prepubertal paw elevation scores were then parsed into five blocks of two days and entered into one-way ANOVAs in order to assess patterns of results over blocks of days. A systematic pattern of results was observed only the control females, who become significantly more right-pawed over days, $F(4,40) = 3.159$, $p < .05$.

Postpubertal paw elevation across days. Postpubertal paw elevation scores were parsed into two blocks of five days in order to assess the reliability of the measure. Pearson r correlations between the two blocks of five days were significantly greater than zero in the control females ($r = .7502$, $n = 10$, $p < .05$), in the control males ($r = .8352$, $n = 10$, $p < .05$), and in the experimental males ($r = .7044$, $n = 11$, $p < .05$), but not in the experimental females ($r = .5176$, $n = 11$, $p > .10$).

Postpubertal paw elevation scores were then parsed into five blocks of two days and entered into one-way ANOVAs in order to assess patterns of results over blocks of days. A systematic pattern of results was once again observed for the control females, who became significantly more left-pawed over days, $F(4,36) = 5.889$, $p < .005$. A


0.80
0.68
0.56
0.44
0.32
0.20
Male P(R)
0.20
0.32
0.44
0.56
0.68
0.80
Female P(R)

pattern of marginal significance was observed for the control males, $F(4,36) = 2.289$, $p = .0782$, but it was not systematic.

Eye Opening

Eye opening scores were derived by assigning the values of 1.0, 0.5, and 0.0 to right first, simultaneous, and left first openings, respectively. Table 2 shows the means and standard errors of these scores for the four groups; a litter's data was included in the calculations only if it contained animals from all conditions.

L^2 tests performed on group data revealed no significant differences in eye opening between treated and control males (L^2 (2) = 0.234, $p > .10$), between treated and control females, (L^2 (2) = 0.099, $p > .10$), or between control males and control females (L^2 (2) = 1.478, $p > .10$).

Eye opening and paw elevation. Eye-opening scores were correlated with paw elevation scores. Although none of the Pearson r correlations were significantly greater than zero (Spearman rho correlations produced the same results), the direction of the correlation between eye opening and prepubertal paw elevation was negative for all four groups; correlations ranged from $r = -.0076$, $n = 10$, $p > .10$ for the female controls, to $r = -.3333$, $n = 11$, $p > .10$ for the female experimentals. The direction of the correlation between eye opening and postpubertal paw elevation was positive in the male and female controls, $r = .2673$, $n = 10$, $p > .10$, and $r = .1086$, $n = 10$, $p > .10$, respectively, but was negative in the male and female experimentals, $r = -.0208$, $n = 11$, $p > .10$, and $r = -.3682$, $n = 11$, $p > .10$, respectively.

Anogenital Distance, Body Weight and Anogenital Proportion

Table 3 shows the means and standard errors of anogenital distance, body weight and anogenital proportion for the four groups; a litter's data was included in the calculations only if it contained animals from all conditions.

Table 2. Proportion of Right Eye Openings.

		N	$\overline{X}$	SE_m
Male				
	Oil	11	0.5455	0.1056
	200 μg	11	0.5455	0.1253
Female				
	Oil	11	0.4091	0.1317
	100 μg	11	0.5000	0.1348

Table 3. Anogenital Distance, Body Weight, And Anogenital Proportion At Day 10 Divided By The Cubed Root Of Body Weight.

	AG Distance			Body Weight			$AG/\sqrt[3]{BW}$		
	N	$\overline{X}$	SE_m	N	$\overline{X}$	SE_m	N	$\overline{X}$	SE_m
Male									
Oil	9	3.8676	0.1137	9	8.8022	0.2699	9	0.1873	0.0044
200 μg	9	4.0036	0.1062	9	8.9578	0.3630	9	0.1930	0.0039
Female									
Oil	9	2.2378	0.0599	9	8.4356	0.3141	9	0.1101	0.0028
100 μg	9	2.4800	0.0886	9	8.1856	0.3736	9	0.1232	0.0037

The anogenital distances of the treated males were not significantly different from those of the untreated males, $F(1,9) = 1.768$, $p > .10$. The anogenital distances of the treated females were significantly larger than those of the untreated females, $F(1,8) = 15.476$, $p < .005$. The anogenital distances of the control males were significantly larger than those of the control females, $F(1,8) = 403.795$, $p < .001$.

The body weights of the treated males were not significantly different from those of the untreated males, $F(1,9) < 1$; because of nonhomogeneity of variance, these data were also analyzed by the Wilcoxon signed ranks test, which confirmed the result, $T = 19$, $n = 10$, $p > .10$. The body weights of the treated females were not significantly different from those of the untreated females, $F(1,8) < 1$; because of nonhomogeneity of variance, these data were analyzed by the Wilcoxon signed ranks test, which confirmed the result, $T = 22$, $n = 9$, $p > .10$. The body weights of the male controls were significantly greater than those of the female controls, $F(1,8) = 6.482$, $p < .05$.

Anogenital distance tended to be positively correlated with body weight. The correlation was significantly greater than zero in the male controls ($r = .7588$, $n = 10$, $p < .05$), was marginally greater than zero in the male experimentals ($r = .6291$, $n = 10$, $p < .10$) and in the female experimentals ($r = .6097$, $n = 9$, $p < .10$), and was not significantly greater than zero in the female controls ($r = .3652$, $n = 9$, $p > .10$).

Although testosterone treatment did not significantly influence body weight, the correlations between anogenital distance and body weight suggest that the measure of anogenital distance may partially reflect differences in body size, hence body weight. Accordingly, the one-way ANOVAs of anogenital distance were conducted again using body weight as a covariate; for these analyses, the variables were between rather than within litters. Analysis of covariance is a superior method of minimizing confounding effects of varying body size (Packard & Boardman, 1987). No results changed. The anogenital distances of the treated males were still not significantly different from those

of the untreated males, $F(1,17) < 1$, $p > .10$, but the anogenital distances of the treated females remained significantly larger than those of the untreated females, $F(1,15) = 8.074$, $p < .05$, and the anogenital distances of the control males remained significantly larger than those of the control females, $F(1,16) = 205.463$, $p < .001$.

Because body weight was not significantly affected by treatment, and because body weight and anogenital distance are to a large extent correlated, it would be appropriate to calculate anogenital distance per unit body weight. However, 1) millimeters/gram is a conceptually awkward unit of measurement and 2) Packard and Boardman (1987) stated that, when possible, body mass should be eliminated from the ratio by raising it to an allometric exponent suggested by the data. The linear measure of anogenital distance suggests that a linear measure of body mass should be used, and one can determine the allometric exponent of body mass by recognizing that the density of water is such that one gram of the substance occupies a volume of one cubic centimeter. Assuming that a gerbil is mostly water, its volume in cubic centimeters is approximately equal to its mass in grams. An approximate measure of body length in millimeters may be obtained by taking the cubed root of the mass (centimeters), and multiplying this number by ten. Thus, anogenital distance as a proportion of body length, or anogenital proportion, may be defined by the ratio of anogenital distance (millimeters) to the cubed root of body mass times ten; the allometric exponent for body mass is therefore 1/3.

It may be contended that anogenital proportion as a derived measure is conceptually flawed. Treated animals may be shorter and wider than their untreated counterparts; although treatment may have not affected body weight, it may have affected body length. However, the results of statistics performed upon anogenital proportion do not contradict the results of statistics performed upon the pure measures of anogenital distance and body weight, and of the analysis of covariance; because the former result converges with the latter results, a ratio is likely an appropriate measure to

use (Packard & Boardman, 1987). Anogenital proportion is useful in practice: It allows anogenital distance to be related to body size along a single dimension, making it in essence an index of correlation.

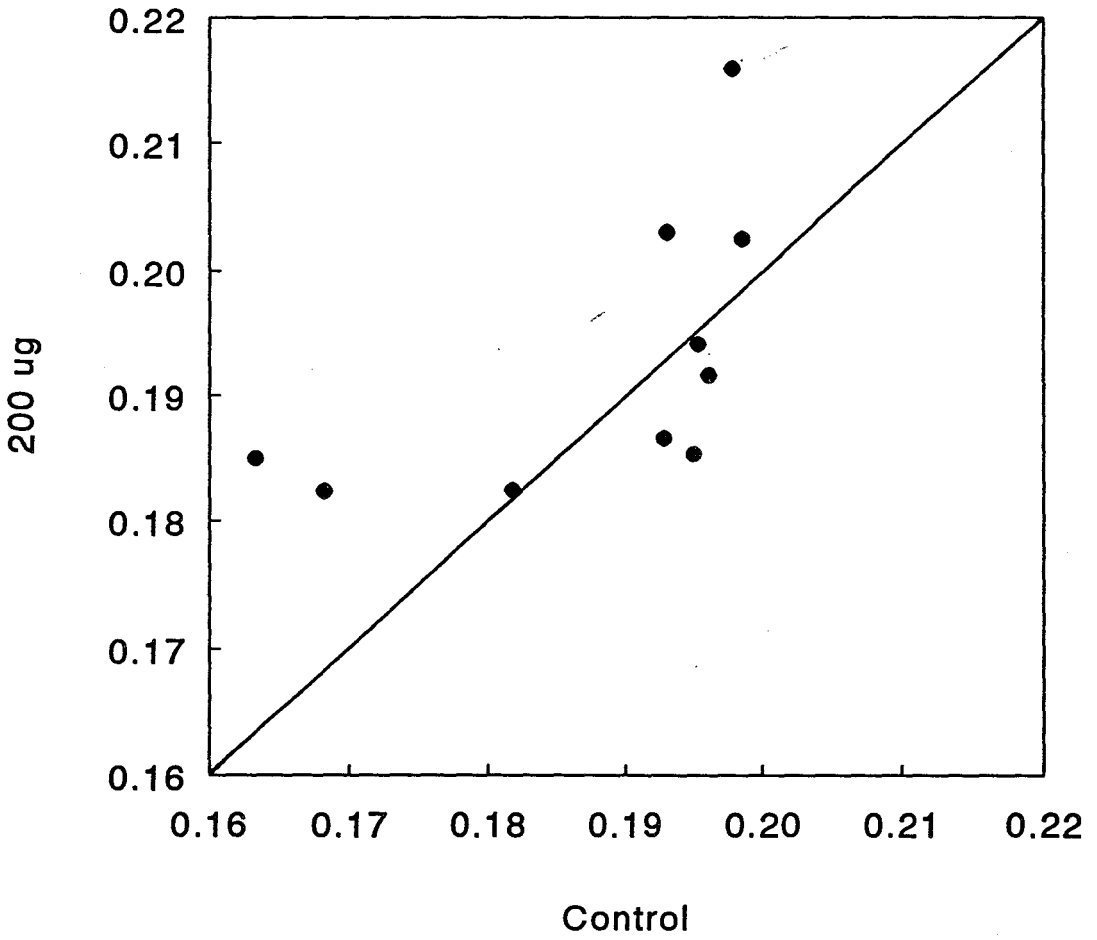
A two-way ANOVA of sex x treatment revealed a main effect of treatment, with treated animals having significantly larger anogenital proportions than untreated animals, $F(1,8) = 47.818$, $p < .001$. A separate one-way ANOVA within the males revealed no significant differences in anogenital proportion between treated and control animals, $F(1,9) = 1.889$, $p > .10$. Figure 7 shows the distribution of male anogenital proportions; in six of the ten litters, the treated male was larger than his control brother, a result that is not significant by the sign test ($p = .377$).

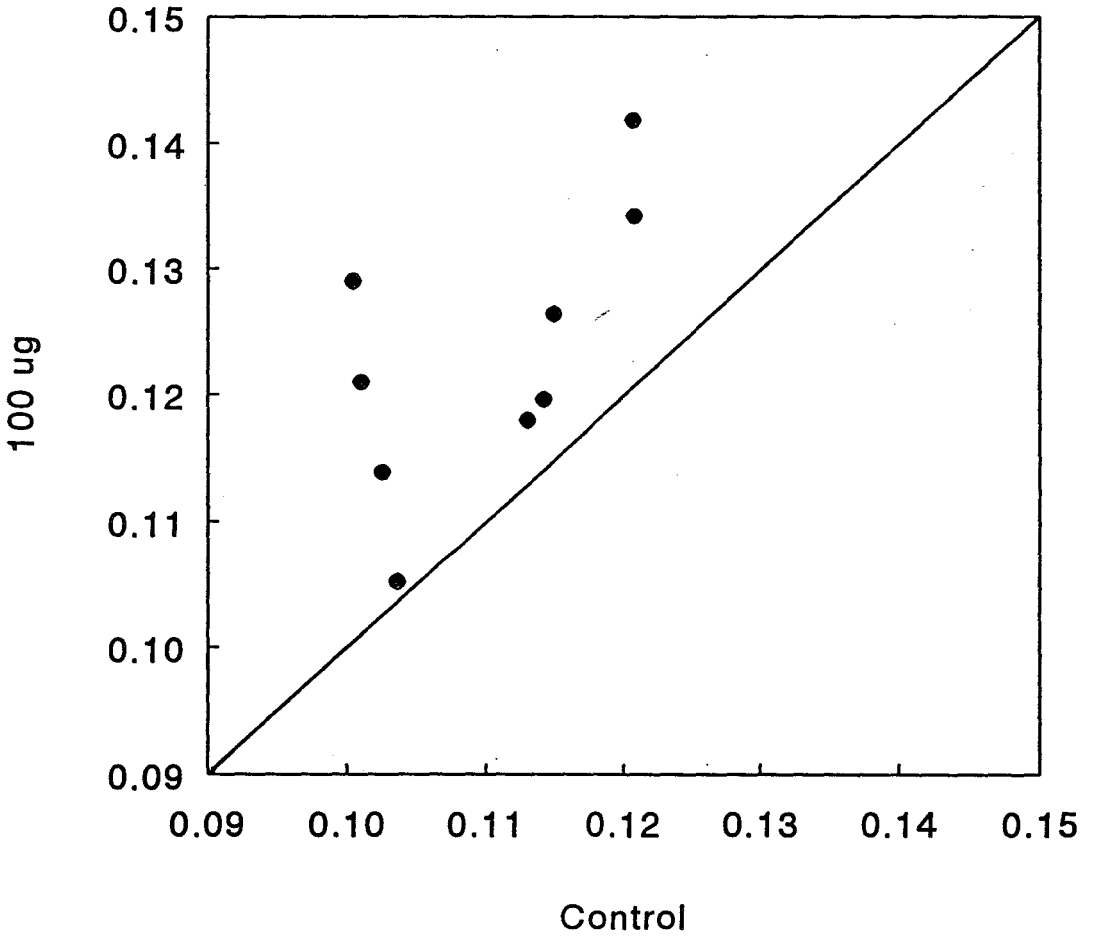
A separate one-way ANOVA within the females revealed that the anogenital proportions of the treated animals were significantly larger than those of the controls, $F(1,8) = 20.108$, $p < .01$. Figure 8 shows the distribution of female anogenital proportions; in all nine litters, the treated female was larger than her control sister, a result that is significant by the sign test ($p = .002$).

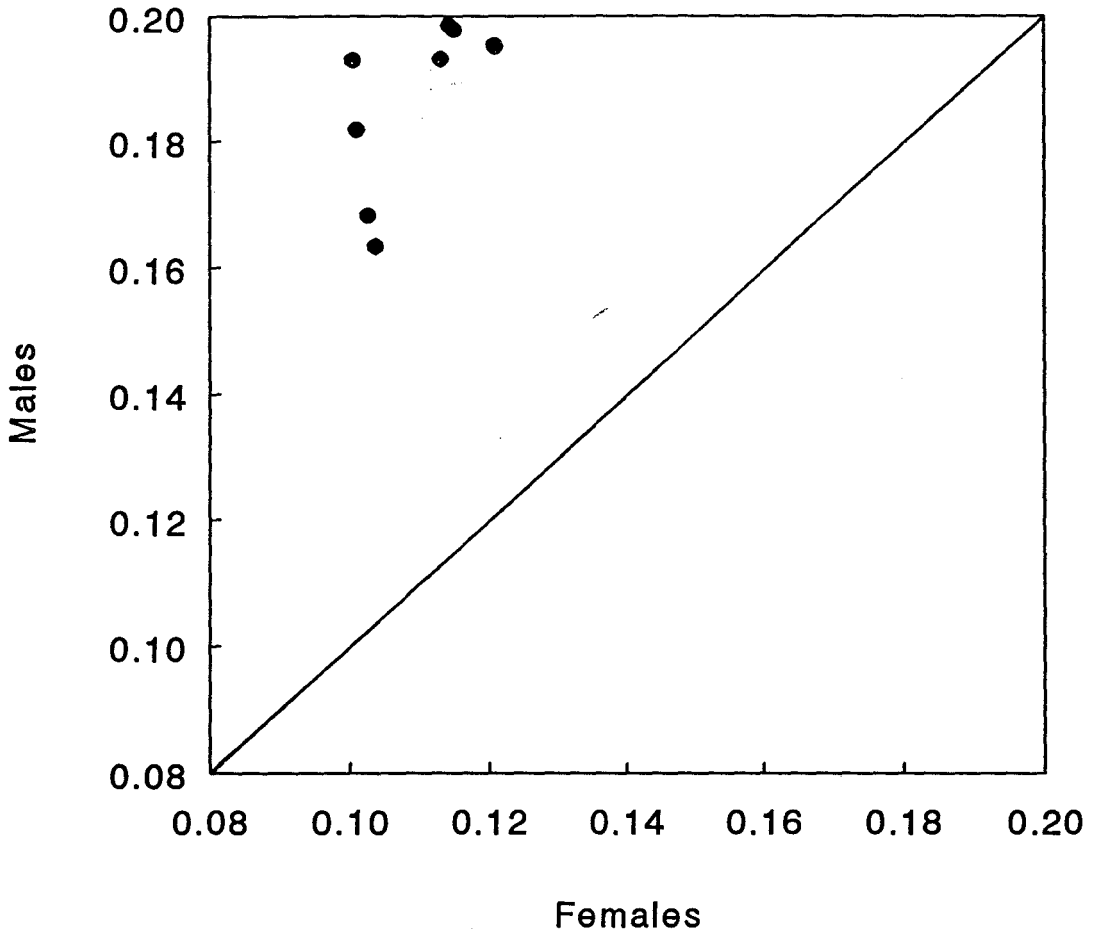
A separate one-way ANOVA within the controls revealed that males had significantly larger anogenital proportions than females, $F(1,8) = 543.486$, $p < .001$. Figure 9 shows the distribution of control anogenital proportions; in all eight of the litters, the male was larger than his control sister, a result that is significant by the sign test ($p = 0.004$).

Scent Marking

The scent marking score is the average number of marks per day for each animal. Table 4 shows the means and standard errors of scent marking scores for the four groups before and after puberty; a litter's data was included in the calculations only if it


0.22
0.21
0.20
0.19
0.18
0.17
0.16
200 ug
0.16
0.17
0.18
0.19
0.20
0.21
0.22
Control


0.15
0.14
0.13
0.12
0.11
0.10
0.09
100 ug
0.09
0.10
0.11
0.12
0.13
0.14
0.15
Control


0.20
0.18
0.16
0.14
0.12
0.10
0.08
Males
0.08
0.10
0.12
0.14
0.16
0.18
0.20
Females

contained animals from all conditions. Scores were extremely variable, both between sexes, and within a sex before and after puberty. Parametric statistics were attempted, but had to be abandoned because of nonhomogeneity of variance. The Wilcoxon signed ranks test, a non-parametric statistic, was chosen so as to allow the matching of littermates. The sign test was also chosen for this reason; in the cases where scores of the control animals equalled those of the treated animals (ties), the probabilities of the most extreme outcomes were averaged.

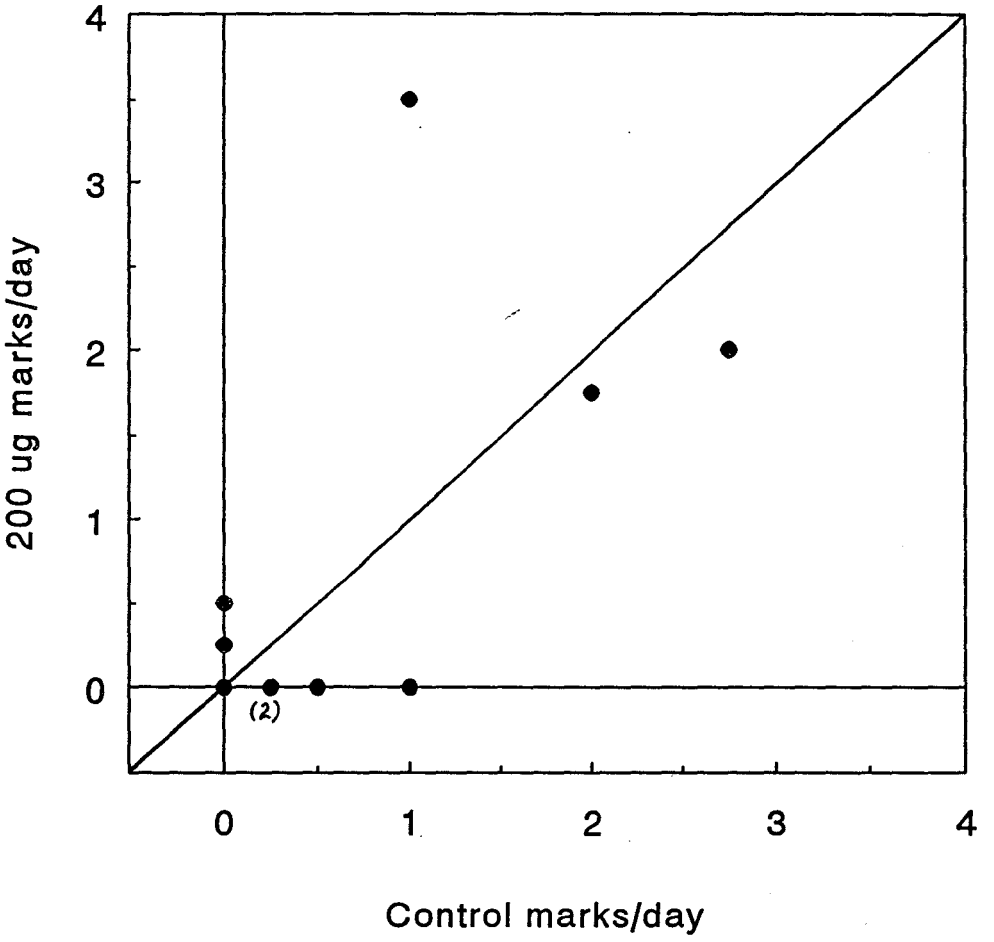
Prepubertal marking. The scores of the treated males were not significantly different from those of the untreated males ($T = 17$, $n = 10$, $p > .10$). Figure 10 shows the distribution of scores for the males: In six litters, the control marked more than the experimental, in three litters, the control marked less than the experimental, and in one litter, control and experimental marked with equal frequency. This result is not significant by the sign test ($p < .275$).

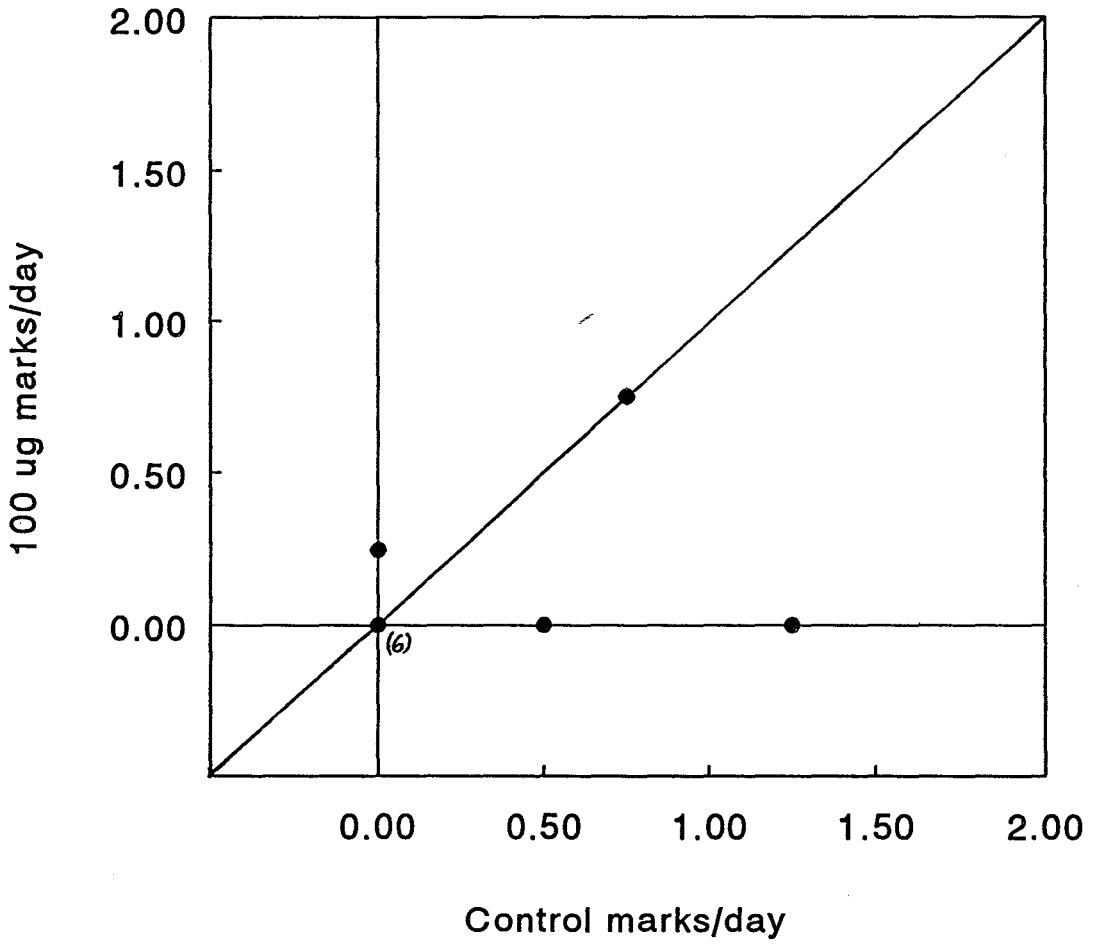
The scores of the treated females were not significantly different from those of the untreated females ($T = 9$, $n = 11$, $p > .10$). Figure 11 shows the distribution of scores for the females: In two litters, the control marked more than the experimental, in one litter, the control marked less than the experimental, and in seven litters, control and experimental marked with equal frequency. This result is not significant by the sign test ($p < .500$).

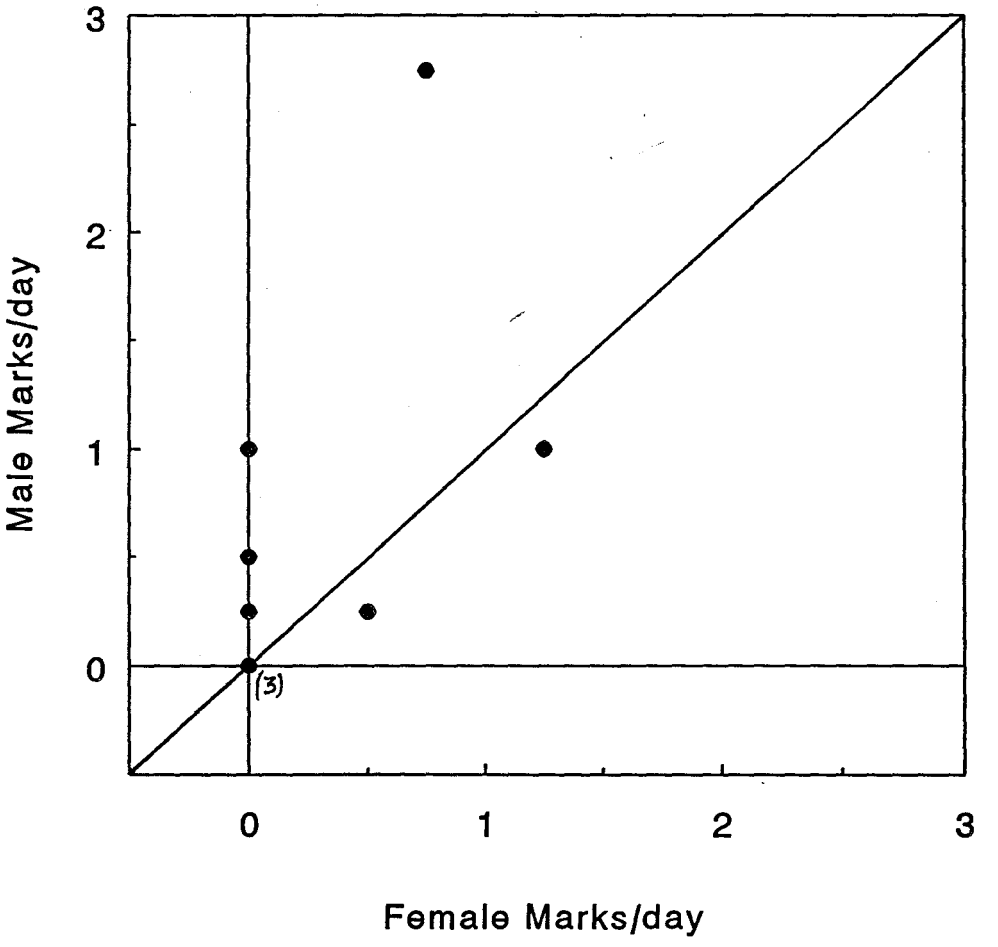
The male controls marked significantly more than the female controls ($T = 0$, $n = 10$, $p < .05$). Figure 12 shows the distribution of scores for the controls: In four litters, the male marked more than the female, in two litters, the male marked less than the female, and in three litters, male and female marked with equal frequency. This result is not significant by the sign test ($p < .418$).

Table 4. Average Number of Marks Per Day Before and After Puberty.

	Before			After		
	N	$\bar{X}$	SE_m	N	$\bar{X}$	SE_m
Male						
Oil	9	0.6389	0.2950	9	5.5278	1.6664
200 μg	9	0.6944	0.4120	9	4.9444	2.5688
Female						
Oil	9	0.2778	0.1528	9	0.5278	0.2682
100 μg	9	0.1111	0.0845	9	0.3889	0.2129


4
3
2
1
0
200 ug marks/day
(2)
0
1
2
3
4
Control marks/day


2.00
1.50
1.00
0.50
0.00
100 ug marks/day
(6)
0.00
0.50
1.00
1.50
2.00
Control marks/day


3
2
1
0
Male Marks/day
(3)
0
1
2
3
Female Marks/day

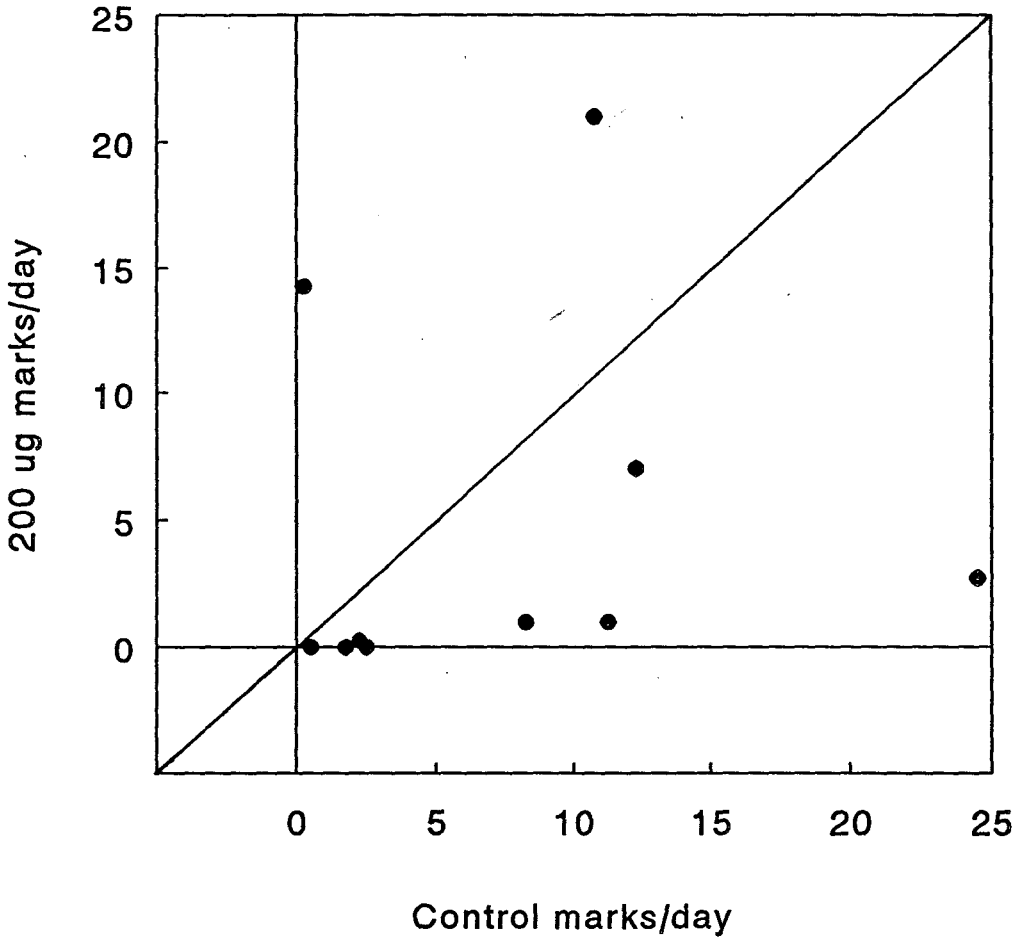
Postpubertal marking. The scores of the treated males were not significantly different from those of the control males ($T = 16.5$, $n = 10$, $p > .10$). Figure 13 shows the distribution of scores for the males: In eight litters, the control marked more than the experimental, and in two litters, the control marked less than the experimental. This result is marginally significant by the sign test ($p = .055$).

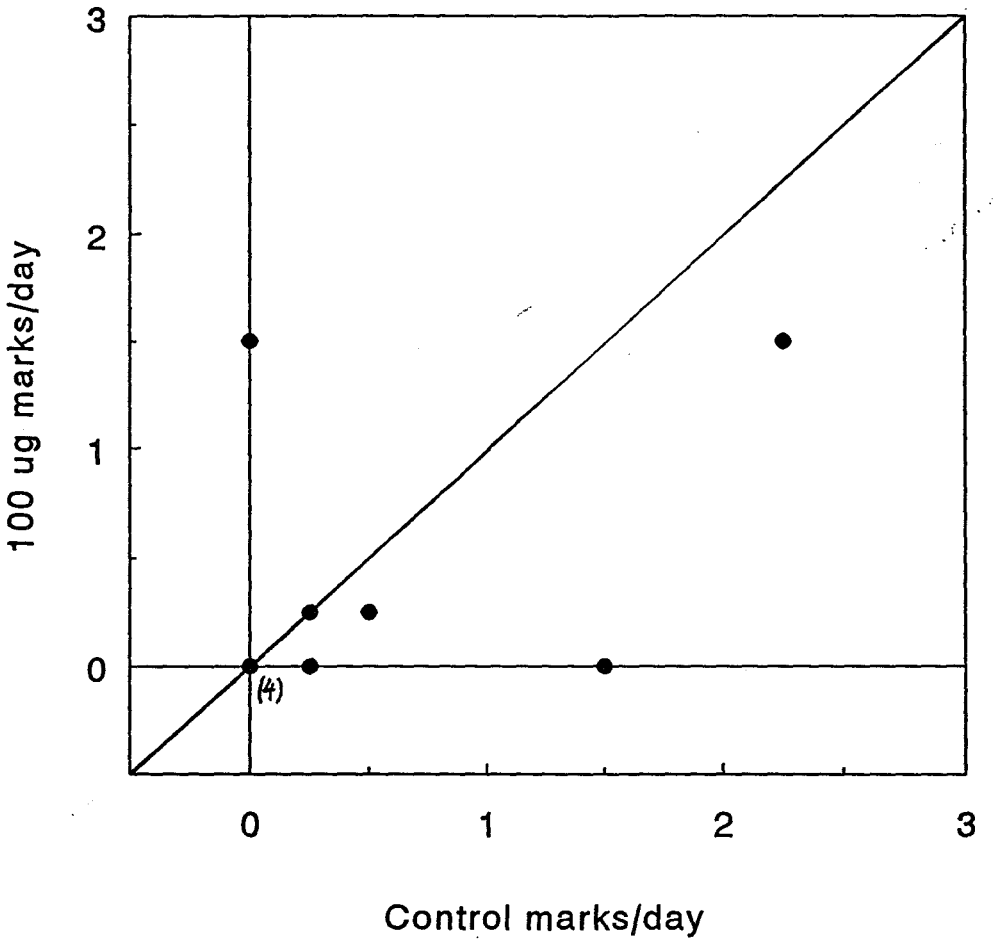
The scores of the treated females were not significantly different from those of the control females ($T = 4.5$, $n = 10$, $p > .10$). Figure 14 shows the distribution of scores for the females: In four litters, the control marked more than the experimental, in one litter, the control marked less than the experimental, and in five litters, the control and experimental marked with equal frequency. This result is not significant by the sign test ($p < .420$).

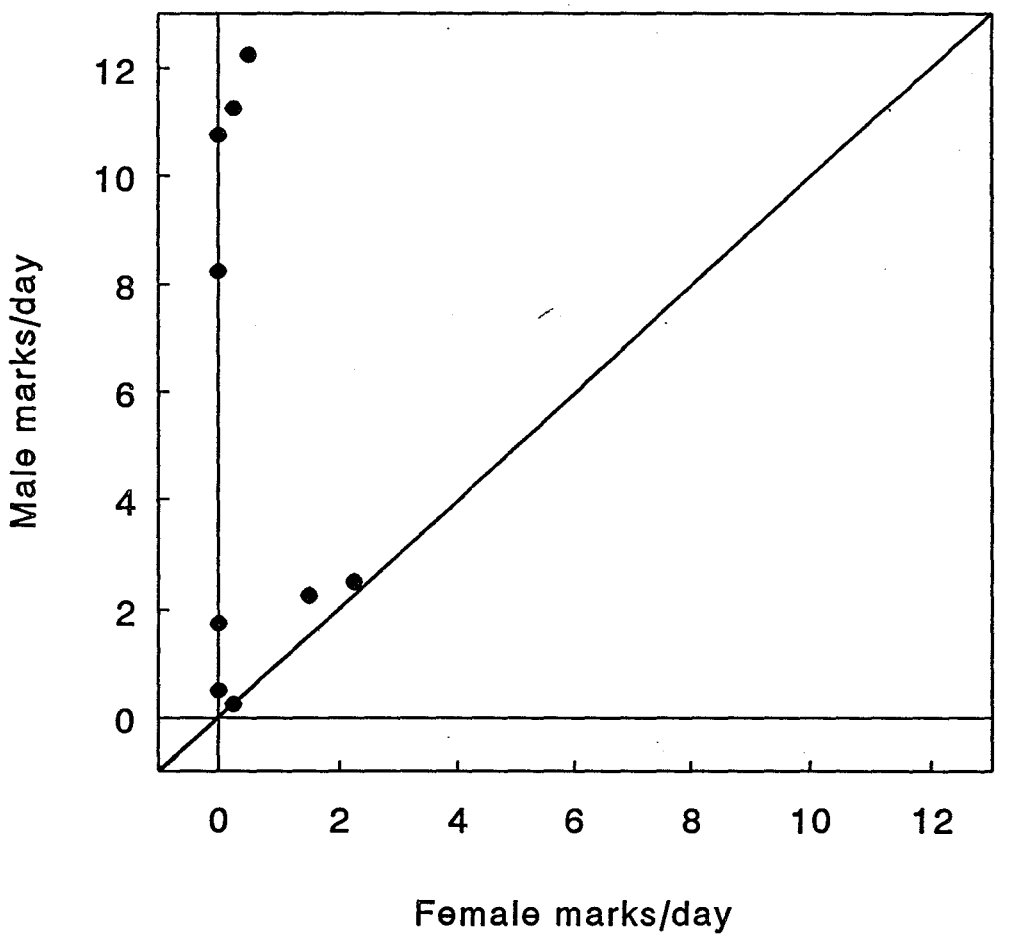
The male controls marked significantly more than the female controls ($T = 0$, $n = 9$, $p < .05$). Figure 15 shows the distribution of scores for the controls: In eight litters, the male marked more than the female, and in one litter, the male and female marked with equal frequency. This result is significant by the sign test ($p < .011$).

To summarize, treated animals in general were more right-pawed before puberty than untreated animals. The treated males were more right-pawed before puberty than the control males, and the treated females were marginally more right-pawed at this time than the control females. Control females did not differ from control males in the direction of prepubertal paw elevation. Control females became more right-pawed over blocks of prepubertal days. Animals in all four conditions showed low consistency in paw elevation across the prepubertal testing period.

Treated animals in general were more right-pawed after puberty than untreated animals. However, treated males did not differ from control males, treated females did not differ from control females, and control males did not differ from control females in


25
20
15
10
5
0
200 ug marks/day
0
5
10
15
20
25
Control marks/day


3
2
1
0
100 ug marks/day
(4)
0
1
2
3
Control marks/day


Male marks/day
Female marks/day
0
2
4
6
8
10
12

the direction of postpubertal paw elevation. Control females became more left-pawed over blocks of prepubertal days. Treated and control males, and control females were consistent in their paw elevation across days; experimental females were not, but their consistency was greater than it was before puberty.

Comparisons between treated and control males, treated and control females, and control males and females did not reveal any significant differences in the direction of eye opening.

Eye opening was negatively correlated with prepubertal paw elevation in all four conditions. After puberty, eye opening was positively correlated with paw elevation in the male and female controls, but remained negatively correlated in the male and female experimentals. None of the correlations was significantly greater than zero.

Treated animals in general had larger anogenital proportions than untreated animals. The anogenital proportions of treated and control males did not differ from each other. Treated females had larger anogenital proportions than control females. Control males had larger anogenital proportions than control females.

The prepubertal scent marking scores of treated animals did not differ from the scores of their same-sex littermates. Control males marked more than control females at this time.

Treated males marked marginally less after puberty than control males. The postpubertal scores of the treated females did not differ from the scores of the untreated females. Control males marked more after puberty than control females.

General Discussion

In accordance with the Geschwind-Galaburda theory's predictions, testosterone treatment affected the direction of gerbil paw elevation. Contrary to the theory's predictions, this direction was not leftward, but rightward.

No sex difference in the paw elevation of the control animals was observed, either before puberty or after. The observation is consistent with Clark et al. (1993), whereby gerbils of both sexes that were situated between two males in utero (2M) were reported to elevate their left paws in the tripedal stance, and gerbils of both sexes that were situated between two females in utero (2F) were reported to elevate their right paws in the tripedal stance. 2Ms of both sexes would have been exposed to testosterone contributed to the intrauterine pool by adjacent brothers in addition to the testosterone that they and 2Fs would have gleaned from other sources. More 2Ms than 2Fs might therefore be expected to be left-pawed, and, in the absence of information about uterine position, roughly as many females should display left paw elevation as males.

The lack of sex difference in the direction of paw elevation appears at first blush to contradict the Geschwind-Galaburda prediction that more males than females will display left appendage use. However, the lack of sex difference is explicable by an inter-species difference. Unlike gerbils, humans are not litter-bearers; babies generally develop as singletons. The brains of human females are therefore not exposed to intrauterine testosterone contributed by adjacent male fetuses, and human females should as a group display less left hand use than human males, whose brains are exposed to higher levels of intrauterine testosterone by virtue of production from their own testes. The exception to this rule is twins, the human equivalent of the litter. If the group to be studied is limited to opposite sex twin pairs (Koch, 1966, in Miller, 1994), the sex difference in left hand use is reduced and becomes aligned with the findings of Clark et al. (1993).

No sex difference was observed in the direction of paw elevation of the treated animals, either before puberty or after. The observation is consistent with Clark et al. (1993) in the sense that testosterone treatment essentially converts gerbils of both sexes into 2Ms, which should display the same direction of paw elevation regardless of sex, but is inconsistent with Clark et al. (1993) and the Geschwind-Galaburda prediction that the direction of paw elevation for both sexes will be leftward.

The Grand Scheme

Paw elevation vs. writing hand use. Asymmetries in human appendage use are task-specific; each human arm task (e.g. unscrewing a jar, dealing cards, writing, etc.) provides a different distribution of asymmetries across the population studied (Annett, 1970). Because the Geschwind-Galaburda theory focuses almost exclusively upon the writing task, the present experiment sought to relate asymmetries in gerbil paw elevation to asymmetries in writing hand use. The relation must, however, be interpreted with caution, for there are several important differences between the two:

1. Although individual gerbils display clear asymmetries in paw elevation, left and right paw elevation tendencies are equally represented in the population (Clark et al., 1993). This stands in contrast to the 90:10 right-left population level split in writing hand use (Geschwind & Galaburda, 1987).
2. Asymmetries in non-human appendage use vary more across tasks than do asymmetries in human appendage use (Hellige, 1993). The mechanism that produces the observed asymmetries in paw elevation may not be as strongly lateralized as the mechanism that produces the observed asymmetries in writing hand use.
3. Paws are (structurally) homologous to hands, but from an evolutionary perspective it is doubtful that the two are (functionally) analogous (Hellige, 1993). The fine motor task of writing may have no parallel in gerbils.

4. Across species, mammalian sexual differentiation is inherently variable (vom Saal et al., 1992). Inasmuch as both paw and hand use asymmetries are determined during sexual differentiation, information about the ontogeny of paw elevation may be a poor guide for inferences about the ontogeny of writing hand use.

Paw elevation in the tripedal stance was chosen as a measure of appendage use because it was seemingly the only forearm task that gerbils would perform. Prior attempts to train the animals to reach for food (which, by comparison, is a "skilled" task) failed, and the even more skilled task of seed-husking revealed no asymmetries in the paw used for husking. Paw elevation has been reported in other species. In the field mouse, paw elevation has been interpreted as a defensive posture that is often temporally associated with batting the face of one's opponent with the elevated paw (Bovet, 1972). Quadrupedal primates elevate their left forearms to perform visually-guided reaching tasks while supporting themselves with their right forearms, but bipedal primates, including humans, tend to use their right forearms for manual movements (Bradshaw & Rogers, 1993).

One hypothesis for the change in the use of the primate forearm is that the evolution of bipedality brought with it a decreased reliance on unimanual predation, and an increased specialization of the right forearm for fine motor movements (MacNeilage, Studdert-Kennedy & Lindblom, 1987). As such, the right hemisphere lost its specialization for left forearm use, and the left hemisphere gained specialization for right forearm use (MacNeilage et al., 1988). Gerbils, which are evolutionarily older than primates, would be more likely to share the adaptations of the quadrupeds than the adaptations of the bipeds. Gerbils would thus be more likely to exhibit right hemispheric dominance for visually guided reaching tasks such as batting one's opponent (gerbils exhibit the tripedal stance more frequently when they are startled), and hence for the elevation of the left paw. From an evolutionary point of view, therefore, comparing

human writing to gerbil paw elevation is like comparing apples to oranges: When the tasks are analyzed, hands and paws do not appear to be analogous.

If the Geschwind-Galaburda theory is relaxed to account for species differences in hemispheric dominance, it predicts that testosterone treatment would render the brain more symmetrical by decreasing the size of the (larger) dominant hemisphere, with a subsequent increase in the size of the (smaller) non-dominant hemisphere. In gerbils, therefore, testosterone should cause all or part of the right hemisphere to decrease in size and all or part of the left hemisphere to increase in size. Because dominance for paw elevation would be just as likely to reside in one hemisphere as the other, treated animals should exhibit more right paw elevations than the controls. The right-dominance theory, however, is not borne out in the controls, where at the population level there is a 50:50 left-right split in paw elevation asymmetry; if the right hemisphere were indeed dominant for paw elevation, the split would be biased towards the left. Perhaps the gerbil brain is essentially symmetrical, with a slight bias towards a larger right hemisphere. Such symmetry and right bias has been reported at a population level in rats (Diamond, 1984). Testosterone treatment may confer further symmetry to the brain by decreasing the size of part or all of the right hemisphere, and may actually increase asymmetry by decreasing the size of part or all of the right hemisphere with the subsequent increase in the size of part or all of the left. Either scenario would result in a population with more right bias in paw elevation than a control population.

There are accounts in the literature of the effects of testosterone on hemispheric size. Stewart and Kolb (1988) provided physiological evidence that the offspring of rats stressed during pregnancy had more structurally-symmetrical brains than did the offspring of control mothers, something that may be attributable to high prenatal testosterone levels (Ward, 1992). Nosten, Roubertoux, Degrelle and Leboyer (1989)

concluded that, in mice, testosterone reduces the degree of pawedness by reducing hemispheric asymmetry.

Eye opening. Neither a sex nor a treatment difference in eye opening was observed. Eye opening may be viewed as an index of the rate of hemispheric development; in rabbits, the optic nerve of the first eye to open is in a more advanced state of myelination than the optic nerve of the second eye to open (Narang, 1977). The problem arises as to which hemisphere is larger by virtue of neuron number and/or more advanced in development: In humans, for example, roughly the same number of fibers in each optic nerve project to the left hemisphere as to the right (e.g. Kolb & Wishaw, 1990). If one assumes that the first eye to open is, like paw use, contralateral to the faster developing, larger cerebral hemisphere, the Geschwind-Galaburda theory as extended to gerbils would predict that male controls and treated animals would display more right eye openings than female controls and untreated animals, respectively, by virtue of the increased size of part of or all of the left hemisphere. The lack of asymmetry in eye opening between male and female controls is explicable by the above argument that, on average, equal numbers of gerbils of both sexes are likely to be 2Ms and 2Fs. However, the lack of asymmetry in eye opening between treated and untreated animals contradicts the Geschwind-Galaburda theory as extended to gerbils.

An insignificant positive correlation was observed between the first eye to open and adult paw elevation for the control animals of both sexes, replicating the direction of correlation reported by Clark et al. (1993), and an insignificant negative correlation was observed between the first eye to open and adult paw elevation for the experimental animals of both sexes. One explanation for the directions of the correlations is that the brains of the controls were more asymmetrical (hence paw elevation and eye opening were observed on the same side of the body) than the brains of the experimental animals (hence paw elevation and eye opening were observed on opposite sides of the body).

Again, this logic assumes that paw elevation and eye opening are governed chiefly by one and the same hemisphere. A second explanation is that weak correlations between paw elevation and eye opening will inevitably occur. The finding that the correlations are not significant suggests that the latter explanation is more likely to be correct.

Appendage use: The direction-degree dichotomy. Genetic theorists (e.g. Bryden, 1987; Bryden & Steenhuis, 1991) distinguished between the direction and the degree of hand use. In the present experiment, only the direction of appendage use has been considered. However, there is some evidence that gerbil paw elevation also varies in degree. The control females exhibited a systematic change in the magnitude of prepubertal paw elevation, becoming more right-pawed over the 10-day testing period; such a change in magnitude of direction may be considered to be a change in degree. The control females also showed a systematic change in the degree of postpubertal paw elevation, becoming more left-pawed over the 10-day testing period. The degree of paw elevation was more consistent in adulthood than in it was infancy; animals in all four groups showed far greater correlations between the two blocks of postpubertal scores than between the two blocks of prepubertal scores.

Collins et al. (1991) argued that in mice, direction at the population level is a function of degree. According to those authors, there are four kinds of mice: Strongly left-pawed, strongly right-pawed, weakly left-pawed, and weakly right-pawed. In a testing environment that pressures the animals to reach for food with their right paws, the weakly left-pawed mice will do so, but the strongly left-pawed mice will not (Collins, 1975). The result would be a 75:25 right-left population level split in paw use. Because in the mouse population, like the gerbil population, left and right paw use are ordinarily equally represented, any shift of direction of paw use at the population level must be the result of the interaction of a biased testing environment with a low degree of

paw use. Collins et al. (1991) concluded that a right-biased world is responsible for the extreme proportion of right hand use seen in the human population.

However, the latter statement begs the question of how the human world became right-biased in the first place. Like Collins (1975), the present experiment reports a population level shift in directional paw use, but this shift occurred in the absence of environmental bias: Paw elevations were recorded only if the gerbil was in the center of the aquarium, away from the potentially biasing walls. Therefore, the interaction of the degree of paw use with features in the outside world is not necessarily the underlying cause of a population-level shift in direction.

The finding that degree is heritable but direction is not (Collins, 1969, 1985; Collins et al., 1991), and the argument that degree is likely not the underlying cause of direction suggest that the two aspects of appendage use are mediated by different mechanisms. A useful framework within which degree and direction may be interpreted is the **organizational-activational hypothesis** (e.g. Beatty, 1992). According to the organizational-activational hypothesis, sex hormones exert two distinct actions upon the central nervous system. The organizational effects of sex hormones are to irreversibly interact with and guide brain development during a brief perinatal "critical" period, and the activational effects of sex hormones are to reversibly interact with and guide mature brain function during the postpubertal period (Beatty, 1992). I propose that the degree of paw elevation is largely an activational effect of sex hormones, and that the direction of paw elevation is largely an organizational effect of sex hormones.

Blood plasma is the chief vehicle for the transportation of potentially activating hormones. In the present experiment, changes in plasma hormone levels at puberty were correlated with changes in the degree of paw elevation. The plasma hormone levels of adult female gerbils fluctuate by virtue of the five-day estrus cycle (Barfield & Beeman, 1968; Gerall & Givon, 1992), while the hormone levels of adult males change only

gradually across the lifespan. In the present experiment, the control females would have experienced at least one estrus cycle over the 10-day postpubertal testing period, but the androgenized females would have not experienced normal estrus (e.g., Dohler, 1987; Gorski, 1967; Gerall & Givon, 1992). The control females were the only group to show a systematic change in the degree of postpubertal paw elevation, which suggests that estrus hormones were exerting activational effects on the brain. For the systematic change in degree of prepubertal paw elevation to be explained by activational effects, the control females would have had to have been sexually mature at the time of prepubertal testing. Vaginal opening is one index of sexual maturity (e.g. Wilson, 1992), and informal observations confirmed that, in most litters, the control females were the first to show it, and did so during that time.

Changes in plasma hormone levels at puberty were correlated with changes in the consistency of the degree of paw elevation; gerbils in all conditions displayed more consistency in the degree of paw elevation as adults than they did as juveniles. A change in degree of paw elevation bracketing puberty is in accordance with the human literature, wherein the motor asymmetries of children are reported to be far less stable than those of adults (Hellige, 1993). Humans also show a shift towards consistent handedness as a function of age, regardless of the initial direction (Bryden, 1987; Porac, 1993). Hormonal changes at puberty may stabilize degree, or at least, direct more consistent changes in degree compared to the seemingly random fluctuations of childhood.

In the present experiment, changes in plasma hormone levels at puberty were not correlated with changes in the direction of paw elevation; the direction of paw elevation in each condition remained the same whether the animals were tested as juveniles or as adults. Specifically, direction could not have been an activating effect of testosterone: After puberty, scent marking frequency was significantly increased in both the male controls and the male experimentals, but the direction of paw elevation remained the

same as it did before puberty. Because the magnitude of scent marking behavior reflects the magnitude of plasma testosterone levels, it can be stated that changes in plasma testosterone levels were not accompanied by changes in direction.

Why might degree of paw use be heritable but not direction? Both direction and degree of paw use may be at least partially determined by the environment, which, in this case, is the hormonal milieu of the brain. The low heritability of direction may be explicable by the tendency for brain-organizing hormones to come from extra-genetic sources (the mother or adjacent fetuses) during the critical period of brain development, and from the inability of animals of all genotypes to cope immediately with an extra-genetic hormonal assault at that time. The high heritability of degree may be explicable by the tendency for brain-activating hormone to come from intra-genetic sources (one's own testes or ovaries) during the postpubertal period. Heritability of degree may also be explicable by the fact that animals of some genotypes may be quicker to restore homeostasis following a hormonal assault in the critical period; because the adult brain's metabolism of and sensitivity to hormones appears to be partially determined by hormone metabolism in the differentiating brain (vom Saal et al., 1992), the phenotype would persist into the postpubertal period.

Hormonal mediation of direction. The path of metabolization of the injected testosterone may be inferred from the measures of anogenital proportion and scent-marking. During the critical period of brain differentiation, testosterone may act in its pure form, or through its parallel conversion to estradiol and DHT (Yahr, 1988; vom Saal et al., 1992). Dihydrotestosterone mediates anogenital distance (Breedlove, 1993); hence, the increase in the anogenital proportions of treated females with respect to control females suggests that the injected testosterone was largely converted to DHT and estradiol. Because the injection (and therefore conversion) occurred during the critical period, pure testosterone was likely not the chief brain-organizing hormone.

Dihydrotestosterone does not appear to affect central nervous system development (Yahr, 1981). Hence, by the process of elimination, estradiol is the most likely candidate for the chief brain-organizing hormone in female gerbils.

The lack of increase in the anogenital proportions of treated males with respect to untreated males suggests that either testosterone was not metabolized to DHT and estradiol, or that the effect of DHT on anogenital distance was at a ceiling. The former explanation may not be correct: Males exhibited the same direction of paw elevation as females; direction is hypothesized to be an organizing effect of hormone; the hormone that organizes female brains was concluded to be estradiol. Thus, the brains of males may also be organized by estradiol, but because in the current experiment, the effects of the DHT that was formed in conjunction with estradiol may have been at a ceiling, the anogenital proportions of treated males were not significantly increased.

The adult scent marking scores of the treated males were marginally lower than those of the untreated males. During the critical period, the brains of males are normally defeminized, which prevents the emergence of behaviors typical of adult females, and masculinized, which promotes the emergence of behaviors typical of adult males (Breedlove, 1993). Low levels of testosterone in castrated males (Baum, 1993) and high levels of estradiol in intact males (Hendricks, 1992) impede the masculinization process. The experimental males did not show female-typical behaviors, i.e., they were defeminized, but they showed a reduction in scent-marking, which at higher rates is a male-typical behavior (Thiessen, Blum & Lindzey, 1969); thus, they were not fully masculinized. Testosterone levels during the critical period were likely not abnormally low because of the surplus provided by the injection; hence, the incomplete masculinization of the treated males may be attributable to abnormally high estradiol levels during this time.

There is also evidence in the literature that estradiol may be the chief organizing hormone responsible for right paw elevation. In mice, the 2M fetus has higher levels of circulating testosterone than the 2F fetus, and 2F fetus has higher levels of circulating estradiol than the 2M fetus (vom Saal et al., 1992). 2M gerbils elevate their left paws, and 2F gerbils elevate their right paws (Clark et al., 1993). Inasmuch as gerbil ontogeny is similar to mouse ontogeny, prenatal exposure of the brain to high levels of estradiol is correlated with right paw elevation. Furthermore, only estradiol receptors are present in the mouse brain before birth; testosterone receptors do not appear until after birth (vom Saal et al., 1992). If paw elevation were an organizing effect of testosterone but not of estradiol, then Clark et al.'s (1993) gerbils should have not have differed in paw elevation with respect to their uterine positions.

Future Research. Future research could better elucidate the hormone responsible for paw elevation via the injection of a testosterone antagonist, estradiol, and an estradiol antagonist during the postnatal critical period. Equivalent dosages should be administered to males and to females so as to eliminate the confounding effects of dosage and sex seen in the present experiment. Prenatal experiments in which the pregnant dam is injected with the above substances could be undertaken. Because of the hypothesized connection between hormone treatment and hemispheric symmetry, the brains of treated animals could be examined for thickness, volume, cytoarchitecture, etc.

Plasma hormone levels could be manipulated both in the adult and in the juvenile. The manipulations would verify that direction and degree of paw elevation are indeed dissociable and that degree is an activating, not an organizing, effect of sex hormone.

Because gerbils appear to be limited to paw elevation as a measure of appendage use, other rodent species that are compatible with more sensitive measures may prove to be a better model of human handedness.

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