# Aggression in Adult Mice: Modification by Neonatal Injections of Gonadal Hormones

Abstract. *Incidence of spontaneous aggression in adult male mice given a single injection of estradiol benzoate (0.4 milligram) when they were 3 days old was less than that of controls injected with oil. Aggressiveness was increased among adult females injected with either estradiol or testosterone propionate (1 milligram) at the same age. The increased aggressiveness noted among females given androgen was further documented during subsequent mating tests, when these females often attacked, wounded, and, in one case, killed naive males.*

The sexual differentiation of particular behavioral or neuroendocrine control systems may be influenced by the presence of gonadal hormones during infancy in rodents (*1*). For example, neonatal administration of androgens to females results in an acyclic, male-like secretion of gonadotropin during adulthood rather than in the cyclic pattern characteristic of normal adult females (*2*). Similarly, sexual behavior of female rats may be masculinized to a degree if they are given neonatal injections of androgen, or that of males may be feminized if they are castrated during infancy, provided that appropriate gonadal hormones are administered during adulthood (*1*, *3*). Estrogens, depending upon the time and dose of their injection, may mimic some of these effects of androgens (*4*). We hypothesized that aggressive behavior could also be modified following treatment with androgens or estrogens during infancy. Our results demonstrate that aggressiveness was increased in adult female mice if they were given either androgen or estrogen as neonates; aggressiveness in adult males was partially suppressed if they were injected with estrogen during infancy.

Table 1. Number of pairs (of same sex) in which fighting occurred at least once during three encounters and total number of fights occurring during all three encounters.

| Neonatal treatment | Fighting at least once (No.) | Fights in three encounters (No.) |
|---|---|---|
| | *Males* | |
| Oil | 23/24 | 51/72 |
| Testosterone | 18/19 | 46/57 |
| Estradiol | 10/20 | 20/60 |
| | *Females* | |
| Oil | 1/24 | 1/72 |
| Testosterone | 5/18 | 10/54 |
| Estradiol | 4/14 | 5/42 |

Table 2. Number of male-female pairs in which severe fighting occurred within the first hour after pairing and number in which wounding of one member occurred within 18 hours. Females had been previously tested in the primary experiment (Table 1), after which they were given progesterone daily for 8 days and then paired with naive males.

| Neonatal treatment of females | Fighting (1st hour) | Wounding (18 hours) |
|---|---|---|
| Oil | 0/20 | 0/20 |
| Testosterone | 12/23 | 5/23* |
| Estradiol | 4/14 | 0/14 |

* One pair in which female was wounded, three pairs in which male was wounded, and one pair in which male was killed.

Complete litters of 3-day-old C57BL/6J mice of both sexes were injected subcutaneously with 0.05 ml of corn oil containing either 1 mg of testosterone propionate, 0.4 mg of estradiol benzoate, or nothing. Mice were weaned at 21 to 25 days of age and housed singly until tested for aggressiveness at 80 to 90 days of age. Spontaneous aggression (*5*) was measured in test chambers (12 by 12 by 6 inches) with removable partitions in the middle. Single mice of the same sex and treatment were placed on either side of the partition. It was removed 20 minutes later and the mice were observed until a fight was initiated, or for a maximum of 15 minutes (Table 1). The same pair of mice was tested once daily for three consecutive days, after which vaginal smears were obtained for five consecutive days from all females. All males and 12 females from each group were then autopsied to verify the expected effects of neonatal injections on reproductive tract morphology. Ovaries, uteri, and testes were weighed and examined histologically. Seminal vesicles were homogenized in water and analyzed for fructose (*6*).

The remaining females from each of the three groups received subcutaneous injections of progesterone (0.3 mg per mouse per day) for 8 days to induce estrous cycles (*7*). On the afternoon of the 8th day, they were paired with naive males in the females' home cages. Our purpose in this secondary experiment was to verify the lack of mating in females treated neonatally with testosterone or estradiol and to follow a suggestion by Barraclough that changes in aggressiveness might be more obvious in such a situation (*8*). Incidence of fighting was recorded for the first hour after pairing, and all pairs were inspected for wounding and presence of vaginal plugs on the following three mornings. Males used in this experiment were about 100 days old, intact, and sexually and experimentally inexperienced; each male had been housed with four or five others since weaning.

The results of the primary experiment, in which mice were given the opportunity to fight only members of the same sex and treatment group, are presented in Table 1. Spontaneous fighting occurred at least once during three encounters in all but one pair of males in each of the two groups that received injections of either oil or testosterone during infancy. Neonatal injections of estradiol reduced the incidence of fighting in adult males to 50 percent ($P<.01$). Only 4 percent of the control females fought, whereas fighting among pairs that had received neonatal injections of either testosterone or estradiol increased to 28 and 29 percent, respectively ($P < .05$ in both cases).

The secondary experiment, in which

Table 3. Body weight, relative (paired) organ weights, and fructose concentrations in seminal vesicles of males treated neonatally with oil, testosterone, or estradiol; body and relative uterine weights of similarly treated females (mean ± standard error).

| Neonatal treatment | Males | | | | | Females | | |
|---|---|---|---|---|---|---|---|---|
| | No. | Body wt. (g) | Testes (mg/g body wt.) | Seminal vesicle (mg/g body wt.) | Seminal vesicle fructose (μg) | No. | Body wt. (g) | Uterus (mg/g body wt.) |
| Oil | 48 | 27.8 ± 0.4 | 7.53 ± 0.52 | 2.42 ± 0.27 | 174.0 ± 6.3 | 12 | 22.6 ± 0.7 | 3.19 ± 0.31 |
| Testosterone | 37 | 27.3 ± 0.5 | 5.93 ± 0.20* | 1.98 ± 0.08* | 137.0 ± 5.4* | 12 | 28.2 ± 1.0* | 5.23 ± 0.67* |
| Estradiol | 40 | 24.4 ± 0.4* | 4.61 ± 0.41* | 1.07 ± 0.15* | 48.2 ± 4.7* | 12 | 23.1 ± 0.8 | 2.37 ± 0.38 |

* Significantly different from oil controls, as determined by analysis of variance, with a probability of at least $P < .05$.

females were injected with progesterone for 8 days and then paired with normal males, revealed marked aggressiveness on the part of females injected neonatally with testosterone (Table 2); fighting among such pairs was often vicious and usually initiated by the females. Females treated with estradiol also fought with males, but both the incidence and severity of fights were lower. No fighting was noted among pairs in which the female had been injected only with oil in infancy. No vaginal plugs were found in any females receiving steroid neonatally, but 55 percent of the females injected with oil had plugs during the 3 days after pairing.

The effects of neonatal injections of estradiol or testosterone on vaginal cycles and reproductive tracts were similar to previous findings (*2*, *4*) and will be reported here only to an extent necessary for correlation with the behavioral data. Neonatal injections of estradiol in males resulted in decreased body and reproductive organ weights and relative aspermia. Injections of testosterone in infancy also decreased weights of male organs but to a lesser extent than that caused by estradiol (Table 3). All vaginal smears obtained from all females injected neonatally with either steroid contained approximately 80 percent cornified cells and 20 percent leukocytes, and ovaries of such females were polyfollicular and devoid of corpora lutea. Body and uterine weights were increased among females injected neonatally with testosterone.

Androgen is a necessary prerequisite for attack behavior in inexperienced male mice (*9*), whereas estrogen administered during adulthood has no effect on aggressiveness of males (*10*). The reduction in spontaneous aggression shown by males injected with estrogen in our study was correlated with large changes in their reproductive tracts, and secretion of testicular androgen was probably considerably reduced. Weights of reproductive organs were also lower in males given neonatal injections of androgen, but they were as aggressive as control males. These facts suggest that those males injected with androgen neonatally probably had sufficient androgen in their circulation during adulthood to permit a high degree of aggressive behavior, whereas those that received estrogen did not. The amount of fructose in seminal vesicles, a good correlate of androgen titers (*6*), was reduced by 72 percent among males given estradiol in infancy compared to that in controls given oil (Table 3). The comparable figure for males receiving testosterone neonatally was only 21 percent and, hence, the postulate appears reasonably good on this basis.

The low incidence of spontaneous aggression found among control females agrees well with observations of other workers using mice (*11*). Androgen will not increase aggressiveness in either immature or mature gonadectomized females (*12*). However, neonatal injections of testosterone, and to a lesser extent estradiol, increase aggressiveness in females after maturity. These effects were significant in both experiments although more dramatic in the uncontrolled secondary experiment where some previously tested females were paired with naive males in the females' home cages after receiving progesterone to induce estrous cycles. Under such conditions mating did not occur, and the females usually attacked and sometimes wounded males. Wounding was sufficiently severe to cause death in one case. The reasons for the dramatic effects observed in this experiment are not readily obvious because of its uncontrolled nature and the data are presented only as an extreme example of a phenomenon observed in the primary experiment. Two investigators have reported that "masculine or aggressive responses" interfered with normal female sexual behavior when rats were treated with estrogen or testosterone in infancy (*13*) but not to the extent shown in the present study with mice.

A reasonable hypothesis to explain the increased aggressiveness of females treated neonatally with gonadal hormones is the alteration of a neural mechanism whose sexual differentiation is normally regulated by androgen in infancy. Such a concept parallels the conclusions of many studies dealing with either sex behavior or the hypothalamic control of gonadotropin secretion, and some degree of experimental mimicking of androgen by estrogen is well documented in this respect. It does not seem reasonable at this time, however, to suspect the hypothalamus at the expense of other neural structures because the number of brain areas known to function in aggression is relatively large (*14*). Furthermore, as evidenced by changes in body weight in both sexes, the effects of early administration of steroids may be widespread.

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**References and Notes**

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15. This investigation was supported in part by PHS grants FR-05545-05 and HD-00767.

21 May 1968

## Lysergic Acid Diethylamide: Sensitive Neuronal Units in the Midbrain Raphe

Abstract. *Units in areas of the midbrain rich in neurons containing serotonin respond to parenteral injections of* d-*lysergic acid diethylamide by a reversible cessation of spontaneous activity. The dose required is at or below threshold for gross behavioral effects. An inhibition of neurons containing serotonin after administration of* d-*lysergic acid diethylamide could account for the decreased metabolism of serotonin produced by this drug.*

In the caudal midbrain raphe we find neurons whose spontaneous firing is reversibly inhibited by small doses of parenterally administered LSD (*d*-lysergic acid diethylamide). The caudal midbrain raphe is the locus of two large aggregates of neurons containing serotonin (5-hydroxytryptamine) (*1*). Although there have been studies on the effects of LSD applied to single units (*2*), these have not dealt specifically with the effects of LSD on units in the midbrain raphe or other brain regions with neurons containing serotonin.

There has been much speculation about possible interactions between LSD and serotonin in the brain (*3*). The notion that serotonin may be involved in the central effect of LSD is derived from observations that LSD could antagonize or facilitate the effects of serotonin in smooth-muscle preparations (*4*). Within the brain, LSD induces a small but consistent increase in sero-