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Pup-Killing in Mice: The Effects of Gonadectomy and Testosterone Administration[1]

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GANDELMAN, R. AND F. S. VOM SAAL. *Pup-killing in mice: the effects of gonadectomy and testosterone administration.* PHYSIOL. BEHAV. 15(6) 647–651, 1975. — The administration of testosterone to intact and to gonadectomized adult female mice induced pup-killing whereas such treatment failed to induce killing in intact males and in males gonadectomized in adulthood. Testosterone treatment was effective in inducing killing when given to adult, neonatally gonadectomized, males. Also, gonadectomy of males performed prior to experience with young reduced the number of animals that subsequently killed. Gonadectomy performed after animals had killed pups did not eliminate killing behavior. Finally, practically all females that exhibited pup-killing in response to the administration of testosterone also displayed intraspecific aggression. However, although pup-killing tended to appear earlier than did fighting, more animals displayed intraspecific aggression than killed pups.

Pup-killing Gonadectomy Testosterone Aggression Fighting

THE killing of mouse young by adult mice is exhibited frequently by males and rarely by females. Approximately 40 percent of adult males of our Rockland-Swiss albino strain (R-S) will kill young as compared to less than 10 percent of the adult females [8]. Mice of either sex that do not kill generally display maternal activities toward the young.

Female mice can be induced to kill pups by the administration of testosterone. Specifically, 5 daily injections of 500 μg testosterone propionate induced pup-killing in about 60 percent of intact and gonadectomized adult females. That the steroid did not produce permanent effects was demonstrated by data showing that pup-killing declined significantly 7 days following the termination of hormone treatment [4]. These data suggest, then, that androgen may provide the basis for the sex difference in the propensity to engage in pup-killing behavior. The following group of experiments is designed to explore further the possible relationship between pup-killing and androgen by seeking to determine whether non-killer males will kill following exposure to testosterone (T), whether gonadectomy will eliminate pup-killing behavior of adult males and/or reduce the number of animals normally expected to kill young, and whether a relationship exists between intraspecific aggression, behavior that is known to be influenced by androgen, and pup-killing.

EXPERIMENT 1

This experiment is designed to determine whether an elevation in levels of T will induce pup-killing in adult males that previously did not kill young. To this end, T was administered to intact and to gonadectomized males and, for purposes of comparison, to intact and to gonadectomized females. The experimental design also permits an assessment of the effect of gonadectomy upon pup-killing behavior.

Method

Animals. R-S male and female mice, 60–70 days old, were housed individually in 11 × 7 × 5 in. translucent cages, the floors of which were covered with pine shavings. The animals were maintained on a free-feeding schedule (Purina Laboratory Chow) and kept upon a 12/12 hr light/dark cycle with lights on between 6 a.m. and 6 p.m. Testing was at 8–9 a.m.

Procedure. Twenty-four hr following isolation housing, half of the animals of each sex were gonadectomized under ether anesthesia while the remaining animals were subjected to sham gonadectomy. The mice then were left undisturbed for 4 weeks. At the end of that period they were screened for pup-killing. This was accomplished by placing three 1 day old R-S pups into each animal's cage. Fifteen min later

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the adults' behavior toward the young was recorded. Animals that killed at least 1 pup were eliminated from the experiment. (It must be noted that in practically all cases those mice that did kill killed all 3 pups and those animals that did not kill always exhibited maternal activites such as retrieval and hovering).

Immediately following the screening procedure, approximately half of the mice of each sex were treated with T. A 10 mm length of Silastic tubing (Dow Corning, 0.062 in. i.d.) containing 5 mg T in a sesame oil vehicle and sealed at each end with Dow Corning Silicone Type A adhesive was implanted under the skin of the back. The surgery was performed with ether anesthesia. Such an implant will maintain seminal vesicle, coagulating gland, and ventral prostate gland weight in gonadectomized R-S mice (B. Goldman, personal communication). The remaining animals were implanted with Silastic tubing that contained only sesame oil. The mice were left undisturbed for 3 weeks, at the end of which time they again were presented with three 1 day olds. Fifteen min later their behavior toward the young was recorded.

Results

Table 1 presents the results of the screening test for pup-killing. Significantly more intact males killed pups than did intact females ($\chi^2 = 7.18$, $df = 1$, $p<0.01$). Furthermore, significantly fewer gonadectomized males killed young as compared to intact males ($\chi^2 = 10.72$, $df = 1$, $p<0.01$).

The number of intact and gonadectomized males and females that killed young and that were maternal toward them following treatment with either T or oil is summarized in Table 2. (It must be remembered that all animals in this phase of the experiment exhibited maternal behavior during the initial screening test.) It can be seen that the administration of T produced pup-killing in intact and in gonadectomized females. Significantly more T-treated intact females killed as compared to oil-treated intact females ($\chi^2 = 25.08$, $df = 1$, $p<0.001$). Similarly, significantly more gonadectomized females administered T killed pups as compared to oil-treated gonadectomized females ($\chi^2 = 17.95$, $df = 1$, $p<0.001$). Testosterone-treated intact and gonadectomized females did not differ from each other with respect to the percentage killing young.

A different pattern of results emerged for the males. There was no significant difference between any of the 4 treatment groups with respect to the percentage of animals killing. Therefore, the killing exhibited by the intact and gonadectomized males could not be attributed to T treatment since approximately the same number of oil-treated animals killed. This suggests, then, that males showed a greater tendency to display spontaneous pup-killing than did females. That is, 25 percent of intact oil-treated males killed as compared to 0 percent of intact, oil-treated females; a statistically significant difference ($\chi^2 = 4.51$, $df = 1$, $p<0.05$). Also, more oil-treated gonadectomized males killed than did their female counterparts. That difference, however, was not statistically significant.

TABLE 1

THE NUMBER AND PERCENTAGE OF INTACT AND GONADECTOMIZED MALE AND FEMALE MICE THAT KILLED AND WERE MATERNAL TOWARD 1 DAY OLD MOUSE PUPS

Group	N	Number Kill	Maternal
Intact female	50	5 (10)*	45
Intact male	60	20 (33)	40
Gonadectomized female	46	3 (7)	43
Gonadectomized male	50	3 (6)	47

*Percentage of animals killing

TABLE 2

THE NUMBER AND PERCENTAGE OF INTACT AND GONADECTOMIZED MALE AND FEMALE MICE ADMINISTERED EITHER T OR OIL THAT KILLED AND WERE MATERNAL TOWARD 1 DAY OLD MOUSE PUPS

Group	N	Number Kill	Maternal
Intact ♀ + T	21	16 (76)*	5
Intact ♂ + T	20	7 (35)	13
Intact ♀ + oil	24	0 (0)	24
Intact ♂ + oil	20	5 (25)	15
Gonadectomized ♀ + T	20	14 (70)	6
Gonadectomized ♂ + T	26	8 (31)	18
Gonadectomized ♀ + oil	23	1 (4)	22
Gonadectomized ♂ + oil	21	6 (29)	15

*Percentage of animals killing

EXPERIMENT 2

The results of the first experiment showed, in part, that the administration of T to intact and to gonadectomized males does not produce an increase in pup-killing above that exhibited by oil-treated animals whereas T-treatment readily induces killing behavior in females. This experiment asks whether the difference between males and females in their responsiveness to T may be due to the males previous exposure to endogenous androgen by administering T to males that had been gonadectomized on the first day of life.

Method

Animals. Eleven litters of R-S mice were reduced to 6 male offspring on the day of parturition. If a litter did not contain the requisite number of males, additional 1 day old males were fostered to it. The offspring of 5 of the litters were gonadectomized on the day of birth. The operations were performed using ether anesthesia. Collodion was applied to the incisions. The animals were kept under a heat lamp until they appeared to be fully recovered from the anesthesia, at which time they were returned to the dam. Six litters of mice were treated similarly except that the testes were not removed.

Procedure. The mice were weaned on Day 21. Ten of the gonadectomized and 1 of the sham-gonadectomized animals

died prior to weaning. Therefore, the gonadectomized group initially consisted of 20 and the sham-gonadectomized group of 35 animals. The mice were kept in littermate groups from Day 21 until Day 60, at which time they were housed individually. Twenty-four hr later each animal was proferred three 1 day old pups. The pups were left with each animal for 15 min. Eleven of the sham-gonadectomized and none of the gonadectomized mice killed pups. The sham-gonadectomized mice that killed were eliminated from the experiment. Twenty-four hr later the remaining animals were implanted with 10 mm lengths of Silastic tubing containing 5 mg T in sesame oil. Three weeks following implantation each animal was given a 15 min test for pup-killing.

Results

As mentioned above, more sham-gonadectomized than gonadectomized animals killed pups on the initial screening test (35 vs 0 percent).

Table 3 summarizes the effects of T upon the non-killer animals. As can be seen, the administration of T to adult, neonatally gonadectomized, males induced pup-killing whereas the hormone treatment failed to induce killing in intact males. The difference in the proportion of killers in the 2 groups is statistically significant ($\chi^2 = 15.06$, $df = 1$, $p<0.001$).

TABLE 3

THE NUMBER AND PERCENTAGE OF T-TREATED NEONATALLY GONADECTOMIZED AND INTACT MALE MICE THAT KILLED AND WERE MATERNAL TOWARD 1 DAY OLD MOUSE PUPS

Group	N	Number	
		Kill	Maternal
Gonadectomized	20	15 (75)*	5
Intact	24	3 (13)	21

*Percentage of animals killing

EXPERIMENT 3

The previous experiments showed that gonadectomy performed prior to males' initial exposure to young tended to reduce the number of animals that killed relative to the number normally expected to kill young. This experiment seeks to determine whether gonadectomy will eliminate pup-killing in males that previously exhibited such behavior.

Method

Fifty 60–70 day old R-S males were housed singly as previously described. Twenty-four hr later each was presented with three 1 day olds and 15 min later the adult's behavior was recorded. Eighteen of the 50 animals killed young. The killers were divided into 2 groups of 9 animals. One group of mice was gonadectomized and the other underwent sham gonadectomy. The animals then were left undisturbed for 4 weeks, at the end of which time each animal again was presented with three 1 day olds and tested in the previously described manner.

Results

Gonadectomy did not eliminate pup-killing. Six of 9 gonadectomized and 8 of 9 sham-gonadectomized animals killed pups.

EXPERIMENT 4

It is well-known that male mice generally exhibit intraspecific fighting behavior and that non-lactating females rarely fight (c.f., [2,6]). Recently it has been shown that the administration of T (or testosterone propionate) to adult females will induce intraspecific aggression similar to that displayed by males [1,14]. Since the exposure of adult females to T induces both pup-killing and intraspecific aggression, one could ask whether a relationship exists between the two behaviors. This experiment is addressed to that issue by attempting to determine whether all females that exhibit T-induced intraspecific aggression will also kill young, whether T-induced pup-killing is exhibited only by females that show intraspecific fighting behavior, and whether the two behaviors are produced simultaneously by the administration of T.

Method

Sixty-seventy day old R-S females were housed singly as described previously and, 24 hr later, were presented with three 1 day old R-S pups. Only those adults that displayed maternal activities such as pup-retrieval and hovering during the 15 min. test were used. Twenty-four such animals were gonadectomized 24 hr following the presentation of young. Immediately after gonadectomy, the animals were implanted with T in the dosage and manner previously described. Five days later the animals were tested for pup-killing and for intraspecific aggression. The tests for pup-killing were the same as those used previously. Tests for intraspecific aggression consisted of placing an olfactory bulbectomized male R-S mouse into the home-cage of each T-treated female. The stimulus male was left there for 15 min or until a fight, defined as persistent biting and chasing, occurred. Olfactory bulbectomized males were used because, although they are attacked by males and T-treated females, they do not initiate attacks or fight back in response to biting attacks [5,14]. Bulbectomized males thus provide a relatively constant source of stimulation. Twelve of the T-treated females always were tested for pup-killing at 8 a.m. and for intraspecific fighting at 2 p.m. while the other 12 animals always were tested in the reverse order. All animals were exposed to T for 20 days and were tested 4 times at 5 day intervals, the first test taking place on Day 5.

Results

The order of testing (i.e., pup-killing followed by intraspecific fighting tests or vice versa) produced no significant effects. Hence, the data for the 12 mice in each of the two order conditions were pooled.

Figure 1 shows the number of animals that killed pups and fought across each of the 4 tests. It must be noted that once an animal killed pups or fought an adult male it always continued to do so on the subsequent tests. Pup-killing generally was observed prior to the display of intraspecific fighting. Eight of the 11 mice that killed pups did so on the first test while only 2 of the 19 animals that fought an adult male did so on the first test day. By the last test day, more animals fought than killed pups. A compari-

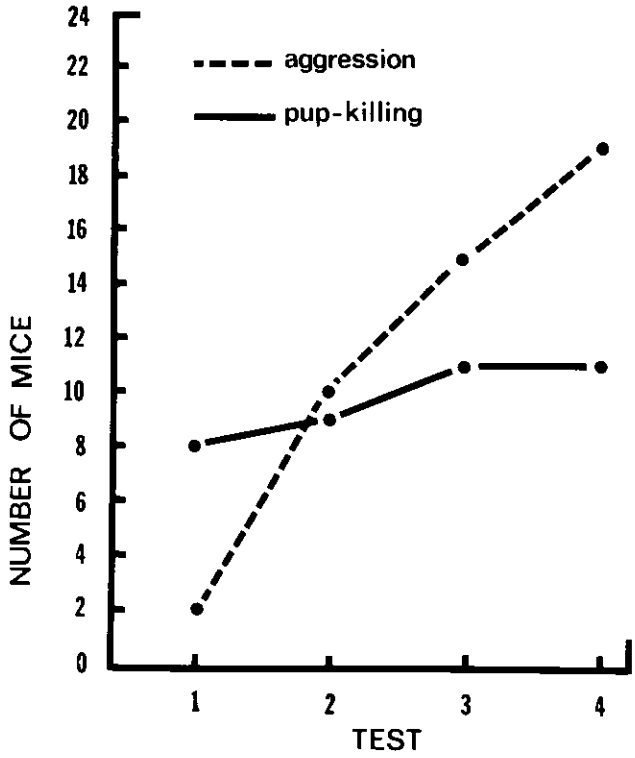

FIG. 1. The number of T-treated female mice that killed pups and attacked an adult male on each of 4 tests. Animals were tested every 5th day for 20 days beginning on the 5th day following the implant of T.

TABLE 4

THE NUMBER OF T-TREATED GONADECTOMIZED FEMALE MICE THAT KILLED PUPS, ATTACKED AN ADULT MALE, AND THAT EXHIBITED BOTH BEHAVIORS ON EACH OF 4 TESTS

	Killed Pups	Attacked Adult Male	Killed Pups and Attacked Adult Male
Test 1	7	1	1
Test 2	7	8	2
Test 3	5	8	6
Test 4	2	10	9

Tests were held once every 5 days for 20 days beginning 5 days after the implant of T.

son of Test 1 and 4 using the Phi Coefficient [15] showed that the increase in the number of animals that fought was significantly greater than was the increase in the number of animals that killed pups ($\phi^2 = 5.64, df = 1, p<0.02$).

Table 4 summarizes the data for each test in terms of the number of mice that either killed pups or fought and the number of animals that displayed both behaviors. On the first test day only 1 animal exhibited both behaviors; 7 animals only killed pups and 1 only fought. However, by Test 4, most of the animals that killed pups also displayed intraspecific fighting (9 of 11) whereas about half of the animals that fought did not kill pups (10 of 19).

DISCUSSION

The results verified previous reports by showing that more male than female mice kill mouse young and that exposure of females to T induces killing behavior. In addition, the data now show that exposure of intact and gonadectomized adult males to T fails to induce pup-killing. This finding could be explained in two ways. First, it could be argued that only a certain proportion of male R-S mice (about 40 percent) are sensitive to the pup-killing-inducing property of T, and that these are the animals that kill young spontaneously. Since the spontaneous pup-killers were eliminated from the first experiment by prior screening for pup-killing, the remaining males may have been those normally insensitive to the pup-killing-inducing property of T. A second explanation is that pup-killing will result when, for the first time in the life of the organism, titers of T increase from relatively low to relatively high levels. Since adult males have had prior exposure to relatively high levels of endogenous androgen, the administration of T will not produce a novel state of high T, as it will in females. This argument receives support from the results of Experiment 3 which showed that 75 percent of adult, neonatally gonadectomized, males killed pups in response to T treatment. Furthermore, it has been shown that males begin to kill pups by Day 32 of life [9]. McKinney and Desjardins [11] have reported for male mice that plasma androgen levels increase 300 percent between Days 20 and 55.

The results also have shown that gonadectomy of males, either neonatally or in adulthood, will reduce the number of animals that kill relative to the number normally expected to kill pups but will not eliminate pup-killing behavior once it already has been exhibited. This suggests that although T may be necessary for the induction of pup-killing, the behavior, once established, will persist in the absence of androgen. Thus, experience in performing the behavior may lessen the sensitivity of the appropriate neural tissue to changes in the levels of androgen, thereby reducing or even eliminating the effects of gonadectomy. Since female mice will stop killing young following the withdrawal of T [4], it would appear that a sex difference exists with respect to the effect of pup-killing experience. Such experience apparently does not serve to maintain killing in the absence of T in the female.

The results of Experiment 4 show that a relationship exists between T-induced pup-killing and intraspecific aggression in that practically all females that killed young also attacked an adult male. The fact that the mice generally killed pups before they attacked the stimulus male suggests that testosterone may be acting upon different neural substrates, each involved in the display of one of the behaviors. However, it also is possible that T is acting upon the same tissue. In either case, it would appear that the threshold for inducing pup-killing is lower than is the threshold for the induction of intraspecific aggression. It also must be noted that a smaller percentage of gonadectomized females killed pups in the 4th experiment (45 percent) as compared to Experiment 1 (70 percent). The 4th experiment consisted of giving the adults 4 presentations of young while the animals of the first experiment were proferred young on only two occasions. It has been shown that experience with young can attenuate the pup-killing-inducing property of T (10). Differential experience with young possibly can account for the smaller percentage of killers in Experiment 4.

The ability of T to induce pup-killing apparently generalizes to other species. Fuller *et al.* [7] reported that the administration of testosterone propionate to Dutch-belted rabbits on Days 17 to 29 of gestation led to a significant increase in cannabalism of young. Pup-killing also has been exhibited by adult female rats that had been administered testosterone propionate on Day 3, 5, 7 and 9 of life and again on Day 40 [13]. Moreover, both pre- and postpubertal castration of male rats will reduce the incidence of pup-killing [12].

In summary, the results of this and of other studies (e.g., [9,10]) suggest that the difference between male and female mice in the propensity to kill young may be caused by androgen. That is, androgen at levels normally found in the male may act to induce pup-killing behavior. In addition to the sex difference with respect to the number of animals that spontaneously engage in killing behavior, a sex difference also appears to reside in the responsiveness to androgen. Testosterone administration will produce high levels of killing in non-killer females but will have little if any effect in the non-killer males. As stated previously, this difference may result from differential experience with relatively high levels of T.

A number of questions remain to be answered concerning T-induced pup-killing; namely, how does the hormone induce such behavior and what is the significance of such behavior in the natural environment. With respect to the latter, it is possible that pup-killing may be one of a number of population-limiting mechanisms. It has been reported that the incidence of killing increases in mouse populations of high density [3]. A careful examination of pup-killing behavior in semi-natural populations of mice and of other rodents may be a fruitful approach to the study of population dynamics.

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