Pup-Killing in Mice: The Effects of Gonaectomy and Testosterone Administration

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GANDELMAN, R. AND F. S. VOM SAAL. Pup-killing in mice: the effects of gonaectomy and testosterone administration. PHYSOL. BEHAV., 10(11) 647–651, 1975. - The administration of testosterone to intact and to gonaecotomized adult female mice reduced pup-killing whereas such treatment failed to inhibit killing in intact males and in males gonaecotomized in adulthood. Testosterone treatment was effective in inducing killing when given to adult, normally gonaecotomized, males. Also, gonaectomy of prepubertal mice to experience with young reduced the number of animals that subsequently killed. Gonaecotomized prepubertal mice that had killed pups did not eliminate killing behavior. Finally, physically all females that exhibited pup-killing in response to the administration of testosterone also displayed hyperthecic aggression. However, although pup-killing tended to appear earlier than male fighting, more animals displayed hyperthecic aggression than killed pups.

Pup-killing Gonaectomy Testosterone Aggression Fighting

EXPERIMENT 1

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

Method

Animals. RR male and female mice, 60–70 days old, were housed individually in 11 x 7 x 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 gonaecotomized under ether anesthesia while the remaining animals were subjected to sham gonaectomy. The mice then were left undisturbed for 4 weeks. At the end of this period they were screened for pup-killing. This was accomplished by placing three 1 day old RR pups into each animal's cage. Fifteen min later

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2 Please address reprint requests to: R. Gandelmann, Department of Psychology, Rutgers University, New Brunswick, NJ 08893, U.S.A.
the adults' behavior toward the young was recorded. Animals that killed at least 1 pup were nominated from the experiment. (It must be noted that in practically all cases those mice that did not kill or at least 1 pup. At the end of breeding. A different group of rats was kept and the experimental results were compared with the 1 day old pups. Fifteen min later their behavior toward the young was recorded.

Results

Table 1 presents the results of the screening test for pop-killing. Significantly more intact males killed pups than did intact females ($X^2 = 7.18, df = 1, p < 0.01$). Furthermore, significantly fewer gonadectomized males killed young as compared to intact males ($X^2 = 10.72, df = 1, p < 0.01$). 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 (Table 1). It can be seen that the administration of T produced pop-killing in intact and in gonadectomized females. Significantly more T-treated intact females killed as compared to oill-treated intact females ($X^2 = 25.08, df = 1, p < 0.001$). Similarly, significantly more gonadectomized females administered T killed pups as compared to oill-treated gonadectomized females ($X^2 = 17.95, df = 1, p < 0.001$). Treatment of T-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 suggest, then, that males showed a greater tendency to display spontaneous pop-killing than did females. That is, 25 percent of intact oil-treated males killed as compared to 0 percent of intact, oil-treated females; statistically significant difference ($X^2 = 4.51, df = 1, p < 0.05$). Also, more oil-treated gonadectomized males killed than did their female counterparts. That difference, however, was not statistically significant.

Experiment 2

The results of the first experiment showed, in part, that the administration of T to intact and to gonadectomized males did not produce an increase in pop-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 androgens by administering T to males that had been gonadectomized on the first day of life.

Method

Animals
Eleven litters of R 5 mice were reduced to 6 males culling 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 clipping of 5 of the litters were gonadectomized on the day of birth. The operations were performed using ether anesthesia. Colloidon 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 examined on Day 21. Ten of the gonadectomized and 1 of the sham-gonadectomized animals

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Kill</th>
<th>Material</th>
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</thead>
<tbody>
<tr>
<td>Intact female</td>
<td>59</td>
<td>5(10)*</td>
<td>45</td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>20(33)</td>
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<tr>
<td>Gonadectomized female</td>
<td>46</td>
<td>3(7)</td>
<td>43</td>
</tr>
<tr>
<td>Gonadectomized male</td>
<td>50</td>
<td>3(6)</td>
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*Percentage of animals killing

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<th>Group</th>
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<tr>
<td>Intact o + T</td>
<td>21</td>
<td>16(76)*</td>
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</tr>
<tr>
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<td>7(35)</td>
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<tr>
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<td>24</td>
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<tr>
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<td>20</td>
<td>5(25)</td>
<td>15</td>
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<tr>
<td>Gonadectomized o + T</td>
<td>29</td>
<td>14(70)</td>
<td>6</td>
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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 androgens by administering T to males that had been gonadectomized on the first day of life.
died prior to weaning. Therefore, the gonadectomized group initially consisted of 20 and the sham-gonadecto- 
mized 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 transferred these 3 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 cm lengths
of Solastic tubing containing 5 mg T in saline 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 9 percent). Table 3 summarizes the effects of T upon the non-killing
animals. As can be seen, the administration of T to adult,
sham-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 $\chi^2 = 15.00, df = 1$, $p < 0.001$

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<th>Kill</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadectomized</td>
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<td>15 (75)*</td>
<td>5</td>
</tr>
<tr>
<td>Intact</td>
<td>24</td>
<td>3 (13)</td>
<td>21</td>
</tr>
</tbody>
</table>

*Percentage of animals killing

EXPERIMENT 2
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 behav-
ior.

Method
Fifty 60-70 day old R.S. males were housed singly as
previously described. Twenty-four hr later each was pre-
sented with three 1 day old 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 old 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-fertile, al-

cates rarely fight (c.f., [2,6,3]). Recently it has been shown that
androstenedione (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
this issue by attempting to determine whether all females that
exhibit 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 females that displayed
maternal behavior such as pup-attention and nursing during
the 15 min test were used. Forty-four such animals were

gonadectomized 24 hr following the presentation of young.
Immediately after gonadectomy, the animals were implant-
ed 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 al-
though they are attacked by males and T-treated females,
they do not initiate attacks or fight back in response to
being attacked [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 compar-
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 ovariectomized 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 R5 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 ovariectomized, 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]. McKimney and Beniardis [11] have reported for male mice that plasma androgen levels increase 200 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 has already 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 is also 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 prefered young only on 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 killer in Experiment 4.

DISCUSSION

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Killed Pups</th>
<th>Attacked Adult Male</th>
<th>Killed Pups and Attacked Adult Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Test 2</td>
<td>7</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Test 3</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Test 4</td>
<td>2</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

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

The results 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 ($\chi^2 = 5.64, d.f. = 1, p = 0.02$).

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).
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-bred rabbits on Days 17 to 29 of gestation led to a significant increase in cannibalism 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 [11]. Moreover, both pre- and postpartum 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 androgens. That is, androgens 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 androgens. Testosterone administration will produce high levels of killing in two-killer females but will have little if any effect on the two-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 under 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.

REFERENCES