Castration Does Not Inhibit Aggressive Behavior in Adult Male Prairie Voles
(Microtus ochrogaster)

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DEMAS, G. E., C. MOFFATT, D. L. DRAZEN AND R. J. NELSON. Castration does not inhibit aggressive behavior in adult male prairie voles (Microtus ochrogaster). PHYSIOL BEHAV 66(1) 59–62, 1999.—The relationship between castration and reduced male aggression is well established. However, anecdotal observations of male prairie voles (Microtus ochrogaster) suggest that castration does not reduce aggressive behavior. To investigate the role of testicular androgens on aggressive behavior, castrated or gonadally intact male prairie voles were paired in a neutral arena with a gonadally intact vole. Castration did not reduce the frequency of intermale aggression. In Experiment 2, aggressive behavior was examined further using resident–intruder, grouped aggression, and aggression against a lactating female models. Again, castration did not affect the frequency of aggression in male prairie voles. Taken together, the results of this study suggest that aggressive behavior may be independent of gonadal steroid hormones in adult male prairie voles. © 1999 Elsevier Science Inc.

Testes Androgens Arvicoline rodents

Castration reduces aggression in males in a wide range of vertebrate species (1,16,18,27). However, this widely accepted relationship between the presence of the testes (and normal circulating testosterone concentrations) and aggression is primarily based upon studies of male–male interactions in only a few domesticated species (2,12,13). For example, castration influences aggressive behavior in house mice (Mus musculus) (12) and Syrian hamsters (Mesocricetus auratus) (27). However, if females are used as stimulus animals, or if males of undomesticated species are studied, then exceptions to the relationship between low circulating testosterone blood concentrations and aggression emerge. For example, castrated male mice are more aggressive toward conspecific, lactating females than gonadally intact male mice (17). Strain differences also exist in the extent to which castration affects aggression (3). Some strains of M. musculus retain aggressiveness for several months after castration while other strains do not (28). Males of several nondomesticated species do not exhibit reduced aggression after castration. For example, castration does not decrease aggressiveness in male red-sided garter snakes (Thamnophis sirtalis parietalis), European starlings (Sturnus vulgaris) (10,22), or Mongolian gerbils (Meriones unguiculatus) (8).

The extent to which testosterone mediates aggressive behavior in arvicoline rodents is unclear. Anecdotal observations in our laboratory and others (C.S. Carter, personal communication) suggest that castration does not reduce aggression in male prairie voles. Among arvicoline rodents, juvenile male and female voles rarely engage in agonistic behavior, but both sexes become increasingly aggressive towards strange males in adulthood (7). Exogenous testosterone treatments of gonadally intact adult Townsend voles (Microtus townsendii) do not affect the frequency or quality of aggressive behavior (21). Conversely, treatment of intact adult prairie voles (M. ochrogaster) with subcutaneous testosterone implants elevates aggressiveness (14). However, aggressive behavior is often not
directly observed in these studies; rather, the number of wounds is assessed as an indirect measure of aggression.

The goal of the present study was to determine the effects of castration on aggressive behavior in male prairie voles. Previous observations in our laboratory indicated that castrated voles displayed equal or increased aggressiveness compared to gonadally intact conspecifics. In Experiment 1, we sought to confirm these anecdotal observations that castrated male voles did not display reduced aggressiveness in a neutral arena. In Experiment 2, we examined several parameters of defensive aggression in castrated and intact male prairie voles.

**EXPERIMENT 1**

**Materials and Methods**

Twenty-four adult (>60 days of age) male prairie voles (Microtus ochrogaster ochrogaster) were obtained from our laboratory breeding colony. The progenitors of these animals were originally trapped near Champaign, IL (40.1° N latitude). All animals were weaned at 21 days of age and housed individually in polycarbonate cages (28 × 17 × 12 cm) in colony rooms illuminated with a 24-h LD 16:8 light cycle (lights on 0700 h EST). Temperature was kept constant at 21 ± 2°C, and relative humidity was maintained at 50 ± 5%. Food (Prolab 2000; Agway; Syracuse, NY) and tap water were provided ad lib throughout the study.

Half of the voles (n = 10) were randomly selected and castrated, while the remaining animals (n = 10) received sham operations. All animals were anesthetized with methoxyflurane vapors (Metofane; Pitman-Moore, Mundelein, IL) prior to surgery. Castrations were performed through bilateral abdominal incisions; both testes were removed, the abdominal wall sutured, and the incision in the skin closed with 9-mm wound clips. Animals that were sham castrated experienced a similar procedure, but their testes were irrigated with saline, then returned to the abdominal cavity prior to closing.

**Aggression Tests**

Aggressive behavior was assessed in a glass aquarium (26 × 32 × 51 cm); the floor of the aquarium was covered with 1 cm of clean pine shavings. A central barrier divided the aquarium into two separate compartments. A male vole was randomly selected from one of the experimental groups and introduced into one of two compartments of the aquarium while an intact male stimulus vole was placed in the other compartment. After a 10-min acclimation period, the central barrier was raised and the animals were allowed to interact for 10 min. The behavior of the voles was recorded with a Panasonic WV-3260 camera and a JVC 9000-U time-lapsed video recorder. Aggressive behavior was analyzed from the videotape record by scorers who were uninformed of the animals' experimental condition (9). The latency to initial attack, the number of attacks, and attack duration were calculated. Each stimulus vole was randomly selected and used only once per test. All behavioral tests were conducted 4 weeks after surgery.

**EXPERIMENT 2**

**Materials and Methods**

Twenty-four adult male prairie voles were obtained from our breeding colony and housed as described above. Half of the voles were castrated while the remaining voles underwent sham castrations. Castrations and sham castrations were performed as described above, and behavioral testing was conducted 4 weeks after surgery.

**Resident–Intruder Aggression**

Resident–intruder aggression was assessed by introducing an adult male stimulus vole (i.e., intruder) into the cage of either a castrated or intact vole (i.e., resident). Intruder voles were marked on the tail with an indelible marker for purposes of identification. The bedding in the home cages remained unchanged for 10 days prior to behavioral testing. The latency to initial attack and the total number of attacks initiated by the resident male were recorded. Aggression tests lasted 5 min and were conducted each day for 3 consecutive days between 1300 and 1500 h. A novel pairing of animals was made for each consecutive test and intruder males were not used more than once per day.

**Grouped Aggression in a Neutral Arena**

Grouped aggression in a neutral arena was assessed by placing either four adult castrated or sham-operated voles into a clear glass aquarium. The latency to the initial attack and the total number of attacks were recorded. Grouped aggression tests lasted 15 min and were conducted between 1300 and 1500 h EST.

**Aggression Against a Lactating Female**

Aggression directed against a lactating female was assessed by introducing a lactating female into the cage of either a castrated male or sham male and recording the number of attacks by the male directed at the female.

**Open-Field Activity**

Relative anxiety levels were determined for both castrated and sham-operated voles using a standard open-field test. Briefly, voles were placed in the center of an open arena (1 m²) for 15 min. A 4-cm border around the perimeter of the apparatus was marked off, and the space beyond this border was operationally defined as the open field. The amount of time the voles spent in the open field as well as the number of defecations were recorded for each animal.

**Statistical Analyses**

Differences between treatment means were assessed using independent Student's t-tests (Sigma Stat, Jandel Inc., San Rafael, CA). In all cases, differences were considered to be statistically significant if p < 0.05.

**RESULTS**

In Experiment 1, castration did not decrease the overall frequency of aggressive behaviors among male prairie voles. Castrated voles displayed comparable levels of aggressiveness to gonadally intact voles (p > 0.05) (Fig. 1).

In Experiment 2, castration did not decrease the frequency of aggressive encounters among male prairie voles in a resident–intruder paradigm. There were no differences between castrated and sham-operated voles in either the latency to initial attack or the number of total attacks (p > 0.05 in both cases) (Fig. 2). Castrated and sham-operated voles also displayed comparable levels of aggression in a neutral arena (p > 0.05). There were no differences in the number of attacks directed at a lactating female between castrated and sham-operated males (p > 0.05). In the open-field test, castrated voles did not display reduced aggressiveness compared to gonadally intact voles (p > 0.05). In the open-field test, castrated voles did not display reduced aggressiveness compared to gonadally intact voles (p > 0.05).
voles spent more time in the open-field compared to sham-operated voles (195.1 ± 33.8 s vs. 95.7 ± 35.21 s, respectively) ($p < 0.05$).

DISCUSSION

Aggressive behavior was not diminished by castration in adult male prairie voles (Microtus ochrogaster). Castration did not decrease the overall occurrence of aggressive behavior among prairie voles. These results do not appear to reflect changes in anxiety levels, because castrated voles spent more time than intact males in the open field, suggesting less anxiety in castrated voles. Taken together, these results strongly suggest that circulating testosterone concentrations do not mediate aggressive behavior in adult male prairie voles. These results are consistent with anecdotal observations from several laboratories that study this species. However, the present results are in contrast to previous findings demonstrating increased aggression in testosterone-treated prairie voles (14). The apparent discrepancy between those findings and the results of the present study may be due to geographic strain differences; the animals used by Gaines (14) originated from Kansas, while those used in the present study were from Illinois. For example, recent research has shown that the Kansas and Illinois strains differ in a variety of ways, including differences in parental behavior (25) and spontaneous aggression (Lee et al., unpublished data).

One potential explanation for the absence of reduced aggressive behavior in castrated prairie voles is that testosterone is necessary for the organization but not the activation of aggressive behavior in male prairie voles. For example, neonatal exposure to elevated concentrations of androgens causes rather large and permanent increases in aggressive behavior in both male and female mice and rats (5,23). Increased aggressive behavior persists into adulthood, even in the absence of substantial androgen concentrations. Additionally, neonatal castration of prairie voles renders these animals slightly more responsive to androgens in adulthood in the context of sexual behavior (26); whether these results extend to aggressive behavior remains to be tested. In the present study, prairie voles were castrated at 60 days of age (i.e., adulthood); thus gonadal steroid hormones (particularly androgens) were present at normal concentrations throughout the organizational period. It is possible that neonatal exposure to androgens was sufficient to organize aggressive behavior permanently in prairie voles. Thus, adult levels of aggression were unaffected by castration in the present study.

Alternatively, androgens may not play an important role in either organization or activation of aggressive behavior in
Prairie voles relative to other rodent species. Adult prairie voles have circulating serum testosterone concentrations an order of magnitude lower than house mice and rats, and roughly four times lower than other vole species (15,19,20). Thus, castration results in a smaller absolute reduction in circulating testosterone concentrations in prairie voles compared to house mice. Generally, gonadal androgens play an important role in the masculinization and differentiation of male physiology and behavior in a variety of rodent species (4,24). The relatively low concentrations of circulating androgens, along with the substantially reduced sexual dimorphism demonstrated in prairie voles (11,20) suggest that androgens may not play an important role in regulating male-typical aggressive behavior in this species. Recent evidence suggests that the neuropeptides arginine-vasopressin and oxytocin are involved in the mediation of male–male aggression in prairie voles (6,29).

In summary, our results suggest that gonadal steroid hormones are not important for the expression of male-typical aggression in adult prairie voles. Neither castration nor testosterone replacement therapy significantly affected aggressive behavior in resident–intruder or neutral arena models of aggression. These results provide support for several anecdotal observations that aggression in prairie voles appears to be independent of testosterone. Further studies are required to determine the role of gonadal steroid hormones in the organization of aggressive behavior in this species.

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References