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Time of Neonatal Androgen Exposure Influences Length of Testosterone Treatment Required to Induce Aggression in Adult Male and Female Mice[1]

FREDERICK S. VOM SAAL, BRUCE SVARE, and RONALD GANDELMAN

Department of Psychology, Rutgers University, New Brunswick, New Jersey 08903

Male and female mice, gonadectomized within the first 12 hr of life, were administered either testosterone propionate (TP) or oil on Postnatal Day 0, 3, 6, or 12. They were implanted with a capsule containing testosterone (T) on Day 60 and tested for fighting behavior against an olfactory bulbectomized male every other day for a maximum of 62 days (30 tests). Virtually all animals fought regardless of whether or not they were exposed to TP neonatally. However, the neonatally androgen-exposed mice fought sooner following the administration of T than did the non-neonatally androgen-exposed animals. Animals exposed to TP on Day 12 required a longer exposure period to T than did the mice given TP on Day 6. Moreover, significantly more neonatally than non-neonatally androgenized mice fought 2 weeks after the T capsule was removed. It also was found that non-neonatally androgen-exposed males fought sooner in response to adult T treatment than did non-neonatally androgenized females. The results suggested that both prenatal and early postnatal androgen exposure influence sensitivity to the aggression-promoting property of androgen in adulthood.

Male mice are much more apt to exhibit intraspecific aggressive behavior than are nonlactating females. The etiology of this dimorphism has been attributed to the production of gonadal steroids during particular periods of development in that it has been reported that mice will display aggression only if exposed to testosterone during both the early postnatal and adult periods (cf. Bronson and Desjardins, 1970; Edwards, 1968). These data have led to the promulgation of the "organization-activation" model of aggressive behavior which states that androgen present perinatally acts to permanently

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organize particular portions of the brain, thereby rendering that neural tissue responsive to the aggression-activating property of testosterone later in life.

The validity of the organization-activation model recently has been questioned by data showing that female mice that are not treated with androgen during the early postnatal period and males gonadectomized on the day of birth will display aggression following chronic exposure to testosterone in adulthood (Svare, Davis, and Gandelman, 1974; vom Saal, Gandelman, and Svare, in press). It appears, then, that neonatal exposure to androgen is not a necessary condition for the display of androgen-induced intraspecific fighting. Such exposure, however, does significantly affect the rapidity with which testosterone induces aggressive behavior in adulthood; animals exposed to testosterone neonatally require a shorter period of adult testosterone exposure to exhibit aggressive behavior than do animals not androgenized neonatally. This can be seen readily if, for example, one compares the data of Svare *et al.* (1974) with those of Bronson and Desjardins (1970). It required an average of 21 consecutive days of exposure to 200 μg testosterone propionate (TP) to establish aggression in non-neonatally androgenized female mice, whereas neonatally androgenized females fought following as few as four daily injections of 200 μg TP. It appears, therefore, that animals exposed to androgen early in life are more responsive to the aggression-promoting property of testosterone than are animals not androgenized during the early postnatal period.

The following experiment was designed to consider two issues. First, does a relationship exist between the time of postnatal androgen exposure and the duration of adult testosterone treatment required to establish aggression? Whitsett, Bronson, Peters, and Hamilton (1972) have presented data which suggest that the sensitivity of a female to testosterone, as assessed by the uptake of labelled testosterone into various areas of the brain, declines significantly between Days 0 and 12 of life. Should the presence of testosterone during the early postnatal period attenuate or arrest the declining sensitivity to testosterone of brain areas mediating aggression, one would predict that animals exposed to the steroid later in neonatal life (at a time when sensitivity to testosterone has markedly declined) would require a longer adult testosterone exposure regime than would mice exposed to the steroid earlier in neonatal life (at a time when sensitivity to the testosterone has not significantly declined).

A second issue considered was whether males and females that were gonadectomized soon after birth and not administered testosterone neonatally would require similar lengths of adult testosterone treatment to establish fighting behavior. *Prenatal* exposure to androgen would be implicated in an animal's later responsiveness to testosterone if males required a shorter treatment period to induce aggression than did females.

METHODS

Fifty litters of Rockland-Swiss albino mice (R-S) were sexed and distributed at birth such that a litter contained only six male or six female pups. If a litter did not contain the requisite number of pups of a particular sex, additional pups were fostered to it. All animals were gonadectomized within the first 12 hr of life. Surgery was performed using ether anesthesia and the wounds were covered with a commercially obtained plastic antiseptic covering (Newskin). The pups were kept under a heat lamp until they recovered from the anesthesia, at which time they were returned to the dam. The litters were housed in 11 X 7 X 5 in. translucent polypropylene cages, the floors of which were covered with pine shavings. Water and Purina Laboratory Chow were available in excess. A 12/12 light/dark cycle was maintained with lights on at 6 AM.

The litters of males and females were divided into two major treatment groups with all members of a litter receiving the same treatment. Animals received either one subcutaneous injection of 300 μg TP dissolved in 0.02 cc sesame oil or an equal volume of oil. The TP and Oil groups were each divided into four subgroups differing as to when the injection was administered. The injections were given either on Day 0 (the day of birth), 3, 6, or 12 of life. A male or female thus could have received either oil or TP on one of four postnatal days. The experimental design thus employed 16 groups. Each group of TP-treated animals was composed of 24 animals while each Oil-treated group was composed of 12 animals. Five deaths occurred before the animals reached adulthood with the result being that three postnatal TP-treatment groups did not consist of 24 animals.

The animals were weaned on Day 21 and housed in littermate groups until Day 60. On that day each animal was anesthetized with ether and implanted subcutaneously with a 10 mm length of silastic tubing (Dow Corning, 0.062 in. id) that contained 5 mg testosterone (T) suspended in sesame oil. The ends of the capsule were sealed with Dow Corning Silicone Type A adhesive. These implants have been shown to maintain seminal vesicle, coagulating gland, and ventral propostate gland weights in castrated R-S mice (B. Goldman, personal communication). Following surgery, the animals were housed singly under conditions previously described.

The first test for aggression was administered 2 days after surgery. An aggression test, occurring between 8 and 11 AM, consisted of placing an olfactory bulbectomized adult male R-S mouse into the homecage of the test animal. The stimulus animal was left there for a maximum of 10 min or was removed as soon as a fight occurred. Olfactory bulbectomized males served as opponents because, although they elicit aggression comparable to that of intact males, they neither initiate fights nor respond to attack by fighting

back (Denenberg, Gaulin-Kremer, Gandelman, and Zarrow, 1973). Thus, they provide a relatively constant source of stimulation for tests of aggression. A fight was considered to have occurred if the T-implanted animal displayed 5 sec of persistent biting and chasing of the stimulus animal. Tests for aggression were administered every other day for a maximum of 62 days (30 tests). As soon as an animal exhibited aggression, it was anesthetized with ether and the silastic capsule was removed. Two weeks later the animal was retested once for aggression. Animals that did not exhibit aggression while exposed to T were not retested in the absence of the T-containing capsule.

RESULTS

Only two of 283 animals failed to exhibit aggression following exposure to T. Each animal was given a score corresponding to the number of days of exposure to T required to establish fighting. The data are summarized in Table 1. The analysis revealed that the means of the groups varied linearly as a function of the standard deviations. Therefore, log transformations were performed (Bliss, 1967). An overall analysis of variance performed upon the transformed data yielded a significant Postnatal Treatment Effect [$F(1, 267) = 330.8$, $P < 0.001$). Thus, regardless of sex, animals administered TP neonatally displayed aggression sooner than did those administered oil. The overall analysis also showed that males displayed aggression sooner than did females [$F(1, 267) = 30.3$, $P < 0.001$]. A significant Sex X Postnatal Treatment interaction was also obtained [$F(1, 267) = 17.3$, $P < 0.001$] which is

TABLE 1

The Proportion of Male and Female Mice Exposed during the Neonatal Period to Either Testosterone (TP) or to Oil that Exhibited Fighting in Response to the Administration of Testosterone during Adulthood and the Mean Number of Days of Adult Testosterone Exposure Required to Establish the Behavior

	Day of TP treatment				Day of oil treatment			
	0	3	6	12	0	3	6	12
Males								
Proportion fighting	24/24	24/24	22/22	24/24	12/12	11/12	12/12	12/12
Mean	4.6	4.0	3.8	5.5		15.5		
Females								
Proportion fighting	24/24	23/23	24/24	22/22	12/12	12/12	12/12	11/12
Mean	4.7	4.5	4.9	7.2		27.6		

accounted for by the fact that the difference between males (mean = 4.47 days) and females (mean = 5.32 days) administered TP neonatally was very small, whereas females administered oil neonatally required a considerably longer exposure to T in order to display aggression (mean = 27.6 days) than did males treated neonatally with oil (mean = 15.5 days). Finally, the overall analysis did not yield a statistically significant Day of Treatment Effect. However, since a prediction was made concerning the effect of the day of neonatal TP treatment (the earlier the exposure, the less adult exposure to T would be required to induce aggression), an analysis of variance was performed upon the transformed data for the four groups of males and four groups of females administered TP during the neonatal period. Critical values ($c.v. = t(2MS/\bar{n})^{1/2}$) were calculated for each sex (Winer, 1971). The data revealed that for both the males and females, the Day 12 treated animals took significantly longer (P 0.05) to exhibit aggression than did Day 6 treated animals. However, the Day 0, 3 and 6 TP-treated animals did not differ in length of adult T treatment needed to induce aggression.

Table 2 presents the proportion of animals in each group that exhibited aggression 2 weeks following the removal of the T implant. Chi-square analyses revealed that a significant number of animals in each group stopped fighting following the cessation of adult T treatment (Ps < 0.001). However, significantly more males and females that received TP during the neonatal period fought following removal of the T implant than did males and females given oil neonatally [$\bar{X}^2(1) = 5.27$, $P < 0.025$, males; $\bar{X}^2(1) = 11.28$, $P < 0.001$, females]. Moreover, significantly more males exhibited aggression on the retest than did females [$\bar{X}^2(1) = 4.1$, $P < 0.05$].

DISCUSSION

The results have verified previous findings (Brain and Evans, 1975; Svare *et al.*, 1974; vom Saal *et al.*, in press) by showing that exposure to androgen

TABLE 2

The Proportion of Male and Female Mice Exposed during the Neonatal Period to Either Testosterone Propionate or to Oil that Exhibited Fighting Behavior 2 Weeks following the Cessation of Adult Testosterone Treatment

	Neonatal treatment		
	TP	Oil	Total
Males	47/94	13/47	60/141
Females	37/93	5/47	42/140
Total	84/187	18/94	102/281

during the early postnatal period is not necessary for the establishment of androgen-induced aggression in male and female mice. It appears, then, that the often reported failure to induce aggression by administering testosterone to adult non-neonatally androgen-exposed mice was due to the relative brevity of the adult testosterone treatment period. Tollman and King (1956), for example, gave one injection of TP and Bronson and Desjardins (1970) gave only four injections.

The fact that animals administered TP on Day 12 of life required a significantly longer adult T regime to establish fighting than did animals given TP on Day 6 and that differences were not observed between animals exposed to TP on Days 0, 3, and 6 suggest that androgen exposure during at least the first 6 days of postnatal life is necessary if the neural mechanisms mediating aggression are to be maximally sensitive to the aggression-eliciting property of T in adulthood. However, the Day 12 TP-treated males and females still exhibited aggression more rapidly following adult T exposure than did neonatally Oil-treated animals. The administration of TP as late as Day 12, therefore, may still serve to attenuate the normal decline in sensitivity to testosterone.

The results also demonstrate that neonatal androgen exposure results in a longer-lasting change in the tendency to exhibit aggression once the behavior has been established in adulthood by T administration. That is, significantly more animals of both sexes receiving TP in infancy displayed aggression 2 weeks after the cessation of adult T treatment as compared to animals given oil neonatally. It thus appears that fighting experience in adulthood in response to T treatment may interact with the neonatal hormonal milieu to modulate an animal's propensity to continue to display fighting behavior following the withdrawal of testosterone.

It also was found that males gonadectomized at birth and administered oil neonatally required significantly less exposure to T in adulthood to establish fighting than did similarly treated females. It is known that the fetal mouse testis is capable of secreting testosterone (Block, Lew, and Klein, 1971; Hall, Eik-Ness, and Samuels, 1963). Exposure to testosterone prenatally, therefore, also may exert facilitatory influences on adult responsiveness to the aggression-eliciting properties of testosterone in mice. However, this interpretation must be viewed with caution. Since gonadectomies were performed following birth, it is possible that the results may have been due to early (within the first 12 hr of life) postnatal androgen exposure. Research exploring the possible role of prenatal androgen in modulating an organism's later responsiveness to testosterone is in progress.

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