Physiology & Behavior, Vol. 17, pp. 53–57. Pergamon Press and Brain Research Publ., 1976. Printed in the U.S.A.

Aggression in Male and Female Mice: Evidence for Changed Neural Sensitivity in Response to Neonatal but not Adult Androgen Exposure[1]

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(Received 25 August 1975)

VOM SAAL, F. S., R. GANDELMAN AND B. SVARE. *Aggression in male and female mice: evidence for changed neural sensitivity in response to neonatal but not adult androgen exposure.* PHYSIOL. BEHAV. 17(1) 53–57, 1976. – Male mice were gonadectomized on Day 0, 2, 4, or 6 of life and w re administered 300 μg testosterone propionate (TP) or oil daily beginning on Day 60. Over 90% of the TP-treated animals in each of the 4 groups exhibited aggressive behavior toward an olfactory bulbectomized male. Animals administered oil did not display fighting behavior. The day of gonadectomy, although not influencing the number of TP-treated animals that fought, did affect the time taken to display aggression in response to TP replacement. Mice gonadectomized on Day 0 required a longer exposure to TP than did animals gonadectomized on Day 2 in order to induce fighting. Similarly, mice gonadectomized on Day 2 required longer exposure to TP than did mice gonadectomized on either Day 4 or 6. There were no differences between mice gonadectomized on Day 4 and 6. In a second experiment, female mice, gonadectomized on Day 60, were given daily injections of either 500 μg TP or oil. Twenty-eight of 30 TP-treated mice fought whereas 2 of 30 Oil-treated mice fought. After a 60 day interval, the animals originally administered TP were re-administered the hormone and again tested for aggression. No differences were found between the amount of TP treatment required to initially induce aggressive behavior and the amount necessary to reestablish the behavior. Mice administered oil initially and then TP required the same amount of hormone treatment to induce aggression as did those animals given two TP treatments.

Gonadectomy Testosterone propionate Aggression

A concept based upon organization-activation has been used to explain how androgen induces aggressive behavior in mice. It is presumed that androgen organizes the brain during the perinatal period thereby rendering the neural tissue responsive to the activational property of androgen later in life. This conceptualization has evolved, in part, from a host of experiments which have shown that the induction of aggression in female mice can be accomplished only by administering androgen during both the perinatal and adult period of life [4, 7, 8].

The organization-activation model assumes the existence of a critical period in development during which androgen is maximally effective in organizing central tissue and during which organization normally occurs. Edwards [8] reported that significantly more male mice gonadectomized on Day 10 of life and given testosterone propionate (TP) replacement as adults fought as compared to males gonadectomized on Day 1 and given TP when adult. Thus, the period of maximal sensitivity to the putative organizing property of androgen declines precipitously between Day 1 and 10 of life. The period of organization was further delineated by Peters, Bronson, and Whitsett [11] who demonstrated that males gonadectomized on the day of birth and given TP when adult did not exhibit aggression, that animals gonadectomized on Day 2, although showing an increase in the incidence of aggression, still fought less than did controls in response to adult TP treatment, and that gonadectomy on Day 6 produced no suppression of aggression. From these data one could conclude that the process or organization normally terminates between Day 2 and 6. In short, the concept of organization-activation is supported by data showing that female mice can be induced to fight only by administering androgen during both the perinatal and adult period of life and that adult androgen-treated males will fight only if they are exposed to androgen during a restricted portion of the perinatal period.

Recently, data were presented which question the organization-activation model. It has been shown that female mice will exhibit aggressive behavior when exposed to TP (or to testosterone) only as adults [2, 3, 12]. These

[1]This research was supported by funds from the Biological Science Support Grant from U.S.P.H.S. and by Grant HD 06863 from N.I.C.H.D., N.I.H. Reprint requests should be addressed to R. Gandelman, Department of Psychology, Rutgers University, New Brunswick, New Jersey 08903, U.S.A.

results, as well as others [5,13], lead to 2 predictions concerning the ability of androgen to induce fighting behavior in adult, perinatally gonadectomized, male mice. First, prolonged exposure to androgen should be effective in inducing fighting behavior in males that have been gonadectomized on the day of birth. This prediction follows from the fact that aggression can be induced in non-perinatally androgenized female mice by long-term exposure to TP. Second, the length of the TP replacement regimen in adulthood necessary to induce aggression should vary as a function of the time of perinatal gonadectomy. That is, those animals castrated earlier should require a longer period of exposure to TP in adulthood before exhibiting aggression than those animals gonadectomized later. The following experiment tests these predictions.

EXPERIMENT 1

Method

Pregnant nulliparous and multiparous Rockland-Swiss albino mice (R-S), having been maintained as an outbred strain in a closed colony, were housed singly in 11 × 7 × 5 in. translucent cages, the floors of which were covered with pine shavings. The animals were given free access to food (Purina Laboratory Chow) and to water and were maintained on a 12/12 hr light/dark cycle, with the lights on between 6 AM and 6 PM.

Litters were reduced to 8 male offspring on the day of parturition. If a litter did not contain the requisite number of males, other newborn males were fostered to it. The litters were assigned to 1 of 4 groups differentiated in terms of the time gonadectomy was performed. Gonadectomy took place either on Day 0 (the day of birth), 2, 4, or 6 of life. The operations were performed under ether anesthesia. A commercially obtained plastic adhesive covering was applied to the wounds. The pups were kept under a heat lamp until they appeared to have recovered fully from the anesthesia, at which time they were returned to the dam.

All animals were weaned on Day 21 and kept in littermate groups until Day 60. On Day 60 the animals were housed singly under conditions described previously. Daily injections were begun 24 hr later. Six animals were selected at random from each of the 4 perinatal gonadectomy groups and given daily SC injections of 0.02 cc sesame oil. The remaining animals were given daily injections of 300 μg TP in 0.02 cc sesame oil. Owing to deaths which occurred between birth and weaning (mortality rate was not related systematically to the time of gonadectomy), the number of animals in each of the 4 TP groups was not equal. The number of TP-treated mice in each of the 4 perinatal castration groups was as follows: Day 0 – N = 25; Day 2 – N = 30; Day 4 – N = 22; Day 6 – N = 21.

All animals received the initial aggression test 48 hr following the first injection and were subsequently tested every other day until a fight occurred or for a maximum of 44 days. Thus, each animal could have received a total of 22 aggression tests and 44 injections. Aggression testing took place between 8 and 11 AM and injections were administered between 2 and 4 PM. An aggression test consisted of placing an olfactory bulbectomized adult male R-S mouse into the home cage of the test animal. The stimulus animal was left there for 10 min or until a fight occurred. Bulbectomized males were used as stimulus animals because, although they elicit aggression comparable to that of intact males, they neither initiate fights nor fight back in response to attack [6]. A bulbectomized male thus serves as a relatively constant source of stimulation for tests of aggression. An encounter was scored as a fight if the test animal exhibited a sustained attack (biting and chasing the stimulus animal) for at least 5 sec. Since the mice were injected every day, the cages were marked to indicate whether a mouse belonged to a TP or to the Oil treatment group. However, the tester was naive with respect to the day gonadectomy was performed.

RESULTS AND DISCUSSION

The proportion of animals that fought in each group is summarized in Table 1. As stated previously, the Oil-treated animals were drawn from each of the 4 perinatal gonadectomy groups. Since time of gonadectomy did not affect the Oil-treated animals, their data were pooled to form 1 Oil-treated group. As can be seen, practically all TP-treated animals exhibited aggression regardless of the day on which gonadectomy was performed. In contrast, only 1 Oil-treated animal fought. The fighting behavior of the TP-treated animals was similar to that normally exhibited by intact adult males. Attacks were preceded by tail-rattling, ano-genital sniffing, and rough grooming. Biting was directed principally toward the flanks.

TABLE 1

THE PROPORTION AND PERCENTAGE OF MALE MICE CASTRATED ON DAY 0, 2, 4, OR 6 OF LIFE THAT EXHIBITED AGGRESSIVE BEHAVIOR IN RESPONSE TO TP TREATMENT BEGUN ON DAY 60. OTHER ANIMALS RECEIVED OIL BEGINNING ON DAY 60. THE OIL-TREATED GROUP WAS COMPOSED OF AN EQUAL NUMBER OF MICE CASTRATED ON DAY 0, 2, 4, AND 6

	GROUP (TP)				OIL
	0	2	4	6	
Proportion Fighting	23/25	28/30	21/22	21/21	1/24
Percentage Fighting	92	93	95	100	04

The cumulative percentage of animals that fought in each group across the days of testing is depicted in Fig. 1. It is apparent that the groups differed with respect to the time taken to the onset of fighting. The data were arranged in blocks of 6 days and a Chi-square test was performed [15]. An overall analysis revealed a significant Group Effect (χ^2 = 36.86, df = 3, $p<0.01$). Since a prediction was made concerning the direction of potential group differences (the earlier castration is performed the longer the TP regimen would have to be to induce aggression), individual comparisions were made using 1-tailed tests of significance. Day 0 castrates required a significantly longer TP regimen to induce aggression than did Day 2 castrates (χ^2 = 3.54, df = 1, $p<0.05$). Day 2 castrates required a significantly longer TP treatment period than did Day 4 (χ^2 = 15.93, df = 1,

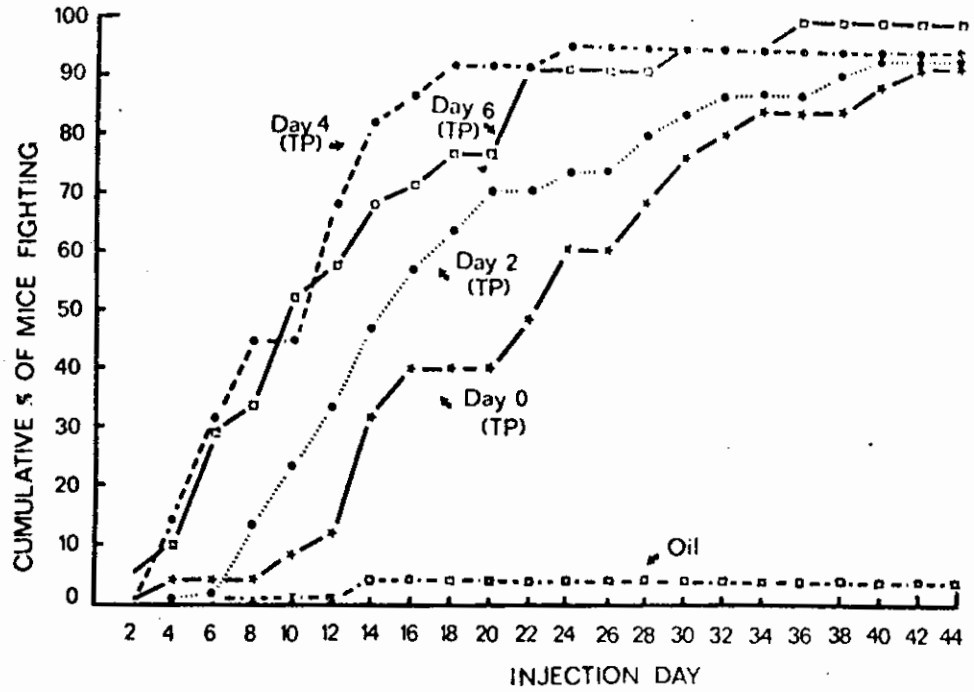

FIG. 1. The cumulative percentage of male mice castrated on Day 0, 2, 4, or 6 of life that exhibited aggressive behavior in response to TP treatment begun on Day 60. Other animals received oil beginning on Day 60. The Oil-treated group was composed of an equal number of mice castrated on Day 0, 2, 4, and 6. Aggression tests were held every other day.

$p<0.001$) and Day 6 castrates ($\chi^2 = 6.08$, $df = 1$, $p<0.01$). Finally, the mice gonadectomized on Day 4 and Day 6 did not differ with respect to the duration of the TP treatment necessary to induce aggression.

The results of this experiment support the 2 predictions made at the onset, namely, that TP treatment would induce aggression in males gonadectomized on the day of birth and that the earlier in the perinatal period gonadectomy was performed, the longer the TP regimen would have to be to induce fighting behavior in adulthood. The data, [illegible] agree with previous reports which show that non-perinatally androgenized female mice will display fighting behavior following chronic exposure to testosterone as adults [3,12]. The present findings also are in agreement with those of Owens, Peters, and Bronson [10] which showed, in part, that gonadectomy of male mice on Day 10 resulted in a prolongation of the time required to respond to adult testosterone treatment. Moreover, the data are in partial agreement with Edwards' finding [8] that a longer period of exposure to TP was required by males gonadectomized on the day of birth than was required to induce aggression in mice gonadectomized on Day 10. However, Edwards reported that only 41% of pairs of mice gonadectomized on the day of birth exhibited aggression while 85% of the Day 10 gonadectomized pairs fought. While the procedure of pairing animals of the same treatment condition leaves in doubt whether one or both of the mice fought, this difference was interpreted as indicating that there was a critical period for organizing the neural substrate which mediates aggression. The present results and the findings that non-perinatally TP-treated females will exhibit aggression if the duration of TP treatment in adulthood is extended for a longer time than was previously attempted [12] indicate that the concept of a critical period for neural organization with its implication that organization is a prerequisite for the induction of aggressive behavior may be incorrect. Instead, the data appear to support the view that androgen present during the perinatal period acts to maintain sensitivity to androgen later in life, a sensitivity which, as suggested by the data of Whitsett, *et al.* [13], declines with advancing age. Moreover, it appears from the present findings that androgen must be present at least for the first 4 days of life in order for maximum sensitivity to be maintained.

It could still be argued, however, that the organization of the neural tissue that mediates aggression can occur anytime throughout life. If the present data were to be interpreted within the framework of the organization-activation model, one would have to propose that when TP is administered chronically to adult, non-perinatally androgen-exposed mice, both organization and activation occur. This would imply that a shortened TP regimen would be required to re-induce aggression at a later time since organization already has occurred. The next experiment is addressed to that issue.

EXPERIMENT 2

Method

Ten litters of R-S mice were separated by sex at birth with 6 females being left in each cage with the dam. If a litter did not contain the requisite number of female

offspring, additional pups were fostered to it. The young were weaned on Day 21 and maintained in littermate groups until 60 days of age. Conditions of housing and maintenance were the same as those described previously.

On Day 60 of life each mouse was gonadectomized under ether anesthesia and housed individually. The mice then were assigned randomly to 1 of the following 3 groups: Group TP-TP (N = 30) was given SC injections of 500 μg TP in 0.02 cc sesame oil during both the Test and Re-test phase of the experiment; Group Oil-TP (N = 20) was injected with 0.02 cc oil during the Test and 500 μg TP during the Re-test phase; Group Oil-Oil (N = 10) was administered 0.02 cc oil during both the Test and Re-test phase. After assignment to the treatment groups, the animals were given the following sequence of tests:

Test. The first injection was given on the day ovariectomy was performed. Animals in Group TP-TP were administered daily injections of TP until a fight occurred or until 38 days had elapsed. TP-TP animals that had not exhibited aggression by Injection Day 38 were eliminated from the experiment. Animals in Group Oil-TP and Oil-Oil were given injections of oil for 38 days. Unlike the animals in Group TP-TP, animals in Group Oil-TP and Oil-Oil that displayed aggression were eliminated from the experiment. Thus, only mice which had not fought while being treated with oil were kept.

Tests for aggression took place every other day, commencing on the 2nd day following gonadectomy (after 2 injections). Therefore, each female was tested for aggression a maximum of 18 times. Aggression tests were conducted between 8 and 11 AM and injections were administered between 2 and 4 PM. Aggression testing was identical to that previously described.

Test – Re-test interval. Injections were terminated for 60 days as soon as animals of Group TP-TP displayed aggression or following the 38 daily injections of oil for mice in Group Oil-TP and Oil-Oil. During this 60-day interval the animals were left undisturbed. However, since isolation housing can augment aggression (c.f. [14]), an intact adult R-S female was housed with every test animal. The companion female was left with the test animal for 58 days of the 60 day Test – Re-test interval.

Preliminary screening for aggression. In order to determine whether animals administered TP during the Test phase would continue to exhibit aggression 60 days following the termination of TP treatment, the animals were given a screening test prior to the readministration of TP during the Re-test phase. (In order to maintain commonality of treatment, animals of Group Oil-TP and Oil-Oil also were given the screening test). The screening consisted of 1 aggression test. If an animal fought during the screening test it was eliminated from the experiment.

Re-test. The Re-test phase was identical to the Test phase. Animals in Groups TP-TP and Oil-TP were administered daily injections of TP while the mice in Group Oil-Oil received oil injections. Each animal could have received a maximum of 38 injections and 18 aggression tests.

RESULTS AND DISCUSSION

The aggression exhibited by the females was qualitatively similar to that displayed by normal males. Attacks usually were preceded by ano-genital sniffing, tail-rattling, and rough grooming.

As summarized in Table 2, 28 of 30 animals in Group TP-TP fought during the Test phase of the experiment. The mean latency to fight was 16 days. None of the TP-TP mice that fought during the Test did so during the Screening Test which occurred just prior to the readministration of TP. Of the 28 mice that displayed fighting behavior during the Test, 26 fought during the Re-test with a mean latency of 14 days. Fifteen of these animals required fewer injections during the Re-test as compared to the Test in order to re-establish aggression, 10 required more injections, and 3 mice fought during the Re-test following the same number of injections required to establish fighting during the Test. A Sign Test revealed that there was no significant difference between the Test and Re-Test with respect to the number of injections required to induce aggression.

TABLE 2

THE MEAN NUMBER OF INJECTIONS NECESSARY TO ELICIT AGGRESSIVE BEHAVIOR AND THE PROPORTION (AND PERCENTAGE) OF MICE IN EACH GROUP THAT FOUGHT DURING THE INITIAL TREATMENT (TEST) AND THE SUBSEQUENT TREATMENT (RE-TEST) WITH TP. THE GROUP NAMES INDICATE THE TREATMENT CONDITION DURING THE TEST AND THE RE-TEST

	TEST		RE-TEST	
Group	Mean*	Proportion & (%) That Fought	Mean	Proportion & (%) That Fought
TP-TP	16	28/30 (93)	14†	26/28 (93)
Oil-TP	20	2/20 (10)	17*	16/17 (94)
Oil-Oil	—	0/10 (00)	8*	2/10 (10)

*Based on the data for the mice that fought.
†Based on the data for all mice that fought during the Test phase.

Of the 20 mice in Group Oil-TP, 2 fought during the Test. The data for these 2 animals were elimianted since neither had received TP injections prior to exhibiting aggression. Also, 1 animal died. Sixteen of the remaining 17 mice, while displaying no aggression during the Screening Test, displayed aggression during the Re-test. The mean latnecy to fight was 17 days. *T*-tests revealed that the number of injections required to induce aggression in animals of Group Oil-TP during the Re-test did not differ significantly from the number of injections required to induce aggression in TP-TP animals during both the Test and Re-test.

None of the 10 animals in Group Oil-Oil fought during the Test and Screening Test. Two of them did fight during the Re-test.

The results confirm the findings of Experiment 1 and of previous experiments which have demonstrated that perinatal exposure to androgen is not a necessary condition for the establishment of androgen-induced aggression [2, 3, 12]. The data also have shown that prolonged exposure to androgen in the adult, non-perinatally androgenized, female apparently does not permanently alter the substrate underlying aggressive behavior since the mice of Group TP-TP required the same duration of TP treatment to re-establish fighting as was required to initially induce the behavior. Further support for this interpretation is provided by the

fact that the latency in days to fight on the Re-test for animals administered TP only once (Group Oil-TP) did not differ from that of the mice administered TP twice. It is interesting to note that Barkley and Goldman [1] showed that ovariectomized adult female mice implanted with silastic capsules containing 10 mg testosterone exhibited aggression, but after a 70 day interval following removal of the capsules were still found to exhibit normal vaginal as well as ovarian cyclicity following an intraocular ovarian graft. This finding provides further evidence that the effects of adult exposure to androgen are not permanent.

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