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The Effects of Pre and Postnatal Gonadal Hormone Administration on the Adult Aggressive-Target Biting in Female Mice

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THE EFFECTS OF PRE AND POSTNATAL GONADAL HORMONE ADMINISTRATION ON THE ADULT AGGRESSIVE-TARGET BITING IN FEMALE MICE

by

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David D. Smith

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INTRODUCTION

It is now well established that androgens and estrogens are intricately involved in influencing the sexual differentiation of the brain, as well as male and female differences of aggressiveness. Evidence is accumulating which indicates that these steroid hormones influence the pattern of nervous connection and organization developing in utero and in the early postnatal period.

To more completely link testosterone's involvement with aggressive behavior, one must examine the voluminous literature which links testosterone with developing neonatal correlates of aggression as well as with adult capacity to display aggressive behavior. In most species the male is more aggressive than the female (Thomson, 1967; Seward, 1945; Ulrich, 1938). Unlike the female of most mammalian species, the male requires androgenization neonatally for a characteristically male type morphological, sexual and aggressive behavioral development. Castrating male mice reduces their aggressiveness and testosterone replacement will restore that aggression (Edwards, 1970; Sigg et al., 1966; Bevan et al., 1958). Castration performed during the first two days after birth effectively blocks aggression in adulthood. As the time of castration advances from birth, testosterone replacement in adulthood becomes progressively more effective in restoring aggression (Peter et al., 1972; Edwards, 1969).

Bronson and Desjardins (1969) demonstrated that when female mice were treated with testosterone on postnatal days 0 through 14 and subsequently ovariectomized at 25 days of age, male-like aggression

occurred in adulthood if testosterone was readministered at that time. Bronson and Desjardins (1970) further concluded that the dose necessary for maximum development of aggression in adulthood was between 100 and 400 µg testosterone proprionate (TP) administered on the first day after birth.

These findings demonstrated a temporal specificity of female mouse sensitivity to testosterone masculinization. This temporal pattern correlated directly with the temporal changes in the male's circulating levels of testosterone. Resco et al (1967) found male rat plasma testosterone levels to be significantly higher during the first 10 days of life than on each of days 10 through 30. At the time of puberty, (40 days of age), these concentrations increased to three times those observed during the first ten days of life. Enhanced neonatal plasma testosterone concentrations (days 1 through 10) correlated directly with the sensitivity of the neonates' brain to testosterone masculinization. The observation was supported by research from Martini and Motta (1977) who in addition produced complimentary findings that fetal rat testes increased testosterone production when exposed to gonadotrophins. The temporal fluctuation in perinatal testosterone levels in males correlated with histochemical evidence showing the production of testosterone by the Leydig cells. Histochemical evidence indicated that Leydig cells were functional during the pernatal period in rats and regressed shortly after parturition until puberty (Niemi and Kormano, 1964).

Early postnatal testosterone injections to female rats and mice impaired LH secretion and resulted in constant adult estrus. Histological examination of the ovaries of these rats indicated a lack of corpora lutea and an excess of polycystic nonovulatory follicles. Adult enhancement of ovarian testosterone and estradiol secretion was observed as a result of early testosterone treatment (Kinel and Magueo, 1965; Weisz and Lloyd, 1965).

Not only did testosterone administered postnatally cause masculinization of the female mouse, but prenatal testosterone administration proved to effectively masculinize the female mouse. Testosterone proprionate (TP) administered to a female mouse on the twelfth day of pregnancy resulted in masculinization and sterilization of the female fetuses. The females were externally indistinguishable from males with an increase in anogenital distance which was similar to the males and is a very sensitive index for the extent of virilization of the female fetus (Austin and Short, 1972; Turner, 1969).

More specifically, testosterone exerts its anatomical masculinizing effects primarily on specific brain tissues. Brain areas which have recently been shown to be target areas for gonadal hormones are the hypothalamic (preoptic), septal, and the amygdaloid areas. The pituitary has also been cited as a major target organ for gonadal hormones (Seigel et al., 1976; Weisz and Gibbs, 1974; Sheridan et al., 1974; Plapinger and McEwen, 1973; Kulin and Reiter, 1972).

Furthermore, biochemical evidence has shown that the brain, like the seminal vesicles and placenta, is able to convert testosterone to

dihydrotestosterone as well as estradiol. Estradiol has been found to be the hormone which actively combines with the nuclear protein receptor in neurons of the hypothalamus, (preoptic), septal, and amygdaloid areas which in turn affects their biochemical activity. The female of the species is protected from the masculinizing effects of its own and its mother's estrogen by alpha-fetoprotein, a molecule which exists in the fetal cerebrospinal fluid. This protective molecule binds estrogen but not testosterone which has free access to the target cell. In normal male development testosterone is then converted in the cytosol to estradiol which then exerts its masculinizing effects (McEwen, 1976; Leiberburg and McEwen, 1975; Plapinger and McEwen, 1973; Plapinger et al., 1973).

The hypothalamus, septum and amygdala in addition to being primary target areas for testosterone and estrogen have been studied by means of electrical stimulation and lesioning and have been accepted as important neural substrates underlying aggressive behavior. (Slotnick and Mullen, 1970; Hutchinson and Renfrew, 1966).

Not only has it been difficult to specify the physiological correlates of aggression, but it has also been difficult quantitatively and qualitatively to define behaviors that are aggressive in nature. Historically, aggression research on mice has utilized a variety of paired-subject designs. Subjects have been paired and observed for frequency of wounding, for severity of wounding, for hierarchy formation and by counting the number of fights, bites, tail rattles, sniffs and urine markings (Leshner et al., 1973; Bronson and Desjardins, 1969; Edwards, 1969). Researchers have been attempting to minimize the con-

founding variables associated with counter-attack, dominance-submissive reactions, pheromones and extensive wounding by carefully selecting an unfamiliar opponent which is approximately the same size, is bulbectomized and muzzled (Simon,1978; Leshner and Moyer,1975). An obvious disadvantage of the paired-subject design is the necessity for subjective quantification and qualification of aggressive behavior. This inherent weakness is somewhat controlled by the use of a validating observer.

It has been found that animals will attack an inanimate target object. Thus, researchers are now able, in a variety of experimental situations,to collect data concerning latency, frequency and force of attack toward a specific inanimate target(Hutchinson,1973; Hutchinson and Emely, 1972; Hutchinson et al., 1966; Azrin et al.,1964). This design utilizes a single restrained subject which eliminates the aformentioned confounding variables resulting from a paired-subject design. Hutchinson et al. (1968) suggests that most investigations which analyze social vs. nonsocial (single-subject designs) paradigms produce similar results. Thus, Azrin et al. (1968) found that shock and intense heat produce a biting attack frequency similar to the frequency of fighting in a paired-subject design.

Wagner et al. (1979) found that it was unnecessary to use shock since restraint in the chamber was an adequate aversive stimulus to produce reliable bite-attack. He also found that there was a significant correlation between total paired fights and total bite-

attacks for individual subjects. In another study Wagner et al. (1979) found that both control males and castrated males with adult testosterone replacement demonstrated higher bite-attack frequencies than did the control or TP or estrogen-treated females. Castrated males bite-attack level was significantly lower than control males. One unexpected result from Wagner's research was that TP injections to normal adult female mice increased their bite-attack frequencies somewhat. Results from both of the formentioned studies indicated that there was no correlation between target biting and free wheel activity in CF1 strain mice.

The purpose of the present study was to observe the effects of prenatal, postnatal and combined pre and postnatal testosterone proprionate treatment on the adult female mouse's capacity to display aggressive behavior. The single subject bite target design pioneered by Hutchinson was the primary method utilized to test for aggression. Paired fighting tests were used to determine if a correlation existed between bite-attack frequency and paired fights. Runwheel activity was monitored concurrently to examine the overall activity as an independent variable possibly correlated with bite-attack behavior. Specific physiological parameters were examined to confirm that masculinization had occurred due to testosterone proprionate administration.

METHODS

Animals

Mice, strain CF1, were obtained from the Upjohn Company (Kalamazoo, Michigan). All female mice were placed in groups of 25 along with 2 stud males which were placed in the cage for one day. Those females which indicated a substantial weight gain in 14 days were considered pregnant. Pregnant females were housed singly and female offspring for these experiments were cross fostered with normal, lactating females from birth until 30 days of age. All animals had ad libitum access to food and water. Colony room lights were on at 07:00 and off at 19:00.

Apparatus

The test apparatus consisted of a transparent Plexiglas cylinder which was 9.0 cm long with an inner diameter of 3.0 cm and a permanent cap at one end. The animal was backed into the tube while sliding the tail through a slot in the Plexiglas base of the cylinder. The animals' tail was then fastened to a Plexiglas rod which extended backwards away from the rear of the cylinder with "Dermicel" tape (Johnson and Johnson, New Brunswick, N.J.). The front of the cylinder was placed into a larger apparatus (Figure 1). This apparatus positioned the animal in front of a plastic bite target which extended inward via a hole in the mobile face cap horizontal to the long axis of the animal approximately 2 cm in front of its mouth. The bite target (0.5 cm wide and 4.0 cm long) was then attached to a microswitch (Model #V31-2426-D8, Honeywell, Minneapolis, Minn.) which when activated with a 1.5

Figure 1. The test apparatus: TR = tail restraint, PC = plastic cylinder, MS = microswitch, BT = bite target.

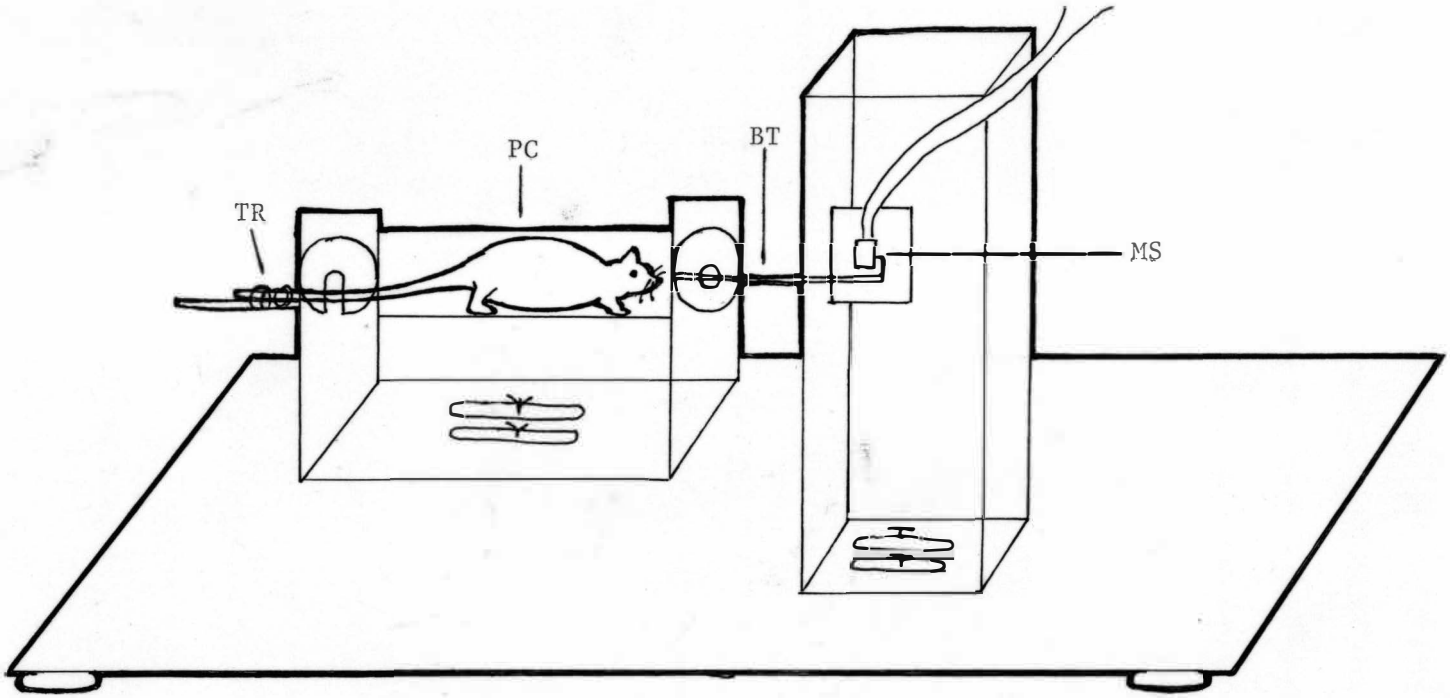

FIGURE 1

gram pull-bite initiated an electrical signal to both electromechanical counters and cummulative recorders. The apparatus was housed in a sound attenuated, temperature and light controlled, ventilated chamber. Animals were removed from the bite-target apparatus and were placed in a 16 cm diameter runwheel which was used to measure general activity. Revolutions were recorded via a mechanical recorder located on the wheel.

Experimental Protocols

The first experiment was designed to determine if postnatal testosterone propionate (TP) administration will enhance adult female target biting as compared with that of male target biting when TP was readministered in adulthood. This experimental approach was designated phase one (Table 1). Experimental females were obatined from pregnant females which received 1 ml sesame oil (vehicle) subcutaneously on each of days 16 through 20 of gestation. The female offspring of the vehicle-treated mother received 400 µg testosterone propionate dissolved in 10 µl of oil subcutaneously on each of days 1 through 6 postnatally. Female offspring were crossfostered on normal lactating females until 30 days of age at which time they were ovariectomized and housed individually until 100 days of age when testing began. At approximately 19:00 each evening a mouse was removed from its home cage and placed in the bite-target apparatus, the chamber temperature was recorded and testing began when the lights in the chamber came on. Sessions were ten minutes long and ended with the chamber lights going off. The animal was then removed and placed into a free

Table 1. Sequence of procedures and experimental manipulations in days for all groups. Numbers above indicate age of subject at which time specific procedures occured. Numbers below indicate testing session spans. The columns of numbers on the left represent the number of mice per group. CAST = castration; OVAR = ovariectomy; V = vehicle injection; TP = testosterone propionate.

PHASE I

Age (days)	Prenatal 16-20	Postnatal 1-6	30		100	
7 ♀	V	TP	OVAR	V	TP	V
4 ♀	V	V	OVAR	V	TP	V
6 ♂	V	V		V	TP	V & CAST
TESTING SESSION				1-11	12-30	31-37

PHASE II

Age (days)	Prenatal 16-20	Postnatal 1-6	30		100	
6 ♀	TP	TP	OVAR	V	TP	V
6 ♀	V	V	OVAR	V	TP	V
TESTING SESSION				1-13	14-26	27-32

PHASE III

Age (days)	Prenatal 16-20	Postnatal 1-6	30		100	
6 ♀	TP	V	OVAR	V	TP	V
6 ♀	V	V	OVAR	V	TP	V
TESTING SESSION				1-9	10-27	28-33

PHASE IV

Age (days)	Prenatal 16-20	Postnatal 1-6	30		100	
12 ♀	V	V	OVAR	V	TP	V
10 ♀	V	TP	OVAR	V	TP	V
TESTING SESSION				1-17	18-35	36-47

TABLE 1

wheel in an adjacent testing chamber. Free wheel activity sessions were also ten minutes long. Animals were weighed following run-wheel activity. The animals then received either a vehicle injection (0.1 ml oil) during the establishment of a pre- and postdrug biting level or an injection of 150 µg of TP dissolved in 0.1 ml of oil when testing adult TP administration effects on bite-attack. Control females in phase one received identical treatment to the experimental females except that the control females received 10 ul oil on each of the first six days postnatally. A control male group was tested concurrently to establish the degree of masculinization of female bite-attacks. Males received identical treatment to the experimental females except that the males did not receive TP on days 1 through 6 postnatally, and were not gonadectomized at 30 days of age. Males were castrated following the normal testing schedule and then were retested to evaluate castration effects on bite-attack response.

The second experiment was designed to determine if prenatal TP administration will enhance adult female target biting and paired fighting when TP is readministered in adulthood. This experiment was designated phase two. Phase two experimental females were obtained from pregnant females which received 250 µg of TP dissolved in 0.1 ml of oil on each of the 16th through 20th days of gestation. The female offspring of these animals were treated with 10 µl of oil on each of the first 6 days after birth. The subsequent experimental manipulations, adult hormonal treatment and testing was identical to that utilized in phase one. Control females received 0.1 ml oil on

each of the days 16 through 20 of gestation and received 10 µl of oil on each of the first six days postnatally. The remaining procedures were identical to all preceding groups.

The third experiment was designed to determine if combined pre- and postnatal TP administration would enhance adult female target-biting and paired fighting when TP was readministered in adulthood. This experimental procedure was designated phase three. Phase three experimental females were obtained from pregnant females which received 250 µg of TP/0.1 ml oil during days 16 through 20 of gestation. Experimental females then received 400 µg of TP/10 µl oil on each of the first six days postnatally. The remaining procedure was identical to that followed in phases one and two. Control female mice were obtained from pregnant females which received 0.1 ml of oil on each of days 16 through 20 of gestation. Control females then received 10 ul of oil on each of the first six days postnatally. The remaining procedures were identical to that performed with the experimental females. Runwheel activity was recorded for all subjects.

The final experiment was deisnged to verify findings of phase one and was designated phase four. Phase four was a replication of phase one with the exception that the bite-target microswitches had a 3 gram pull rather than the 1 gram pull used in the telegraph key, bite-pull device used with phase one mice. The microswitch bite-pull in phases two and three had a 1.5 gram pull. Only the female experimental and control groups were repeated from phase one and runwheel activity was not monitored.

Intragroup paired fighting tests (Edwards, 1970) were administered to all females in phases two, three and four. Subjects were paired intragroup on the final day of adult TP administration and on the final postdrug (vehicle) test day.

Mice were killed following testing by means of ether exposure. The testes and uteri of appropriate animals were fixed in a 10% neutral buffered formalin and prepared for histological examination. In addition, the weights of uteri, adrenals, seminal vesicles and prostate glands were recorded.

Statistical analysis of bite-attack frequency utilized a two-way analysis of variance with a level of significance set at 0.05. Paired fighting and bite-attack correlation data were obtained by using the Pearson product-moment correlation coefficient. Levels of significance were again set at the 0.05 level of significance.

RESULTS

Phase I

In phase one (Figure 2) there was no significant difference in the bite-attack frequency of control males as compared with postnatal TP, ovariectomized females (PN-TP, Ov) or with postnatal oil, ovariectomized females (PN-oil, Ov) during the initial 11 sessions with vehicle only (Figure 2). Among the group means from days 1 through 5, the range of bite frequencies was 261 to 396 with no significant differences between groups. Comparison of mean bites per session on days 6 through 11 evidenced no significant difference in bite-attack means among groups.

Figure 2. Plot of bite-attack means over sessions for phase I. Adult treatments are indicated above graph in appropriate time sequence. TP = testosterone propionate; OVAR = ovarectomy; ●—● = Ovar., postnatal TP females; ○—○ = Ovar., postnatal oil females; ▽—▽ = normal males, oil postnatally; ☆ = castration during adult testing.

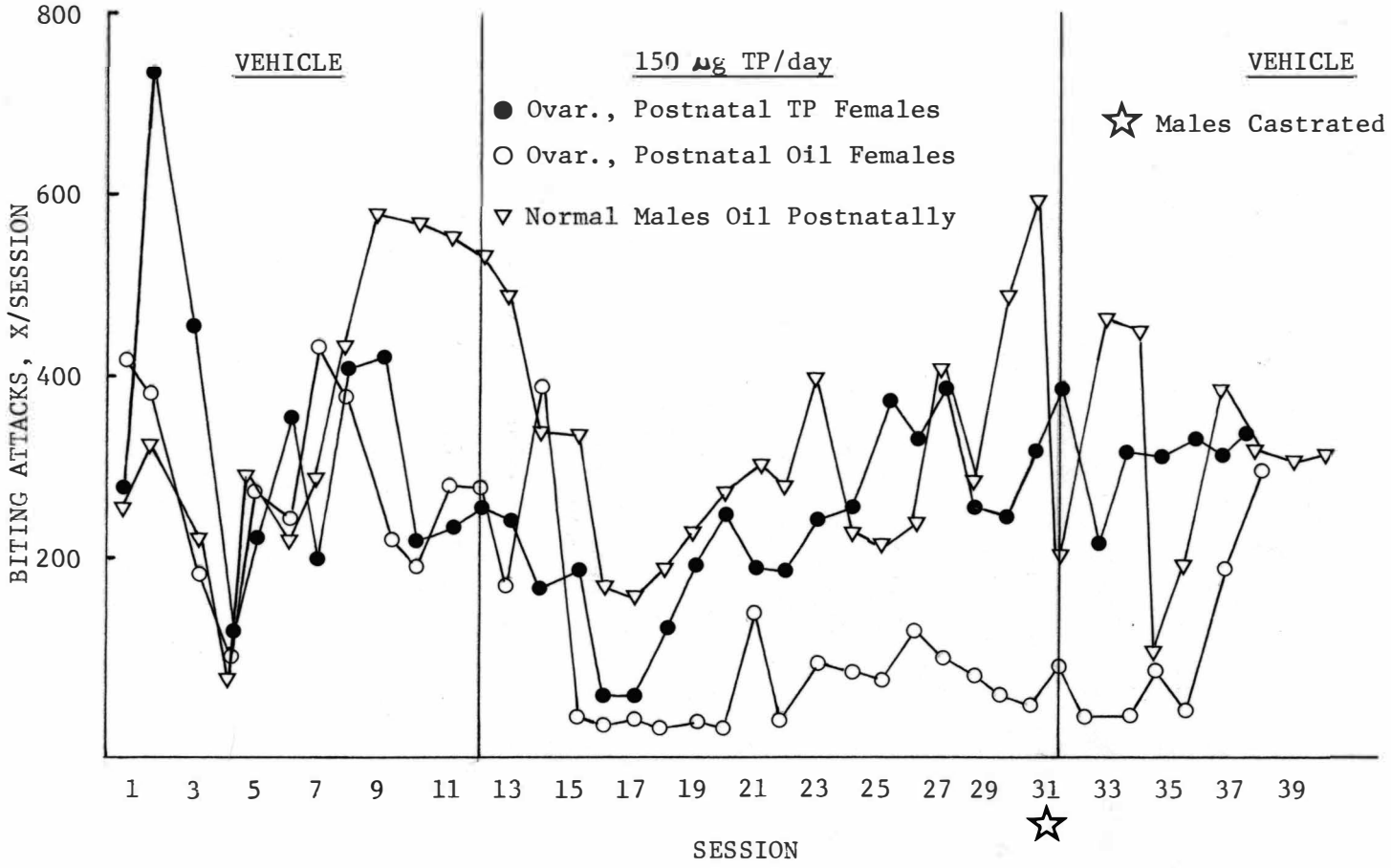
800
600
400
200
BITING ATTACKS, X/SESSION
VEHICLE
150 µg TP/day
VEHICLE
● Ovar., Postnatal TP Females
○ Ovar., Postnatal Oil Females
▽ Normal Males Oil Postnatally
☆ Males Castrated
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
SESSION

FIGURE 2

During the next twenty sessions testosterone propionate was administered daily following each session. Again there was no statistical difference in mean bite frequencies when a comparison was made among the three groups. The control male bite frequency was the greatest. Analysis of mean bites per session on days 12 through 17 revealed control males having the greatest at 314, PN-oil, Ov females 183 and PN-TP, Ov females 133. The mean bite frequency for control males was 337, for TP-PN, Ov females was 208, and for PN-oil, Ov females was 81. This relationship was not altered in sessions 24 through 30. The reinstatement of vehicle injections during sessions 31 through 37 again did not alter this relationship and again there was no significant difference among mean bite-attack levels.

Males were castrated on day 33 of testing and were allowed one week for recovery. Castration did not significantly alter mean bite-attack levels. Preoperative mean bite-attacks on days 24 through 30 were 357 and postoperative mean bite-attacks from days 33 through 40 were 292.

Phase II

In phase two there was no significant difference between pre- and postnatal TP, ovariectomized females (PPN-TP, Ov) and pre- and postnatal oil, ovariectomized females (PPN-oil, Ov) during the initial 12 sessions (Figure 3). The mean bite frequency from sessions 1 through 6 for PPN-TP, Ov females was 297 and for PPN-oil, Ov females was 286. In the following six sessions the mean bite-attack frequency for PPN-TP, Ov and PPN-oil, Ov females was 110 and 44 respectively. Daily 150 ug TP injections (sessions 13-25) significantly increased bite-attack

Figure 3. Plot of bite-attack means over sessions for phase II. Adult treatments are indicated above graph in appropriate time sequence. TP = testosterone propionate; OVAR = ovariectomy; ●—● = Ovar.,pre & postnatal TP females; ○—○ = Ovar., pre & postnatal oil females.

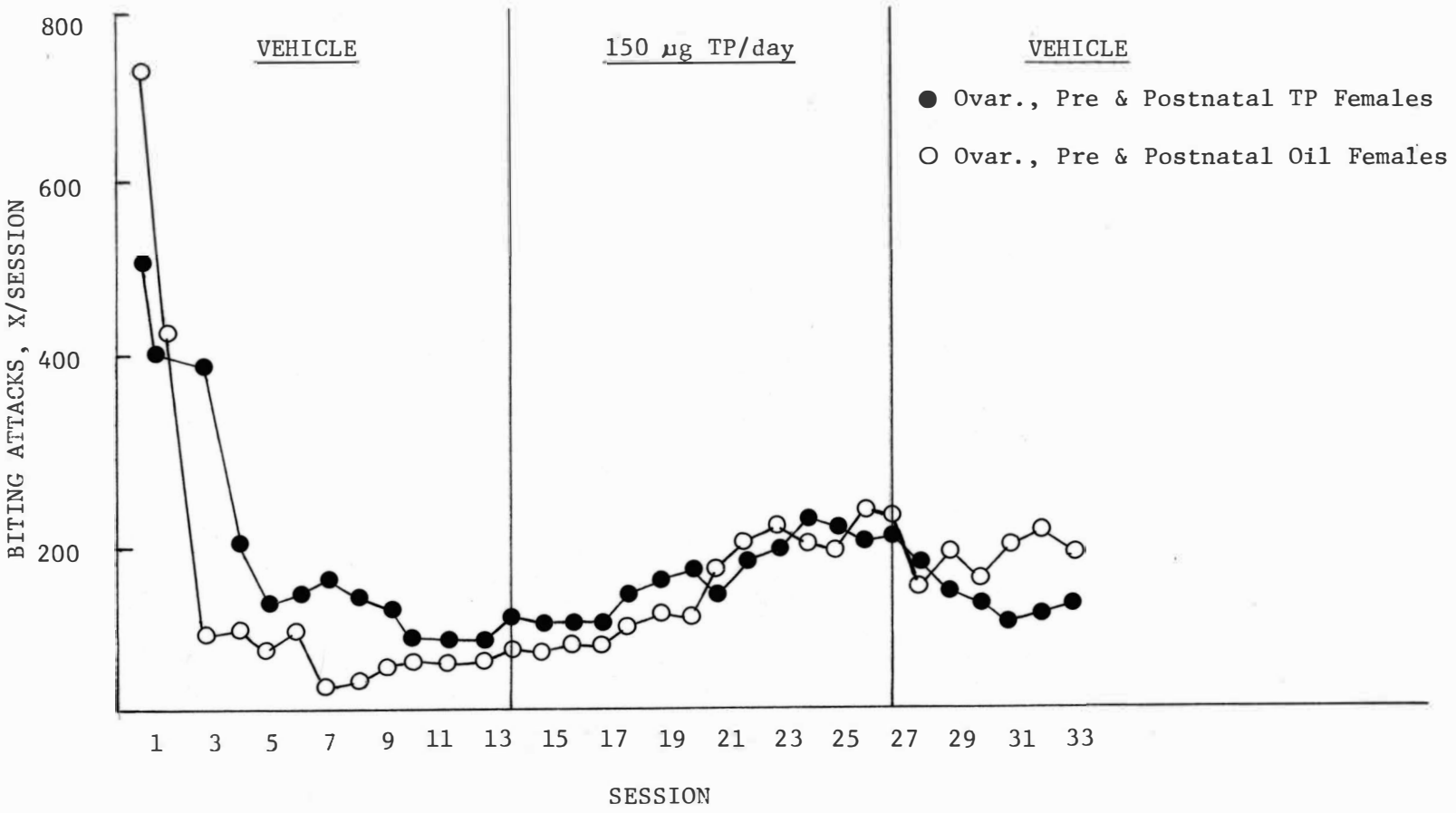
800
600
400
200
BITING ATTACKS, X/SESSION
VEHICLE
150 µg TP/day
VEHICLE
● Ovar., Pre & Postnatal TP Females
○ Ovar., Pre & Postnatal Oil Females
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
SESSION

FIGURE 3

levels in both groups as compared to predrug bite-attack levels. However, the groups did not significantly differ from one another. During sessions 22 to 24, TP dosages were increased from the standard 150 ug/day to 250 ug/day. Increased TP dosage did not significantly alter bite-attack level as compared to the bite frequency of mice treated with vehicle only. The final six sessions of vehicle administration revealed a significant reduction in bite frequency of the PPN-oil, Ov females from the previous five session mean of 200 to 104, but no significant change over time for PPN-oil, Ov females. There was not a significant difference between the two group means,

Phase III

Phase three (Figure 4) was a comparison of bite-attack frequencies among prenatally TP treated, ovariectomized females (PN-TP, Ov) and prenatally oil treated, ovariectomized females (PN-oil, Ov). During sessions 1 through 9 there was no significant difference between animals that had been exposed to TP or oil prenatally. There was a significant reduction in the mean bite-attack frequency for the PN-TP, Ov females over time. The means for days 1 through 4 of PN-TP, Ov females and the PN-oil, Ov females were 78 and 84 respectively. The means for sessions 5 through 9 were 2 for the PN-TP, Ov group and 87 for the PN-oil, Ov group.

Testosterone injections began on session 10 and continued through session 25. There was a significant decrease in PN-TP, Ov females' bite-attack frequencies as compared with predrug levels. Finally, there was a significant difference between the mean bite-attack

Figure 4. Plot of bite-attack means over sessions for phase III. Adult treatments are indicated above graph in appropriate time sequence. TP = testosterone propionate; OVAR = ovariectomy; ●—● = Ovar., prenatal TP females; ○—○ = Ovar., prenatal oil females.

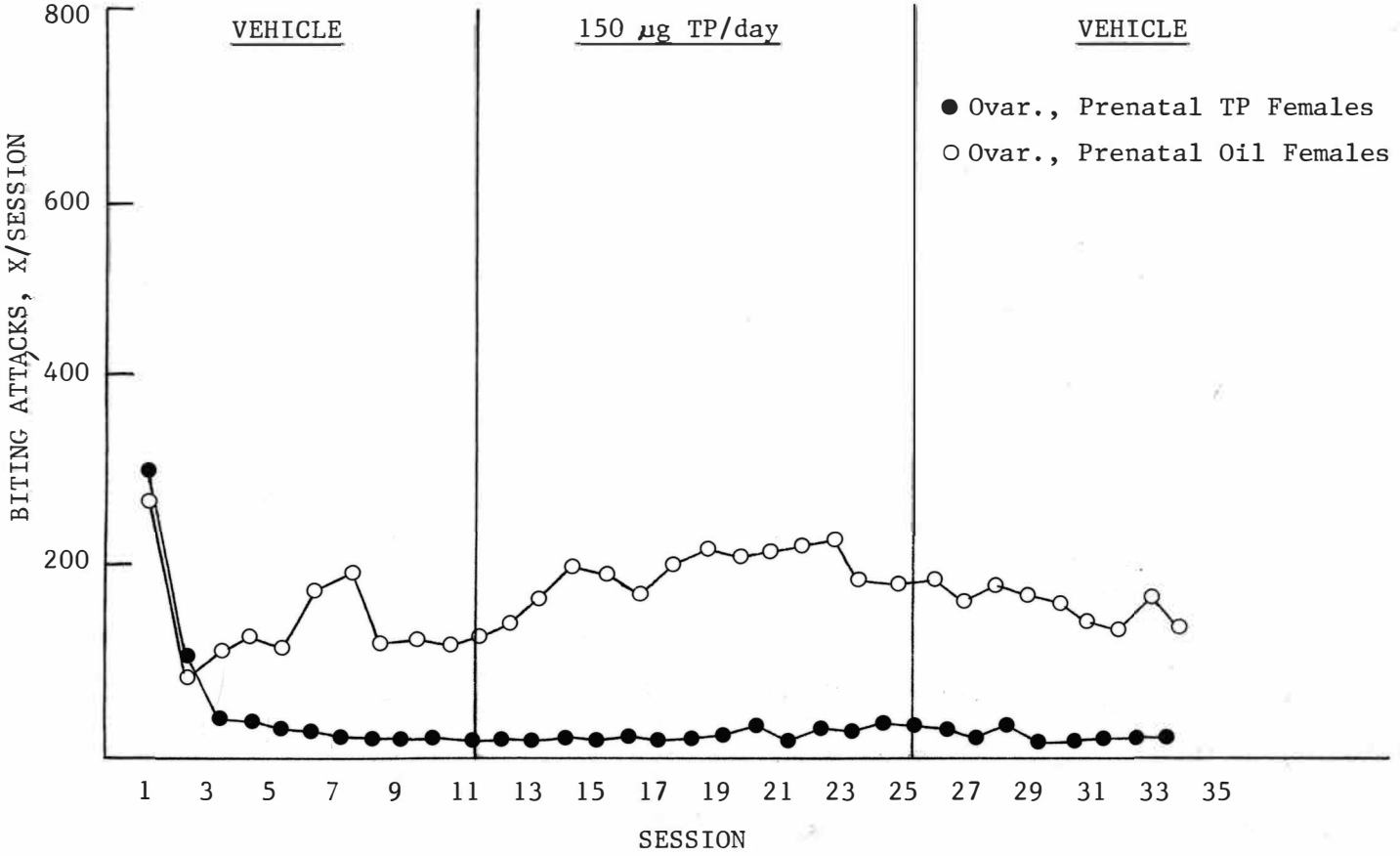
VEHICLE
150 µg TP/day
VEHICLE
● Ovar., Prenatal TP Females
○ Ovar., Prenatal Oil Females
BITING ATTACKS, X/SESSION
800
600
400
200
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
SESSION

FIGURE 4

levels of the two groups. Analysis of sessions 10 through 17 means found PN-oil, Ov group's mean 161 and PN-TP, Ov group's mean 10. Sessions 18 through 24 means for PN-TP, Ov females and PN-oil, Ov females was 24 and 209 respectively. This relationship was not altered when vehicle injections were reinstated. There remained a significant difference between PN-TP, Ov females' and PN-oil, Ov females' bite-attack means in sessions 26 through 33. Mean bite-attack frequency for PN-TP, Ov females was 24 and PN-oil, Ov females, 195.

Runwheel Activity

Runwheel activity was not significantly altered by any hormone administration. Figure 5 represents phase one runwheel activity; there were no significant differences in phase two and three in relation to runwheel activity. There also was no significant difference between the activity of any group in the first three phases except following the surgical castration of the control males in phase one. Castration produced a significant reduction in runwheel activity. There was a significant increase over time in the runwheel activity of all groups.

Paired Fighting Tests

The results from the paired fighting tests are summarized in Table 2. Paired fighting was examined for phases two, three and four. The correlation between total paired fights and total biting attacks was $r = 0.00$ for phase two and $r = 0.41$ for phase three. Correlation results were considered significant at an 0.05 alpha level. The correlation value for phase four was 0.00.

Figure 5. Plot of free wheel revolution means over sessions for phase I. Adult treatments are indicated over graph in appropriate time sequence. TP = testosterone propionate; OVAR = ovariectomy; ●—● = Ovar., postnatal TP females; ○—○ = Ovar., postnatal oil females; △—△ = normal males, oil postnatally; ☆ = males castrated during adult testing.

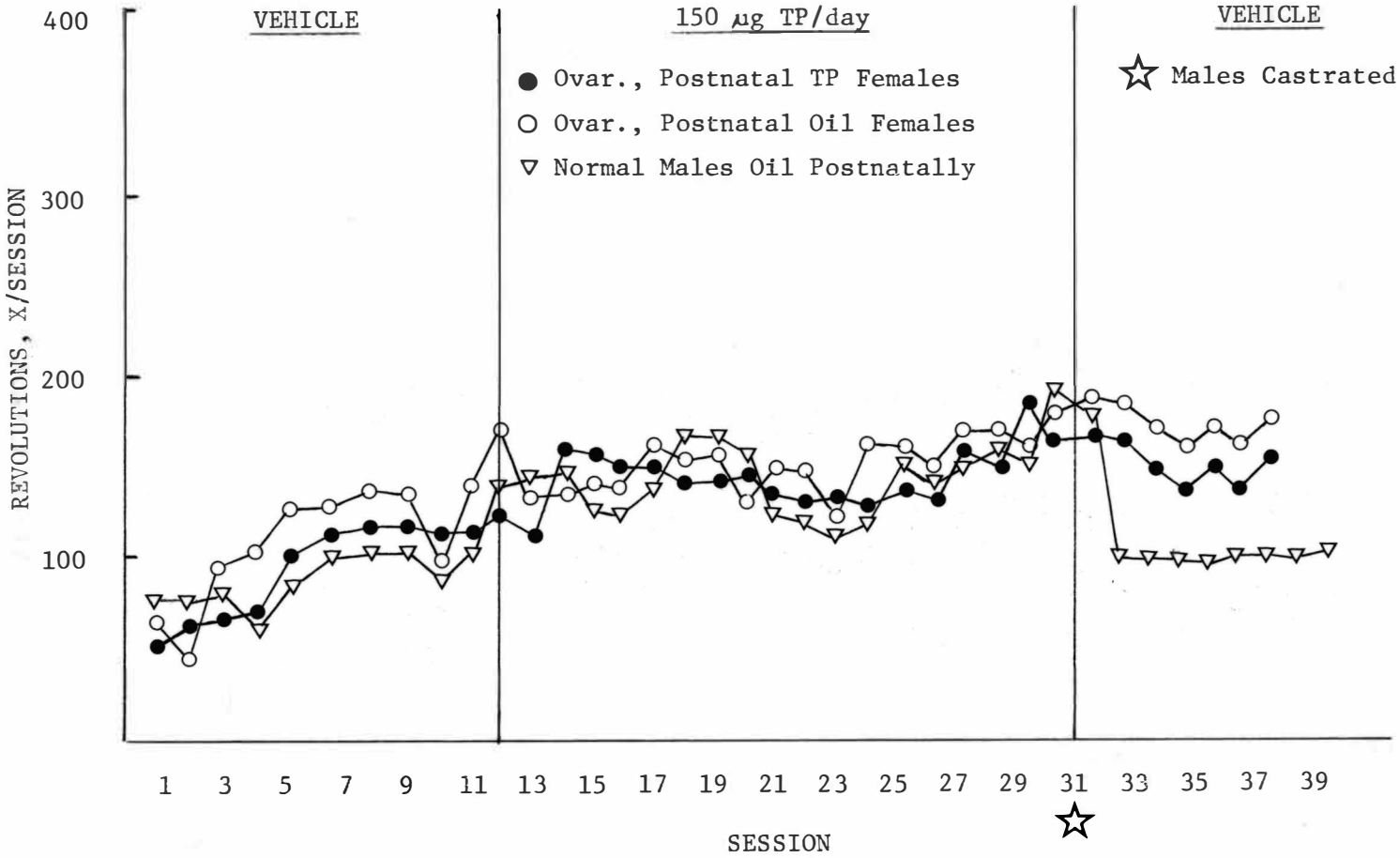
400
300
200
100
REVOLUTIONS, X/SESSION
VEHICLE
150 µg TP/day
VEHICLE
● Ovar., Postnatal TP Females
○ Ovar., Postnatal Oil Females
▽ Normal Males Oil Postnatally
☆ Males Castrated
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
SESSION

FIGURE 5

TREATMENT	NO. PAIRS	AVERAGE # OF FIGHTS ADULT TESTING		AVERAGE LATENCY	
		VEHICLE	TP	VEHICLE	TP
PHASE II Pre & Post-natal TP	3	0	0	300 $\pm$ 0	300 $\pm$ 0
PHASE II Pre & Post-natal oil	3	0	0	300 $\pm$ 0	300 $\pm$ 0
PHASE III Prenatal TP	3	0	45	300 $\pm$ 0	63 $\pm$ 5
PHASE III Prenatal oil	3	0	0	300 $\pm$ 0	300 $\pm$ 0
PHASE IV Postnatal TP	3	0	0	300 $\pm$ 0	300 $\pm$ 0
PHASE IV Postnatal oil	3	0	0	300 $\pm$ 0	300 $\pm$ 0

Table 2. Averaged latency and pair-fighting data from phases two, three and four. The scores were averaged from two observers. Latency values are in seconds. A fight response was scored if either mouse lunged at its opponent in a biting attempt. TP = testosterone propionate.

Organ Weights

The effects of hormonal and surgical manipulations are summarized in Table 3. In phase one, PN-TP, Ov females' adrenal and uterus weights were not significantly different than those of the PN-oil, Ov females.

In phase two, PPN-TP, Ov feamles' uterus weights were significantly greater than PPN-oil, Ov female controls, whereas adrenal weights were not significantly different from the controls.

In phase three PN-TP, Ov females had a nonsignificant reduction in uterine weight as compared with PN-oil, Ov females. Again, there was no significant difference in two groups' mean adrenal weight.

Histological examination of all ovaries revealed that there was an increase in the number of observed vesicular and cystic follicles and a decrease in the number of corpora lutea in the TP treated females as compared to control females.

Phase IV

Phase four postnatally treated with TP, ovariectomized females (PN, TP, Ov) as compared with postnatally treated with oil ovariectomized females (PN-oil, Ov) had no significant differences in mean bite-attacks in sessions 1 to 5, 6 through 11, or 12 through 17. TP injections began on day 18 and continued daily through day 35 of testing. Analysis of group means on days 18 through 23 resulted in no significant differences. Analysis of mean bite-attacks on days 24 through 29 and 30 through 35 revealed a significant increase in PN-TP, Ov female mean bites as compared with that of PN-oil, Ov females. Means for PN-TP, Ov females

Table 3. Body and organ weights of all animals from phases one, two and three. All weights were in milligrams (mg) with the exception of body weight which is in grams (g). Measurements were taken following adult bite-attack testing. $\bar{X}$ = mean; R = range; TP = testosterone propionate.

PHASE I

		BODY WT. (g)	ADRENAL WT./PR. (mg)	UTERUS WT. (mg)	SEMINAL VESICLE WT. (mg)	PROSTATE WT. (mg)
Postnatal treated TP females	$\bar{X}$	35.1	1.0	15.5		
	R	31-40	0.7-1.5	11.5-20.8		
Postnatal treated TP females	$\bar{X}$	38.4	0.7	38.0		
	R	34-41	0.4-1.0	11-50.4		
Normal male & adult castration	$\bar{X}$	44.8	1.1		3.8	9.9
	R	42-46	0.6-1.4		2.2-7.0	7.8-13

PHASE II

Pre & post-natal TP females	$\bar{X}$	41.4	1.35	49.7		
	R	37-46	1.2-1.7	21-80		
Pre & post-natal oil females	$\bar{X}$	36.1	0.8	22.7		
	R	30.9-39.8	0.6-1.2	18.7-28.5		

PHASE III

Prenatal treated TP female	$\bar{X}$	35.9	1.6	5.4		
	R	28.7-41.7	1.3-1.9	4.6-6.7		
Prenatal treated oil female	$\bar{X}$	44.3	1.4	13.8		
	R	41-48.7	1.3-1.5	11.6-18.3		

Table 3

and PN-oil, Ov females from days 20 through 25 was 72 and 41, respectively. That relationship was not immediately altered when TP injections were discontinued and vehicle injections reinstated. There remained a significant difference between bite-attack means of the PN-TP, Ov females and the PN-oil, Ov females during sessions 36 through 41. Respective means were 64 and 24. Means from sessions 42 through 47 revealed that again there was no significant difference between groups (Figure 6).

Paired fighting tests (Table 3) revealed that no animal in any pair from either the PN-TP, Ov female group or the PN-oil, Ov female group fought. The resultant statistical correlation value was 0.00. This was consistent whether or not TP was administered during adult testing.

All PN-TP, Ov females were morphologically virilized which was characterized by a decreased vaginal opening and a hypertrophied clitoris. PN-oil, Ov females were not genitally masculinized.

Figure 6. Plot of bite-attack means over sessions for phase IV. Adult treatments are indicated above graph in appropriate time sequence. TP = testosterone propionate; OVAR = ovariectomy; ●—● = Ovar., postnatal TP females; ○—○ = Ovar., postnatal oil females.

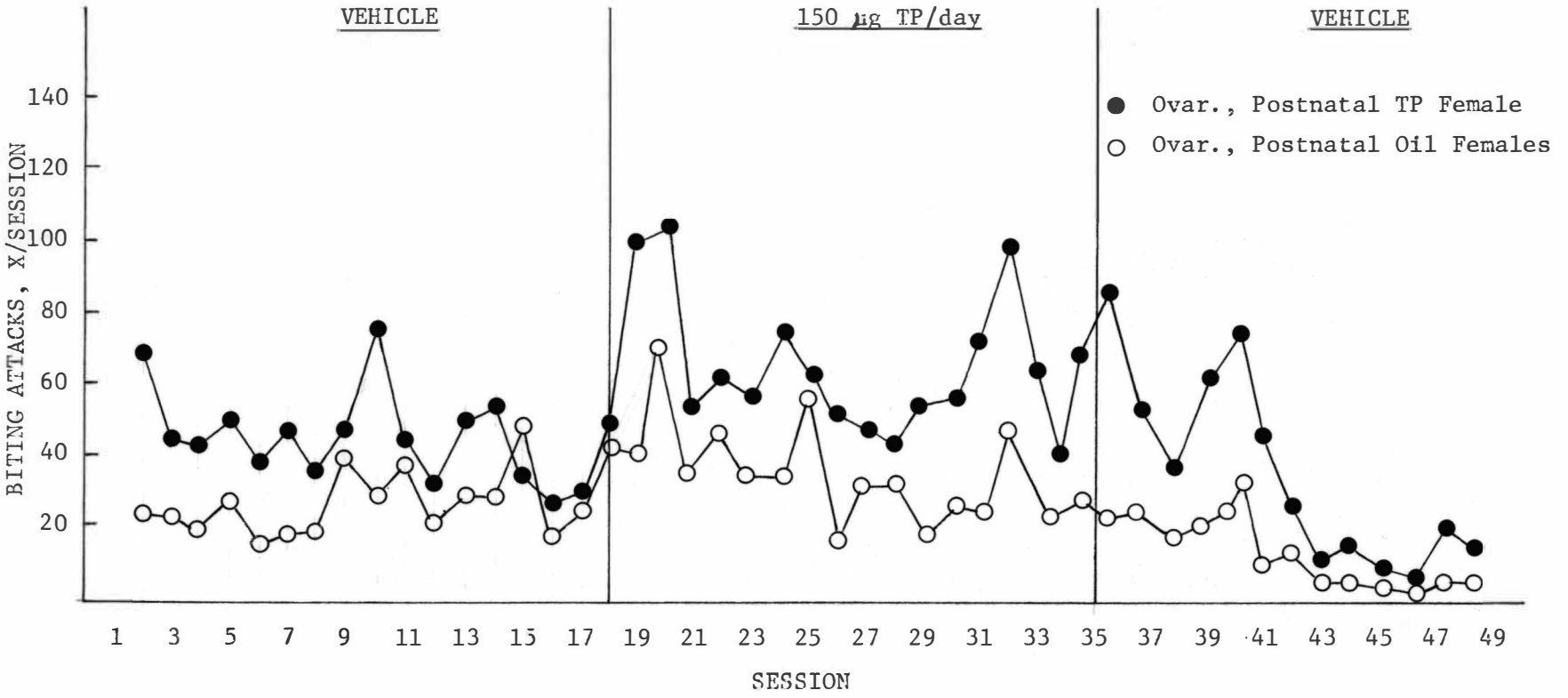
VEHICLE
150 µg TP/day
VEHICLE
Ovar., Postnatal TP Female
Ovar., Postnatal Oil Females
BITING ATTACKS, X/SESSION
140
120
100
80
60
40
20
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49
SESSION

FIGURE 6

DISCUSSION

The purpose of this study was to examine the single subject design's ability to measure aggressive behavior which historically has been measured with the paired subject design. The single subject design provided for efficient measurement of large numbers of subjects' bite-attack behavior.

Graphical representation of phase one results suggest that there was a difference among groups' daily mean bite-attack, but statistical analysis did not reveal a difference among means. This result was partially due to a large amount of intragroup variability of individual bite-attacks during a given session. This variability may have resulted from a difficulty in maintaining a consistent 1.5 gram sensitivity of the modified telegraph key was replaced by a 1.5 gram pull microswitch. There also existed a variation in individual subject response to the experimental dosage of TP as indicated by both behavioral and morphological criteria. The large range of weights of uteri and adrenals from both experimental and control groups tends to negate any consistencies between the two groups physiological response to TP. A possible explanation for this inconsistency may be that either there existed a significant difference in the manner in which some animals respond to similar quantities of exogenous testosterone, or that adrenal estrogens were compenstaing uterine development in some animals in response to the lack of ovarian estrogen. The adrenal is a known source of progesterone, estrogen and androgens, although

the latter is thought to be secreted in minute amounts (Baird, 1972).

Phase four was designed to repeat phase one's experimental design with the exception that a microswitch was substituted for the modified telegraph key. The microswitch had an activation sensitivity of 3 grams. The results more clearly demonstrated that there is a significant difference between the bite-attack frequency of females receiving TP during the perinatal sexual differentiation period and those only receiving oil during the same period. The resulting low correlation (0.00 and 0.41) between the bite-attack means and the paired fighting data (females did not pair fight, but they did bite-attack) may indicate that the bite-attack is a more sensitive measure for individual aggressive potential than intragroup paired fighting. It could also mean that the bite-attack response has behavioral components beyond those commonly thought to originate due to aggression. Individual variations in response to pheromones and other variables affecting primarily sexual behavior may also be affecting the bite-attack response. Also, the size of the animal in relation to the aversiveness of the restraint apparatus and the general exploratory behaviors need to be considered as variables modifying bite-attack.

Phase two results indicated that combined pre and postnatal TP administration to female mice did not significantly alter their bite-attack frequency regardless of whether or not TP was readministered in adulthood. One possible explanation for these results is that the experimental levels of TP administered prenatally may have suppressed the development of the neural correlates of female aggressive behavior rather than promoting the development of the male type. This prenatal

suppression may then act to desensitize the animal to the neurological masculinization effects of postnatal TP. However, this research offers no neurophysiological data to support or refute this hypothesis. Again, there was no significant difference in uterus and adrenal weights between experimental and control groups. Paired fighting showed that no animal fought when paired. These results may further support the idea that the bite-attack response is a very sensitive measure of aggression as compared with the more subjective measurement of paired-fighting.

Phase three results indicate that prenatal TP administration significantly lowered the bite-attack response of PN-TP, Ov female mice as compared to the response of the PN-oil, Ov female mice. Results were consistent regardless of whether TP was readministered during adult testing. Paired fighting data indicated that neither group would fight when receiving vehicle injections in adulthood. TP injections during adult testing altered the paired fighting, in that prenatally TP-treated animals did pair fight in adulthood. The control females derived from mothers treated with oil during preganancy did not pair-fight under TP or vehicle treatment. This low correlation between pair fighting and bite-attack frequency is somewhat contradictory to Wagner's (1979) data except that he examined a male CF1 mouse population.

It is possible that extended exposure to the aversive stimulus of the restraint chamber may cause the animal to habituate its response to the bite target. This was substantiated by the finding that control

males bite-attack frequency did not change following castration, as was expected on the basis of Wagner's findings. Males had been tested in the apparatus for 31 days prior to castration. Wagner's (1979) findings that males reduced bite-attack following castration resulted from a limited exposure to the apparatus of 5 days prior to castration. The proposition of habituation is further supported by the findings that in phases 1 through 3 bite-attack level did not significantly change when TP injections were discontinued and vehicle reinstated. It may be that the sensitivity of the single subject design is limited to a specific duration of continuous testing.

All phases confirmed Wagner's findings that runwheel activity is not dependent on or related to bite-attack frequency. Runwheel activity was found not to be related to the presence of circulating androgen in the masculinized or nonmasculinized female mouse.

The results of the present experiment suggest that the critical period for masculinization of the female mouse aggressive bite-attack is more likely to be closer to the time of birth than during days 16 through 20 of gestation. It is also necessary to consider that the perinatal TP dosage may have been an insufficient amount to potentiate complete masculinization. TP sensitization on days 1 through 6 postnatally was adequate to produce elevated bite-attacks above that of controls when tested in adulthood. This is consistent with previous paired fight data from Edwards (1969). Edwards found that female mice given TP on the first day following birth demonstrated more paired fighting than did control females and that females receiving TP on the

tenth day following birth also demonstrated more aggressiveness than did control females.

It is still unclear whether the low correlation between bite-attack data and paired fight data is largely due to extended sensitivity of the single subject bite-attack behavior paradigm picking up a low level of aggression not detectable in a five minute paired-fight test or whether the bite-attack behavior has components other than those aggressive behaviors observed in the paired-fight paradigm.

There is a need to decrease intragroup variation. In order to eliminate the high degree of intragroup variation one might preselect from a specific strain those animals which demonstrate a high level of either bite-attack or paired fighting. It would be productive to incorporate a more exact means to monitor individual circulating hormone levels and resultant physiological effects. It may prove necessary to monitor the circulating levels of TP in the maternal circulation and in the newborn. Thus, it would be possible to control more of the variables contributing to the observed individual variance in both the physiological and behavioral responses to TP. There also may be a need to adrenalectomize the newborn to reduce the complications in masculinization generated by adrenal synthesized steroid hormones.

This research also indicates that female mice demonstrate bite-attack means similar to that of control males during their initial exposure to the confinement of the restraint apparatus. This observation also contradicts a well-documented fact that female mice are not as aggressive in a paired-fight situation as are males. In all

studies immediate exposure to the restraint tube produced similar bite-attack responses from both male and female mice.

This study further confirms that neonatal exposure to TP does enhance the bite-attack response of female mice if TP is readministered in adulthood. It is also suggested that prenatal exposure to TP may actually act to suppress the development of neurobehavioral correlates underlying adult aggressiveness. Finally, this study clearly demonstrates the need to further examine concomitantly other possible dependent variables that may be affecting the specificity of bite-attack as a measure of aggression.

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