EFFECTS OF EARLY HORMONAL AND REARING CONDITIONS ON THE MATING BEHAVIOR OF THE MALE RAT.

by

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In a search for similarities in the disrupting effects of early androgen-deprivation and social isolation on the male rat's sex behavior, forty-eight male hooded rats were studied in a 2 x 2 x 2 factorially designed experiment under the following conditions: sham vs. anti-androgenic injection administered pre- and early postnatally, non-castration vs. castration, and group-rearing vs. isolation. All abnormal treatments reduced mating. Early androgen-deprivation was found to have an effect on the ejaculatory response. Isolation induced the occurrence of non-mating behaviors and had an additive effect when combined with other abnormal treatments. The findings were discussed with reference to the neural organizational role of early androgen, the competing response hypothesis, and the role of sensory feedback on mating. It was concluded that early androgen and social contact, rather than having an organizational role, both increase the probability of mating by providing optimal conditions for the facilitation of "prewired" neural structures.

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INTRODUCTION

The mating behavior of a normal male rat is a well-organized, biologically effective sequence of separate motor reactions (Beach and Holz, 1946). Upon encountering an estrous female, the male engages in a prolonged investigation of the female. He usually starts by nibbling the neck region of the female and then proceeds to sniff and lick her genitals. During this period, there is a visible increase in the state of arousal of the male which culminates in his mounting the female and achieving intromission. Once mounting has taken place, other activities subside and the male proceeds to perform a series of mounts, each followed by autogenital grooming. Ejaculation occurs after several intromissions (usually from five to twenty) and terminates the mating sequence. Following ejaculation, the male waits about five min. before engaging in mating again. Each new series of mounts is preceded by a brief period of neck-biting and genital sniffing and is terminated by ejaculation. In the normally treated male rat, neither initial susceptibility to sexual arousal nor overt copulatory acts appear to depend

on the animals having had previous sexual experience (Beach, 1942). Adult male rats can copulate successfully during their first encounter with an estrous female. There is, however, a period in late fetal and early postnatal life during which the development of the behavior can be disrupted. During this period sexual differentiation is taking place. The first sign of differentiation, and that which is under genetic control, is the proliferation of the primitive gonads into either ovaries or testes. In a series of experiments Jost (in Jones and Scott, 1958) showed that the gonads appear to be responsible for a considerable amount of further differentiation into either the male or the female type. It is the gonadal hormones which regulate the differentiation of the external genital system (Burns, 1961). Furthermore, there is a growing body of evidence that the gonadal hormones have an effect on the developing neural structures in the central nervous system which control the adult patterns of gonadotropic secretion and sexual behavior. Simply stated, the basic findings are that in the absence of testicular hormones (androgens) during these periods of development, both genetically male and female organisms develop female external genitalia, a cyclic pattern of gonadotropic secretion, and easily elicitable

female behavior patterns. When androgens are present during these critical periods, male external genitalia develops, the gonadotropic functioning becomes acyclic (or the male pattern), male behavior patterns are predominant and female behavior patterns are rarely elicitable (Phoenix, Goy, Gerall, and Young, 1959; Grady and Phoenix, 1963; Feder and Whalen, 1965; Harris and Levine, 1965).

It has been suggested (Harris, 1964; Young, 1961; and Young, 1965) that gonadal hormones act on the central nervous system in different ways at different stages of development. During fetal life, the presence or absence of these hormones organizes the brain with regard to gonadotropic secretion and sexual behavior. Levine and Mullins (1966) suggested that the organization is accomplished by sensitizing the tissues of those parts of the central nervous system involved in the integration of sexual acts. During adult life, the presence of gonadal hormones activates the already organized brain structures, and, in the presence of appropriate stimuli, elicits the responses that have been programmed earlier.

These conclusions are based on experiments which involve the use of two techniques for interfering with normal hormonal conditions and hence with normal mating behavior:

either the administration of exogenous hormones pre- and postnatally or the removal of hormone producing endocrine glands during the early postnatal period. By the use of these methods, a large body of evidence has been accumulated on the effect of hormonal interference on the mating behavior of the male rat. In one set of experiments, an anti-androgenic substance was administered to male rats. Anti-androgenic substances block the action of testicular androgen during the administration of the substance, but it does not block the action of androgen after the withdrawal of the substance. (Neumann, Elger and von Berswordt-Wallrabe, 1967). Neumann and Elger (1966) reported that pre- and postnatal anti-androgen treatment to male rats resulted in inadequate male mating behavior. Females were pursued, and the genitals were sniffed and licked. Mounting took place, but it was said to be less purposeful than in normal animals. Ejaculation was reduced. Such anti-androgenically treated males were reported to behave bisexually; in the presence of a mounting male they behaved as females and were regarded as such by male animals. Zucker (1966) reported that although anti-androgenically treated male rats showed the components of normal male sexual behavior, they had a low incidence of ejaculation.

In another set of experiments castration was employed to disrupt normal male mating behavior. Beach and Holz (1946) castrated groups of male rats at the ages of day 1, 25, 50, 100 and 350. Day 1 castrates treated with androgens in adulthood, although they displayed a high degree of excitement and interest in an estrous female, were unable to mount and clasp the female properly, had a low incidence of complete mounts, and almost never ejaculated. Similar findings were reported by Gerall (1967) for one-day old castrated males. Further evidence for the role of androgen comes from a study in which neonatally castrated rats given a single injection of exogenous androgen within a few hours after castration were compared to non-injected castrates. When both groups were tested in adulthood after androgen replacement, the animals having the single neonatal injection of androgen exhibited patterns of complete copulation more often than the non-injected group. However, neither group ejaculated (Mullins and Levine, 1968). These experiments seem to indicate that early androgen deprivation, although it does not abolish mating behavior completely, does interfere with the normal pattern. It should be noted, however, that male animals castrated before three days of age or treated with anti-androgenic substances

pre- and postnatally show retardation of genital development (Beach and Holz, 1946; Neumann and Elger, 1966; Gerall, 1967). A recent experiment by Beach, Noble and Orndoff (1969) investigated the relation between genital anatomy and coital reactions. Groups of neonatally castrated male rats were injected with androgen on two consecutive days at different days of life. When adult, they were given further androgen injections and were rested for male sex behavior. The external and internal sex organs were examined. The finding was that animals given the postnatal androgen treatment early in life showed more intromissions and had more developed penes than animals receiving the same treatment later. There was a high correlation between the size of the penis and the frequency of intromissions. Thus, any explanation of the behavioral effects of these treatments must take into account the possible role of the presence or absence of early androgens on both neural functioning and external genital development.

There is another line of evidence which indicates that interfering with hormonal function is not the only way to interfere with normal male sex behavior. Although Beach (1942a, 1958) and Kagan and Beach (1953) reported normal sexual behavior in adult male rats raised from

weaning in individual cages, several recent investigators have demonstrated that early rearing conditions can affect the occurrence and proficiency of male sex behavior. For example, Folman and Drori (1965) showed that male rats reared in isolation were inadequate copulators as compared to animals reared in groups. Isolated animals, however, exhibited lively interest in the female; they nuzzled and sniffed the female, and circled them with increasing speed. Similar findings were reported by Gerall, Ward and Gerrall (1967a); isolated animals were found to be inadequate copulators, but they showed high interest in the female. In another experiment (Drori and Folman, 1967) male rats reared with males were found to be less competent copulators than male rats reared with females. It is important to note that in this latter experiment the males reared with males had less developed genitals than the males reared with females, and that there was a significant correlation between penis weight and the number of ejaculations. The reasons for the conflicting findings between the earlier and later investigations in rats are not clear at this stage. The disrupting effect of isolation on mating behavior has been reported for other species. Valenstein, Riss and Young (1955) have evidence from guinea-pigs and there is a growing

body of evidence that in higher mammals early social deprivation can cause irreparable damage to adult sexual behavior. Male rhesus monkeys raised under extreme social deprivation have been reported to display inadequate and disoriented mounting and copulation patterns (Mason, 1960).

One common feature of the behavior of anti-androgen treated or early castrated male animals and of those reared in isolation is the persistent interest in estrous females even when copulatory behavior is inadequate. It would seem that some of the neural mechanisms underlying male sex behavior are activated by circulating androgens in the presence of the female, but that there is a deficiency in the integration of motor sequences involved in the copulatory act. The question then arises: is there a common factor that might account for the poor motor integration observed in both the hormone-treated and the isolated animals? The evidence regarding anatomical differences between the genitalia of hormone-treated and normal animals suggests that the lack of normal sensory feedback from the genitals may deprive these animals of an important source of sensory stimulation essential to the integration of copulatory motor sequences.

To assess the role of feedback from the genitals

in sexual behavior in the male rat, Beach and Holz (1946) subjected experienced normal adult animals to an operation in which part of the bone was removed from the shaft of the penis. The number of incomplete copulatory attempts increased and the number of ejaculations decreased in post-operative sex tests as compared to preoperative tests. Similar results were obtained by Adler and Bermant (1966) by applying local anesthesia to the glans and shaft of the penis of sexually experienced male rats. Therefore, sensory feedback from the genitals appears to be an important mediator of normal male sex behavior in the rat, and it is possible that inadequate copulation is at least partially attributable to the abnormality of sensory feedback.

It can be hypothesized that in order for adequate adult male copulatory behavior to occur, animals must have the opportunity to receive sensory stimulation. This stimulation is altered by the abnormal development of the penis in the case of anti-androgen treated and castrated animals. While in the case of isolated animals, possible morphological abnormalities and the absence of sensory stimulation arises from lack of bodily contact with other animals.

The purpose of the present study was to investigate the effects of hormonal and environmental conditions during development on the sex behavior of the adult male rat. Specifically, the experiment was designed to study the similarities or differences produced by the influence of androgenic hormones at two different periods of development and by the influence of rearing conditions. The use of an anti-androgenic compound made it possible to deprive male rats of androgens during late fetal and early postnatal life, that period during which androgens have been said to have profound effects on neural development. Castration at ten days of age, while not interfering with neural development, served to retard the growth of external genitalia. The absence of other animals during rearing reduced the possibility of sensory feedback from bodily contact. Although these factors have been separately investigated by various experimenters, the exact behavioral components affected by each condition have not been clarified. In the present study it was possible to undertake a detailed analysis of the mating pattern and compare the effect of the above variables within the same experiment.

METHOD

Subjects

Forty-eight male hooded rats of the Royal Victoria Hospital strain, born in the laboratory of Sir George Williams University were divided into eight groups and were given treatments as described in the section so entitled. From birth until weaning (about 21 days), the animals were raised with their mothers and littermates of both sexes. They were then housed in living cages either in isolation or two to a cage. The cages, measuring 8 in. wide, 8 in. high and 10 in. long, had solid metal walls on three sides, and wire-mesh fronts (Wahman Mfg. Co., Baltimore, Md., model number A-4107-11). The maintenance of the animals (cleaning, feeding, and watering) was done without disturbing or handling the animals. They were maintained on an ad libitum diet of Purina laboratory chow throughout the experiment. Tap water was available at all times, with the exception of a three-week period during which an antibiotic (Terramycin, Pfizer) was added in a prophylactic dose. The subjects were housed in a room where females used as lures were

also housed and were handled and exercised daily on a wooden stand covered with sawdust. Animals living in isolation were exercised alone, while the others were exercised with their cage-mates. The animals had contact with strange animals (females) only during the tests. The room temperature was kept constant at 74° Fahrenheit. The animal colony was maintained under a reversed light-dark cycle, the room being darkened from 8 a.m. to 8 p.m. and lighted artificially with overhead light during the other 12 hours. At the termination of the experiment the animals were sacrificed and an autopsy was performed. The external and internal reproductive organs were removed and the following measurements were taken: testicular weight, penis weight and length, combined weight of prostate, coagulating gland and seminal vesicle.

Treatment

Twenty-four of the animals were treated with an anti-androgenic substance, cyproterone (Schering A.G.), before and after birth. The prenatal treatment consisted of injections of 10 mg cyproterone per day in 0.2 cc of sesame oil given subcutaneously to the mothers for five days prior to parturition. The postnatal treatment started

on the day of birth and consisted of injections of 0.3 mg of cyproterone per day in 0.05 cc of sesame oil given subcutaneously to the pups for 21 days. The other 24 animals and their mothers were similarly treated with sham injections of 0.05 cc of sesame oil. The injections to the mothers and to the pups were given by inserting a 25-gauge needle under the skin at the nape of the neck. To prevent leakage, a drop of collodion was placed on the skin and then a 5-min. waiting period was allowed before returning them to their mothers so that the animals would not carry the odor of collodion which interferes with normal maternal responses.

At 10 days of age, half of the animals in the anti-androgen treated group and half of the animals in the sham injection treated group were castrated. These rats were anesthetized with ether and the testes were removed through two small lateral-ventral incisions. The incisions were then sutured and the animals were washed with gauze pad soaked in saline solution to remove the odor of the anesthetic. One hour after the operation the pups were returned to their mothers. The remaining animals were anesthetized, but not operated upon.

At 21 days of age, all the animals were weaned. Half of each group was housed two to a cage and half in isolation, thereby giving eight groups. The treatments given to each group are summarized in Table 1.

After initial testing, the castrated animals were given daily subcutaneous injections of 500 μg of testosterone proprionate in 0.1 cc of sesame oil as replacement therapy until the termination of the experiment.

Apparatus

A 12 x 12 x 12 in. observation box with transparent plastic walls and cover and a plywood floor was used for all testing. The tests were conducted in an air-conditioned room which was illuminated by a 60-watt red light bulb placed above the observation box, and by a 60-watt white light bulb placed against the wall to allow sufficient light for recording. A 20-db white noise served as masking throughout the experiment. A metronome with a signal every 2 sec. was used for taking time measures.

Procedure

On the day prior to the first test, all the animals were habituated to the observation box for a 15-min. period. Each mating test was further preceded by an additional 5-min. adaptation period before the female was introduced.

TABLE 1

SUMMARY OF TREATMENT

Designation of Group		Treatment	N
N	Normal	Sham injected	6
INT	Intact	Non-castrated	
GR	Group-reared	Reared two to a cage	
N	Normal	Sham injected	6
INT	Intact	Non-castrated	
IS	Isolated	Reared in isolation	
N	Normal	Sham injected	6
CAS	Castrated	Castrated at 10 days of age	
GR	Group-reared	Reared two to a cage	
N	Normal	Sham injected	6
CAS	Castrated	Castrated at 10 days of age	
IS	Isolated	Reared in isolation	
A-A	Anti-androgen treated	Anti-androgen injected	6
INT	Intact	Non-castrated	
GR	Group-reared	Reared two to a cage	
A-A	Anti-androgen treated	Anti-androgen injected	6
INT	Intact	Non-castrated	
IS	Isolated	Reared in isolation	
A-A	Anti-androgen treated	Anti-androgen injected	6
CAS	Castrated	Castrated at 10 days of age	
GR	Group-reared	Reared two to a cage	
A-A	Anti-androgen treated	Anti-androgen injected	6
CAS	Castrated	Castrated at 10 days of age	
IS	Isolated	Reared in isolation	

Throughout the experiment, mating tests were conducted during the dark phase of the reversed light-dark cycle. From the age of 80 days, the animals were given tests for male sex behavior in the presence of an estrous female at four-day intervals. Each test lasted for 15 min. Females in natural heat showing good receptivity were used. All animals received an initial series of five mating tests and, after 14 days of testosterone treatment, the castrates were given five additional tests at four-day intervals.

During each test, the behavior of the male animals was manually recorded on a mimeographed sheet. At each 2-sec. click of the metronome, the experimenter wrote down the type of behavior which had predominated during the interval. Thus the frequency of occurrence of different behaviors, the duration of time spent in any particular behavior, and the sequential record of behavior could be obtained from the data. The behavior categories regularly noted and counted included the following:

Neck-biting: the male nibbles the fur of the female sometimes on the back, but most often around the neck region.

Genital sniffing: The male sniffs and licks the genital region of the female. He usually approaches her from the back, but occasionally from the side.

Tail-sniffing: the male investigates the tail region of the female either by nibbling, by holding the tail with the forepaws or by following her while she locomotes.

Side-pushing: the male approaches the side of the female. He places his nose close to her hind quarters under her belly and makes quick, repeated, upward movements with his head.

Undercrawling: the male places his nose at the side of the female, pushes his head and then his body under her belly, and crawls out from under on the other side. His body is rubbed against the floor and the female. He often repeats this sequence, going back and forth in quick succession. He occasionally stays under the female, his body being squeezed between the floor and the female.

Sideling: the male approaches the side of the female. He presses his body sidelong against her and, maintaining this position, he follows her when she locomotes. This behavior is often accompanied by exploratory movements of the head.

Jumping: the male performs stationary jumps often accompanied by contortion of the body. These jumps usually occur after brief and violent neck-biting.

Darting: the male darts in a manner similar to an estrous female soliciting a male. He approaches the female usually from the front, nibbles her fur and then suddenly turns around and jumps ahead. When he comes to a halt, he is facing away from the female in a semi-sitting position, with hindlegs slightly apart.

Circling: the male locomotes quickly around his own axis or around the female, his head being turned to the side.

Leaping: the male makes a quick jump across the back of the female and rubs his underside against her back.

Climbing: the male approaches the female from the back. Standing on his hindlegs, he pushes his body on top of her and rubs his underside on the back of the female. He pushes his body until his frontlegs and hindlegs hang inertly on the side of the female, thus covering her body completely.

Phantom mounting: the male approaches the female either from the front or from the back, he presses his body against hers, lifts up one of the hindlegs, and draws it across her head or back. The sequence is always done in slow motion.

Holding: the male places both forepaws on the back of the female, but does not mount or palpate.

Incomplete copulation: the male clasps the female with his forepaws usually in the lateral-lumbar region but occasionally about the head or side, and exhibits rapid palpating movements with the forepaws. At the same time, he slowly moves his hindquarters in and out in piston-like fashion. The pelvic thrusts are terminated by the male relaxing the clasp and slipping backward.

Complete copulation: the pattern is similar to that of incomplete copulation. However, instead of slipping weakly off the female following the slow pelvic thrusts, the male terminates the pelvic thrusts with a more vigorous thrust in which his pelvic region strikes against the female's perineum. During this final forceful pelvic thrust, penis insertion (intromission) is achieved. Intromission is followed by a backward thrust; the male abruptly releases the female, lunges backward, and comes to rest in a sitting position.

Ejaculation: when intromission occurs, the termination of intromission is somewhat modified. The male does not release the female after the final pelvic thrust, but instead presses his hindquarters against the female and simultaneously slowly raises both forepaws. During this

prolonged intromission, ejaculation takes place and a vaginal plug is formed. Following ejaculation, the backward thrust, as described earlier, occurs.

Genital grooming: following copulation, the male sits and manipulates his own genital region. He uses his forepaws and mouth to lick the penis and the scrotum. This reaction is observed usually after complete copulation, but occasionally it accompanies incomplete copulation.

Grooming: the male licks all parts of his own body with the exception of the genital region. It is done usually in a sitting position.

Exploration: the male investigates the floor, the side, or the top of the observation box while sitting or rearing. At the same time, quick movements of the whiskers are observed. Occasionally, chewing of the floor or of litter on the floor occurs.

No activity: the male stays stationary in a sitting position and occasionally turns his head.

A few other behaviors were observed, but they did not occur with sufficient frequency to warrant detailed description. These behaviors were: fighting, rolling on

the back, pushing the female, and bending foreward in a sitting position which occasionally occurred following incomplete or complete copulation.

For the purpose of analysis, it was decided to group some of the behaviors into categories. The principle for grouping was based on the components of copulatory behavior as observed in a normal male. Thus, the following categories were established:

Behaviors exhibited by normal copulating males

1. Incomplete copulation.

2. Complete copulation.

3. Ejaculation.

4. Female-directed acts: neck-biting, genital sniffing, tail sniffing, and side pushing. These behaviors are characterized by the male's persistent investigation of the female. Contact with the female is made through the head of the male.

Behaviors rarely exhibited by normal copulating males

1. Abnormal copulation: leaping, climbing, phantom mounting, and holding. The elements of copulation are present in these patterns of behavior and contact with the female is established through the genital region of the male.

2. Contact behavior: sideling, and undercrawling. In these behaviors, the elements that are characteristic of copulation are not present. Neither the head, nor the genital region of the male is brought into contact with the female.

3. Arousal behavior: jumping, darting and circling. These behaviors do not involve contact with the female.

RESULTS

The first data to be considered are those from the five tests in which all the animals were observed for mating behavior under the influence of either endogenous (testicular) or exogenous (injected) androgen. The analysis of the five pre-injection tests for the castrated animals will follow. The treatment of scores and the analysis used to test the effects of the three main variables: normal vs. anti-androgen treatment, group-rearing vs. isolation, and non-castration vs. castration, and their interactions will be discussed under each activity.

Incomplete and complete copulations and ejaculations

The number of incomplete copulations, complete copulations and ejaculations during the five tests were totalled for

each animal. The group means, and the number of animals which engaged in each of these activities are shown in Figure 1. It can be seen that the number of animals which engaged in complete copulation was approximately the same for all groups. The observed differences tested with χ^2 were not significant. There was, however, a significant difference between the groups when the proportion of ejaculators among the complete copulators was examined. Among normal animals seven out of nine ejaculated, whereas among the anti-androgen injected animals, only one out of eight animals ejaculated. This difference was significant (Fisher Exact Probability Test, p=.025, one-tailed test). A similar analysis showed that more intact than castrated animals ejaculated. Among the intacts, five out of 6 animals ejaculated, whereas, among the castrates, only three out of eleven animals ejaculated (p=.05). A comparison between animals raised in groups and animals raised in isolation did not show significant differences.

In the case of the number of complete copulations, the three main effects were tested by comparing the scores of the groups using the normal approximation to the distribution of U of the Mann-Whitney U test. Table 2 gives means and the probabilities associated with the observed differences. The

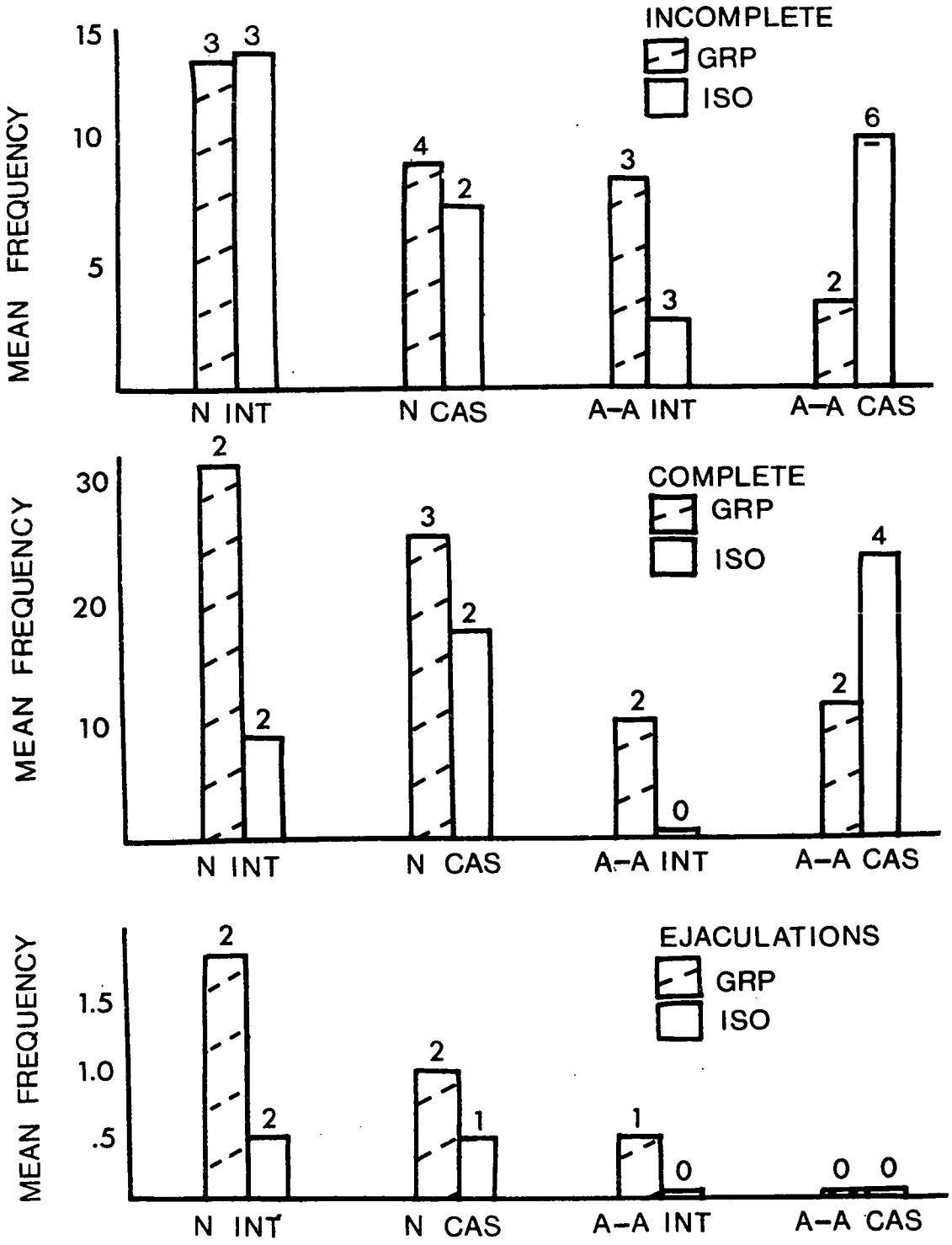

Fig.1. Mean number of incomplete copulations, complete copulations, and ejaculations per group in five mating tests. The number above the bar represents the number of animals in that group engaged in each activity.

TABLE 2

COMPLETE COPULATIONS UNDER THE INFLUENCE OF ANDROGEN
MANN-WHITNEY U TEST

Main effects tested

Group	Mean	Group	Mean	z	p
Normal	21.21	vs. Anti-Androgen treatment	11.04	.257	.3974
Non-Castration	13.29	vs. Castration	18.96	1.542	.0618
Group-rearing	20.04	vs. Isolation	12.21	.303	.3821

only comparison which approached significance was between intact and castrated animals ($p=.06$). Castrated animals engaged more frequently in complete copulations than intact animals.

The incidence of incomplete copulations was not analyzed separately. The inspection of Figure 1 suggests that the number of incomplete copulations varied concurrently with the number of complete copulations in all groups. A test for correlation indicated a high, positive relation between the two measures of copulatory behavior and yielded an $r=.90$ (Pearson product-moment correlation coefficient, $df=94$, $p<.01$, two-tailed test).

In summary, normal animals ejaculated more frequently than anti-androgen treated animals. Among all the groups, normal, intact, group-reared animals showed the highest number of complete copulations and ejaculations.

Female-directed acts

The scores for female-directed acts consist of the total time in sec. spent by each animal in these acts during the five tests. The group means are shown in Figure 2. Although there appears to be some tendency for normal animals to spend less time in female-directed acts than anti-androgen treated animals, a three-way analysis

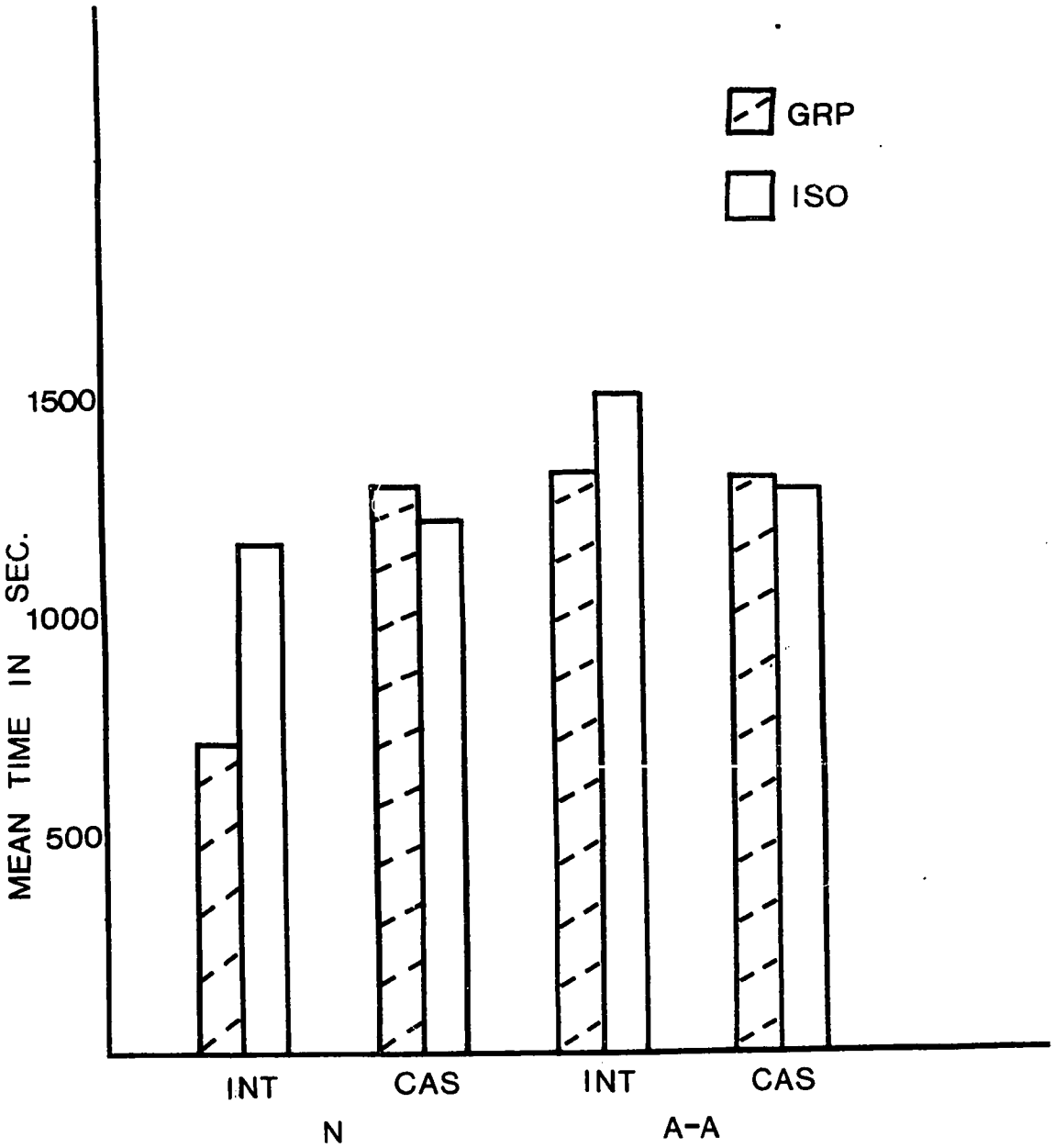

Fig. 2. Mean time spent in female-directed acts per group in five mating tests under the influence of androgen.

of variance did not yield any significant effects (Table 3).

Inspection of Figure 2 shows that among all the groups, the normal, intact, group-reared group spent the least time in female-directed acts. The mean for this group was 705.67 sec., and the next lowest mean was 1168.00 sec. for the normal, intact, isolate group. A comparison was made between the mean of the normal, intact, group-reared group and the combined weighted mean of all the other groups by the orthogonal technique. The comparison yielded a significant difference ($F=6.42$, df-1/40, $p<.05$, two tailed test). One further finding was that the group with the highest score for female-directed acts, the anti-androgen treated, intact, isolated group, was the only group having zero incidence of complete copulation. A comparison was made between the mean for this group (1506.00 sec.) and the mean for the non-copulating animals in the normal, intact, group-reared group (743.00 sec). The difference was significant ($t=2.78$, df=8, $p<.05$, two-tailed test).

Inspection of the data also showed that there were qualitative differences between the groups. In both, normal and anti-androgen treated groups, castrated, isolated animals showed abnormal behavior when they investigated the female. There was a high incidence of violent neck-biting,

TABLE 3

FEMALE-DIRECTED ACTS UNDER THE INFLUENCE OF ANDROGEN
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	802,901.40	2.73
Non-Castration vs. Castration (B)	1	118,803.00	
Group-Rearing vs. Isolation (C)	1	212,534.10	
A X B	1	591,852.10	2.04
A X C	1	40,368.00	
B X C	1	417,387.06	1.42
A X B X C	1	86,190.64	
N-INT-GRP - Group vs. All other Groups	1	1,863,394.30	6.42*
Error	40	290,399.84	

*$p < .05$

fur, ear, or leg-pulling along with normal nibbling of the neck region. In one case, a normal, castrated, isolated animal bit the genital of the female as could be judged from the squealing and fighting on the part of the female.

Abortive copulations

The abortive copulation score for each animal consists of the total frequency of occurrence of abortive copulations in the five tests. The mean scores for each group are shown in Figure 3. It is apparent that isolated animals engaged more often in abnormal copulatory behavior than group-reared animals. A three-way analysis of variance presented in Table 4 indicates that the only significant effect was group-rearing vs. isolation ($F=8.11$, $df=1/40$, $p<.05$, two-tailed test).

Contact-acquiring behavior.

The contact-acquiring score for each animal consists of the total frequency of occurrence of this behavior in the five tests. The mean score for each group is shown in Figure 4. A three-way analysis of variance presented in Table 5 yielded a significant group-rearing vs. isolation effect ($F=28.77$, $df=1/40$, $p<.01$, two-tailed test). Iso-

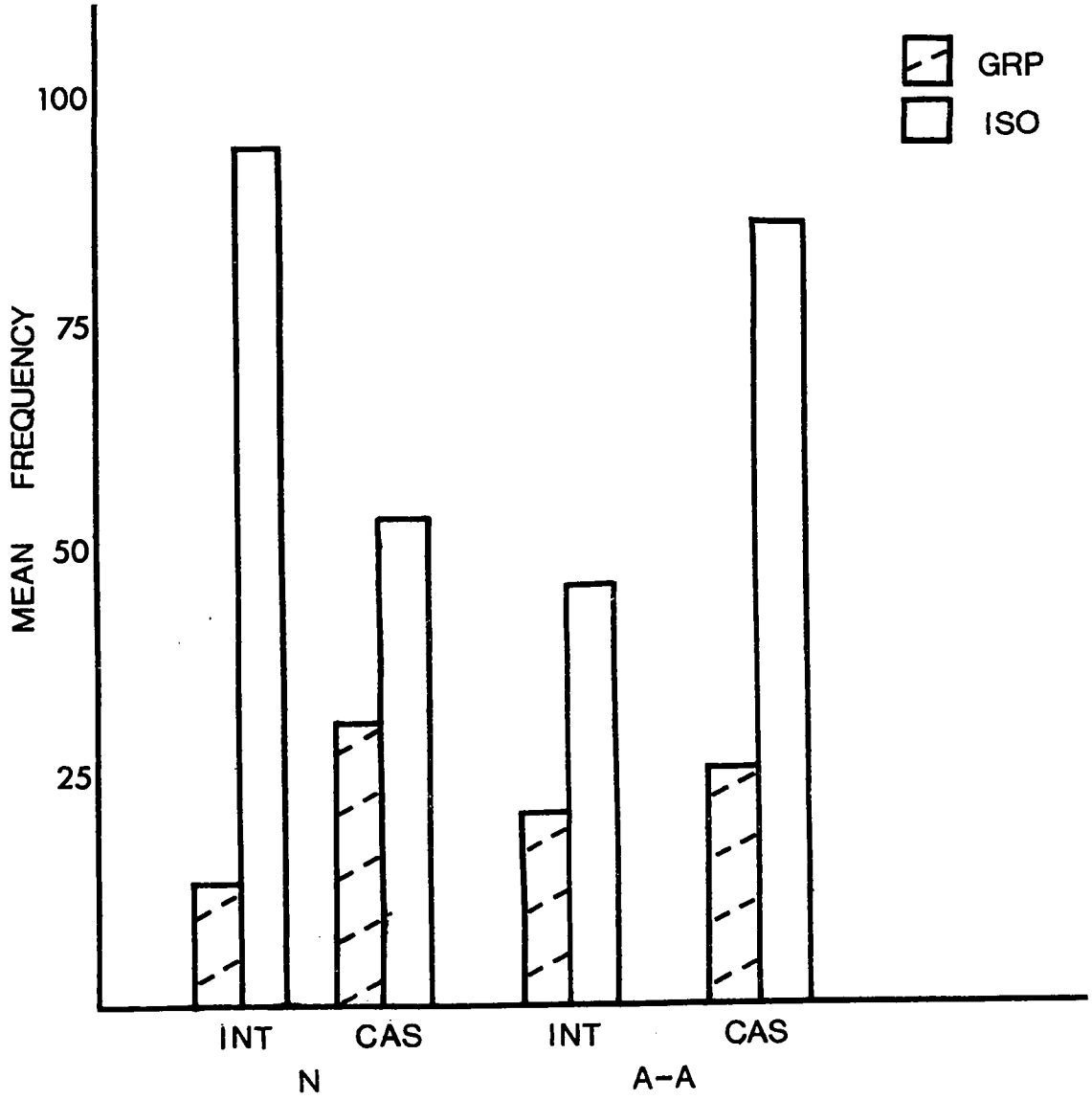

Fig. 3. Mean frequency of abortive copulations per group in five mating tests under the influence of androgen.

TABLE 4

ABORTIVE COPULATIONS UNDER THE INFLUENCE OF ANDROGEN
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	123.43	
Non-Castration vs. Castration (B)	1	346.68	
Group-Rearing vs. Isolation (C)	1	26,367.18	8.11*
A X B	1	3,451.11	
A X C	1	247.62	
B X C	1	450.20	
A X B X C	1	6,323.93	1.95
Error	40	3,249.57	

*$p < .05$

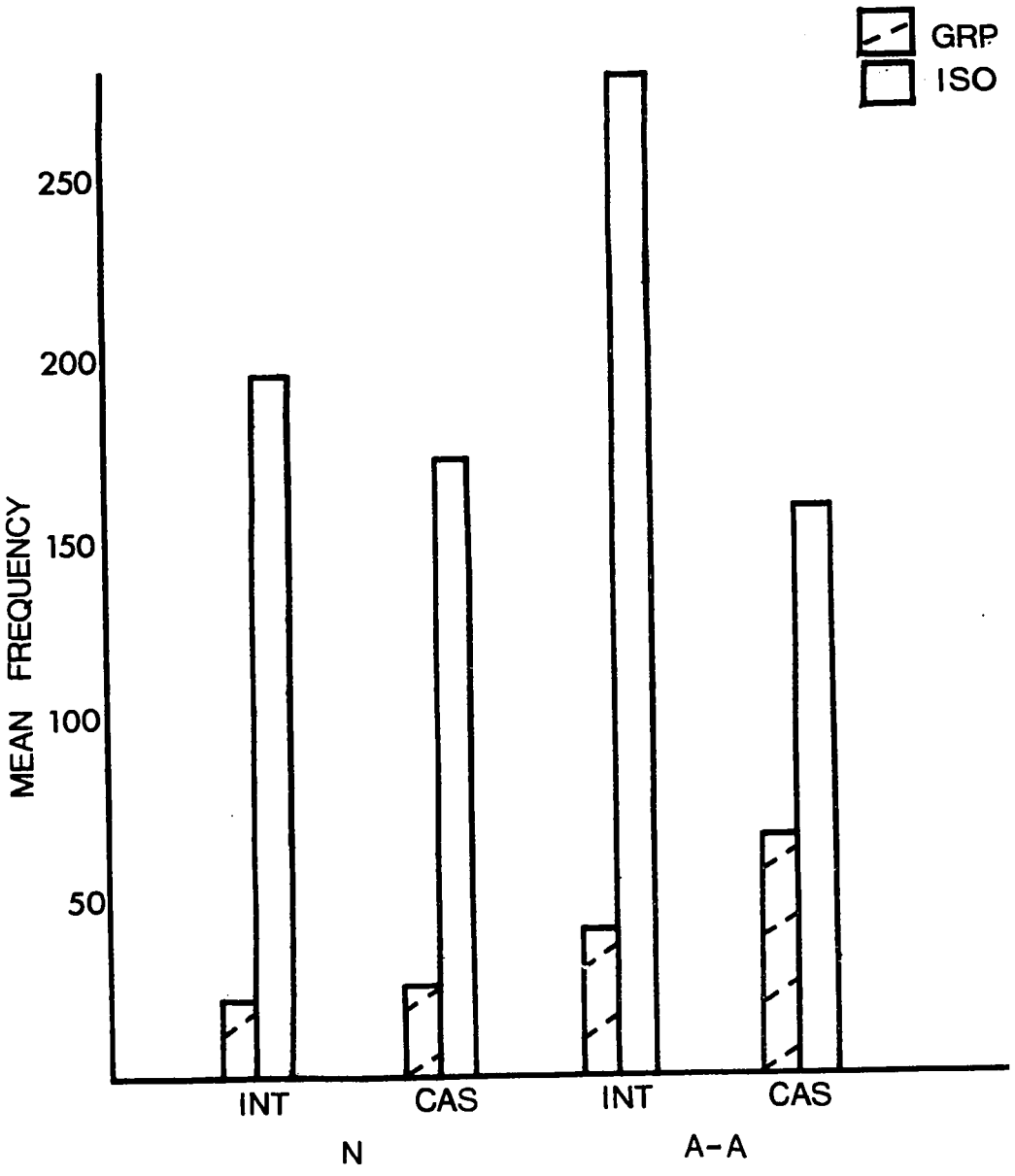

Fig. 4. Mean frequency of contact-acquiring behavior per group in five mating tests under the influence of androgen.

TABLE 5

CONTACT-ACQUIRING BEHAVIOR UNDER THE INFLUENCE OF ANDROGEN
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	12,642.52	1.15
Non-Castration vs. Castration (B)	1	9,605.02	
Group-Rearing vs. Isolation (C)	1	316,063.02	28.77**
A X B	1	4,125.52	
A X C	1	111.02	
B X C	1	22,403.52	2.04
A X B X C	1	10,650.52	
Error	40	10,985.93	

**$p < .01$

lates engaged more often in contact-acquiring behavior than did group-reared animals. The group which obtained the highest contact-acquiring score, i.e., the anti-androgen treated, intact, isolated group, is the same group which never engaged in complete copulation and which had the highest score in female-directed acts.

Arousal Behavior

The arousal behavior score for each animal consists of the total frequency of occurrence of arousal behavior during the five tests. The group means are shown in Figure 5. A three-way analysis of variance presented in Table 6 yielded a significant group-rearing vs. isolation effect ($F=42.21$, df-1/40, $p<.01$, two-tailed test). As can be seen in Figure 5, isolates engaged in a greater amount of arousal behavior than did group-reared animals.

Analysis of pre- and post-injection tests for castrated groups

In this section, those data will be considered which were obtained when castrated animals were tested in the absence of androgen. They will be compared with the data obtained when the same animals were tested under the influence of androgen. Two-way analyses of variance with repeated measures allowed for testing the effects of

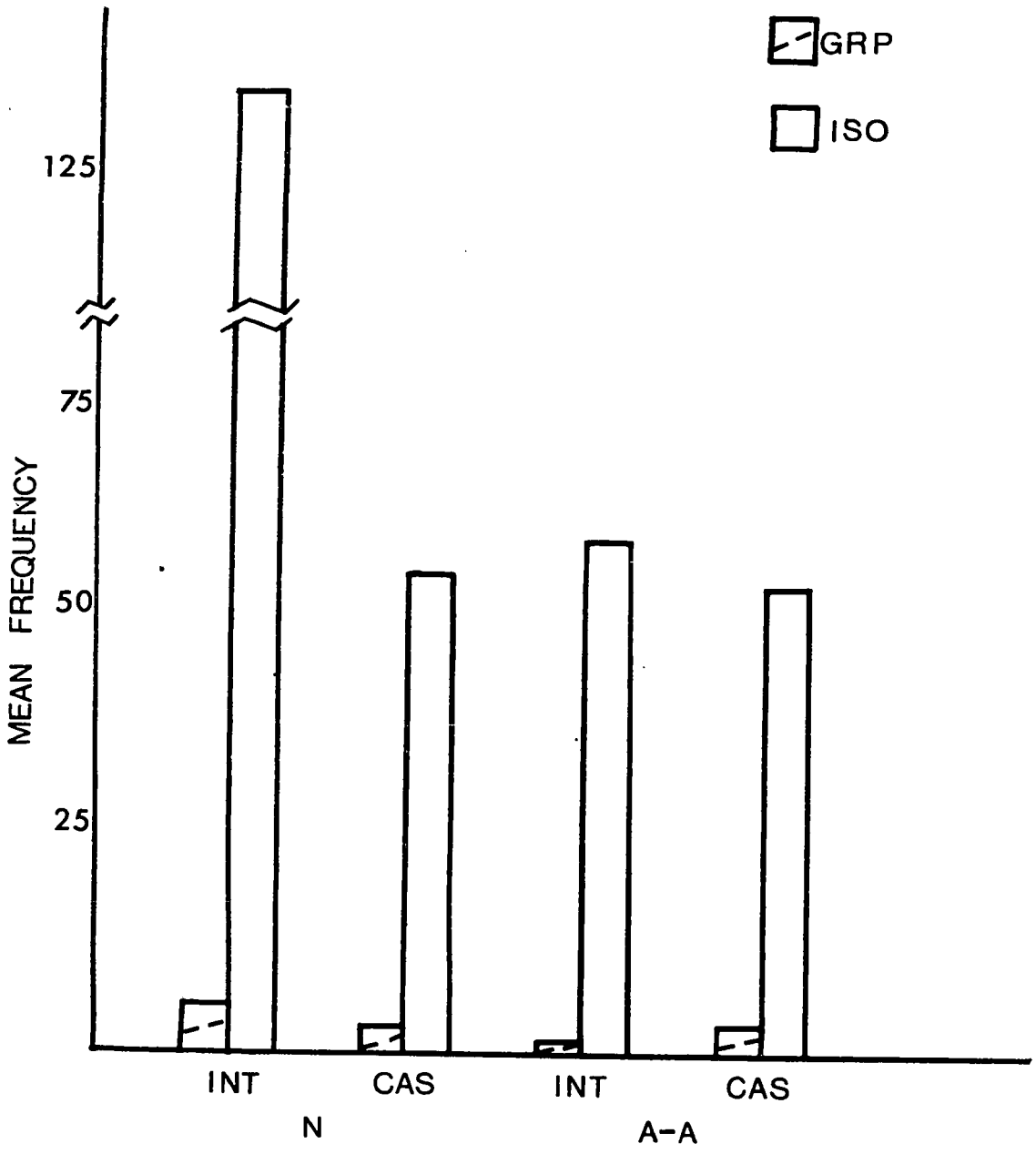

Fig. 5. Mean frequency of arousal behavior per group in five mating tests under the influence of androgen.

TABLE 6

AROUSAL BEHAVIOR UNDER THE INFLUENCE OF ANDROGEN
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	4,351.02	2.52
Non-Castration vs. Castration (B)	1	5,611.69	3.25
Group-Rearing vs. Isolation (C)	1	72,774.19	42.21**
A X B	1	3,692.52	2.14
A X C	1	3,383.52	1.96
B X C	1	4,981.68	2.89
A X B X C	1	2,806.02	1.63
Error	40	1,724.28	

**$p < .01$

the following variables: normal vs. anti-androgen treatment, group-rearing vs. isolation, pre-injection vs. post-injection tests. These analyses were done for female-directed acts, abnormal copulation, contact-acquiring behavior and arousal behavior. The scores in these analyses were obtained similarly to the scores in the data previously discussed; the total amount of time spent or the total frequency of occurrence during the five pre-injection tests were calculated for each animal.

The mean time spent in female-directed acts by each of the four groups is shown in Figure 6. The analysis presented in Table 7 indicates that there was a significant pre- vs. post-injection effect ($F=6.41$, $df=1/40$, $p<.05$, two-tailed test). Animals spent more time in this activity during post-injection tests than during pre-injection tests. The interaction between pre- vs. post-injection and group-rearing vs. isolation was also significant. ($F=14.02$, $df=1/40$, $p<.01$, two-tailed test). As Figure 7 illustrates, the amount of time spent in female-directed acts during pre- and post-injection tests varied as a function of rearing condition. Group-reared animals showed an increase in the amount of time spent in female-directed

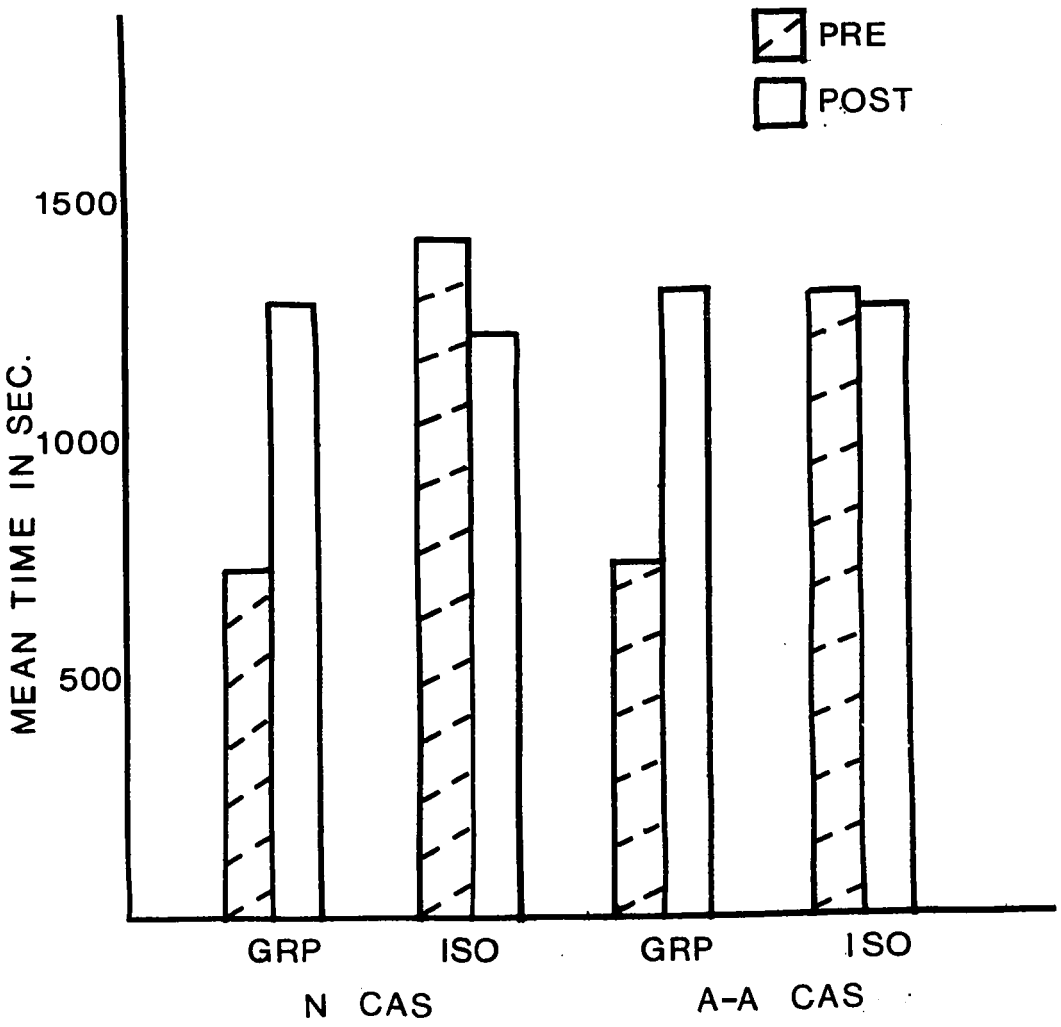

Fig. 6. Mean time spent in female-directed acts per group during pre- and post-injection tests.

TABLE 7

FEMALE-DIRECTED ACTS DURING PRE- AND POST-INJECTION TESTS
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	690.10	
Group-Rearing vs. Isolation (B)	1	1,022,584.10	2.34
Error (1)	20	437,873.09	
Pre- vs. Post-Injection Tests (C)	1	655,201.30	6.41*
A X B	1	5,166.10	
A X C	1	23,408.40	
B X C	1	1,431,061.40	14.02**
A X B X C	1	27,075.50	
Error (2)	20	102,083.22	

*$p < .05$
**$p < .01$

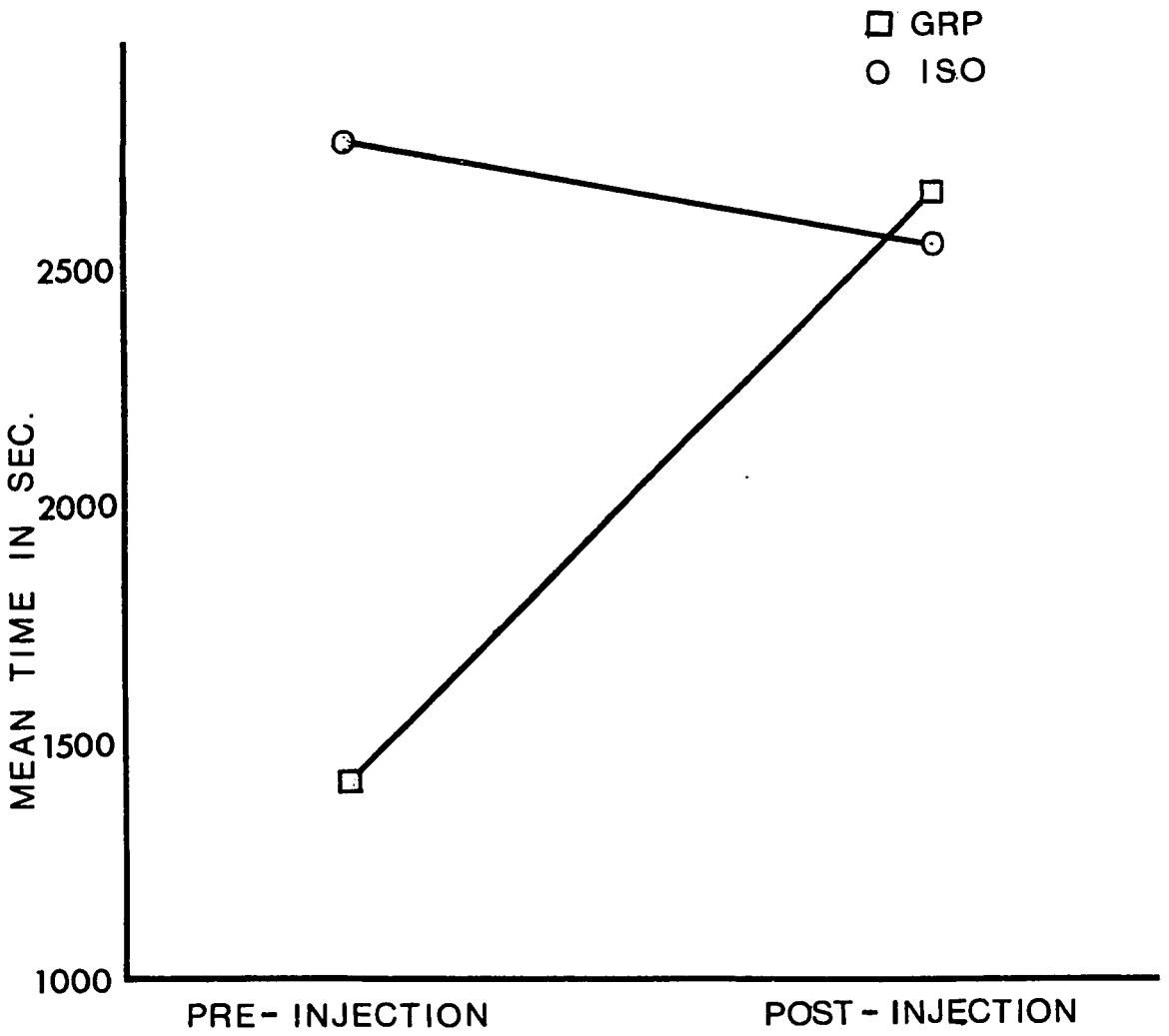

Fig. 7. Mean time spent in female-directed acts per group averaged over normal and anti-androgen treatment during pre- and post-injection tests.

acts between pre- and post-injection tests. Isolated animals, on the other hand, showed little change in this activity from one set of tests to the other.

The mean frequency of occurrence of abortive copulations for each group is shown in Figure 8. The only significant effect yielded by the analysis (Table 8) was group-rearing vs. isolation ($F=9.38$, $df=1/40$, $p<.01$, two-tailed test). As it was found in the results presented earlier for this activity, isolates engaged in a greater number of abnormal copulations than group-reared animals. There was no significant change in this activity between pre- and post-injection tests.

The mean frequency of occurrence of contact-acquiring behavior for each group is shown in Figure 9. The analysis presented in Table 9 yielded a significant effect of grap-rearing vs. isolation ($F=26.83$, $df=1/40$, $p<.01$, two-tailed test). Isolates engaged more often in contact-acquiring behavior than group reared animals. This finding is in accordance with the analysis presented earlier for this activity. Further, it is apparent from Figure 9 that contact-acquiring behavior attenuated over tests; animals engaged in less contact-acquiring behavior during the post-injection tests than during the pre-injection tests. However,

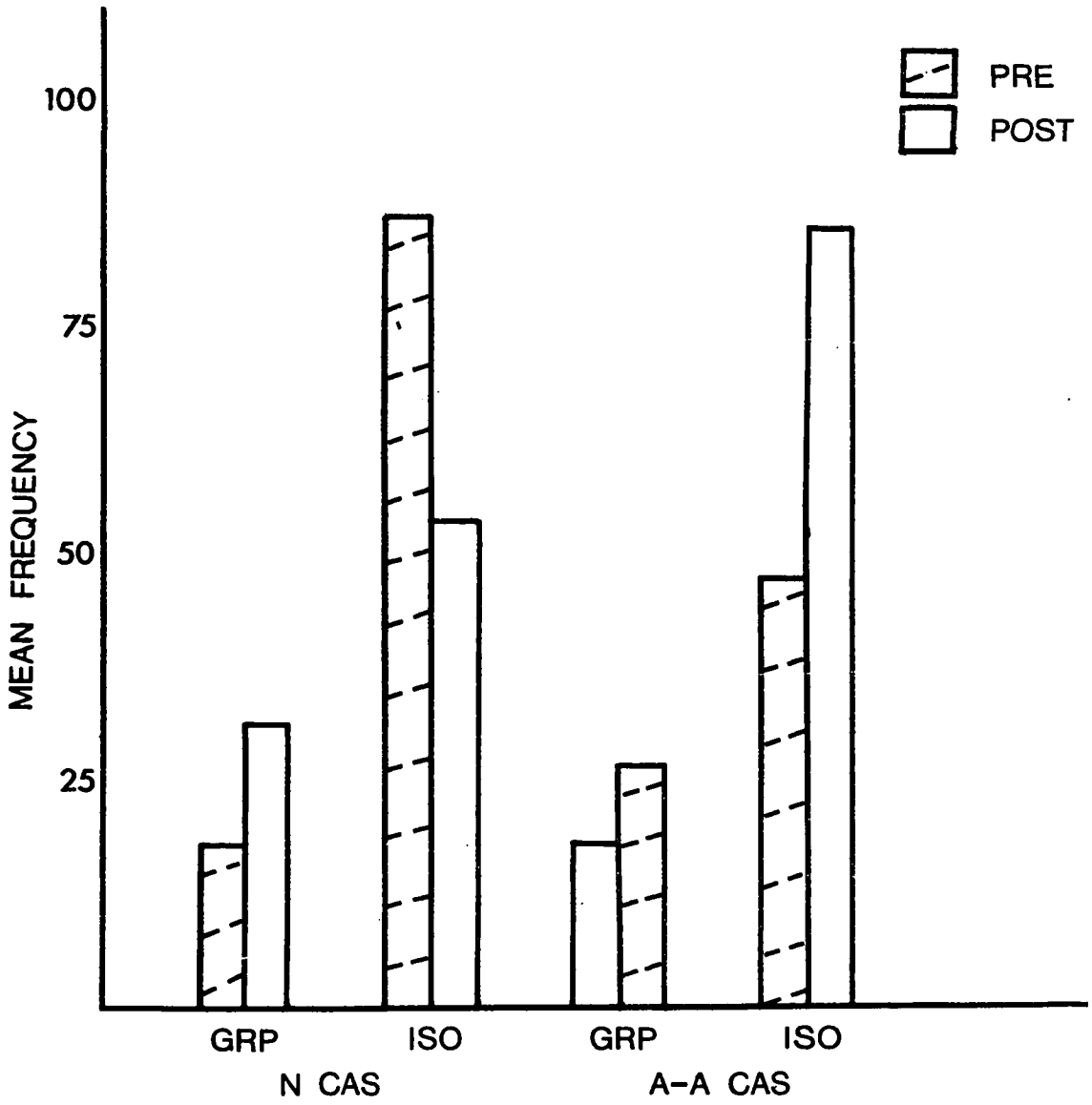

Fig. 8. Mean frequency of abortive copulations per group during pre- and post-injection tests.

TABLE 8

ABORTIVE COPULATIONS DURING PRE- AND POST-INJECTION TESTS
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	117.19	
Group-Rearing vs. Isolation (B)	1	24,345.02	9.38**
Error (1)	20	2,595.18	
Pre- vs. Post-Injection Tests (C)	1	581.02	
A X B	1	9.19	
A X C	1	3,417.19	2.63
B X C	1	221.02	
A X B X C	1	4,466.02	3.44
Error (2)	20	1,298.06	

**$p < .01$

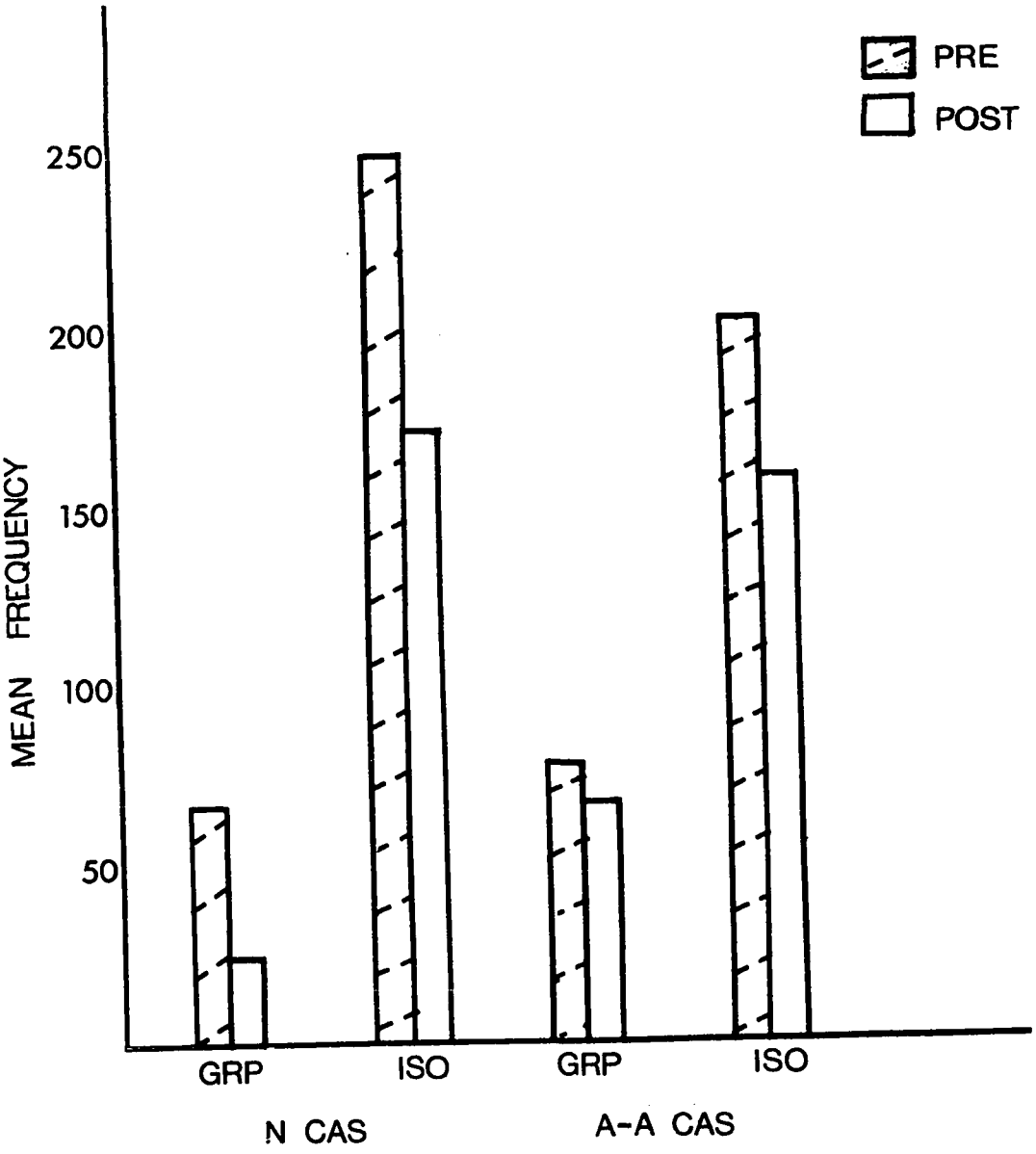

Fig. 9. Mean frequency of contact-acquiring behavior per group during pre- and post-injection tests.

TABLE 9

CONTACT-ACQUIRING BEHAVIOR DURING PRE- AND POST-INJECTION TESTS
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	38.52	
Group-Rearing vs. Isolation (B)	1	223,177.69	26.83**
Error (1)	20	8,318.41	
Pre- vs. Post-Injection tests (C)	1	22,663.52	4.80
A X B	1	9,268.52	1.11
A X C	1	2,961.02	
B X C	1	3,588.02	
A X B X C	1	13.02	
Error (2)	20	4,722.05	

**$p < .01$

the observed differences did not reach an acceptable level of significance.

The mean frequency of occurrence of arousal behavior for each group is shown in Figure 10. Again, the effect of group-rearing vs. isolation was significant ($F=21.76$, $df=1/40$, $p<.01$, two-tailed test, Table 10). There was no significant change in this activity from pre- to post-injection tests.

Autopsy data

Appendix A contains individual data on body weight and on organs examined. The most obvious findings were that anti-androgen treated animals were smaller in body size, had lighter and shorter penes, lighter testicles, and lighter seminal vesicles.

DISCUSSION

Male mating behavior can be said to be successful when the behavioral sequences involved in copulation are terminated in ejaculation, and, consequently, in the impregnation of the female. Therefore, the presence or absence of the ejaculatory behavioral pattern is the most important indicator of the disruption produced by the various treatment conditions.

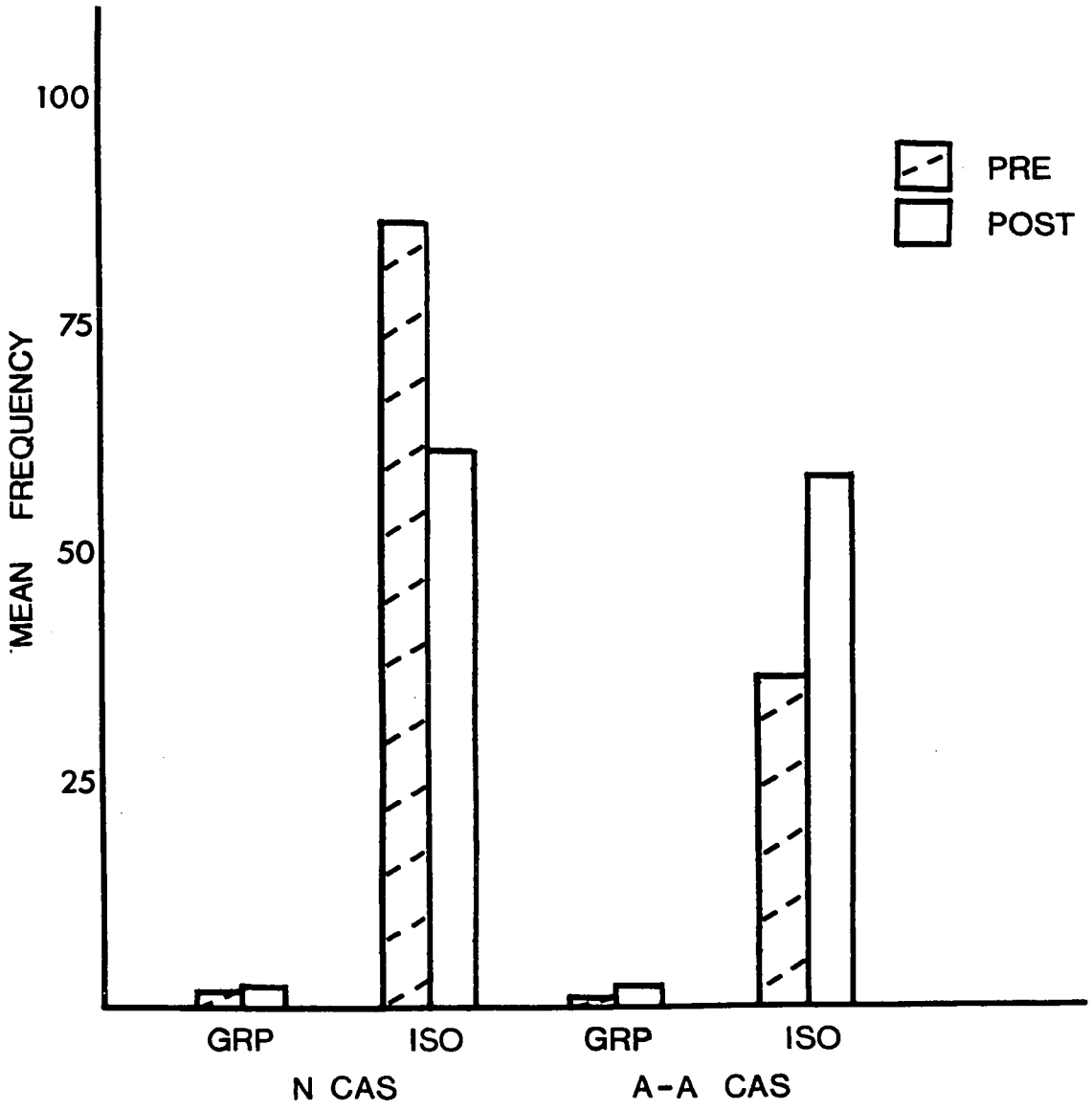

Fig. 10. Mean frequency of arousal behavior per group during pre- and post-injection tests.

TABLE 10

AROUSAL BEHAVIOR DURING PRE- AND POST-INJECTION TESTS
ANALYSIS OF VARIANCE

Source	df	MS	F
Normal vs. Anti-Androgen Treatment (A)	1	2,227.69	
Group-rearing vs. Isolation (B)	1	41,713.02	21.76**
Error (1)	20	1,917.22	
Pre- vs. Post-Injection Tests (C)	1	4,69	
A X B	1	2,067.18	1.08
A X C	1	1,764.18	2.25
B X C	1	25.52	
A X B X C	1	1,621.70	2.08
Error (2)		782.72	

** $p < .01$

The most important finding of the present study was that the presence of gonadal hormones in the male during pre- and early postnatal life is crucial to successful mating. The anti-androgen treated animals, although they engaged in various components of mating behavior, did not ejaculate. This finding is in accordance with those of previous investigators (Neumann and Elger, 1966) who have suggested that it indicates that pre- and early postnatal gonadal androgen somehow prepares the central nervous system for the effect of testosterone in adult life and thus makes mating possible. If endogenous androgen is not present during these critical periods, the development of the nervous system is altered in such a way that it will not respond to postpuberal androgens, and, as a result, normal male mating behavior will be deficient (Phoenix, Goy, Gerall, and Young 1959; Grady and Phoenix, 1963; Feder and Whalen, 1965; Harris and Levine, 1965). Whether the action of pre- and early postnatal androgen is organizational and whether the action is strictly on the central nervous system, as it has been thought to be, is questionable. It is evident from the present study and from other investigations that all the components of the mating pattern

with the exception of ejaculation are present in early androgen-deprived animals. Organization is manifested by the orderly appearance or disappearance of the different motor components involved in mating. Early androgen-deprived animals, even in the absence of adult circulating androgen, engage in those components of the sex act which are the first to appear in a normal copulating male, the nibbling of the female's neck region and the sniffing of her genitals. Such early androgen-deprived animals, when they have adult circulating androgen, proceed to mount the female after the foreplay of the sequence, but they do not ejaculate. The fact that normal vs. anti-androgen treated animals could not be differentiated by either the number of incomplete or the number of complete mounts is strong evidence for the emerging view (Hart, 1967, 1968; Beach, Nobel, and Orndoff, 1969) that the primary effect of early androgen-deprivation on male sexual responses is on the ejaculation response. Hart has evidence that at least some components of the mating pattern are organized below the encephalic level and that the spinal cord also mediates sexual responses. The ejaculatory responses are partially controlled by reflex mechanisms at the spinal level. On the basis of his work, he has suggested that

neonatal testicular androgen has an organizational influence on neural tissues mediating sexual reflexes at the spinal level. Beach, Nobel, and Orndoff have questioned the very notion of the neural organizing action of early androgen. On the basis of a high positive correlation between intromission frequency and the penis size, as affected by the age when castrated animals were exposed to the "organizing" effect of androgen, Beach has suggested that the early androgen effect can best be described as that of sensitizing the penis during a crucial period of development in such a way that it will react to later androgenic stimulation by increasing to its normal size. In the present study the difference found between the morphology of normal and anti-androgen treated animals is a strong support of Beach's argument.

This view of the primary effect of early androgen-deprivation does not discount possible secondary effects, such as persistent interest in the female, high mount rates, which could arise from an inability to ejaculate. In the present experiment anti-androgen treated, intact, group-reared animals spent a greater amount of time in female-directed acts than did normal, intact, group-reared animals. Apart from ejaculation, it was in this respect

only that the two groups differed. Furthermore, it was only in female-directed acts that the anti-androgen treated, intact, group-reared animals were similar to all the other abnormally treated groups. Thus, early androgen-deprivation seems to affect the mating behavior of the male rat primarily through the disruption of ejaculatory response and only secondarily does early androgen deprivation effect other components of the mating behavior.

The combined effect of anti-androgen treatment and castration is difficult to interpret. Both, the group-reared and isolated castrated subgroups engaged in more copulatory behavior than their intact counterparts. This difference may have been due to the dose of injected androgen, which was administered to the adult castrates. It was probably much greater than that circulating in the intact, anti-androgenically treated animals.

While the early androgen-deprivation effect was manifested in the ejaculation response, the general effect of isolation was manifested by the high scores of all isolated animals on contact-acquiring behavior, arousal behavior and abortive copulation. These behaviors are not necessarily part of the normal mating pattern, rather, they appear to be indicative of a high level of arousal, and have been observed

in isolates by other investigators (Gerall, 1967a; Folman and Drori, 1965). Only isolated animals obtained high scores for contact-acquiring behavior, arousal behavior, and abortive copulation, whereas all abnormally treated animals had comparably high scores on female-directed acts, acts which are part of the normal mating sequence. Regarding the effect of isolation on the copulatory acts themselves, it seems that for at least some, otherwise normally treated, male rats early socialization is not necessary for successful mating. However, the incidence of complete copulations and ejaculations was lower in the normal, intact isolates than in the controls. How, then, does isolation interfere with successful copulation? Kagan and Beach (1953) and Gerall (1965) have formulated a hypothesis to explain the disrupting effect of isolation on mating behavior. According to this hypothesis, the high incidence of non-copulatory activities observed in isolated animals competes with and intrudes into the normal male copulatory pattern. The normal, intact, isolated animals in the present experiment had the highest incidence of arousal behavior, and they also obtained high scores on contact-acquiring behavior and abortive copulations. On the other hand, findings of the present experiment on the

morphology of isolated animals indicate that these animals had somewhat smaller penes than group-reared animals. Whether the reduction in the frequency of mating behavior produced by social isolation is due to the emergence of competing responses in the test situation, or is due to the lack of sensory feedback from underdeveloped genitals resulting from lack of bodily contact, is not clear. The indications of the present study are that probably both factors have a contributing effect.

Considering the effect of isolation when combined with other abnormal treatments, on copulation, it appears merely to accentuate the effects brought about by these other treatments. This is evident in the normal, castrated, isolated group and the anti-androgen treated, intact, isolated group, both of which had lower copulatory and ejaculatory scores than their group-reared counterparts. Isolation alone seems to reduce the likelihood that the animal will engage in the normal copulatory sequence, and to increase the likelihood that other forms of behavior will occur in the test situation. Isolation in combination with other treatment exaggerates the effects produced by these other treatments.

These findings clarify to some extent the differences

and similarities between the effects of early androgen-deprivation and abnormal social rearing conditions on male sex behavior in the rat. It seems that neither of the abnormal treatments truly disrupts the sequence of male sex behavior. Early androgen-deprivation, however, interferes with ejaculation. Whether early androgen-deprivation affects the central nervous system itself or reduces the effects of sensory excitation by retarding penis growth, is not known. The reasoning presented by Beach, Noble, and Orndoff (1969) in regard to peripheral sensitization of tissues by androgen, and whether the same reasoning can be applied to the effects of androgen on mechanisms in the central nervous system, is open to investigation. Abnormal social rearing reduces the likelihood that the appropriate motor acts will be executed. Thus it may be that the neural organization is sound after these treatments and that the only problem is to have it activated in adulthood. The fact that female rats tested for male sex behavior after androgen treatment engage in most of the components of the sequence with the possible exception of ejaculation pattern would support the views of Glickman and Schiff (1967), that certain species specific behavioral patterns are organized in a "prewired" form in the brainstem

of the member of the species. In a normal animal, the occurrence of these motor patterns is dependent upon the activation of motor pathways by information arriving through the appropriate sensory channels in conjunction with already ongoing activity in the brain. It may be more accurate, then, to say that early androgen and social contact both increase the probability that male sex behavior will occur by providing conditions under which it is most likely to be facilitated, such as normal morphological development, sensory innervation, and practice of parts of the mating sequence during prepuberal play.

SUMMARY

In a search for similarities in the disrupting effects of early androgen-deprivation and social isolation on the male rat's sex behavior, forty-eight male hooded rats were studied in a 2 x 2 x 2 factorially designed experiment under the following conditions: sham vs. anti-androgenic injection administered pre- and early postnatally, non-castration vs. castration, and group-rearing vs. isolation. All abnormal treatments reduced mating. Early androgen-deprivation was found to have an effect on the ejaculatory response. Isolation induced the occurrence of non-mating

behaviors and had an additive effect when combined with other abnormal treatments. The findings were discussed with reference to the neural organizational role of early androgen, the competing response hypothesis, and the role of sensory feedback on mating. It was concluded that early androgen and social contact, rather than having an organizational role, both increase the probability of mating by providing optimal conditions for the facilitation of "prewired" neural structures.

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APPENDIX A

BODY WEIGHT IN GRAMS

ANIMAL	NORMAL				ANTI-ANDROGEN TREATED			
	INTACT		CASTRATED		INTACT		CASTRATED	
	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated
1	352	382	310	304	180	330	254	312
2	378	314	310	340	290	-	270	324
3	324	288	292	296	346	306	356	214
4	310	380	-	302	312	284	308	-
5	348	332	350	318	280	328	320	304
6	368	294	322	250	254	270	296	328
TOTAL	2,080	1,990	1,584	1,810	1,662	1,518	1,804	1,482
MEAN	345	332	317	302	277	304	301	295

APPENDIX A (cont'd).

WEIGHT OF TESTICLES IN GRAMS

ANIMAL	NORMAL				ANTI-ANDROGEN TREATED			
	Intact		Castrated		Intact		Castrated	
	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated
1	3.30	3.65	-	-	2.62	2.58	-	-
2	3.43	3.70	-	-	3.23	-	-	-
3	3.32	3.05	-	-	3.00	2.64	-	-
4	3.31	3.22	-	-	1.72*	3.12	-	-
5	3.04	3.55	-	-	3.00	2.95	-	-
6	2.98	2.30	-	-	2.70	2.94	-	-
TOTAL	19.38	19.47			16.27	14.23		
MEAN	3.23	3.25			2.71	2.84		

*(right) testicle only.

APPENDIX A (Cont'd.)

WEIGHT OF PENES IN GRAMS

ANIMAL	NORMAL				ANTI-ANDROGEN TREATED			
	INTACT		CASTRATED		INTACT		CASTRATED	
	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated
1	.30	.30	.30	.27*	.20	.17	.19**	.15
2	.30	.33	.32	.36	.31	-	.21**	.20
3	.25	.32	.27	.36	.27	.15	.20	.16
4	.30	.35	-	.30	.24**	.15	.20	-
5	.35	.41	.30	.31	.25**	.15	.21	.12
6	.37	.33	.20	.31	.22*	.23	.15	.20
TOTAL	1.87	2.04	1.39	2.01	1.49	.85	1.16	.83
MEAN	.31	.34	.28	.34	.25	.17	.19	.17

* glans penis was open on dorsal side

** penis was open on dorsal side

APPENDIX A (Cont'd.)

PENIS LENGTH IN MILLIMETRES

ANIMAL	NORMAL				ANTI-ANDROGEN TREATED			
	INTACT		CASTRATED		INTACT		CASTRATED	
	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated
1	28	25	28	16	20	20	16	20
2	25	24	26	27	23	-	16	21
3	26	24	26	21	23	20	21	16
4	28	25	-	26	16	21	20	-
5	28	24	25	25	20	24	20	18
6	27	25	25	26	15	23	16	23
TOTAL	162	147	130	141	117	108	109	98
MEAN	27.0	24.5	26.0	23.5	19.5	21.6	18.2	19.6

APPENDIX A (Cont'd).

COMBINED WEIGHT OF PROSTATE, SEMINAL VESICLE, AND COAGULATING GLAND IN GRAMS

	NORMAL				ANTI-ANDROGEN TREATED			
	Intact		Castrated		Intact		Castrated	
ANIMAL	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated	Group Reared	Isolated
1	1.93	1.99	4.37	2.33	.70	2.12	2.62	2.37
2	2.73	2.54	3.50	3.36*	1.94	-	2.87	3.12
3	2.57	2.27	2.60	4.80	1.75	1.43	4.33	2.42
4	2.88	2.07	-	4.41	1.94	1.45	3.90	-
5	2.62	2.04	4.00	4.26	1.40	1.80	3.42	3.00
6	2.91	1.80	2.80	3.75	1.10	1.25	2.34	3.35
TOTAL	15.64	12.71	17.27	22.91	8.83	8.05	19.48	14.26
MEAN	2.61	2.12	3.56	3.82	1.47	1.61	3.25	2.85

*one of the seminal vesicles was atrophied.