PERIPHERAL SEXUAL MORPHOLOGY, SENSITIVITY AND ANDROGENIZATION: THEIR EFFECTS ON MALE SEXUAL BEHAVIOR IN RATS AND HAMSTERS

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ABSTRACT

PERIPHERAL SEXUAL MORPHOLOGY, SENSITIVITY AND ANDROGENIZATION: THEIR EFFECTS ON MALE SEXUAL BEHAVIOR IN RATS AND HAMSTERS.

By

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Bilateral section of the dorsal nerve of the penis (DNP) in male rats increased mount frequency but eliminated intromission and ejaculation. Unilateral section of the DNP caused a transient decrease in intromission and ejaculation frequency. Treatment with fluoxymesterone (FM) in castrated unsectioned males maintained mounting, intromission and ejaculation, but FM was not as effective as testosterone propionate (TP. The effects of fluoxymesterone on mounting were abolished by sectioning the DNP suggesting that fluoxymesterone exerts its faciliatory effect upon mounting via an influence upon genital morphology.

In a second experiment, male and female hamsters castrated on day 1 (day of birth considered day 1) were treated neonatally with fluoxymesterone, testosterone or the hormone vehicle. At adulthood, mounting behavior and lordosis behavior were assessed. While the degree of penile or clitoral virilization did not differ between testosterone and fluoxymesterone treated animals, behavioral differences occurred. Neonatal treatment with fluoxymesterone failed to "masculinize" or "defeminize" behavior. Neonatal treatment with testosterone induced mounting and intromission. It was concluded from these results and those of others that fluoxymesterone has little or no central neural effects.

A third experiment employed male hamsters castrated on the day of birth and treated with TP or estradiol benzoate (EB) on days 2, 3 and 4 of life. Neonatal treatment with TP but not EB induced penile virilization as adults. TP induced sexual behavior in these animals was assessed prior to and following sectioning of the DNP. Penile desensitization had little effect upon hamster sexual behavior regardless of the degree of penile virilization. Replacement with either fluoxymesterone or sesame oil caused a decline in sexual behavior but no statistical differences were observed among sectioned or sham animals or among neonatal treatment groups. Fluoxymesterone treated animals in all neonatal groups tended to mount longer than sesame oil treated animals but this trend was nonsignificant. Penile size or sensitivity in hamsters appears to have little effect upon the maintenance of sexual behavior.

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By

Archie J. Vomachka

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GENERAL INTRODUCTION

While the importance of gonadal hormones for the control of male mating behavior is recognized, where gonadal hormones act to control sexual behavior has been debated extensively. While there is evidence to indicate that the gonadal steroids stimulate behavior by action on the central nervous system, there is also reason to suspect gonadal hormones acting upon genital (Peripheral) structures, exert control over sexual behavior. The problem has been summed up most effectively:

> Tell me, where is fancy bred,
> In the heart or in the head?
> In the glands (of which the're legions)
> Or is it in the pelvic regions?
>
> (Kohn, 1971)

The following series of experiments examine the role of penile size and sensitivity in the sexual behavior of rats and hamsters. Experiments were designed to test hypotheses related to the peripheral control of sexual behavior.

EXPERIMENT 1

Introduction

Experimental evidence suggests that there are two modes by which gonadal hormones may facilitate male sexual behavior in the rat; 1) by direct action upon the central nervous system, 2) by action upon genital morphology. In support of a central action, it was found that testosterone implanted into the preoptic area restored mating behavior in castrated rats when given in quantities far below the amount required for stimulation of seminal vesicles (Davidson, 1966; Lisk, 1967). Electrical stimulation of the posterior hypothalamus or lateral preoptic area lead to stimulus bound copulation or activation of sexual behavior (Caggiula & Hoeble, 1966; Caggiula, 1970; Madlafousek, Freund & Grofova, 1970, Caggiula & Szechtman, 1972). By the same token, discrete lesions in the medial forebrain area (Hitt, Hendricks, Ginsberg & Lewis, 1970, Hitt, Byron & Modianos, 1973) and medial preoptic area (Giantonio, Lund and Gerall, 1970; Singer, 1968) abolished male sexual behavior in the presence of exogenous androgens.

Experimental decreases in genital sensory feedback or penile structure in rats, cats, and monkeys reduced or eliminated intromission and ejaculation behavior but did not cause immediate decreases in mounting (Beach & Holz, 1946; Carlsson & Larsson, 1964; Adler & Bermant, 1966; Aronson & Cooper, 1966 & 1968 & 1969; Whalen, 1968; Herbert, 1972; Larsson and Sodersten, 1973). Evidence for hormonal effects upon sexual behavior via genital morphology was suggested by Beach & Levinson (1950)

who correlated the decreased size of penile spines following castration with a decrease in ejaculation and the "tendency to copulate."

Nadler (1969) gave cyproterone acetate, an anti-androgen, prenatally to rats and attributed the lack of male copulatory behavior in the offspring at adulthood to deficient penile development. In addition, neonatal castration of male rats resulted in a reduction in penile size and weight. Limited androgen administration neonatally and/or in adulthood did not overcome this effect of castration on genital size (Beach & Holz, 1946; Grady, Phoenix & Young, 1965; Whalen, 1968). Likewise, neonatal treatment of male rats with gonadotropin antiserum produced animals with penes of a reduced size and a smaller number of penile spines than normal control animals (Goldman, Quadagno, Shryne & Gorski, 1972). These neonatal manipulations with antiserum resulted in animals which mounted a receptive female but failed to intromit or ejaculate. While these experiments suggest the importance of penile structure in male sexual behavior, the treatments used to achieve a reduction in penile development may also have resulted in a reduced central neural androgenization or virilization. Consequently, we cannot establish the relative contribution of central and peripheral factors to male sexual behavior.

In an attempt to circumvent some of these problems, Hart (1972) gave fluoxymesterone or TP to day 4 castrated male rats and tested these animals for sexual behavior as adults in response to testosterone treatment. Fluoxymesterone is known to have strong virilizing effects on genital morphology. While animals treated neonatally with TP show high levels of mounting, intromission and ejaculation, fluoxymesterone treated animals showed a marked reduction in ejaculation. However, the

penes of the fluoxymesterone treated animals were not visibly different from those of the TP treated animals. Hart therefore suggested, that "the behavioral difference between the groups could not be attributed to peripheral factors" (p. 845). In a study of adult rats, Beach & Westbrook (1968) gave fluoxymesterone to non-copulating, castrated males and although mating behavior was not reinstated, the penile spines and papillae as well as seminal vesicle morphology were restored to the level of testosterone treated animals. The data from these experiments suggest that fluoxymesterone may not have strong effects upon the central nervous system even though it is a powerful androgen for penile tissues.

In the present study we provide experiments to determine whether penile sensory feedback may facilitate mounting, even though it may not be necessary for this behavior. We surgically sectioned the dorsal nerve of the penis (DNP) in male rats and tested them for copulatory behavior. These animals with desensitized penes were placed on various hormone regimes. Testosterone propionate was used as an androgen with central neural activity as well as an agent to maintain penile morphology. Fluoxymesterone was used to provide androgenic stimulation of peripheral genital structures, presumably in the absence of strong central effects upon male sexual behavior.

EXPERIMENT 1, PART 1

Methods and Materials

The subjects for this study were 47 male rats of the Long-Evans strain. These animals were 90-100 days old at the beginning of the experiment and were housed in groups of 3-5 in stainless steel cages

with food and water available ad libitum. Lights in the colony were off from 1100 hr. to 2100 hr.; testing began 1-3 hours after the lights had gone off. Temperature and humidity were maintained at a constant level. All rats were castrated under ether anesthesia 2 weeks prior to the beginning of testing, and were placed on daily injections of testosterone propionate (TP): 500 µg in 0.1 cc sesame oil.

At 13-18 days post-castration, animals were selected for the experiment on the basis of sexual performance in a selection test in which each animal was placed with a sexually receptive stimulus female. Stimulus females were ovariectomized and brought into sexual receptivity with exogenous estrogen and progesterone. Animals which failed to mount the female in 10 minutes or failed to ejaculate within 20 minutes were excluded from the study. In order to establish a baseline of sexual activity, two pretests of 20 minutes each (tests 1 and 2) were given to the experimental animals 4 and 8 days after the selection test. In each pretest, a sexually receptive female was placed into a 2'x2' plexiglass arena with the experimental male after the male had been allowed to adapt to the arena for at least 5 minutes. All subsequent testing followed the same procedure.

At the completion of the second pretest, animals were randomly divided into 2 groups. In one group, a 3-5 mm section of the dorsal nerve of the penis (DNP) was removed bilaterally from each animal. Animals in the remaining group received a sham operation. The operations were performed under ether anesthesia. The skin was incised and the penis retracted. The fascia was removed to expose the DNP, which was then lifted and a section removed. For the sham operated animals,

the DNP was similarly exposed, however, it was neither lifted nor sectioned.

Animals were allowed a recuperation period of 14-17 days before receiving 3 postoperative tests (tests 3-5) at 4 day intervals. Upon completion of the third postoperative test, the 2 groups were subdivided into a total of 6 groups and assigned to the following hormone treatments:

1) TP Cut, n=8. These animals with DNP section continued to receive 500 µg TP daily.
2) TP Sham, n=8. These were Sham operated animals which received 500 µg TP daily.
3) Fluoxymesterone Cut, n=8. These animals with DNP section as well as all of the following groups, received no further TP administration. Replacement in this group was 500 µg of fluoxymesterone (Halotestin. Upjohn) daily, suspended in 0.1cc sesame oil.
4) Fluoxymesterone Sham, n=8. These Sham operated animals received 500 µg fluoxymesterone daily in 0.1cc sesame oil.
5) Oil Cut, n=7. These animals with DNP section received 0.1cc sesame oil (the hormone vehicle used in this study) daily.
6) Oil Sham, n=8. These Sham operated animals received 0.1cc sesame oil daily.

Following the onset of these group assignments, animals were tested on day 4, 8, 12, 17, 21, 28, 35, 42, 49 and 56 (tests 6-15).

For each 20 minute test, all mounts, intromissions and ejaculations were recorded on an Esterline-Angus Event Recorder. This provided a temporal sequence of behaviors and the following measures were derived

from the record:

1) Mount Latency. The time in seconds from introduction of the stimulus female at the beginning of the test until the first mount by the male, up to 1200 seconds for an animal which failed to mount.
2) Intromission Latency. The time in seconds from the beginning of the test until the first intromission response by the male, or 1200 seconds for animals which did not intromit.
3) Ejaculation Latency. The time in seconds from the first intromission to ejaculation, or in subsequent ejaculations, time from first intromission in a series to ejaculation. In animals which failed to ejaculate, ejaculation latency was taken as 1200 seconds minus the intromission latency.
4) Mount Frequency. Number of mounts without intromission.
5) Intromission Frequency. Number of mounts with intromission.
6) Ejaculation Frequency. Total number of ejaculations in a 20 minute test.
7) Post-ejaculatory Interval. Time in seconds from ejaculation to the reinitiation of copulatory activity.
8) Mounts Per Minute. Mount frequency divided by total test time (20 minutes) minus total time in post-ejaculatory interval.
9) Intromissions Per Minute. Intromission frequency divided by total test time minus total time in post-ejaculatory interval.

Following ejaculation, the male rat enters a post-ejaculatory interval (PEI) lasting 200-300 seconds. During this period, he shows no copulatory behavior. Since DNP cut animals rarely ejaculated, they

seldon entered a PEI. Animals with no ejaculations often mounted regularly throughout the entire 20 minute test, while animals with multiple ejaculations were sexually active for a much shorter amount of time. In such a situation, elevated mount frequencies for cut groups per 20 minute test would reflect a lack of the PEI normally seen in ejaculating males. To correct for this, comparisons of mount frequencies and intromission frequencies were made on the basis of frequencies per minute active. Thus, for animals achieving ejaculation, the PEI was subtracted from the 20 minute tests, and dividing mount frequency or intromission frequency by this number gives behaviors per minute active.

At the completion of testing, all animals were sacrificed and penes were removed. Penes were fixed in Bouin's solution, divided into glans and shaft portions and embedded in parafin. The tissue was sectioned at 10 μ and representative sections were mounted on glass slides and stained for histological examination.

Results

Ejaculations. The mean ejaculation frequencies for each group across all tests are shown in Figure 1. Comparison of ejaculation frequency scores from the 2 pretests with the ejaculation frequency scores from the postoperative tests 3-5 showed that sectioning the DNP nearly abolished ejaculation (Sign Test, $p<0.001$). In tests 3-5, sham animals showed a statistically significant increase in ejaculation frequency over the pretest scores (tests 1 & 2) (Sign Test, $p=0.002$).

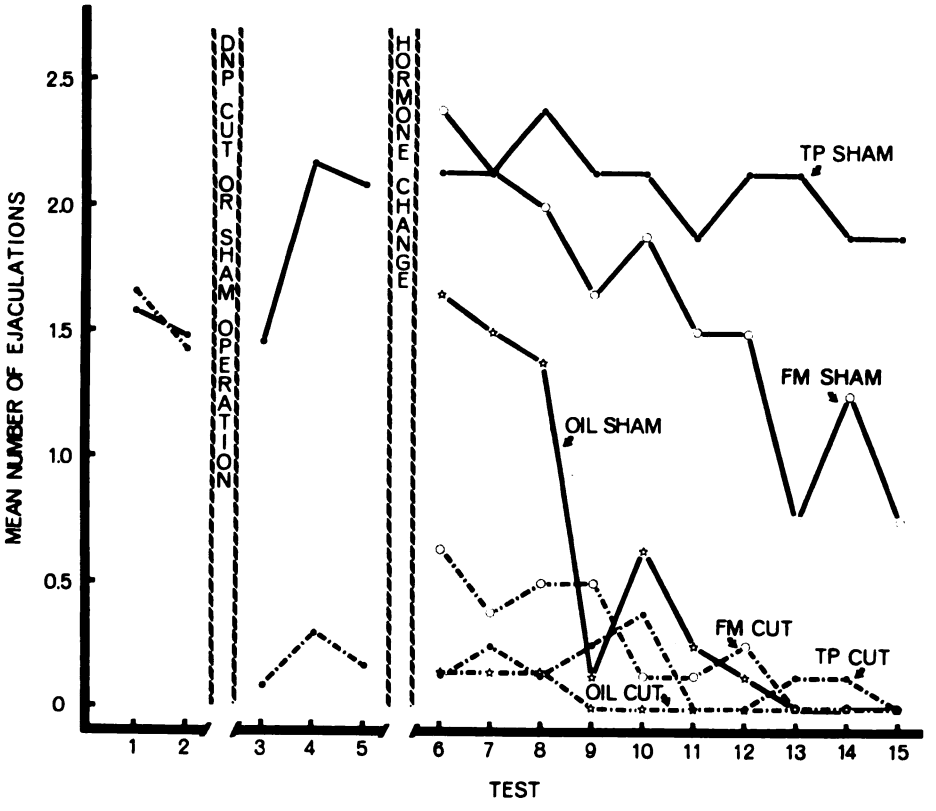

Figure 1. Effects of penile desensitization and hormone replacement on ejaculation in male rats.

The effect on ejaculation frequency of replacing TP with either fluoxymesterone or oil in cut and sham groups is also shown in Figure 1, tests 6-15. In all DNP section groups, ejaculation frequency remained low throughout the experiment. The TP Sham group showed no significant change in ejaculation frequency between tests 6 and 15, whereas there was a significant decrease in ejaculation frequency between tests 6 and 15 (Wilcoxon Matched Pairs, Signed - ranks Test, $p<0.01$ and $p<0.02$ respectively) for both the Fluoxymesterone Sham and Oil Sham groups. At test 6, the scores for the sham groups were not significantly different, however, by test 15, a statistical difference was obtained among groups (Kruskal-Wallis, 1 way AOV by ranks, $p<0.01$). Ejaculation frequency in the TP Sham group was significantly higher than in the Fluoxymesterone Sham group (Mann-Whitney U-test, $p<0.01$); likewise, the Fluoxymesterone Sham scores were significantly greater than were those of the Oil Sham group (MWU, $p<0.01$).

Intromissions. Mean intromission scores per non-PEI minute for each group are shown in Figure 2. DNP section resulted in a significant decrease in intromission scores in tests 3-5 as compared with tests 1 and 2 (Sign Test, $p<0.001$). For the sham group, intromission scores did not differ significantly between pre- and post-operative tests.

Comparison of test 6 scores with test 15 scores showed that both Fluoxymesterone Sham and Oil Sham animals had significant decreases in intromission scores over time (Wilcoxon, $p<0.002$), while TP Sham animals showed no change in intromission frequency. At test 15, TP, Fluoxymesterone, and Oil Sham animals were significantly different on intromission measures (K-W, $p<0.01$), whereas, in test 6, these groups

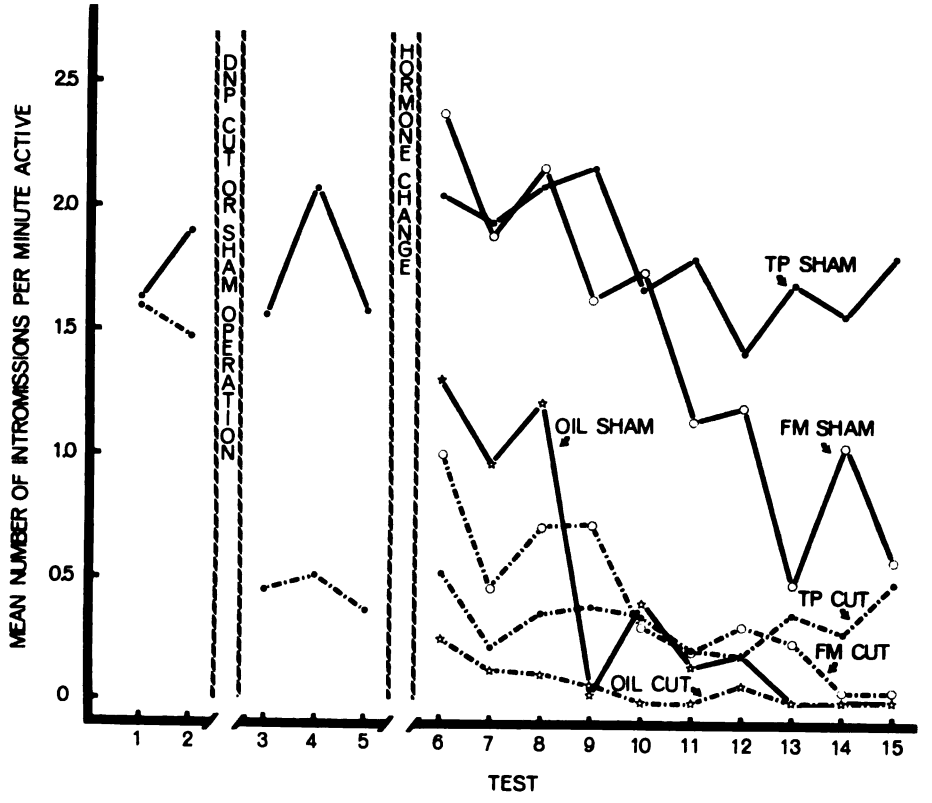

Figure 2. Effects of penile desensitization and hormone replacement on intromissions per minute active in male rats.

were not different from each other. On test 15, TP Sham animals achieved intromission at a higher rate than the Fluoxymesterone Sham animals (MWU, $p<0.05$) and likewise, Fluoxymesterone Sham animals had a significantly higher intromissions-per-minute score than did the Oil Sham animals (MWU, $p=0.002$). Intromission scores for the cut groups remained low throughout tests 6-15.

Mounts. Mean mount frequency per non-PEI minute for each group is shown in Figure 3. Mount scores in the DNP section groups showed a significant increase between pre- and post-operative tests (Sign Test, $p=0.034$), however, sham groups showed no change when comparing tests 1 and 2 with tests 3-5.

In Fluoxymesterone Sham and Fluoxymesterone Cut groups, mount scores decreased significantly between tests 6 and 15 (Wilcoxon, $p<0.05$ and $p<0.002$, respectively), as did Oil Sham and Oil Cut groups (Wilcoxon, $p<0.002$ and $p=0.002$, respectively). All TP animals remained unchanged over time. Although the 6 groups did not differ significantly at test 6, a statistically significant difference in mount scores was seen among the 6 groups in test 15 (K-W, $p<0.01$). In test 15, TP Sham animals had higher mount scores than did the Fluoxymesterone Sham animals (MWU, $p<0.05$) and Fluoxymesterone Sham animals had significantly higher mount scores than the Oil Sham group (MWU, $p<0.02$). In the cut groups, TP Cut animals achieved significantly higher mount scores at test 15 than did the Fluoxymesterone Cut animals (MWU, $p<0.002$), however, Fluoxymesterone Cut animals were not different from Oil Cut animals.

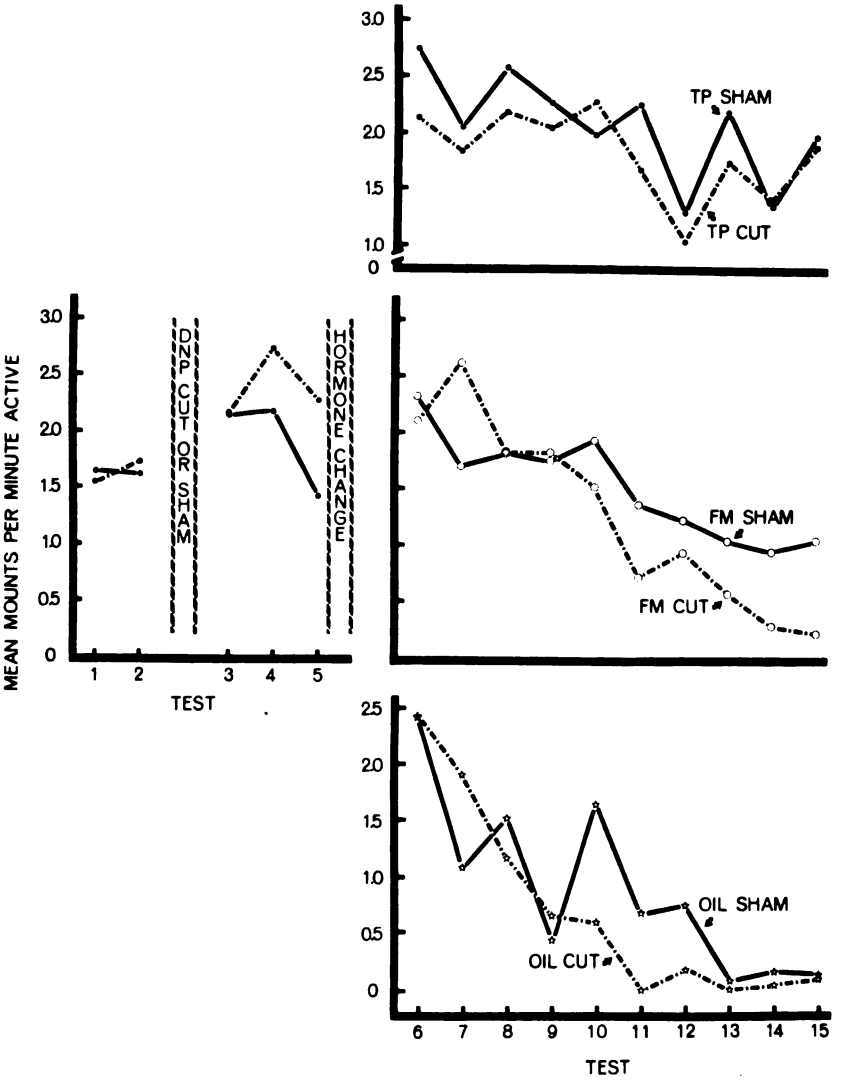

Figure 3. Effects of penile desensitization and hormone replacement on mounts without intromission per minute active in male rats.

Latency Measures. Results of dorsal nerve section on latency measures can be seen in Table 1. Sectioning the DNP caused a significant increase in ejaculation latency (Sign Test, $p=0.002$) with no change in mount latency or intromission latency for pre- and post-operative scores. In sham operated animals, a statistically significant decrease in ejaculation latency was observed (Sign test, $p<0.034$) with no changes in mount latency or intromission latency between these same tests.

Latency scores for tests 6 and 15 can be seen in Table 2. Following the change in hormones in test 6, mount latency and intromission latency were not significantly different among the 6 groups, although ejaculation latency did differ among the groups (Kruskal-Wallis, $p<0.02$). By test 15 a significant difference for all measures was noted among groups (K-W; EL, IL, & ML $p<0.001$). Comparison of test 6 latency scores with test 15 scores within groups gives an indication of changes over time. TP Sham animals showed no change in mount latency or intromission latency measures but did exhibit significant increases in ejaculation latency (Wilcoxon, $p<0.05$). TP Cut animals showed no change in mount latency or ejaculation latency but did increase in intromission latency (W., $p<0.05$) in a comparison between the 6th and final test. Fluoxymesterone Cut and Sham animals as well as Oil Cut and Sham animals all exhibited statistically significant increases in mount latency, intromission latency and ejaculation latency between tests 6 and 15 (W., all p values<0.02).

Table 1. Mean Latency Measures for Male Rats Prior to and Following Section of the Dorsal Nerve of the Penis.

			Test 2	Test 5
Mount	Cut	M	18.3	9.5
Latency (sec.)		SE	± 3.7	± 1.7
	Sham	M	10.1	6.5
		SE	± 2.3	± 0.6
Intromission	Cut	M	56.0	187.9
Latency (sec.)		SE	±17.7	±73.4
	Sham	M	34.1	29.8
		SE	±12.3	± 9.4
Ejaculation	Cut	M	518.0	1047.0
Latency (sec.)		SE	±71.6	±53.5
	Sham	M	460.9	356.8
		SE	±75.3	±70.2

Table 2. Effects of Hormone Manipulation on Mean Latency Measures (seconds) of Male Rats With or Without Section of the Dorsal Nerve of the Penis.

		Test 6			Test 15		
		Mount Latency	Intromission Latency	Ejaculation Latency	Mount Latency	Intromission Latency	Ejaculation Latency
TP Sham	M	6.3	21.6	273.6	12.4	43.8	416.5
	SE	1.1	5.9	82.7	3.2	17.2	95.9
TP Cut	M	10.0	48.8	1109.1	11.3	172.6	1028.6
	SE	2.4	13.2	45.3	4.8	57.3	57.4
FM Sham	M	5.3	13.0	211.4	175.9	406.0	610.9
	SE	0.8	5.1	45.8	146.5	148.8	114.7
FM Cut	M	8.0	67.6	841.8	913.8	1058.3	1191.8
	SE	1.4	33.8	129.4	187.4	141.7	8.2
Oil Sham	M	9.4	24.4	491.1	747.9	1200.0	1200.0
	SE	3.3	9.4	156.4	181.9	0.0	0.0
Oil Cut	M	8.6	111.1	1051.1	1042.0	111.4	1117.1
	SE	1.3	63.6	100.4	157.7	88.6	82.9

Between group comparisons of test 15, latency scores revealed that although Fluoxymesterone Cut animals were not different from Oil Cut or Sham animals in ejaculation latency, intromission latency or mount latency, Fluoxymesterone Sham animals showed significantly shorter latencies in all measures than did Fluoxymesterone Cut, Oil Cut or Oil Sham animals (Mann Whitney U, $p<0.02$ in all comparisons). Scores of Fluoxymesterone Sham animals compared to the TP Sham animals at test 15 showed that the TP group had a significantly shorter mount latency (MWU, $p<0.05$) and intromission latency (MWU, $p<0.05$) but that TP and Fluoxymesterone Sham animals did not differ with respect to ejaculation latency. On test 15, TP Cut animals had a shorter mount latency than the Fluoxymesterone Sham animals (MWU, $p<0.05$) while intromission latency did not differ between these groups. Fluoxymesterone Sham animals had a significantly shorter ejaculation latency than did the TP Cut animals on test 15 (MWU, $p<0.05$).

Histology. Microscopic examination of cross sections of the glans penis confirmed earlier observations (Beach and Levinson, 1950; Beach and Westbrook, 1968) in that penes of animals treated with TP and Fluoxymesterone were indistinguishable, while those animals treated with daily sesame oil showed a marked regression of papillae and a nearly complete lack of penile spines. Within hormone treatments, no difference could be seen between DNP Cut and Sham animals. Although a large difference in behavior was seen between cut and sham animals, no histological differences were evident upon examination of the shaft.

The following study was run to assess the effects of partial penile desensitization on male sexual behavior in rats.

EXPERIMENT 1, PART 2

Methods and Materials

Subjects for this experiment were 19 male rats of the Long-Evans strain, approximately 120 days old at the beginning of the study. They were housed under the same conditions as were those in Experiment 1, Part 1. All animals were castrated as adults and given TP replacement of 500 µg daily. Behavioral measures and conditions were identical to those in Experiment 1, Part 1.

A selection test was given two weeks after castration. At 4 and 8 days after the selection test, animals were given a 20 minute pretest with a sexually receptive female to establish a baseline of copulatory behavior. Following a second pretest 1 day later, animals were randomly assigned to a cut or sham group.

DNP sectioned animals were divided into 2 groups of 6 animals each. One group received a surgical section of the left dorsal nerve of the penis; in the second group, the right dorsal nerve of the penis was sectioned. Following a 2 week recuperation period, all animals were tested 3 times at 4 day intervals (Tests 3-5). After test 5, surgery was again performed to section the remaining nerve. Following a two week recovery period, the males were given the final 3 tests at 4 day intervals (Tests 6-8). Sham animals received 2 sham operations and were tested similarly. Since unilateral sectioning of the left and right produced scores which were not different, the data were pooled for statistical analysis.

Results

Ejaculations. The effects of cutting one of the DNP and then the other on ejaculation frequency are shown in Figure 4. Surgical section of one nerve caused a decrease in ejaculation frequency in test 3, when compared to pretest levels. However, this decrease was followed by a recovery in test 5. Ejaculation frequency for DNP section animals and sham animals was not significantly different in test 1 and test 5. However, recovery of ejaculation frequency was not seen following sectioning of the second nerve. Test 8 scores for ejaculation frequency were significantly lower in the sectioned animals than in the sham operated animals (MWU, $p<0.002$). Within groups, comparing test 1 with test 8, sectioned animals showed a significant decrease in ejaculation frequency (Wilcoxon, $p<0.002$), while scores for the sham animals showed no change between tests 1 and 8.

Intromissions. Intromission scores on tests 1-8 are shown in Figure 4. Unilateral sectioning caused a decrease in intromission scores, but intromission frequency returned to the level of the Sham group by test 5. Bilateral sectioning caused a sustained decrease in intromissions-per-minute, as seen in Experiment 1. Sectioned animals had significantly lower intromission-per-minute scores than did the sham animals on test 8 (MWU, $p<0.002$). Sectioned animals showed a significant decrease in intromissions-per-minute on test 8 as compared to the pretest score on test 1 (Wilcoxon, $p<0.002$). Sham animals did not change in intromissions-per-minute between tests 1 and 8.

Mounts. Mount scores for all tests are given in Figure 4. Sectioning of one of the dorsal nerves had no effect on mount scores

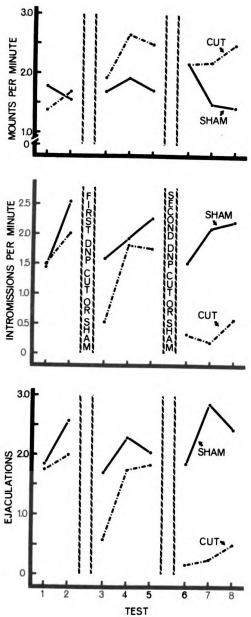

Figure 4. Effects of partial and complete penile desensitization on mounting, intromissions and ejaculations in the male rat.

when compared to sham animals on test 5. However, following the second nerve section, a significant increase in mounts-per-minute was noted on test 8 (MWU, $p<0.01$). Sectioned animals showed a significant increase in mounts-per-minute between tests 1 and 8 (Wilcoxon, $p<0.002$), but sham animals had scores on tests 1 and 8 which did not differ.

Discussion

Androgenic steroids and their metabolites regulate male sexual behavior by action at various sites. In the adult male rat, evidence indicates that the central nervous system, particularly the medial preoptic area (MPOA) and its connections represent a major site of androgen action. Intracerebral implants of testosterone into the MPOA will reinstate mating behavior in castrated animals (Davidson, 1966; Lisk, 1967; Christensen & Clemens, 1974). However, minute quantities of testosterone in the brain enter the peripheral circulation in amounts high enough to cause stimulation of penile tissue and growth of penile spines and papillae (Kierniesky & Gerall, 1973). While this penile stimulation might be regarded as only a side effect of the presence of central androgen, it renders conclusions based on these findings difficult to interpret in terms of strict central control of male behavior.

Intracerebral implants of estrogen, the aromatized metabolite of testosterone, are also very effective in restoring male behavior in male rats, however, no penile virilization is seen with estrogen implants (Christensen & Clemens, 1974). Stimulation of behavior without concommitent penile stimulation, ejaculatory maintenance by systematic injections of EB (Södersten, 1973), studies of estrogen uptake (Whalen & Littge, 1970) and in vitro studies of brain tissue

metabolism of steroids (Naftolin, Ryan & Petro, 1971 a,b) have been noted. These findings have led to the hypothesis that estrogenic metabolites of androgens may be the centrally active compounds responsible for male mating behavior.

It has been suggested that testosterone's action may be achieved by its metabolic products; 5α reduced androgens appear necessary for morphological maintenance of penile and sexual accessory tissues, while estrogenic metabolites or their precursors function centrally (Parrott, 1974). That androgens may influence sexual behavior at a peripheral locus has been suggested by the finding that the administration of presumably peripherally active non aromatizable androgens such as dihydro-testosterone (DHT) presents or slows down the loss of ejaculatory performance in castrated male rats (Parrott, 1974 a,b). Although androgen metabolites such as DHT will stimulate penile and sexual accessory tissues, DHT, even in large doses will not reinstate male mating behavior (McDonald et al. 1970; Whalen & Luttge, 1971). Brain implants of DHT are also ineffective (Johnston & Davidson, 1972).

Recent evidence has shown that administration of estrogen, in combination with DHT or fluoxymesterone, to castrated male rats will induce the full complement of male sexual behavior (Baum & Vreeburg, 1973; Larsson, Södersten & Beyer, 1973a,b; Feder, Naftolin & Ryan, 1974; Johnson & Tieffer, 1974). Presumably, such treatment provides a peripherally active androgen for appropriate stimulation and androgen maintenance of the genitalia, coupled with activation of central neural tissues which mediate sexual behavior.

Studies of male sexual behavior utilizing estroge, DHT combination indicate that the administration of EB + DHT to castrated male

rats with the DNP sectioned yielded results similar to those of intact rats (Clemens & Vomachka, unpublished observations). However, upon withdrawl of estrogen treatment, animals with desensitized penes still receiving DHT showed a more rapid decline in mounting behavior than intact animals receiving only DHT. The more rapid decline in rats with desensitized penes suggests an important behavioral role of penile tissues.

In the present study, fluoxymesterone administration after TP withdrawl produced a effect similar to that seen with EB + DHT following desensitization. Animals with desensitized penes showed a more marked decline in behaviors than animals with intact penile afferent nerves. While these experiments suggest a synergistic influence of brain and genital mechanisms, it is apparent that genital influences are neither necessary nor sufficient for mounting behavior. Likewise, treatment with testosterone propionate maintained mount frequencies at a high level even after section of the DNP. These findings with TP and fluoxymesterone suggest that sensory feedback from the penis may have a facilitative influence upon behavior, but in the presence of centrally active steroids, this influence is masked.

Studies of the influence of other sensory processes on sexual behavior have suggested that sensory effects are additive in terms of their influence on sexual behavior and that like penile sensitivity, the other sensory channels are not individually essential for the initiation of mating (Beach, 1942, Adler & Bermant, 1966; Carlsson & Larsson, 1964). For example, the effects of deprivation of different sensory modalities, including blinding, anosmia and deafening, on male

sexual behavior were explored in a classic study by Beach (1942). Loss of each modality individually does not eliminate copulation in sexually vigorous males. However, elimination of two or more of these did cause a reduction in copulation. Given the additive nature of sensory stimulation, it might be predicted that loss of penile sensations combined with loss of other sensory modalities would result in decreased sexual behavior.

The question remains as to how penile afferent sensations, supported by androgenic agents, might exert an effect on sexual arousal. An analogy may be drawn to the effects of intromissions on ejaculation. It has been suggested that the input of information from each intromission adds to the general excitatory state of the animal's central nervous system or maintains the excitatory state until the ejaculatory mechanism and ejaculation occurs (Beach, 1956; Sachs & Barfield, 1974). An animals readiness to mount a receptive female may also be influenced by penile stimulation in a similar manner. Day to day stimuli from the fluoxymesterone treated penis, whether from contact with the penile sheath, grooming, copulation, or non-specific tactile input, may increase a central excitatory state. This state then becomes manifest in the form of mounting when the proper stimulus condition is met. In the normal animal with a high androgen titer in the CNS, these penile contributions to sexual arousal, seen as shorter latency and higher frequency measures, are masked and only act to augment a highly stimulated motivational mechanism. In the present preparation, with reduced central androgenization but with selective androgenic support of penile tissue, a possible contribution of penile stimuli to behavioral maintenance becomes evident.

While fluoxymesterone treatment maintains penile morphology and facilitates mounting, the effects of FM on penile sensitivity are not known. Beach and Levinson (1950) postulated a sensory role for penile spines and papillae. However, in a series of neurophysiological experiments, Cooper and Aronson (1974) found that genital sensory fields and sensory thresholds were not different between intact and castrated cats. Assuming that this finding in another species applies to rats as well, where might androgen act to facilitate penile sensory processes? One site of androgen action could be at the level of the spinal cord.

Possibly fluoxymesterone maintained penile input to the CNS through spinal pathways to a greater degree than that seen in oil treated animals or animals with the DNP severed. Hart (1967, 1968, 1973) has investigated the decrease in sexual reflexes in spinal male rats following castration. Such decreases may reflect changes in the neural functioning of the spinal cord involved in the transfer of information from the genitalia to the CNS. Cooper and Aronson (1974) report that castration alters central processing of genital sensory input, in that stimulation of penile afferent nerves of castrates and non castrates resulted in differences in various measures of neocortical activity. The "thalamus or cortex or other sensory relay or integrative centers" were proposed as possible areas involved in the observed differences between castrates and non-castrates. In this light, the spinal pathways, devoid of androgen stimulation could conceivably alter penile afferent sensations and thereby alter behavior.

Of course, the possibility remains that fluoxymesterone is actually acting at other central neural sites to maintain sexual

behavior. Although this seems unlikely, the following experiment was designed to test the central neural androgenicity of fluoxymesterone in the developing animal.

EXPERIMENT 2

Introduction

Androgenic steroids which stimulate growth of genital and sexual accessory tissues do not always stimulate male sexual behavior. Dihydrotestosterone (DHT) is one such peripherally active compound. This steroid has been shown to have little influence upon behavior but is a powerful virilizing agent peripherally (Feder, 1971; Johnston and Davidson, 1972; Parrott, 1974 a,b, and c, 1975; Christensen, Coniglio, Paup, and Clemens, 1973; Feder, Naftolin and Ryan, 1974). Another such compound is fluoxymesterone which was originally administered clinically to humans (Reilly and Gordon, 1961). When given to stimulate growth in boys of short stature, a concomitant phallic enlargement was noted without "evidence of stimulation of libido or masturbation". In rats fluoxymesterone would not restore the loss of mating in long term castrated males but did stimulate the normal size of seminal vesicles (Beach and Westbrook, 1968). Likewise, the penile epithelium, which generates and becomes smooth following castration, was maintained with papillae and cornified penile spines by fluoxymesterone.

Johnson and Tiefer (1974) also found that fluoxymesterone, given to castrated male rats, did not maintain mounting, intromission or ejaculation any better than did sesame oil. However, combination of estradiol benzoate (EB) treatment with fluoxymesterone was highly

effective in maintaining sexual behavior at a level above that achieved with either EB or fluoxymesterone alone. Similar findings with DHT in combination with EB (Baum and Vreeburg, 1973; Larsson, Södersten and Beyer, 1973 a and b; Feder, Naftolin and Ryan, 1974) have strengthened the hypothesis that sexual behavior is the result of both central neural activation of behavior (by estrogenic steroids) and maintenance of genital tissues (by peripherally active steriods).

In an experiment designed to determine the extent to which penile development is influential in the development of male sexual behavior, Hart (1972) administered either testosterone propionate (TP) or fluoxymesterone to neonatal male rats and tested their sexual behavior and sexual reflexes as adults. Comparison between the sexual behavior of animals which had been castrated on day 4 and received TP on days 5, 7, 9, and 11 and those which were castrated and received fluoxymesterone showed that fluoxymesterone animals achieved fewer ejaculations. While the fluoxymesterone treated animals did mount and intromit, vehicle control animals achieved significantly fewer intromissions. Since gross penile development and reflexes were not different between the androgen treated groups, Hart could not attribute the behavioral differences observed to peripheral factors. The results support the concept that ejaculation behavior requires the influence of androgen during neonatal development (Hart, 1972).

That Hart could not find differences between testosterone and fluoxymesterone treatment neonatally on mounting and intromission does not mean that these behaviors develop independent of steroid influences. Lack of differences may have been the result of the nature of the

animal with which he was working. In the rat, testicular differentiation occurs on about day 13 (post coital age), while behavioral sexual differentiation occurs between days 18 and 27 (see Clemens, 1973). When castrated on day 4 of postnatal life (assuming that birth occurs on day 21 post coital) neonatal male rats have already been exposed to their own endogenous androgens for at least 7 days. Conclusions at to the "organization" of mounting and intromission based on post natal hormone administration to rats are tenuous at best.

Since the hamster has a relatively short gestation period (16 days) it offers a better model for studying androgenic control of behavioral development. While testicular differentiation occurs at about 11.5 days post coital, behavioral differentiation appears to be entirely post natal, beginning at 16 days post coital age (Nucci and Beach, 1971; Clemens, 1973). The male hamster castrated on the day of birth (day 1) will not show mounting, intromission, or ejaculation as an adult even when given large doses of androgen as an adult. On the other hand, the female or Day 1 castrated male hamster which receives gonadal hormones during the first few days of life will show mounting and suppressed lordosis behavior at adulthood (Carter, Clemens and Hoekema, 1972; Paup, Coniglio, and Clemens, 1972, 1974; Coniglio, Paup, and Clemens, 1973; Coniglio, 1973). Consequently, the estent to which neural or genital virilization influence behavioral sexual differentiation and later behavior may be more easily examined in the hamster.

The following experiment:

1. tests the virilizing effects of fluoxymesterone upon behavior and genital tissue when administered to day 1 castrate male or

female hamsters.

2. tests two alternate hypotheses regarding the mechanism of androgen influence upon development and the control of mating:

a) Does treatment neonatally with fluoxymesterone induce a behavioral differentiation similar to that seen with testosterone,

and

b) is male sexual behavior in the hamster programmed prior to the neonatal organization of peripheral tissues.

3. provides comparative data on the development of sexual behavior in rodents.

The timing of behavioral sexual differentiation in the hamster makes it a more suitable subject than the rat to study the androgenicity of certain compounds (Vomachka, Paup, Coniglio, McManus and Clemens, 1974), the control of sexual differentiation (Clemens, 1973) or the extent to which sexual behavior is controlled by central or peripheral stimuli (see Experiment 3).

Methods and Materials

Subjects

The subjects of this experiment were 27 male and 36 female hamsters (Mesocricetus auratus) born and raised in the Hormones and Behavior Laboratory at Michigan State University. All animals were given food and water ad libitum and were maintained on a reversed day-night

light cycle of 14 hours light, 10 hours dark with lights out at 11:00 hours. All animals were weaned at 21 days of age and housed in unisexual groups of 2-6 animals. Cages used were plexiglass with removable stainless steel tops with dimensions of 26X20X15 cm or 35X30X17 cm depending upon the size of the group.

Treatment Groups

On the day of birth (day 1), males were castrated through a mid-ventral incision using hypothermal anesthesia. The incisions were sutured and sealed with flexible collodion and the pups were returned to their mothers. On days 2, 3, and 4 of postnatal life, all animals in each litter received a subcutaneous injection of one of the following substances: fluoxymesterone (100µg/day) suspended in sesame oil, testosterone (100µg/day) free alcohol form, or the hormone vehicle, sesame oil (0.03 cc/day). Subcutaneous injections were administered under the skin of the back with needle entry in the posterior back region and the bolus of hormone being deposited under the skin of the neck. Upon withdrawl of the needle, puncture sites were sealed with flexible collodion and animals were inspected for leadage of hormone before being returned to their mothers. At 60 days of age, all animals were earpunched for identification and females were ovariectomized under ether anesthesia. Ovaries were fixed in Bouin's solution, embedded in paraffin, sectioned and stained with hematoxylon-eosin for histological examination.

Test Procedures

Mounting Behavior:

At approximately 75 days of age, all animals began weekly testing for mounting behavior. Testing (1300-1700 hr.) was conducted in an air conditioned annex to the animal quarters which were dimmly illuminated. Observation arenas were 10 gal. aquaria with Sani-cell^{r} (ground corn cobs) covering the floor. All mounting behaviors during a 10 minute test were recorded on an Esterline-Angus even recorder. Tests followed a 3 minute adaptation period during which the experimental animal was placed alone in the observation arena. After the adaptation period, a sexually receptive stimulus female was introduced into the arena and behaviors were recorded. Receptivity was induced by administration of 12µg EB for 3 days and 0.5 mg progesterone to ovarectomized females on the morning of testing. Stimulus females were proven receptive prior to experimental use by placing them with vigorous nonexperimental males.

Male and female experimental hamsters were tested weekly for 5 consecutive weeks. Following the first test for mounting (pretest) daily administration of 300µg TP was used to induce mounting. All mounts and intromissions were recorded and mounts without intromission were further divided into catagories as to whether they were directed to the head or side, or to the rear of the receptive stimulus female. A mount was scored as such only if it was accompanied by a clasp and pelvic thrusting. In addition, the length of time to the first mount and/or intromission was measured (mount latency and intromission latency). If no sexual behavior was observed during a particular test, a latency

measure of 600 seconds (the length of the test) was assigned for that test. Following the final test for mounting, TP administration ceased.

Lordosis Behavior:

At approximately 30 and 44 days following the fifth test for mounting behavior, 10 minute tests for lordosis behavior were given to all experimental animals. Lordosis tests were preceded by daily administration of 6 μg EB for 3 days and an injection of 0.5 mg progesterone on day 4, 4-6 hours prior to testing. Testing occurred between 1300 and 1700 hours. Measurement of lordosis behavior entailed placing the experimental animal into an observation arena (10 gal. aquarium) with a sexually vigorous indicator male and recording the lordosis behavior of the experimental animal. Indicator males which did not mount were replaced. Measures derived from the Esterline-Angus recorder record included the frequency of lordosis exhibited, the total amount of time spent in lordosis and the duration of the longest lordosis response during each 10 minute test. A mean for each of these measures was taken for each animal.

Morphological Measures

Approximately one month after the final lordosis test, all animals were sacrificed and inspected for the presence of testicular or ovarian tissue. If gonadal tissue remained, the data from that particular animal were eliminated from the study. Anogenital distance was measured in all animals. Penile or clitoral bone and cartilage was dissected out and the total length of these was measured.

Statistical Analysis

The data were evaluated using one way analysis of variance and further comparisons were made using the Student-Newman-Keuls test for multiple comparisons among means (Sokal and Rohlf, 1969).

Results

Mounting Behavior of Males

Twenty eight days of TP administration induced high levels of mounting in males treated neonatally with testosterone (Fig. 5 and 6, Table 3) but not in animals treated neonatally with fluoxymesterone or sesame oil. By test 4 (28 days) analysis of variance revealed a significant difference among groups for mount latency, head and side mount frequency, and rear mount frequency. While animals treated neonatally with T showed significantly higher head and side mount scores and rear mount scores than the other groups ($p<0.05$), animals treated neonatally with fluoxymesterone or sesame oil did not differ statistically ($F=6.58$, $df=2,24$, $p=0.005$; $F=11.91$, $df=2,24$, $p<0.01$; and $F=3.64$, $df=2,24$, $p<0.05$ respectively). Similarly, fluoxymesterone and sesame oil treated animals showed significantly longer latencies to the first mount than did the neonatal testosterone group ($p<0.05$). Fluoxymesterone and sesame oil groups did not differ in mount latency. The only group to show any intromission patterns was the neonatal testosterone group (mean intromission frequency = 1.8 on test 4).

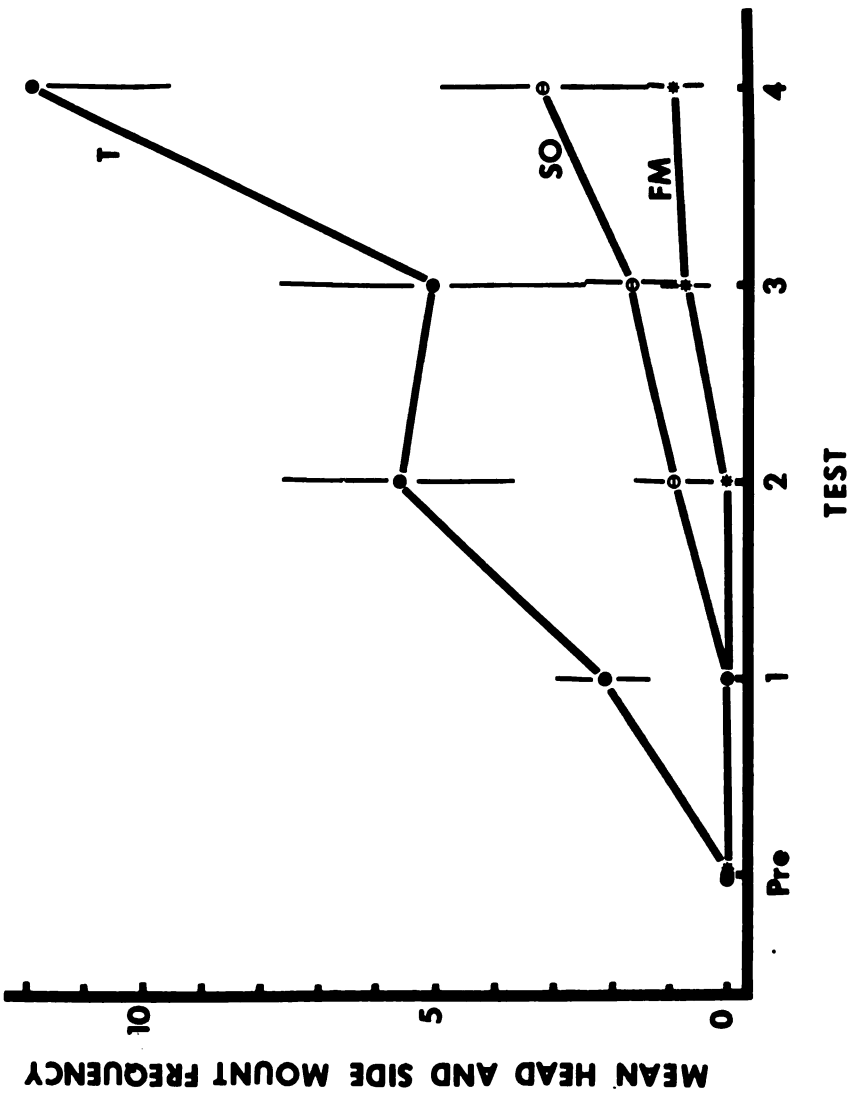

Figure 5. Effects of neonatal administration of fluoxymesterone, testosterone or sesame oil on mean head and side frequencies in castrated male hamsters.

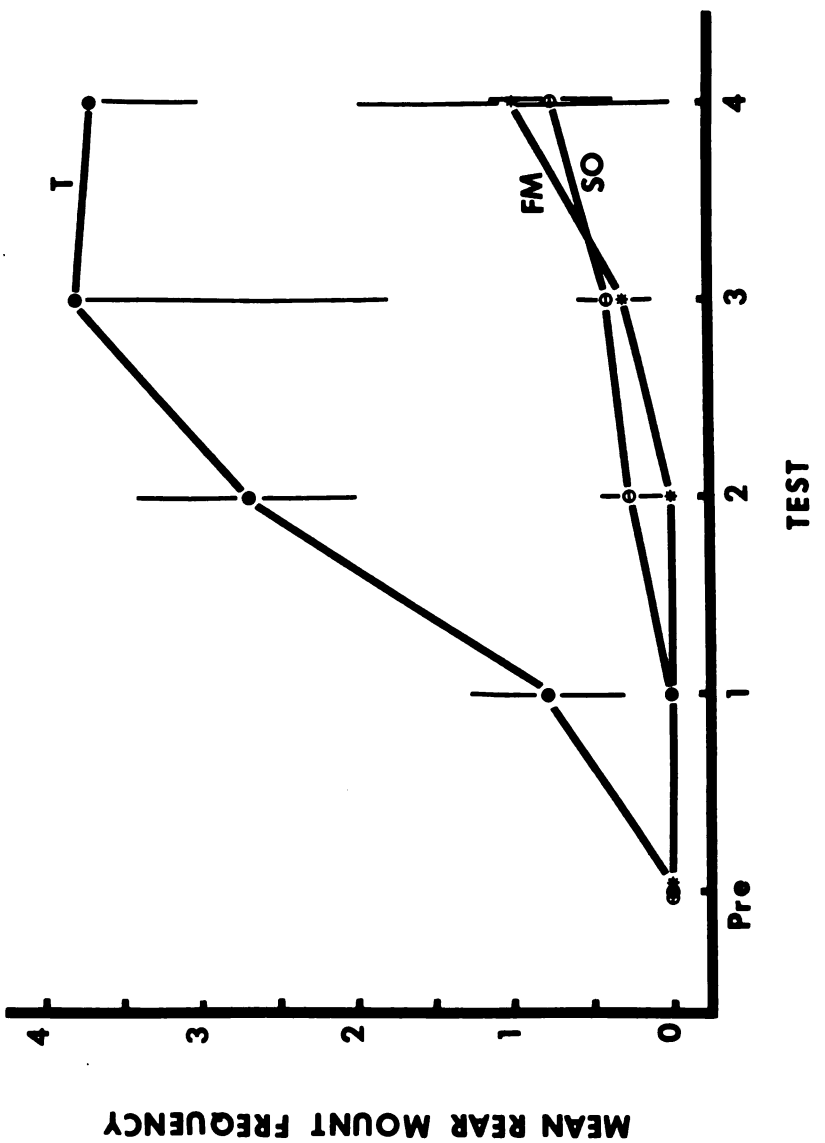

Figure 6. Effects of neonatal administration of fluoxymesterone, testosterone or sesame oil on mean rear mount frequencies in castrated male hamsters.

Table 3. Mean mount latency for male hamsters castrated on the day of birth and treated neonatally with fluoxymesterone, testosterone or sesame oil (in seconds ± s.e.).

Neonatal Treatment (d2-3-4)	Weekly Tests				
	Pretest	1	2	3	4
Fluoxymesterone (100 µg/day)	600 ±0	600 ±0	600 ±0	573 ±20	458 ±73
Testosterone (100 µg/day)	600 ±0	389 ±80	310 ±85	261 ±86	106 ±50
Sesame Oil (0.03 cc/day)	600 ±0	600 ±0	454 ±74	389 ±84	373 ±92

Mounting Behavior of Females

At 28 days of daily TP treatment, females treated neonatally with testosterone showed higher head and side mount and rear mount frequencies than fluoxymesterone or sesame oil treated females ($p<0.05$). Fluoxymesterone and sesame oil treated females were not different from each other in mount frequencies (Fig. 7&8). Fluoxymesterone and sesame oil groups did not differ from each other in latency to the first mount (Table 4). However, both groups took a longer time to the first mount than did the testosterone treated females, (H&SMF, $F=8.83$, $df=2,33$, $p<0.01$; RMF, $F=15.49$, $df=2,33$, $p<0.001$; and ML, $F=7.05$, $df=2,33$, $p<0.005$). Intromission patterns were not seen in any of the treatment groups.

Lordosis Behavior of Males

Testosterone treated animals showed more lordoses than did fluoxymesterone treated animals ($F=4.24$, $df=2,23$, $p<0.05$) but the treated animals were not different from sesame oil animals (Figure 9). Likewise, fluoxymesterone and sesame oil animals were not different from each other in lordosis frequency.

Lordosis Behavior of Females

Females treated neonatally with testosterone showed more lordoses than sesame oil or fluoxymesterone treated animals (Figure 10) (L.F. $F=5.20$, $df=2,33$, $p<0.025$, and Longest L $F=4.53$, $df=2,33$, $p<0.025$) while sesame oil and fluoxymesterone animals were not significantly different. In contrast to lordosis frequency, the mean longest lordosis for testosterone

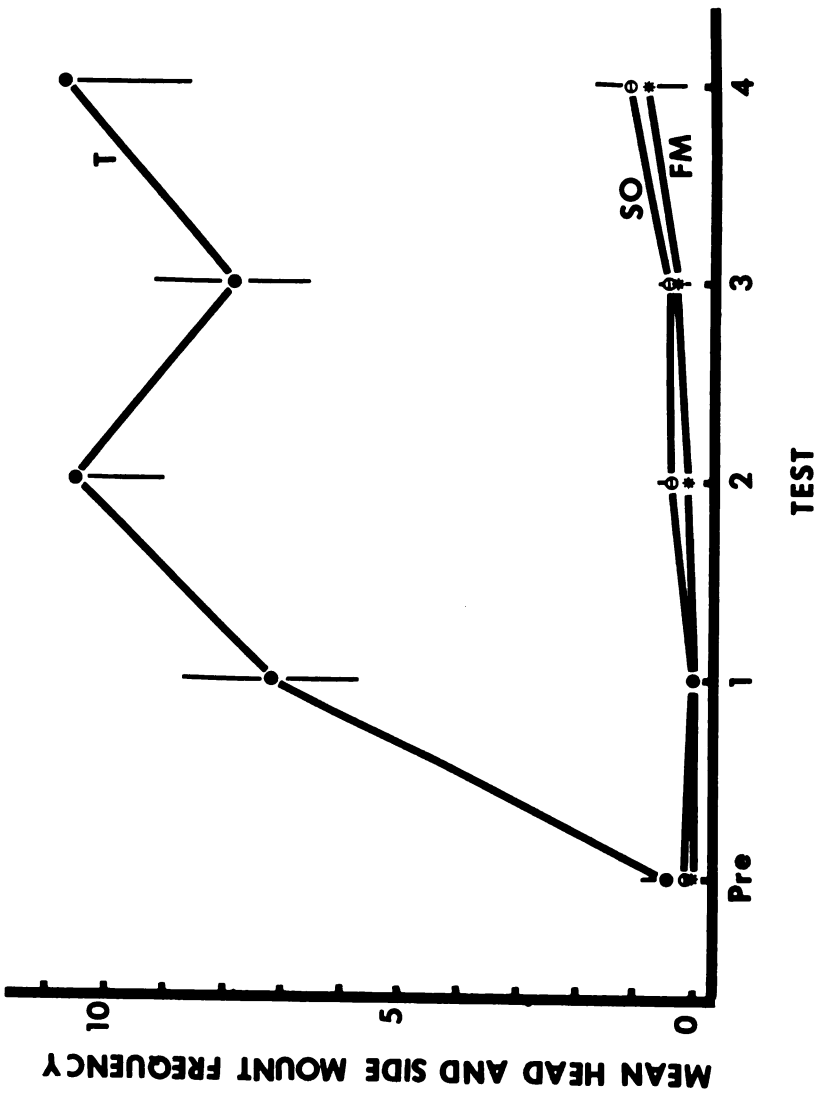

Figure 7. Effects of neonatal administration of fluoxymesterone, testosterone or sesame oil on mean head and side mount frequencies in female hamsters.

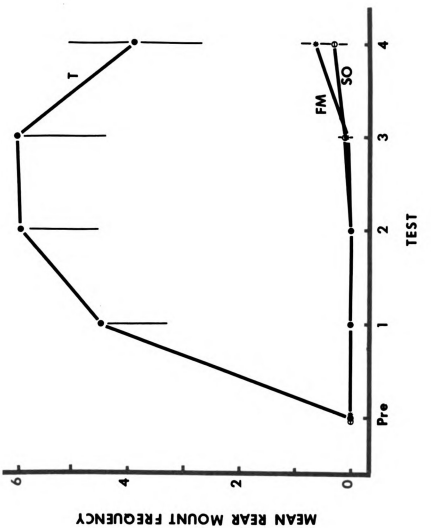

Figure 8. Effects of neonatal administration of fluoxymesterone, testosterone or sesame oil on mean rear mount frequencies in female hamsters.

Table 4. Mean mount latency for female hamsters treated neonatally with fluoxymesterone, testosterone or sesame oil (in seconds ± s.e.)

Neonatal Treatment (d 2-3-4)	Weekly Tests				
	Pretest	1	2	3	4
Fluoxymesterone (100 µg/day)	600 ±0	600 ±0	571 ±29	528 ±49	445 ±60
Testosterone (100 µg/day)	461 ±61	170 ±66	123 ±55	49 ±10	132 ±53
Sesame Oil (0.03 cc/day)	550 ±50	600 ±0	535 ±76	473 ±76	457 ±75

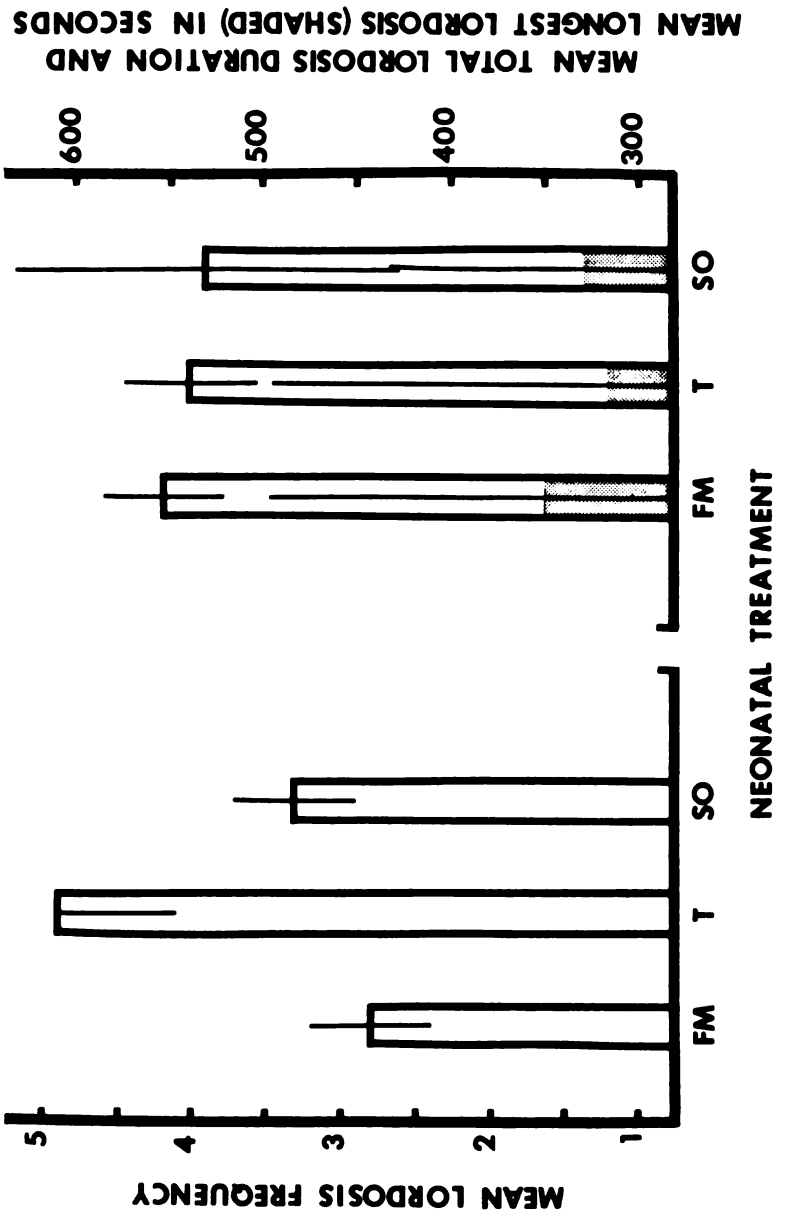

Figure 9. Paremeters of lordosis behavior in castrated male hamsters treated neonatally with fluoxymesterone, testosterone or sesame oil.

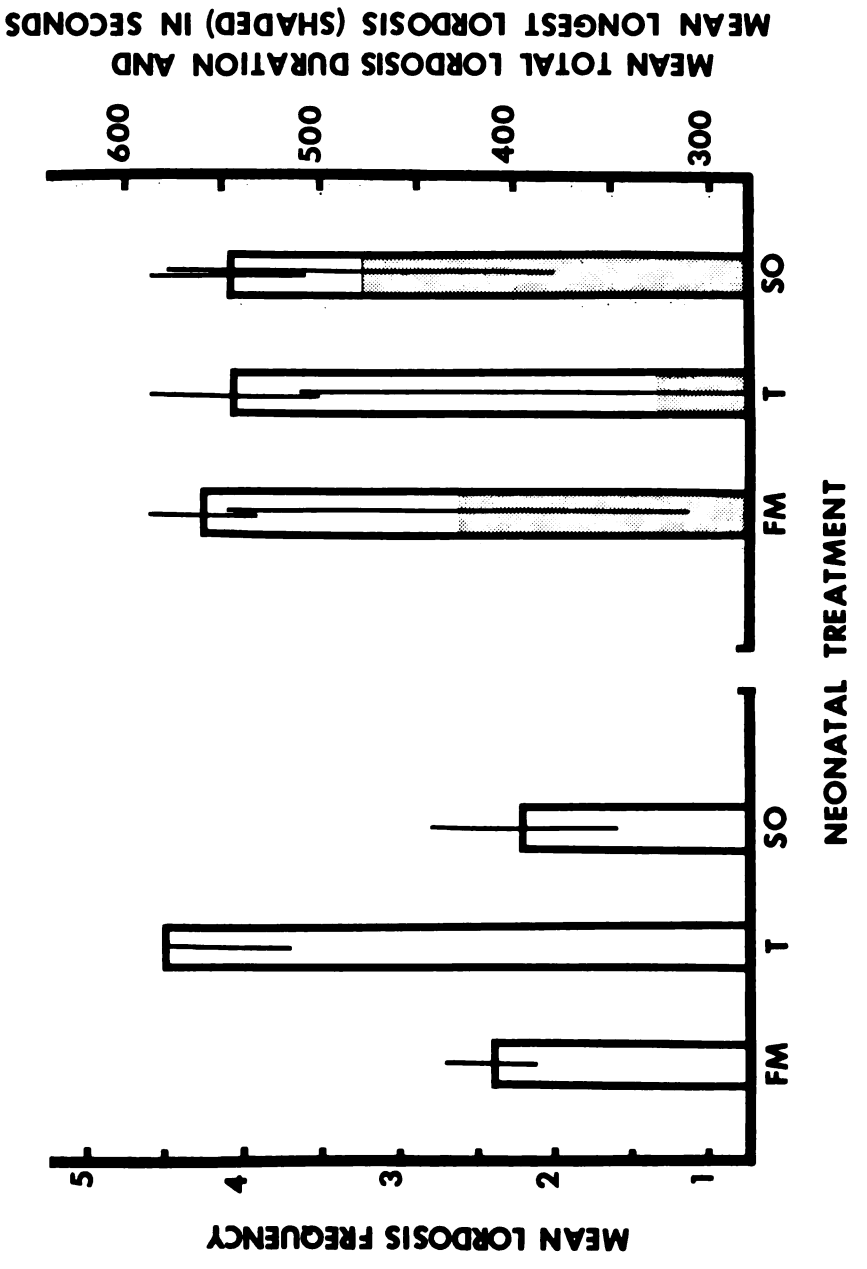

Figure 10. Parameters of lordosis behavior in female hamsters treated neonatally with fluoxymesterone, testosterone or sesame oil.

treated females was shorter than that of either fluoxymesterone or sesame oil treated females.

Morphological Measures

Males:

While anogenital distance did not vary significantly among the three groups, analysis of variance did reveal a significant difference among treatments in length of penile bone and cartilage ($F=58.63$, $df=2,24$, $p<0.01$). Neonatal fluoxymesterone and testosterone treatment caused a significant increase in penile length when compared to sesame oil treated animals (Table 5) ($p<0.05$). Fluoxymesterone and testosterone treated males did not differ from each other significantly.

Females:

Neonatal treatment with fluoxymesterone on testosterone resulted in a longer anogenital distance than did sesame oil treatement ($F=69.5$, $df=2,33$, $p<0.001$); fluoxymesterone and testosterone treated animals were not different from each other (Table 6). Fluoxymesterone and testosterone treated females, while not different from each other, showed greater clitoral virilization than did sesame oil treated females ($F=58.41$, $df=2,30$, $p<0.001$).

Ovarian Histology

None of the neonatal treatments, fluoxymesterone, testosterone, or sesame oil disrupted ovarian function. Ovaries possessed corpora-lutea and developing follicles, and appeared normal.

Table 5. Morphological measures of male hamsters castrated on the day of birth and treated neonatally with fluoxymesterone, testosterone or sesame oil.

Neonatal treatment (d 2-3-4)	A-G distance mean length in mm (± s.e.)	Penile bone and cartilage mean length in mm (±s.e.)
Fluoxymesterone (100 µg/day)	16.0 ±0.4	4.6 ±0.0
Testosterone (100 µg/day)	15.2 ±0.4	4.5 ±0.0
Sesame Oil (0.03 cc/day)	14.7 ±0.2	3.4 ±0.0

Table 6. Morphological measures of female hamsters treated neonatally with fluoxymesterone, testosterone or sesame oil.

Neonatal treatment (d 2-3-4)	A-G distance mean length in mm (±s.e.)	Clitoral bone and cartilage mean length in mm (±s.e.)
Fluoxymesterone (100 µg/day)	10.2 ±0.2	4.0 ±0.0
Testosterone (100 µg/day)	10.3 ±0.2	3.9 ±0.0
Sesame Oil (0.03 cc/day)	7.2 ±0.2	3.0 ±0.0

Discussion

While neonatal administration of fluoxymesterone or testosterone to castrated male and female hamsters induced similar patterns of penile and clitoral growth in response to androgen at adulthood, only testosterone achieved significant masculinizing influence upon behavior. Fluoxymesterone neither "masculinized" nor "defeminized" behavior. These findings as well as those of others (Reilly and Gordon, 1961; Beach and Westbrook, 1968; Johnson and Tiefer, 1974; Hart, 1972) strengthen further the idea that fluoxymesterone is a peripherally acting androgen with little or no central neural actions.

The absence of mounting and intromission in fluoxymesterone treated animals in spite of marked genital virilization agrees with other work in which a dissociation of sexual behavior and genital virilization was found (Coniglio, 1973; Paup et al., 1974). The fact that the present results with hamsters do not agree entirely with those Hart obtained with the rat may be attributable to species or dosage differences. Indeed, male rats castrated neonatally normally show male sexual behavior (Larsson, 1966) when given androgen as adults, while hamsters do not.

Although the neonatal dosages of hormone given did not induce ejaculation in any group, mounting and intromission were induced by testosterone given neonatally. This suggests that in the hamster, mounting and intromission are not organized independent of androgen as has been speculated for the rat (Beach and Holz, 1946; Whalen, 1968).

Whalen (1968) argued that in the rat, the reduced incidence of intromission in males castrated neonatally reflects inadequate penile development. Similar conclusions have been reached by Nadler (1969) and Goldman et al., (1972). Loss of intromission after various manipulations including removal of sections of penile bone (Beach and Holz, 1946), the glans penis (Whalen, 1961) anesthetization of the penis (Carlsson and Larsson, 1964; Adler and Bermant, 1966) and removal of the dorsal nerves of the penis (Larsson and Södersten, 1973) lend credence to the hypothesis that successful intromission is a function of an adequate phallus. Although the phalli of the testosterone and fluoxymesterone groups in this as well as in Hart's study (1972) were not different, intromission behavior always occurred with greater frequency in the testosterone treated groups.

It is proposed that testosterone, prenatally in the rat, and postnatally in the hamster programs the CNS for mounting. FM on the other hand lacks this effect, but shares with T the ability to virilize the genital morphology. The differences in T & FM in intromission scores reflect the differential effect of these hormones on mounting behavior. Since intromissions cannot occur without a mount, an androgen which does not program the CNS for mounting cannot induce intromissions.

In the present study, higher doses of testosterone neonatally or more prolonged administration might have induced adult ejaculations. It is doubtful that increased fluoxymesterone would have, since ejaculation was not induced in rats with much higher doses of fluoxymesterone.

EXPERIMENT 3

Introduction

Evidence that sensory feedback from the penis is necessary for intromission and ejaculation has accumulated for several species (Adler and Bermant, 1966; Whalen, 1968; Aronson and Cooper, 1966, 1968; Herbert, 1973; Larsson and Södersten, 1973). However, manipulations which result in changes in penile sensitivity, size, and structure of the penis do not have as profound an effect on mounting as they do on intromission (Beach and Holz, 1946; Adler and Bermont, 1966; Whalen, 1968; Nadler, 1969; Goldman et al., 1972).

The fact that penile sensitivity has little influence on mounting is not entirely clear. Beach and Levinson (1950) postulated that the loss of mounting behavior subsequent to castration in rats was correlated with a loss in penile sensitivity. Even though electrophysiological research suggests that castration does not cause a decrease in penile sensitivity in cats (Cooper and Aronson, 1974), it has been shown that decreases in peripheral sensitivity produced by local anesthesia or nerve section can lead to decreases in mounting over time (Carlsson and Larsson, 1964; Aronson and Cooper, 1968; Herbert, 1973). Experiment 1 of this thesis showed that penile sensory feedback may have a facilitatory effect upon mounting as well as intromission and ejaculation in rats. In order to extend the work of Experiment 1 and to entend the comparative basis for theories of

the peripheral influence on male mating behavior, the hamster was chosen for further work. The questions asked in this experiment are:

1) Does penile sensory feedback facilitate male mating behavior in the hamster?
2) Does sexual behavior vary as a function of penile size in the hamster?

Sexual differentiation of male mating behavior in hamsters is presumably under the control of gonadal steroids present during some "critical period" in development (Clemens, 1973; Eaton, 1970; Coniglio, 1973). The presence of estrogens or androgens which can be aromatized to estrogens during the period of behavioral sexual differentiation leads to an animal which will show mounting as an adult. In the absence of such androgen or estrogen the female behavior pattern of receptivity (lordosis) but not mounting behavior are evident at adulthood. Gonadal steroids also affect the growth potential of the genital tissue of the male. The presence of androgens during development results in a phallus which will respond with growth to pubertal increases or exogenous administration of androgens at adulthood (Bruner and Witschi, 1946; Beach, Noble, and Orndoff, 1969; Coniglio, 1973). However, estrogens, which will cause behavioral masculinization do not increase the potential for genital virilization (Coniglio, 1973, Paup, Coniglio and Clemens, 1974). This dissociation of behavioral and morphological development with estrogens and the concomitant

behavioral and morphological virilization with androgens can be used to create an experimental model to study the influence of penile sensory feedback on behavior. Neonatally androgenized and estrogenized animals have a similar potential for mounting but a different potential for phallic growth when given TP as adults.

It was presumed that sensory feedback from the penis in neonatally androgenized hamsters would be greater than the sensory input from the unvirilized neonatal estrogenized animal. The present experiment attempted to reveal differential feedback effects on the maintenance of mounting behavior. Fluoxymesterone maintains penile size and integrity but not sexual behavior while section (DNP) of the dorsal nerve of the penis provided a presumably complete reduction in penile sensations.

Methods and Materials

Subjects

The subjects of this study were 107 male golden hamsters (*Mesocricetus auratus*) born and raised in the Hormones and Behavior Laboratory at Michigan State University. Animals were maintained as outlined in Experiment 2.

Treatments and Behavior Testing

All males were castrated on the day of birth (day 1) using the procedures described in Experiment 2. On days 2, 3, and 4 of life all animals received subcutaneous injections of one of the following hormones: testosterone propionate, either 100µg or 25µg per day (TP 100 or TP25) or estradiol benzoate 25 µg/day (EB25). Injections were administered under the same procedural paradigm as Experiment 2.

At 60 days of age all animals were earpunched for identification. When the animals were 75 days of age they entered a weekly testing schedule for mounting behavior with test procedures and measures identical to those in Experiment 2. The first 5 weekly tests were the same as those in Experiment 2, namely, a 10 minute pretest prior to TP treatment, daily injections of 300µg TP and 10 minute tests at 7 day intervals for 28 days.

Following the 5th test for mounting behavior, animals from each of the neonatal treatment groups were randomly assigned to one of two groups; to be called Cut or Sham. Males in the Cut groups were subjected to bilateral removal of a 2-4 mm section of the dorsal nerve of the penis (DNP). Procedures used were the same as in Experiment 1.

Animals were allowed a recuperation period of 10 days with daily TP injections before receiving 3 weekly postoperative tests (tests 6-8). Following the third postoperative test, TP injections were terminated and animals from cut and sham groups were assigned to one of the following two hormone conditions. Condition 1 consisted of daily administration of 300µg fluoxymesterone. Condition 2 was no exogenous hormone at all but daily injections of the sesame oil vehicle (0.05cc). All animals were then tested for mounting at weekly intervals for 8 weeks (tests 9-16). Behavioral measures were the same as those taken for male behavior in Experiment 2. The final groups for data analysis are as follows:

<u>TP 100µg neonatal</u>

1) FM CUT: DNP section; fluoxymesterone for the final 8 tests. n=9

2) FM SHAM: sham operated males; fluoxymesterone for the final 8 tests. n=11

3) OIL CUT: DNP section; sesame oil for the final 8 tests. n=10

4) OIL SHAM: sham operated; oil vehicle for the final 8 tests. n=10

TP 25 µg neonatal

1) FM CUT n=8

2) FM SHAM n=6

3) OIL CUT n=10

4) OIL SHAM n=10

EB 25 µg neonatal

1) FM CUT n=9

2) FM SHAM n=9

3) OIL CUT n=8

4) OIL SHAM n=7

These 12 final groups were sacrificed 1-2 days after the completion of test 16.

Morphological Measures

At sacrifice all animals were inspected for completion of gonadectomy. Any evidence of remaining testicular tissue eliminated the animals from the experiment. Ano-genital distances were measured in all animals. Penile bone and cartilage were removed as a unit and measured in all animals. Portions of the glans penis were fixed in Bouin's solution for histological examination. Penile glans tissues were embedded in paraffin, sectioned at 10 µ, stained with hematoxylin-eosin and examined for the presence of spines and/or papillae.

Statistical Analysis

Data were evaluated using one way analysis of variance. Further comparisons were made using the Student-Newman-Keuls test for multiple comparisons among means (Sokol and Rohlf, 1969) or Mann-Whitney U-test. Although not listed in results section, S-N-K and M-W-U $p<0.05$.

Results

Results of neonatal treatment (Tests 1-5), penile desensitization (Tests 6-8) and hormone manipulation (Tests 9-16) on parameters of male mating behavior will be considered separately. Appendix Tables A1-A4 give means ± standard errors for various copulatory behaviors. (Tables A1, mean latency to first mount; Table A2, mean head and size mount frequency. Table A3, mean rear mount frequency, and Table A4 mean intromission frequency. Tables A5-A7 list the percentage of animals exhibiting either head and side mounts, rear mounts or intromissions for each neonatal treatment group.)

Effects of Neonatal Treatments (Tests 1-5)

Twenty eight days of TP administration in adulthood caused an increase in mating behaviors in all groups (Table A 1 and Figures 11-13, tests 1-5). Analysis of variance of scores from test 5 revealed no differences within any of the neonatal treatment groups for mean rear mount frequency, latency to mount or mean intromission frequency. Comparison of test 5 scores among the 3 neonatal treatment groups revealed no differences in rear mount frequency, however TP 100 and TP 25 animals achieved significantly more intromissions than did neonatally EB 25 treated animals ($f=4.730$, $df=2,33$, $p<0.025$; S-N-K $p<0.05$).

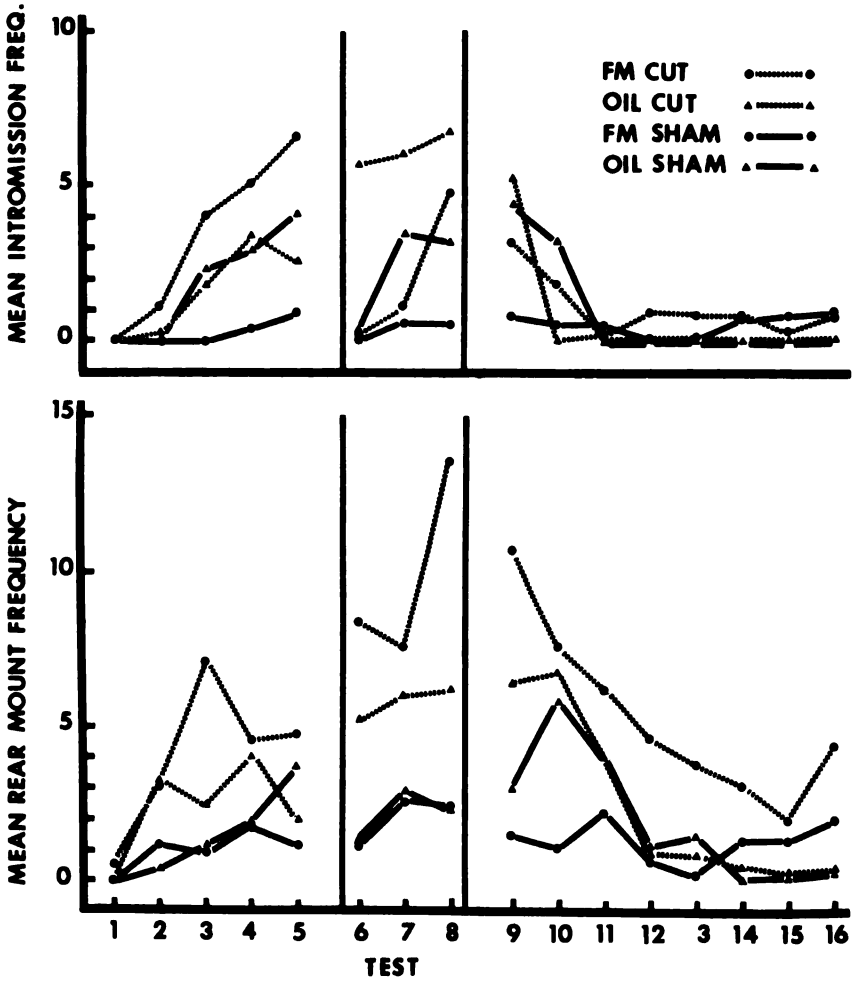

Figure 11. Rear mount frequency and intromission frequency in day 1 castrated male hamsters treated with TP100 neonatally and given various adult treatments.

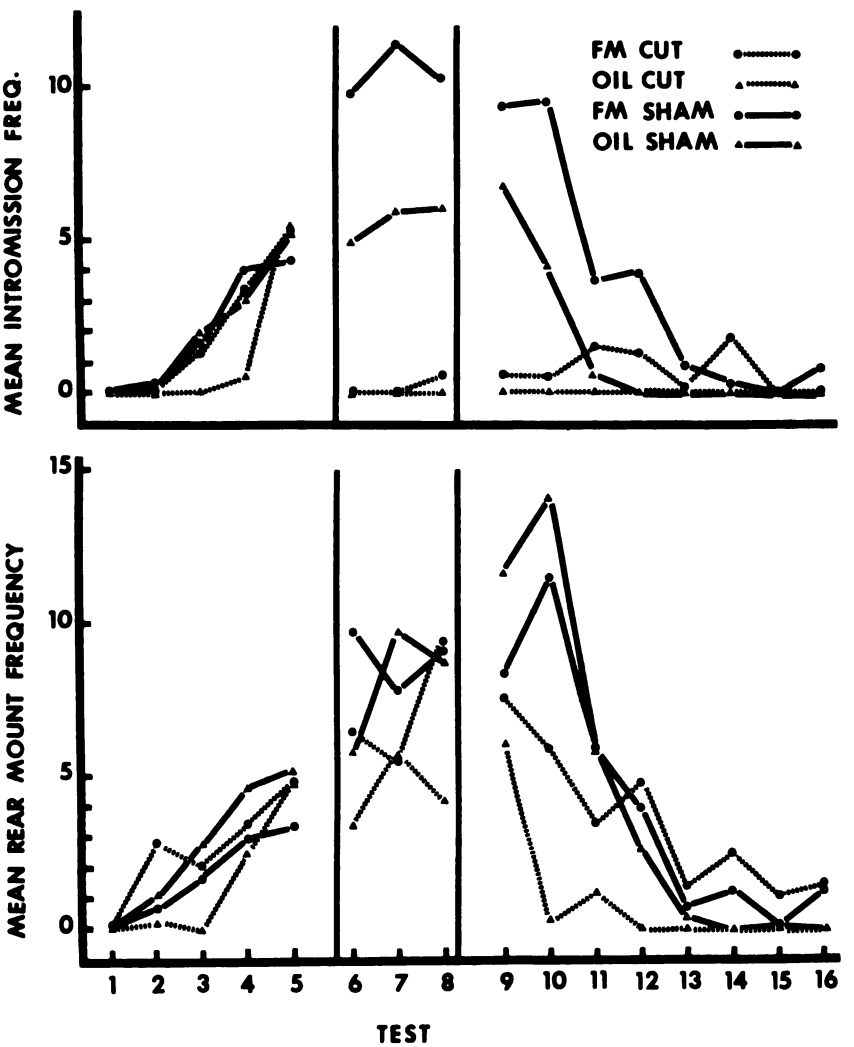

Figure 12. Rear mount frequency and intromission frequency in day 1 castrated male hamsters treated with TP25 neonatally and given various adult treatments.

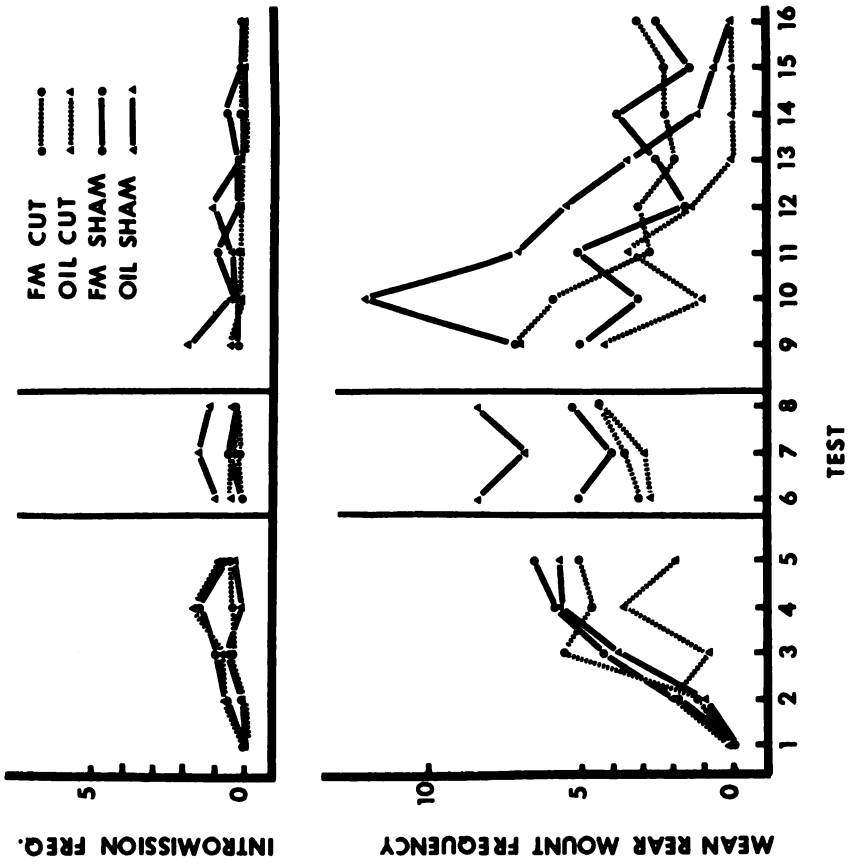

Figure 13. Rear mount frequency and intromission frequency in day 1 castrated male hamsters treated with EB25 neonatally and given various adult treatments.

Ejaculations were infrequent in all neonatal treatment groups. Only 7.5% of TP 100 animals, 18% of TP 25 animals and 0% of EB 25 animals achieved ejaculation on test 5.

Effects of Penile Desensitization (Tests 6-8)

For the 4 groups treated neonatally with TP 100, fluoxymesterone sectioned animals had significantly more mounts on test 8 than the other 3 groups ($p<0.05$) while those groups did not differ from each other (Figure 11). Analysis of latency measures showed that the TP 100 sectioned groups had shorter latencies to mount (Table A1) than sham animals (RMF $f=4.752$, $df=3,36$, $p<0.01$ and ML $F=5.346$, $df=3,37$, $p<0.005$). Mean intromission frequency on test 8 did not differ statistically among the 4 groups of TP 100 animals. Animals treated neonatally with TP 25 (Figure 12) did not differ among groups on test 8 in rear mount frequency. While fluoxymesterone sham and oil sham groups did not differ in intromissions, fluoxymesterone sham animals did intromit more than cut groups ($F=3.509$, $df=3,35$, $p<0.05$). The 4 groups of animals treated neonatally with EB 25 were not different from each other in either mount latency, rear mount frequency or intromission frequency on test 8.

As can be seen in appendix Tables A5-A7, a considerable variation in the number of animals mounting occurred on each test. To compensate for animals which did not mount on test 8, analysis of mount positive animal scores was run:

$$\text{Mount + rear mount frequency} = \text{RMF} \times \frac{1}{\%\ \text{mounting.}}$$

When only animals which mounted were considered, no differences on

test 8 rear mount scores were observed for animals receiving neonatal TP 100, TP 25 or EB 25.

Effects of Hormone Manipulation (Tests 9-16)

Following withdrawal of daily TP treatment and substitution of either daily fluoxymesterone or sesame oil, a marked decline in mounting and intromission was observed in all 3 neonatal treatment groups (Table A1, Figure 11-13, Tests 9-16). Analysis of test 9 scores for rear mount frequency or intromission frequency gave results identical to those of test 8 without consistent differences among treatment conditions in each neonatal treatment group. By test 16, mounts and intromissions were at a very low level in all groups in each neonatal treatment. Analysis of variance revealed no differences in rear mount or intromission frequency within neonatal treatment groups. Likewise, analysis among neonatal treatment groups revealed no differences. However, there was a trend that within each neonatal treatment group, fluoxymesterone treated animals showed more mounts and intromissions than oil treated animals but this was not statistically significant. By test 16, no oil treated animal in any group exhibited intromissions. The only oil treated animals to show rear mounting on test 16 were one animal in TP 100 oil cut group and one animal in TP 100 oil sham.

Morphology

Measures for anogenital (AG) distances and penile bone plus cartilage were analyzed for differences both within, each neonatal treatment group and among neonatal treatments. Results of these comparisons will be considered seperately.

Within Neonatal Treatments:

NEONATAL TP 100 GROUP. (Table 7) For AG distance, oil cut, oil sham and fluoxymesterone cut animals did not differ statistically. Fluoxymesterone sham animals exhibited significantly longer AG distances than any other group (AG $F=8.792$, $df=3,35$ $p<0.001$). Mean bone and cartilage lengths for oil cut and oil sham animals did not differ from each other. Oil cut animals had significantly smaller penile bone and cartilage lengths than did fluoxymesterone treated animals (B & C $F=3.437$, $df=3,36$, $p<0.05$). Oil sham, fluoxymesterone cut and sham groups did not differ from each other.

NEONATAL TP 25 GROUP. (Table 7) Within the neonatal TP 25 group oil sham and oil cut animals did not differ in AG distance, however both groups had significantly shorter AG distances than did the fluoxymesterone cut group ($F=14.531$, $df=3,30$, $p<0.001$). Fluoxymesterone cut animals had significantly shorter AG distances than did the fluoxymesterone sham group ($p<0.05$). Fluoxymesterone sham animals showed greater penile virilization than did any of the other groups ($F=6.350$, $df=3,30$, $p<0.005$) which did not differ from each other.

NEONATAL EB 25 GROUP. (Table 7) For the neonatal EB 25 treatment group, bone and cartilage lengths were uniformly small for the 4 treatment conditions. Analysis of the AG distances revealed that the oil sham group had significantly shorter AG distances than the other 3 groups ($F=5.016$, $df=3,29$, $p<0.01$) which did not differ from each other.

Table 7. Mean anogenital distance, penile bone plus cartilage lengths (in mm) and penile condition in male hamsters treated neonatally with TP100, TP25 or EB 25.

	AG distance ± s.e.		Bone + Cartilage length ± s.e.		Penile spines and papillae at sacrifice
TP100					
FM Cut	15.9	0.6	5.5	0.1	+
Oil Cut	15.0	0.3	5.2	0.0	0
FM Sham	18.2	0.6	5.6	0.0	+
Oil Sham	15.7	0.4	5.4	0.0	0
TP25					
FM Cut	18.0	0.6	5.4	0.0	+
Oil Cut	16.3	0.7	5.3	0.1	0
FM Sham	19.5	0.5	5.8	0.0	0
Oil Sham	5.4	0.3	5.5	0.0	0
EB25					
FM Cut	15.5	0.7	3.5	0.2	+
Oil Cut	14.7	0.3	3.3	0.1	0
FM Sham	14.8	0.5	3.7	0.0	+
Oil Sham	12.8	0.2	3.4	0.0	0

Among Neonatal Treatments:

Comparing neonatal TP 100, TP 25 and EB 25 animals revealed that EB 25 animals, whether from fluoxymesterone or sesame oil groups, had significantly shorter AG distances than either of the TP groups (fluoxymesterone $F=16.760$, $df=2,50$, $p<0.001$) (sesame oil $F=9.902$, $df=2,50$, $p<0.001$). TP 100 and TP 25 animals did not differ from each other. Mean length of penile bone and cartilage across neonatal treatment groups also revealed highly significant differences whether the final hormone condition had been fluoxymesterone ($F=199.966$, $df=2,51$, $p<0.001$) or sesame oil ($F=230.337$, $df=2,48$, $p<0.001$). Neonatal EB 25 treated animals showed significantly less penile virilization than either neonatal TP treated group regardless of final hormone condition ($p<0.05$). TP100 and TP 25 induced the same amount of penile virilization.

Penile Spines and Papillae:

Because of the procedures used for removal of penile bone and cartilage, glans penis tissue was too damaged for quantitative measurement of penile spines and papillae. However, histological examination of portions of the glans revealed that all fluoxymesterone treated animals possessed penile spines and papillae while sesame oil treated groups had none (see Table 7). Microscopic examination did reveal subtle differences among the neonatal treatment groups after adult fluoxymesterone administration. Neonatal TP groups, whether TP 100 or TP 25, exhibited many long spines and deep, regular papillae. Neonatal EB 25 animals had spines and papillae which were more irregular and

misshapen than those of neonatal TP animals. The general impression is that fluoxymesterone treatment of neonatal EB animals induced fewer and smaller spines.

Discussion

The present study demonstrates that penile desensitization yields no clear evidence of an effect of genital sensations on male sexual behavior in hamsters. Sectioning the DNP caused no consistent loss of intromission as has been reported for rats (Experiment 1; Larsson and Södersten, 1973), cats (Aronson and Cooper, 1968) and monkeys (Herbert, 1973). Thus for the hamster although the 3 neonatal hormone treatments used in the present study induced different degrees of penile virilization, few behavioral differences were observed. This was contrary to the prediction based upon data on penile feedback effects in rats. TP administration in adulthood induced similar mounting levels in neonatally TP or EB treated animals but intromissions were fewer in the neonatal EB group. This dissociation of behavioral and genital virilization has been previously reported (Coniglio, 1973; Paup et al., 1974).

The general decline in mating behaviors for all groups when TP treatment was withdrawn and sesame oil or fluoxymesterone was substituted is consistent with results reported by other workers. (Christensen, Coniglio, Paup & Clemens, 1973). Although fluoxymesterone has not been used previously in hamsters the decline in sexual response observed was similar to that seen in male rats (Johnson and Tiefer, 1975; Experiment 1). The trend for animals treated with peripheral androgens to continue mounting longer than non-treated castrated animals has been previously reported in rats (Parrott, 1974b).

However, fluoxymesterone does not have dramatic behavioral effects on castrated male hamsters suggesting that penile integrity is not as important for maintenance of sexual behavior in hamsters as it is in rats. The general decline following the hormone change was not correlated with differences in penile size. Fluoxymesterone did maintain penile integrity in the form of penile spines and papillae.

Since the role of penile sensations in the control of male mating behavior seems to be different for rats and hamsters, a consideration of possible reasons for this apparent difference is in order. That section of the DNP in hamsters was less effective than DNP section of rats in blocking intromissions may reflect anatomical species differences. The DNP of rats branches extensively as it approaches the distal end of the penis (Calaresu, 1970). The position of the nerve cut along the penile shaft was made as close to the proximal end of the penis as possible to avoid this branching. While the branching pattern of the hamster's DNP has not been studied, it is possible that the hamster has more extensive branching at the proximal end of the penile shaft. This would result in incomplete desensitization and would account for the persistence of intromission in nerve cut groups. Likewise, if both cut and sham groups continued to receive penile sensations through undamaged portions of the nerves, the similar behavioral decline which was seen would not be surprising. Experiment 1 with rats and work by Herbert (1973) in monkeys have shown that partial desensitization does not eliminate intromission. Further anatomical work would help us understand this problem.

Differences in behavioral decline that were seen with the rats in Experiment 1 and not in the present experiment may have been related to the level of masculinization in these hamsters. Male mating behavior in rodents might be viewed as a continuum with sexual arousal at one end and ejaculation at the other. An animal must be sexually aroused in order to mount a receptive female. Intromission cannot occur without a mount and intromission must preceed ejaculation. In the present study the experimental model used was at the lower end of the continuum. The specific neonatal treatments used in this experiment resulted in males which were sexually aroused, mounted, intromitted occasionally and seldom ejaculated. Experiment 1 employed experimental animals which encompassed the whole range of the continuum. If the hamsters in the present study had also shown the whole range of behaviors, more consistent differences among groups may have resulted.

Another possible explanation for the discrepancy seen between rats and hamsters is the difference in mating pattern. In the rat, a basic unit of sexual behavior is the mount bout. The mount bout is defined as a sequence of mounts with or without intromission, uninterrupted by any behavior other than genital autogrooming. A series of mount bouts leads to ejaculation. Reducing genital sensations with local anesthetic in rats increases mount frequency, mount bout length and the number of mounts per mount bout (Sachs and Barfield, 1970). In Experiment 1, continued copulation in the fluoxymesterone treated animals may have been related to this phenomenon instead of genital feedback. The male hamster shows a somewhat different pattern with mount bouts interrupted only by ejaculation. Our inability to find differences between cut and

sham animals may be related to this pattern since these hamsters were not programmed for ejaculation.

The discrepancy seen between the results of Experiments 1 and 3 with rats and hamsters emphasizes the need for comparative work. Predictions based upon findings with the rat did not hold for the hamster. Likewise behavior seen in these hamsters following nerve section appeared to be different from work with cats and monekys. Further comparisons between species will add more to our knowledge of the control male sexual behavior.

Conclusion

In the rat, penile sensations are involved in the expression of intromission and ejaculation. However, previous experiments have shown little effect of these sensations on mounting behavior. In Experiment 1 we were able to demonstrate for the first time that sensory feedback from the penis is involved to a degree in the maintenance of mounting.

Other investigations have tried to show that penile virilization at some critical period in development is necessary for later male behavior. Contrary to this position, Experiment 2 showed that fluoxymesterone treated hamsters which had the capacity for penile growth at adulthood exhibited no capacity for male behavior. These results confirmed and extended those of others in establishing that penile virilization during development is not sufficient to bring about adult sexual behavior.

Penile sensations in adult hamsters seem to be less important for male mating behavior than in rats. In Experiment 3 reduction of penile

sensory feedback in male hamsters caused little interruption of intromission behavior. The decline in behaviors seen with fluoxymesterone was not related to genital size or sensitivity.

Clearly, penile sensations via the dorsal nerve of the penis are not among the most important sensory mediators of male mating behavior. Whether sensations from deep pressure receptors, or other perigenital nerve endings are involved to a greater degree is not known. Further work along these lines may lead to other conclusions. The old central vs. peripheral controversy is little more than academic. That central neural stimulation and maintenance of behavior has far more import than peripheral sensation has been shown many times. The present series of experiments which was designed to explore the genital feedback effects on behavior lends little support to the peripheral side of the central-peripheral debate.

APPENDIX

Table A1. Mean latency to first mount (in seconds) for castrated male hamsters treated neonatally with TP or EB and given various adult treatments.

		TEST														
	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TP 100	FM Cut	512	265	53	64	142	122	136	89	136	70	117	287	282	297	355	261
		64	84	13	18	75	61	56	25	62	23	32	99	81	96	97	89
	Oil Cut	595	163	158	147	200	190	199	205	256	274	271	314	408	485	468	356
		4	66	67	68	71	79	78	77	83	83	81	76	73	64	69	85
	FM Sham	460	322	325	344	307	366	384	448	396	432	429	482	541	434	483	440
		73	83	93	82	91	83	89	76	87	86	87	64	59	84	65	82
	Oil Sham	600	380	326	437	373	424	392	388	429	302	431	450	494	600	566	488
		0	90	92	84	93	77	86	87	85	83	77	79	71	0	34	75
TP 25	FM Cut	517	331	53	104	135	199	199	197	167	147	302	299	319	333	399	505
		54	102	6	32	79	82	63	85	67	71	96	93	76	87	98	71
	Oil Cut	600	458	363	325	264	251	260	250	107	426	418	600	600	600	600	600
		0	92	238	98	101	112	109	111	24	96	115	0	0	0	0	0
	FM Sham	545	262	164	171	160	72	74	160	142	137	319	364	511	526	531	495
		55	77	68	67	54	17	18	53	58	58	88	77	39	52	53	70
	Oil Sham	541	355	154	142	145	95	134	102	90	94	72	245	302	431	442	554
		59	84	67	67	63	36	56	34	57	57	26	74	85	69	81	46
EB 25	FM Cut	600	379	196	227	190	121	225	246	185	411	218	354	402	405	500	427
		0	86	78	88	83	67	74	90	81	85	95	97	87	96	65	88
	Oil Cut	578	404	356	315	388	282	330	315	222	286	388	444	477	427	541	578
		22	79	96	92	88	87	90	165	95	90	87	79	78	88	59	22
	FM Sham	600	272	189	165	156	143	159	123	97	193	116	228	192	276	320	327
		0	81	81	85	80	79	72	96	72	91	72	89	91	97	106	103
	Oil Sham	600	393	200	172	119	148	140	135	199	230	207	257	247	443	416	482
		0	84	103	92	81	81	78	82	103	117	98	91	102	91	103	81

Table A2. Mean head and side mount frequency in castrated male hamsters treated neonatally with TP or EB and given various adult treatments.

		TEST														
	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TP 100	FM Cut	1.3	3.6	6.2	6.3	6.8	6.0	5.8	8.1	5.6	5.3	2.7	2.3	3.0	2.2	1.2	2.3
		0.94	1.0	2.0	0.8	2.0	1.5	1.2	1.9	1.3	1.5	0.3	1.0	0.9	1.1	0.8	0.8
	Oil Cut	0.3	6.8	9.8	8.0	9.2	7.5	6.6	7.5	5.5	5.9	6.4	2.5	2.5	1.0	1.5	1.7
		0.2	1.8	2.5	2.6	3.2	3.0	2.8	3.2	2.5	3.0	3.4	1.7	1.2	0.6	0.8	1.0
	FM Sham	0.7	2.6	2.9	3.5	1.7	3.2	1.4	1.7	1.6	1.8	1.2	0.8	0.2	1.1	0.5	0.9
		0.4	0.8	1.1	1.6	0.7	1.3	0.8	0.9	0.9	1.1	0.6	0.5	0.2	0.6	0.3	0.5
	Oil Sham	0	2.4	2.5	1.4	2.1	1.7	1.8	2.0	1.5	3.4	2.8	2.9	1.4	0	0.2	1.2
		0	1.2	1.0	1.0	0.8	0.9	1.0	1.2	1.4	2.0	1.5	1.9	1.2	0	0.2	0.8
TP 25	FM Cut	0.8	8.5	13.8	6.4	11.7	8.6	6.6	4.4	5.5	4.5	4.6	2.1	3.3	3.0	2.4	1.3
		0.5	3.5	3.5	2.2	3.2	1.9	1.6	1.7	1.8	2.2	2.8	1.0	1.4	1.5	1.6	0.9
	Oil Cut	0	3.5	2.2	3.2	5.8	5.8	4.7	3.0	11.7	1.2	0.3	0	0	0	0	0
		0	3.1	1.3	1.2	1.9	3.0	2.0	1.0	3.0	0.5	0.2	0	0	0	0	0
	FM Sham	0.1	3.2	8.5	6.6	6.7	5.4	7.0	3.0	4.0	3.0	2.3	1.9	0.6	1.2	0.7	0.4
		0.1	1.7	2.5	1.5	1.6	1.0	1.1	0.8	0.8	1.1	0.9	0.7	0.3	0.8	0.5	0.3
	Oil Sham	0	3.5	7.9	5.8	7.4	5.6	5.4	6.7	6.4	10.2	7.9	5.2	1.4	0.8	1.0	0.1
		0	1.9	1.2	1.1	1.7	1.2	1.5	1.3	2.3	1.7	1.7	1.8	0.5	0.4	0.6	0.1
EB 25	FM Cut	0	3.4	9.2	7.0	7.0	5.9	6.0	6.4	7.1	4.8	5.6	3.4	2.3	1.1	0.7	3.0
		0	1.4	3.2	2.7	2.3	2.7	2.3	2.9	2.4	2.4	2.5	2.5	1.3	0.7	0.5	2.0
	Oil Cut	0.2	2.7	1.9	3.2	1.6	1.7	2.0	3.0	8.7	3.0	5.0	2.3	0.8	0.4	0.1	0.1
		0.2	1.6	0.9	1.3	1.0	0.6	0.8	1.9	2.8	1.2	2.6	1.3	0.4	0.2	0.1	0.1
	FM Sham	0	7.8	5.4	10.8	10.6	11.3	11.8	15.5	16.8	14.6	12.6	7.9	5.5	9.5	5.3	6.6
		0	3.0	2.0	3.3	3.0	3.0	3.2	6.8	6.7	5.8	5.2	3.6	2.6	4.0	2.8	2.7
	Oil Sham	0	5.0	9.4	11.1	9.9	9.4	8.9	8.4	10.3	9.2	7.3	8.3	6.6	1.3	1.4	1.6
		0	4.0	3.5	4.2	2.8	3.1	3.3	2.2	4.7	3.1	4.2	5.6	4.4	0.7	0.1	1.4

Table A3. Mean rear mount frequency in castrated male hamsters treated neonatally with TP or EB and given various adult treatments.

Group	TEST 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
FM Cut	0.6	3.1	7.1	4.6	4.8	3.4	7.6	13.6	10.7	7.6	6.2	4.6	3.8	3.1	2.0	4.4
	0.4	1.8	3.6	1.4	1.4	2.4	2.2	2.8	3.1	2.0	2.3	2.3	1.8	1.6	1.4	1.7
Oil Cut	0	3.2	2.4	4.0	2.0	5.2	6.0	6.2	6.4	6.7	4.0	0.9	0.8	0.4	0.3	0.4
	0	1.5	0.8	1.3	0.7	1.9	2.4	2.2	1.9	3.1	1.6	0.5	0.6	0.3	0.3	0.4
FM Sham	0	1.2	1.0	1.8	1.2	1.2	2.6	2.4	1.5	1.1	2.2	0.7	0.2	1.3	1.3	2.0
	0	0.7	0.6	1.2	0.9	1.0	1.7	2.0	1.2	0.6	1.7	0.5	0.2	1.1	1.3	1.9
Oil Sham	0	0.4	1.1	1.9	3.7	1.3	2.9	2.3	3.0	5.8	4.0	1.2	1.4	0	0.1	0.2
	0	0.4	0.7	1.3	1.7	0.6	1.5	1.6	1.7	2.9	2.3	0.8	1.4	0	0.1	0.2
FM Cut	0.1	2.8	2.1	3.5	4.8	6.5	5.5	9.4	7.6	5.9	3.5	4.8	1.4	2.5	1.1	1.5
	0.1	4.4	1.6	1.5	1.7	3.2	2.0	3.1	3.4	2.9	1.5	2.5	0.9	1.2	0.9	1.5
Oil Cut	0	0.2	0	2.5	4.8	3.5	5.7	4.2	6.0	0.3	1.2	0	0	0	0	0
	0	0.1	0	2.1	3.3	2.9	3.1	3.3	2.4	0.3	0.7	0	0	0	0	0
FM Sham	0	0.7	1.7	3.0	3.4	9.8	7.8	9.1	8.4	11.5	5.8	4.0	0.7	1.3	0.1	1.3
	0	0.5	0.5	0.8	1.1	1.8	1.5	2.2	2.2	2.6	2.2	1.6	0.6	1.2	0.1	1.1
Oil Sham	0.1	1.1	2.8	4.6	5.2	5.8	9.7	8.7	11.7	14.1	5.8	2.6	0.3	0	0.1	0
	0.1	0.6	1.0	1.2	1.7	1.1	2.7	2.7	3.4	3.8	1.6	1.4	0.2	0	0.1	0
FM Cut	0	1.2	5.6	4.7	5.1	3.1	3.6	4.4	7.1	5.9	2.7	3.1	1.9	2.2	2.2	3.1
	0	0.8	1.6	2.6	2.1	1.6	1.8	2.1	4.2	4.2	1.8	1.7	1.2	1.1	2.1	2.1
Oil Cut	0.2	2.0	0.9	3.6	1.9	2.7	2.9	4.4	4.2	1.0	3.4	1.4	0	0	0	0
	0.2	1.5	0.6	1.4	1.0	1.4	1.3	2.2	1.7	0.7	2.1	0.8	0	0	0	0
FM Sham	0	1.9	4.3	5.9	6.6	5.1	4.0	5.3	5.0	3.1	5.1	1.5	2.5	3.8	1.4	2.5
	0	1.1	2.7	2.3	2.5	1.6	1.3	1.8	1.9	1.4	1.9	0.6	0.8	1.5	0.9	1.6
Oil Sham	0	1.0	3.8	5.7	5.7	8.4	6.9	8.4	7.0	12.0	7.0	5.4	3.4	1.1	0.6	0
	0	0.8	1.7	2.7	1.7	3.0	2.4	5.0	3.2	8.0	5.4	3.2	2.8	0.8	0.4	0

Table A4. Mean intromission frequency in castrated male hamsters treated neonatally with TP or EB and given various adult treatments.

	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TP 100	FM Cut	0.1	1.1	4.0	5.1	6.6	0.2	1.1	4.8	3.1	1.8	0.2	0.9	0.8	0.8	0.3	0.8
		0.1	0.8	2.2	1.8	2.4	0.1	0.6	2.2	1.9	1.0	0.2	0.8	0.6	0.6	0.3	0.5
	Oil Cut	0	0.2	1.8	3.4	2.5	5.7	6.0	6.7	5.2	0	0.1	0	0	0	0	0
		0	0.1	1.0	1.6	2.0	3.0	3.5	4.1	3.5	0	0	0	0	0	0	0
	FM Sham	0	0	0	0.4	0.9	0.1	0.6	0.5	0.8	0.5	0.5	0	0	0.7	0.8	0.9
		0	0	0	0.4	0.9	0.1	0.5	0.5	0.8	0.5	0.5	0	0	0.7	0.8	0.9
	Oil Sham	0	0	2.3	2.9	4.1	0.3	3.4	3.2	4.4	3.2	0	0	0	0	0	0
		0	0	2.3	2.9	3.0	0.2	2.8	3.1	3.2	2.2	0	0	0	0	0	0
TP 25	FM Cut	0	0.1	1.3	3.4	5.3	0	0	0.6	0.6	0.5	1.5	1.3	0.1	1.8	0	0
		0	0.1	0.8	1.7	1.8	0	0	0.5	0.6	0.4	1.0	1.0	0.1	1.0	0	0
	Oil Cut	0	0	0	0.5	5.5	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0.5	4.0	0	0	0	0	0	0	0	0	0	0	0
	FM Sham	0	0.3	1.6	4.0	4.3	9.8	11.4	10.3	9.4	9.5	3.7	3.9	0.9	0.3	0	0.8
		0	0.3	0.8	2.5	2.1	2.1	2.8	3.1	2.8	2.2	2.0	2.2	0.9	0.3	0	0.8
	Oil Sham	0	0.2	1.9	3.0	5.3	4.9	5.9	6.0	6.8	4.2	0.6	0	0	0	0	0
		0	0.2	1.0	1.0	1.5	1.8	2.2	2.6	2.7	2.3	0.3	0	0	0	0	0
EB 25	FM Cut	0	0	0.4	0.3	0.4	0	0.1	0.3	0.1	0	0	0	0	0	0	0
		0	0	0.3	0.3	0.2	0	0.1	0.3	0.1	0	0	0	0	0	0	0
	Oil Cut	0	0.6	0.7	1.6	0.8	0.4	0.3	0.3	0.4	0	0	0	0	0	0	0
		0	0.5	0.4	1.1	0.6	0.4	0.2	0.2	0.4	0	0	0	0	0	0	0
	FM Sham	0	0.6	0.9	1.5	0.6	0	0.4	0.2	0.1	0.1	0.8	0	0.1	0.4	0	0
		0	0.6	0.5	0.8	0.4	0	0.2	0.1	0.1	0.1	0.4	0	0.1	0.4	0	0
	Oil Sham	0	0	0.5	0.1	0.3	0.9	1.4	1.1	1.7	0.3	0.3	0.9	0	0	0	0
		0	0	0.3	0.1	0.2	0.5	1.1	0.7	1.2	0.2	0.3	0.9	0	0	0	0

Table A5. Percentage of male hamsters showing head and side mount, rear mount or intromission for behavioral tests. Animals were castrated on day 1, given TP100 neonatally, and received various adult treatments.

	FM Cut	n=9		Oil Cut	n-11		FM Sham	n=10		Oil Sham	n=10	
Test	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I
1	22.2	22.2	11.1	18.2	0	0	30	0	0	0	0	0
2	77.8	55.6	22.2	72.7	45.5	18.2	60	50	0	40	10	0
3	100	85.7	57.1	81.8	72.7	45.5	50	30	0	50	40	10
4	100	100	66.7	81.8	63.6	54.5	60	40	10	30	30	10
5	88.9	66.7	55.6	81.8	63.6	36.4	60	30	10	50	40	30
6	88.9	88.9	22.2	72.7	63.6	36.4	50	20	10	30	40	20
7	88.9	100	44.4	72.7	63.6	36.4	40	30	20	40	40	20
8	100	100	44.4	63.7	63.6	36.4	40	20	10	40	30	20
9	88.9	77.8	33.3	54.5	63.6	36.4	40	20	10	20	40	20
10	77.8	88.9	55.6	54.5	54.5	0	30	30	10	50	40	20
11	88.9	88.9	11.1	54.5	54.5	9.1	30	20	10	30	40	0
12	44.4	55.6	22.2	63.6	27.3	0	30	30	0	30	20	0
13	66.7	55.6	33.3	45.5	18.2	0	10	10	0	20	10	0
14	44.4	44.4	22.2	36.4	18.2	0	30	20	10	0	0	0
15	44.4	33.3	11.1	27.3	9.1	0	30	10	10	10	10	0
16	66.7	66.7	22.2	45.5	9.1	0	30	20	10	20	10	0

Table A6. Percentage of male hamsters showing parameters of male mating behavior. Animals were castrated on day 1, given TP25 neonatally and received various adult treatments.

	FM Cut	n=8		Oil Cut	n=6		FM Sham	n=10		Oil Sham	n=10	
Test	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I
1	25	12.5	0	0	0	0	10	0	0	0	10	0
2	50	50	12.5	33.3	16.7	0	70	20	10	50	30	10
3	100	75	37.5	50	0	0	90	60	50	100	57.1	57.1
4	87.5	75	50	66.7	50	16.7	100	80	40	90	70	60
5	85.5	85.7	57.1	83.3	33.3	33.3	90	90	70	88.9	77.8	77.8
6	100	87.5	0	66.7	50	0	100	90	90	90	100	60
7	87.5	87.5	0	66.7	50	0	100	100	80	90	90	70
8	75	100	25	66.7	83.3	0	80	70	70	90	70	50
9	87.5	62.5	12.5	100	66.7	0	90	90	70	80	90	60
10	87.5	50	25	50	16.7	0	80	80	80	90	90	50
11	75	62.5	25	33.3	33.3	0	60	60	40	100	90	30
12	50	62.5	25	0	0	0	60	60	30	80	40	0
13	75	25	12.5	0	0	0	30	20	10	60	20	0
14	50	50	50	0	0	0	20	20	10	50	0	0
15	37.5	25	0	0	0	0	20	10	0	30	10	0
16	25	12.5	0	0	0	0	20	20	10	10	0	0

Table 7A. Percentage of male hamsters showing parameters of male mating behavior. Animals were castrated on day 1, given EB25 neonatally and received various adult treatments.

	FM Cut	n=9		Oil Cut	n=9		FM Sham	n=8		Oil Sham	n=7	
Test	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I	H&SM	RM	I
1	0	0	0	11.1	11.1	0	0	0	0	0	0	0
2	55.6	18.2	0	44.4	33.3	11.1	75	62.5	12.5	57.1	28.6	0
3	77.8	77.8	22.2	44.4	22.2	22.2	87.5	57.1	42.9	85.7	71.4	33.3
4	77.8	44.4	11.1	55.6	44.4	44.4	87.5	75	50	85.7	71.4	14.3
5	77.8	77.8	33.3	44.4	44.4	22.2	87.5	75	37.5	85.7	85.7	28.6
6	55.6	77.8	0	66.7	55.6	11.1	87.5	75	0	85.7	71.4	42.8
7	88.9	66.7	11.1	55.6	22.2	22.2	100	87.5	25	85.7	85.7	28.6
8	66.7	55.6	11.1	50.0	50.0	22.2	83.3	83.3	16.7	85.7	57.1	28.6
9	77.8	55.6	11.1	66.7	66.7	11.1	87.5	87.5	12.5	71.4	57.1	28.6
10	27.3	22.2	0	66.7	33.3	0	75	50	12.5	66.7	66.7	33.3
11	66.7	22.2	0	44.4	33.3	0	87.5	75	37.5	85.7	57.1	14.3
12	36.4	33.3	0	33.3	33.3	0	75	62.5	0	85.7	42.9	14.3
13	55.6	22.2	0	33.3	0	0	75	62.5	12.5	71.4	42.9	0
14	22.2	44.4	0	33.3	0	0	62.5	62.5	12.5	42.9	28.6	0
15	22.2	22.2	0	11.1	0	0	62.5	37.5	0	28.6	28.6	0
16	33.3	33.3	0	11.1	0	0	50	50.0	0	28.6	0	0

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