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# GENETIC, HORMONAL, AND NEUROANATOMICAL CORRELATES OF SEXUAL BEHAVIOR IN THE HOUSE MOUSE (*MUS MUSCULUS*)

By

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ABSTRACT

Genetic, hormonal, and neuroanatomical correlates of sexual behavior in the house mouse (Mus Musculus)

By

Beth Eva Ferguson Wee

The behavioral response to castration and hormone replacement varies among species. Many animals show a rapid decline in sexual behavior following castration, with a return to precastration levels after hormone replacement. However, B6D2F1 male mice retained the ejaculatory reflex for more than one year after castration (McGill and Manning, 1976). The present study investigated the influences of genotype and gonadal hormones on this retention phenomenon and on the morphology of a sexually dimorphic nucleus in the spinal cord associated with reproductive behaviors.

Masculine sexual behaviors, levels of plasma testosterone (T), and characteristics of the spinal nucleus of the bulbocavernosus (SNB) were examined in male B6D2F1, DBA/2J, and C57Bl/6J house mice. Gonadally intact males did not differ in their levels of plasma T or SNB neuronal area, but DBA males had significantly fewer SNB neurons than males of the other two strains. Both SNB neuronal number and size were reduced in the B6D2F1 castrates. In contrast to the rapid decline in copulatory behaviors by castrated males of the parental strains, approximately 30% of the F1 castrates retained the ejaculatory reflex for 25 weeks after surgery. Thus, the results extend previous findings of genotypic

influences on the retention of sexual behavior after castration to include genotypic influences on SNB morphology.

A behavioral heterogeneity was observed among the genetically homogeneous F1 castrates. Some males (the continuers) retained the ejacualtory reflex throughout the 25 weeks; other castrates (the noncontinuers) did not. No significant differences in levels of plasma T or nuclear estrogen receptors in the medial preoptic area of the hypothalamus, or SNB characteristics were found between these two groups of F1 castrates. Both testosterone propionate and estradiol benzoate restored masculine sexual behaviors in castrated B6D2F1 males that did not retain the ejaculatory reflex for 29 weeks after surgery.

Although B6D2F1 mice are genetically homogeneous, two behavioral phenotypes seem to exist within the castrates of this strain : one phenotype, the continuers, exhibits the ejaculatory reflex in the absence of gonadal hormones; the other phenotype, the noncontinuers, requires testicular hormones for the display of masculine copulatory behaviors.

**This dissertation is dedicated with love and gratitude to Mom and Jim.**

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The person whose name could be tacked on to almost every list mentioned above, and many more lists of contributions, is my husband, Jim Wee. His never-ending support and love can not be acknowledged enough, but, perhaps, the knowledge that he is a major part of my accomplishments will be a small reward.

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## ABBREVIATIONS

| | |
|---|---|
| AA | AndrostAnedione |
| AE | AndrostEnedione |
| B6D2F1 | the F1 hybrid resulting from the cross between a C57B1/6 female and a DBA/2 male |
| BC | BulboCavernosus (muscle) |
| DHT | DiHydroTestosterone |
| DHTP | DiHydroTestosterone Propionate |
| DLN | DorsoLateral Nucleus |
| E2 | Estradiol |
| EB | Estradiol Benzoate |
| EL | Ejaculation Latency |
| HRP | HorseRadish Peroxidase |
| IC | IschioCavernosus (muscle) |
| IF | Intromission Frequency |
| IL | Intromission Latency |
| LA | Levator Ani (muscle) |
| MF | Mount Frequency |
| ML | Mount Latency |
| MPOA | Medial PreOptic Area (of the hypothalamus) |
| NER | Nuclear Estradiol (Estrogen) Receptor |
| P | Progesterone |

| | |
|---|---|
| PG | Propylene Glycol |
| PEI | PostEjaculatory Interval |
| RDLN | RetroDorsoLateral Nucleus |
| SNB | Spinal Nucleus of the Bulbocavernosus |
| T | Testosterone |
| TP | Testosterone Propionate |

# INTRODUCTION

The behavioral response to castration and subsequent hormone replacement varies among species. In many animals, castration greatly reduces masculine sexual behavior and hormone replacement restores these behaviors to presurgical levels. However, some exceptions to this general trend have been noted. For example, males of one hybrid strain of house mouse have been shown to retain the ejaculatory reflex for several months after castration (McGill and Manning, 1976). The retention of masculine sexual behavior after castration in these mice was examined in the present study. Therefore, a review of similar studies with other animals will provide the background for this series of experiments.

The degree of dependence upon gonadal hormones varies with the species, and, among mammals at least, Beach (1947) noted that the phylogenetically lower animals (rodents, lagomorphs) were more dependent upon gonadal hormones than the more advanced mammals (cats, dogs, primates). Beach suggested that this trend is correlated with an evolutionary increase in encephalization, particularly neocorticalization of the control of sexual functions (Beach, 1958). However, due to the variation observed among animals of the same species, some authors were skeptical of this phylogenetic

hypothesis (Hart, 1968). Although this hypothesis is no longer widely accepted, it provided the basis for comparative studies that examined the behavioral response to castration and hormone replacement (see reviews by Beach, 1958; Young, 1961; Hart, 1974; Beyer, 1979).

One problem with comparing different studies lies in the ambiguity in the use of the word "copulation" (Young, 1961). Young noted that in much of the work on the effects of castration, ejaculation was not the endpoint (see general description of mammalian sexual behavior below). Some studies used mounting and/or intromission as the copulatory behavior measured before and after castration. In other cases, a score was assigned to represent the level of sexual activity. This score may have been based on latencies, whether or not specific behaviors occurred, the duration or performance of each behavior, or some combination of these measures. Thus, careful examination is needed to make appropriate comparisons among the different studies.

A problem that arises with taking a species comparative approach is that some behaviors are species- or class-specific. For example, in birds, the mating act is called cloacal contact; in amphibians, the clasping reflex, or amphiplexus, is used to measure sexual activity. The relation of these behaviors to mounting behavior or ejaculation in mammals is not always apparent. Consequently, because the research presented later involves measures of ejaculation, intromission, and mounting behaviors, the first sections of this literature review will focus on the effects

of castration and hormone replacement on these three behaviors. Although other behaviors (such as spawning in fish, the amphibian clasping reflex, cloacal contact in birds, and courtship in several species) have been examined in nonmammalian species, they will not be reviewed here. However, several review articles have been published which describe sexual behaviors in nonmammalian species (Liley, 1969; Arnold, 1975; Silver et al., 1979; Adkins-Regan, 1981; Crews and Silver, 1985; Ingle and Crews, 1985; Crews and Moore, 1986). In order to keep the review to a manageable size, it will emphasize more recent studies (primarily since Young's 1961 review) and only on mating behavior in males of nonhuman, mammalian species and only in response to postpubertal (as opposed to prepubertal) castration. Furthermore, studies that assigned scores to copulatory behaviors are not described here, because they can not be compared to the results presented in this dissertation. The last sections of the literature review will deal briefly with sex differences in the nervous system, with a thorough review of the specific sexually dimorphic system that was studied in this dissertation research.

## General description of mammalian sexual behavior

Normal male mammals exhibit the following generalized pattern for masculine sexual behavior. After a period of initial investigation, the male will mount the female and achieve vaginal penetration (penile insertion)

(intromission). This vaginal penetration is accompanied by pelvic thrusting, which may continue for an extended period of time until the male ejaculates. Ejaculation may occur during the first intromission, or the male may dismount, groom, then achieve subsequent and repeated intromissions before ejaculation.

Castrated males do not release seminal fluid and sperm. Therefore, the more appropriate term for the behavioral pattern which they exhibit is an ejaculatory reflex. Similarly, because intromission appears to require testicular hormones for maintenance of erection, the correct terminology to describe the behavioral pattern displayed by castrates is an intromission-like response or pattern. However, for brevity, the terms ejaculation and intromission may be used for both castrated and gonadally intact animals.

Some of the behavioral measures that are taken during tests for sex behavior are the following : mount latency (ML) : time from introduction of the female to the male until the male's first mount; mount frequency (MF) : number of mounts by the male; intromission latency (IL) : time from introduction of the female to the male until the male's first intromission; intromission frequency (IF) : number of intromissions by the male; ejaculation latency (EL) : time from first intromission to ejaculation; postejaculatory interval (PEI) : time from ejaculation to next intromission; interintromission interval (III) : the average time between intromissions.

## Effects of Castration and Hormone Replacement on Sexual Behaviors

### Castration and the ejaculatory reflex

The extent of retention of the ejaculatory reflex after castration varies both among species and among individuals of the same species. By the fourth week after gonadectomy, 80% of deer mice (Peromyscus maniculatus bairdi) failed to ejaculate (Clemens and Pomerantz, 1981). Davidson (1966) reported a similar decline in the percent of castrated rats (Rattus norvegicus) displaying the ejaculatory pattern, but one male retained this response for five months after surgery. A group of "low active" male rats (determined prior to castration) stopped copulating by 25 postoperative days (Larsson, 1966). In contrast, the last ejaculations exhibited by the "high active" males occurred 75 days after castration. The ejaculatory reflex was eliminated immediately in red deer (Cervus elaphus) (Fletcher and Short, 1974), in rabbits (Oryctolagus cuniculus) by 53 days (Stone, 1932), guinea pigs (Cavia porcellus) by 9 weeks (Grunt and Young, 1953), gerbils (Meriones unguiculatus) by three weeks (Yahr et al., 1979), golden hamsters (Mesocricetus auratus) within four to seven weeks (Whalen and DeBold, 1974), CD2F1 hybrid house mice (Mus musculus)

within one week (Champlin et al., 1963), DBA/2J mice by three to eight days, B6D2F1 mice by three to sixty days, and C57Bl/6J mice immediately after castration (McGill and Tucker, 1964).

The variable length of persistence of ejaculation also has been observed in phylogenetically higher animals. Clegg et al. (1969) reported that one ram (Ovis aries) ceased at five weeks, two rams continued for approximately one year, and a fourth ram was intermediate between these extremes. In a prior study with sheep, ejaculation did not occur at all after castration (Banks, 1964). Although some dogs (Canis familiaris) ceased mating within a month or two, others still mated 250 days after castration (Hart, 1968). In another study with dogs, various males were tested from approximately two to more than five years after surgery (Beach, 1970). Only one of the seven dogs exhibited complete loss of the ability to insert and lock (the copulatory lock is associated with ejaculation), and this dog's last lock occurred 18 months after castration. Goats (Capra hircus) retained the ejaculatory reflex for 18 weeks to one year after surgery (Hart and Jones, 1975). One rhesus monkey (Macaca mulatta) ceased ejaculating within five postoperative weeks, but another ejaculated intermittently for 109 weeks (Michael and Wilson, 1974), and, in a similar study, 50% and 30% of castrated males ejaculated at least once during postoperative weeks 21-25 and 51-55, respectively (Phoenix et al., 1973).

As mentioned above, castrated B6D2F1 mice show a wide

range of day of last ejaculation (McGill and Tucker, 1964). The phenomenon of retention of the ejaculatory reflex after castration was examined further in a diallelic design using DBA/2J, C57Bl/6J, and BALB/c strains plus the hybrids resulting from the six possible crosses between them (McGill and Manning, 1976). When tested one year after surgery, approximately 40% of the B6D2F1 castrates were still displaying the ejaculatory reflex fairly regularly. In contrast, only one D2B6F1 castrate and none of the castrates from the parental or other hybrid strains ejaculated. Thus, the B6D2F1 mouse resembles the phylogenetically higher species, such as the dog, goat, and rhesus monkey in its retention of sex behavior after castration. If one considers that the average life span of a house mouse is about two years, and that of a rhesus monkey is a few decades, the retention phenomenon displayed by the B6D2F1 mouse is even more remarkable.

Hormone replacement and the ejaculatory reflex

The facilitative actions of testosterone and its androgenic and estrogenic metabolites on sexual behaviors have been reviewed extensively by Luttge (1979) and Södersten (1979). Testosterone propionate (TP) restored or maintained the ejaculatory reflex in castrated deer mice (Clemens and Pomerantz, 1981, 1982), rabbits (Macmillan et al., 1969), rats (Beach and Holz-Tucker, 1949; Bloch and Davidson, 1968; Whalen and Luttge, 1971), guinea pigs (Grunt

and Young, 1953), house mice (Champlin et al., 1973; McGill et al., 1976), gerbils (Yahr et al., 1979), hamsters (Whalen and DeBold, 1974), sheep (Clegg et al., 1969), dogs (Beach, 1970), and rhesus monkeys (Phoenix et al., 1973; Michael and Wilson, 1974) . In his review of the effects of hormone replacement on masculine sexual behavior, Larsson (1979) stated that "No effect of testosterone has ever been reported before 24 hr after the injection, and usually behavioral effects are delayed for several days. Usually testosterone is administered over a period of time and the effects obtained represent a result of dosage, time of treatment, the particular ester administered, and the procedures of administration."

Although TP administered immediately after castration prevented the loss of sexual behavior and maintained it at the level of intact males, a higher dose of TP was required to restore sexual behavior in the gerbil once it had ceased (Yahr et al. 1979). In several species, more TP is needed to reinstate mating behavior than to maintain it (Davidson, 1972).

Individuals differ in their response and/or sensitivity to androgens. Guinea pigs (Grunt and Young 1952, 1953; Riss and Young, 1954) and rats (Whalen et al., 1961; Larsson, 1966) exhibited different levels of sexual activity (i.e. presence/absence or duration of specific behaviors, or numbers of ejaculations per test, etc.) prior to castration, and these levels were retained with hormone replacement after castration. After TP treatment, those animals having a

high level of sexual performance prior to castration remained high; low active or noncopulator males continued to show low levels of sexual behaviors. Further, supramaintenance doses of TP did not increase the levels of sexual activity above precastration levels (Riss and Young, 1954; Larsson, 1966).

Testosterone propionate treatment decreased latency to ejaculate in rats (Beach and Holz-Tucker, 1949), guinea pigs (Grunt and Young, 1953), and hamsters (Whalen and DeBold, 1974). In contrast, McGill et al. (1976) observed increases in ejaculation latency with increasing doses of TP given to B6D2F1 males at the time of castration. McGill (1978a) suggested that the differences between hormone replacement studies may not reflect a species difference, but rather that animals that continue to mate in the absence of gonadal and adrenal hormones may respond differently than do those genotypes that lose sexual behavior soon after castration. Evidence in support of McGill's suggestion was reported for castrated CD2F1 male mice (Champlin et al., 1963). These mice lost their sexual responsiveness within one week after surgery, but sexual behavior was maintained equally by both low and high doses of TP, and no differences in ejaculation latency were observed for each of the TP doses. However, when the study was replicated with more animals (McGill, 1978a), CD2F1s also exhibited longer latencies to larger hormone doses (as the B6D2F1 castrates had done in the McGill et al. study). Thus, McGill (1978a)

concluded that house mice respond differently to exogenous hormones than do other rodents.

Treatment with dihydrotestosterone propionate (DHTP) was as effective as TP in restoring the ejaculatory reflex in deer mice (Clemens and Pomerantz, 1981, 1982), and DHTP increased the percent of rhesus monkeys ejaculating (Phoenix, 1974). In the hamster, only the highest dose (1000 ug/day) of dihydrotestosterone (DHT) maintained the percent of castrates ejaculating near the precastration levels (Whalen and DeBold, 1974). Reduction of the DHT dose resulted in reduced performance of the DHT group. In contrast, androstenedione (AE) was substantially more effective than DHT in restoring ejaculatory behavior and was slightly more effective than T at certain doses. In addition to testosterone (T) and AE, androstenediol (Beyer et al., 1973) and 19-hydroxytestosterone (19HT) (Parrott, 1976), all aromatizable androgens, elicited ejaculatory behavior in castrated rats, but the nonaromatizable androgens dehydroepiandrosterone, androstanedione (AA), 5a-androstanediol, 5B-androstanediol, and 11B-hydroxyandrostenedione (Beyer et al., 1973) and 5a-19-hydroxytestosterone (5a19HT) (Parrott, 1976) were either much less effective or failed to elicit ejaculatory behavior in castrated rats. Silastic capsule implants of E2 or DHT were not as effective as T or 19-nortestosterone (an aromatizable androgen) for stimulating sex behavior after castration in the gerbil (Yahr et al., 1979).

Estradiol benzoate (EB) restored the ejaculatory

pattern in some long-term castrated rats (Davidson, 1969), but did not facilitate ejaculatory responses in deer mice (Clemens and Pomerantz, 1981). However, in a later study with deer mice, an inhibitor of the enzyme that aromatizes testosterone to estradiol, 1,4,6-androstatriene-3,17-dione (ATD), prevented activation by TP of ejaculatory behavior (Clemens and Pomerantz, 1982). Based on this finding, and because DHTP induced ejaculatory behavior, the authors suggested that the metabolism of T to both estrogens and 5a-reduced androgens is obligatory for T reliably to stimulate masculine sexual behavior in castrated deer mice. Subcutaneous pellets of estradiol-17B implanted before the onset of the normal mating season reinstated ejaculatory behavior in castrated red deer (Fletcher and Short, 1974).

Reviewing studies which used a combination of hormones, Larsson (1979) concluded that DHT in combination with either estradiol or estrone (an estrogen which is interconvertible with estradiol) is highly active in eliciting ejaculatory behavior in rats.

In summary, castration reduces the display of the ejaculatory reflex in all mammals studied to date, but the extent of this reduction varies among species and among animals of the same species. In most rodents and lagomorphs, ejaculatory behaviors persisted after castration for a couple of months, at most, whereas some castrated dogs, sheep, rhesus monkeys, and goats retained this response for many months or years. The B6D2F1 house mouse does not follow

the trend of other rodents, as some castrates retained the ejaculatory reflex for over half of their life time. In all mammals examined, treatment with TP increased the percent of animals ejaculating, a measure that was reduced by castration. Based on hormone replacement studies (described above), one might conclude that T acts directly and/or via one or more of its metabolites (ex. E2, DHT, AE, etc.) to facilitate ejaculation.

Castration and intromission

Castration and hormone replacement may affect intromission differently than they affect ejaculation, as observed in the gerbil (Yahr et al., 1979) and guinea pig (Grunt and Young, 1953). Sexual behavior in these animals decreased after castration as a loss of ejaculation, followed by a loss of intromission, and finally a loss of mounting. Androgen therapy reinstated mounting first, followed by intromission, and then ejaculation. In the rat, these patterns of loss and restoration of behaviors were not observed (Davidson, 1966; Bloch and Davidson, 1968). However mounting behavior did remain after other elements of the mating behavior had disappeared (Bloch and Davidson, 1968), and mounting was the behavior that was most sensitive to an increase in androgen level (Whalen et al., 1961).

Only ten percent of castrated deer mice achieved intromissions by the eighth postoperative week (Clemens and Pomerantz, 1981). Citing unpublished work by Contreras and Beyer, Larsson (1979) stated that although sexual activity

decreased within ten days after castration, some rabbits intromitted sporadically for six months after surgery. "High active" male rats achieved intromissions up to postoperative day 105, but intromissions ceased by 25 days in "low active" male rats (Larsson, 1966), four weeks in gerbils (Yahr et al., 1979), four to seven weeks in hamsters (Beach and Pauker, 1949; Whalen and DeBold, 1974), and 6, 35, 41, and 109 weeks in rhesus monkeys (Michael and Wilson, 1974). Phoenix et al. (1973) reported that 50% of the castrated rhesus monkeys achieved intromission one year after surgery. Castration reduced the percent of guinea pigs displaying intromissions to zero, but the change was not as abrupt as it was for ejaculation (Grunt and Young, 1953).

Only two of the six minimally experienced cats (Felis domesticus) achieved intromission after castration, and then only during the first postoperative week (Rosenblatt and Aronson, 1958). In contrast, more than half of the cats that had been permitted several intromissions during precastration tests showed intromissions during the fifth preoperative week, and three castrates continued to achieve intromissions for 34, 88, and 139 postsurgical weeks. Rosenblatt and Aronson noted a bimodal distribution in the retention of intromission among these maximally experienced cats. Approximately 25% of these castrates retained the ability to display intromissions for a long time after castration; other males exhibited no more than five intromissions within a three month period following surgery.

The authors suggested that if a male continued to intromit beyond the three months, he was likely to continue the behavior for many more months.

Hormone replacement and intromission

The relative effectiveness of testosterone and three of its naturally occurring metabolites in stimulating intromission was examined in castrated Swiss Webster (SW) and CD-1 mice (Luttge and Hall, 1973). Testosterone induced mounting and intromission in both strains, but was slightly more effective in SW castrates. Although DHT was incapable of stimulating male sex behavior in CD-1 castrates, in SW castrates it was only slightly less effective than T in eliciting intromissions. Only the highest dose of AE stimulated intromissions and only in SW mice. Androstanedione (AA) did not induce sex behavior in either strain. This study was extended using other 5a-reduced androgens and either propylene glycol vehicle (PG) or oil vehicle (Luttge et al., 1974). When dissolved in PG, the pattern of effectiveness in stimulating male sex behavior in SW mice was : T > 3B-androstanediol > 3a-androstanediol; no facilitation of sex behavior was observed with DHT in PG. However, with the oil vehicle, DHT was nearly as potent as T.

The two studies described above used sexually naive mice. When proven sexually competent CD-1 castrates were treated with DHT, they displayed intromissions in a dose-related fashion (Wallis and Luttge, 1975). Thus, experience

could have accounted for differences between the two studies. In addition, hormone replacement started 36 days after castration in the first study and on the day of castration in the later study. Because less hormone is needed for maintenance than for restoration of sex behavior in related species such as the rat (Davidson, 1972), Wallis and Luttge suggested that the different results for CD-1 castrates might reflect a shift in hormone threshold after castration.

In contrast to the greater potency of DHT than AE observed in SW mice, both DHT and androsterone had very weak actions in maintaining intromissions, and T and AE maintained high levels of intromission in castrated male hamsters (Christensen et al., 1973). Rabbits more closely resembled mice than hamsters, as DHT and DHT plus EB induced intromissions in 62% and 85% of castrated rabbits, respectively (Beyer et al., 1975). Both EB and DHT plus lower doses of EB (than EB alone) maintained intromission in CD-1 castrates (Wallis and Luttge, 1975). These results demonstrate the synergistic effects of DHT and EB in stimulating masculine sexual behavior in male rabbits and mice.

The effectiveness of hormone replacement on the percent of castrated CD-1 mice displaying intromissions was the following : E2 > T, TP > DHT > E2 + DHT > oil > EB (Dalterio et al., 1979). Only E2 restored the percent of males achieving intromission to precastration levels, and this was

a single dose of E2, without androgen maintenance. The authors suggest that the prior exposure to androgen may have affected the behavioral response to T. Furthermore, because EB is considered to be the longer lasting form of estradiol (due to slow release from the injection site), the authors considered that estrogen activation of sexual behavior requires rapid distribution in this species, and, therefore, E2 was more potent than EB. Dalterio et al. concluded that an appreciable, but not necessarily sustained, elevation of E2 levels in the brain is critical for facilitation of masculine mating behavior in mice.

To summarize, intromission follows the general patterns of reduction after castration (and persistence by some animals) and increase by hormone replacement described above for ejaculation. However, in some animals such as the gerbil, guinea pig and (high active) rat, intromission behavior is retained longer and is restored, or at least increased, earlier after hormone replacement than is ejaculation.

Castration and mounting behavior

Mounting behavior persisted after castration in rabbits for 212 days (Stone, 1932), in high active rats for 105 days, but only for about a month in low active rats (Larsson, 1966), in 40% of guinea pigs for 14 weeks (Grunt and Young, 1953), in deer mice for 12 weeks (Clemens and Pomerantz, 1981), in only a few CD2F1 mice one week after surgery (Champlin et al., 1963), and in 75% of hamsters for

six weeks (Beach and Pauker, 1949). In another study, hamsters mounted sporadically throughout 30 weeks of postoperative testing (Whalen and DeBold, 1974). Gerbils ceased mounting by five postoperative weeks (Yahr et al., 1979). Using very short test periods, Banks (1964) reported that castrated sheep decreased mount frequency (number of mounts/test) within the first postoperative month and ceased altogether by 52 days. Mount frequency also was reduced in castrated rhesus monkeys, but mounting continued for the remainder of a 45 week observation period (Michael and Wilson, 1974).

The proportion of cats that mounted without achieving intromission during the first 15 postsurgical weeks was consistently higher in the experienced group (see above) and some of these castrates showed intermittent mounting for one and a half to five and a half years (Rosenblatt and Aronson, 1958). Rosenblatt and Aronson noted that the upper limits represented the termination of the testing procedures, and all indications were that the animals would have continued to mount, had they been given the opportunity.

An analysis of individual males' performance after castration provided the basis for dividing the castrated cats into three types or classes : (a) those castrates that continued to copulate for a long period of time after surgery; (B) castrates that ceased copulating soon after surgery; and (C) an intermediate group that retained some

behaviors (ex. mounting) for a long time, but lost other behaviors (ex. intromission) shortly after castration. Hart (1968) also noted three types of castrates among the dogs he tested.

Hormone replacement and mounting behavior

The aromatizeable androgens T and AE stimulated mounting significantly more than the nonaromatizable androgens androsterone (An) and DHT, but An, but not DHT, maintained mounting behavior above the oil-treated group of castrated hamsters (Christensen et al., 1973). In contrast, AE was less potent than either T or DHT in CD-1 and Swiss Webster mice, but the effects on mounting behavior were greater in SW castrates (Luttge and Hall, 1973). Treatment with AA failed to induce mating behavior in either strain.

In contrast to the findings by Dalterio et al. (1979) of ineffectiveness of EB in stimulating mounting in CD-1 mice, Edwards and Burge (1971) observed EB-induced mounting in approximately 50% of castrated SW mice, and prolonged EB treatment effectively facilitated masculine sexual behavior in CD-1 mice (Wallis and Luttge, 1975).

In conclusion, mounting behavior persisted after the loss of the ejaculatory reflex and intromission, and was the first to be restored by hormone treatment. Classification of conspecifics into groups based on their behavioral response to castration, may provide the basis for understanding the mechanisms responsible for individuals differences (see experiment 3 below).

# Factors Affecting the Retention of Masculine Sexual Behaviors After Castration

## Plasma testosterone levels in inbred mice

Hormone replacement studies show that in most species and most strains of mice, androgens are important for the display of masculine copulatory behaviors. Therefore, the differences in sex behavior exhibited by individuals, or by different strains, may be related to differences in circulating androgen levels. For example, McGill (1978b) cited unpublished work by Albeda that BALB/c males have 2.5 - 3 times as much endogenous androgen as do B6D2F1 males, and BALB/c males had ejaculation latencies that were approximately three times longer than those of B6D2F1 males (McGill 1962; 1970). To examine the relationship between sex behavior and circulating androgen levels, Batty (1978a) measured plasma T levels and copulatory behaviors in eight strains of house mice. Within strains, there were no correlations between plasma T levels and behavior. However, among strains, all measures of masculine sexual behaviors were significantly negatively correlated with mean plasma T levels. For example, strains which showed high levels of mounting (but low mount latencies), such as the B6D2F1, had low circulating levels of T.

In the study described previously, blood samples were taken from animals three days after their last behavioral test, i.e. in a non-sexual context. When blood samples were obtained during and after copulation, the effect of the sex

behaviors themselves were found to influence the levels of plasma T (Batty, 1978b). Close proximity to a receptive female resulted in rapid increases in plasma T in males, and this was most evident in strains with low "basal" T levels and a high probability of showing sex behavior. Within each strain, animals which showed sexual responses of at least mounting had higher T levels than animals showing no sexual responses, and animals that intromitted or ejaculated had increased T over animals which only mounted. Blood samples taken at particular stages of copulation suggested that T levels were greatest at initiation of mounting and declined during copulation. Thus, the negative correlation between sexual activity and plasma T level observed between strains in a non-sexual context, breaks down when examined in a sexual context, due to a marked increase in T levels in some of the strains when mating is initiated. Furthermore, the behaviors that were directly related to T levels were associated with initiation of mating behavior (mount frequency and latency), but no relationship was found between T levels and measures associated with the execution of mating (ejaculation frequency and latency). Batty noted that changes in T levels are the product of a neuroendocrine sequence of events, and any preceding links (pituitary or hypothalamic actions) may be important in the control of sexual behavior.

Reviewing the relationship between genetics and hormonal control of masculine sexual behavior, Shrenker and

Maxson (1983) noted the importance of estrogenic influences on mating behavior. In trying to interpret Batty's results, these authors stated that the inability at times to correlate strain differences in sex behavior with circulating T levels might be due in part to strain differences in the rate of testosterone metabolism or the degree of dependence on the different metabolites of T.

Thus, the hormone levels, themselves, may not be good indicators of levels of sexual activity. Rather, an animal's responsiveness or sensitivity to existing hormone may be a more important factor. In other words, one animal may readily respond to low levels of hormone with much sexual activity, but another animal might only show that same level of sexual behavior under conditions of very high levels of hormone.

Genetic factors

One way to study the influence of genotype on sexual behavior is to use inbred strains of animals, which result from at least 20 generations of brother-sister matings to achieve genetic homogeneity. Masculine sexual behaviors of inbred mice were described by McGill (1962) and Levine et al. (1966). These behaviors have been analysed for a variety of inbred strains and the hybrids resulting from crosses of parental strains (McGill, 1970; Vale and Ray, 1972, McGill and Manning, 1976).

The persistence of sex drive after castration varies with genotype within a species (McGill and Tucker, 1964).

Genetic homozygosity (in the parental strains, C57 and DBA) was associated with a rapid loss of the ejaculatory reflex after castration. In contrast, heterozygosity (in the F1 hybrid) resulted in the retention of the ejaculatory response for a maximum of 60 postsurgical days. McGill and Tucker suggested that the species differences in retention of sex behavior may be due to the degree of heterozygosity in the samples. Prior studies which showed a retention of sex behaviors in carnivores had used mongrel cats and dogs; studies with rodents which did not retain sex behavior after castration used animals from relatively inbred strains of laboratory colonies. Although a study by Champlin et al. (1963) demonstrated that one hybrid strain of mice, the CD2F1 (BALB/c female X DBA/2J male), lost the ejaculatory reflex within one week of castration, McGill and Tucker explained that this discrepancy may have resulted from retention of homozygosity at critical loci in the CD2F1 hybrid, maternal effects, or procedural differences.

The hypothesis that degree of heterozygosity at particular genetic loci is associated with retention of sexual behavior in male mice was examined by McGill and Haynes (1973). This hypothesis was supported by the finding of a significant positive correlation between days to last postcastrational ejaculatory response and percent heterozygosity (produced by testing males of the parental strains (C57 and DBA), the B6D2F1 hybrid, and F2 and backcrosses). However, although the castrated B6D2F1 males

(with 100% heterozygosity) maintained the ejaculatory reflex the longest, and males of the parental strains (with 0% heterozygosity) ceased ejaculating the earliest, the trend did not hold for every comparison; the BDF2 males (resulting from a cross between two B6D2F1s) with only 50% heterozygosity retained the reflex longer than backcrossed individuals with 75% heterozygosity. Thus, the degree of heterozygosity, itself, may not be as important as heterozygosity at specific genetic loci (McGill and Manning, 1976). McGill and Haynes did not rule out environmental factors such as perinatal hormone levels and maternal effects, which may influence this retention phenomenon, as the genotypically homogeneous group of B6D2F1 castrates exhibited a wide range of days to loss of the ejaculatory reflex.

The idea that heterosis (hybrid vigor) per se, is not an adequate explanation for the retention phenomenon was shown by results from the diallelic design employed by McGill and Manning (1976), in which the only genotype that exhibited prolonged retention of sex behavior was the B6D2F1 hybrid. If heterosis was the correct explanation, then one would expect the D2B6F1 hybrid (resulting from a cross between a DBA/2J female and a C57Bl/6J male) to retain the copulatory behaviors to the same extent as the B6D2F1 (C57Bl/6J female X DBA/2J male). However, the performance of D2B6F1 males resembled that of the parental strains, with a more rapid decline of sexual behavior after surgery than that exhibited by the B6D2F1 males.

B6D2F1 males receive their Y chromosomes from a DBA male, but D2B6F1 males receive their Y chromosome from a C57 male. To examine the possibility that a gene on the Y chromosome affects copulatory behavior in male mice, Shrenker and Maxson (1984) examined the sexual behavior of males of the DBA/1Bg, DBA/2Bg, and DBA/2.DBA/1-YBg congenic strains. The latter two strains, which differ only in their source of Y chromosome (the DBA/2.DBA/1-YBg is a DBA/2 with a DBA/1 Y chromosome), differed significantly in the proportion of males that mount, but not the proportion that intromitted or ejaculated. The investigators cited their previous findings that the DBA/1 and DBA/2 Y chromosomes were identical in their effect on intermale aggression and propose that the mouse Y chromosome may have at least two genes which affect sexually dimorphic behaviors.

In conclusion, the genetic factor(s) that might account for the prolonged retention of sex behaviors after castration may be associated with : (1) the Y chromosome; (2) autosomes (non-sex chromosomes) expressed under conditions of heterozygosity at specific loci; or (3) some combination of these, for example, the Y chromosome of a DBA with autosomes from the C57. However, the variability observed among members of the same species and the same strain is an indication that environmental factors also are important for the retention phenomenon.

Adrenal androgens

The retention of copulatory behaviors after castration does not result from the persistence of adrenal androgens, since adrenalectomy did not alter this retention phenomenon in B6D2F1 mice (Thompson et al., 1976). These results were consistent with those found in the rat (Bloch and Davidson, 1968), cat (Cooper and Aronson, 1958), hamster (Warren and Aronson, 1956), and dog (Schwartz and Beach, 1954; Beach, 1970).

Experience

Sexual experience during the postweaning period when the males were gonadally intact was not crucial for the performance of the ejaculatory reflex in castrated B6D2F1 mice (Manning and Thompson, 1976). However, lack of social contact before surgery resulted in marked suppression of the group's performance during the first five weeks of testing, and social experience with females led to a significantly faster rate of development of the ejaculatory reflex after castration.

Citing unpublished work by Contreras and Beyer, Larsson (1979) states that no relation existed between preoperative sexual experience and postoperative sexual activity of New Zealand white rabbits.

Rosenblatt and Aronson (1958) examined the influence of sexual experience prior to castration on the degree of retention of sex behavior after castration in cats. Maximally experienced cats were superior to minimally

experienced cats on the following measures : (1) the presence or absence of intromissions, (2) mount and intromission frequency per test, and (3) number of weeks after castration that any component of mating behavior was observed. The results from the experiment led to the conclusion that despite low levels of testicular hormone, the retention of copulatory behaviors after castration is facilitated by sexual experience prior to castration.

The role of prior experience on retention of sexual behaviors following castration was examined in the dog (Hart, 1968; Beach, 1970) and rat (Larsson, 1966, 1979; Bloch and Davidson, 1968). In contrast to the results observed in the cat, sexually experienced adult male beagles were not superior to sexually inexperienced males after castration for any of the behavioral measures. Similarly, rats with preoperative mating experience did not differ significantly from sexually inexperienced rats for any of the behavioral measures taken during five to seven weeks after castration (Bloch and Davidson, 1968).

In order to evaluate the role of sexual experience in the maintenance of sexual activity after castration, Larsson (1979) measured sex behavior in castrated rats that had either been housed singly from weaning or lived with females up to 70 days of age. At the first postoperative test session, 75% of the sexually experienced, but none of the single-housed males ejaculated. However, 15 days after surgery no group differences in sexual activity were

observed. Although prior sexual experience influenced the display of sex behavior initially, it did not prevent animals from ceasing to copulate. Larsson concluded that sexual experience is only of minor importance for maintenance of masculine sexual behavior in the rat.

Larsson (1966) noted that because both high and low active rats had equal amounts of presurgical sexual experience, the differences in retention of copulatory behavior after castration can not be attributed to variation in experience. However, Hart (1968) pointed out that the amount and type of sexual experience in the various studies were not equivalent, and these procedural differences might explain the different conclusions about precastration experience. In any case, the persistence of mating behavior after castration, even at times when all testosterone has been metabolized, and the fact that individual differences in copulatory behavior persist after castration, are all indications of important non-hormonal factors which influence sex behavior (Larsson, 1966).

In summary, the response to castration and subsequent hormone replacement appears to depend upon species and strain, prior sexual experience and/or sexual vigor of the castrate, hormone dose, time after castration when hormone is administered, and vehicle used with the replacement hormone. These factors also may affect the specific gonadal hormone or metabolite which is most effective in stimulating sexual behavior, as well as which specific behavior is stimulated or restored.

# Sex differences in CNS Morphology

## Introduction

Hormones modify behaviors, in part, by acting on specific populations of neurons. In addition to their effects on behavior, hormones also influence characteristics of the nervous system such as the number of cells; size of the neuron, its nucleus or nucleolus; neuronal connectivity; regional nuclear volume; cortical development; or plasticity (Gorski, 1983). Because the hormonal milieu varies between the sexes, sexual dimorphisms in these neural characteristics exist. In the last 15 years or so, sex differences in the nervous system have been investigated extensively, starting with the observation of sex differences in the hypothalamus by Raisman and Field (1971; 1973). These investigators observed that the number of non-amygdaloid synapses on dendritic spines in the preoptic area of the hypothalamus was higher in the normal female rat than in the male. Further, these sex differences in types of dendritic synapses could be reversed by manipulation of neonatal androgens.

Sexually dimorphic regions have been identified in a variety of species and may or may not be steroid-induced (Arnold and Gorski, 1984). Sex differences in the preoptic area of the hypothalamus have been observed in several species : rat (Gorski et al., 1978), hamster (Greenough et al., 1977; Bleier et al., 1982), guinea pig (Bleier et al.,

1982; Hines et al. 1985), gerbil (Commins and Yahr, 1984), mouse (Bleier et al., 1982; Robinson et al., 1985), ferret (Tobet et al., 1986), macaque monkey (Ayoub et al., 1983), and humans (Swaab and Fliers, 1985). Sex differences in other areas of the nervous system include : vocal control regions of the songbird brain (Nottebohm and Arnold, 1976), hypothalamic arcuate nucleus (Matsumoto and Arai, 1980), preoptic anterior hypothalamic area (Dörner and Staudt, 1968), ventromedial nucleus of the hypothalamus (Matsumoto and Arai, 1983), amygdala (Bubenik and Brown, 1973; Mizukami et al., 1983), hippocampus (Loy and Milner, 1980), vomeronasal organ (Segovia and Guillamon, 1982), accessory olfactory bulb (Valencia et al., 1986), hypogastric ganglion (Suzuki et al., 1983), superior cervical ganglion (Wright and Smolen, 1983a, 1983b), cerebral cortex (Diamond et al., 1981), thoracolumbar intermediolateral nucleus of the spinal cord (Calaresu and Henry, 1971; Henry and Calaresu, 1972), spinal nucleus of the bulbocavernosus in the lumbar spinal cord (Breedlove and Arnold, 1980), and cerebellum and overall brain weight (Yanai, 1979).

## Model Systems

Among the observed sexually dimorphic areas, four model systems have been studied most extensively : (1) vocal control regions of the brain in songbirds, (2) the sexually dimorphic nucleus of the preoptic area (SDN-POA), originally observed in the rat, (3) the sexually dimorphic area (SDA)

in the gerbil hypothalamus, and (4) the spinal nucleus of the bulbocavernosus (SNB). The first three models will be described briefly and the last model, the SNB, will be described much more extensively, since it was examined as part of the author's doctoral research. More detailed descriptions of these model systems may be found in reviews by Gorski (1983), Arnold and Gorski (1984), Arnold (1985), and Arnold and Breedlove (1985).

In songbirds, vocal control regions of the brain are significantly larger in males than in females, and these sex differences are correlated with sex differences in vocalizations (Nottebohm and Arnold, 1976; Arnold, 1985). In the rat, the volume of the SDN-POA of the hypothalamus is larger in the male than in the female (Gorski et al., 1978). This sexual dimorphism is significantly influenced by the perinatal, but not by the adult, hormonal environment. Although the function of the SDN-POA itself is not known, portions of the POA are intimately involved in the control of ovulation and sexual behavior : two sexually dimorphic traits (Arnold and Breedlove, 1985). The shape of the SDA of the gerbil hypothalamus differs between the sexes, and females lack the dense subgroup of cells, the SDA pars compacta, found in males (Commins and Yahr, 1984; Yahr et al., 1986). SDA cells accumulate both estrogens and androgens and may be involved in the hormonal control of mating and scent marking, because discrete lesions of the SDA impair both of these behaviors in adult males.

# The Spinal Nucleus of the Bulbocavernosus

## Introduction

The spinal nucleus of the bulbocavernosus (SNB) (Figure 1) was first described in the rat (Breedlove and Arnold, 1980), but has been observed in mice, hamsters, golden-mantled ground squirrels, and white-footed mice (Breedlove, 1984a). In the rat, the SNB contains large, multipolar, densely staining motoneurons which innervate muscles of the perineal area, the bulbocavernosus (BC) and levator ani (LA) (Hayes, 1965; Breedlove and Arnold, 1981). In males, the neurons are larger and more abundant than in females, which lack the target musculature found in the males (Hayes, 1965; Cihak et al., 1970; Breedlove and Arnold, 1980). Male rats that have a genetic mutation for androgen insensitivity (testicular feminization [tfm], which results in reduced androgen receptors, but normal estrogen receptors, in XY males) have a remarkably feminine SNB (Breedlove and Arnold, 1980; 1981). Based on hormonal manipulation studies (described below) and the observations in tfm males, the sex differences in SNB morphology appear to be mediated by interactions of androgens, but not estrogens, with their receptors early in development, rather than the adult hormonal state or the presence of the Y chromosome.

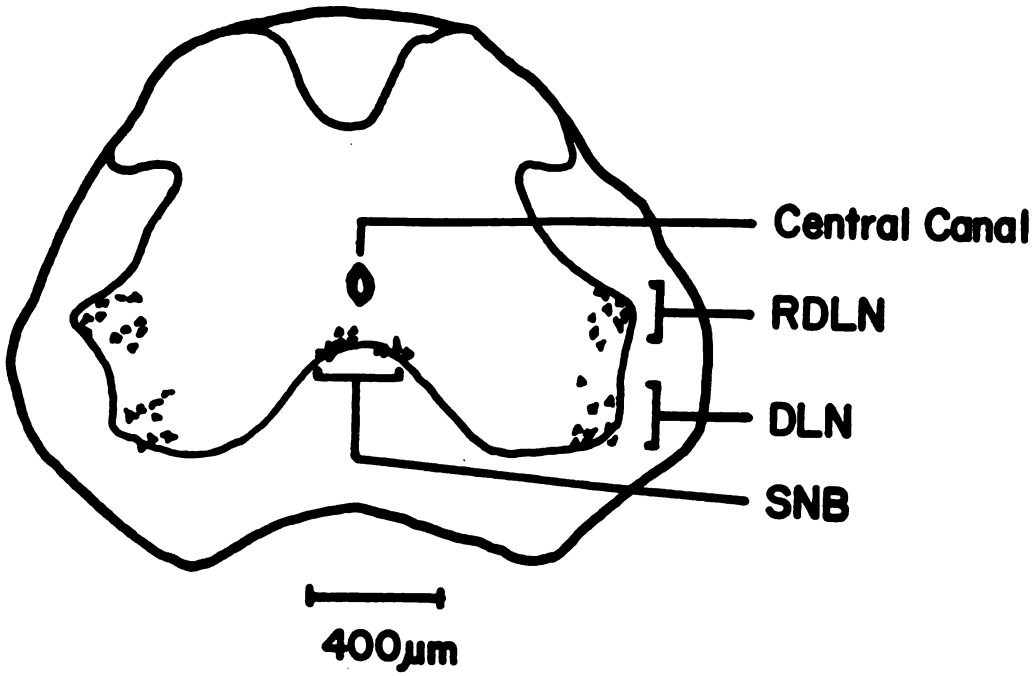



Figure 1. Camera lucida drawing of cross section through male mouse spinal cord at spinal levels L5-L6. SNB = spinal nucleus of the bulbocavernosus, DLN = dorsolateral nucleus, RDLN = retrodorsolateral nucleus.

A second nucleus in the lumbar spinal cord is the retrodorsolateral nucleus (RDLN) (Figure 1) whose neurons innervate the non-sexually dimorphic leg muscles (Shroder, 1980) via the sciatic nerve (Navaratnam and Lewis, 1970). No significant sex differences were observed in the size or number of RDLN motoneurons (Jordon et al., 1982). A third motor nucleus, the dorsolateral nucleus (DLN) (Figure 1), exhibits an intermediate degree of sexual dimorphism (Breedlove, 1984a). In both sexes, most DLN neurons innervate leg muscles (Shroder, 1980), but only in males, a subpopulation of DLN neurons innervate the ischiocavernosus (IC) muscle (Breedlove and Arnold, 1980). Therefore, a distinct DLN was found in both sexes, but there were significantly more motoneurons in the DLN of male than of female rats (Jordon et al., 1982).

Function of SNB and DLN target musculature

Because motoneurons of the sexually dimorphic SNB and DLN are androgen sensitive (Breedlove and Arnold, 1980; 1983c; Jordan et al., 1982), and because their target musculature is homologous in other mammalian species to muscles associated with erection and ejaculation (see reviews in Sachs, 1982; Breedlove, 1984a), the SNB and DLN may have reproductive functions. The striated penile muscles which are innervated by SNB and DLN motoneurons are attached to the base of the penis, and are absent in females (Hayes, 1965). Castration reduces the size of these muscles, and testosterone treatment increases the perineal musculature in

females and castrated males in rats (Wainman and Shipounoff, 1941; Cihak et al., 1970) and in castrated mice (Venable, 1966a; b). The striated penile muscles have been studied for their role in the mediation of penile reflexes (Sachs, 1982; Hart and Melese-d'Hospital, 1983). In addition, the effects of perinatal androgen treatment on these penile responses, the associated muscles and motoneurons, and other behavioral parameters have been determined for male and female rats (Sachs and Thomas, 1985).

The role of the BC and IC muscles in the control of penile reflexes was examined in both intact and spinally transected male rats (Sachs, 1982; Hart and Melese-d'Hospital, 1983). Contractions of the BC occur almost exclusively in association with penile erections, and contractions of the IC primarily are associated with penile flips. Intense erections that produce a cup-like flaring of the distal glans penis apparently require the mechanical action of the BC muscle contraction, because removal of the BC virtually eliminated erections with cups.

In attempted copulation, males lacking IC rarely gained intromission, and in males that did achieve intromission and ejaculate, ejaculation rarely occurred in the vagina (Sachs, 1982). Apparently vaginal penetration requires penile flips, which were eliminated by removal of the IC. Although intromissions and intravaginal ejaculations were achieved by males with excised BC and LA (levator ani) muscles, only one fifteenth of the females mated to these males became

pregnant. Sachs attributed the males' infertility in part to their inability to form a penile cup. Without the formation of a cup, a large portion of the seminal plug is withdrawn from the vagina after ejaculation, and the plug presumably is prevented from being tightly lodged against the cervix. Copulatory plugs are important for transport of sperm (Matthews and Adler, 1978), which is essential for successful impregnation. In addition to causing suboptimal deposition of the copulatory plug, the reduced capacity for erection may have resulted in reduced vaginal stimulation of the female (Sachs, 1982), which hinders the success of pregnancy (Wilson et al., 1965). Further, intact BC and IC musculature appears to be necessary for the removal of previously deposited copulatory plugs (Wallach and Hart, 1983). In multi-male groups, males commonly alternate copulations (McClintock et al., 1982). Therefore, the occurrence of intromission prior to ejaculation and subsequent removal of previously deposited plugs plays an important role in sperm competition (Wallach and Hart, 1983).

In summary, the SNB and DLN are associated with a variety of reproductive functions. The SNB-innervated BC is associated with penile cups, and the DLN-innervated IC is associated with penile flips. Thus the SNB and DLN are involved in the following reproductive functions : (1) vaginal penetration necessary for intromission and intravaginal ejaculation. (2) vagino-cervical stimulation essential for maintenance of pregnancy, (3) removal of

previously deposited copulatory plugs, and (4) formation of a new copulatory plug over the female's cervix to facilitate sperm transport through the uterus.

Effects of Gonadectomy

Gonadectomy in adulthood reduced the size of SNB neurons in the rat, but had no effect on neuronal number in either sex (Breedlove and Arnold, 1981; Kurz et al., 1986). Dendritic length was reduced by 56 % in males castrated in adulthood (Kurz et al., 1986). The SNB was demasculinized in males that were castrated at birth and had been given prenatal injections of the nonsteroidal, anti-androgen, flutamide, which does not interfere with estrogen receptors, (Breedlove and Arnold, 1983a). Although flutamide did not reduce mounting or intromission behavior (ejaculations were not measured), it reduced the number and size of SNB neurons and the number of target muscle fibers.

Effects of hormone replacement

Adult gonadectomized male and female rats that received TP replacement had much larger somal cross-sectional areas than gonadectomized rats that received oil injections (Breedlove and Arnold, 1981). Similarly, both somal area and dendritic length were restored in castrates given testosterone-filled Silastic capsules (Kurz et al., 1986). Hormone replacement in adulthood did not affect the number of neurons in either sex (Breedlove and Arnold,

1981). In males exposed prenatally to flutamide and castrated on the day of birth, postnatal TP treatment masculinized the SNB system, and these masculinizing effects were significantly more pronounced in males given flutamide prenatally than in males given vehicle prenatally (Breedlove and Arnold, 1983a). Breedlove and Arnold concluded that the presence of androgen either prenatally or postnatally can significantly compensate for a lack of androgens during the other time period.

Females treated with TP on day 2 of life, had more SNB neurons in adulthood than oil treated controls, although the size of individual neurons was not increased by the TP treatment (Breedlove et al., 1982). Treatment of females on day 2 with estradiol benzoate (EB) had no masculinizing effect on either cell size or number of the SNB in adulthood, which suggests that the aromatization of testosterone to estradiol is not sufficient for dimorphic development of the SNB.

The critical periods for androgen-induced masculinization of the SNB system were investigated by administering TP or dihydrotestosterone propionate (DHTP) to females during prenatal or postnatal periods and examining the SNB system in adulthood (Breedlove and Arnold, 1983b). Both pre- and postnatal androgen treatments are able to masculinize the SNB system in female rats, and two critical periods for these androgen actions were revealed. Although late postnatal treatment with either TP or DHTP masculinized neuronal size, such treatments had no effect on neuronal

number. The critical period for the masculinization of neuronal number begins prenatally and ends by about postnatal day 5. The critical period for the masculinization of neuronal size extends beyond postnatal day 5, but was not established in the study. The finding that neuronal size and number have different, albeit overlapping, critical periods, suggests that the mechanisms responsible for organizational masculinization of these two parameters are somewhat independent (Breedlove and Arnold, 1983b).

Breedlove (1985) injected horseradish peroxidase (HRP) into the BC muscles of adult females treated with androgens prenatally and determined that prenatal DHTP treatment caused the BC to be innervated by motoneurons located outside their normal anatomical locus in the SNB. In females given TP prenatally, HRP injections in adulthood revealed that most motoneurons innervating the BC were found in the medial motoneuronal column, the same location as the SNB in males. Although HRP injections into the BC of adult males virtually never labeled lateral neurons, these motoneurons were labeled after HRP injection of BC in adult females treated prenatally with DHTP. This lateral motor column corresponds to the DLN, located 500 um away from the SNB. Based on the different configurations of innervation produced by prenatal TP and DHTP, Breedlove (1985) suggests that the normal pattern of connectivity in the SNB system may be determined by the relative contributions of each hormone.

Steroid accumulation

In both sexes, motoneurons in the DLN, RDLN, and SNB accumulated substantial amounts of label after injection of tritiated DHT, but accumulated very little label after E2 injection (Breedlove and Arnold, 1980; 1983c). In contrast, T accumulation was much greater in males than in females for all three nuclei, and was greater in the SNB than in the DLN and RDLN. This variable accumulation between motor nuclei after T, but not DHT, injection, suggests that the activity of 5a-reductase, the enzyme that irreversibly converts T to DHT (Selmanoff et al., 1977), varies between motoneurons (Breedlove, 1985). Breedlove (1985) suggests that the development of the normal innervation pattern of the SNB may require the regional inhibition of T's conversion to DHT.

Androgenic regulation of sex differences

The various manipulations described above show that sex differences in the SNB system result from differences in androgens during critical periods in development. The androgen-induced sex differences in neuronal number might be explained by one of three possible mechanisms (Breedlove, 1984a). Androgens may increase neuronal number by (1) increasing neurogenesis, (2) causing the redifferentiation of non-SNB cells into SNB cells, or (3) preventing programmed cell death. These possibilities have been examined, and the third possibility seems to be the most likely.

Breedlove et al. (1983) determined that neurogenesis of

motoneurons of the SNB, DLN, and RDLN is complete by gestational day 14. Because testosterone production in the rat occurs after day 14 (Warren et al., 1973; Picon, 1976; Feldman and Bloch, 1978) and plasma T levels do not exhibit sex differences until gestational day 18 (Weisz and Ward, 1980), neurogenesis of the SNB ends before the onset of T production. These findings rule out the possibility that androgens affect neuronal number by promoting neurogenesis. Further, as described above, neuronal number was decreased or increased by androgen treatment more than a week after the end of neurogenesis (Breedlove and Arnold, 1983a, b; Breedlove et al., 1982).

The possibility that androgens direct the differentiation of immature neurons into motoneurons, which then innervate the SNB target musculature, was ruled out by a thymidine autoradiographic study (Breedlove, 1984b). Female rats were given a single exposure to tritiated thymidine on day 12 of gestation and, on the day of birth, either they were sacrificed to examine thymidine labelling of motoneurons or non-motoneurons, or they were treated with TP. In the females sacrificed on day 1, most of the heavily labelled cells were motoneurons. In adulthood, the "androgen-induced" SNB neurons retained thymidine label. Because TP was administered at the time when most motoneurons were heavily labelled, the SNB neurons observed in adulthood probably had differentiated into motoneurons before androgen treatment. Apparently neonatal androgen

treatment in females adds SNB cells, not by redifferentiating non-motoneurons into motoneurons, but rather by constructing the SNB from pre-existing motoneurons (Breedlove, 1984a).

The third explanation for androgen-induced increases in neuronal number is that androgens prevent or attenuate the normally occurring cell death. This mechanism of androgen action in the SNB system has been supported by counts of SNB motoneurons and pycnotic (dying) neurons in males, females, and prenatally TP-treated females (Nordeen et al., 1985). In all three groups, the number of SNB motoneurons increased dramatically between prenatal day 18 and 22, then exhibited a significant loss of SNB cells between prenatal day 22 and postnatal day 10. Males and androgenized females had a greater increase in cell number during the first phase and less neuronal cell death during the second phase of development than was observed for the untreated females. These results support the hypothesis that sex differences in SNB neuronal number result from androgenic regulation of normally occurring cell death. The perinatal androgen manipulations that permanently altered SNB morphology (Breedlove and Arnold, 1983a; b) were performed during the time of cell death.

Nordeen et al. (1985) point out that their results do not exclude the possibility that sex differences may arise from androgenic influences on the migration of SNB motoneurons. A lateral-to-medial migration which produces the prenatal increase in SNB neuronal number has been

suggested by results from a perinatal HRP study (Sengelaub and Arnold, 1984). Their results indicated that the formation of early projections from the SNB is comparable in both sexes, and that the differential death of motoneurons and the resultant dimorphism in cell number do not result from a failure of SNB neurons to send their axons to the periphery in the female.

In conclusion, androgens, but not estrogens, act both pre-and postnatally to influence the sexually dimorphic development and maintenance of adult function of the SNB system. At maturity both the SNB motoneurons and the BC muscles, which have reproductive functions, bind androgen and respond morphologically to hormonal manipulations (Breedlove, 1984a). Thus, the SNB system is a useful model for investigating the mechanisms by which hormones influence both behavior and neural structure throughout ontogeny.

## Statement of Purpose

The overall objective of the research reported here is to understand the physiological and environmental factors that influence the retention of copulatory behaviors after castration. To address some of these issues, the following five experiments were designed to investigate the influences of genotype, plasma hormone concentrations, and possible neuroanatomical sites of hormone action on the retention of copulatory behaviors in male house mice. Each experiment will be described separately, and then a general discussion will emphasize certain points that relate to one or more of the experiments.

## EXPERIMENT 1

## Effect of Genotype on Retention of Sex Behavior After Castration

### Introduction

Males from the B6D2F1 hybrid strain, but not from the parental strains (C57Bl/6J and DBA/2J), retained the ejaculatory reflex for several months after castration (McGill and Manning, 1976). The objective of this first experiment was to replicate the findings reported by McGill and Manning in our own laboratory. If the phenomenon could be replicated, further analyses, such as those described in the later experiments, would aid in the understanding of the physiological mechanisms or other factors which influence the retention of sexual behavior after castration in house mice.

### Methods

#### Subjects

Adult male mice (approx. 90 days old at the start of the experiment) purchased from Jackson Labs, Bar Harbor, ME were individually housed and maintained on a 14:10 reverse light-dark cycle, with lights off between 1100 and 2100

hours. Commercial chow and water were available ad libitum. Males from the following strains were used : DBA/2J, C57Bl/6J and B6D2F1 (the hybrid resulting from the cross between a C57Bl/6J female and a DBA/2J male). A total of 85 males successfully completed the 38 week experiment.

Adult B6D2F1 female mice, group housed in the same room as the males, were used as stimulus females. They were brought into behavioral estrus by sequential intramuscular injections of estradiol benzoate (50 ug) and progesterone (.5mg), both administered in sesame oil vehicle.

Behavioral testing

Testing was conducted under red illumination between 1200 and 1700 hours, following procedures modified from those described by McGill and Manning (1976). Males were placed individually in the testing arenas (10 gallon aquaria, with a substrate of sanicel bedding) for 30 minutes prior to the onset of testing. Testing began by placing a hormone-treated stimulus female into the male's testing cage. If 10 minutes passed without an intromission or an intromission-like response, the first female was replaced by another stimulus female. If the male failed to intromit with the second female within the next 10 minutes, a third and final female was introduced. Failure to intromit within the 30 minutes resulted in the termination of that male's test.

If intromission did occur, the test continued until (1) the male ejaculated, (2) 40 minutes elapsed between any two successive intromissions, or (3) 4 hr passed, at which time

the test was terminated even if the male was still sexually active. If the stimulus female became unreceptive during the course of testing, she was replaced by another stimulus female. Each male was tested once every 2 weeks.

Masculine copulatory behaviors were recorded with a TRS-80 Model 4P computer using a program described by Rakerd et al. (1985). The following behavioral parameters were recorded : mount latency (ML, time from introduction of the first female into the test arena to first mount); intromission latency (IL, time from introduction of first female into test arena to first intromission); intromission frequency (IF, the number of intromissions until ejaculation or until termination of the test) and ejaculation latency (EL, time from first intromission to ejaculation). Since castrated animals do not show seminal emission, they do not exhibit an ejaculation per se. However, they do exhibit an ejaculatory pattern or reflex, that normally is associated with seminal emission in the gonadally intact animals. Intromission refers to intromission-like behavior, since ventral viewing was not used for every test.

One week after their seventh sexual behavior test, males from each of the three strains were either castrated or sham operated under Metofane (methoxyflurane; Pittman-Moore, Inc.) anesthesia. Behavioral testing resumed one week later and continued for an additional 13 biweekly tests (25 weeks). In contrast to the protocol used by McGill and Manning (1976), in which males were no longer tested after

the criterion for loss of the ejaculatory reflex was met, males of all groups were tested throughout the study to investigate any possible recovery by the parental strains.

At the termination of the behavioral experiment, the males were sacrificed, and blood samples were taken for radioimmunoassay. At this time castration or sham operation were confirmed by noting the presence or absence of testes; in addition, accessory sex organs were examined.

### Statistics

The percent of animals showing each behavior was compared among strains for the same surgical treatment (to determine genotypic influences) and within strains (to determine the effect of castration) using Kruskal-Wallis and Mann-Whitney U tests (Siegel, 1956). These two nonparametric tests also were used to analyze behavioral latencies for the intact F1s and a subpopulation of the castrated F1s (defined in the results section). The percent of tests with behaviors and behavioral latencies were compared statistically between pre- and postcastration with the Wilcoxon nonparametric test. In all statistical analyses, for all the experiments described in this dissertation, significance levels of $p < .05$, and two-tailed tests, where applicable, were used.

## Results

Prior to castration or sham surgery, males of all six groups showed high levels of masculine copulatory behavior. After castration, all three strains showed a significant

decline in the percent of animals showing ejaculations (Figure 2), intromissions and mounts ($p < .001$ for all behaviors for all strains). However, the decline in the percent of F1 castrates showing these behaviors was less than that for castrates of the two parental strains (Appendix 1). The F1 castrates continued to copulate for many more weeks after castration than did castrates of the parental strains, and approximately 30% of the F1 castrates continued to display ejaculatory reflexes regularly 25 weeks after castration. In addition, the decline in the percent of castrates showing sexual behaviors was much more rapid in the DBA and C57 strains than for the F1s (eg. compare Figures 2a, b with 2c). Intact males of all three strains continued to copulate throughout the experiment. However, some variability was observed in the intact males of the DBA and C57 strains, with respect to the percent ejaculating. In contrast, all of the intact F1 males consistently ejaculated throughout the experiment (compare Figure 2c with 2a, b). The percent of tests with mounts, intromissions, and ejaculations following surgery in all three strains are presented in Appendix 1. These measures for sham operated and castrated F1 males during different postsurgical periods are presented in Appendix 2.

The effects of sham operation or castration on behavioral latencies are shown in Figures 3, 4, and 5. An analysis of the variability within each group on each test (by drawing separate graphs with variability of the means)

revealed that the apparent rhythmic pattern for the C57's mount latency (Figure 3b) and intromission latency (Figure 4b) is due to large within-group variability and does not reflect a weekly rhythmicity of latencies.

The within-group variability was examined for latencies of different groups of F1 castrates that differed in their behavioral response to castration. The peaks in mount and intromission latency observed for all castrated F1s (Figures 3a, 4a) are attributed to those F1 castrates that eventually stopped ejaculating or showed reduced levels of copulatory behaviors (these castrates were designated as noncontinuers and intermediates). In other words, these peaks are not observed in graphs of mount and intromission latencies for just the animals that continued to show high levels of sexual behaviors (the continuers).

Comparisons of behavioral latencies between intact F1s and the castrates that continued to show masculine sexual behavior (continuers) are presented in Appendix 3. These comparisons examined the behavioral latencies for pre- versus post-surgical weeks in both groups of F1s as well as between groups. Sham operation had no effect on any of the behavioral latencies $p > .15$). All behavioral latencies were significantly longer after castration than after sham operation, except for ejaculation latency (EL) during the first seven postsurgical weeks (Post 1; $p = .064$). However, EL during Post 1 was significantly longer than presurgical EL ($p < .02$). Because very few of the castrates in the parental strains showed copulatory behavior after

castration, no conclusions can be made about the effect of castration on latencies in these strains.

Discussion

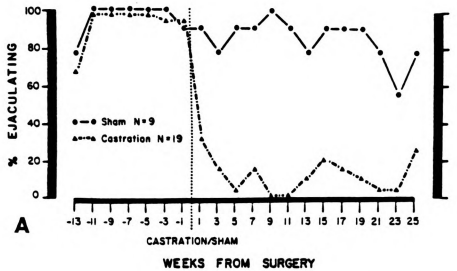
Results from the behavioral tests confirm earlier reports of prolonged retention of copulatory behavior following castration in male B6D2F1 mice (McGill and Manning, 1976). Castrated males of the parental strains did not retain sexual behavior to the same extent as castrates of the hybrid strain. The loss of sexual activity in the parental strains soon after castration, as compared to the retention of copulatory behaviors up to 25 weeks after castration by some of the F1s, indicates that genotype influences the retention of sexual behaviors following castration.

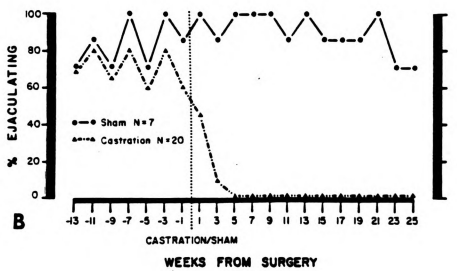
In the study by McGill and Manning, ejaculation latencies for the B6D2F1 castrates increased during postcastration weeks 4-7 and declined rapidly during weeks 7-12, after which they gradually returned to the level of postcastration week 1. McGill and Manning referred to the time of increased ejaculation latencies as a "difficult period". Although an overall increase in ejaculation latency (EL) resulted from castration, (comparisons of pre- versus postcastration latencies, Appendix 3), no difficult period occurred in this experiment. The data from individual animals and the variability within each subgroup of F1 castrates were examined to analyse the increase in EL

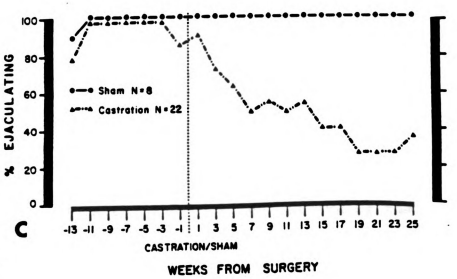
between 13 and 21 postsurgical weeks for all castrates (Figure 5a). The analysis showed that this peak resulted from long ejaculation latencies exhibited by a few of the continuers on random weeks, but not by the same animals each week.

Castration of F1s increased the mean mount and intromission latencies in a similar fashion to the increase seen in EL (Figures 3-5; Appendix 3). In contrast, Manning and Thompson (1976) found that castration increased the mean latency to ejaculation, but had no effect on mean intromission latency or mean mount latency. The discrepancy between these two studies can be explained by the fact that most of the F1 castrates in Manning and Thompson's study retained sexual behavior for a long time and probably would have been selected as continuers in our study. As described above, the peaks in mount and intromission latencies observed for all F1 castrates (Figures 3, 4) were due to the noncontinuers and intermediates that showed those behaviors on random weeks. Because Manning and Thompson had a small percent of noncontinuers and intermediates, the data from these animals probably would not have affected the overall latencies as much as the data from noncontinuers and intermediates in this study did.

**Figure 2. Percent of animals showing ejaculatory reflex after sham operation or castration in three strains of house mice. A. DBA/2J, B. C57Bl/6J, C. B6D2F1.**


% EJACULATING
Sham N=9
Castration N=19
A
CASTRATION/SHAM
WEEKS FROM SURGERY


% EJACULATING
Sham N=7
Castration N=20
B
CASTRATION/SHAM
WEEKS FROM SURGERY


% EJACULATING
Sham N=8
Castration N=22
C
CASTRATION/SHAM
WEEKS FROM SURGERY

2

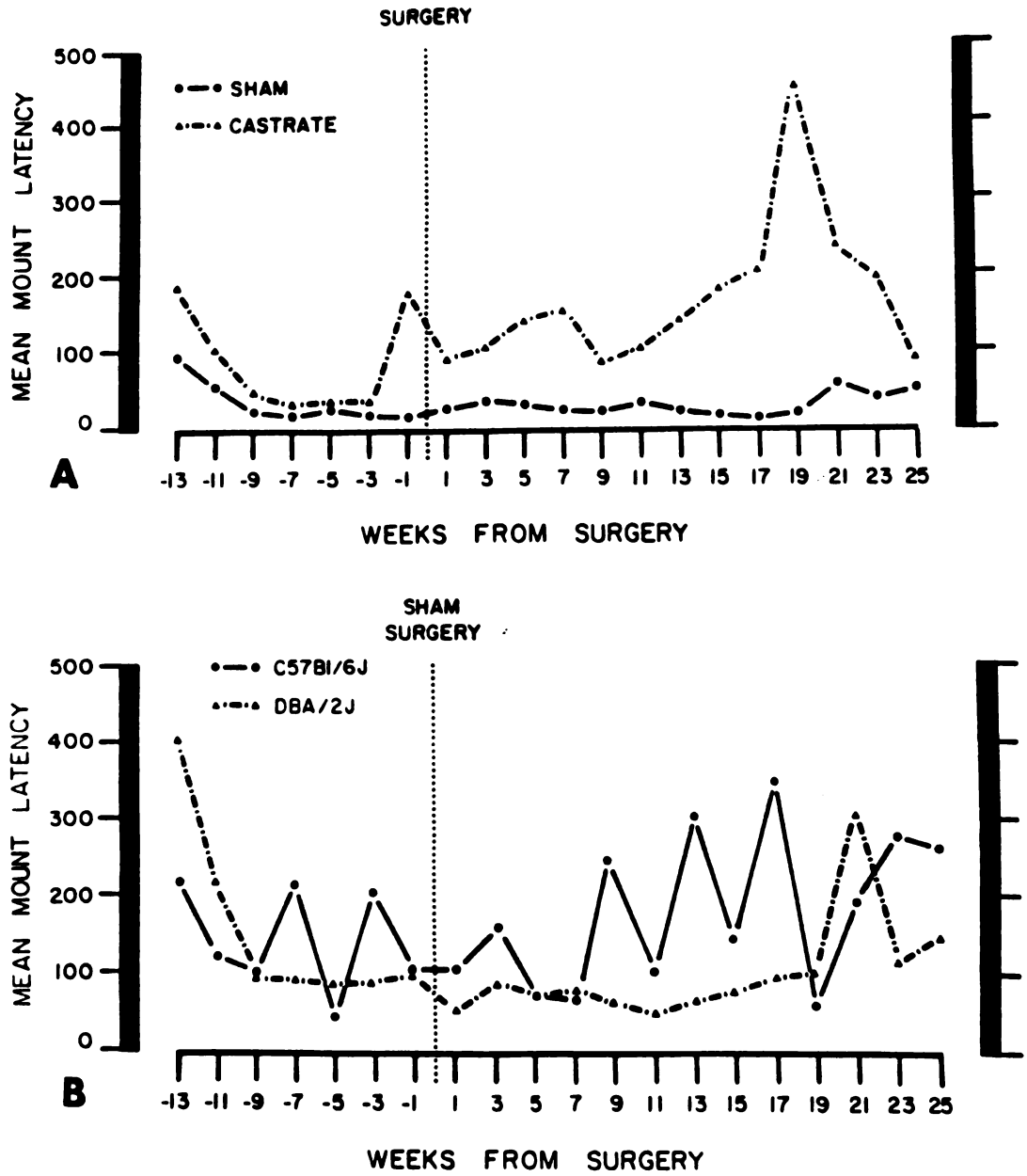



Figure 3. Influence of castration or sham operation on mount latency in house mice. A. Sham operated and castrated B6D2F1 males. B. Sham operated C57Bl/6J and DBA/2J males. The repeated pattern of the C57s is due to large within-group variability and does not reflect a weekly rhythmicity of latencies.

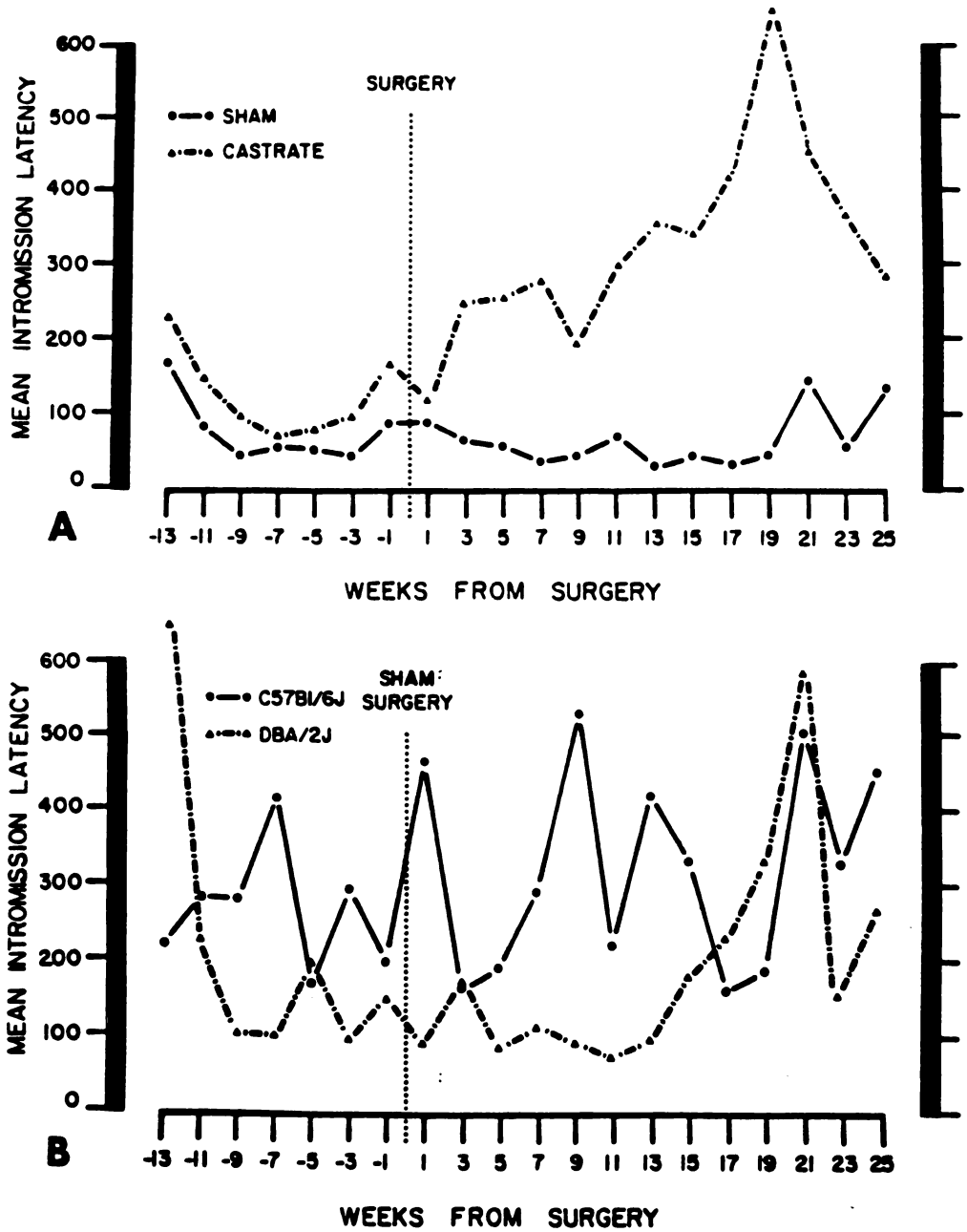



Figure 4. Influence of castration or sham operation on intromission latency in house mice. A. Sham operated and castrated B6D2F1 males. B. Sham operated C57Bl/6J and DBA/2J males. The repeated pattern of the C57s is due to large within-group variability and does not reflect a weekly rhythmicity of latencies.

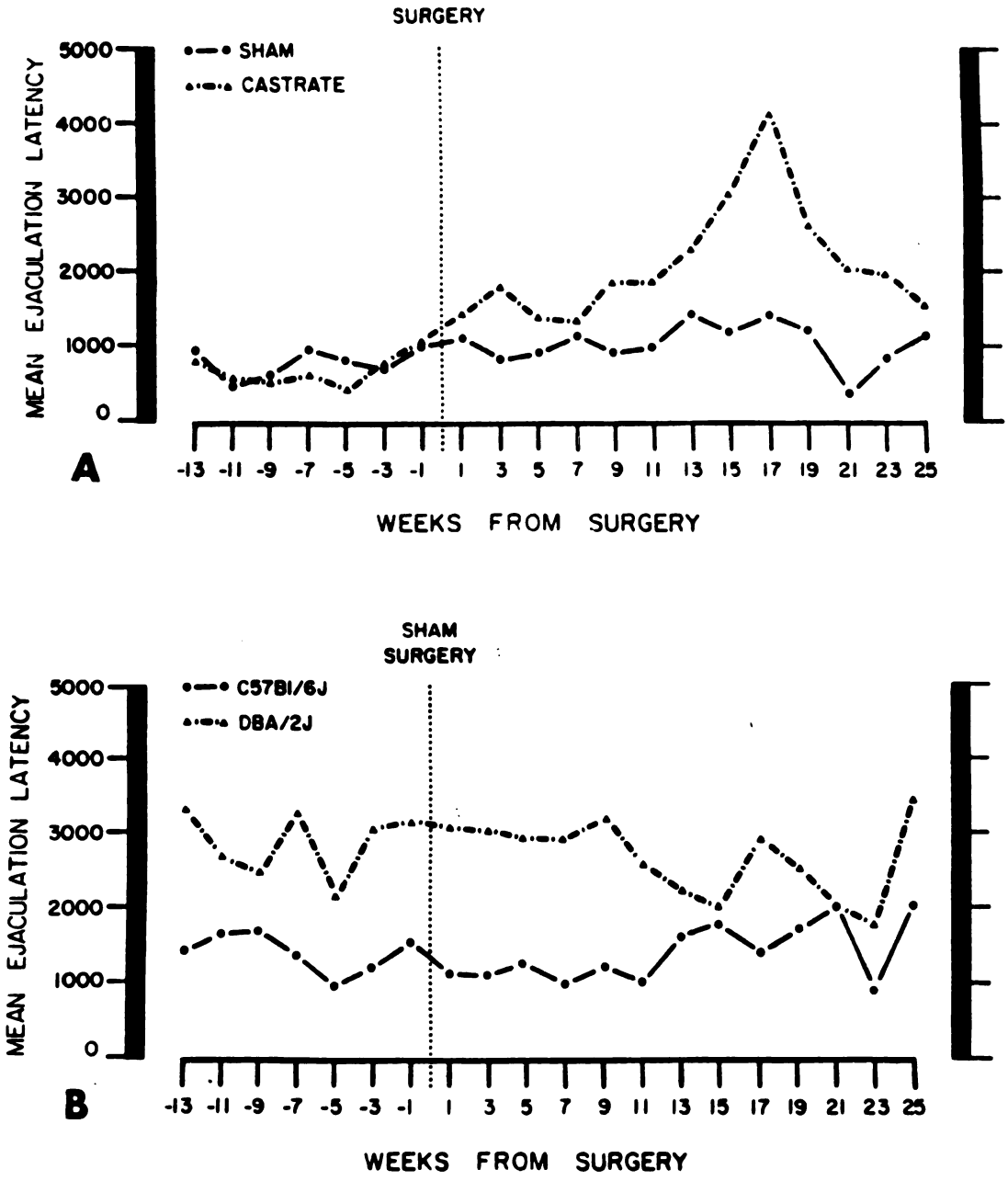



Figure 5. Influence of castration or sham operation on ejaculation latency in house mice. A. Sham operated and castrated B6D2F1 males. B. Sham operated C57Bl/6J and DBA/2J males.

# EXPERIMENT 2

## Radioimmunoassay of Plasma Testosterone Levels

### Introduction

One possible explanation for the retention of sex behaviors after castration is that androgens produced by the adrenal gland or stored in the body after castration maintain sexual behaviors. Further, although the adrenal glands of B6D2F1 mice produce only small amounts of androgens, Thompson et al. (1976) suggested that B6D2F1s might possess an extremely high sensitivity to androgens, such that the adrenal androgens would be sufficient to retain the copulatory behaviors. However, based upon the finding that adrenalectomy does not prevent the retention of sexual behaviors after castration, these authors suggested that adrenal androgens play no part in the maintenance of sexual behavior in castrated B6D2F1s. In addition, residual T levels probably do not maintain sexual behaviors after gonadectomy, as plasma T levels were significantly reduced within hours after castration of rhesus monkeys (Resko and Phoenix, 1972).

To extend the findings of Thompson et al. that retention of copulatory behaviors after castration is not due to adrenal androgens, or to some as yet undetermined extra-adrenal testosterone source, plasma testosterone levels were measured in the present study.

## Methods

### Subjects

Sham operated (intact) and castrated males from all three strains tested for copulatory behaviors in experiment 1 were used in this experiment.

### Radioimmunoassay

At the termination of the behavioral testing, males were sacrificed via cardiac puncture or decapitation. Blood samples were taken for radioimmunoassay and sent to the lab of Dr. Bruce Goldman at the Worchester Foundation. Procedures followed those described by Vitale et al. (1985). The sensitivity of the assay was 4-6 pg testosterone per test tube. Each test tube contained 100 ul of the serum extract. Thus, the lowest detectable levels were 40-60 pg/ml. Cross reactivity of the antibody with dihydrotestosterone was 67% and with other androgens was < 0.5 %.

### Statistics

Data were analysed by Kruskal-Wallis (nonparametric one-way ANOVA), followed by Ryan's Procedure for ordered data.

## Results

In all three strains of mice, plasma testosterone levels in castrated males were significantly lower than plasma testosterone levels of sham operated (intact) males

(Figure 6a). Testosterone levels in sham operated males of one strain did not differ significantly from those of another strain. Similarly, T levels in the three strains of castrates did not differ from each other. None of the subgroups of castrate B6D2F1s (continuers, noncontinuers, and intermediates) differed significantly from each other (Figure 6b).

## Discussion

The results from the radioimmunoassay are consistent with the morphological observations of atrophied accessory sex organs following castration. Reduced plasma testosterone levels in all the castrates (as compared to the sham operated mice of the same strain) indicate that the retention of copulatory behavior after castration can not be attributed to an alternate (nongonadal) testosterone source. The results from the radioimmunoassay are in agreement with findings by Thompson et al. (1976), who showed that the retention of the ejaculatory reflex after castration in the B6D2F1s is not dependent upon adrenal androgens. Further, the lack of differences in T levels across strains for intacts and for castrates supports the idea that differences in sexual activity across strains are not due to differential plasma testosterone levels. This topic was studied by Batty (1978a, b) and reviewed in the literature review section.

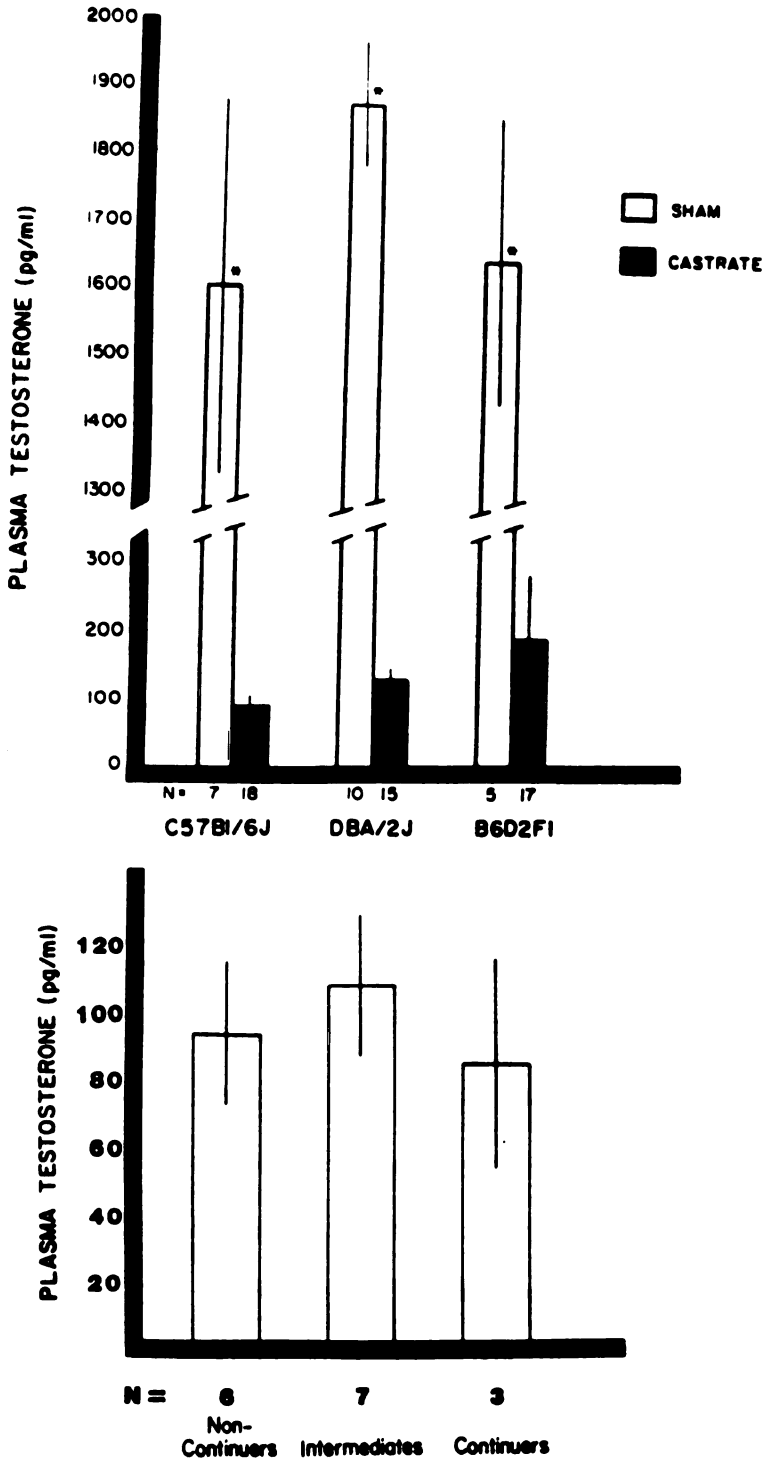



Figure 6. Plasma testosterone levels in house mice. A. Influence of genotype and castration or sham operation, * = significantly different from castrates of that strain, B. Comparison among three groups of B6D2F1 castrates that differ in their behavioral response to castration.

# EXPERIMENT 3

## Nuclear Estrogen Receptor Assay

### Introduction

McGill and Manning (1976) and McGill and Haynes (1973) noted striking individual differences among the B6D2F1 males. Similarly, the F1 males in experiment 1 were far from homogeneous in their response to castration. Although some F1 castrates stopped copulating, other castrates continued to copulate throughout the entire experiment. Because all B6D2F1s are genetically identical, the variability in the behavioral response to castration cannot be explained in terms of individual genetic differences. In order to examine this behavioral variability further, two groups of F1 castrates were selected on the basis of their behavior after castration. One group, the "continuers", continued to show the ejaculatory reflex throughout the 25 week postcastration period. The second group, the "noncontinuers", did not continue to exhibit the ejaculatory reflex. The behavioral performance of males from these selected groups in their last 3 tests is summarized in Figure 7a. These two selected groups of F1 castrates were examined in experiment 3 to determine whether the behavioral differences were associated with differences in gonadal hormonal processes. Some of the F1s that did not fit into either group, the intermediates, were not included in experiment 3.

Considerable evidence supports the idea that testosterone's activation of masculine sexual behavior

involves its aromatization into estradiol in steroid target tissues (Coniglio et al., 1973; Christensen and Clemens, 1974; 1975; Naftolin et al., 1975; Luttge, 1979; Södersten, 1979). One possible target site for estrogenic stimulation is the medial preoptic area (MPOA) of the hypothalamus, a brain region important in the control of masculine sexual behavior in rodents and other mammals (Christensen and Clemens, 1974; Hart, 1974; Clemens and Weaver, 1985). The behavioral difference between the two groups of B6D2F1 castrates may result from differences in estrogenic stimulation of the MPOA between these two groups. The source of this estrogenic stimulation could be either testosterone, estradiol or other aromatizable androgens (eg. androstenedione).

Steroid hormones such as testosterone or estradiol affect behavior by interacting with specific receptors. The steroid hormone-receptor complex binds to the DNA in the cell's nucleus and alters protein synthesis; presumably these changes eventually result in an alteration of behavior (O' Malley and Means, 1974; McEwen et al., 1979). Because the steroid is first bound to the receptor before the receptor binds to the DNA (Jensen et al., 1968), the level of nuclear receptors bound to the DNA is a direct indication of stimulation of the target cell by the steroid hormone. Therefore, to determine whether the retention of copulatory behaviors after castration was due to estrogenic stimulation, nuclear estrogen receptor levels in the MPOA

were measured in the F1 males described in experiment 1.

## Methods

### Subjects

All intact F1 males plus the continuer and noncontinuer F1 castrates selected from experiment 1 were used in this experiment. Those castrated F1 males that did not fit into either category were not included in the nuclear estrogen receptor (NER) assay.

The continuers and noncontinuers were selected on the basis of their performance on behavioral tests after castration. Continuers (studs) ejaculated on the majority of postoperative tests (at least 85% in experiment 1) and ejaculated on at least four of the last six tests. The noncontinuers (duds) ceased ejaculating much sooner after surgery than the continuers and, with a few exceptions, only mounted or did not exhibit any behavior in the last six tests. The intermediates were those castrates whose sex behavior was inconsistent (ex. ejaculation one week, no behavior the next week) and, therefore, they could not be classified as either continuers or noncontinuers. Two investigators (the author and David Weaver) independently made the group assignments (studs/intermediates/duds), which were confirmed later.

### NER Assay

This experiment was performed in the lab of Dr. Edward Roy at the University of Illinois. Prior to this experiment,

Dr. Roy had run a NER assay on gonadally intact (unoperated) F1 males from our lab to obtain normal levels of nuclear estrogen receptors. One week after the last behavioral test (26 weeks after surgery) males were sacrificed by decapitation, and the brains were removed rapidly and placed on ice in preparation for the NER assay. Estrogen receptors were measured in cell nuclei of the medial preoptic area of the hypothalamus by an exchange assay described by Roy and McEwen (1977).

Statistics

Data were analysed with the Students' t test.

Results

Nuclear estradiol receptor (NER) levels in the MPOAs of both groups of F1 castrates were significantly lower than the NER levels in the MPOAs of the sham operated F1s (Figure 7b), which were not different from the levels measured in the gonadally intact (unoperated) animals (not shown). NER levels of the two behaviorally different groups of F1 castrates did not differ significantly from each other or from zero. The sham operated F1 males were found to have 21.8 +/-2.8 fem. NER/mg DNA nuclear estradiol receptors in their MPOAs, and the unoperated mice had 21.4 +/- 2.1 fem. NER/mg DNA. In contrast, the castrate-continuers and castrate-noncontinuers had 2.34 +/- 0.92 fem. NER/mg DNA and 2.85 +/-1.2 fem. NER/mg DNA in their MPOAs, respectively.

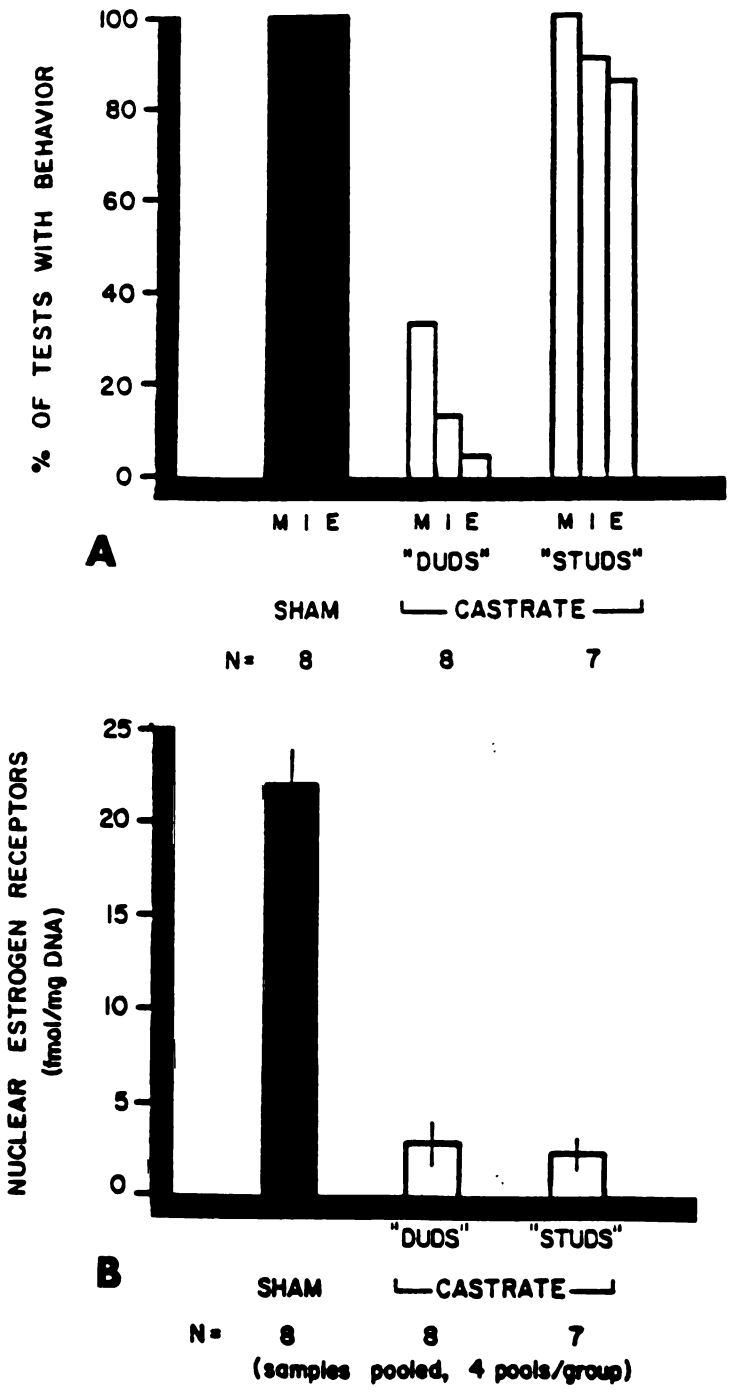



Figure 7. Comparison of behavior and nuclear estrogen receptor levels in B6D2F1 mice. A. Percent of tests during weeks 21-25 after castration or sham operation, in which males displayed mounts (m), intromission (i), or ejaculation (e), B. Nuclear estrogen receptor levels in the medial preoptic area of the hypothalamus in sham operated and castrated B6D2F1 mice, "STUDS" = continuers, "DUDS" = noncontinuers.

Discussion

Although the F1 castrates could be distinquished as those that continued to copulate and those that ceased copulating after castration, this behavioral difference was not associated with a difference in nuclear estradiol receptor levels in the MPOA. Based on these findings, we suggest that the behavioral differences were not due to differences in estrogenic stimulation of the MPOA. These data contrast with results from a study of NER levels in gonadally intact rats in which sexually nonresponsive males had significantly reduced levels in the MPOA compared to sexually responsive males (Clark et al., 1985).

The decision to measure estradiol receptors rather than receptors for testosterone or other metabolites was based on the following factors. First, estradiol has been suggested as the active metabolite of testosterone for facilitation of masculine sexual behavior (see literature review in Introduction and Experiment 4). Second, the radioimmunoassay (Experiment 2) would have detected any significant differences in testosterone levels that might exist among the groups of F1 castrates. Third, the study by Clark et al., (1985) suggested that behavioral differences may be explained by differences in estrogenic stimulation of the medial preoptic area which could be evaluated by measuring nuclear estradiol receptor levels.

# EXPERIMENT 4

## Hormone Replacement to Noncontinuers

### Introduction

McGill et al. (1976) found an inhibitory effect of TP in castrated B6D2F1 male house mice. An increase in ejaculation latency (EL) was observed that was proportional to the amount of hormone injected daily to both prepubertally and postpubertally castrated males. Increased ejaculation latencies also were observed in neonatally androgenized B6D2F1 females receiving TP therapy in adulthood. McGill (1978a) reported that castrated CDF1 males also exhibited longer ejaculation latencies in response to larger doses of TP, indicating that house mice respond differently to exogenously administered TP than do other rodents. In guinea pigs, androgen replacement restores sexual performance to precastration levels (Grunt and Young, 1952; 1953), but suprathreshold doses do not improve the behavior above precastration levels (Riss and Young, 1954). Lowered ejaculation latencies are produced with proportionately larger doses of testosterone propionate in castrated rats (Beach and Holz-Tucker, 1949) and with increasing doses of testosterone, androstenedione (AE), or dihydrotestosterone (DHT) in castrated hamsters (Whalen and DeBold, 1974).

In addition to the effects of TP, McGill (1978a) examined the response of B6D2F1 castrates to DHT, AE, and estradiol benzoate (EB) and found that increasing amounts of

DHT increased EL, while the opposite was true for AE and EB. These hormone treatments were given to males at the time of castration and maintained copulatory behaviors. However, hormone replacement has never been given to long-term castrated B6D2F1s, and, therefore, its role in restoring sex behaviors (which have ceased or have longer latencies in response to castration) has not been investigated. The objective of the following experiment was to examine the retention of sex behavior in a larger number of B6D2F1s and to evaluate the effects of hormone replacement in long-term castrates that no longer exhibited the ejaculatory reflex.

## Methods

### Subjects

Adult male B6D2F1 mice, housed and fed under the conditions described for experiment 1 were used in this experiment. After the initial testing of all F1 males (29 weeks after castration), only those castrates that did not continue to copulate after surgery (the noncontinuers) were given hormone replacement.

### Behavioral testing

Except for the testing schedule, the procedures used in this study were identical to those described in experiment 1. All males received four weekly precastration tests during the four weeks prior to castration or sham operation. One week after surgery, testing resumed and continued for 14

additional tests. These tests were spaced at 2 week intervals, except for the interval between tests 9 and 10 which were spaced 4 weeks apart, during which time the males had no sexual contact.

Six weeks after their last postcastration test (35 weeks after surgery), the noncontinuers were given one behavior test prior to hormone replacement, followed by four weekly tests during hormone replacement.

## Hormone Replacement

The noncontinuers received daily intramuscular injections of either testosterone propionate (20 ug), estradiol benzoate (5 ug), or sesame oil vehicle (.03 cc), beginning the morning after each male's pre-hormone replacement test and continuing for four weeks. After the fourth post-hormone replacement test, the animals were sacrificed, and seminal vesicle and body weights were measured.

## Statistics

The percent of animals showing mounts, intromissions, and ejaculations during the initial behavioral tests were compared using Mann-Whitney U for comparisons between sham operated and castrated males and Wilcoxon for comparisons of each group before and after surgery. The effects of hormone replacement (comparisons before and after treatment) on percent of animals showing each of the behaviors was analysed by Cochran's Q (nonparametric) test. Hormone

effects on response latencies were analysed by Friedman's two-way ANOVA (Siegel, 1956). Comparisons between the last two hormone replacement tests and the last two precastration tests were analysed by McNemar's test for significance of changes, for percent data, and by Wilcoxon matched pairs signed ranks test, for latency data. Effects of the specific hormones (ex. oil versus TP) on latencies were analysed by comparing the last two weeks of each hormone treatment, using Mann Whitney U. Seminal vesicle and body weights were analysed by Kruskal-Wallis and Mann-Whitney U.

## Results

The gonadally intact males continued to show the full pattern of sexual behavior (including mounts, intromissions, and ejaculations) throughout the initial behavior tests. Although castration reduced the percent of animals showing all three behaviors ($p < .001$; Appendix 4), some (approx. 20%) castrates continued to copulate when tested with a sexually receptive female 29 weeks after surgery. Some of these continuers ejaculated on all of their behavioral tests. In contrast, other castrate continuers may have shown decreased levels of copulatory behaviors initially after surgery, but they later "recovered" their ability to show the ejaculatory reflex later in the experiment.

Behavioral latencies from only those castrate continuers that continued to show the behaviors throughout the experiment (i.e. not the "recoverers) were analysed

statistically. Over the course of the postoperative testing, the continuer castrates showed elevated latencies to mount ($p < .03$), intromit ($p < .03$), and ejaculate ($p < .008$) (Figure 8; Appendix 5). Although mount and intromission latencies of the gonadally intact males remained constant over time, their ejaculation latencies also increased with time ($p < .008$).

Hormone replacement restored masculine copulatory behaviors to precastration levels. Both TP and EB significantly increased the percent of animals showing mounts, intromissions, and ejaculations when compared to pre-hormone replacement levels (Cochran's Q, $p < .001$; Figure 9). Treatment with the sesame oil vehicle did not significantly alter the percent of animals showing mounts or ejaculations, but did significantly increase the percent of animals achieving intromission (Cochran's Q, $p < .05$). However, none of the behaviors were restored to precastration levels (McNemar's, $p < .01$).

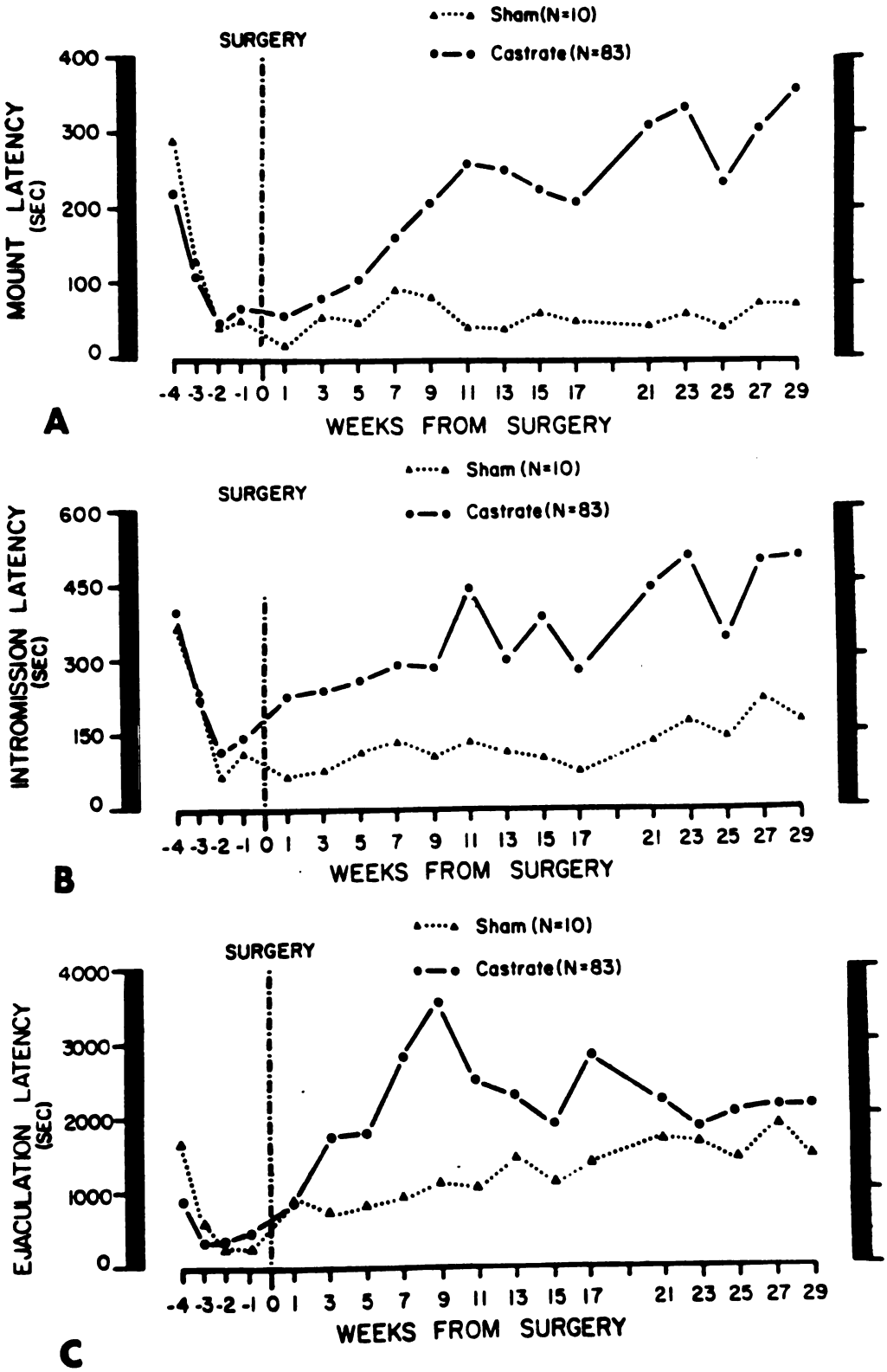
For the most part, hormone replacement reduced latencies from castration levels back to precastration levels (Figure 10). Comparison of values before and after hormone replacement revealed that TP significantly reduced ML, IL, and EL, and EB significantly reduced ML and EL (Friedman's, $p < .001$), but IL was only significant at $p < .1$. Latencies on the last two tests after TP treatment were not significantly different from latencies on the last two precastration tests. Similarly, after EB treatment, ML

and IL were not significantly different from precastration scores. However, after EB treatment, EL was significantly longer than precastration EL (Wilcoxon, $p < .001$). Mount and intromission latencies did not differ significantly between TP and EB treated animals, but, in the last two hormone replacement tests, EL was significantly longer after EB than after TP (Mann-Whitney U, $p < .002$).

Because oil treated animals failed to show much sex behavior after oil treatment, only mount latencies could be considered. ML was significantly reduced by oil treatment as compared with pre-oil treatment (Friedman's, $p < .001$). However, when the last two oil tests were compared to the last two tests after EB or TP, ML was significantly longer for oil treated versus EB or TP treated animals (Mann-Whitney U, $p < .002$). Similarly, oil treated animals showed MLs which were significantly longer than prior to castration (Wilcoxon, $p < .007$).

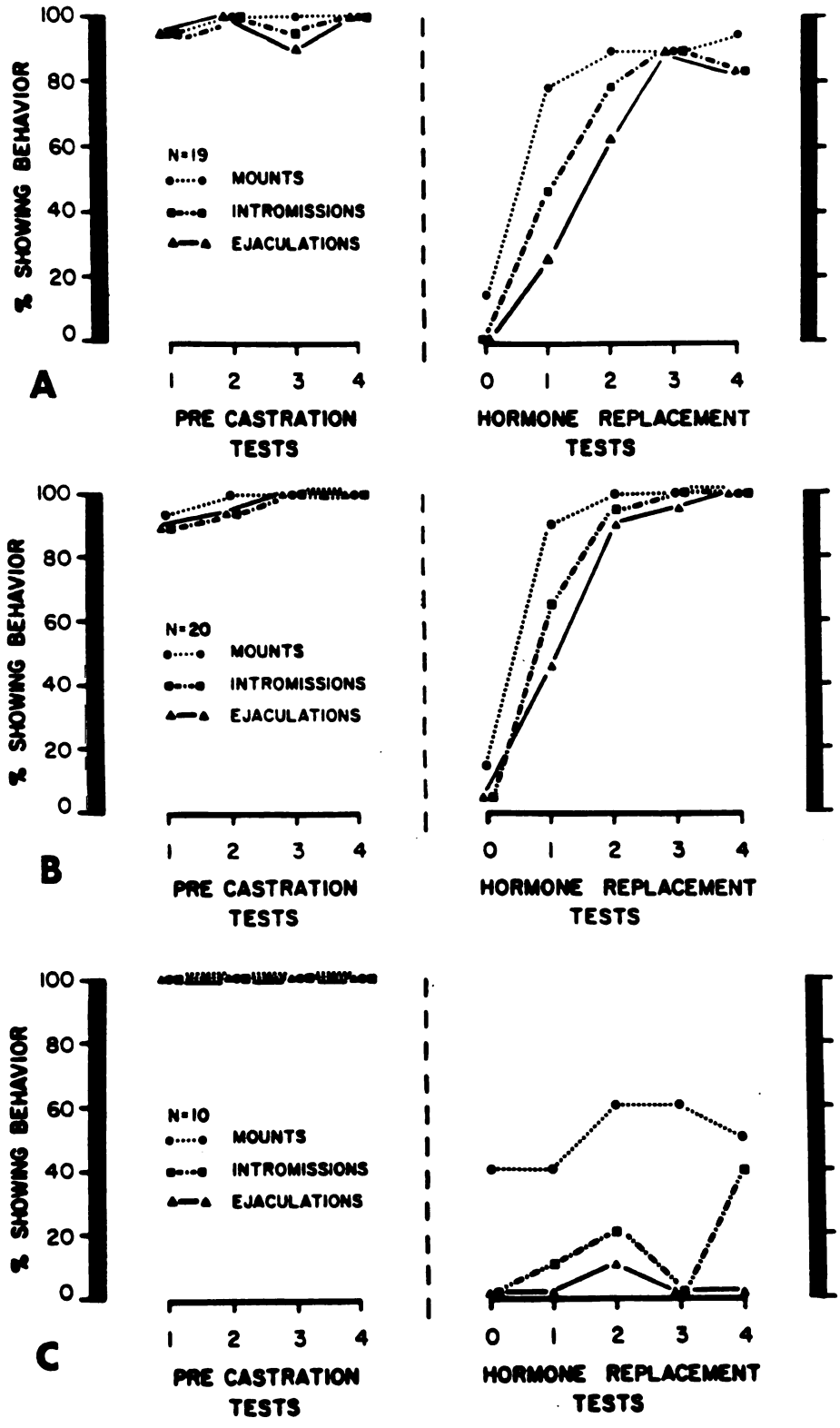
Seminal vesicle and body weights are presented in Table 1. Because the levels of significance were the same for actual weights as for seminal vesicle weights presented as a percentage of body weights, only actual weights are presented. Body weights did not differ significantly between the three treatment groups. The seminal vesicle weights of both the TP and EB treated animals were significantly different from the oil treated animals and the EB and TP animals were significantly different from each other.

Figure 8. Influence of castration or sham operation on behavioral latencies in B6D2F1 male mice. A. Mean mount latency, B. Mean intromission latency, C. Mean ejaculation latency.


Sham(N=10)
Castrate(N=83)
SURGERY
MOUNT LATENCY (SEC)
400
300
200
100
0
-4 -3 -2 -1 0 1 3 5 7 9 11 13 15 17 21 23 25 27 29
WEEKS FROM SURGERY
A
Sham (N=10)
Castrate(N=83)
SURGERY
INTROMISSION LATENCY (SEC)
600
450
300
150
0
-4 -3 -2 -1 0 1 3 5 7 9 11 13 15 17 21 23 25 27 29
WEEKS FROM SURGERY
B
Sham (N=10)
Castrate(N=83)
SURGERY
EJACULATION LATENCY (SEC)
4000
3000
2000
1000
0
-4 -3 -2 -1 0 1 3 5 7 9 11 13 15 17 21 23 25 27 29
WEEKS FROM SURGERY
C

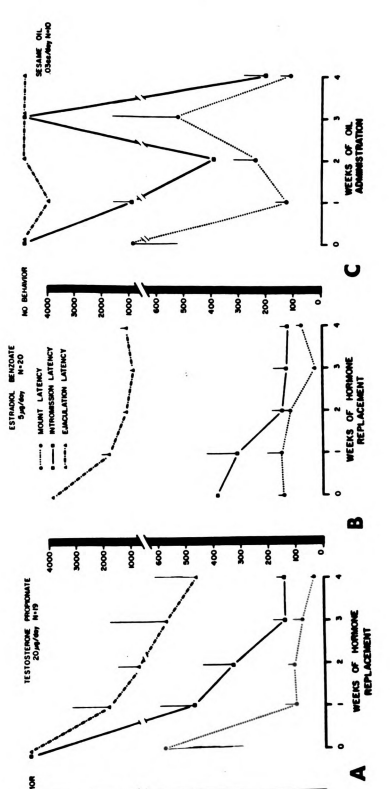
8

Figure 9. Effects of hormone replacement on percent of animals showing masculine sexual behaviors in B6D2F1 noncontinuer castrates. A. 20 ug/day testosterone propionate, B. 5 ug/day estradiol benzoate, C. .03 cc/day sesame oil vehicle


% SHOWING BEHAVIOR
100
80
60
40
20
0
N=19
MOUNTS
INTROMISSIONS
EJACULATIONS
A
1 2 3 4
PRE CASTRATION TESTS
0 1 2 3 4
HORMONE REPLACEMENT TESTS
% SHOWING BEHAVIOR
N=20
MOUNTS
INTROMISSIONS
EJACULATIONS
B
PRE CASTRATION TESTS
HORMONE REPLACEMENT TESTS
% SHOWING BEHAVIOR
N=10
MOUNTS
INTROMISSIONS
EJACULATIONS
C
PRE CASTRATION TESTS
HORMONE REPLACEMENT TESTS

9

Figure 10. Effects of hormone replacement on mean behavioral latencies in B6D2F1 noncontinuer castrates. A. 20 ug/day testosterone propionate, B. 5 ug/day estradiol benzoate, C. .03 cc/day sesame oil vehicle


LATENCY IN SECONDS
NO BEHAVIOR
4000
3000
2000
1000
600
500
400
300
200
100
0
TESTOSTERONE PROPIONATE
2mg/day N=9
ESTRADIOL BENZOATE
2mg/day N=20
MOUNT LATENCY
INTROMISSION LATENCY
EJACULATION LATENCY
SESAME OIL
0.5cc/day N=10
0
1
2
3
4
WEEKS OF HORMONE REPLACEMENT
WEEKS OF OIL ADMINISTRATION
A
B
C

10

TABLE 1. Seminal vesicle and body weights in castrated B6D2F1 mice following hormone replacement

| Treatment | Seminal Vesicle Wt. (mg)# | Body Wt. (g)# |
|---|---|---|
| TP | 193 (10.9) b, c | 37.1 (1.14) |
| EB | 20.7 (1.44) a, c | 34.0 (.890) |
| oil | 10.6 (.835) a, b | 35.0 (1.13) |

# Values given as mean (S. E. M.)
a = signif. diff. from TP-treated mice (MWU, $p < .001$)
b = signif. diff. from EB-treated mice (MWU, $p < .001$)
c = signif. diff. from oil-treated mice (MWU, $p < .001$)

## Discussion

Three conclusions can be drawn from this study. First, some F1 male mice continued to copulate when tested up to 29 weeks after castration. This finding confirms earlier reports of the long-term retention of copulatory behaviors after castration in these mice (McGill and Manning, 1976; experiment 1). As observed in experiment 1, two groups of F1 castrates, the continuers and noncontinuers, could be selected on the basis of their behavioral response to castration. Second, testosterone propionate restores copulatory behaviors in F1 males that did not continue to copulate after castration. Third, the conversion of testosterone to estradiol may be important in this restoration, because treatment with estradiol benzoate also restored sexual behavior to precastration levels. The

suggestion that TP inhibits masculine sexual behavior in castrated B6D2F1 mice (McGill et al., 1976) was not supported by the results from this experiment.

## EXPERIMENT 5

### Effects of Genotype on Spinal Cord Morphology

#### Introduction

The spinal nucleus of the bulbocavernosus (SNB), first described in the rat (Breedlove and Arnold, 1980), exhibits a sexual dimorphism between males and females. The purpose of this study was to compare the effects of castration and genotype on masculine sexual behavior with their effects on the number and size of SNB neurons.

#### Methods

##### Subjects

Spinal cords from the mice tested for masculine sexual behavior in experiment 1 were used in this experiment.

##### Tissue Preparation

At the end of the sex behavior tests (25 weeks after surgery), mice were sacrificed with an overdose of Metofane (methoxyflurane; Pittman-Moore, Inc.) and perfused intracardially with saline followed by neutral phosphate buffered 10% formalin. The spinal cords from approximately

spinal levels L4 to S1 were removed and postfixed with formalin and sectioned 60 um thick in the transverse plane with a freezing microtome. Alternate sections were mounted on gel-coated glass slides and stained with thionin. Sections from animals in all six treatment groups were stained simultaneously to avoid differences due to staining.

SNB neurons were distinquished by their large somata, prominent Nissl substance, and anatomical position (Figure 1, 11). In the mouse, the SNB is located approximately 100 - 350 um ventral to the central canal and within approximately 200 um from the midline. This region corresponds to the position of the SNB in the rat.

In all animals, all SNB neurons containing distinct nucleoli were counted in every second 60 um thick section. These neurons were traced through a camera lucida, and the areas of the cell bodies were measured using a digitizing pad and microcomputer. The raw counts of cells, taken from alternate sections, were doubled to obtain the correct number of SNB cells.

## Statistics

Data were analysed with One-way Analysis of Variance for comparisons among the six treatment groups (three strains, intacts and castrates of each strain), and data from the three groups of F1 castrates were analysed with Kruskal-Wallis and Mann-Whitney U nonparametric tests.

Results

The data for cell counts from all six treatment groups and the three groups of F1 castrates are presented in Table 2. In addition, data for the number of cells based on a sampling method and the Konigsmark (1970) correction are presented in histogram form (Figure 12a). As described above, the total counts of SNB neurons were adequate, and the sampling procedure and Konigsmark correction did not provide any additional insights. The results reported below are based on analyses of raw counts, but are similar to those obtained using the sampling and corrected counts.

For the gonadally intact males, the number of SNB cells varied among strains [$F(2, 17) = 4.33$, $p < .05$]. The DBAs had significantly fewer SNB neurons than either the C57s [$F(1, 11) = 4.97$, $p < .05$] or the F1s [$F(1, 12) = 8.48$, $p < .05$], which did not differ significantly from each other [$F(1, 11) = .099$, $p > .7$]. In contrast to the strain effect on the number of cells, neuronal area was not influenced by genotype [$F(2, 17) = 2.66$, $p > .08$] (Figure 12b). No significant differences were observed among castrates of each strain for either number of neurons [$F(2, 45) = 1.01$, $p > .3$] or somal area [$F(2, 45) = .953$, $p > .3$].

The effect of castration varied with genotype. Castration significantly reduced neuronal area in the F1 hybrid [$F(1, 16) = 37.7$, $p < .001$] and DBA (the paternal strain) [$F(1, 22) = 15.0$, $p < .001$], but not in the C57 [$F(1, 24) = 2.07$, $p > .1$], and significantly reduced the number of neurons in the F1 [$F(1, 16) = 6.16$, $p < .05$] and

C57 (the maternal strain) [$F(1, 24) = 5.61$, $p < .05$], but not in the DBA [$F(1, 22) = .045$, $p > .8$].

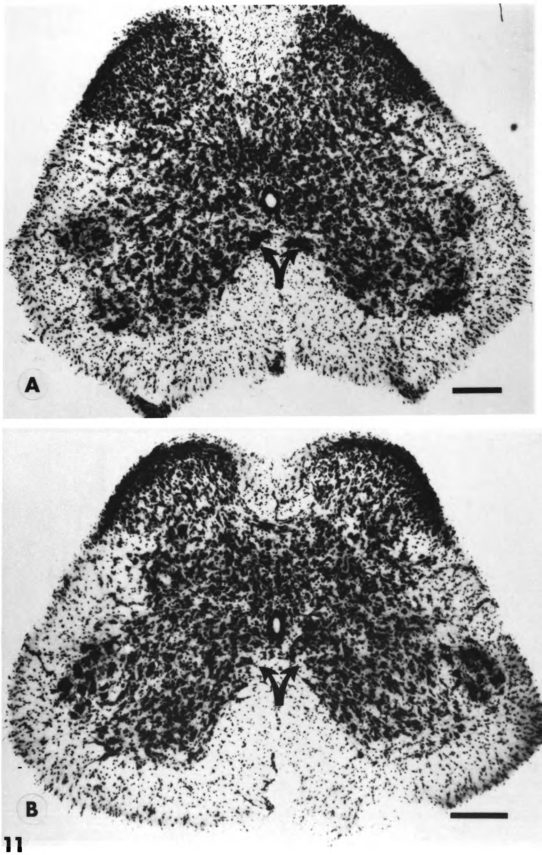
Neither somal area or neuronal number differed significantly among the three groups of F1 castrates (Table 2).

TABLE 2. Characteristics of the spinal nucleus of the bulbocavernosus in three strains of house mice #

| Treatment Group | N | Mean Somal Area square um | Mean Number of SNB cells |
|---|---|---|---|
| C57Bl/6J intact | 6 | 570 (31.9) | 113 (10.6) |
| C57Bl/6 castrate | 20 | 515 (17.2) | 92.4 (3.63) |
| DBA/2J intact | 7 | 668 (40.0) | 84.0 (7.93) |
| DBA/2J castrate | 17 | 530 (16.4) | 86.2 (5.93) |
| B6D2F1 intact | 7 | 653 (18.3) | 117 (8.16) |
| B6D2F1 castrate | 11 | 488 (17.8) | 96.4 (4.26) |
| continuers | 1 | 542 | 126 |
| intermediates | 7 | 469 (25.0) | 97.7 (3.66) |
| noncontinuers | 3 | 515 (13.5) | 83.3 (2.40) |

# Values given as mean (S. E. M.)

Figure 11. Cross sections of mouse spinal cord. Abbreviations as in Figure 1. Bar = 200 microns A. male, B. female.




11

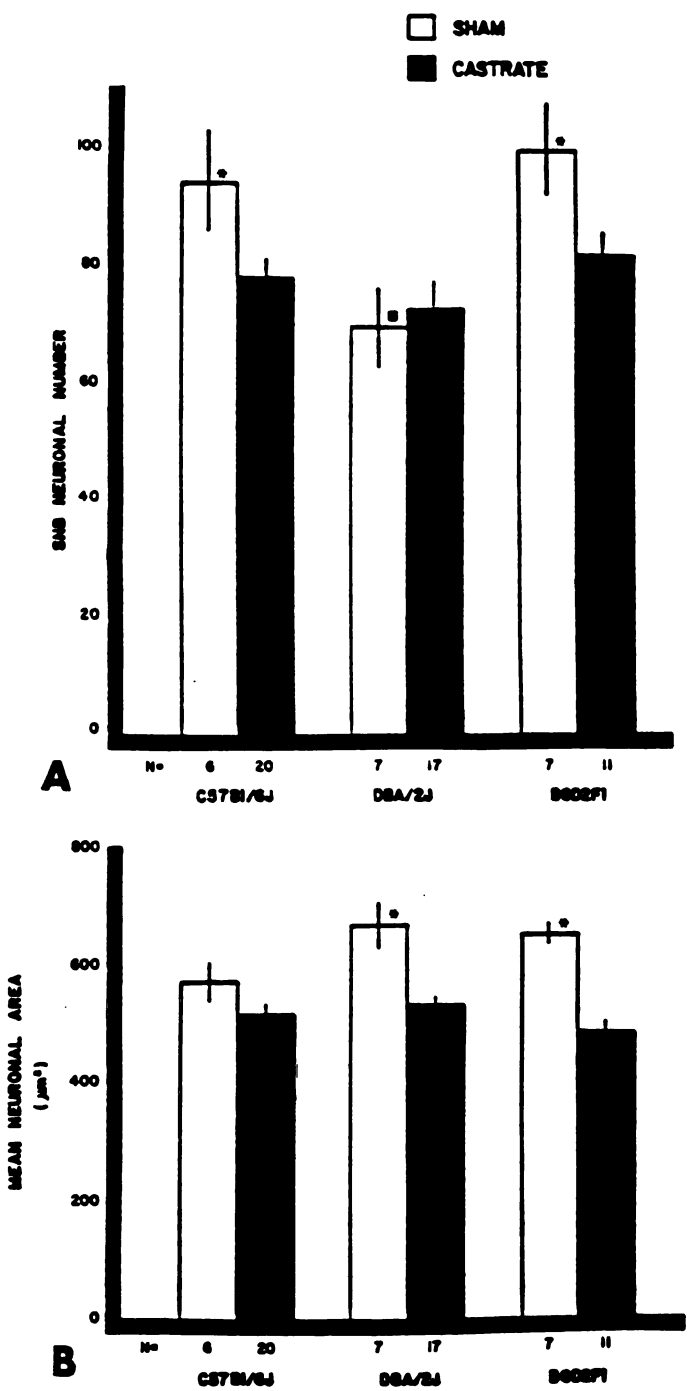



Figure 12. Influence of genotype and castration on characteristics of SNB. A. Number of SNB cells (calculated with Konigsmark formula), B. Mean somal area, * = significantly different from castrates of that strain, ■ = significantly different from C57Bl/6Js and B6D2F1s.

Discussion

Some of the observations in the house mouse are at variance with the findings in the rat. Although somal area was reduced by castration, Breedlove and Arnold (1981) reported that neither castration nor treatment with testosterone propionate in adulthood had a significant effect on the number of SNB neurons.

In the present study, darkly stained motoneurons were counted only if the nucleolus was seen within the nucleus. In addition, alternate 60 um sections were used. Thus, two sequential sections were 120 um apart. Nucleoli have diameters of approximately 1.5 to 2.0 um, and the mean diameter of the neuronal nuclei in these mice only ranged from approximately 10 - 15 um. Even if the nuclei of these motoneurons contained more than one nucleolus, the nucleus would not be observed in more than one section. Thus, the possibility that cells were counted twice (i. e. observed in two sections) was ruled out.

Breedlove and Arnold (1981) used a sampling method in which only 10 - 12 cells were drawn for each animal, and the mean diameter of the nuclei was used to calculate a value for the number of cells that corrects for the possibility of split nucleoli (Konigsmark, 1970). However, due to the relatively small number of SNB cells per animal in the present study, all cells were counted and drawn, and no sampling procedures were required to obtain the number of cells. Because alternate 60 um sections were used, no corrections for split nuclei were needed.

In the initial study of the SNB in the rat, Breedlove and Arnold (1980) injected horseradish peroxidase (HRP) into the target muscles and identified the SNB motoneurons by HRP label in the spinal cord. Subsequent studies with the rat and the present study did not employ this technique, because the SNB motoneurons could be identified on the basis of their location, size, and dark thionin staining; characteristics of the HRP labelled cells in the first report of the SNB. However, in the later studies, counting errors may have resulted from incorrect identification of SNB cells. The conclusions presented here, and for other studies that did not include HRP injections, are based on the assumption that SNB neurons of all treatment groups stain with equal intensity. In addition, the HRP-labelled SNB cells in the rat were the largest cells; probably alpha-motoneurons. The possibility that other motoneurons, such as the smaller gamma-motoneurons, are part of the SNB, but were not counted due to their small size and/or less dense staining cannot be excluded. However, because the same criteria for identifying cells as SNB neurons were used for all animals, the relative size and number of cells among all treatment groups should not be affected, even if the absolute values are inaccurate.

The lack of significance observed among the three groups of F1 castrates may have been due to the small number of animals used in two of the groups (Table 2). Although no significant differences were observed between the studs and

duds, the results presented in Table 2 suggest that larger sample sizes might result in significant differences between these two groups with respect to SNB cell number. Such group differences might influence the overall comparison between intact and castrated F1s. This comparison showed that castration reduced the size and number of SNB neurons (Table 2). In contrast, when these features were compared between the continuers and sham-operated males from experiment 4 (data not presented), castration significantly reduced cell size, but not cell number. Thus, the differences between intact and castrated F1s in the present experiment may be due to the fact that most of the F1 castrates, whose spinal cords were examined, were noncontinuers and intermediates, with fewer cells than the continuers.

The effects of castration on SNB morphology observed in the hybrid occurred in one, but not both of the parental strains. Therefore, the sensitivity to effects of castration on neuronal size appears to be inherited independent of that for the number of neurons. The observation of strain differences in the number of SNB cells further demonstrates the influence of genotype on SNB morphology.

## GENERAL DISCUSSION

The experiments described here investigated the role of genotype and gonadal hormones in the retention of copulatory behaviors following castration in the house mouse. Unlike the parental strains that lose sexual responsiveness soon after castration, up to 30% of B6D2F1 males retained the ejaculatory reflex for 25 and 29 weeks after castration. Levels of nuclear estrogen receptors (NER) in the medial preoptic area (MPOA) of the hypothalamus and plasma testosterone (T) were not significantly different between the B6D2F1 castrates that retained the ejaculatory reflex (continuers) and those castrates that did not (noncontinuers). Therefore, differential estrogenic stimulation of the MPOA, or presence of different amounts of testosterone do not appear to be mechanisms for continued copulatory behavior in males of the continuer castrate group.

Although the continuers displayed mounts, intromissions, and ejaculations long after hormone levels were significantly reduced, the noncontinuer F1s required gonadal hormones for the display of these behaviors. When treated with testosterone propionate or estradiol benzoate,

the noncontinuers showed an increase in masculine sexual behaviors and a decrease in the latencies to these responses. Thus, although B6D2F1 mice are genetically homogeneous, two behavioral phenotypes seem to exist among the castrates of this strain : one phenotype, the continuers exhibits the ejaculatory reflex in the absence of gonadal hormones; the other phenotype, the noncontinuers, requires testicular hormones for the display of masculine copulatory behaviors.

In addition to influences on the retention of sex behaviors after castration, genotype also affected anatomical characteristics of the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) of the spinal cord. In male mice, gonadally intact DBA/2Js (the paternal strain) had fewer SNB neurons than C57Bl/6Js (the maternal strain) or B6D2F1 (hybrid) males. Genotype also influenced the effect of castration on SNB characteristics : the number of neurons was reduced in castrated C57s and F1s; neuronal area was reduced in castrated DBAs and F1s.

Possible explanations for behavioral heterogeneity among F1 castrates

The results from experiments 2 and 3 showed that the noncontinuers and continuers, which differed in their behavioral response to castration, did not differ with respect to levels of plasma T or NERs in the MPOA. Although these findings rule out some explanations for the retention phenomenon eg. presence of alternate androgen sources,

additional stimulation of the MPOA, other explanations need to be considered.

The possibility that continuers are responding to residual steroid levels cannot be excluded. Although the radioimmunoassays showed that androgens are reduced by castration, these assays did not measure levels of aromatizable T metabolites. The possibility still exists that residual androgen or estradiol is acting on other areas of the brain, or even specific parts of the MPOA that were not detected by the NER assay.

Another explanation for the behavioral heterogeneity between continuers and noncontinuers is that the males of these two groups differed in their intrauterine location relative to their other siblings. Anatomical and behavioral differences have been observed in different groups of female rats (Clemens, 1974; Meisel and Ward, 1981) and mice (vom Saal and Bronson, 1978) depending upon their location <u>in utero</u> relative to male siblings. For example, females residing between two male fetuses (2M females) had longer anogenital distances (a masculine characteristic) and showed greater tendency for masculine behaviors (when administered androgens and tested as adults) than females residing between two females (0M females) or between one male and one female (1M females). Male CF-1 mice that developed <u>in utero</u> between two females (0M males) had higher concentrations of estradiol in their amniotic fluids, were more sexually active as adults, less aggressive, and had smaller seminal

vesicles than males that developed between two male siblings (2M males) (vom Saal et al., 1983). Thus, the early hormone environment can alter the adult phenotype in mice. Possibly the continuer males in the present study were 0M males and the noncontinuers were 2M males. The intermediate group of castrates, which do not retain or lose the ejaculatory reflex to the extent of either the continuers or noncontinuers, might represent males located between only one male and one female or in some other "intermediate" location.

The hypothesis that the retention of masculine sexual behavior after castration is related to intrauterine location has its limitations. The percent of castrates that are continuers, intermediates, or noncontinuers, is not consistent with the probability of having been an 0M, 1M, or 2M male. This probability depends upon a number of factors, such as number of offspring per litter and number of pups per uterine horn. Males that are not located at the cervical or ovarian end of the uterus, have one sibling on either side, and the probability of being located in the uterus next to a female is one-half; the probability of being located next to a male is one-half. If all males had a sibling on either side, such individuals would have a 25% probability of residing between two males (2M), a 25% probability of residing between two females (0M), and a 50% probability of residing between one sibling of each sex (1M). However, a male that is located at either end of the uterine horn has a sibling on only one side and, thus, has

no chance of residing between two siblings. Hypothetically, if the litter is composed of 10 offspring, (the average number of offspring per litter in mice), and if each uterine horn has 5 pups, one pup is located at the cervical end (.20 probability), another pup is located at the ovarian end (.20 probability), each next to only one pup, and three pups are in the middle (3 x .20 = .60 probability), with a pup on either side. Thus, the probability of residing in the middle and being located between 2 males is .60 x .25 = .15. Similarly, the probabilities of being 0M or 1M males are .15 (.60 x .25) and .30 (.60 x .50), respectively. If one considers that the number of pups per uterine horn is not always equal, and that litter size varies, then the probabilities of being an 0M, 1M, or 2M also will vary. Therefore, the hypothesis that retention of sex behavior after castration is related to intrauterine location, should be evaluated by determining the intrauterine location of males (by delivering them by Cesarean section) that are later tested for sex behavior after surgery in adulthood.

(An)other possible mechanism(s) responsible for prolonged retention of sexual behavior by the continuer castrates is(are) related to their retention of SNB cells after castration. As mentioned in the discussion for experiment 5, the small sample sizes prevented any solid conclusions about differences between continuers and noncontinuers with respect to characteristics of the SNB. If further study shows that the continuers have more SNB cells

than the noncontinuers, this would indicate that the number of SNB cells is less affected by castration in the continuers. As described in the literature review, the muscles innervated by the SNB motoneurons are associated with penile erections and cups involved in reproductive functions. One might speculate that retention of motoneurons, even with reduced somal areas, and the associated target musculature, enables the continuer castrates to retain the copulatory behaviors. Whether or not the continuers still show erections, as well as the physiological bases for retention of either the anatomical structures or the behaviors themselves remains to be elucidated.

To summarize, the behavioral heterogeneity observed in the F1 castrates may be due to previously undetected differences in amount of, or response to, gonadal hormones or their metabolites, intrauterine location, or differential changes in neural structure in response to castration.

Retention of sexual behavior after castration

In the present study, males of the parental strains lost the ejaculatory reflex soon after castration. These results are in agreement with those reported earlier (McGill and Manning, 1976). However, a major difference between this study and previous studies is the percent of F1 castrates that retain the ejaculatory reflex at the end of the experiment. In experiments 1 and 4 only 30-40% and 20-30% of the B6D2F1 castrates were still showing the reflex 25 and 29

weeks after castration. In comparison, McGill and Manning (1976) reported that 70% of the B6D2F1s were ejaculating 52 weeks after castration and Manning and Thompson (1976) and Thompson et al. (1976) reported that 80% of the F1 males were ejaculating 10 weeks (20 tests) after castration. This difference may be due to procedural differences. McGill and Manning tested their animals under normal room illumination in the animal's home cages. A preliminary study in our lab has shown that the display of the ejaculatory reflex is restored in some noncontinuers tested in their home cages.

The differences between the studies presented here and those reported in the literature also may be due to differences in the amount of behavioral testing that each group of animals received. In the present study the animals were tested only once every two weeks, in order to test a larger number of animals. In comparison, the animals in the other studies were tested twice per week. Experience may play a large role in determining the extent to which the ejaculatory reflex is retained after castration. While sexual experience before castration is not essential for maintenance of the ejaculatory reflex after castration, it does influence the frequency of the ejaculatory reflex (Manning and Thompson, 1976). Such findings, along with the relative lack of differential hormone activity in continuers and noncontinuers, supports the idea that the variability in behavior between the two groups has an environmental component to it.

Because phenotype is influenced by both genotype and the environment, and the genetic component is held constant due to the use of inbred strains, the procedural differences between the various studies may accentuate the environmental component. The differences in testing conditions might reflect differences due to response to pheromones. If an animal is tested in its home cage, his prior scent marking may influence his display of sexual behavior. The differences among studies that are related to frequency of testing may emphasize a greater dependence upon prior experience in some animals than in others.

In summary, one of the major findings of this study, that genotype influences the retention of sex behavior after castration, is consistent with other studies. The differences that exist between the present study and earlier work appear to result from procedural differences, such as testing conditions and testing schedule, which may accentuate influences by environmental factors.

Hormone replacement

The results from experiment 4 are in general agreement with the concept that testosterone replacement restores copulatory behaviors after castration. The finding that estradiol also restores these behaviors is consistent with the reports of estradiol treatment in other strains of house mice (Edwards and Burge, 1971; Wallis and Luttge, 1975; McGill, 1978a; Dalterio et al., 1979). However, the role of estradiol in restoring copulatory behaviors after castration

may not be universal since it is not important in restoring sex behavior in rabbits (Larsson, 1979; Luttge, 1979) and guinea pigs (Alsum and Goy, 1974), but it does appear to be important in other mammals such as deer mice (Clemens and Pomerantz, 1982), rats (Luttge, 1979; Södersten, 1979), and red deer (Fletcher and Short, 1974).

When given to house mice at the time of castration, TP sustained masculine copulatory behaviors in the CD2F1 hybrid strain (Champlin et al., 1963), and estradiol prevented an initial decline in copulatory behavior (the difficult period) following castration in B6D2F1 males (McGill, 1978a). Champlin et al. noted that unlike previous studies with guinea pigs and rats in which ejaculation latencies were shown to decrease with TP replacement, no such decrease was observed in the CD2F1s. In fact, when McGill and his coworkers extended the study by Champlin et al., they found that CD2F1 males exhibited longer ELs when given larger doses of TP (McGill, 1978a). The effect of TP replacement on ejaculation latency (EL) was further examined by McGill et al. (1976), who, using the B6D2F1 hybrid strain, reported that TP increased EL in neonatally androgenized females and both pre- and postpubertally castrated male mice. In addition, the effects of testosterone's metabolites on EL were examined in B6D2F1 mice (McGill, 1978a). As observed for TP, increasing doses of dihydrotestosterone increased EL. In contrast, McGill observed an inverse dose-response relationship for both

estradiol and androstenedione. Castrated mice that received lower doses of these estrogenic metabolites had higher ELs than castrates treated with higher amounts.

The results from experiment 4 do not support the conclusion that TP inhibits masculine sexual behavior in B6D2F1 mice (McGill et al., 1976). The discrepancy between the present findings and previous work may result from two procedural differences : dose of hormone used and time of hormone replacement. In the present study, all TP treated males received 20 ug/day, and their ELs were reduced to the levels observed prior to castration. This return to precastration levels also was observed for mount and intromission latencies. McGill and his coworkers reported a dose dependent increase in EL, with doses of 60 ug or more resulting in significantly longer ELs than those from the intact control group. In their studies, males treated with 20 ug/day did not differ from the intact males. Consequently, the increases in EL that McGill et al. observed may have resulted from the very large doses of TP used in their studies and may not reflect a physiological situation. In fact, doses of 50 or 100 ug TP were found to be hyperphysiological as measured by their effects on seminal vesicle weights in DBA/1Bg male mice (Shrenker et al., 1985).

The differences noted above between this study and the work by McGill and his coworkers also may have resulted from differences in the times at which the hormone was administered. In the present study, the animals that

received the hormone had already stopped showing the ejaculatory reflex. None of the treated animals showed ejaculatory responses on a regular basis at the time treatment was initiated. In contrast, previous studies employed a maintenance paradigm, in which the animals received hormone treatment starting at the time of castration. Consequently, a distinction between continuers and noncontinuers could not be made. Testosterone and its metabolites may have differential effects on continuers versus noncontinuers. Possibly TP exerted an inhibitory effect on the ELs of the continuers by some as yet undetermined mechanism. However, such an effect would not have been observed in experiment 4 because the continuers were not given hormone replacement. Because the proportion of continuers was often much greater than the proportion of noncontinuers in McGill's studies (see above), the effect of TP that decreases EL in the noncontinuers may have been masked by an inhibitory effect (increasing EL) of TP on the continuers.

In experiment 4, none of the latencies (ML, IL, or EL) measured after hormone replacement was reduced below those measured prior to castration. In other words, hormone replacement at the dosage used, after long-term castration did not improve the animals' sexual performance. Similarly, suprathreshold doses of androgen replacement did not improve copulatory behaviors in guinea pigs (Riss and Young, 1954) and rats (Larsson, 1966). In contrast, McGill et al. (1976)

described an unpublished observation that during weeks 17-26 the castrates ejaculated significantly faster than intact control males.

To summarize, both TP and EB restored masculine sexual behaviors in castrated F1s that did not retain the ejaculatory reflex. For the most part, both the percent of animals copulating and behavioral latencies were restored to preoperative levels. Discrepancies between these results and those reported previously probably result from procedural differences such as dose and time of hormone replacement.

SNB

In experiment 5, the SNB was examined in castrated and gonadally intact house mice from three inbred strains. The effect of genotype on SNB morphology was demonstrated by the fact that the number of neurons in the SNB differed between males of the two parental strains. If SNB cell number is a trait that exhibits no dominance/recessiveness, then one would expect the hybrid to have the same or similar number of neurons as the midparent, i.e. half way between the number of cells observed in the DBA and C57. However, in this study, the B6D2F1 hybrid had more neurons than would be expected for the midparent and more closely resembled the maternal strain. This effect of genotype on the number of cells was abolished by castration, as shown by the fact that castrates of each of the three strains did not differ from each other.

The reduction in the number of SNB neurons as a result

of castration suggests that these cells degenerated; thus, evidence of degeneration should be observed in the spinal cords of the castrates. However, these animals were sacrificed 25 weeks after surgery, by which time degenerated neural tissue would have been cleared from the system. Further evidence of castration-induced degeneration would be provided by electron microscopic examination of spinal cords within a few weeks after castration. Alternatively, the number of glial cells in the SNB could be compared between intact and castrated mice. If the number of glial cells in the SNB is greater in the castrates than in the intacts, indicating that the glial cells had taken over the spaces left by the degenerated SNB neurons, then one could conclude that the SNB neurons had degenerated in response to castration. However, because glial cells do not always proliferate in response to nerve degeneration, the lack of an increase in number of glial cells does not eliminate the possibility of neuronal degeneration.

The assumption that the staining intensity is the same for both intacts and castrates is tenuous. Thionin staining requires the presence of Nissl substance (ribosomes), which is associated with the metabolic activity of the cell. Thus, castrated animals, with reduced metabolic activity of SNB neurons, have fewer ribosomes, and, therefore, stain lighter than intacts, with more ribosomes. If the SNB neurons are identified on the basis of their size and dark stain, then some small, lightly stained SNB neurons may not have been

counted. Such inaccuracies in identification and counting would lead to false conclusions about reduction in the number of cells due to castration.

The number and size of SNB neurons appear to be inherited separately. Although genotype influenced neuronal number in the gonadally intact animals, no strain differences were observed for neuronal area. Further, the effect of castration varied among strains for these two measures. Neuronal area was reduced in the paternal strain (DBA), but not in the maternal strain (C57); neuronal number was reduced by castration in the maternal, but not paternal strain. The F1 hybrid inherited both effects of castration.

The idea that cell size and number are inherited independently is supported by three findings. First, these two characteristics are masculinized in the rat during different critical periods (Breedlove and Arnold, 1983b). The critical period of "masculinization" of cell number is complete by approximately postnatal day 5; somal area is "masculinized" in females treated with either TP or DHTP through postnatal day 7 - 11. Second, cross fostering of pups, exposed prenatally to propylene glycol or the anti-androgen, flutamide, altered SNB soma size, but not neuronal number (Breedlove and Arnold, 1983a). Third, in female rats, a single injection of TP increased the numbers of SNB neurons, but not their mean size (Breedlove et al., 1982).

In contrast to the results of experiment 5, Breedlove and Arnold (1981) reported that castration in adulthood reduced neuronal size, but had no effect on the numbers of

cells in rats. The differences between the two studies may reflect species differences. Because the number and size of cells appear to be inherited independently, the inheritance patterns of each of these characteristics may vary between the house mouse and the rat. In addition, species differences for neuronal size and number are noted in relation to the body size of the animals. For example, the SNB neurons in the mice examined in experiment 5 are significantly smaller and less abundant than the SNB neurons in rats from Breedlove and Arnold's studies, but are much more similar to the SNB neurons reported for the white-footed deer mouse (Peromyscus leucopus) (Forger and Breedlove, 1984). A positive log relationship between body size and number of cells was observed for neurons in the superior cervical ganglion (Purves et al., 1986) and with neuronal size and number in the supraoptic nuclei (Hatton et al., 1972). Thus, morphological characteristics in both the peripheral and central nervous system vary among species.

The finding that SNB neuronal area is influenced by the adult hormonal milieu indicates that the SNB is not a static entity. In other words, androgen-dependent, reversible anatomical plasticity (Kurz et al., 1986) is demonstrated by the finding that somal area and dendritic length are reduced by decreased levels of circulating androgen and are increased with androgen treatment (Breedlove and Arnold, 1981; Kurz et al., 1986). Several possible outcomes may result from, or cause, changes in neuronal morphology, and

these possibilities will be discussed below.

First, a reduction in the size of a neuron would result in an increase in input resistance and a decrease in the membrane capacitance, which affects the synaptic current. Because voltage (V) is influenced by both current (I) and resistance (R) (V = IR), the increased resistance with a constant current would produce a greater voltage change. This increase in efficacy of synaptic currents could lead to increased or decreased excitability, depending upon the nature of the inputs.

Second, the decrease in cell size may affect synaptic density. On one hand, the decrease in membrane surface area might result in increased synaptic density, if the same number of synaptic contacts are crowded into a smaller area of the membrane. Alternatively, because dendritic length also decreases in response to castration (Kurz et al., 1986), the synaptic density may actually be decreased if fewer terminals are making synaptic contact with the dendrites of SNB neurons. Furthermore, the decrease in cell size may not produce these changes in synaptic density, but rather may result from the reduction of synaptic contacts. In other words, neurons that are synapsing upon the SNB neurons might be affected by changes in the hormonal environment, and altered presynaptic input might result in altered neuronal size.

Changes in cell size also may reflect the amount of metabolic activity of the cell. For example, hypothalamic neurosecretory neurons increase in size when metabolic

activity increases due to dehydration or lactation (Hatton, 1985). The mechanism of steroid hormone action involves the binding of hormones to appropriate receptors that interact with the DNA to alter protein synthesis (O'Malley and Means, 1974; McEwen et al., 1979). Androgens bind to both SNB neurons and their target musculature (Breedlove and Arnold, 1980; see review by Breedlove, 1984a). Presumably the increased metabolic activity (i.e. protein synthesis that results from the androgen-receptor-DNA interaction) and accompanying changes in the amounts of ribosomes, polyribosomes, Golgi, etc. would result in increased cell size. Such increases in the size of SNB neurons have been observed in female rats treated with TP (Breedlove and Arnold, 1981). Conversely, the absence or reduction of circulating androgen with resultant decrease in metabolic activity presumably results in the reduction of somal area.

In addition to androgenic influences on SNB neurons, androgens also act on the target muscles. Castration reduced the muscle mass of BC, LA, and IC perineal muscles in adult rats, and TP prevented these effects of castration (Wainman and Shipounoff, 1941). Similar effects were observed in adult mice by Venable (1966a), who noted that the weight changes in the muscle were accounted for solely by changes in the size of the muscle fibers and not by alterations in the number of cell nuclei or the amount of DNA. Considerable evidence (see review by McComas, 1977) supports the idea that muscles exert a trophic influence on motoneurons.

Changes in the size of muscles influence the size of the motoneurons that innervate those target muscles (via feedback mechanisms). Thus, as described above for synaptic connectivity, cell size may not be the causative agent, but rather may be altered as a result of changes in the target musculature.

The perineal muscles are involved with penile reflexes which are important for male reproductive success (see INTRODUCTION). The frequency of these reflexes was decreased by castration and restored by testosterone replacement (Hart, 1967), and DHTP, but not EB, potentiated the reflexes (Hart, 1979). Androgenic facilitation of these penile reflexes may be at the level of the muscle or the SNB neurons. These possibilities were reviewed by Breedlove (1984a) who stated that the presence of androgen receptors on the muscle would permit potentiation of penile reflexes by androgenic action on the muscles themselves. However, Hart and Haugen (1968) demonstrated that local implants of testosterone into the spinal cord augmented the reflexes, but did not affect male accessory organs, indicating that androgen augments the reflexes by acting on the spinal cord directly, with little systemic action. The fact that the reflexes are sensitive to T and DHTP, but not EB, the same steroids which SNB neurons accumulate (Breedlove and Arnold, 1980; 1983c), supports the idea that androgen's action in the spinal cord is mediated by the swelling of SNB somas. Such swelling causes physiological or morphological changes which augment penile reflexes (Breedlove, 1984a).

To summarize, the adult hormonal milieu affects the size and dendritic length of SNB neurons, the mass of the target musculature, and the display of penile reflexes. Changes in cell size result in altered input resistance and, thus, altered voltage changes. These changes in somal area probably result from altered metabolic activity and may influence or be influenced by changes in synaptic connectivity and/or muscle mass. Any or all of these changes may account for changes in penile reflexes and associated copulatory behaviors. Finally, penile reflexes may be augmented by androgenic action on the muscles, SNB neurons, or both.

The preceding discussion has dealt with the effects of the adult hormonal milieu on cell size, but not on the number of cells. The implications of alterations in number of neurons in adulthood have not been examined previously, because, in the rat at least, the number of SNB neurons was thought to be determined by perinatal androgenic influences and not by hormonal changes in adulthood. In the present study castration reduced the number of cells in the C57 and F1 males, but not in the DBA males. The differential effects of castration for different strains of mice suggest that the dependence of SNB neurons upon gonadal androgen varies among strains. Although the number of cells was not reduced significantly in DBA castrates, these males, as well as C57 and F1 castrates, showed reduced levels of plasma testosterone (Figure 6). Furthermore, differences in the

number of neurons were observed among gonadally intact males of the three strains; yet these mice did not differ from each other in their levels of circulating testosterone. Thus, variations in the numbers of neurons can not be explained solely by variations in levels of circulating androgens. Rather, the sensitivity of neurons to fluctuations in androgen levels as occurred with castration, and would occur, for example, at puberty and senescence (Kurz et al., 1986) appears to be influenced, in part, by genotype. Because the SNB in the rat has only been examined in Sprague-Dawley rats obtained from Simonsen Labs, no effects of genotype have been reported previously. These rats resemble DBA mice, in that castration does not reduce the number of SNB neurons in either DBAs or Sprague-Dawleys. Examination of the effects of castration in other strains of rats might show genotypic influences on the number of SNB neurons.

The loss of cells after castration may result from or produce changes in synaptic connections and target musculature as described above for cell size. Such cell loss might result in reorganization of synaptic connections between surviving motoneurons and the perineal muscles. Although successful reorganization of these connections would permit the continued SNB-innervation of the target muscles, complete reorganization is unlikely. Thus, the loss of neurons, as well as the reduction in cell size, would contribute to reduced display of penile reflexes.

In summary, characteristics of the SNB are influenced

by androgens and genotype. The number and size of neurons may be inherited independently, a fact which might explain differences between the results of this study and those reported for the rat. Strain differences related to body size also may account for differences between studies. Changes in the size and number of SNB neurons in response to castration and hormone replacement are discussed in relation to changes in physiological and anatomical factors which alter the display of penile reflexes associated with copulation.

## SUMMARY AND CONCLUSIONS

The behavioral, physiological, and anatomical responses to castration, which vary among animal species, were examined in three strains of house mice (Mus musculus). In contrast to the rapid decline in masculine sexual behavior by castrated males of the two parental strains, C57Bl/6J and DBA/2J, up to 30% of the castrated B6D2F1 hybrid males retained the ejaculatory reflex for 25 and 29 weeks after surgery. The behavioral differences among strains could not be explained by differences in residual testosterone levels or by differences in anatomical characteristics of a sexually dimorphic spinal nucleus, the spinal nucleus of the bulbocavernosus (SNB).

Although B6D2F1 mice are genetically homogeneous, two behavioral phenotypes seem to exist among the castrates of this strain : one phenotype, the continuers, exhibited the ejaculatory reflex in the absence of gonadal steroids, as determined by thier retention of sex behaviors with reduced levels of plasma testosterone and nuclear estrogen receptors in the medial preoptic area of the hypothalamus; the other phenotype, the noncontinuers, required testicular hormones for the display of masculine copulatory behaviors. The

noncontinuers did not retain the ejaculatory reflex after castration, but treatment with either testosterone propionate or estradiol benzoate restored this response to the levels observed prior to castration.

The SNB was examined in the gonadally intact and castrated males that were tested for sexual behavior. Sham operated DBA males had significantly fewer SNB neurons than either the C57 or F1 males. Neuronal area was not influenced by genotype. No strain differences were observed for neuronal size or number among the castrates. The effect of castration varied with genotype. Castration reduced neuronal number in C57 and F1, but not DBA, males and neuronal size in DBA and F1, but not C57, males. No significant differences were observed for either neuronal area or number among the behaviorally heterogeneous groups of F1 castrates.

The results support the findings by McGill and Manning (1976) of genotypic influences on the retention of masculine sexual behavior after castration. Although some of the possible explanations for the observed behavioral differences among strains and among groups of F1 castrates have been ruled out by the experiments presented here, other factors such as experiential and prenatal influences still need to be examined.

# APPENDICES

**APPENDIX 1. Mean Percent of Tests With Behaviors in Male Mice Tested Before (Tests 2 - 6) (Pre) and After (Tests 1 -13) (Post) Surgery in Experiment 1. @**

A. Mean Percent of Tests with Mounts.

| | Sham | | Castrate | | |
|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | |
| C57Bl/6J | 91.4 (4.04) | 96.7 (3.3) | 93 (3.0) | 31.9 (5.21) | a,b |
| DBA/2J | 100 | 94.9 (2.87) | 100 | 27.6 (5.07) | a,b |
| B6D2F1 | 100 | 100 | 100 | 73.8 (6.47) | c |

B. Mean Percent of Tests with Intromissions.

| | Sham | | Castrate | | |
|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | |
| C57Bl/6J | 82.9 (9.20) | 91.2 (6.59) | 83 (4.42) d,e | 9.62 (2.16) | a,b |
| DBA/2J | 100 | 90.6 (4.93) | 100 | 17.8 (4.37) | a,b |
| B6D2F1 | 100 | 100 | 100 | 58.1 (6.91) | b |

C. Mean Percent of Tests with Ejaculations

| | Sham | | Castrate | | |
|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | |
| C57Bl/6J | 82.9 (9.20) | 90.1 (6.43) | 73 (5.48) a,d | 4.23 (1.18) | a,b |
| DBA/2J | 100 | 83.8 (6.83) | 99 (1.0) | 12.2 (4.45) | a,b |
| B6D2F1 | 100 | 100 | 100 | 49.0 (7.43) | b |

@ values given as mean percent (S.E.M.)
a = signif. less than F1 (Mann-Whitney U, $p < .001$)
b = signif. less than sham (Mann-Whitney U, $p < .001$)
c = signif. less than sham (Mann-Whitney U, $p < .01$)
d = signif. less than DBA (Mann-Whitney U, $p < .01$)
e = signif. less than F1 (Mann-Whitney U, $p < .01$)

APPENDIX 2. Mean Percent of Tests With Behaviors in B6D2F1 Male Mice Tested During Postsurgical Tests 1 - 7 (Post 1) and Postsurgical Tests 8 - 13 (Post 2) in Experiment 1. @

| | Sham | | Castrate | |
|---|---|---|---|---|
| | Post 1 | Post 2 | Post 1 | Post 2 |
| % M | 100 | 100 | 83.1 (6.21) a | 62.9 (7.83) b,c,d |
| % I | 100 | 100 | 69.5 (7.36) b,c | 44.7 (8.23) b,d,e |
| % E | 100 | 100 | 62.3 (7.55) b,c | 33.3 (8.84) b,d,e |

@ values given as mean percent (S.E.M.)
a = signif. less than precastration [see APPENDIX 1] (Wilcoxon, $p < .02$)
b = signif. less than precastration [see APPENDIX 1] (Wilcoxon, $p < .002$)
c = signif. less than sham (Mann-Whitney U, $p < .02$)
d = signif. less than post 1 (Wilcoxon, $p < .003$)
e = signif. less than sham (Mann-Whitney U, $p < .001$)

APPENDIX 3. Behavioral Latencies for B6D2F1 Males Tested During Presurgical Tests 2 - 6 (Pre), Postsurgical Tests 1-7 (Post 1), Postsurgical Tests 8 - 13 (Post 2), and All Postsurgical Tests (1 - 13) (All Post) in Experiment 1. @

A. Mean Mount Latencies.

| Group | Pre | Post 1 | Post 2 | All Post |
|---|---|---|---|---|
| sham | 27.5 (3.33) | 26.9 (3.65) | 31.1 (5.46) | 29.0 (4.02) |
| cast | 51.7 (13.9) | 93.6 (23.5) a | 145 (45.0) a | 97.4 (29.1) a |

B. Mean Intromission Latencies.

| Group | Pre | Post 1 | Post 2 | All Post |
|---|---|---|---|---|
| sham | 55.1 (6.67) | 55.6 (7.10) | 76.4 (16.0) | 66.0 (11.3) |
| cast | 98.6 (25.6) | 191 (41.3) a | 330 (115) a | 261 (75.3) a,# |

C. Mean Ejaculation Latencies.

| Group | Pre | Post 1 | Post 2 | All Post |
|---|---|---|---|---|
| sham | 741 (153) | 1068 (205) | 1024 (122) | 1046 (143) |
| cast | 616 (115) | 1820 (218) * | 2921 (1008) b,* | 2371 (470) a,* |

@ values given as mean latency in seconds (S.E.M.)
a = signif. longer than sham (Mann-Whitney U, $p < .008$)
b = signif. longer than sham (Mann-Whitney U, $p < .02$)
* = signif. longer than precastration (Wilcoxon, $p < .02$)
# = signif. longer than precastration (Wilcoxon, $p < .05$)

**APPENDIX 4. Mean Percent of Tests With Behaviors in B6D2F1 Male Mice Tested During Presurgical Tests 2 - 4 (Pre) and Postsurgical Tests 1 - 14 (Post) in Experiment 4. @**

| | Sham | | Castrate | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| % M | 100 | 97.9 (1.09) | 100 | 68.4 (2.64) a,b |
| % I | 100 | 96.4 (1.92) | 99.2 (.585) | 47.1 (3.05) a,b |
| % E | 100 | 95.7 (2.43) | 98.8 (.712) | 38.1 (2.79) a,b |

@ values given as mean percent (S.E.M.)
a = signif. less than sham post (Mann-Whitney U, $p < .001$)
b = signif. less than precastration (Wilcoxon, $p < .001$)

APPENDIX 5. Behavioral Latencies for B6D2F1 Males Tested During Presurgical Tests 2 - 4 (Pre), Postsurgical Tests 1-7 (Post 1), Postsurgical Tests 8 - 14 (Post 2), and All Postsurgical Tests (1 - 14) (All Post) in Experiment 4. @

A. Mean Mount Latencies.

| Group | Pre | Post 1 | Post 2 | All Post |
|---|---|---|---|---|
| sham | 74.6 (17.3) | 53.7 (7.25) | 59.0 (5.55) | 56.4 (5.31) |
| cast | 68.1 (32.3) | 89.8 (18.7) | 175 (25.1) a,#,+ | 132 (18.9) b,# |

B. Mean Intromission Latencies.

| Group | Pre | Post 1 | Post 2 | All Post |
|---|---|---|---|---|
| sham | 139 (29.3) | 104 (12.3) | 145 (18.4) | 125 (12.1) |
| cast | 139 (40.5) | 240 (35.3) a,# | 371 (48.7) a,#,+ | 306 (32.5) a,# |

C. Mean Ejaculation Latencies.

| Group | Pre | Post 1 * | Post 2 *,+ | All Post * |
|---|---|---|---|---|
| sham | 469 (91.4) | 1017 (182) | 1502 (157) | 1259 (162) |
| cast | 244 (34.4) | 1897 (181) a,* | 2101 (272) * | 1999 (199) b,* |

@ values given as mean latency in seconds (S.E.M.)
a = signif. longer than sham (Mann-Whitney U, $p < .009$)
b = signif. longer than sham (Mann-Whitney U, $p < .02$)
* = signif. longer than precastration (Wilcoxon, $p < .008$)
# = signif. longer than precastration (Wilcoxon, $p < .03$)
+ = signif. longer than post 1 (Wilcoxon, $p < .04$)

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