Effects of androgen treatment, neonatally and in adulthood, on adult sexual preference in house mice (Mus domesticus)

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Effects of androgen treatment, 
neonatally and in adulthood, 
on adult sexual preference 
in house mice (Mus domesticus)

by

Lynn E. Hanninen

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Abstract

This thesis examines the extent to which hormones are important in the development of adult sexual preference in house mice. Olfactory preferences are used to infer sexual preference. Ordinarily, males prefer to sniff female odors more than male odors and females prefer to sniff male odors more than female odors. My hypothesis is that testosterone administered neonatally will masculinize adult sexual preference profiles in female mice and that the lack of testosterone neonatally will demasculinize sexual preference profiles in male mice. Subsequently, adult administration of TP should activate behavior to demonstrate the organizational effect of perinatal TP.

The findings indicated that: 1) Male mouse sexual preference was affected by both neonatal and adult levels of testosterone. Neonatally castrated males did not prefer either male or female urinary odors. Subsequent administration of TP resulted in castrated males exhibiting female-typical preference profiles. 2) Female mice were relatively insensitive to the organizational effects of testosterone. Neonatally androgenized and non-androgenized females did not differ significantly in adult anogenital distances or in their olfactory preferences, indicating that neonatal TP failed to affect morphology and behavior.

I conclude that, testosterone affects neonatally castrated adult, male house mice in two ways: 1) its absence
acted to organizationally demasculinize sexual preference; and
2) its presence acted to activationally feminize sexual
preference. More work is necessary to draw conclusions about
the effects of androgen on sexual preferences in females.
Chapter 1: Literature Review

I. Introduction

In this thesis I will examine the extent to which hormones are important in the development of adult sexual preference in the house mice. Sexual preference is defined in terms of tendencies to engage in sexual behaviors with the same or opposite sex conspecific. Homosexuality typically results in the choice of an inappropriate partner, although in some animal models it can result in inappropriate gender-typical behaviors, and provides an important method for interpreting normal sexual preferences. Thus, before describing my research, I will define sexual preference in reference to homosexuality & heterosexuality. Next, I provide a review of sociobiological, psychodynamic and sociobiological theories of homosexuality (with a strong emphasis on human studies). In the third section of chapter 1, human and non-human physiological studies are described. I also discuss the methodology used in the physiological study of preference, and the hypothesis underlying this thesis.

The research is described in chapter 2. Chapter 3 presents the results of my study and chapter 4 concludes with a discussion of these results and suggestions for further research.
II. Measuring Homosexuality/Heterosexuality

A. Theoretical Definitions of Homosexuality

One of the problems with studying sexual preference and homosexuality is that many definitions of homosexuality exist. Theoretical definitions are based on differences that are known or are hypothesized to exist between homosexuals and heterosexuals. These differences can be grouped into three general categories: Behavioral, Psychological, and Physiological.

1. Behavioral

Homosexuality can be defined in humans and other animals as the tendency to engage in sexual behavior with a member of the same sex, where heterosexuality involves sex with a member of the opposite sex. The emphasis (of this definition) is on behavior; how the subject behaves.

Male and female sexual orientation (in humans and non-humans) has been assessed with respect to sex-typical behaviors. For example, heterosexual females typically exhibit behaviors that heterosexual males do not. If a male were to exhibit this behavior, he would be labeled homosexual, or if a female failed to exhibit the behavior, she would be labeled homosexual. The behavior could also be male-typical, rather than female-typical. In this case, a female who exhibited the male-typical behavior or a male who failed to exhibit it, would be labeled homosexual. Such sex-typical
behaviors are often associated with courtship and/or mating, but not in all cases.

In non-human subjects sexually dimorphic behaviors are generally more easily distinguished. Thus, homosexuality or ambisexuality, as Denniston (1980) refers to atypical sexual behaviors, can be ascribed to non-human animals not only when they appear to have a preference for the same sex but also when they display opposite sex behaviors.

An example might demonstrate the difference in this operational definition. Morris (1955, as cited in Denniston, 1980) observed that after several unsuccessful attempts at copulating, a male zebra finch will often display female-typical receptive behavior and then will be mounted by his female mate. Both the male and female are exhibiting atypical sexual behaviors but with the appropriate partner. In this case, the nature of the behaviors might be called homosexual, while the nature of the encounter is heterosexual.

Although the previous example is ambiguous, sexually dimorphic behaviors are often more clearcut. For example, male house mice (Mus domesticus) emit 70 kHz vocalizations (outside the range of human hearing) in response to the presentation of a female, female odors, or conditioned sexual odors (Byatt and Nyby, 1986; Nyby and Zakeski, 1980; Beach, 1975; Nyby, Whitney, Schmitz, and Dizinno, 1978). Females, in contrast, rarely emit this vocalization in response to males or their odors (Nyby, Dizinno and Whitney, 1977). These
vocalizations are thought to be sexually motivated (Nyby, 1983) and may serve to reduce female aggression toward males (Whitney, Coble, Stockton & Tilson, 1973) during courtship. A mouse might be described (under some circumstances) as fitting a homosexual profile if either a) a female emits 70 kHz vocalizations, or if b) a male fails to produce 70 kHz vocalizations.

In contrast, identifying sex-typical behavior in humans is difficult as male and female sexual behaviors are often indistinguishable. No evidence exists that homosexuals adopt opposite sex behaviors when engaged in a sexual encounter (Symons, 1979).

Some psychodynamic and some social theories designate incorrect gender identification as primary in determining adult human sexual orientation. Implicit in these theories is the notion that those who take on opposite sex roles will exhibit opposite sex behaviors (e.g. male homosexuals will act like heterosexual females). Very little evidence can be gathered to support this perspective.

On the other hand, Symons (1979) argues that homosexuality and heterosexuality, in humans, differ only in the preferred sexual object. Male and female behaviors, on the other hand, are different, regardless of sexual orientation. Symons predicts that a homosexual would approach a relationship in much the same manner as would a heterosexual; and that male approaches regardless of the
male's sexual orientation are different from female approaches. As evidence he notes similarities between male homosexual and heterosexual behaviors, and how they contrast to female homosexual and heterosexual behaviors. For instance, regardless of orientation, males are sexually excited by visual stimuli while females are not. Another difference is that males tend to have several partners, while females do not. When a choice exists between sex and love, males are more likely to choose sex, and women, love. Symons hypothesizes that a male is a male, and a female is a female, and that homosexuality is simply an indication of the flexibility of human behavior.

A fundamental question arises: is homosexuality best defined in terms of gender-typical behavior or in terms of partner preference? As previously noted, sex-typical behaviors are difficult to find in humans. Thus, I would not be comfortable applying animal models of sexual preference based on observed sex-typical behaviors, to human populations. Because I would like to develop an animal model, my research will look at partner preferences.

2. Psychological

Sexual orientation can also be described in terms of differences in attitudes, self-concepts, or cognitive constructs (Cass, 1984; Bell, Weinberg, and Hammersmith, 1981). In this case, individuals are considered homosexual if
they perceive themselves as homosexual, regardless of the
gender-typical behavior(s) exhibited or sex partner choice.

Some theorists argue that a distinction should be made
between the cognitive and behavioral components of sexual
preference (Cass, 1984). In addition, Whitam and Mathy (1986)
point out that homosexual persons and homosexual acts differ
in the extent to which they are affected by cultural norms.
In general culture affects overt behavior much more than
internal cognitive mores.

In fact, in certain cultures pre-adolescent and/or
prenuptial homosexuality is not only tolerated but is often
couraged. In Melanesian societies homosexual activity among
young men is the norm, perhaps, in part, because males and
females are isolated from one another for various reasons.
Homosexuality among men may be pursued simply as an
alternative to heterosexual activity (Davenport, 1965, 1977;
Dizinno, 1983), or for sexual experience. In this case, a
difference exists between the behavioral and cognitive aspects
of the act. The behavior is homosexual in nature but these
men would not be considered homosexual in preference. A
distinction is made between obligate (i.e. exhibiting
homosexual behavior under all circumstances) and facultative
(i.e. exhibiting homosexual behavior when a heterosexual
partner is unavailable) homosexuality.

In other cultures (e.g., U.S.A.), homosexual acts are
considered morally abhorrent, and in some states, illegal
(Wasserstrom, 1979). There is not as clear a distinction between the behavior and how it is perceived. But while legal or moral deterrents may decrease the number of homosexual acts and limit the development of a homosexual sub-culture, no evidence exists that the number of obligate homosexual persons is similarly affected (Whitam and Mathy, 1986). In fact, obligate homosexuals are found in all societies in relatively equal proportions. However, facultative homosexuality differs across cultures (Whitam and Mathy, 1986; Dizinno, 1983).

Given that both behavioral and cognitive views can in some cases fall short of adequately identifying homosexual individuals, it is not surprising that most recent human studies use both behavioral and cognitive measures of sexual orientation when screening potential subjects (Bell, Weinberg and Hammersmith, 1981; Whitam and Mathy, 1986).

3. Physiological

Physiological theories assert that homosexuals and heterosexuals are different as a result of differences in physiological function or structure. Homosexuality might be defined in terms of sexual dysfunction (Sakheim, Barlow, Beck, and Abrahamson, 1985), endocrinological irregularities (Meyer-Bahlberg, 1984), neuroanatomic abnormalities (Gladue, Green, & Hellman, 1984), or genetic inheritance (Heston and Shields, 1968 as cited in Futuyama and Risch, 1984), to name a few. Physiological theories do not deny that cultural and cognitive factors influence sexual preference. Instead, the emphasis is
on those physiological factors which may predispose an individual to assume a homosexual identity and/or to engage in homosexual behavior(s).

III. Theories of Homosexuality

I next present some rationale for 3 broad theoretical views of human sexual preference. In certain cases I use animal models to elucidate the following theories.

A. Sociobiological Theories of Homosexuality

Natural selection occurs within a species through the differential reproduction of individuals and, ultimately, of their genes, across generations. For example, some individuals of a given species possess characteristics which better enable them to reproduce than others. If the traits which made these individuals better reproducers are genetically based, their offspring will also possess the same characteristics. After many generations of differential reproduction (natural selection), the majority of individuals in a population would acquire the behavioral and/or physiological characteristics associated with reproductive success. Any genetically determined behavioral or physiological mechanism that contributes to reproductive success would be selected for by natural selection.

To produce offspring, a sexually reproducing organism must mate with a conspecific of the opposite sex. Any organism that wastes time and energy attempting to mate with individuals of another species or a conspecific of the same
sex would be "wasting" their genes (Mayr, 1966). Heterosexual conspecific mate choice is a behavioral mechanism which will contribute to reproductive success; and would be expected to evolve as a consequence of natural selection. By the same argument, natural selection would be expected to select against homosexuality.

Although exclusive homosexuality occurs infrequently, relative to heterosexuality, descriptions of homosexual behaviors are found in all species that have been studied (Wilson, E.O., 1975; Barash, 1982). This fact is intriguing, and a large body of research has attempted to determine the underlying mechanisms of sexual preference. Despite its seeming incompatibility, attempts have been made to reconcile homosexuality with sociobiological theory in a variety of ways.

1. Sociobiological Theories and Genetic Considerations

Five sociobiological theories which attempt to explain homosexuality as a product of natural selection are heterozygous advantage, kin selection, group selection, parental manipulation and inter-male competition.

Most sociobiological theories stipulate that homosexuality be genetically transmissible. Unfortunately, no conclusive evidence exists that homosexuality is under genetic control. The twin paradigm is a common method of study employed to assess the genetic influence on behavior in
humans. Monozygotic twin pairs are compared to dizygotic twin pairs. Monozygotic twins are genetically identical while dizygotic are no more genetically similar than any two siblings. Consequently, a genetically influenced behavior should occur more often between individuals of a monozygotic twin pair than between individuals of a dizygotic twin pair.

There is a conspicuous lack of twin studies on investigating the genetic influence on human homosexuality. Kallmann (1958) reported a 100% concordance rate for homosexuality between twins of monozygotic pairs. A 100% concordance rate is suspiciously high and has been questioned by several investigators (Futayama & Risch, 1984). Bailey & Pillard (1991) reported a 52% concordance rate for homosexuality between 56 pairs of male monozygotic twins and Bailey, et al. (1993) reported a 48% concordance rate in 71 pairs of female monozygotic twins. Dizygotic twins in both studies exhibited a significantly lower concordance rate. (Interestingly, dizygotic twins had a significantly higher concordance rate than non-twin pairs of siblings. We would predict no difference since dizygotic twins are no more genetically similar than non-twin siblings. Perhaps this difference reflects how twins are treated differently from non-twin siblings.) Of 11 other studies, N never exceeds 6, thus their results remain questionable, although taken together they suggest a genetic basis for homosexuality.
Adding up the results any number of ways (e.g., not factoring in studies where N = 1, and/or not including Kallman's results, etc.), shows that the concordance rate for homosexuality between twin males of monozygotic pairs lies in the 50% - 70% range. This range differs significantly from the range found for dizygotic twins.

The most recent twin studies comparing monozygous and dizygous twins, suggests that a genetic component influences sexual preference (Bailey & Pillard, 1991; Bailey & Benishay, 1993; Bailey, Pillard, Neale & Agyei, 1993; see King & McDonald, 1992, for a dissenting twin study). But researchers have yet to isolate an individual gene or cluster of genes responsible for sexual preference.

Unfortunately, the previous findings are confounded by all twin pairs being raised together. Some researchers argue that since monozygotic pairs are more alike than dizygotic pairs, the monozygotic pairs are more likely to be subject to similar environmental effects. Consequently, monozygotic twins are more likely to develop similar adult behaviors than are dizygotic twins. On the other hand, it can be argued that monozygotic twins seek to individualize themselves by seeking out different environments, and developing different adult behaviors. The choice is idiosyncratic and may account for a small portion of the concordance rate, but a large concordance rate for homosexuality between twins of monozygotic pairs, as compared to dizygotic twins, would seem to suggest a strong
genetic component of human homosexuality. In any case, the relative importance of genes versus environment has yet to be definitively determined.

Keep in mind that these studies apply mostly to males. In addition, to date there exists no evidence at the molecular level that a specific gene or cluster of genes regulate the expression of sexual preference in humans or other animals.

a. Heterozygous Advantage

Heterozygous advantage occurs when an individual possesses two different alleles at the same locus (e.g. one gene for homosexuality and another for heterosexuality), whose combination confers some reproductive advantage. Individuals who are homozygous for heterosexuality might not be as reproductively fit as their heterozygous counterparts. Homosexuality would be simply a side effect of selection for heterozygosity. The gene for homosexuality would be maintained in the population because the reproductively successful heterozygous individuals will produce offspring that would be homozygous for homosexuality at about the same rate that they would produce offspring which would be homozygous for heterosexuality. Reproductive advantage is greatest if the aberrant gene occurs infrequently; natural selection will select against a frequently occurring maladaptive gene.

There are two drawbacks to the heterozygous advantage theory. First, while heterozygous advantage is possible, only
one well documented case of the phenomenon exists (i.e. sickle-cell anemia), suggesting that this phenomenon is rarely found in nature. Sexual behavior is complicated, in terms of its sensory, integrative and motor aspects, and undoubtably reflects the expression of multiple loci. Heterozygous advantage is a single locus phenomenon.

b. Kin Selection

Natural selection is now known to affect populations through more than just affecting individuals. Kin selection can occur. In kin selection natural selection selects family member characteristics rather than a single individual. Some of an individual's genes are represented in his/her relatives. Therefore an evolutionary advantage accrues to individuals who aid kin.

According to Barash (1982): The sum of an individual's fitness as measured by personal reproductive success and that of relatives, with those relatives devalued in proportion to their genetic distance (i.e. as they share fewer genes). Inclusive fitness is the accumulated consequences of kin selection for an individual.

Inclusive fitness increases for a homosexual individual if he/she uses the time and energy saved by not reproducing to help his/her family to produce more and healthier offspring. According to this reasoning homosexuality is selected through kin selection because homosexual individuals provide a valuable service to their kin. For homosexuality to evolve,
kin selection would have to operate in opposition to individual selection.

E.O. Wilson (1975) contends that homosexuals may carry altruistic genes which facilitate social organization. Thus we might expect homosexuals to have special talents which would assist them in assuming altruistic roles. Evidence for this theory can be found in that homosexual men score higher than heterosexual men on I.Q. tests. Wilson also suggests that homosexual men are more likely to seek out professional careers, are more successful in their careers, and are more upwardly mobile.

Kin selection would also predict that homosexuals would be more likely to choose professions that would confer social advantages on their families. For instance, in several North American Indian tribes male "berdaches" (i.e. very often homosexual) dress as women and perform chores primarily reserved for women and are shunned. At the same time, they are often tribal medicine men and are thus considered sacred and wield considerable power (Roscoe, 1987).

On the other hand, Futayama and Risch (1984) argue that several cultures exist in which: 1) homosexual males do not hold positions of power, and, 2) even if a homosexual male is privileged, there is no indication that economic gain leads to increased reproductive fitness of kin.

Futayama and Risch (1984) imply in the first part of their conclusion that homosexuality can occur in the absence
of "privileged" homosexuals. Even in the absence of holding a powerful position in society, the homosexual can help his/her kin by caring for the children of relatives, thereby allowing the parents to spend more time finding necessary resources. They make a valid point. In the second part of their conclusion they state that no obvious link exists between economic advantage and increased fitness. I would suggest that economic advantage implies increased access to those resources necessary for survival and reproduction.

Futuyama and Risch (1984) also point out that kin selection does not adequately account for female homosexuality, since females are rarely allowed to assume prominent positions in society or easy access to resources. But if merely helping one's relatives to raise children is sufficient to overcome the disadvantage of not reproducing, then female homosexuality could be explained in terms of kin selection. However, I currently know of no research assessing if altruism toward kin compensates for lack of reproduction in homosexuals.

c. Group Selection

Group selection theory asserts that natural selection will exert its influence at the group level if the absence of reproduction in certain individuals contributes to the increased fitness of the group regardless of the genetic relatedness of the group. Suppressed reproduction in
homosexuals may present them with increased time and energy to help increase group reproduction.

It is important to understand that individual selection pressures are almost always stronger than group selection pressures. Thus, individual selection for heterosexuality would almost always work against group selection for homosexuality (Barash, 1982). Since group selection can evolve only under very limited conditions, this theory is unlikely to explain the prevalence of homosexuality across so many different cultures.

d. Parental Manipulation

Earlier, mention was made that homosexuality can be maintained in the population through kin selection (assuming it is under genetic control). Parents might also influence some of their children to adopt a homosexual lifestyle in the hope that these children will help rear other siblings. (The influence could be conscious or unconscious. Natural selection will elect to pursue some biologically advantageous course whether or not it occurs is consciously or unconsciously. Selection acts on consequences, not on how the consequences arise.)

One method of parental manipulation would be by encouraging males to enter traditionally "feminine" professions, associated with homosexuality, which might confer advantage on his family. Similarly, certain females might be encouraged to assume traditionally "masculine" professions,
associated with homosexuality, that might confer advantage on her family. Assuming such professions exist (a debatable point), the parental manipulation argument implies that assuming certain roles leads to assuming certain sexual preferences, assumedly through improper gender identification.

But assuming that parental manipulation can affect the development of sexual preference, two predictions should follow. First, parents would be more likely to attempt to influence their last child's sexual orientation, since the parents' genes are already represented in their other children. Second, the later a couple waits to have their first child the less likely the parents will be to try to manipulate that child's sexual orientation; since an older couple is less likely to produce enough children to offset the cost of having one homosexual child. No relevant research exists for or against these predictions.

Additionally, in another section evidence is presented that improper gender identification is not a causal factor in the development of homosexuality. Thus, little evidence exists that parental manipulation, in general, exerts much influence on sexual orientation (Bell, Weinberg and Hammersmith, 1981).

e. Inter-male Competition

Other research has assessed the interaction between evolutionary and environmental pressures. Dizinno (1983)
hypothesized that most males have the capacity for facultative homosexuality and that it emerges only under certain conditions. He noted that in social groups where males must compete for females, some males will be unsuccessful. Such failure, however, does not result in diminished sex drives (since at some later point they may gain access to mates). Dizinno (1983) suggested that to gain experience, or to maintain the libido, these males engage in homosexual behaviors. According to this argument, natural selection has selected for males who have the potential for heterosexual preference under normal conditions and homosexual preference when there are no appropriate sex partners (facultative homosexuality).

Since access to mates is often based upon intermale competition, Dizinno predicted that lowered availability of the resources necessary to make one competitive, would be correlated with an increased incidence of homosexuality.

For instance, in non-human animals, polygynous mating systems usually evolve because males compete for dominion over a few territories containing vital resources. Those males which control the resources are sought out by females because these "dominant" males can better provide for the female and her offspring. Since one male monopolizes the resources, several females in his territory mate with him. Those males who lose in the competition are generally left without mates until they can acquire one of the sought after territories.
In addition, Dizinno theorized, if a male lives in a culture where acquiring resources (i.e. "competing" for them) is a prerequisite to marriage, some males may do better to postpone marriage until they have the necessary resources. And no matter how old the male, he would choose the youngest, reproductively capable bride possible because the younger the bride, the more reproductive potential she possesses. Thus, intermale competition for resources should lead to large age differences between males and females marrying for the first time.

One consequence of delaying marriage is that some males experience self-induced mate denial for extended periods of time. Once again, we might predict an increase in the number of homosexual males in these cultures. Putting it all together, one of Dizinno's predictions was that male homosexuality would be more prevalent in societies where the average age difference between a man and his wife was greatest.

Dizinno evaluated correlations between male homosexuality and various factors, across sixty cultures. Seven predictions received some support and led to the following conclusions. Homosexual behavior appears to be related to: 1) the degree of polygyny across cultures; 2) the importance of resource holdings; 3) the degree of male competition; 4 & 5) differences in marriage ages of males and females; 6) the status of the man involved; or 7) the degree of permanance of
the homosexuality's expression. Two predictions did not receive support and led to the following conclusions. Homosexual behavior was not positively related to the 1) degree of stature dimorphism; or 2) degree of sex-role specialization of tasks. While inter-male competition may be the cause of some facultative homosexual behaviors, Dizinno's results do not establish that inter-male competition is the cause of obligate homosexuality. In all probability, inter-male competition can account for only part of the cause and/or maintenance of male homosexuality in general.

2. Conclusions

One of the drawbacks to sociobiological theories is that they usually focus on male homosexuality. Female homosexuality seems more difficult to explain (perhaps because there is less genetic evidence). This distinction might suggest that male and female homosexuality have different underlying mechanisms of action, as Symons (1979) seems to imply. Nevertheless, both types of homosexuality are undoubtedly the result of the interaction of physiological, environmental, and sociobiological factors.

A second drawback is that most theories, except heterozygous advantage (the least likely possibility) lack generality. At best, these theories have only limited explanatory power and cannot account for both obligatory and facultative homosexuality in both sexes.
B. Psychodynamic Theories of Homosexuality

In general psychodynamic theories are concerned with how family dynamics can affect the development and/or maintenance of human homosexuality.

1. Theory

Psychodynamic theories emphasize parental traits and/or parent-child relationships as causal factors in the development of homosexuality. An adult male homosexual is thought to be a product of a family where the mother is dominant and the father submissive. If a young boy inadvertently identifies with his domineering, over-protective mother, he models himself as a woman, thereby developing a sexual preference for men. An adult female homosexual identifies with her father, in an attempt to take her mother's place as the center of her father's attention. The girl thinks that being like her father will make them closer. Consequently, the girl acquires her father's preference for women. Both the boy and girl are said to suffer from improper gender identification.

2. Conclusions

Perhaps the biggest drawback to psychodynamic theories is their subjectivity (relative to other theories). What exactly is a domineering mother? While you can quantify this characteristic, one wonders about its scientific validity.

Part of any good theory is its predictive power. In general, psychodynamic theories are less predictive than other
theories. Their strength lies in clinical application. But if they are based on nongeneralizable data, clinical treatment may be inappropriate.

C. Sociological Theories of Homosexuality

Sociological theories are interested in determining how social relationships affect sexual preference. This approach suggests that social isolation and/or poor peer relationships may result in homosexuality.

1. Theory

Sociologists propose that those children who do not interact well with others lack the social skills necessary for forming opposite sex relationships. Another such theory suggests that preadolescent labeling of a child as homosexual will lead that child to adopt a homosexual role in adulthood. Early atypical sexual experience involving the opposite sex has also been hypothesized to lead to homosexuality. Examples of atypical sexual experience include (but are not limited to) rape; parental punishment for childhood, heterosexual play; or seduction by an older child or adult (all theories reviewed in Bell, Weinberg, and Hammersmith, 1981).

2. Conclusions

Bell, Weinberg, and Hammersmith (1981) conducted an extensive study comparing 979 exclusively homosexual individuals and 477 exclusively heterosexual individuals. Factors compared include (but are not limited to) parental traits, parental relationships, parent-child relationships,
sibling identification, gender conformity, age of onset of sexual activity, and attitudes toward early homosexual and heterosexual activity. Using path analysis the authors found that no single developmental path could account for the occurrence of homosexuality. While the authors do not deny that social pressures can influence sexuality, they conclude that "homosexuality is a pattern of feelings...that cannot be traced back to a single social or psychological root."

D. Overall conclusions

We have seen that theories of homosexuality abound but cannot adequately account for all instances of homosexuality. Two of the difficulties in researching homosexuality is that the theories don't generalize across different cultures and environments and many don't apply to females. Recently, scientists have started moving away from the medical model, which sees homosexuality as a curable disease, to a biological model, which proposes that homosexuality is controlled by irreversible, physiologically controlled mechanisms. The biological model tends to be more generalizable across different cultures and environments and better at explaining female homosexuality. Research driven by this model has taken many directions but before enumerating them, it might be helpful to see how the topic is approached.
IV. Physiological Theories of Homosexuality

A. Introduction

The rest of this literature review is mainly concerned with investigating the physiological factors involved in sexual preference, and thus is apropos to my own research. All studies use non-human subjects, unless otherwise noted.

Physiological theories have taken several paths, as previously mentioned. I will review a few pertinent theories.

B. Physiological Research

1. Dysfunction of Behavioral Isolating Mechanisms/Recognition (e.g. Anosmia in Animals)

In rats, olfactory bulbectomy virtually eliminates male copulatory behavior and the usual preference for estrous vs non-estrous females (Kondo, Shimoda, Yamanouchi & Arai, 1990; Arai, 1984; Arai & Gorski, 1968). The authors suggest that odor recognition may be crucial to rodent sexual behavior. Perhaps the animal simply cannot recognize females as conspecífics or sexual motivation is initiated by the perception of certain odors.

Odor perception clearly is an important factor in rat (and other rodents') sexual behavior. Humans, on the other hand, are relatively poor odor perceivers. Laing & Francis (1989) found that human subjects cannot reliably identify common odors. Humans could not isolate odors if more than 3 distinct substances were mixed. The authors point out that
for a species, such as humans, which is bombarded with odors, it is surprising that the olfactory system is not more discerning.

This finding would suggest that olfaction is an historically primitive form of communication. As humans developed other forms of communication, olfactory functions became less important for survival, and failed to be maintained in humans. In other animals, olfaction remained important and continued to evolve.

2. The Organizational-Activational Hypothesis

Most physiological theories agree that endogenous hormones are involved in sexuality. In contrast, early investigators rejected the idea of hormones predisposing an individual toward sex-typical behaviors because hormone manipulation in adulthood affected only the amount of sex-typical behavior but not the type of sex-typical behavior. Phoenix, Goy, Gerall, & Young (1959) were the first to formulate a developmental theory of hormonal action on behavior: the "organizational-activational" theory. This theory stipulates that perinatal hormones can "organize" or differentiate the neural tissue mediating sexual behavior and that such tissue can be later hormonally activated. The theory further contends that hormones exert their organizational effects early in life during critical periods.

The original conception of the organizational-activational hypothesis specified that perinatal hormones
permanently organized CNS tissue into sexually dimorphic neural systems; a male brain or a female brain. In adulthood these neural systems mediate sex-typical behaviors. Phoenix, Goy, Gerall, and Young (1959) demonstrated that prenatal androgen exposure "masculinized" and "defeminized" female guinea pigs. In other words, these females as adults were less likely to exhibit "feminine" responses to E (estrogen) + P (progesterone) injections and were more likely to exhibit masculine responses to injections of T (testosterone) as compared to control females. Adult, male siblings showed little or no effect of perinatal masculinization.

Perinatal androgen exposure also masculinizes non-neural morphology (e.g. ano-genital distance). Interestingly, smaller doses of androgen were needed to masculinize behavior than morphology. Masculinization of morphology is used as standard check for the behavioral effectiveness of an androgen.

Numerous studies following the Phoenix, et al. (1959) paper demonstrated comparable findings in a variety of vertebrates and across several behaviors (Goy and McEwen, 1980 provides numerous references to such studies).

Since 1959, the hypothesis has been refined, although it's relevancy remains current. Arnold & Breedlove (1985) specify criteria which have been invoked to determine whether an effect is activational or organizational. They are:
1. Permanency - organizational effects can last for weeks months or years; activational effects are usually measured in hours or days.

2. Age at which it occurs - organizational effects occur perinatally and activational effects occur usually in adulthood.

3. There is a critical or sensitive period during which organizational effects can occur.

4. Organization implies structural changes.

5. Organization is asymmetric with respect to the sexes. Androgens, in the male, and the absence of androgens, in the female, organize. On the other hand, activation is symmetric. That is, testosterone and its metabolites activate male-typical behaviors, while estrogens and progestins activate female-typical behaviors.

In general the organizational-activational hypothesis allows for hormones to developmentally affect behavior in three different ways:

1. Combined organizational and activational: perinatal hormones organize neural tissue such that adult hormonal activation is necessary for the expression of behavior. An example of this type of behavior is aggression in adult male and female mice (Vom Saal, Svare and Gandelman, 1976). Neonatally gonadectomized male mice require extended T treatment in adulthood to show aggression (unless implanted with TP shortly after birth).
2. Organizational: perinatal hormones organize neural tissue such that adult hormonal activation is not necessary for the expression of behavior. An example of this type of behavior is activity in adult mice. Castration alone does not affect activity in adult mice (Broida & Svare, 1984).

3. Activational: perinatal hormones do not organize neural tissue such that only hormonal activation is necessary for the expression of behavior. Both males and females have the same capacity. The only difference is the activating hormone. An example of this type of behavior is male-typical mounting in mice (Edwards and Burge, 1971). Both castrated males and females will mount if injected with testosterone.

While copulatory behaviors (e.g. male-typical mounting and female-typical lordosis) are the most often studied gender-typical behaviors, other sexually dimorphic behaviors include aggression, (Beach, 1975) activity levels, (Broida & Svare, 1984) micturition patterns in dogs, (Berg, 1944) ultrasonic vocalizations in rodents, (Nyby and Whitney, 1978) and sexual preference in mice (Bean, Nyby, Dahinden, & Kerchner, 1986; Nyby, Kay, Bean, Dahinden, and Kerchner, 1985). In large part, the use of behaviors other than copulatory activities have extended our knowledge of the hormonal control of sexually dimorphic behaviors. While organizational-activational effects are well-documented, Arnold & Breedlove (1985) suggest that some assumptions may not be strictly correct, and that the hypothesis should be
looked upon as a flexible framework. A few of their examples are:

1) Steroids can change neural structures permanently in adulthood (at relatively arbitrary times); e.g., T-treated females possess more synapses in the RA (in birdthere s, only) region of the brain, than do controls (they acknowledge that this is probably a "long-term" effect rather than a permanent one).

2) Some permanent effects can occur in a critical period beyond the neonatal period.

3) Steroids have long-term reversible effects in adulthood.

4) Ovarian hormone, long thought to be irrelevant in the process of sexual differentiation, may in fact play a role.

In addition, more similarity exists between the neonatal and adult neural systems than previously expected. The systems are hypothesized to be similar in:

1) neuron number;

2) how steroids influence neurite growth;

3) neuronal size & form;

4) sex steroid influences on biochemical processes;

5) steroid-induced changes in steroid binding & accumulation.

Clearly, one must be careful in designing studies using activational-organizational principles because strict adherence to these principles can cause one to overlook or disregard what appear to be experimental artifacts.

3. Hormone Blood Titers and Sexual Preference
A group of studies investigated the effects of sex hormones (e.g. T, P, E, etc.) on sexual preference. Hormones have long been established to play a role in sexual behavior (Young, Goy, & Phoenix, 1976). Castration, in adulthood, for example, usually leads to decreases in libido and sexual activity in a variety of male mammals, including man (Beach, 1942). Thus, sexual orientation has been postulated by some to be dependent on T levels.

However, in only 3 of 27 studies (Meyer-Bahlberg, 1984) which assessed T levels in heterosexual and homosexual human males were any significant differences found in hormone level, between groups, and two of those three were confounded by other variables. While Meyer-Bahlberg asserts that adult T levels may contribute they are obviously not primary factors human sexual preference.

4. Sexual Dysfunction

Sexual dysfunction can affect males and females in at least two ways which could affect the development of homosexuality. First, if early heterosexual experiences are accompanied by sexual dysfunction (impotence, premature ejaculation, frigidity, etc.) the individual may believe that he/she is unable to perform because he/she is homosexual (Symons, 1976). After many unsatisfying heterosexual experiences, the person may cease all sexual activities. During this period of time he/she may become functional. If
a homosexual experience should follow and the person is functional, reinforcement for homosexuality can occur.

A second theory suggests that homosexuals are similar to sexually dysfunctional males, in another way. Past research (reviewed in Sakheim, Barlow, Beck and Abrahamson, 1985) indicated that homosexuals and sexually dysfunctional males suffer from the inability to correlate accurately their physical arousal with their subjective feelings of arousal. Heterosexual males appeared to be better at correlating physical and psychological arousal. Homosexual males, like dysfunctional males, reported significantly lower feelings of mental arousal when physically aroused. Thus, it was concluded that homosexual males should be clinically treated like sexually dysfunctional males; the implication being, that homosexuality could be cured. Once again, we confront the medical model of homosexuality. Researchers were more interested in curing an abhorrent behavior, than investigating sexual preference.

However, when pleasantness of stimuli was properly controlled between groups of heterosexual and homosexual males, no difference in correlating mental and physical arousal was found between the two groups was found (Sakheim, et al., 1985). Previous researchers assumed that both heterosexual and homosexual males would be able to correlate mental and physical arousal (as measured by a mechanical penile strain gauge) when exposed to the exact same stimuli.
In reality, homosexual males are equally as good at correlating mental with physical arousal as are heterosexual males, when homosexuals are exposed to stimuli depicting homosexual sexual encounters and heterosexual males are exposed to heterosexual stimuli.

5. Neuroanatomic Abnormalities

Initial research hypothesized how adult sex differences in CNS organization were affected by steroids, by observing the behavioral effects of endogenous and exogenous steroids. Later studies actually looked at the CNS itself in searching for differences in the neuroanatomical structures of males and females, of various species. Some examples of discoveries made by this later type of research include: differences in gross neural structures, effects on neuronal growth and structure (e.g. neuron size and number, number of dendritic spines, etc.), sex dependent changes in biochemical processes (e.g. enzymatic systems), and differences in steroid-receptor binding patterns (references available in Arnold and Breedlove, 1985).

6. Endocrinological Irregularities

One of the most well known endocrinological irregularities is the unexpected positive LH surge that some homosexual males experience, in response to estrogen treatment. Typically, human females (and other female mammals) respond with a surge in their levels of luteinizing hormone (a signal to the ovaries to start progesterone
production) upon being primed with estrogen. Most males, in contrast, do not exhibit this surge.

Dorner, Gotz, and Rohde (1975), Dorner, Docke, Gotz, Rohde, Stahl, & Tonjes (1987), and Gladue, Green, & Hellman (1984) demonstrated that while exclusively heterosexual males do not display an LH surge, following estrogen treatment, homosexual individuals respond midway between females and heterosexual males.

LH response patterns might be useful in two ways: a) to identify individuals who are biologically predisposed to homosexuality, and b) as a biological marker to be used in other biological studies. However, whether homosexuality causes this abnormal LH surge or whether the abnormal LH surge is related to the cause of homosexuality is not clear.

7. The Intrauterine Position Phenomenon

Short gestation period mammals, such as mice or rats, are not entirely sexually differentiated until ten days after birth. Consequently they are ideal subjects for studies examining the physiological and behavioral effects of perinatal steroids on neuroanatomy, phenotype, and behavior (e.g. sexual preference).

Vom Saal (1980) has demonstrated that the intrauterine position of a fetus relative to his/her brothers and sisters can affect various behavioral and/or morphological characteristics. For example, a female mouse who is positioned between 2 male mice (in utero) exhibits more male-
like behavior & morphology, than a female located between 2 other females. Significant behavioral and morphological effects have been found for blood and amniotic fluid titers and other prenatal hormone differences by female fetuses. Adult females differ in morphology, & physiology. In males, estradiol levels of fetal blood & amniotic fluid were different. Adult males, exhibited sexual behaviors that were affected by intrauterine position.

The implication is that prenatal hormone exposure can organize neuroanatomy and/or phenotype in ways affecting adult sexual preference. Research from our laboratory and others, however, does not support the existence of the intrauterine position effect (Jubilan, 1992; Cologer-Clifford, Simon, & Jubilan, 1992).

C. Physiological Factors: Operational Definitions and Problems of Measurement

Finding a sexually dimorphic behavior indicative of sexual preference, which also correlates well with physiological factors, can present problems. The basic paradigm is as follows:

1. Choose a relevant behavior;
2. affect the physiological system of interest;
3. observe how the behavior changes;
4. make inferences concerning the causal physiological factors.
One behavior that has been used to assess sexual preference is copulatory behavior, since it is clearly a sexual behavior which can be changed through physiological means, and is often sexually dimorphic (in non-humans). Females usually exhibit lordosis (an arching of the back to present the hindquarters to a male) and males respond by mounting (McGill, 1961). If you suspect that the presence or absence of testosterone (T) affects sexual orientation you might expect T to affect sexually dimorphic behaviors like copulatory behavior. Inferences about how T affects sexual orientation can then be drawn.

However, the appropriateness of using copulatory behavior in a behavioral assay for studying sexual preference is debatable. Sexual dimorphism in copulatory behavior may not be as precise an indication of sexual orientation as might be thought. Under certain circumstances, and depending on the species and strain, spontaneous mounting in females or lordosis in males is not unusual (Beach, 1975).

Some researchers have manipulated T level perinatally to assess how such exposure affects adult behavior. Typically, perinatal exposure causes anogenital distance to increase in length.

Care must be exercised in interpreting results from such experiments because T can affect morphology and fail to affect adult behavior. All subjects should be gonadectomized at birth to control postnatal T levels. To determine if the
behavior requires activational T to elicit the behavior, subjects should be tested as adults without, and subsequently with, T replacement.

Neonatal castration removes the T producing organs (i.e. the testes) early in life and produces adult male subjects with undeveloped external genitalia. This outcome (i.e. underdeveloped external genitalia) may have effects on adult sexual behavior (Gerall, Hendricks, Johnson, and Bounds, 1967).

In order to avoid T-induced morphological confounds, studies have used T-dependent behaviors which are relatively independent of morphological effects. For example, studies can be run so that a single subject is monitored while the subject responds to (e.g. sniffs or investigates) an immobile stimulus (e.g. an anesthetized conspecific, a cotton swab soaked in urine from a conspecific, etc., thus by-passing the need to have two interacting subjects).

Alternatively, instead of monitoring a male-typical behavior, researchers can monitor a female, T-independent behavior (in mice) such as sexual receptivity (defined below).

Thus, researchers using mice as subjects have monitored odor preference, ultrasonic vocalizations, and receptivity. I will elaborate on each method.

1. Odor Preference

The primary method utilized in my research to assess the effects of hormones on sexual preference, is to evaluate how
different hormone regimens affect the animal's inclination to
sniff a conspecific's odor. Unlike ultrasonic vocalizations
and receptive behavior (described below), investigation of
objects via olfaction normally occurs in both males and
females. In addition, there are characteristic male and
female olfactory preference profiles. In mice, females spend
more time sniffing odors associated with a male than with
those associated with a female. The reverse is true for
males.

Mice are very much guided in their social/sexual behavior
by the sense of smell and this sense undoubtedly plays a large
role in their choice of sex partners. Therefore, the salience
of a particular odor can be assessed by monitoring the amount
of time a subject spends sniffing it (John Nyby, personal
communication, 1992). Early research demonstrated that a
variety of bodily secretions of the female could induce
sexually motivated behaviors (e.g. ultrasonic vocalizations,
mounting, investigation) in the male mouse (Nyby, 1983).
Initial research suggested that the recipient animal responded
to pheromones manufactured by the donor and secreted in
donor's body fluid(s) (Beauchamp, Doty, Moulton, & Mugford,
1976).

Urine is a good cue to choose to study sexual preference
in mice, for practical, as well as scientific reasons. The
components of mouse urine which appear to be responsible for
some of the urine's cue value does not need to be touched. In
addition, the volatile component of urine apparently carries sufficient quantities of the cue used in gender identification (Nyby, 1982). Pragmatically, urine is easily obtainable, either manually or in metabolic cages, in sufficient quantities for experimental use.

2. Ultrasonic Vocalizations (UV)

Adult male and neonate male & female housemice emit a 70 kHz vocalization which is outside the range of human hearing. These sounds are produced most often when in the presence of an adult mouse. Neonates have been suggested to emit these sounds to minimize rough handling by a parent. These vocalizations also serve as a cue for retrieval. This neonatal defense mechanism disappears at about the age of weaning. In males the vocalizations reemerge in adulthood when courting and copulating with females. In contrast, adult females, rarely emit spontaneous vocalizations in a sexual context. Whitney, et al. (1973) hypothesized that adult male UV also serve to reduce aggression in adult females. He speculated that the adult vocalizations may pacify the female before and during copulation. The behavior could have evolved as a result of natural selection if it promotes conception.

Male vocalizations usually occur during investigation, increase with mountings, decrease across intromissions, and cease after ejaculation (Nyby, 1983). Vocalizations are T dependent (Nyby, Wysocki, Whitney and Dizinno, 1977). Intact,
adult, socially experienced males emit vocalizations to adult females and various body fluid odors (Nyby and Whitney, 1980).

Ultrasonic vocalization quantity is not the best way to measure sexual preference because it can't be used to measure the preference of females. Females do not typically produce vocalizations to conspecifics (absence of a behavior does not necessarily imply sexual preference).

3. Receptivity

Receptivity refers to the female's willingness to engage in copulatory activity. In mice, receptivity is operationally defined as the female "presenting" her hind quarters to the male, with the back arched and tail raised; also known as lordosis (McGill, 1961). The male then mounts the female. Mounting does not necessarily guarantee intromission, ejaculation, and pregnancy, but greatly increases the probability of conception and subsequent delivery. Thus, receptive behavior is subject to natural selection, and is an important behavior to study.

Lordosis is not the optimal behavior to use as an index of sexual preference because lordosis is operationally defined as a female-typical behavior. Induction of female-typical behaviors in a male is not necessarily an indication of sexual preference. While both males & females have been observed exhibiting both mounting & lordotic behavior, these behaviors can be directed at either males or females.

4. Hypothesis of Study
By experimentally determining how long male and female mice spend sniffing male, female, and neutral odors we can have a quantitative measure of sexual preference. As noted previously, olfaction has been shown to be associated with sexually motivated behavior and thus may be used as a measure of sexual preference.

My hypothesis is that exogenous, perinatal testosterone or lack thereof will masculinize or demasculinize adult sexual preference as measured by an odor preference assay. I predict that perinatal exposure to testosterone propionate (TP) will produce adult female subjects who will exhibit masculine preference profiles. In addition, neonatally castrated, adult male subjects who are not given perinatal TP will fail to exhibit masculine preference profiles. Subsequently, adult administration of TP should activate behavior to demonstrate the organizational effect of perinatal TP.
Chapter 2 Methods

I. Subjects

The experiment occurred in two phases. In phase I, animals received intraperitoneal injections of either testosterone propionate (TP) or oil vehicle during the first 5 consecutive days after birth. In phase II these animals were tested in adulthood for odor preference. In experiment II of phase II, these same animals were implanted with silastic capsules of TP or blank capsules and then retested for odor preference.

Three groups of mice participated in the experiment: social experience animals, stimulus donors, and experimental animals (i.e. subjects).

A. Social Experience Animals

Social experience (S.E.) animals consisted of adult male and female CK mice, bred in our laboratory. These animals were group housed, and were 4-6 months old on the first trial. The S.E. animals were used to give adult social experience to the experimental animals, before testing began. Social experience was necessary because it has been demonstrated that sexually motivated behavior such as ultrasonic vocalization in adulthood is facilitated by social experience (Dizinno, G., Whitney, G., and Nyby, J., 1978).

B. Stimulus Donors
Stimulus donors donated the urine used as the stimulus in the experiment. S.E. animals doubled as urine donors. (see Stimulus Collection, below)

C. Subjects

The subjects, consisted of 44 male and 36 female, CK mice, bred in our laboratory. All animals were housed in 28 x 18 x 13 cm cages, with food available ad libitum, and on a 12-12, on/off, light-dark cycle. All procedures occurred during the light portion of the light-dark cycle. Subjects as infants were group-housed with their mothers, in translucent cages to insure against visual contact between litters. Subjects were between 64 and 72 days of age on the first trial.

To insure that all subjects were exposed to similar levels of S.E., all animals were weaned pre-pubertally at 21 days of age, and group housed as siblings. At 55 days of age, all subjects were individually housed. Eight days later, animals received S.E. for 8 consecutive days.

1. Grouping and Identification Procedures

Animals were split into two main groups, by age; 1) group Q animals were born between 2/2 and 2/10, N = 65; and group Z animals were born between 2/20 and 2/26, N = 15. These animals were the first and second litters of the same group of females. Since there were so many animals in the first group, it was split into two groups: (1) group X, N = 33 (females #501-507, #531-537 & males #601-610, #631-639); and (2) group
Y, N = 32 (females #508-515, #538-532 & males #611-616, #640-652).

All experimental animals were toe-clipped, for identification purposes, within 24 hours of birth, as follows: a right paw clip indicated neonatally androgenized females (#501-530) or neonatally castrated males (#601-630); a left paw clip indicated neonatally oil treated, control females (#531-560) or neonatally sham castrated, control males (#631-660).

Anogenital distance was measured with a micrometer caliper, at 55 days of age, to assess the effectiveness of the TP as an androgenizing agent.

II. Experimental Treatment Procedure

A. Neonatal Surgery

Within 24 hours after birth, male and female pups were gonadectomized or sham gonadectomized. The pups were first placed on a clean paper towel, and then, anesthetized by being placed in a freezer. (Chilling the pups took the place of chemical anesthesia which is difficult to administer because of the pups' size.) After approximately 5 minutes, the pup was removed from the freezer. The skin was nearly transparent, and the gonads easily identified. The gonads were removed with a mosquito forceps. The procedure rarely resulted in bleeding. Sham gonadectomized animals experienced all aspects of the procedure except the gonads were not removed. Pups were toe-clipped and then warmed on a heating
pad, set on low, for approximately 30 minutes, before being returned to their mothers.

**B. Hormonal Treatment**

Pups received intraperitoneal injections of either .05 ml of a suspension of 100 ug/.1 cc of TP in peanut oil, or an injection of plain oil, for the first 5 consecutive days after birth. Injections began immediately following surgery. Four groups received treatment: 1) neonatally androgenized females (received TP); 2) control females (oil treated); 3) castrated males (received TP), and 4) sham castrated males (oil treated). After hormonal treatment the animals matured to puberty, at which time they were individually housed. Upon reaching adulthood each subject received social experience and apparatus experience.

**C. Social Experience and Apparatus Experience Procedures**

S.E. consisted of exposing the test animals to both a male and female S.E. animal sequentially, for 3 minutes a day. The order of presentation of S.E. animal was counterbalanced across the 8 days. Males were presented via the tail dangling method of Scott (1966), to ensure that dominance hierarchies could not be formed. (Dominance has been shown to affect related behavior such as UV, Nyby, Dizinno, & Whitney, 1976) Female S.E. animals were given free run of the test subject's cage. An eight day S.E. regimen was chosen (i.e. the duration of 2 estrous cycles in the S.E. females) to control for effects that the females' estrous cycle might have on later
behavior in subjects receiving S.E. S.E. was begun on three different days, according to the group to which the subject belonged (groups explained later). S.E. animals had been used in two previous experiments as S.E. animals.

Subjects were also given Apparatus Experience (A.E.), for 8 consecutive days, the day after completing the S.E. regimen. A.E. consisted of placing each test animal, in his or her home cage, for 3 minutes, under the odor-testing apparatus, in the testing room. This procedure was executed, without stimuli present, to allow the test subjects to become acclimated to the test environment.

D. Experiment I

Experiment I did not require exogenous hormone treatment. Animals were tested to determine if neonatal androgenization had an organizing effect on the neural tissue mediating adult sexual preference. Adult subjects were presented with stimuli carrying neutral or sex-typical odors (as described later, see Figure 1) and were observed for amount of time spent sniffing the stimuli.

E. Experiment II

On the day after each group completed experiment I, subjects were implanted with either a silastic capsule containing TP or a blank capsule. Silastic capsules were made on the day before implantation. Dow Corning silastic tubing with an inside diameter of .062 inches and an outside diameter of .125 inches was cut into 14 mm lengths. Cotton swab sticks
FIG. 1. Odor-testing apparatus. The odor-testing apparatus was made entirely of glass and was designed to sit on top of a standard mouse cage. Odors were presented on cotton-tipped surgical swabs and were carried into the cage on an air stream created by a vacuum pump.
were cut into 2 mm lengths, and inserted into both ends of the tubing as plugs. Surface area of exposed tubing containing hormone was 10mm (2mm on each end for the wooden plugs).

One end of each capsule was initially closed off. The open end was tamped into powdered TP. This action packed the TP inside the capsule. After each capsule was filled, the open ends were plugged. Both ends were sealed with silicon medical grade adhesive (Dow Corning Silastic brand). All of the capsules were soaked in a beaker of physiological saline overnight, to allow TP concentrations to reach equilibrium. The beaker was covered with parafilm, to prevent contamination. Blank capsules were made and treated in the same fashion, except TP was omitted.

The animals were injected with 0.1 ml of sodium pentobarbital for every 10 g of body weight. Once the mouse was anesthetized, a small (approximately 0.5 cm) incision was made into the skin of the neck, dorsally, above the muscle tissue. The skin was gently pulled away from the muscle and a silastic capsule was inserted into the resultant cavity. The wound was sutured with 2 stainless-steel, surgical wound-clips. The animals recovered from the anesthetic within 30 minutes of surgery, and were returned to their individual cages.

III. Apparatus

Cages were washed immediately before testing, in hot, soapy water and allowed to air dry. Animals were transferred
to the clean cages, immediately before testing began, so that some animals were exposed to the clean cages longer than others. For behavioral testing, animals were placed in clean, (29 x 18 x 13), transparent, plexiglass cages, with approximately half an inch of clean woodshavings on the floor, to insure that all subjects were exposed to the same neutral odors.

Immediately prior to behavioral testing, a subject's cage top was removed and replaced by a clean, transparent odor-testing apparatus. (See Figure 1) After each trial the odor-testing apparatus was washed in 100% alcohol on a cotton swab, held by forceps. Care was taken not to touch the surface of the glass or the tubes. Only the far edges (those not exposed to test subjects) of the apparatus were touched. The apparatus was then blown dried with a standard 100 watt hot air blower.

Air was circulated through the test cage by a vacuum pump attached by rubber tubing to the odor-testing apparatus. The vacuum pump was housed in the adjacent room. There was a continuous stream of air drawn through the test cage, upon which volatile odors could travel. While the test chamber was not absolutely airtight, the experimenters were careful not to use cracked or chipped cages, so that extraneous odors could not enter or leave the chamber. The odor-testing apparatus lay flush across the top of the cage. The door between the
two rooms was kept closed to minimize the effects(s) of the noise produced by the pump.

IV. Testing Procedures

A. Stimulus Collection

Stimuli consisted of urine collected immediately before testing, in 1 ml glass syringes. Care was taken not to allow anything to come into contact with the urine. Urine was obtained by grasping the animal dorsally by the nape of the neck. Usually this contact caused the animal to urinate. If not, the bladder was gently palpated. The urine was collected in a glass collection vial and transferred to the syringes. Male and female urine were kept separate, and collected separately.

1. Stimulus Preparation

Urine was stored in 1 ml glass syringes. Stimulus preparation occurred in a room adjacent to the testing room (in the same room in which the animals were born and bred). A sterile, 6 inch, single ended cotton swab was injected with .1 ml of urine. Care was taken not to allow anything except the perforated plastic cover used in testing to come in contact with the last 2 inches of the swab. The swab was suspended through the perforated plastic cover. An alligator clip was used to hold the swab in place. The excess swab stick was cut off, so the resulting swab was
approximately 1.5 inches long. Blank swabs were prepared in the same manner, with the exception that no urine was injected.

Once prepared, the two stimuli were placed over the odor-testing apparatus tubes, with the cotton-tipped end suspended inside the glass tube (see Figure 1). The experimenter was careful to touch only the outside edges of the perforated covers. Fresh stimuli were prepared for each subject. Used stimuli were disca

B. Experiment I & II Procedures

Subjects were tested in 3 groups, based on age and size of the group, as described earlier. Immediately before testing, cages were washed, M and F urine was collected, the apparatus was cleaned and made ready, animals were individually housed in the clean cages, the stimulus preparation area was cleaned and made ready, and stimulus presentation assignments were randomly chosen.

1. Presentation Assignments

Each subject animal received two trials: 1) male (M) urine vs a blank stimulus, and 2) female (F) urine vs a blank stimulus. Trials were conducted 2 days apart. Order of presentation of stimuli were randomly chosen by flipping a coin. Order of presentation of animals to the test situation was also randomly chosen. The research accomplices were blind as to the sex of the subject, to the subject's group, and to the stimulus being presented.
2. Preference Testing

Each animal was tested individually. The cage top was removed and immediately replaced by the odor-testing apparatus. A 2-min habituation period followed, without stimuli present, to allow the animal time to become familiar with this environment. During this 2-min period, the stimulus presenting experimenter, prepared the stimuli. After the 2 minutes, the research accomplice was asked to close her eyes while the two stimuli (one urine and one blank) were positioned. The accomplice, using two stop-watches manually timed how long each subject sniffed each stimulus. The subject was timed anytime its nose was within a centimeter of the odor tube. Individuals were monitored for 3 minutes. The odor-testing apparatus was cleaned between subjects, as described earlier. Sniffing was timed to 0.1 sec.
Chapter 3: Results

I. Anogenital Distances

As seen in figure 2, neonatally sham-castrated adult males had significantly longer anogenital lengths than neonatally castrated adult males (F (1,41) = 43.01, p < .00001). Surprisingly, neonatally ovariectomized (ovx) adult females which had received neonatal TP injections (androgenized) did not have significantly different anogenital distances than females which had received neonatal oil injections (non-androgenized; F (1,34) = 2.43, p = .125).

II. Male Sexual Preference

Initially, N = 44 male subjects were used. Subjects which did not reach a criterion level of sniffing (at least 5 seconds in a 3 minute screening trial) were not included in statistical analyses. Eight subjects were excluded.

As seen in figure 3, in adulthood, neonatally sham castrated males sniffed male and female odors significantly more often than neonatally castrated males (F (1,36) = 3.54, p = .02). Male subjects in general preferred to sniff female odors over male odors (F (1,36) = 15.22, p = .0002). In addition, there was a significant group by stimulus interaction. That is, sham-castrated males preferred sniffing female odors significantly more than did castrated males (F (1,36) = 7.01, p = .009).

After initial preference tests, males were implanted with a capsule containing TP or a blank capsule. Following
Figure 2: Anogenital distances in adulthood of the four groups of neonatally manipulated subjects prior to adult hormone treatment.
Figure 3: Olfactory preferences of males receiving neonatal castrations (CASTRATE) and males receiving neonatal sham castrations (SHAM).
implantation, male preference was reevaluated in the same manner as before preference test. Various animals were not included in the second preference test due to death.

As seen in figure 4, males receiving TP implants did not sniff male and female odors significantly more often than males with blank implants ($F(1,24) = 1.07, p = \text{n.s.}$). Male subjects in general did not prefer to sniff female odors more than male odors ($F(1,24) = 1.05, p = \text{n.s.}$). However, there was a significant group by stimulus interaction. That is, males receiving TP implants preferred sniffing female odors significantly more than males receiving blank implants ($F(1,24) = 14.55, p = .0008$).

Upon further analysis sham-castrated males receiving TP implants preferred female odors significantly more than male odors ($F(1,15) = 11.33, p = .004$). And, castrated males receiving TP implants preferred male odors significantly more than female odors ($F(1,9) = 7.39, p = .024$).

### III. Female Sexual Preference

Because anogenital distance data for females indicated that neonatal androgenization had not affected morphology, it was inferred that substrates mediating preference may not have been androgenized. The preference data are consistent with this conclusion (see Figures 5 & 6). ANOVA showed no main effect for subjects ($F(1,34) = .28, p = \text{n.s.}$; i.e. females did not significantly differ regardless of neonatal treatment). There was a significant effect for stimulus, but
**Figure 4:** Olfactory preferences of males receiving neonatal sham castrations and then either subcutaneous TP Silastic caspsules in adulthood (SHAM + TP) or empty capsules (SHAM + blank) and males receiving neonatal castrations and then either TP capsules (CASTRATE + TP) or empty capsules (CASTRATE + blank) in adulthood.
Figure 5: Olfactory preferences of adult females neonatally treated with either oil (OIL) or testosterone propionate (ANDROGEN).
Figure 6: Olfactory preferences of adult females receiving neonatal oil treatment and then either TP Silastic capsules in adulthood (OIL + TP) or empty capsules (OIL + blank) and females receiving neonatal treatment with testosterone propionate and then either TP capsules (ANDROGEN + TP) or empty capsules (ANDROGEN + blank) in adulthood.
in the wrong direction ($F (1,24) = 8.61, p = .006$; i.e. females exhibited male-typical preference profiles). The interaction was not significant ($F (1,24) = 3.28, p = n.s.$). Given the anogenital length results and inconsistent preference profiles, further analysis was deemed unwarrented. The data from female subjects was not included in the discussion of results.
Chapter 4: Discussion

I. Introduction

My study was designed to examine effects of neonatal androgenization on adult sexual preference in mice. As reported, anogenital distance of neonatally androgenized females was not affected, thus data concerning female trials is not conclusive and is not included in the following discussion. I will first discuss the implications of the data collected on the male mice. I will then offer suggestions as to why the females did not respond as expected to neonatal androgenization. Finally, I will propose future research.

II. Male Sexual Preference

Anogenital distances were significantly longer in adult males that had been neonatally androgenized versus those that had not, indicating that morphological androgenization had occurred. Thus, I expected to find differences in behavior (i.e. in preference profiles of the two groups of males). In fact, intact males preferred to sniff female urinary odors for a significantly longer amount of time than did the castrated males. The intact males apparently either 1) could better discriminate between male and female odors than could castrated males; or 2) preferred to sniff female odors more than did castrated males. The first possibility was minimized by presenting male and female odors separately with a neutral odor (i.e. water). Therefore, subjects did not make a choice
between male and female odors. It appeared that the second possibility was more likely.

Although castrated males did not appear to prefer male or female urinary odors, they also did not exhibit the female preference profile. Thus neonatal androgenization affected preference but lack of androgen was insufficient to reverse the gender preference profile completely.

Subjects were then implanted with T or blank capsules to determine if circulating T in adulthood would stimulate male-typical preference profiles. In all males sniffing behavior occurred much less frequently (perhaps because the subjects had been tested previously, had grown accustomed to the conditions and were somewhat disinterested). Intact males demonstrated preference for female versus male urinary odors whether they received TP or blank implants. Interestingly, castrated males exhibited a female-typical preference profile after TP implant. Blank implants did not affect preference.

It appeared that testosterone affected male preference in two manners. During the neonatal period, lack of testosterone seemed to result in loss of male-typical adult preference. Since absence of testosterone during fetal development results in a female phenotype, one might expect lack of T to result in a female-typical preference profile. Apparently, in adulthood, this female-typical preference profile does not become evident until testosterone activates it. Thus,
testosterone had both organizational and activational effects on male mouse sexual preference.

III. Female Sexual Preference

Unfortunately, morphological assessment indicated that the female subjects did not experience neonatal androgenization. Adult preference profiles confirmed this suspicion. There are several reasons why neonatal injections of T failed to androgenize female subjects. First, incorrect pup gender identification at birth could have led to pups receiving incorrect hormone/oil treatment. However, adult identification confirmed birth identification. Second, errors of measurement of anogenital distance may have occurred because the caliper was not accurate enough to detect differences between female groups. This possibility seems unlikely since the caliper used reads to accuracy of .02 mm.

Jubilan (1992) reports that several other labs find conflicting results of post-natal hormone manipulation on morphology and behavior in varieties of rodents. Some find no effects for post-natal hormone. Jubilan notes that increasing frequency of contradictory findings leads to a suspicion of Type I error. (Type I error occurs when incorrectly rejecting the null hypothesis.)

Primates also show incomplete androgenization effects. For example, Eaton, Worlein, & Glick (1990) looked at high and low dose, pre-natally androgenized females, versus androgenized males, normal males and normal females. They
observed approach, withdrawal, display, mounting, displacement of another, threatening behavior, proximity to a neighbor, contact, grooming, following another, play, distress, retrieval of infants, & distance from mother (among other behaviors). They found that high androgen dose females differed from normal females only in mounting behavior. Low androgen dose females exhibited no observable differences in behavior from normal females. These scientists do suggest that the small sample sizes could have obscured differences between androgenized and normal females. But this study suggests that primate females are relatively insensitive to the androgenizing effects of testosterone.

The lack of female response to androgen may be entirely normal since Jubilan (1992) found that vocalizations of androgenized females were not significantly affected by intrauterine position, suggesting that no significant difference exists in the sensitivity of females of different uterine position to the androgenizing effect of testosterone. In addition, Jubilan states (personal communication) that he has run studies using perinatal (both prenatal and postnatal periods) treatment using TP, and his results were not consistent (anogenital distances). This finding suggests that either Jubilan's or my methodology for administering TP neonatally was flawed or that there is no effect on preference for perinatal TP in females.
IV. Future Studies

My study suggests that T affects the organization and activation of sexual preference in male mice. This finding should be replicated in another strain of mice. Upon replication, it would be interesting to see if similar findings could be demonstrated in primates. T may not be the sole determinant of sexual preference. However, perinatal and adult hormone levels may act to direct sexual preference in one direction or the other.

Additionally, I would try running another group of females this time correcting for body weight since T can affect body weight (Kinsley, Miele, Wagner, Ghiraldi, Broida & Svare; Gentry & Wade). If there are still no morphological differences between groups of females, then perhaps masculinization affects on morphology are prenatal in mice (unlike in rats, in which it occurs perinatally; Gerall, Hendricks, Johnson, & Bounds, 1967; Beach, Noble & Orndoff, 1969), but behavioral effects of hormone could have a wider organizational period (Brand and Slob, 1991). Morphology is more permanent than is behavior. Thus it is appropriate for the window for behavioral organization to be wider than that for morphology.

V. Conclusion

In conclusion, the study indicated: 1) male mouse sexual preference was affected by both neonatal and adult levels of testosterone; and 2) female mice were relatively insensitive
to the organizational effects of testosterone. Testosterone affected males in 2 ways: 1) its absence acted to organizationally demasculinize preference profiles; and 2) its presence in adulthood acted to activationally feminize preference profiles.
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