

A STUDY OF THE EFFECTS OF PRENATAL STRESS ON ADULT  
SEXUAL BEHAVIOR IN MALE MICE OF THE STRAIN AB-Y

Submitted in Partial Fulfillment of the Requirements for  
Graduation with Honors to the Department of Biology at  
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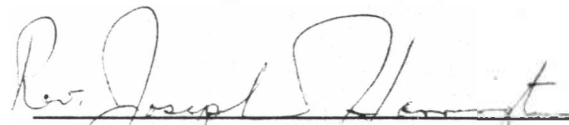
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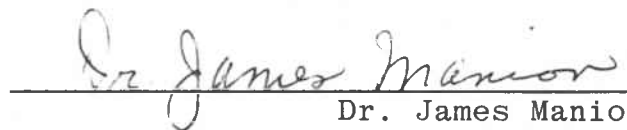


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April 1, 1985

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## ABSTRACT

Pregnant female mice of the strain AB-Y were subjected to psychological and physiological stress during the last two weeks of pregnancy. Stress was provided by subjecting the animals to bright lights, confinement, and temperature increase. The male offspring of these stressed mice were then compared with normal control males of non-stressed mothers to test for deviance in male sexual behavior of the prenatally stressed males. The two groups were tested by placing them with estrus lure female mice and observing the male sexual behavioral characteristics displayed. It was found that the prenatally stressed males showed lower levels of male sexual behavior than the control males.

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## INTRODUCTION

In mammals the sex of an individual is generally said to be determined by the chromosomal content. The female possessing two X chromosomes and the male having both an X and a Y chromosome. In practice, the sex of an individual is determined from the appearance of the external genitalia rather than the chromosomal status. To each sex is ascribed a characteristic pattern of reproductive behavior typical of that species. It has been well confirmed over the years that hormones play an important role in establishing both physical and behavioral sex characteristics (Phoenix, et. al., 1959; Harris, 1964; Levine, 1966). First a brief account will be given of how sexual differentiation of the central nervous system (CNS) is accomplished and how it establishes behavioral patterns. Then the causes and effects of stress will be examined in relation to how they might influence sexual differentiation of the CNS causing behavioral abnormalities.

In many species there exists a marked sex difference in the control of endocrine function and behavior by the central nervous system. These mechanisms play an integral part in the reproductive process, including selecting a sexual partner, mating behavior, and subsequent production

and rearing of offspring. Sex differences in the CNS are the outcome of several factors, most importantly the production of hormones from the gonads of the developing animal. The gonads in turn are developed under genetic expression of either an XX or XY chromosomal pattern (Gorski, 1980).

Inherently, the brain as well as the reproductive system of mammals is female. In order for the brain to assume masculine properties of behavior and gonadotropin secretion it must be stimulated by androgen. This androgen stimulation must take place during a critical period in the development of the brain, which in rodents takes place just prior to and just after birth (Harris, 1964). The androgens necessary to masculinize the brain are produced in the developing testes of the male fetus and are largely composed of the steroid hormone testosterone. Independently of brain masculinization, testosterone also serves to defeminize the brain by suppressing the behavioral and neuroendocrine patterns characteristic of the female (Levine, 1966).

The role that testosterone plays in sexual differentiation is twofold. First, it has an organizational or inductive effect on the brain. This original inductive mechanism results in an adult brain which produces a tonic rather than cyclic release of gonadotropic hormones. It also results in the potential for male sexual behavior patterns. The second role that testosterone plays on brain differen-

tiation is to establish an activational or excitatory mechanism. This neural mechanism is active in regulating adult sexual behavior. The activational mechanism is dependent upon the level of testosterone in the mature animal and is triggered by an environmental stimuli such as the presence of a mate. It has been shown that both the organizational and activational effects of testosterone take place in the hypothalamus of the animal brain (Harris, 1964).

Androgens mediating sexual differentiation appear to act directly on the tissues of the CNS. Specific target areas for testosterone have been located in the brains of several species. The preoptic area of the anterior hypothalamus seems to be a major target site for testosterone in the prenatal brain (MacLusky and Naftolin, 1981). It was earlier shown that this area of the brain controls the cyclic release of gonadotropin which causes ovulation (Barraclough and Gorski, 1961). One of the major effects of testosterone on the brain appears to be the irreversible alteration of the ovulation controlling preoptic region of the hypothalamus from the inherent feminine state to a masculine state.

There are a number of known morphologic sex differences present in the central nervous system. Most of these differences appear to be the result of early stimulation by gonadal androgen. Several suggestions have been made with respect to how androgen stimulation alters neural

development in the perinatal brain. One suggestion is that exposure to androgen somehow alters the function or renewal of steroid receptor molecules in the brain. Another theory is that neurotransmitter production may be altered by androgen exposure. Finally, it has been noted that androgen exposure may alter the morphologic connectivity of neurons in certain brain regions (Gorski, 1980). Any or all of these changes in the neural structure or function could lead to the noted sexual behavior differences between males and females of a species.

A final point on the function of testosterone in sexual differentiation of the CNS must be noted. Recently it has been proposed that in order to elicit the observed effects on the neural tissues, the testosterone must first be converted into estrogen. Evidence has shown that it is estrogen instead of testosterone which acts directly on the neural tissues of the CNS. The conversion hypothesis suggests that once testosterone reaches the specific target areas in the brain that it is aromatized into estrogen. The higher levels of estrogen then act directly in masculinizing and defeminizing the CNS of the animal (Clemens, 1974).

It has been noticed for many years that stress causes a wide variety of behavioral and physiological modifications. In a number of species, stressors applied to a pregnant female result in behavioral abnormalities of the resulting offspring. Specifically, the male offspring

of stressed mothers show demasculinization and feminization in a number of species studied. Demasculinization consists of a decrease in male characteristic sexual behavior patterns. Feminization consists of a male exhibiting female type behavior when placed with a normal sexually active male (Hemming, 1980). In order to gain a better understanding of how stress might influence adult sexual behavior, it is necessary to examine the causes of stress in the laboratory animal and the physiological ramifications of this stress.

Production of psychological and physiological stress in laboratory rodents may be achieved in many ways. One of the most common methods of inducing stress is exposing the animal to bright light. This effect is enhanced if the light exposure is carried out during the dark phase of the animal's light-dark cycle (Ward and Weisz, 1972). Another stressor commonly used is temperature fluctuation. An ambient temperature increase to around 34°C for a period of 45 minutes (Herrenkohl, 1979) or a temperature decrease to about 4°C for 3 to 6 hours (Ciaranello, et. al., 1972) both result in significant stress in the animal. Injection with adrenocorticotropin (ACTH) is also used for stress production. Other stressors include: restraint, nutritional deprivation, overpopulation, and electric shock.

The physiological effects of stress are numerous and cover a wide range of organs and systems. The most significant effects of stress in laboratory animals are

on the endocrine system. The amounts of many hormones are either increased or decreased due to stressful conditions. Plasma levels of epinephrine, cortisol, ACTH, prolactin, and growth hormone are all increased as a result of stress (Sacher, 1980). A significant increase in plasma corticosterone concentration has been observed both in stressed adult animals as well as in the fetuses of pregnant animals (Ward and Weisz, 1980). Catecholamines released from the adrenal medulla and sympathetic nerves are also increased under stress (Kopin, 1980). Sacher (1980) has proposed that the levels of LH are decreased during stress which would account for the observed decrease in estrogen and testosterone which it stimulates. Adrostenedione, an androgen produced from both adult and fetal adrenal cortices has been shown to dramatically increase under stress (Ward, 1972).

The most important of these hormonal changes in regard to sexual differentiation of the CNS are those involving androgen concentrations. From the above discussion on hormonal control of sexual differentiation of the CNS, we may realize the importance of stress mediated changes of androgen levels in the developing fetus. It has long been known that stress causes a decrease in plasma testosterone concentration. More recently, it has been shown that stress causes alterations in the ratio of adrenal to gonadal adrenogens during critical stages of sexual differentiation. Specifically, a decrease is seen in

gonadal androgen including testosterone and an increase is seen in the levels of adrenal androgens including androstenedione (Ward, 1972). The decrease in the concentration of testosterone in the fetal plasma is thought to be due to a decrease in LH production (Sacher, 1980), a decrease in testicular size (Ward, 1972), and an increase in epinephrine and adrenocorticoids released from the mother which cross the placenta and reduce androgen production by the fetal testes (Ward, 1977). Comparing the adrenal and gonadal androgens, it has been shown that androstenedione is a much less potent androgen than is testosterone. It is suggested that the potency of the androgen may directly affect the level of differentiation of the CNS tissues which mediate sexual behavior. A theory has been suggested by Ingeborg Ward (1972) that these two androgens, due to their similar structure, may compete for the same receptor sites in the brain. Thus a higher concentration of less potent androgen would result in a lesser degree of male sexual differentiation.

More recent studies have shown that in addition to the overall decrease in plasma testosterone concentrations in the male rat fetus, the characteristic pattern of testosterone levels throughout gestation is altered by maternal stress. In particular, a surge of testosterone which normally occurs on day 18 of gestation in the male rat fetus takes place prematurely on day 17 in prenatally stressed male fetuses (Ward and Weisz, 1980). It is sug-

gested that this desynchronization of androgen levels in the fetal male affects the sequential hormonal events which determine normal masculine differentiation of adult sexual behavior. As a result of this alteration in testosterone levels, masculine sexual differentiation of the male offspring is impaired. The male offspring of stressed mothers show a characteristic prenatal stress syndrome involving a marked decrease in male copulatory behavior and an increase in female sexual behavior as adults.

Prenatal stress syndrome has been observed in several animal species including hamsters, rats, and mice. The laboratory rat has been the most extensively studied due to the more complete knowledge of its physiology and its easily observed sexual behavior. The period of sexual differentiation of the CNS in the rat extends from day 17 of gestation to between days 5-10 postpartum (Harris, 1964). Subjecting the male fetuses to stress during days 17 to birth results in adult males showing prenatal stress syndrome. These males may have significantly reduced copulatory rates measured by the number of mountings, intromissions and ejaculations achieved over a given period of time together with an estrous female. Stress occurring after the day of birth does not seem to affect the adult sexual behavior of the rat or the mouse (Milkovic, 1966 as cited in Ward, 1977).

The laboratory mouse has not been studied nearly to the extent which the rat has. A number of questions

still exist with regard to the effects of prenatal stress on adult sexual behavior in male mice. The results of some of the experimentation with prenatally stressed mice have been inconclusive or contradictory to similar studies on rats. This experiment will attempt to test the effects of maternal stress upon differentiation of adult sexual behavior in male mice of the strain AB-Y.

## LITERATURE REVIEW

Some of the earliest experimentation involving the effects of hormones on sexual differentiation were carried out by Carroll Pfeiffer in the mid 1930's (cited in Levine, 1966). Pfeiffer demonstrated that sexual differentiation took place very early in the course of a mammal's development by exchanging the sex organs of newborn rats. He found that males with ovaries in place of testes showed female sexual cycles and corpora lutea formation. Females given testes instead of ovaries failed to show any sign of estrus cycling. From these findings Pfeiffer concluded that the controlling factor in sexual differentiation seemed to be the presence or absence of testosterone, and that the presence of testosterone induced a permanent sexual differentiation of the pituitary. This differentiation included a change from the inherent female cyclic release of gonadotropin to a tonic release characteristic of the male. Harris, in 1955 (cited in Harris, 1964), showed that transplanting the pituitary of a male under the hypothalamus of a female resulted in the reproductive functions and behavior remaining female. This suggested that differentiation took place in the hypothalamus rather than the pituitary.

Following the hypothesis presented by Pfeiffer that testosterone caused sexual differentiation, studies were begun using the direct application of testosterone rather than gonad transplants. In 1937, Green and Ivy reported the production of intersexuality in female rats prenatally treated with testosterone. These studies established the fact that testosterone played a role in the differential development of the external genitalia. Extensive experimentation by Charles Phoenix and his co-workers (Phoenix, et. al., 1959) on guinea pigs gave further insight into the neural aspects of sexual differentiation. They treated pregnant guinea pigs with large doses of testosterone propionate resulting in genetically female hermaphroditic offspring having external genitalia similar to newborn males. These hermaphrodites were gonadectomized shortly after birth and were later used to test their responsiveness to estrogen, progesterone and testosterone. The sexual behavior of the hormone treated animals was observed in the presence of both male and female partners. An increase in male-like mounting behavior and a decrease in female lordosis behavior was observed. It was concluded that androgen administered prenatally has an organizing action on the neural tissues mediating mating behavior by producing a responsiveness to exogenous hormones in adulthood.

Many similar experiments were subsequently done supporting this theory and extending it to other species. The majority of these experiments have been carried out



































Figure 3: Effects of prenatal stress on the average number of mountings performed by those mice attempting mounting. The mountings were observed over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers were stressed by exposure to bright light during the last two weeks of pregnancy. The mice used were of the strain AB-Y.

Group C-1 = Control males, first mating  
Group C-2 = Control males, second mating.  
Group E-1 = Experimental males, first mating  
Group E-2 = Experimental males, second mating

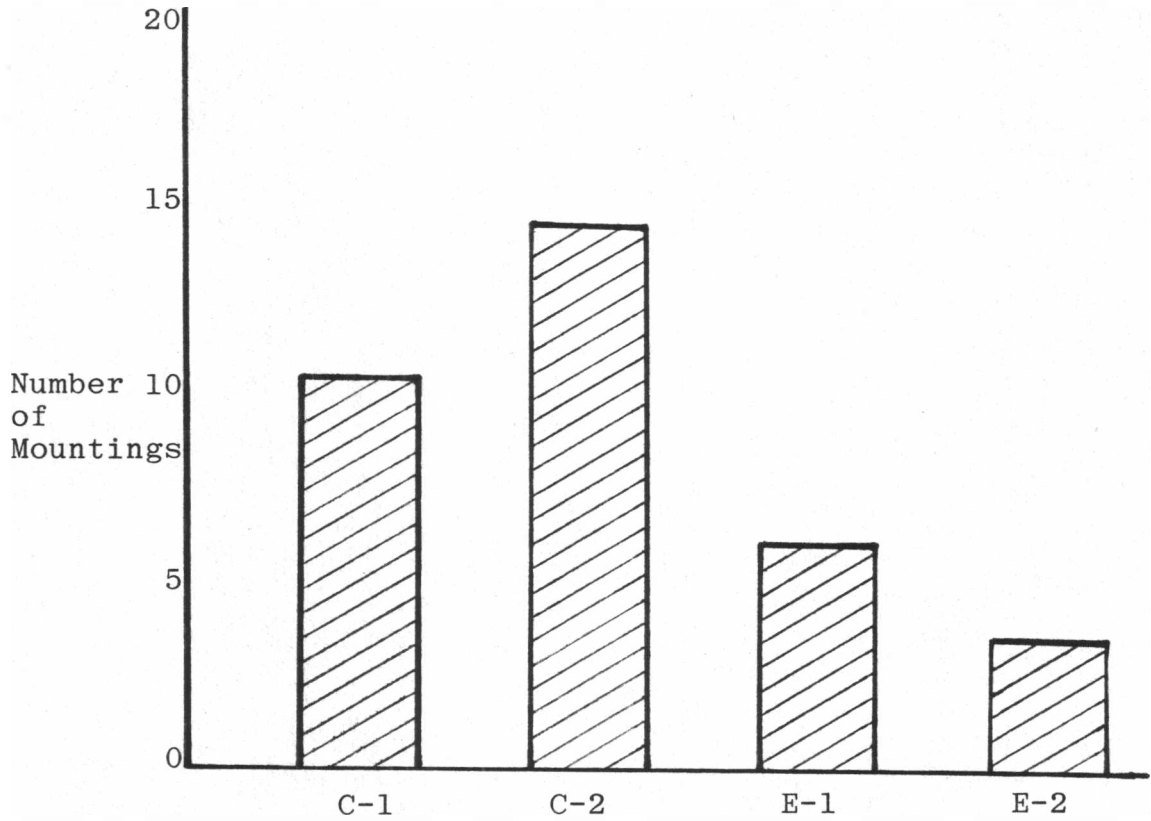


Figure 4: Effects of prenatal stress on the time elapsed (in minutes) before the first mounting in those mice attempting mounting. The mountings were observed over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers were stressed by exposure to bright light during the last two weeks of pregnancy. The mice used were of the strain AB-Y.

Group C-1 = Control males, first mating  
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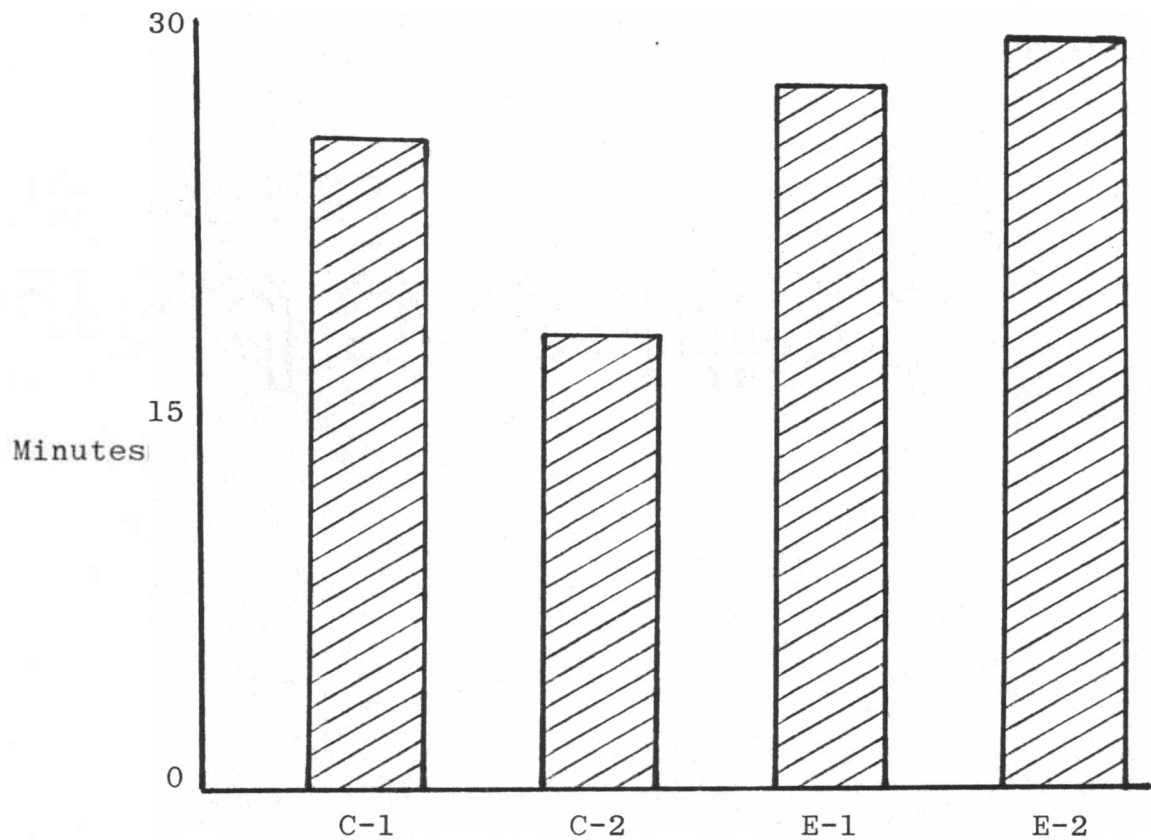


Figure 5: Effects of prenatal stress on the average duration of mountings (in seconds) by mice attempting mountings. The mountings were observed over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers were stressed by exposure to bright light during the last two weeks of pregnancy. The mice used were of the strain AB-Y.

Group C-1 = Control males, first mating  
Group C-2 = Control males, second mating  
Group E-1 = Experimental males, first mating  
Group E-2 = Experimental males, second mating

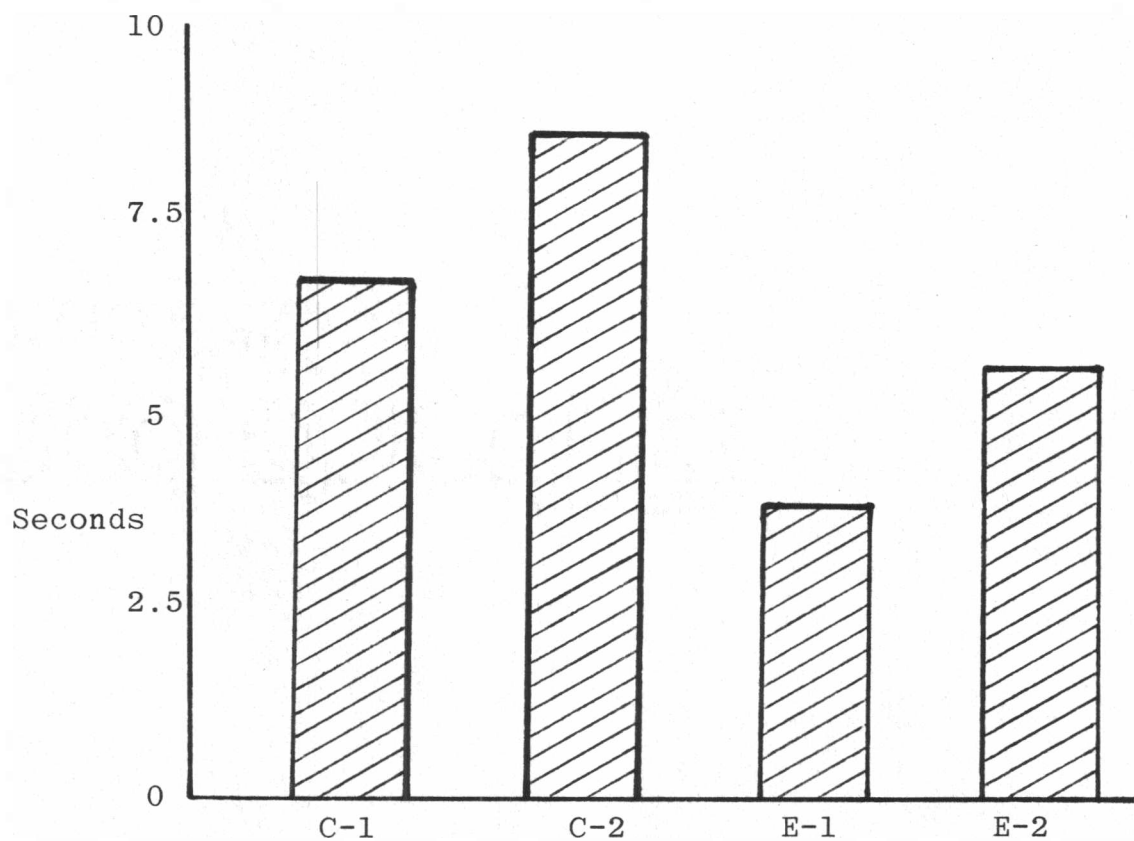


Figure 6: Effects of prenatal stress on the total mounting time (in minutes) by those mice attempting mounting. The mountings were observed over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers were stressed by exposure to bright light during the last two weeks of pregnancy. The mice used were of the strain AB-Y.

Group C-1 = Control males, first mating  
Group C-2 = Control males, second mating  
Group E-1 = Experimental males, first mating  
Group E-2 = Experimental males, second mating

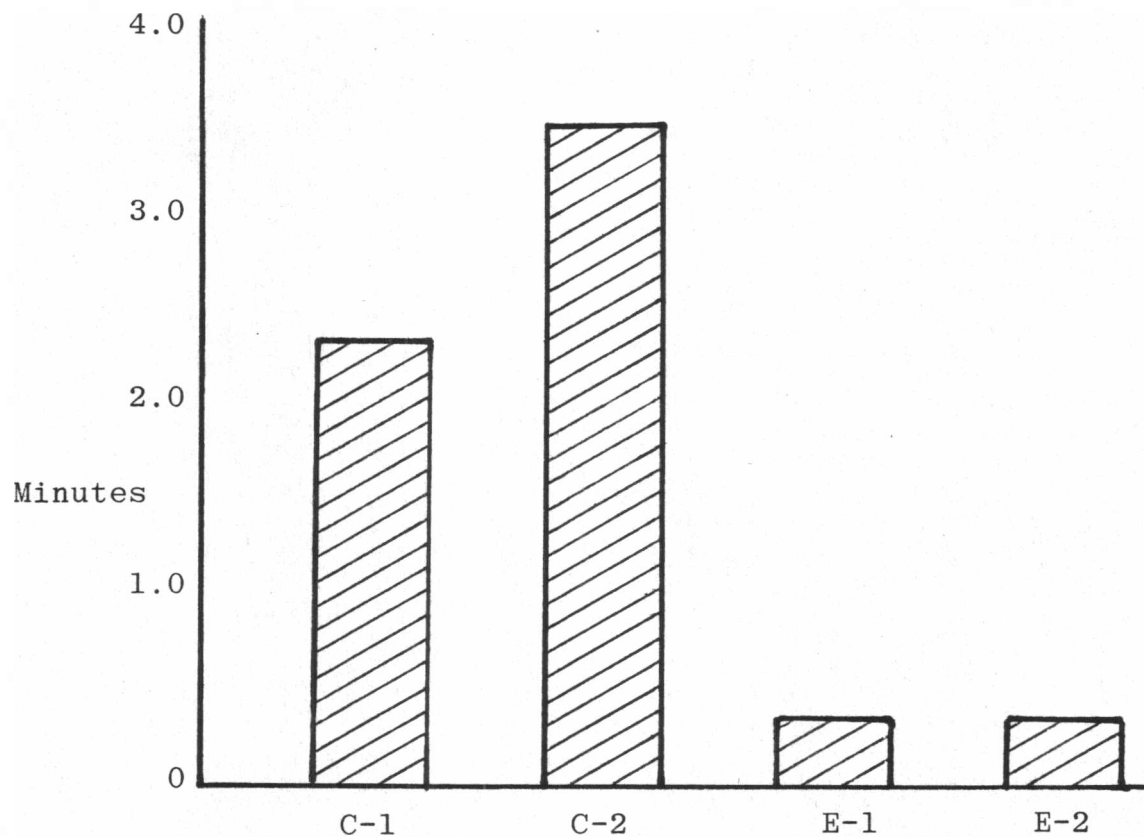


Table 2. Initiation approach behavior of control and prenatally stressed male mice.

Group	Mean Approach Rate/minute + S.E.M.*	Time Elapsed Before First Sexual Approach (minutes) + S.E.M.*
Group C (control group all matings)	2.93 ± 6.98	3.85 ± 3.05
Group C-1: (control group first mating)	1.01 ± 0.97	4.06 ± 3.17
Group C-2: (control group second mating)	1.03 ± 0.75	4.11 ± 3.1
Group E: (experimental group all matings)	0.31 ± 0.34	13.1 ± 18.2
Group E-1: (experimental group first mating)	0.25 ± 0.32	18.8 ± 23.8
Group E-2: (experimental group second mating)	0.38 ± 0.36	6.72 ± 4.63

Note: The mothers of the experimental group were subjected to stress during the last two weeks of pregnancy. This stress consisted of exposure to bright light, confinement, and temperature increase. The number of mounting attempts was observed and recorded during 60-minute trial periods. The mice used were of the strain AB-Y.

\*S.E.M. = Standard error of the mean

Figure 7: Effects of prenatal stress on the initiation approach rate in male strain AB-Y mice. The approach behavior was recorded over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers were stressed by exposure to bright light during the last two weeks of pregnancy.

Group C-1 = Control males, first mating  
Group C-2 = Control males, second mating  
Group E-1 = Experimental males, first mating  
Group E-2 = Experimental males, second mating

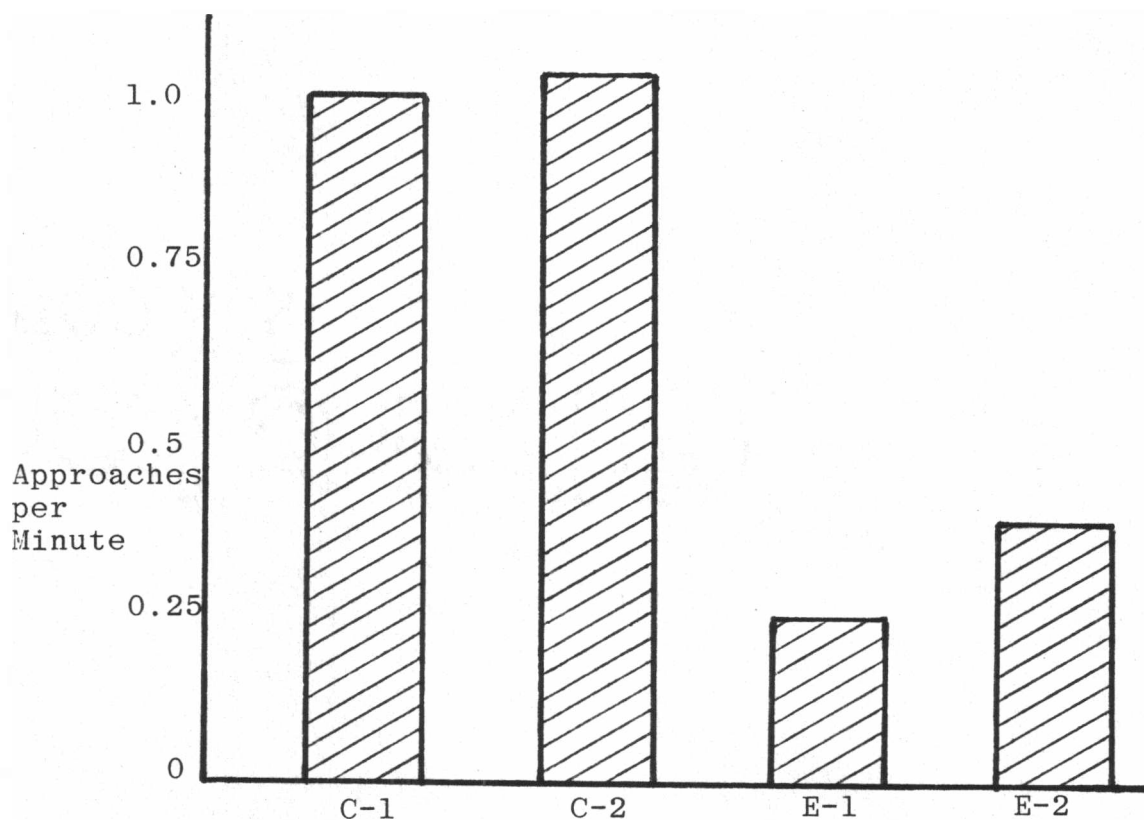
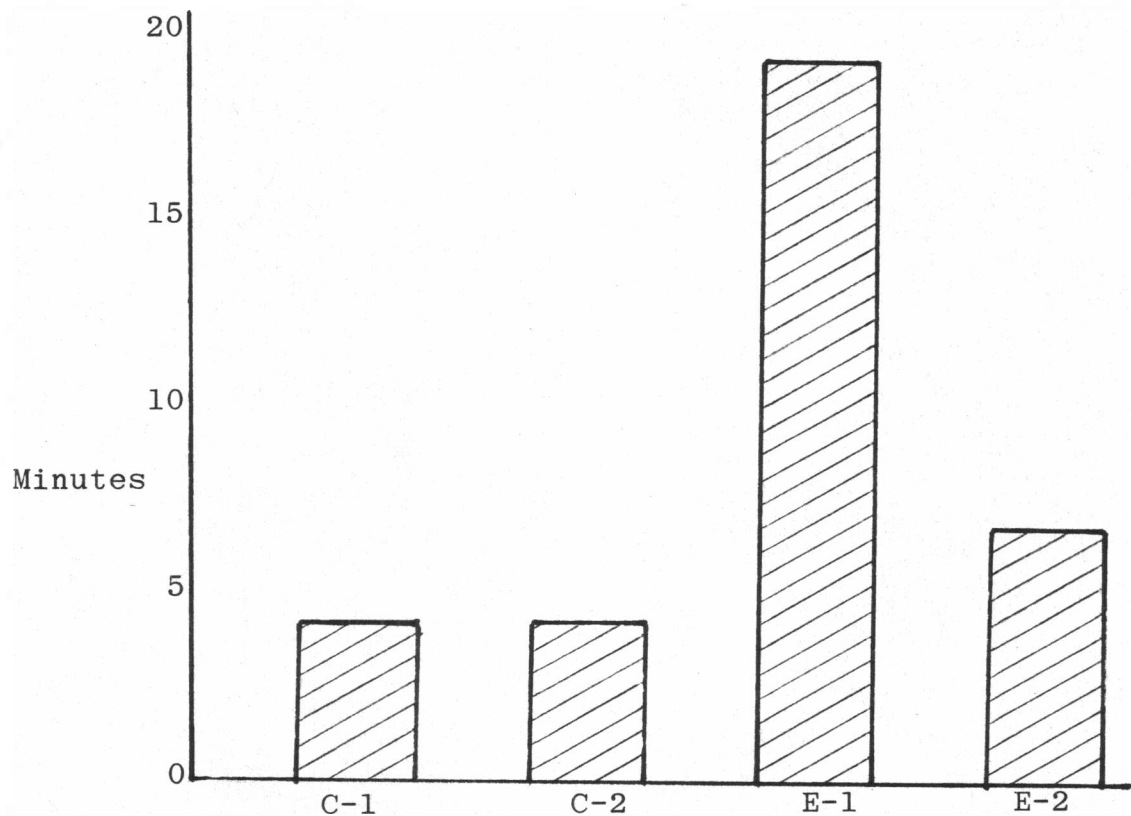


Figure 8: Effects of prenatal stress on time elapsed (in minutes) before the first sexual approach in male mice of the strain AB-Y. The approach behavior was recorded over 60-minute observation periods with estrus females. The experimental group was composed of prenatally stressed males whose mothers had been exposed to bright light during the last two weeks of pregnancy.

Group C-1 = Control males, first mating  
Group C-2 = Control males, second mating  
Group E-1 = Experimental males, first mating  
Group E-2 = Experimental males, second mating



## DISCUSSION AND CONCLUSION

### Effects of Prenatal Stress on Adult Sexual Behavior

The purpose of this experiment was to determine if prenatal stress causes a decrease in adult sexual behavior in male mice. Various statistical tests were used in analyzing the difference in sexual behavior between the prenatally stressed and control groups. Two major aspects of male sexual behavior were examined. These were the effects of stress on mounting behavior and on the sexual initiation approach. The tests showed significant differences between the experimental and control groups in adult male sexual behavior.

Mounting Behavior: Statistically significant differences were observed between the experimental and control groups in many aspects of mounting behavior. There were significantly less mountings attempted by the prenatally stressed males. Although the actual percentage of mice attempting mountings in each group was not statistically significant, there was obviously a higher percentage of control males which mounted. In addition, there was significant difference between the two groups in the amount of time it took the male to mount the female after introduction. There was also significant difference between the

control and experimental groups in the total time spent in the mounting position. There are a number of comparisons in mounting behavior between the two groups which did not show to be significantly different. As mentioned above, the chi-squared analysis of the proportion of mice from each group did not show any significant difference. The mean values of several aspects of mounting did not prove to be significantly different between the control and experimental groups. These include such aspects as the number of mountings, elapsed time before the first mounting, average length of mounting, and total time spent in the mounting position. In addition to these mean values, there was no significant difference between the variance levels in either the time elapsed before the first mounting or the individual mounting duration. The lower levels of mounting behavior seen in the experimental group suggests that the mice were demasculinized by prenatal stress.

Sexual Initiation Approach: The second aspect of male sexual behavior which was observed and compared between the control and experimental groups was that of the sexual initiation approach. This measurement of sexual behavior is generally not needed in mating studies done on the rat due to the more clearly defined mounting behavior. It was described in Hafez (1970) for the mouse and was used to supplement the behavioral comparison between the control and prenatally stressed male mice. This was necessary due to the low levels of copulatory behavior observed

in the control males in preliminary mating trials of this experiment. The time, after introduction, of the first approach was recorded as well as the number of approaches. The number of approaches were recorded up until the first mounting or for the remainder of the 60-minute trial period if mounting did not occur. The recording of approaches was stopped after mountings began due to the difficulty in recording both mounting and approach behavior. Both the approach rate and the elapsed time before the first approach showed significantly less male behavior in the prenatally stressed experimental group. This presents additional evidence that the males resulting from stressed mothers were demasculinized.

#### Interpretation of the Results

Although some of the behavioral characteristics analyzed did not show to be significantly different under the 5% confidence interval used, enough difference was seen between the experimental and control groups to support some general conclusions. The decrease in both mounting and sexual initiation approaches in the prenatally stressed males suggests that they underwent some demasculinization. It appears that the male mice of the strain AB-Y are capable of showing some aspects of the prenatal stress syndrome seen in various strains of rats studied. This evidence supports the theory that the prenatal stress syndrome also applies to mice. The data also seems to support

the theory that the mouse parallels the rat in the timing of the critical period for sexual differentiation of the CNS. The extent of demasculinization observed in the prenatally stressed mice was by no means complete. This supports the theory that varying amounts of stress can produce differential demasculinization in rodents.

#### Possible Improvements in Experimental Technique

The failure to display statistically significant differences between the experimental and control groups in mounting behavior may be attributed to the very small sample size. This small sample size for the mounting behavior comparison is partially due to the unexpectedly low percentage of mice mounting in both the control as well as the experimental groups. These small sample sizes were also the result of a very low survival rate among the prenatally stressed litters. Only 34% of the prenatally stressed pups lived longer than 30 days. In the control litters, 76% of the offspring lived past day 30. This significant decrease in survival rate may be due to delinquent mothering behavior in the stressed females (Herrinkohl, 1979). The results of the experiment could be made more conclusive by using a much larger number of prenatally stressed male mice. This could be accomplished by starting with a larger number of stressed pregnant females. Another method of achieving more conclusive results would be more extensive observation of mating behavior in order to make

a better comparison between the control and experimental groups. This could be done by using longer mating periods or by recording more trials than were recorded in this study (two). A final improvement that could be made would be to let the males grow accustomed to the breeding chamber before introducing the females for the mating trials.

#### Possible Continuations or Advancements of the Experiment

The study was limited by the lack of materials and equipment. Further studies could be carried out on mice involving perinatal treatment with hormones. The reported studies involving perinatal treatment of mice with androgen seem inconclusive. Another important study that could be done would be the direct measurement of the effects of perinatal stress on testosterone levels in mice. Radio-immunoassays for testosterone could be employed to monitor the change in testosterone levels during stress.

#### Purpose of the Prenatal Stress Syndrome

The prenatal stress syndrome characterized by demasculinization and feminization of male offspring resulting from stressed mothers is thought by some to serve an ecological purpose. This purpose may be in the form of population reduction in conditions of overcrowding. It has been demonstrated that overcrowding produces significant physiological stress in rodents. This stress could cause demasculinization of the resulting male offspring. Demasculinization of a significant number of males in a population

would greatly decrease the population growth (Ward, 1972).

Extention of Prenatal Stress Syndrome to Humans

The question arises as to the possible extrapolation of the rodent prenatal stress syndrome to human beings. It was initially thought that stress in humans may play a role in homosexuality which would be analogous to demasculinization and feminization in rodents. Studies on the effects of androgen exposure on the human brain and primates, however, have failed to show any effects on differentiation of the control system governing cyclic gonadotropin secretion or behavior (Treloar, et. al., 1972; Karsch, et. al., 1973; and Resko, 1974).

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