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The anterior commissure : the effects of sex and prenatal stress

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Abstract

The effects of prenatal stress (light/restraint) on the development of the anterior commissure (AC) were investigated. The AC is known to be a sexually dimorphic structure of the brain not directly involved in reproductive behavior; unlike hypothalamic structures and nuclei, however, little is known about its development. The present work examines two factors, sex and stress, known to influence other brain areas. Pregnant rats were assigned to prenatal treatment and control groups. The treatment group was stressed thrice daily for thirty minutes using light /restraint during the third trimester (day 14-21). Control dams remained undisturbed. Male and female offspring were killed between days 90-100 of life. The brain was coronally sectioned and stained with thionin. The AC of each animal was measured for area (mm^2)and volume (mm^3). Results indicated control females had a greater AC area and volume relative to control males. Prenatally stressed males had a significantly greater area and volume of AC relative to control males. The neuroanatomical differences support the hypothesis that factors operating in the prenatal environment affect sexually dimorphic structures in the brain not directly involved in reproduction.

[Model Approval Page]

I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts/Science.

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The Anterior Commissure: The Effects of Sex and Prenatal Stress.

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The Anterior Commissure: The Effects of Sex and Prenatal Stress

Gonadal hormones exert powerful organizing and activating influences on the sexual differentiation of the mammalian brain. The brain is ultimately responsible for sexual dimorphism in behavior and reproductive physiology. Recently, attention has been focused on the neuroanatomical sex differences that may underlie these functional disparities. In specific areas of the brain responsible for sexually dimorphic function, structure, nuclei and nerve cells differ between the sexes (Pfaff, 1966; Dorner & Staudt, 1968). Further, these sexually dimorphic results appear across species. Sexually dimorphic dendritic branching patterns of neurons in the preoptic area (POA) have been observed in the rat (Greenough, Carter, Steerman & DeVoogd, 1977; Hammer & Jacobson, 1987), hamster, rhesus monkey (Meyers & Gordon, 1982) and macaque monkey (Ayoub, Greenough, & Juraska, 1983). Other intracellular and ultrastructural differences in the rat include synaptic organization in the POA (Raisman & Field, 1973), arcuate nucleus (Matsumoto & Arai, 1981) and medial amygdala (Nishizuka & Arai, 1981). Other sexual dimorphisms have been recognized in the bed nucleus of the stria terminalis in the rat (BNST) (Del Abril, Segovia, & Guillamon,1987) and guinea pig (Hines, Davis, Coquelin, Goy & Gorski, 1985). There is also a striking sex difference in the morphology of the rat medial preoptic area (MPOA) (Gorski et al., 1978, 1980; Hsu, Chen & Peng,1980; Bleier, Byne & Siggelkow, 1982; Young, 1982). One nucleus in this area, the

sexually dimorphic nucleus of the preoptic area (SDN-POA), is much larger in male rats relative to females (Gorski, Gordon, Shryne & Southam, 1978). The sex difference is so apparent that it can be observed in stained sections without the benefit of magnification. The difference appears due to the number of neurons in the area (Gorski, Harlan, Jacobson, Shryne & Southam,1980). A similar sexual dimorphism has been demonstrated in the guinea pig (Hines, Davis, Coquelin, Goy & Gorski, 1985), gerbil (Yahr & Commins, 1982), ferret (Tobet, Gallagher, Zahniser, Cohen & Baum, 1983) and quail (Panzica, Viglietti-Panzica, Calacagni, Aneselmetti, Schumacher & Balthazart,1987).

Most of these sexual differences have been observed to result from the action of steroid hormones during a critical and sensitive period of perinatal sexual differentiation. In these areas, there are a large number of neurons which bind steroids during early development (Sheridan, Sar & Stumpf, 1975; Vito, Weiland, & Fox, 1979). Hormonal manipulations at the same time influence the sex differences in these neural regions (Raisman & Field, 1973; Matsumoto & Arai, 1981; Nishizuka & Arai, 1981). The size of the SDN-POA is determined by the perinatal environment. Evidence for this was demonstrated by Dohler, Coquelin, Davis, Hines, Shryne & Gorski (1982) with the administration of testosterone to female rats during the late prenatal and early postnatal life. Results indicated a SDN-POA that is similar in size to that of a control male rat. Manipulations of hormones in adult rats were not found to affect the size of the nucleus (Gorski et al., 1978).

The scientific community has recently investigated structural differences in brain regions not directly related to sexual function. Sexually dimorphic patterns of hippocampal and cortical asymmetries are present in the rat (Diamond, 1988). Additionally, sexually unique asymmetry in the midsagittal area of the corpus callosum (CC) of the rat was observed (Berrebi, Fitch, Ralphe, Denenberg & Denenberg, 1988). The number of axons and the extent of myelinization (Juraska & Kopick, 1988) in the CC varied depending upon the sex of the animal.

Another structure that may be sexually dimorphic and not directly related to sexual function is the anterior commissure (AC). This fiber structure is a distinct white-matter tract with a shape that resembles a mustache. The AC transects the midline at the upper end of the lamina terminalis, immediately anterior to the anterior columns of the fornix. On either side of the midline, the AC curves antero-inferolaterally and then postero-inferlaterally through the lateral nucleus of the globus pallidus to course with the white matter of the external and extreme capsules toward the middle and inferior temporal gyri (Naidich, Daniels, Pech, Haughton & Pojunas,1986). In summary, the AC is a fiber tract of axons connecting the right and left hemispheres (Fox, Fisher, & Desalva, 1948; Jouandet & Gazzaniga, 1979).

The subcortical path of the AC consists of three branches, the pars olfactoria, pars interhemispherica and the commissural component of the stria terminalis. The third limb of the AC has been clearly mapped out for the rat (De Olmos & Ingram, 1972) the rabbit (Van Alphen, 1969) and primate (Jounandet & Gazzaniga, 1979). The pars olfactoria and pars

interhemispherica have been investigated in the human (Klinger & Gloor, 1960) and rhesus monkey (Fox, Fisher & Desalva, 1948) but has yet to be examined in the rodent. In summary, although the third limb of the AC has been examined in various mammals and, in the rat, the bed nucleus of the stria terminalis (BNST) is sexually dimorphic (Hines et al., 1985) much work remains to be done exploring the development and size of the entire AC.

Development of the AC

The development of the AC in humans has been observed to appear during the eighth embryonic week (Muller & O' Rahilly, 1990). However, it is not known when the AC sexually differentiates in humans or if the entire AC is sexually dimorphic in rodents. In animal models, the perinatal projections of the AC were examined in hamsters. By the 15th gestational day, the fibers crossed the midline entering the opposite hemisphere and reaching the borders of their target regions (Lent & Guimaraes, 1991). In the mouse, the AC was labeled with a fluorescent dye, DiI, on embryonic day 16. The embryos that were allowed to develop to term indicated that the early connections were similar to those of adult connections in their selection specificity of a target area (Snyder, Coltman, Muneoka & Ide,1991). These early yet distinct connections may exert a powerful influence on the formation of future connections during postnatal development. Although information exists about the period of development of the AC, questions still remain as to the exact factors which lead to the difference in AC size in the human (Allen & Gorski, 1992) and possibly the rat. Differences in neuronal axons, myleninization, connective tissue, or glia, may reflect the discrepancy in size between the sexes. In

human subjects, differences in AC were reflected in the number of axons not their density (Tomasch, 1957). However, in the rhesus monkey, the size of the AC did not correlate with number of axons (LaMantia & Rakic, 1990).

Although the exact time at which the AC sexually differentiates has not been demonstrated, genetic factors, environmental influences and or gonadal hormone levels may play a role in its sexual differenentation (Allen & Gorski, 1992). Sexually dimorphic structures in rats such as the MPOA, SDN-POA, cerebral cortex, CC and BNST are influenced by prenatal and postnatal environmental factors. Prenatal stress feminized the cerebral cortical asymmetry of the male. The cortices of the prenatally stressed males were more symmetrical resembling those of control females (Fleming, Anderson, Kinghorn & Bakaitis, 1986). The alteration of cerebral asymmetry in the males was aleviated with exposure to an enriched environment. The males exposed to alcohol exhibited a greater cerebral asymmetry, resembling that of control males, after postnatal exposure to an enriched environment (Diamond, 1988). The CC has additionally been found to be altered by prenatal exposure to alcohol (Zimmerberg & Scalzi, 1989). Prenatal alcohol exposure feminized the size of the CC in the midsaggital plane. Prenatal alcohol exposure reduced the callosal size in males only. Each of these environmental factors significantly affected the males and left the females unaltered.

The differing results of environmental factors on males and females suggest neonatal hormones play a role in the sexual differentation of structures. Sexually dimorphic structures examined thus far are

influenced by perinatal exposure to gonadal hormones. Alcohol and prenatal stress of light/restraint alter the normal testosterone surge in male fetuses on days 18 and 19 of gestation (McGivern, Raum, Salido & Redei, 1988). Therefore, environmental factors influence the AC in a sexually dimorphic manner by disrupting the perinatal hormones.

Since there is an uptake of gonadal hormones in the cerebral cortex of the maturing rat (Sheridan, Sar, & Stumpf 1975; MacLusky, Naftolin & Goldman-Racik, 1986) and a depletion of neurons and their axons during development (Berlucchi, 1981), it is possible that gonadal hormones influence the number of axons and neurons of the AC. It is also of consideration that sex differences in the AC may reflect different influences of gonadal hormones upon myelination (Curry & Heim, 1966) during development, since 5-alpha reductase, the enzyme responsible for converting testosterone to dihydrotestosterone, is inversely related to myelination (Celotti, Melcangi, Negri-Cest, Ballabio & Martini, 1987).

Functional Significance of the AC

The purported sexual dimorphism of the AC, CC and cerebral hemispheres are less pronounced and more difficult to explain compared to the sexually dimorphic nuclei that are believed to underlie sexually dimorphic reproductive function. The sexually dimorphic nuclei are greatly dimorphic as are the functions they regulate. Sexual differences in areas of the brain not directly related to reproductive function are more difficult to explain since the functional significance of these structural sex differences are not known as yet. It is recognized that the AC and CC are in the areas of the brain which regulate cerebral lateralization and cognitive skills that

display subtle sex differences between males and females. Allen and Gorski (1992) examined the area of the AC in heterosexual males and females in addition to homosexuals of both genders. They observed a heterosexual sex difference in that women had larger ACs relative to men. In the homosexual population, males exhibited a larger AC when compared with heterosexual men and women. The results supported the hypothesis that early developmental influences on sexual differentiation are global. It was possible that differences in the AC represent no significant functional difference but are a result of different metabolic influences of estrogen (Curry & Heim, 1966) and testosterone (Celotti et al., 1987) on myelination. Sex differences in numbers of axons in the AC may reflect differences in transfer of information and may underlie sex differences in asymmetries (McGlone, 1980; Beaton, 1985; Kimura, 1987) and or cognitive function (Kimura, 1987).

LaMantia & Rakic (1990) examined the structural composition of the AC and CC. Using monkeys, the researchers found that axon composition in the AC is uniform and like that of callosal sectors that contain association projections. The CC and the AC, the two largest of the four cerebral commissures, interconnect the frontal, parietal, temporal and a smaller part of the occipital cortices of each cerebral hemisphere. The interhemispheric pathways play a major role in higher cortical function. Further, increases in interhemispheric connectivity were proposed to explain differing extents of hemispheric lateralization and interhemispheric processing in males versus females.

The composition of the AC sparked the emergence of anatomical and cytological questions that emerge from intriguing and controversial issues. These questions include: What are the precise boundaries of the AC and how many axons does it contain? Are there differences between the types of axons and other cellular elements within the AC? LaMantia & Rakic (1990) discovered there is a simple organization of glial cells in the AC. In addition, the AC has predominately small and medium sized myelinated axons, occasional large axons and a small percentage of unmyelinated fibers. By dividing the sample taken along the orthogonal diameter in the anterior to posterior dimension or the dorsal to ventral dimension no differences in axon density were present. After dividing the AC into quadrants, systematic sampling further revealed there were no significant differences in axon density, the proportion of myelinated axons or mean axon size.

Further, the researchers found a significant correlation between axon number and area of the AC (LaMantia & Rakic, 1990). The relationship between axon diameter and conduction velocity has been established as a physical mirror of functional organization (Gasser, 1950).

The AC has been documented to transfer interhemispheric sensory information in primates and man. In humans, the AC governs the interhemispheric transfer of visual, auditory and olfactory information (Risse, LeDoux, Springer, Wilson & Gazzaniga, 1978). In macaque monkeys the AC plays a role in inhibiting bilateral formation of engrams, thereby increasing both functional asymmetry and pneumonic storage capacity of the brain by preventing repetition (Black & Myers, 1964; Doty &

Overman, 1977). The AC may be responsible for discrimination difficulties involving information about left-right mirror images, since monkeys with lesioned ACs are able to differentiate more accurately between left-right mirror images (Achim & Corballis, 1977). It is presently unclear, however, whether any of these functions is sexually dimorphic or whether the actual number of neurons and or midsagittal area of the AC has a bearing upon these functions.

The role of the AC in rodents is less known. It has been demonstrated that the AC is comparable to the hippocampal commissure and the neocortical commissural system, the CC. The anterior wing of the rodent AC connects the two main olfactory bulbs. The fibers of the AC originate in the contralateral accessory olfactory nucleus (AON) and terminate mainly in the internal plexifom (ipl) and granule cell (gcl) layers of the olfactory bulb, where they synapse with granule cells (Macrides & Davis, 1983). In a study conducted by Nickell & Shipley (1993) they demonstrated that AC terminals are regulated by cholinergic action and are therefore responsible for coordinating the transfer of information between the two olfactory bulbs.

Although we know that the AC plays a role in the interhemispheric transfer of olfactory information (Bennett, 1968), the extent to which the rodent is dependent on the AC for the normal transfer of olfactory information is less clear. In an early study by Swann, (1934) three rats had lesions of the olfactory tract. One, with bilateral destruction of the lateral olfactory tract (LOT) and unilateral transection of the anterior limb of the AC had only slight deficits in odor discrimination. The other subjects

had bilateral damage to the LOT and bilateral damage to the AC and had much more severe deficits. Based on his results, Swann suggested that the AC was more critical than LOT for olfaction. Bennett (1968) has also demonstrated that bilateral lesions of the AC reduced olfactory acuity. Lesions of only the anterior limb of the AC produced slight deficits in odor discrimination (Slotnick & Schoonover, 1992). In contrast, Long and Tapp (1970) have failed to find deficits in simple odor discrimination after a bilateral lesion of the AC. Differences in the findings may be explained in two different ways. Olfactory function may be spared after lesions to different parts of the system due to the simplicity of the task or high concentrations of odors. In addition, the plasticity of the olfactory sense may be due to the mass organization of the olfactory projections to the olfactory cortex. Anatomical examinations indicated that the olfactory bulb projections to the olfactory cortex are not topographically organized but are broad and overlapping (Luskin & Price, 1982). It is possible, then, that olfactory information is encoded in the cortex in a manner in which any one odor would activate many cells and each cell would participate in the coding of many odors. The slight deficits in impairment and resilient olfactory system may be demonstrated behaviorally in the above studies and reflect the redundancy inherent in an ensemble coding mechanism (Slotnick et al, 1992). In summary, there are multiple pathways for the projection of olfactory impulses from the olfactory bulb to the forebrain. These projections to the olfactory cortex appear to be relatively diffuse and may be responsible for the limited success of the prior studies. One statement which can be drawn from previous investigations is that the AC

is involved in the transmission of olfactory information. Since the AC plays a role in the transfer of olfactory information, and rodents are highly dependent upon olfactory cues for sexual behavior, there may be a difference in performance of sexual and nonreproductive behaviors if maximal lateralization is dependent upon normal maturation.

Prenatal Stress

In numerous species there is evidence of sex differences in the control of endocrine function and behaviors of the reproductive process via the central nervous system (CNS). Sex differences in the CNS are a result of an interaction between gonadal hormone secretion and the character of the substrate on which it acts (MacLusky & Naftolin, 1981). In mammals, the "default" pattern of development is female. Masculine differentation, gonadotropin secretion and male typical behavior, occurs only with the exposure to testicular hormones during critical periods of development (Gorski, 1971). Species differ in the extent to which CNS functions are mediated by perinatal gonadal hormone exposure. For rodents, early exposure to gonadal hormones from the maturing testes permanently supresses the cyclic female pattern of gonadotropin secretion and facilitates the tonic release pattern of the typical male. There are two independent processes in the supression of female functions and the expression of male characteristics. The supression of neuroendocrine patterns and behaviors typical of the female is known as defeminization, whereas the enhancement of typical male patterns is masculinization (Beach, 1971). The absence of androgens or alterations in the timing of exposure have effects on the sexual differentation of the mammal. One

mechamism which disrupts the exposure to androgens necessary for male differentaion is prenatal stress.

Prenatal stress exhibits long-lasting effects on numerous behaviors and structures in a sex-dependent manner. In male rodents, prenatal stress affects intermale aggression (Kinsley & Svare, 1986), copulation (Ward, 1972) and parental behaviors (Kinsley & Bridges, 1988). Female rodents exposed to stress in utero display alterations in sexual behavior, pregnancy-induced postprtum aggression (Politch & Herrenkohl, 1979) and parental behavior (Kinsley & Bridges, 1988). Specifically, pregnant female animals subjected to a standardized stress regime of light/restraint stress thrice daily during the last trimester of gestation (days 14-21) demonstrated altered neonatal hormone levels causing a premature surge of circulating testosterone and then an absence of the hormone at the critical time needed for sexual differentiation in the brain (Ward, 1984). The malfunction in hormone fluctuation was linked to the demasuclinized and feminized sexual behavior of the male rat. Other hallmarks of sexual differentiation were also examined. An important enzyme utilized for the synthesis of testosterone by Leydig cells, 5-alpha-3*B*-hydroxysteroid dehydrogenase, has been observed to exhibit the same abnormal pattern as that of testosterone. The alteration in testosterone levels are believed to be the result of abnormalities in the steroidogenic activity of the fetal gonads. Stress changes the 5-hydroxytryptamine (5-HT) receptor binding in many brain areas of the adult offspring and modifies the behavioral responses to 5-HT receptor agonists. Specifically, maternal free

tryptophan, 5-HT, and 5 hydroxyindoleacatic acid were at abnormal levels in the brains of prenatally stressed offspring (Peters, 1990).

The standardized stress regime used in the many of the previous laboratory experiments consisted of a combination of restraint and light, both of which are noxious to the rat. Commencing on day 14 of pregnancy (1st day of pregnancy = 0), and continuing until day 21, pregnant rats are placed three times daily for 30 minutes into plexiglass restraint tubes illuminated by flood lights delivering 200 foot candles of light. To verify that the treatment was stressful, plasma levels of corticosterone were measured in unstressed mothers and their male and female fetuses, as well as in unstressed mothers and offspring sacrificed in the middle of a stress session on days 18 and 21 of gestation (Ward & Weisz, 1984). The researchers observed elevated levels of corticosterone in the stressed animals as compared to the unstressed controls. Therefore, it was concluded that the treatment imposed upon the mother stresses her and by some as yet unknown mechanism she transmits the stress to her fetuses.

Additional investigations of how prenatal stress affects behavior, physical characteristics and brain development in the rat have yielded some intriguing results. Stressed rats produced male offspring that demonstrate differences in the sexually dimorphic nuclei in the spinal cord (Grisham, Kerchner & Ward, 1991). These nuclei in the spinal cord house motor neurons innervating muscles in the perineum that produce penile reflexes during erection and ejaculation (Breedlove & Arnold, 1983). It was found that there was a decrease in the number of dorsolateral nucleus

bulbocavernous neurons in prenatally stressed animals (Grisham, Kerchner & Ward, 1991). This morphological difference may underlie the alterations in the mechanics of the sexual behavior of prenatally stressed male rats.

Further, researchers have shown a morphological difference in the preoptic area of the brain known as the sexually dimorphic area of the preoptic area (SDN-POA) (Gorski, Harlan, Jacobson, Shryne & Southam, 1980). It was demonstrated that a reduction of perinatal androgen, via castration, will cause the SDN-POA to retain its inherent female character (Raisman & Field,1973). Prenatal stress which alters fetal testosterone levels in males, was found to be a factor in reducing the SDN-POA by 50% in males whereas females SDN-POA was not affected (Anderson, Rhees & Fleming, 1985).

Behavioral observations of prenatally stressed animals reveal feminized juvenile play behavior of males (Ward & Stehm, 1991). It has been suggested that the hormonal events early in development affect brain asymmetry differently in the sexes. In rodents, prenatal stress affects brain lateralization. Alonso, Castellano & Rodriguez (1991) reported a right -bias of lateralization in the control group, with females exibiting less absolute laterality relative to males. Prenatal stress decreased the degree of absolute laterality in males but increased it in the females. Therefore, the results suggest that lateralization is influenced by prenatal levels of gonadal steroids.

Other behavioral measures such as an open field test demonstrated a significant increase in emotionality, evidenced by higher

incidence of defecation, in both sexes of the stressed offspring (Wakshalk & Weinstock, 1990) and a decrease in ambulatory behavior (Suchecki & Neto, 1991).

Prenatally stressed rats were able to more quickly detect the odor of their home cage relative to their control counterparts (Szuran, Zimmerman, Pliska & Pfister, 1991). One explanation for the superior olfactory performance by the stressed rats may be found in the role of the AC. Pups between 6 and 12 days have olfactory cross-projections housed in the AC that mature and give a functional vehicle for the transfer of olfactory learning (King & Hall, 1990). Findings by Kucharski, Burka and Hall (1990) that access to contralateral olfactory memories are mediated by the anterior limb of the AC provide further support for King and Hall's observations.

The AC may additionally serve to inhibit aggression in that lesion-induced hyperdefensiveness was exhibited in subjects with lesions in the anterior portion of the BNST which is the crossover region in the AC. This area is significant in the regulation of hormone dependent aggression (Albert, Petrovic, Walsh & Jonik, 1989).

Although it is unknown what effect stress might have on the number of neurons in sexually dimorphic regions of the brain, it is suggested that the elimination of neurons in the sexually dimorphic nuclei may be determined by the presence or absence of gonadal hormones during a critical period of development (Nordeen, Nordeen, Sengelaub, & Arnold, 1985).

The vomeronasal organ (VNO), accessory olfactory bulb (AOB) and BNST which carry olfactory information and play indirect roles in reproductive behavior are influenced by sex steroids (Del Abril, Segovia & Guillamon, 1987). The AC which also transfers olfactory cues may be influenced by perinatal androgens. Given that prenatal stress alters androgen exposure leading to differences in brain morphology and sexual and non-reproductive behaviors in the rat, we investigated the possible influence prenatal stress may have on the development of the AC. The demasculinizing effect on male offspring of mothers exposed to stress during their last week of gestation is due to a premature surge followed by an absence of testosterone during critical periods of brain development. The early elevation and subsequent absence of hormones alters the morphology of the brain without disturbing the external physical characteristics of the animal. The present study examined the hypothesis that there was a sex difference in the area and volume of AC and that in prenatally stressed animals the sex difference was in the opposite direction in the offspring of control dams.

Method

Subjects

Ten virgin female Sprague-Dawley rats 60 days of age were timed-mated (plug date = day 0 of pregnancy) and randomly assigned to prenatal stress or control groups. They were housed in 24 x 32 x 16 inch plastic maternity tubs with an ambient room temperature of 24 c and exposed to a 14:10 hour light: dark cycle. Light onset was 0500 to 1900. The lights

were turned off from 1900 to 0500 hours. Purina lab rat chow in pellet form and water were provided ad lib.

Design and Procedure

On day 14 of pregnancy the stressed animals were placed in a 6 3/4 cm Plexiglas tube and exposed to bright light (two 150 watt lights; approximately 200 lm/m) three times a day (0830 h, 1230 h and 1630 h) for thirty minutes from days 14 through 21 of gestation. The control animals were left undisturbed until parturition. After birth, (day 0) all pups were kept with their own litter mates until day 21 of age. At weaning, day 21, prenatally stressed offspring were housed in same sex pairs as were controls. In order to control for litter effects, only one male and one female from each litter were subjects in the experiment. The remaining animals were utilized in other laboratory investigations. Between 90 and 100 days of age, all males and females were weighed, overdosed with sodium pentobarbital and perfused through the left ventricle of the heart under gravity, first with physiological saline, followed by 10% formalin. Equal perfusion pressures were maintained for all animals. Following removal from the cranium, brains were fixed in a 10% formalin solution. The brains were blocked using a brain matrix (Kopf instruments) and a coronal section was removed from 1 cm anterior to the optic chiasm to 1 cm posterior the optic chiasm following the Paxinos and Watson (1986) rat brain atlas. Ten serial sections were made at 40 *u*m using a Zeiss cryostat maintained a -17 degree temperature. Specimens were mounted on slides subbed with gelatin, stained with thionin and coverslipped.

Following the histological procedures, two independent investigators, naive to the condition of the subject, traced the boundaries of each section of the AC at a magnification of 25 x, using a Bioquant imaging system, OS/2 software package. For greater sensitivity of measurement, a Summagraphics digitizing pad was employed to trace the perimeter of the AC. Boundary tracing commenced with the first section section of the AC observed to transect the midline. This section and the following three serial sections were used to obtain values of area and volume. The inter-rater reliability was assessed to ensure reliable measurements. The area, in microns (*u*m), of the AC was obtained by averaging the boundaries of the 4 individual nuclear tracings for each animal. The two measurments of the tracers were averaged and the subsequent number was adjusted into (mm^2) and used for analysis. The volume, (*u*m), of the AC was obtained by summing the 4 tracings of the individual tracers, multiplying by the section thickness (40 *u*m), averaged and converted into (mm^3).

Statistical Analysis

Correlations. Pearson's r correlations were conducted between the measurements of the two tracers.

MANOVA. A 2 x 2 between-subjects multivariate analysis of variance (MANOVA) was performed on the dependent variables of area and volume of the AC and animal body weight. Post-Hoc examinations using Duncan-t tests were performed on significant interactions. SPSSX MANOVA was utilized for the analyses. There were no univariate or multivariate within-cell outliers at = .0001. Evaluations of assumptions of

normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory.

Results

Body Weight.

The mean body weights for each sex and prenatal treatment group are shown in Table 1.

Table 1 about here

The MANOVA indicated a significant effect of sex on body weight, $F(1,36) = 150.02$, $p<.001$. Male body weights ($M = 641.43$) were larger than female body weights ($M = 328.7$). Prenatal treatment did not significantly affect body weight $F(1, 35)= .737$, $p = .486$. There was not an interaction between sex and stress conditions for body weight, $F(1,36) = .117$, $p = .734$.

Area .

A Pearson's correlation coefficient demonstrated a significant correlation between the two rater's measurements of area ($r= .90$, $p < .001$). For the dependent variable of area, no main effects of stress $F(1,36) = .783$, $p = .382$, or sex $F(1,36) = .177$, $p = .676$, were observered. There was a significant interaction between sex and prenatal treatment $F(1, 36) = 7.92$, $p<.008$.

Insert Table 2 about here

As seen in Figure 1, this interaction had several interesting components. First, control females had a significantly greater AC area (M = 2.01) compared to control males (M = 1.73), p<.05. The area of the AC of prenatally stressed males (M = 2.04) was significantly greater relative to control males (M = 1.73), p<.005. Prenatally stressed males (M = 2.04) and females did not differ. No differences were found between control (M = 2.01) and stressed females. Finally, control males (M = 1.73) and stressed females were not different nor were there differences between stressed females and control males (M = 1.73).

Insert Figure 1 about here

Volume.

Using Pearson's correlation coefficient there was a significant correlation between the two rater's measurements of volume (r =.90, p<.001). Table 3 demonstrates there were no main effects for the conditions of stress F(1,36) =.813, p =.373 or sex F(1,36) = .160, p =.69.

Insert Table 3 about here

Figure 3 demonstrates a significant interaction between sex and prenatal treatment was observed for the dependent variable of volume (mm^3) F(1,36) = 8.283, p =.007.

Insert Figure 3 about here

Specifically, control females had a significantly greater AC volume (M = 8.21) compared to control males (M = 6.91), $p<.05$. The volume of the AC of prenatally stressed males (M = 8.04) was significantly greater relative to control males (M = 6.91), $p<.005$. Prenatally stressed males (M = 8.04) and females (M = 7.60) did not differ. No differences were found between control (M = 8.21) and stressed females (M = 7.60). Finally, control males (M = 6.91) and stressed females (M = 7.60) were not different nor were there differences between stressed females (M = 7.60) and control males (M = 6.91).

Discussion

The present work demonstrates a sex difference in the AC of the rat. Specifically, control females have a greater AC area and volume in the coronal plane relative to control males. Prenatally stressed males had a greater AC area and volume relative to control males. Our results support the notion that maternal stress during the third week of gestation influences the morphological differentiation of the AC. The area and volume of AC, therefore, appears to be sensitive to hormonal alterations and may be determined by the hormonal environment prenatally.

The present study shows that maternal stress during the third trimester of gestation influences the ultimate morphological differentation of the male AC in the oppposite direction from that observed in control males. This adds additional evidence to the growing body of literature which has

found alterations in male sexual (Ward, 1972), aggressive (Kinsley & Svare, 1986) and male and female parental behavior (Kinsley & Bridges, 1988) as a result of prenatal stress. Alterations due to prenatal stress have also been found in brain structures responsible for sexual functions. The cross-sectional volume of the SDN-POA in stressed males was reduced relative to control males (Anderson et al., 1985). These results are not surprising, since disruptions of gonadal hormones may be the underlying mechanism for the observed effects of prenatal stress (Ward, 1984) and changes in perinatal hormonal environments alter SDN-POA morphology (Jacobson, Csernus, Shryne & Gorski, 1981). Additionally, hormonal disruptions appear to be the determining factor for the presence or death of sexually dimorphic spinal nuclei during a critical period of development (Nordeen et al., 1985). These data from independent laboratories provide empirical evidence that modifications in male behavior and morphology results from a failure to undergo the surge of testosterone during critcal periods necessary for sexual differentation. Thus, prenatal stress appears to demasculinize male behavior and morphology.

It has been well documented that many brain structures involved in sexual reproduction are sexually dimorphic (Pfaff, 1966: Dorner & Staudt, 1968; Gorski et al., 1978; Hines et al., 1985). Further, these structures are influenced by perinatal gonadal hormone exposure, and any alteration of the hormonal milieu, such as that produced by prenatal stress, influences males and females differently. Recently, other structures not directly related to reproduction were reported to be sexually dimorphic in the rat and altered by prenatal stress. Cerebral cortical asymmetry was reported

to be altered by prenatal stress (Fleming et al., 1986). The CC may also be influenced by the stress of maternal alcohol ingestion (Zimmerberg & Scalzi, 1989). Each of these environmental factors affected males and females differently.

It is plausible that gonadal hormones influence the area of the AC by influencing the area's number of axons or neurons. In addition, the size difference of the AC may mirror the different impact gonadal hormones have on myelination. Support for this hypothesis is found in the investigation of Curry and Heim (1966), who reported that estradiol increases myelination. Other work suggests a differential distribution of 5-alpha-reductase in the white matter structures of the brain; subcortical white matter such as the corpus callosum and optic chiasm were three times as effective as the cerebral cortex in converting testosterone to 17-beta-hydroxy-5-alpha-androstan-3-one (DHT) (Celotti, Melcangi, Negri-Cest, Ballabio & Martini, 1987).

Alterations of the in utero hormonal environment have led to alterations in brain structures and the behavior they govern. Specifically, prenatal stress has produced male offspring that display a demasculinization and partial feminization of sexual behavior. It has been suggested that the prenatal stress regimen serves as a model for investigating the mechanisms through which typical sexual differentiation are accomplished. Given that (1) during development, 5-alpha-reductase, the enzyme responsible for converting testosterone to dihydrotestosterone, is inversely related to myelination, (2) aromatization of androgen to estrogen may be required for the masculinization of the nervous system,

and (3) prenatally stressed rats have significantly lower levels of brain aromatase activity on days 18, 19 and 20 of gestation relative to controls (Weisz, Brown & Ward, 1982), it would be of interest in the future to examine the effect prenatal stress has on the testosterone conversion capability of the AC in the rat. The sexually dimorphic differences observed in the area of the AC may be a result of the main components of the white matter which have yet to be investigated in detail. For instance, if myelin sheaths, oligodendrocytes cell bodies, or axons convert testosterone into its metabolites, then perhaps differences in these structures might be quantified later in life and may be more abundant in females and prenatally stressed males in such structures as the AC.

The Prenatal Stress regime serves as a useful tool for investigating the mechanisms by which sexual differentation is achieved. Additionally, it has demonstrated a number of ways in which environmental stressors alter the pregnant dam's hormonal milieu in which the fetuses differentiate, resulting in altered behavior and anatomy in the male offspring. If tempered with caution, human applications for the prenatal stress model are interesting to speculate about.

For instance, in heterosexual humans, females demonstrate larger ACs relative to males. Additionally, homosexual males had larger AC's compared to both heterosexual men and women (Allen & Gorski, 1992). The results supported the idea that early developmental influences differentiate the structures of the brain in a global rather than specific fashion.

Our present observations are, to the best of the author's knowledge, the first demonstration of neuroanatomical evidence in the rat model which is in a similar direction to that of the human neuroanatomical evidence. Both appear to support the hypothesis that alterations in sexual behavior are related to alterations in prenatal hormone exposure which affect the brain in a global manner.

The conclusion that the prenatal stress model provides a direct explanation for homosexuality is premature without conclusive data. Extreme caution should be exercised when observing similarities in data among rodents, nonhuman primates and humans. There are unique mechanisms governing sexual differentiation of behavior in the rat and primate. In the rat, aromatization of testosterone to estradiol primarily directs the defeminization and masculinization of behavior potentials. The aromatization step is not required for the primate (Goy, 1981). Differences in sexually dimorphic behaviors after exposure to DHT between species may reflect the distinction in the necessity for aromatization. Fetal female rats administered DHT have masculinized reproductive morphology but feminized sexual behavior (Luttake & Whalen, 1970). In contrast, neonatal female rhesus monkeys exhibit masculinized morphology and behavior (Goy, 1981).

Although nonhuman animals appear to have a sexually dimorphic AC, it is recognized that animal models may pose difficulties for studying the AC in humans since the structure may change with advancing evolutionary development. For example, the rodent AC consists predominantly of the anterior limb, which contains primarily axons that

form cell bodies of the olfactory system; however, in primates, the anterior limb may be reduced to a few strands and the posterior limb, which contains a majority of axons from cell bodies of the temporal cortex (Fox et al, 1948; Jouandet & Gazzaniga, 1979). However, advancements in the resolution of imaging techniques may make it possible to more precisely quantify size and neuronal density allowing the correlation of the area of the AC with behavioral function and gonadal hormone exposure, thereby leading to an understanding of how environmental factors and or gonadal hormones sexually differentiate both the structure and function of the human brain.

In summary, the present investigation has demonstrated that there is a sex difference in the AC measured in the coronal plane. Control females have a greater AC area and volume than control males. Further, prenatal stress alters the area and volume of the AC. Although there was a tendency for prenatal stress to decrease the area of the AC in females, male offspring were most affected. Prenatal stress increased the area of the AC in males relative to control males. It appears, therefore, that prenatal stress- and its accompanying alterations of gonadal and adrenal hormones and neurotransmitters- modifies structures in the mammilan brain. Future investigations are needed to replicate our findings, in addition to elucidating the functional significance of the AC, possible mechanisms responsible for the altered sexual differentiation, and the many behavioral consequences.

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Table 1.

Mean Body Weights ± S.E.M. (g) in Adult Offspring From Control and Prenatally Stressed Treatment Groups.

Group	n	Body Wt. (g)
Control Males	10	632.5 ± 39.90*
Control Females	10	310.9 ± 12.90
Stressed Males	10	670.4 ± 25.40**
Stressed Females	10	346.4 ± 16.60

* Control male mean significantly different from control female mean
** Stressed male mean significantoly greater from stressed female mean

Table 2.

Means + S.E.M. for the Area (mm2) of Anterior Commissure in 4 Coronal Sections Control Males, Control Females, Prenatally Stressed Males and Prenatally Stressed Females.

Group	n	Area, mm^2	Body Wt. (g)
Control Males	10	1.73±1.23* ^	632.5±39.90
Control Females	10	2.01±2.36	310.9±12.90
Stressed Males	10	2.04±3.43	670.4±25.40
Stressed Females	10	1.84±2.89	346.4±16.60

* Statistically different from stressed males, $p<.0005$

^Statistically different from control females, $p<.05$

Table 3.

Volume (mm^3) of Anterior Commissure in Mean of 4 Coronal Sections & Body Weight (g), for Control males, Control Females, Prenatally Stressed Males and Prenatallly Stressed Females.

Group	n	Volume, (mm^3)	Body Wt. (g)
Control Males	10	6.88±.082 *^	632.5 ±39.90
Control Females	10	7.03±2.56	310.9 ±12.90
Stressed Males	10	8.21±1.39	670.4±25.40
Stressed females	10	7.32±1.19	346.4±16.60

* Statistically different from stressed males, $p<.0005$

^Statistically different from control females, $p<.05$

Figure Caption

Figure 1. Mean ± S.E.M. area (mm^2) of the AC in the coronal plane for control and stressed males and females using four serial sections, beginning with the first section in which the AC transected the midline. Control males (M =1.73) are statistically significant from control females (M = 2.01), $p<.0005$ and stressed males (M =2.04), $p<.05$.

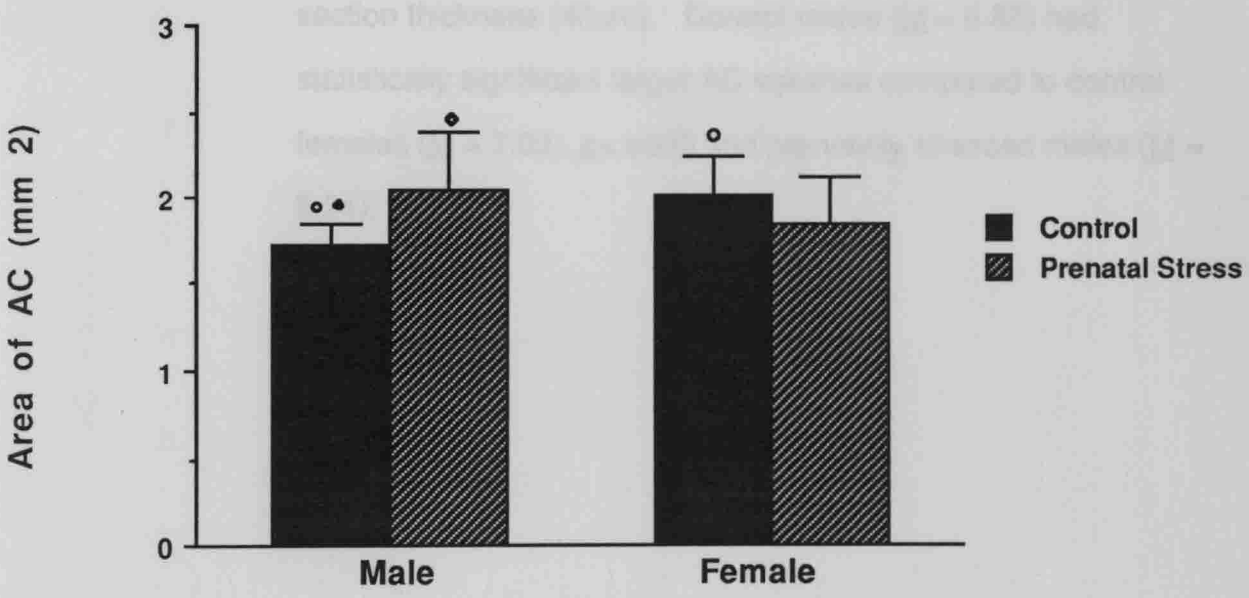
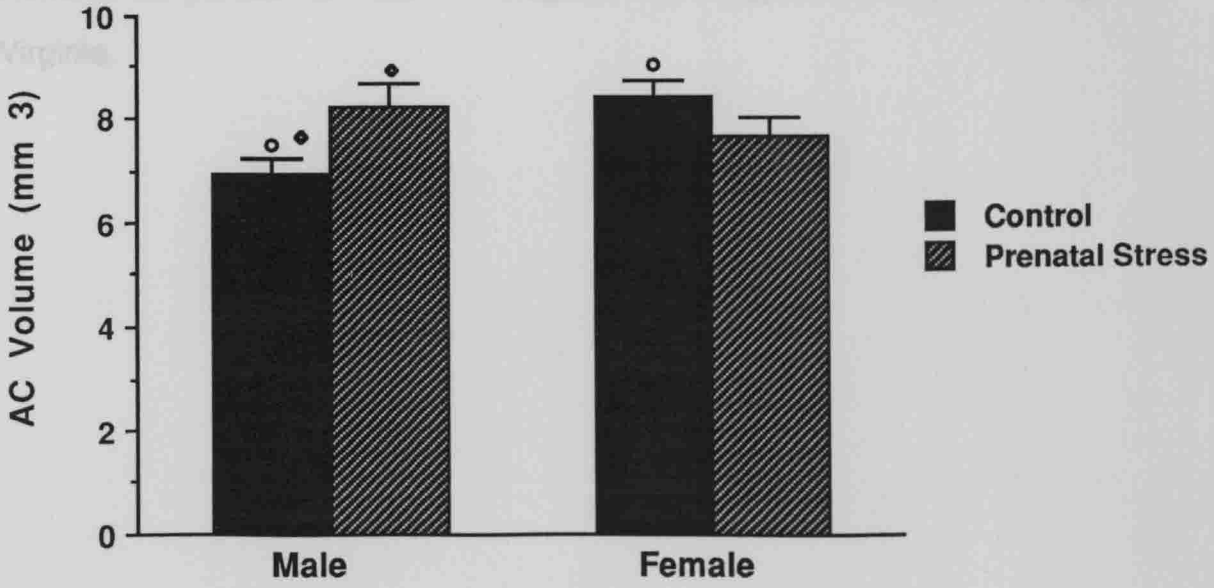

Area of AC (mm 2)
3
2
1
0
Male
Female
Control
Prenatal Stress

Figure Caption

Figure 2. Mean ± S.E.M. Volume (mm3) of the AC in the coronal plane for control and prenatally stressed males and females. Volume was obtained by multiplying the average area of two tracers (um) by section thickness (40um). Control males (M = 6.88) had statistically significant larger AC volumes compared to control females (M = 7.03), p<.0005 and prenatally stressed males (M = 8.21), p<.05.


AC Volume (mm 3)
10
8
6
4
2
0
Male
Female
Control
Prenatal Stress

Vita

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