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# The effects of prenatal stress on the size of the corpus callosum

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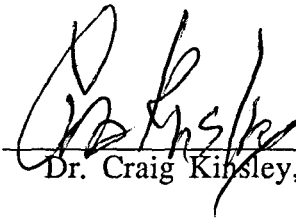
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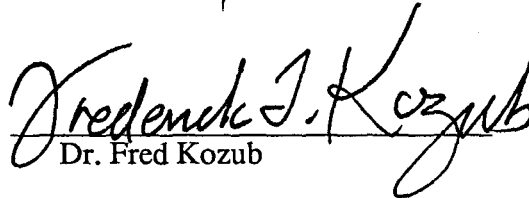
### Abstract

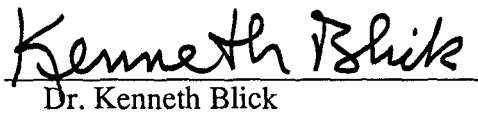
The effects of prenatal stress on the size of the corpus callosum in rats was investigated using a prenatal heat, light, and restraint stress paradigm that influences the fetal hormonal milieu (Ward, 1972). Females were stressed thrice daily from Day 15 of pregnancy until parturition. Control females were left unstressed throughout their pregnancies. In adulthood ( $M = 223.16$  days of age), male and female offspring from the two groups were sacrificed. Area, perimeter, and length of the corpus callosum were determined from sagittal sections of each brain. Results showed a sex difference for area, perimeter, and length of the corpus callosum, with males having larger values on all three measures. However, when body weight was taken into consideration, these sex differences disappeared. These findings are consistent with Berrebi et al's (1988) study that showed no sex differences in the corpus callosum of older rats (215 days old) as opposed to younger rats (110 days old) when brain weight was controlled. Questions, though, remain as to the importance of hormonal influences on body and brain size and the relationship of these same hormones to the size of structures in the brain. No stress effects or sex by stress interactions existed, suggesting that prenatal stress effects on the corpus callosum either diminish with age or do not exist for that particular structure.

THESIS APPROVAL

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.

  
Dr. Craig Kinsley, Advisor

  
Dr. Fred Kozub

  
Dr. Kenneth Blick

The Effects of Prenatal Stress on the Size  
of the Corpus Callosum

By

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The Effects of Prenatal Stress  
on the Size of the Corpus Callosum

Questions concerning the behavioral repertoire of humans and animals have intrigued scientists throughout history. Disparities between the behavior of males and females, especially, have spurred numerous lines of research concerning the basis for human and animal behavior. The question of which behaviors are sexually dimorphic has led to questions of why behaviors are different in the two sexes. Researchers have posited a number of interesting evolutionary explanations for differences in behavior, and many scientists now are attempting to identify the underlying, proximal biological mechanisms. Explanations ranging from differences in morphology to differences in hormonal exposure to differences in social influences, as well as interactions of all three have been entertained. Although these possibilities have been considered, studies in the area have yet to exhaust all contingencies.

Behavioral differences between males and females are for the most part obvious to the observer. What may be responsible for these striking differences is, however, not as apparent. To explain sexual differentiation, scientists have begun to focus on the brain, beginning with subtle differences in brain anatomy between sexes. A number of studies have found anatomical differences in regions of the brains of rats known to control sexually dimorphic functions. Pfaff (1966) and Dorner and Staudt (1968) found sex differences in neuronal size both intracellularly and ultrastructurally. Other studies have found sex differences in the synaptic organization of such structures as the preoptic area of the hypothalamus (Raisman & Field, 1971), the medial amygdala (Nishizuka & Arai, 1981), and the arcuate nucleus (Matsumoto & Arai, 1981). Dendritic branching patterns of neurons in the preoptic area have also been shown to be sexually dimorphic (Hammer & Jacobson, 1984). Not only have areas within the brain been shown to be sexually dimorphic in rats, but also other nuclei within the central nervous system such as the spinal nucleus of the bulbocavernosus (Breedlove & Arnold, 1983a, 1983b) and the dorsolateral

motor nucleus (Jordan, Breedlove, & Arnold, 1982) both of which are located in the spinal cord.

Gorski, Gordon, Shryne, and Southam (1978) were among the first researchers to demonstrate gross morphological sex differences of larger structures (as opposed to individual neurons) in the brains of rats. Their study looked at the medial preoptic nucleus of the brain which is thought to be involved in sexual behavior and determined that independent of adult hormonal concentrations, the medial preoptic nucleus of males was significantly larger than that of females when brain weight is taken into consideration. Also, by comparing neonatally castrated males to males castrated at the time of weaning, they narrowed the explanation for sexual differences to the influence of hormones during the perinatal period. It was later found that this same area of nuclei had a much higher neuronal density and contained larger cells and neurons in the male rat brain than in the female brain (Gorski, Harlan, Jacobson, Shryne, & Southam, 1980). The authors of the latter study proposed that the group of nuclei be termed the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA). It was suggested that the increased density of these nuclei led to the original findings of a sexual dimorphism in the SDN-POA.

Sex differences in the brain morphology of rats have also been found when looking at other structures in the brain. The volumes of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis have been reported to be almost twice as large in males as in females (Hines, Allen, & Gorski, 1992; del Abril, Segovia, & Guillamon, 1987). These nuclei are involved in the regulation of aggression, gonadotropin secretion, integration of olfactory information, and some sexual behavior, all of which are sexually dimorphic functions. It is interesting to note that these two groups of nuclei are included with the SDN-POA in a neural circuit that relays olfactory information to regions of the hypothalamus known to regulate reproduction. Consistent with explanations offered for sex differences in other parts of the brain, the authors suggest that perinatal hormones may play some role.

Two major fiber tracts within the rat brain have been reported to be sexually

dimorphic as well. Jones (1994) discovered that the anterior commissure, a fiber tract involved mainly in transporting olfactory information, is larger in females than in males. The corpus callosum, another major interhemispheric fiber tract, has also been shown to be sexually dimorphic in rats (Berrebi et al., 1988; Fitch, Cowell, Schrott, & Denenberg, 1991; Denenberg, Berrebi, & Fitch, 1989; Fitch, Berrebi, Cowell, Schrott, & Denenberg, 1990; Juraska & Kopcik, 1988). Berrebi et al. (1988) was the first study to show sex differences in the corpus callosum. The authors found that callosal area, perimeter, and length, as well as certain regional widths were larger in males than in females. Even when the experimenters controlled for brain weight, significant differences remained for area, perimeter, and a number of the widths. Juraska and Kopcik (1988) found no sex differences in the corpus callosum when looking at gross size measures such as area and perimeter. However, when they investigated the ultrastructure of the corpus callosum, they discovered that males had larger axons within the structure but females had a significantly greater number of axons. Again, explanations for such differences centered around the influence of hormones perinatally.

Findings concerning sex differences in brain morphology have not been confined to rats. Sex differences exist in the dendritic branching patterns of neurons in the preoptic area in both the hamster (Greenough, Carter, Steerman, & DeVoogd, 1977) and the macaque monkey (Ayoub, Greenough, & Juraska, 1983). Sexual dimorphisms were also found when investigating the volume of nuclei involved in vocal communication in songbirds (Nottebohm & Arnold, 1976), and in the bed nucleus of the stria terminalis in guinea pigs (Hines, Davis, Coquelin, Goy, & Gorski, 1985). Finally, differences in the volume of nuclei in the preoptic area have been discovered in gerbils (Yahr & Commins, 1982), guinea pigs (Hines et al., 1985), ferrets (Tobet, Zahniser, & Baum, 1986), and quail (Panzica et al., 1987).

Humans have also been the subject of studies concerning sexual differentiation and brain morphology. Although research has concentrated more on the brains of animals, differences between human males and females have been found in the anterior commissure



and massa intermedia (Allen & Gorski, 1991), the bed nucleus of the stria terminalis (Allen & Gorski, 1990), and in some of the interstitial nuclei of the anterior hypothalamus, (Allen, Hines, Shryne, & Gorski, 1989). The human corpus callosum is another structure found to be sexually dimorphic in humans (de Lacoste-Utamsing & Holloway, 1982; Elster, DiPersio, & Moody, 1990; Steinmetz et al., 1992), although questions still remain as to whether these differences are in measurements of the structure as a whole or of specific parts.

If sex differences in behavior are due to differences in brain structure, then what is responsible for these differences in brain anatomy? Current theories of sexual differentiation suggest that normal masculinization of the central nervous system requires adequate amounts of the testicular androgen testosterone to reach sexually dimorphic structures at critical stages of development (Goy, 1970). Weisz and Ward (1980) determined that day 18 and possibly day 19 post conception is the critical period during which the central nervous system of the male rat is primed by testosterone. In their study, they found that plasma testosterone levels in fetal male and female rats were very similar from day 17 post conception until day 5 post partum except for days 18, 19, and 21 post conception at which time testosterone levels were significantly higher in males than in females. The greatest difference existed at day 18. Such findings suggest that the increased testosterone exposure in males on day 18 sensitizes the male to testosterone later on in development which, after this critical period, is not significantly different than testosterone levels in females (Weisz & Ward, 1980).

One way in which hormones “prime” the brain for development is by playing a role in the cell death of sexually dimorphic structures (Nordeen, Nordeen, Sengelaub, & Arnold, 1985). Nordeen et al. (1985) studied the effect that androgens have on cell death in the sexually dimorphic spinal nucleus of the bulbocavernosus of rats, a neuron group that controls penile reflexes important in copulatory behavior. Although motoneuron numbers are similar in males and females in early development, greater cell death in females during the first few weeks of postnatal life leads to the sex difference in motoneuron

number that is apparent in adults (Breedlove & Arnold, 1980). Nordeen et al. (1985) showed that perinatal androgen treatment in females attenuated postnatal cell death in the spinal nucleus of the bulbocavernosus, suggesting that a primary role of androgens in the development of sexually dimorphic structures is to prevent normally occurring cell death. Prevention of cell death can also result from the effects of neurotrophic factors such as nerve growth factor (Frim et al., 1993; Van der Zee, Fawcett, & Diamond, 1992; Thoenen & Barde, 1980) and the growth hormone thyroxine (Nicholson & Altman, 1972). Although these compounds prevent cell death and in some cases increase the size and number of cells (Thoenen & Barde, 1980), there is no conclusive evidence to suggest that these factors play a part like that of sex hormones in the sexual differentiation of structures within the brain.

Because prenatal hormone levels appear to be important to the normal development of offspring, research has been done to investigate the environmental factors that could disrupt sexual differentiation and lead to abnormalities in normal sexually dimorphic behavior. Prenatal stress has been identified as a variable that can disrupt sexual differentiation. Ward (1972) showed that stress administered to female rats during the last third of pregnancy leads to reduced levels of copulatory behavior and increased levels of lordotic behavior in male offspring. Ward's (1972) procedure involving the presentation of light and restraint stress three times a day throughout the last trimester of pregnancy has become the standard prenatal stress procedure in literature on the subject. Other studies have attempted to identify the precise effects that prenatal stress has on hormone levels, behavior, and brain anatomy.

Maternal stress alters testosterone levels at the critical period of days 18 and 19 post conception (Ward & Weisz, 1980, 1984). Whereas normal males experience a testosterone surge on day 18, prenatally stressed males are exposed to high levels of testosterone on day 17 but experience a drastic reduction in testosterone levels on days 18 and 19 (Ward & Weisz, 1980). Therefore, if the structural and behavioral differences in males and females are a product of a critical period for testosterone exposure, then any outside variable (i. e.

prenatal stress) that disrupts fetal testosterone exposure is likely to affect the development of sexually dimorphic behaviors and structures. Numerous studies have confirmed Ward's (1972) findings of the effects of prenatal stress on sexual behavior (Dahlof, Hard, & Larsson, 1977; Meisel, Dohanich, & Ward, 1979).

Besides Ward's (1972) finding that prenatal stress decreases copulatory behavior and increases lordotic responding in male rats, a number of other studies have shown that prenatal stress attenuates other sexually dimorphic behaviors. Herrenkohl (1979) investigated the effects of prenatal stress on female offspring and found that prenatally stressed adult females experienced fewer conceptions, more spontaneous abortions and vaginal hemorrhaging, longer pregnancies, and fewer viable young than nonstressed females. She hypothesized that the dysfunctions in adulthood were a result of the influences of prenatal stress on the balance of hormones at a critical stage of hypothalamic differentiation (Herrenkohl, 1979). A similar study on reproductive functions also showed that prenatally stressed females exhibited vaginal opening at a later date, had longer estrus cycles, and had higher median quality receptivity scores than non-prenatally stressed females (Politch & Herrenkohl, 1984). Again, explanations revolved around prenatal hormone disruption. Prenatal stress has also been shown to disrupt other behaviors mediated by hormonal exposure and balance such as mother-infant interactions (Moore & Power, 1986; Power & Moore, 1986; Kinsley & Bridges, 1988), aggression (Politch & Herrenkohl, 1979; Kinsley & Svare, 1986, 1988), and play behavior (Takahashi, Haglin, & Kalin, 1992; Ward & Stehm, 1991).

The literature concerning the effects of prenatal stress on brain morphology is much less complete. Prenatal stress has been shown to reduce the size of the SDN-POA in male rats (Anderson, Rhees, & Fleming, 1985; Anderson, Fleming, Rhees, & Kinghorn, 1986), without affecting the size of the structure in females, and to reduce the size of the spinal nucleus bulbocavernosus and the dorsolateral nucleus of the spinal cord in male rats (Grisham, Kerchner, & Ward, 1991). Prenatal stress has also been shown to increase the size of the anterior commissure in male rats (Jones, 1994). No work, though, has been

undertaken to examine the effects of prenatal stress on the corpus callosum. While some researchers have found that hormones and handling affect the size of the corpus callosum (Fitch et al, 1990; Fitch, Cowell, Schrott, & Denenberg, 1991), and that rearing environment affects the ultrastructure of the rat corpus callosum (Juraska & Kopcik, 1988), little work has been done to determine the effects of prenatal conditions on the size of the corpus callosum. Zimmerberg and Mickus (1990) and Zimmerberg and Scalzi (1989) administered alcohol throughout the pregnancy of rats and found that prenatal alcohol exposure decreased the area of the corpus callosum and abolished the sexual dimorphism of the structure. Their study, however, did not use the typical prenatal stress paradigm of light, heat, and restraint stress and did not limit the stress exposure to the last third of pregnancy.

Specifically according to Paxinos and Watson's brain atlas (1986), the corpus callosum of the rat is a fiber tract whose most anterior portion, known as the genu, begins at the posterior segment of the anterior olfactory nucleus and the anterior portion of the medial forebrain bundle, about 2.7 mm anterior to Bregma. At its most anterior point in the brain, the corpus callosum does not cross mid-sagittally. The two sides of the structure come together about 1.1 mm posterior to its origin. The corpus callosum crosses the midline for about 7.2 mm moving posteriorly. Also, the more posterior the structure the more dorsal it becomes in the brain. Around the posterior region of the medial mammillary nucleus, about 6.1 mm posterior to Bregma, the corpus callosum again separates and extends another .8 mm before ending at the point where the trochlear root nerve can be seen and where the superior colliculi begin to diminish. From the anterior end (the genu) to the posterior end (the splenium), the corpus callosum extends about 9.2 mm. For almost the whole length of its existence, the structure extends laterally between 4.5 and 5 mm from the midline just to the base of the cerebral cortex. At the point where the corpus callosum contacts the cortex, it is slightly more ventral than the area closer to the midline (Paxinos & Watson, 1986).

The primary purpose of the corpus callosum in rats is the bilateralization of sensory

information that is being processed. Specifically, in the somatic sensory cortex, areas corresponding with callosal input are associated to midline representations, while areas that do not have callosal input are associated with vibrissae, hands, and feet representations (Akers & Killackey, 1978). In the primary visual cortex, callosal fibers are present in the area which corresponds to the vertical midline of the visual field (Montero, Rojas, & Torrealba, 1973). With respect to the primary auditory cortex, callosal projections are not as discontinuous as in the primary visual cortex and the somatic sensory cortex, suggesting that the corpus callosum may have more of a multi-purpose job in the lateralization of auditory information (Vaughan, 1983).

Therefore, as the corpus callosum is sexually dimorphic, and it is clear sexual dimorphism is due to prenatal androgen exposure (which is altered by prenatal stress), this study investigated the effect of prenatal stress on the size (area, perimeter, and length) of the corpus callosum in male and female rats. Based on previous research suggesting that prenatal stress demasculinizes the behavior and brain structure of male rats and defeminizes the behavior and brain structure of female rats (e.g. Ward, 1972; Jones, 1994; Herrenkohl, 1979), it was hypothesized that the callosal size of prenatally stressed males would be smaller than control males (more like females) and that the callosal size of prenatally stressed females would be greater than control females (more like males).

## Method

### Animals

Ten adult nulliparous female Sprague-Dawley rats approximately 60 days of age were timed mated in our laboratory. The day that sperm was observed in the vaginal smear was designated Day 0 of pregnancy. At this time the females were isolated in 20 x 45 x 25 cm polypropylene cages whose floors were covered with wood shavings. Food (Purina rat chow) and water were available ad libitum and all animals were housed in light (on from 0500-1900 h)- and temperature (22°C)-controlled testing rooms for the duration of the experiment.

### Prenatal Stress Procedure

Half of the females were randomly assigned to one of two groups. One group [prenatally stressed (P-S)] of pregnant animals ( $n = 5$ ) was exposed to a regimen of heat and restraint stress comprised of placing the female into a 6 3/4 cm Plexiglas restraint tube in a separate room from where the animals were housed. Two 150-watt flood lights were placed over the restraint tube. This procedure produces close to 350 foot candles of illumination and an ambient temperature within the restraint tube of approximately 38° C. The stress procedure began on Day 14 of pregnancy and continued through parturition (approximately Day 21). All females delivered on Day 21 or Day 22. The subjects were exposed to three 30-min stress sessions each day at 0830, 1230, and 1630 h. The second group of females [non-prenatally stress (Control);  $n = 5$ ] was left undisturbed for the duration of their pregnancies, except for routine maintenance. At parturition, offspring remained with their litter until they were weaned at 21 days of age. At that time, animals were maintained in same-sex groups of 2-4 until testing.

### Histology

In adulthood (from 184 to 337 days of age,  $M = 223.16$ ), all animals were weighed, administered an overdose of sodium pentobarbital and perfused intracardially with saline followed by a 10% formalin solution. Brains were removed and stored in a 10% formalin solution. A total of 42 animals were sacrificed. Thirty-one of the brains were analyzed (7 P-S males, 8 P-S females, 8 Control males, and 8 Control females). Four brains (2 P-S females and 2 Control females) were used as practice brains, and the remaining 7 brains (3 P-S males, 1 P-S female, and 3 Control males) were either lost during sectioning or staining. At the time of sectioning, brains were blocked by hand along the midline. Brains were then sectioned in the sagittal plane at a thickness of 100  $\mu$ m on a Vibratome Series 2000 (Technical Products International, Inc., 1985). The 3 sections adjacent to the midline in each hemisphere were mounted onto gelatin-coated slides and stained with thionin, which stains primarily cell bodies blue and leaves fiber tracts white. The first section on each side of the midline of each subject was chosen for analysis. The

two sections from each brain were traced by 2 experimenters (naive to the treatment groups) at x25 on a Zeiss Axioplan microscope via a NeuroLucida imaging system and software package (MicroBrightField, Inc., 1993) and a computer mouse. The NeuroLucida package automatically computed callosal area (squared mm) and perimeter (mm). Two separate experimenters used the software package to go back and determine callosal length (the longest length from one end of the structure to the other). Each experimenter made length measurements of all tracings that were used to compute area and perimeter. The NeuroLucida package then computed the actual length. This sectioning method is a variation of the one used by Fitch, Berrebi, Cowell, Schrott, & Denenberg (1990).

## Results

### Initial Analyses

*Body Weight.* A 2 x 2 (Sex x Stress) analysis of variance for body weight revealed a main effect for sex,  $F(1, 27) = 338.98, p < .001$ . Males were significantly larger

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Insert Table 1 about here

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than females. No main effect existed for stress,  $F(1, 27) = .785, p = .383$ , nor was there a sex by stress interaction,  $F(1, 27) = 1.449, p = .239$ .

*Callosal Size.* The three tracings made by each experimenter on the first section of each brain (both right and left hemispheres) was averaged to come up with a mean area and perimeter for both the right and left hemispheres of each subject. Those means were then averaged to produce a mean area and perimeter for each subject for each of the two experimenters. These numbers were used to compute alpha coefficients for interrater reliability. An average of the mean area measure of each of the two experimenters was computed to give a total area measurement for each subject. The mean perimeter measures for each experimenter were also combined to give a total perimeter measurement for each subject. Two other experimenters (also blind to the treatment groups) went back and made measurements of length for all (both hemispheres and both initial experimenters) of the

tracings made on the first section of each brain. Again averages of the hemispheres, brains, and the initial experimenters were calculated to produce an average corpus callosum length for each of the second two experimenters. Alpha coefficients were again computed and the mean of the two experimenters' measurements was calculated to give a total length for each subject.

*Area.* Interrater reliability (alpha) for the area measurements was .912. A 2 x 2 (Sex x Stress) analysis of variance showed a main effect for sex,  $F(1, 27) = 15.705$ ,  $p < .001$ . Overall, males had a larger callosal area than females.

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Insert Table 2 about here

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However, there was not a significant effect for stress,  $F(1, 27) = .048$ ,  $p = .829$ . The sex by stress interaction also was not significant,  $F(1, 27) = .651$ ,  $p = .427$ .

*Perimeter.* Interrater reliability (alpha) for the perimeter measurements was .251. A 2 x 2 (Sex x Stress) analysis of variance revealed a main effect for sex,  $F(1, 27) = 7.968$ ,  $p = .009$ . Again, males had bigger perimeters than females. But again there

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Insert Table 3 about here

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was no main effect for stress,  $F(1, 27) = .371$ ,  $p = .548$ , nor was there a sex by stress interaction,  $F(1, 27) = .049$ ,  $p = .826$ .

*Length.* Interrater reliability (alpha) for the length measurements was .916. A 2 x 2 (Sex x Stress) analysis of variance of callosal length showed a main effect for sex,  $F(1, 27) = 7.521$ ,  $p = .001$ . Males had a larger average length than females.

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Insert Table 4 about here

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The main effect for stress was not significant,  $F(1, 27) = .045$ ,  $p = .834$ . The sex by



stress interaction was also not significant,  $F(1, 27) = .000$ ,  $p = .994$ .

#### Analyses Using Body Weight as a Covariate

Due to the significant main effect of sex on body weight (males larger than females), a second set of analyses was conducted to take this difference into account. The following analyses of variance were performed isolating body weight as a covariate.

*Area.* When taking into account body weight, the main effect for sex on the area of the corpus callosum disappeared,  $F(1, 26) = .438$ ,  $p = .514$ . Significance for the main effect of stress,  $F(1, 26) = .283$ ,  $p = .599$ , and the sex by stress interaction,  $F(1, 26) = .179$ ,  $p = .675$ , continued to be nonexistent.

*Perimeter.* Similarly, by covarying out body weight the main effect for sex on the perimeter of the corpus callosum also disappeared,  $F(1, 26) = 1.657$ ,  $p = .209$ . There was again no main effect for stress,  $F(1, 26) = .256$ ,  $p = .617$ , nor an interaction,  $F(1, 26) = .121$ ,  $p = .731$ .

*Length.* The second analysis, however, did not have as great an effect on the significance for the sex effect on length,  $F(1, 26) = 1.861$ ,  $p = .184$ , as it did on the measures of area and perimeter. The stress effect,  $F(1, 26) = .011$ ,  $p = .919$ , and the interaction effect,  $F(1, 26) = .024$ ,  $p = .878$ , remained not significant.

#### Discussion

The lack of significant effects in this study could be due to a number of explanations. Berrebi et al. (1988) had previously found no sex differences in size of the corpus callosum when looking at 215 day old rats and controlling for brain weight. The fact that 110 day old rats did show a sex difference in the corpus callosum suggests that the corpus callosum develops not only at least partially throughout the course of a life, but also at differing rates for males and females. Because the rats in the current study were older ( $M = 226.13$  days of age) compared to the rats in which sexual differences were found in the corpus callosum (e.g. Berrebi et al., 1988; Fitch et al., 1991), the developmental change in callosal size may have already taken place by the time the animals were sacrificed.

A major question arises when discerning the control of body or brain weight when analyzing the size of specific brain structures. If the timing of androgen (testosterone) exposure prenatally is the main determinant of sexual differentiation (Weisz & Ward, 1980), how can one separate the effects of that exposure? Specifically, how can one control for body weight or brain weight when looking at the size of the corpus callosum when sex differences in body weight, brain weight, and callosal size or all apparently caused by this same testosterone exposure? A possible remedy for such a situation might be to castrate animals immediately after birth to reduce the effects of hormones on body and brain size, but this still does tease apart the weighted effects of prenatal testosterone exposure on differentiating brain structure size and brain or body size. The current findings of sex differences without controlling for body or brain weight may be a valid analysis and the results meaningful. There is a possibility that measures of body or brain weight and callosal size are not just highly correlated measures in which it is assumed that body and brain weight in themselves contribute to callosal size, but that all three measures are a result of the same cause.

The most obvious explanation for the current results is that sex differences are not present in the measures taken. While most of the research on sex differences in brain structures has involved gross measurements such as area, perimeter, and length, researchers like Juraska and Kopicik (1988) who found no sex effects on larger measures of the corpus callosum, did find differences in the ultrastructure of the structure (larger axons in males and a greater number of axons in females). The sex differences in the corpus callosum of the rats in the current study may not be apparent because measures of the ultrastructure (actual neurons) were not taken.

While the lack of a sex effect can be attributed to a developmental influence in this study, the lack of a prenatal stress effect has no easy explanations. In relation to the possible lifelong development of the corpus callosum as shown by Berrebi et al. (1988), the timing of the stressor may not have had as pronounced an effect in the present case as it did with structures such as the anterior commissure (Jones, 1994) and the SDN-POA

(Anderson et al., 1985, 1986). A reasonable explanation is that the developmental changes that apparently occur in the corpus callosum between 110 and 215 days of age in the rat were enough to override any effect that prenatal stress may have had on the initial differentiation of the structure. This makes logical sense considering that these changes were enough to diminish sex differences in the corpus callosum and prenatal stress acts at the same time that these natural sex differences are being initialized. The findings of Zimmerberg and Mickus (1990) showing that prenatal alcohol exposure and maternal undernutrition affected the size of the corpus callosum in rats sacrificed at 110 days of age suggests that they were able to precede the changes that occur at some point between 110 and 215 days of age. It could be speculated that prenatal heat and restraint stress may have the same effect if subjects were examined at an earlier age.

Another possible reason for the lack of a stress effect may simply be due to the size of the structure in question. Relatively speaking, the corpus callosum is much larger than both the SDN-POA, the anterior commissure, and the sexually dimorphic nuclei within the spinal cord. Because of its size, the corpus callosum may require more than the timed exposure to testosterone that is needed to differentiate other structures in the brain. The initial analysis of area in which there was a sex difference and prenatal stress drove male means in the direction of females and female means in the direction of males (the hypothesized results) suggests that sex and stress were interacting to produce an effect, but that the effect with the current procedure was a minor one at best. Heat and restraint stress thrice daily throughout the last third of pregnancy may not have been enough of a prenatal stressor to do the job of disrupting sexual differentiation of such a large structure. However, the age of sacrifice and the influence of lifelong changes in the corpus callosum's development seem to be more plausible explanations.

While an effect for sex in the current study would have bolstered the already large number of studies finding sex differences in the size of the corpus callosum, the lack of a sex effect gives some support to the lifelong development of the corpus callosum and the importance of time (within a life) of measurement. These results also suggest that the

corpus callosum may be a structure high in plasticity. An interaction of sex and stress would have supported numerous reports such as Ward's (1972) study on the demasculinizing and defeminizing effects of prenatal stress. However, the current findings do point out the importance of researching development as a possible influence on brain structure size and of considering measurements other than large gross morphological measurements such as area, perimeter, and length.

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Table 1

Mean Body Weights as a Function of Sex and Prenatal Stress

Condition	Body Weight (g)	+/-	S.E.M.
Males			
Control (n=8)	719.56		19.90
Prenatally Stressed (n=7)	712.49		34.23
Females			
Control (n=8)	334.44		9.55
Prenatally Stressed (n=8)	374.75		19.19

Table 2

Mean Area of the Corpus Callosum (mm<sup>2</sup>) as a Function of Sex and Prenatal Stress

Condition	Area (mm <sup>2</sup> )	+/-	S.E.M.
Males			
Control (n=8)	3.92		0.24
Prenatally Stressed (n=7)	3.71		0.19
Females			
Control (n=8)	2.98		0.14
Prenatally Stressed (n=8)	3.09		0.20

Table 3

Mean Perimeter of the Corpus Callosum (mm) as a Function of Sex and Prenatal Stress

Condition	Perimeter (mm)	+/-	S.E.M.
Males			
Control (n=8)	16.80		0.22
Prenatally Stressed (n=7)	16.54		0.36
Females			
Control (n=8)	15.87		0.20
Prenatally Stressed (n=8)	15.75		0.40

Table 4

Mean Length of the Corpus Callosum (mm) as a Function of Sex and Prenatal Stress

Condition	Length (mm)	+/-	S.E.M.
Males			
Control (n=8)	7.49		0.32
Prenatally Stressed (n=7)	7.46		0.48
Females			
Control (n=8)	7.11		0.26
Prenatally Stressed (n=8)	7.08		0.38

## Biography

The author is a native of Johnson City, Tennessee who grew up in Columbia, South Carolina. He graduated in 1992 from Furman University in Greenville, South Carolina with a B.A. in psychology. After graduation with an M.A. in General Psychology from the University of Richmond, Mr. Rowe will take a year off before pursuing the Ph.D. degree.