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Modifications of nitric oxide and sexual behavior in prenatally stressed male rats

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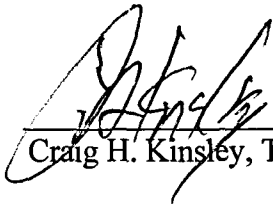
Abstract

Normal male sexual differentiation is the culmination of perfectly timed, prenatal

gonadal hormone release. Prenatal stress (PS) has a detrimental effect upon this process,

obstructing the natural development of brain structures and sexual behavior. Prenatally-

stressed male rats exhibit many physiological and neuroendocrinological differences

when compared to control males. PS has a particularly harmful effect upon male sexual

behavior, to which the neurotransmitter nitric oxide (NO) has been shown to be

intimately involved. The present experiment examined whether PS reduces nNOS, the

rate limiting enzyme of NO, in the medial preoptic area (mPOA) of male rats, and

whether administration of the NO substrate, L-arginine, ameliorates the detrimental

effects of PS upon sexual performance. PS male rats had significantly less nNOS

immunoreactivity in the mPOA than controls. Following L-arginine administration, PS

males reached ejaculation significantly more than PS males injected with the vehicle.

These combined results suggest that the resultant loss of nNOS from PS may be a factor

in PS-induced sexual deficits.

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 in Psychology.

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Modifications of Nitric Oxide and Sexual Behavior in

Prenatally Stressed Male Rats

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Modifications of Nitric Oxide and Sexual Behavior in

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A human embryo has the biological potential to develop into either a male or female adult, depending upon whether the father donated an X or Y chromosome. The process through which one sex system takes precedence and continues development while the other degenerates is termed sexual differentiation. Prenatal and early postnatal sexual differentiation of the brain determines the quality and quantity of future behavior, physiology, and anatomy. Normal sexual differentiation in the male requires the release of prenatal gonadal hormones at precisely timed intervals. These hormones, particularly testosterone (T), have a massive impact on development in specific regions of the brain (Kerchner, Malsbury, Ward, & Ward, 1995). This vital developmental period is not without risks however. Contrary to some opinions, the fetus is not beyond harm in the protective womb; many forms of insult can cause irreparable damage to the maturing organism. Prenatal development and sexual differentiation occurs along a finite timeline, and if a certain developmental stage is missed or interfered with along the way, the window of opportunity passes, often with no chance of reclamation.

Prenatal stress (PS) can have a profound effect upon these developing neural systems, disrupting the normal sequence of sexual differentiation and impacting adult behaviors like aggression and sexual activity (Harvey & Chevins, 1984; Kinsley & Svare, 1986; Kinsley & Bridges, 1987). The types and consequences of PS are, however, variable. Among humans, for instance, one individual may find verbal confrontations to be highly stressful, while another person may enjoy arguing. Velazquez-Moctezuma,

Dominguez Salazar, and Cruz Rueda (1993) found that for male rats whose mothers underwent immobilization or REM sleep deprivation, there was an impairment of male sexual behavior. Conversely, in the same experiment, the male offspring of pregnant rats who underwent immersion in cold water actually exhibited an increase in male sexual behavior. While there are different types of PS, not all adult behaviors are affected, regardless of the stressor, indicating that while the brain areas responsible for one set of behaviors mature postnatally, regions responsible for sexual performance develop, at least in part, prenatally. Sexual behavior, particularly in males, is often affected by PS, whereas behavior like open-field performance is not (Meisel, Dohanich, & Ward, 1979). However, behavioral abnormalities occurring from PS are not necessarily permanent. Aberrant sexual behavior from PS can be partially ameliorated by social and environmental conditions. Social rearing conditions and neonatal handling have the ability to reverse prenatal damage to neurological organization (Ward & Reed, 1985; Wakshlak & Weinstock, 1990). However, detrimental postnatal social conditions can interact with PS and can lead to greater cumulative damage to the offspring than PS alone (Dunlap, Zadina, & Gougis, 1978).

Ingeborg Ward (1977) showed that copulatory behavior is drastically handicapped in PS male rats, and with the introduction of exogenous androgens, these PS males even display female sexual postures. Evidently, PS demasculinized and feminized these males and prevented their normal sexual function. Dahloef, Hard, & Larsson (1977) provided comparable evidence that PS elicits feminine sexual behavior in males; however, they did not find the concurrent demasculinization of behavior as well. This difference may be due

to differences in PS paradigms. Nonetheless, prenatal stressors have consistently been shown to impact future adult behaviors.

Since PS alters the normal levels of endogenous hormones and neurotransmitters, there are several neurological pathways through which PS can elicit its effects. When a pregnant female is subjected to stress, her male offspring are exposed to much lower levels of T than are necessary for sexual differentiation. As a result, such offspring lack typical male-like sexual behavior and their physiology is significantly and irreversibly altered. Normally, adult male rats will continuously engage in copulatory behavior when presented with a female in estrous. However, Ward (1972) showed that when a PS male is introduced to a sexually-receptive female, he exhibits virtually no interest in mating with her. Evidently, fetal masculinization is in part dependent upon ambient levels of T, disruptions of which impede natural development. In rats, fetuses are lined up in the dual uteruses of the female, and, depending upon the intrauterine position, some male fetuses receive more or less T than their littermates (Lephart, Fleming, and Rhees, 1989). Pups that are in contact with higher levels of T are more aggressive and masculine in adulthood, whereas littermates which were exposed to less T display less masculine behavior. Similarly, the resultant low levels of T from PS have long lasting effects into adulthood, impacting normal sexual behavior (Ward, 1972). Research has shown that these reduced levels of fetal T may be the result of diminished amounts of the testicular steroidogenic enzyme, delta-5-3-beta-hydroxysteroid dehydrogenase (3β-HSD) (Ward, 1984). Amounts of 3β-HSD in the fetal Leydig cells of PS animals are lower than normal on gestational days 18 and 19, and higher than normal immediately two days before and

two days after this period. These alterations of the enzyme occur at a point when T is critical for neuronal sexual differentiation and future behavioral development.

However, T is not the only endogenous substance to impact adult sexual behavior. Though Crump and Chevins (1989) found that PS males had significant difficulty with copulation, the researchers did not find evidence that T levels were also affected. Other hormones, though, are equally important in the regulation of sexual behavior, and might be altered more in adulthood by PS. Kinsley & Bridges (1987) investigated the effects of PS upon prolactin (PL) release, and found that PS subjects had a decreased ability to release PL in response to estradiol. Quadros et al. (1997) found that the number and concentration of estrogen receptors in the anterior hypothalamus are smaller in PS males as compared to controls. It was also shown that a PS male exposed to a sexually-receptive female does not demonstrate the accelerated pattern of luteinizing hormone (LH) release characteristic of control animals (Kinsley, Mann, & Bridges, 1992), illustrating that endocrinologically, the PS male is not aroused by a female's presence.

Neurotransmitter systems also play a significant role in the modulation of sexual motivation and behavior. One group in particular, the catecholamines, have been shown to control several aspects of sexual behavior. Norepinephrine (NE) is an important mechanism in the control of sexual arousal and copulatory performance (Mallick, Manchanda, & Kumar, 1996). Direct injections of NE into the medial preoptic area (mPOA) of male rats enhances arousal and ejaculation. Moyer, Herrenkohl, and Jacobwitz (1978) investigated the effects of PS upon adrenergic systems in the male rat

and found that there was 38 percent less NE in the mPOA and 49 percent less in the

median eminence of PS males when compared to controls.

Dopamine (DA), another catecholamine, is also important in the induction of

sexual behavior and copulation. DA has been found to play an important role in

motivation and facilitation of sexual behavior in the adult male rat (Melis & Argiolas,

1995). Hull (1995) found that extracellular DA levels rose when a male was presented

with a stimulus female, and levels continued to climb as copulation occurred. The

elevated DA levels were isolated to sexual behavior, since other locomotor activity or

exposure to another male did not affect extracellular DA levels.

How then does PS affect this neurotransmitter system that is essential for sexual

behavior? Henry et al. (1995) found that PS changes DA receptor systems in the brain,

with densities of D2 and D3 DA receptor subtypes altered in the shell and core of the

nucleus accumbens. Not only are receptors affected, but DA concentrations are also

altered in PS animals. While DA turnover rates are elevated in the right prefrontal cortex,

resulting in cerebral asymmetry, there are lower DA levels in the medial preoptic area

(mPOA) (Fride & Weinstock, 1988, 1989; Fride, Dan, Gavish, & Weinstock, 1985).

PS also has significant effects upon serotonergic systems in the brain. Serotonin

(5-HT) is understood to direct neuron development in the fetus (Peters, 1990), inhibit

sexual behavior in the adult male (Lorrain, Matuszewich, Friedman, & Hull, 1997), and

influence the processes of learning and memory (Hayashi et al., 1998). Peters (1986a)

showed that PS rats exhibited increased locomotion in open-field tests and increased

behavioral reactions to 5-hydroxy-L-tryptophan. Later, Peters (1986b) found that 5-HT

binding and synthesis were modified in the adult PS male rat. While 5-HT binding was elevated throughout the cerebral cortex, 5-HT binding and synthesis was reduced in the hippocampus. Additional research provided evidence that the critical period for prenatally altering serotonergic system was between gestational day 15 and birth in the rat (Peters, 1988). Recent studies by Hayashi et al. (1998) have investigated the effects of PS on 5-HT in the hippocampus of the juvenile rat. Concurrent with Peters, Hayashi et al. found that 5-HT levels and synaptic density were decreased by 17% and 32%, respectively.

Endogenous opioids play important roles in the process of sexual differentiation by communication with gonadal steroids, and in the regulation of divers behaviors. PS appears to interfere with behaviors that are mediated by opiates. Opioid regulated behaviors like locomotion and rearing were found to be significantly reduced in adult PS rats (Poltyrev & Weinstock, 1997). Smith, Stevens, Torgerson, & Kim (1992) investigated the effects of PS upon human infants and their reaction to sucrose, a natural analgesic. Infants who had experienced chronic prenatal stress required a higher concentration of sucrose solution before exhibiting any signs of analgesia. Concurrent studies show that PS male rats are also significantly less analgesic than controls (Kinsley, Mann, and Bridges, 1988), indicating that PS fundamentally alters opioid function and hence, behavior. Building upon this research, Insel, Kinsley, Mann, and Bridges (1990) also found less mu-opiate receptor binding in the striatum.

Investigations using opioid inhibitors and blockers shed light upon the unique relationship between PS and abnormal sexual behavior. Endogenous opioids work to

inhibit sexual activity in the male rat, hence it is logical that sexual deficiencies may stem in part from the opioid system. When injected with naloxone, an opiate antagonist, control animals that were sexually inactive previously increased their sexual activity. However, PS males had no reaction to naloxone and remained sexually inactive (Rhees, Badger, & Fleming, 1983). Keshet and Weinstock (1995) investigated whether the effects of PS could be ameliorated by administering the opiate antagonist naltrexone in combination with prenatal stress. The naltrexone prevented the reduction in anogenital distance in PS males and decreased their anxiety in maze studies. Likewise, the feminization of behavior from PS can be reduced by opiate antagonists. Ward, Monaghan, and Ward (1986) showed that PS males did not exhibit lordosis if the maternal dams were injected with naltrexone before stressing. Evidently, opioid systems play a large role in the relationship between PS and abnormal sexual behavior.

PS affects not only hormone levels and neurotransmitter systems, but changes the fundamental anatomy of the brain as well. As the brain develops in the fetus, neurons migrate from their areas of creation to the regions where they will remain and carry out their functions. However, PS intervenes and changes the blueprints of the neuroanatomy, causing alterations in brain matter that will translate into abnormal behavior. Sexually dimorphic areas of the brain are affected by PS. Normal male rats have a right>left thickness asymmetry in the cerebral cortex, while PS males have a nonsignificant pattern, reminiscent of female brains (Fleming, Anderson, Rhees, Kinghorn, & Bakaitis, 1986). It was reported that the anterior commissure, a forebrain fiber tract that conveys information regarding sexual odors between brain hemispheres, was feminized in PS

males, affecting their receipt of important mating cues (Jones et al., 1997). One area of

the brain that appears markedly different between controls and PS animals is the sexually

dimorphic nucleus of the medial preoptic area (SDN-mPOA). The SDN-mPOA has been

shown to be 50 percent smaller in PS males (Anderson, Rhees, & Fleming, 1985),

possibly as a result of the suppression of T during prenatal development (Kerchner &

Ward, 1992). Alterations in neuroanatomy due to PS can also be seen in the spinal cord,

specifically, the spinal nucleus bulbocavernosus (SNB) and the dorsolateral nucleus

(DLN), two nerve bundles that control erections and ejaculation. Grisham, Kerchner, &

Ward (1991) noted that these nuclei were significantly smaller in PS males, providing

further evidence that PS alters the basic neuroanatomy responsible for sexual behavior.

Certain areas of the brain have been identified as being crucial in the regulation of

sexual behavior. In male rats, the mPOA is regarded as a necessary component in sexual

motivation and stimulation. Research has shown that the mPOA plays a large role in

regulating male sexual behavior (Humm, Lambert, & Kinsley, 1995). Electrical

stimulation of the mPOA has been linked to activation of neural pathways controlling

penile erection (Giuliano et al., 1996). Also, administration of gonadal steroids like

testosterone directly to the mPOA increases a male's interactions with females, number of

mounts, and reduction of the latency to first mount (Wood & Newman, 1995).

Conversely, the expression of male sexual behavior can be effectively inhibited by

implanting a protein synthesis inhibitor directly into the mPOA (McGinnis & Kahn,

1997). The mPOA is the nexus for male sexual behavior, and as such, is innervated with

neurotransmitter systems. DA, as discussed previously, has been shown to be an

important regulator of sexual behavior, and evidence suggests that most of the neurotransmitter activity occurs in the mPOA (Hull, Du, Lorrain, & Matuszewich, 1995).

In summary, PS has a detrimental effect upon neurochemistry, neuroanatomy, and adult sexual behavior. The current work attempts to expand the knowledge of PS by examining its effect on a gaseous neurotransmitter called nitric oxide (NO) (Snyder, 1992). NO is found throughout the central nervous system (Snyder & Bredt, 1991) and is involved in many disparate behaviors and physiological activities. Gathering research shows that NO is necessary for the expression of male sexual behavior (Hadeishi & Wood, 1996), and if concentrations of this neurotransmitter are too low, copulation is reduced. For example, administration of a NO synthesis inhibitor like N-nitro-L-arginine methyl ester (L-NAME), effectively inhibits the male rats' ability to achieve ejaculation (Bialy, Beck, Abramczyk, Trzebski, Przybylski, 1996). Hull, Lumley, Matuszewich, & Dominguez (1994) provide comparable evidence , finding L-NAME impaired copulation and decreased the number of erections.

NO appears to stimulate sexual behavior by interaction with the mPOA. Several lines of evidence show that reduction of NO in the mPOA results in loss of normal male sexual behavior and copulation. Mice lacking NO in the mPOA have sexual deficits (Nelson et al., 1995). Also, administration of the NO synthesis inhibitor NG-monomethyl-L-arginine (L-NMMA) in the mPOA reduces the occurrence of mounting, while administration of the NO precursor L-arginine significantly increases the mount rate of male rats (Sato, Horita, Kurohata, Adachi, & Tsukamoto, 1998).

The present study seeks to investigate a research area that has been virtually unexplored, the connection between PS and NO, and their effect upon adult male sexual behavior. The enzyme responsible for NO production in the brain is neuronal nitric oxide synthase (nNOS) (Price, Mayer, & Beitz, 1993), and is also the primary means by which NO activity is quantified in the brain through cytochemical methods. We have two hypotheses: 1) PS will cause a reduction in the number of nNOS containing neurons in the mPOA of adult male rats, and 2) that administration of the NO precursor, L-arginine, to adult PS male rats will reverse the detrimental effects of PS upon sexual behavior. Pilot data collected in our lab indicated that PS male rats have approximately half the number of nNOS containing neurons in the mPOA compared to controls. Experiment 1 concerns our first hypothesis and seeks to confirm the findings of the pilot study to determine whether the difference seen between PS and control animals is significant. Moses and Hull (1994) showed that a NOS inhibitor is able to reduce the amount of copulation a male engages in. If PS lowers the amount of nNOS available in the mPOA, administration of a NO agonist such as L-arginine should ameliorate the sexual behavior deficits caused by PS. Experiment 2 attempts to answer our second hypothesis and determine to what degree PS interacts with NO to inhibit sexual behavior.

Method

Animals:

Adult nulliparous female Sprague-Dawley rats were time mated in our laboratory. The day that sperm was observed in the vaginal lavage was designated day 1 of pregnancy, at which point the females were isolated in cages. Food and water was

administered ad libitum, and all animals were housed in light (12:12 hr) and temperature

(23° C) controlled rooms, left undisturbed except for routine maintenance.

Prenatal Stress Procedure:

The PS procedure used is an adaptation of that employed by Kinsley and Bridges

(1988). On gestation day (G) 15, females were randomly assigned to one of two groups.

One group of pregnant animals, henceforth designated the PS group, was exposed to a

regimen of heat and restraint stress. The PS procedure consisted of placing a female in a

Plexiglas restraint tube with a 100-watt flood light positioned 6 in. directly above the

animal. Stress began on G15 and continued through G22. Subjects were exposed to stress

three times a day (9:00, 13:00, & 16:00 hrs.) at 30-minute intervals. Temperature

readings were taken regularly to ensure the ambient temperature never rose above 100° F.

The second group of females, which comprised the control group, were not handled

throughout pregnancy and birth. At 21 days of age, the pups from both groups were

weaned and placed in separate cages, 2-4 animals per cage until the time of separation

and testing.

<u>Experiment One</u>

Surgery and Immunocytochemistry (ICC):

Nine PS males and nine control males (90-120 days of age) were anaesthetized

and sacrificed with an overdose of pentobarbital sodium and perfused for 3 min with cold

phosphate buffered saline (PBS), followed by 500 ml of 4% paraformaldehyde in 0.1 M

phosphate buffer. The brains were removed and post fixed in 4% paraformaldehyde for

60 min at 4°C, then cryoprotected overnight in a 20% sucrose solution at 4°C. The

following day the brains were blocked in an area isolating the mPOA, frozen sectioned at 30μm on a Zeiss cryostat, and placed in PBS for 10 min. The sections were collected in a 24 well microplate and washed 3 times 5 min each in PBS on a shaker at room temperature, followed by a 30 min wash in 1.5% non-fat dry milk. The tissues were processed for nNOS immunohistochemistry by means of exposure to the primary antibody (anti nNOS 1:15,000, DiaSorin Corporation-Stillwater, MN, http://www.diasorin.com) for 24 hrs at 4°C. The sections were washed 3 times in PBS followed by incubation in the biotinylated secondary antibody for 60 min (anti-rabbit IgG 1:200, Vector Labs, Burlingame, CA, http://www.vectorlabs.com) on a shaker at room temperature. Next, the sections were washed 3 times in PBS and incubated at room temperature for 60 min on a shaker in the avidin-biotin complex solution (Vector Labs). Following another 3 PBS washes, the sections were exposed to $DAB/NiSO_4/H_2O_2$ for 5 min, then washed 3 times in distilled H_2O. The sections were then mounted on chrom-alum-coated slides, dehydrated in 70%, 90%, and 100% alcohols, cleared in xylene, and coverslipped using Permount.

Image Analysis and Quantification:

The nNOS immunoreactive (IR) cells in the mPOA were counted in 10 consecutive sections per animal. As done by Nelson et al. (1995), a 490μm x 460μm area on both the left and right hemispheres of the dorsal mPOA located 700m ventral to the anterior commissure has been chosen for analysis. The sections for each animal were analyzed and the total number of nNOS IR cells, defined as darkly stained perikarya, were determined at 400x on a Zeiss Axioskop microscope, fitted with a Hitachi CCD

camera. nNOS cells were counted using a Bioquant (Nashville, TN,

http://www.bioquant.com) software package. Each slide's identification was concealed

until after completion of analysis to ensure blind measurement.

Experiment Two

Drugs and Treatment Groups:

L-arginine was purchased from Calbiochem (La Jolla, CA,

http://www.calbiochem.com), dissolved in saline, and diluted to a 50 mg/kg

concentration immediately before use. Intraperitoneal (i.p.) injections were in a volume

of 0.1 cc / 100 g b.w., administered 15 min before testing. The eight experimental PS

animals received only one treatment of L-arginine, while the eight control PS animals

received only one administration of the vehicle.

Behavioral Testing:

Copulatory behavior tests of the experimental and control males were performed

during the period of darkness (between 13:00 and 18:00 hr) in a quiet room, under red

light conditions. After a 10-min adaptation period in a rectangular glass observation cage

(10 x 20 x 12 in.), a sexually receptive female was presented to the male by placing her in

the cage. Copulatory behavior was videotaped and three behavioral variables were later

scored (number of intromissions, latency to first intromission, and latency to ejaculation).

Tests were terminated if intromission did not occur within 15 min of female introduction,

if ejaculation latency exceeded 30 min, or immediately after ejaculation. To ensure blind

measurement, the copulatory videos were scored by a trained assistant naïve to the

experiment.

Results

For each of the nine animals per group in Experiment 1, NOS IR cell counts

across the ten consecutive brain sections of the mPOA were summed. These data were

then analyzed with a one-way analysis of variance (ANOVA). PS males had significantly

less nNOS IR in the mPOA ($\underline{X}$ = 1054.67) than control males ($\underline{X}$ = 1870.33) ($\underline{F}$(1, 16) =

85.35, $\underline{p}$ < 0.05, Fig. 1). The variables from Experiment 2, number of intromissions,

latency to first intromission, and latency to ejaculation, were analyzed with a multivariate

analysis of variance (MANOVA). Whether or not the animal ejaculated was analyzed

with a Chi Square (χ^2). PS males who were given injections of L-arginine prior to

copulation tests reached ejaculation significantly more than PS males who received the

vehicle injection (χ^2(1, $\underline{N}$ = 8) = 4, $\underline{p}$ < 0.05, Fig. 2). There were no differences in number

of intromissions, latency to first intromission, or latency to ejaculation (Fig. 3 & Fig. 4).

Discussion

PS has a wide range of effects upon a developing organism, altering

neuroanatomy, neuroendocrine and neurotransmitter systems. The hypotheses that PS

would reduce the number of nNOS IR neurons in the mPOA and that administration of

the NO substrate, L-arginine, would ameliorate the detrimental effects of PS upon sexual

behavior were supported. Male PS rats had significantly fewer nNOS containing cells in

the mPOA as compared to controls. Also, six of the eight PS males given an injection of

L-arginine reached ejaculation, in comparison to the two out of eight PS males given a

saline injection.

It is not surprising that PS could influence the levels of nNOS in adult male rats considering the PS effects upon other neurotransmitter systems (Moyer, Herrenkohl, & Jacobwitz, 1978; Peters, 1986b, 1988; Henry et al., 1995). Still, the differences in nNOS IR levels seen in Experiment 1 are striking, with control animals having on the average almost twice the number of nNOS IR as PS rats (Fig. 4). The results from Experiment 1 combined with those gathered in Experiment 2, indicate that NO plays a significant role in the PS-induced attenuation of sexual performance. Benelli et al. (1995) investigated the role of NO in sexual behavior, concluding that increasing the production of NO caused male rats to copulate more vigorously and to ejaculate more frequently, while limiting NO production led to impaired sexual response and ejaculation. The current data parallel Benelli et al. in that PS reduces sexual performance possibly by decreasing nNOS and effectively limiting NO production. Also, these detrimental effects of PS are alleviated by administering a substance that increase NO production. The present data suggests that the resultant loss of nNOS from PS may be a factor in PS-induced sexual deficits.

The current experiment examined the number of mPOA cells containing nNOS IR in PS animals. However, other alterations may be taking place that could modify NO activity and affect sexual behavior. Researchers have found that decreased levels of NO correlate with decreased sexual performance (Hull, Lumley, Matuszewich, & Dominguez, 1994; Hadeishi & Wood, 1996; Bialy, Beck, Abramczyk, Trzebski, & Przybylski, 1996), yet there are other reasons why nNOS levels could be lower in PS males. Considering that PS alters the anatomy of the mPOA (Anderson, Rhees, &

Fleming, 1985), it may be possible that the cell morphology of neurons in this area are also affected, thus lowering nNOS levels. Although it is possible that changes in cell structure may play a part in this reduction, it was not considered in this experiment, and may even be undetectable at the magnifications used. Likewise, there may be differences between PS and control animals in the storage and synthesis of nNOS that rudimentary cell counts would not have addressed.

As this research area is undeveloped, the results from Experiment 2 are interesting in that they indicate that NO may be a link between PS and abnormal sexual behavior. However, not all of the behavioral variables scored were significant. Though number of intromissions were non-significant, there was a trend in the data with the control group having on the average, more mounts ($\underline{X}$ = 25.5) than the experimental group ($\underline{X}$ = 19.8). This trend may simply be due to the control PS animals having been with the females for a longer time, since the majority of these rats did not ejaculate and therefore were tested the entire 30 minutes. Though Benelli et al. (1995) found significant differences in latency to first intromission and latency to ejaculation after the L-arginine injection, the current study did not. This may be the result of the PS since undoubtedly, more than NO pathways were affected in our PS males, including 5-HT, DA, LH, and endogenous opioids. However, the current experiment only attempted to compensate for the NO deficiency. Benelli et al. administered the NO substrate to non-stressed rats, presumably animals that had normal neurochemistry and neuroanatomy. Those non-significant behavioral variables in the current experiment may have been dependent on a pathway other than NO, and therefore were not affected by L-arginine.

Though most of the present results were significant, they must nonetheless be interpreted cautiously. The combined data suggest that inadequate amounts of NO play a role in the behavioral abnormalities of PS. However, other chemical pathways should also be considered. Lorrain et al. (1996) found that NO enhances mPOA DA release during copulation. As stated previously, DA is understood to be important for sexual motivation and is released in greater quantities during copulation (Hull, 1995). In the present experiment, the greater ejaculation rate of the animals receiving L-arginine may be due to increased DA release, and indirectly to NO. Though this would indicate that NO is an intermediary neurotransmitter between PS and abnormal sexual behavior and not the direct cause, the results are still meaningful. Restoring sexual performance to a normal level in a PS male with L-arginine, highlights the overall importance of NO, regardless of its exact role in sexual behavior.

Further research might focus on the mechanics of NO and its modulation of sexual behavior. The current study used the NO substrate, L-arginine, to counteract the effects of PS upon sexual behavior; however, L-arginine is converted to NO only by the enzyme nNOS, which was found to be reduced in PS males. It would be interesting to use a NO donor, which is not dependent upon nNOS to generate NO, on PS male rats and see whether that has a greater beneficial effect upon their sexual behavior. The present experiment takes the neuroscience community one step closer to defining the exact link between PS and abnormal sexual behavior.

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Wood, R. I., & Newman, S. W. (1995). The medial amygdaloid nucleus and medial preoptic area mediate steroidal control of sexual behavior in the male Syrian hamster. *Hormones & Behavior, 29*(3), 338-353.

Figure Caption

Figure 1. Mean cumulative total of nNOS IR neurons in the mPOA.

Figure 2. Number of animals that ejaculated as a function of treatment group.

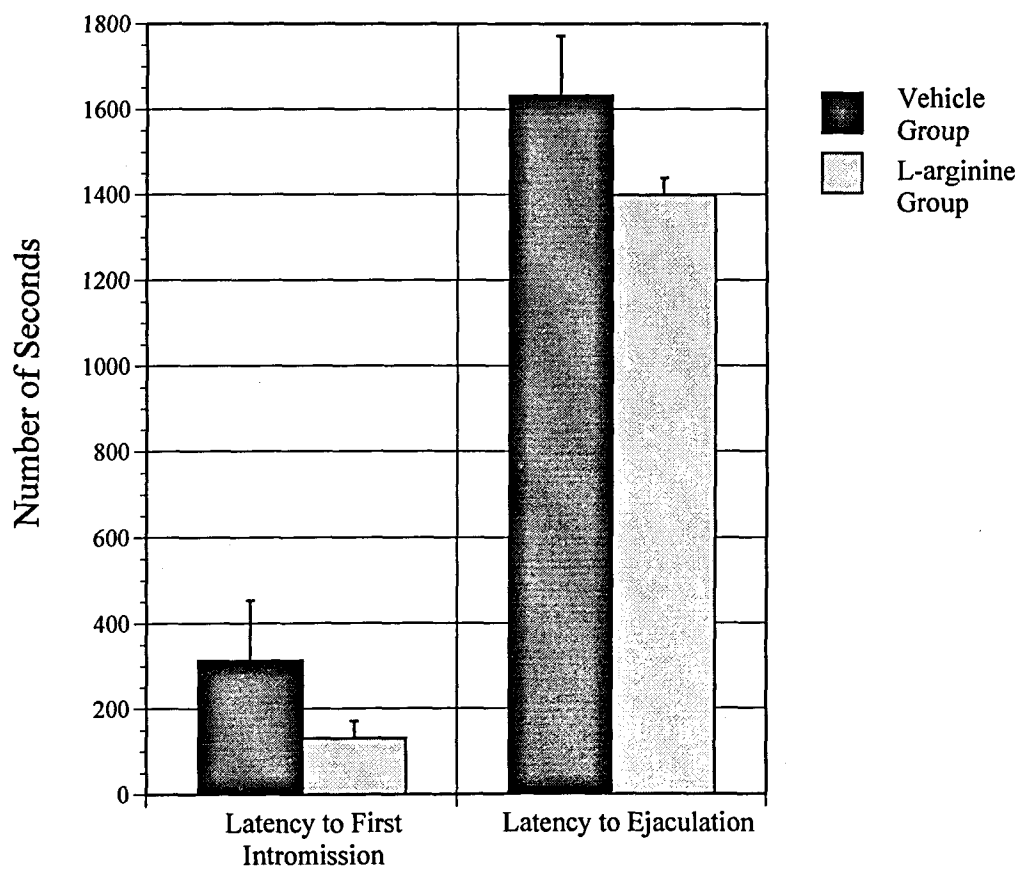Figure 3. Number of intromissions as a function of treatment group.

Figure 4. Latency to first intromission and latency to ejaculation as a function of
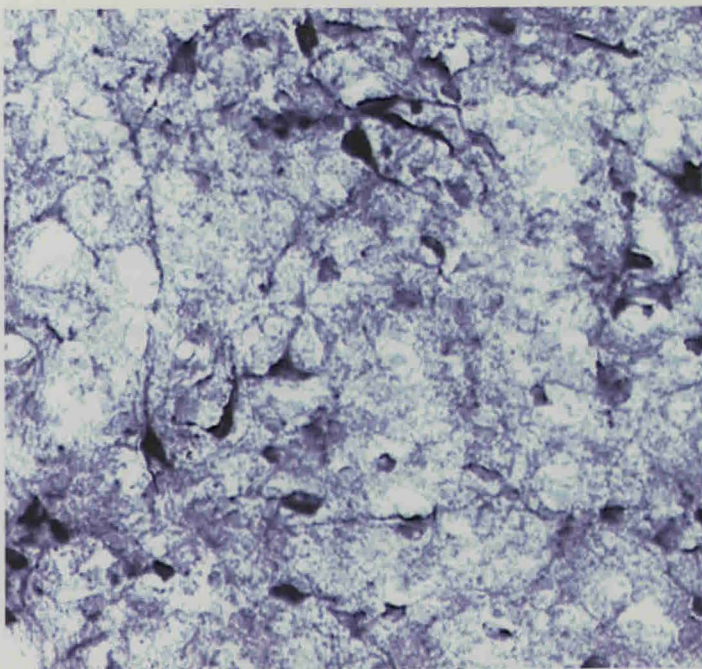
treatment group.

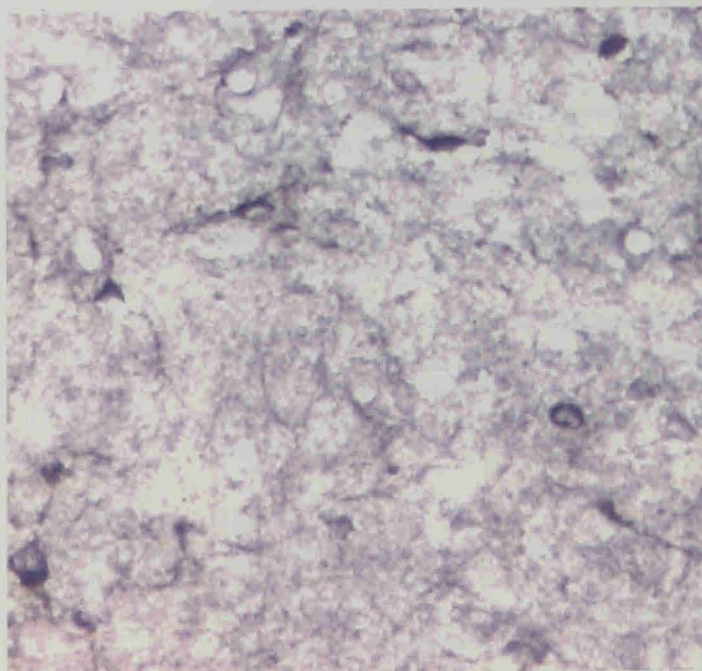Figure 5. Example of nNOS IR in a section of the mPOA in control and PS animals

(400x).

Number of animals that ejaculated

8
7
6
5
4
3
2
1
0

L-arginine Group   Vehicle Group

PS groups
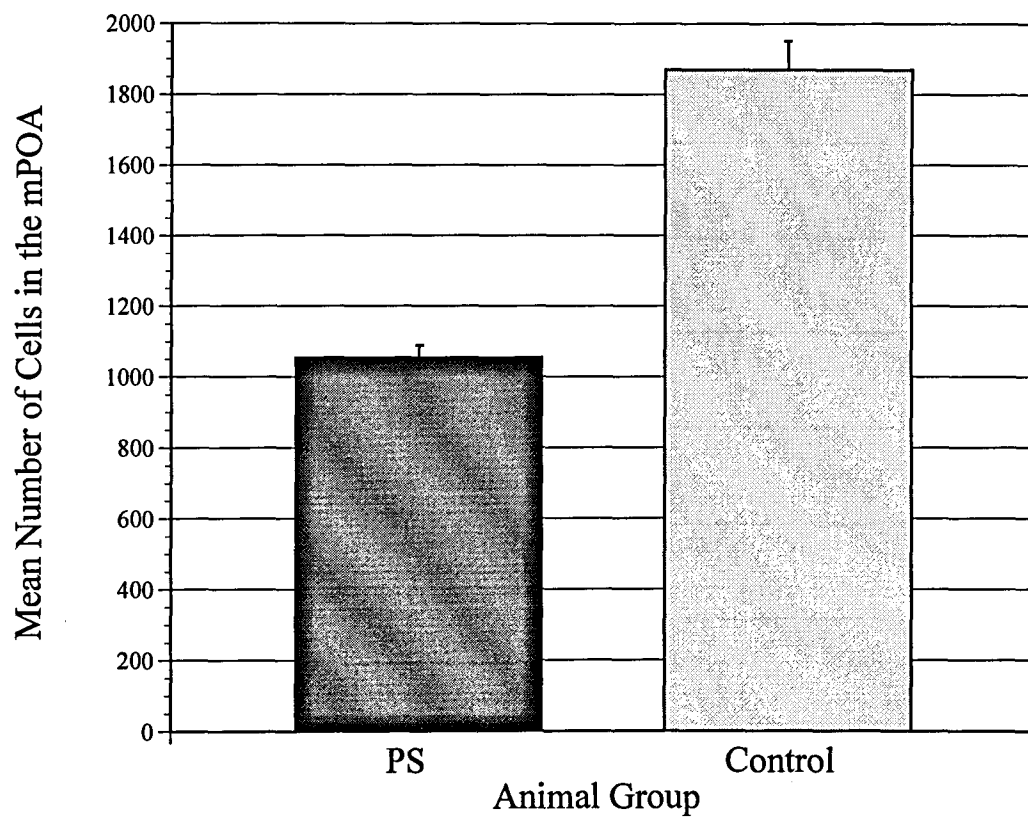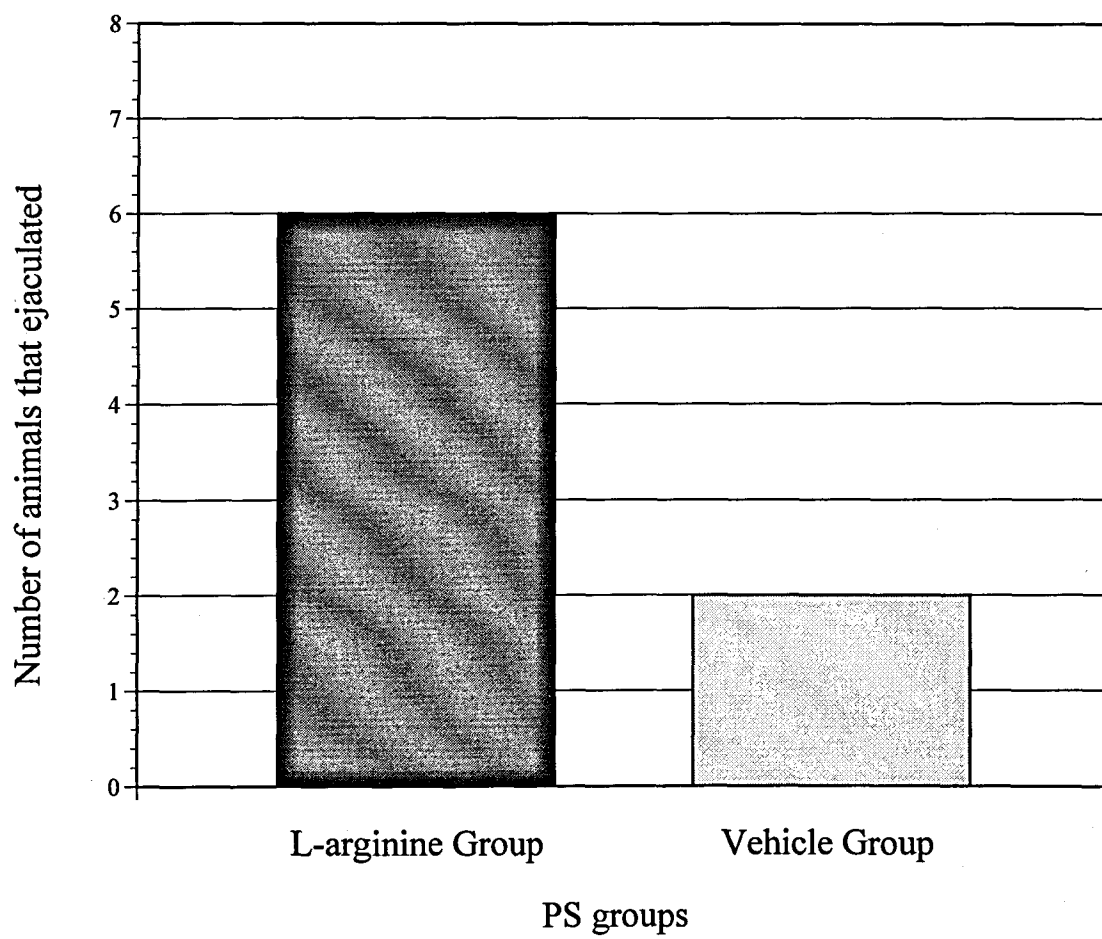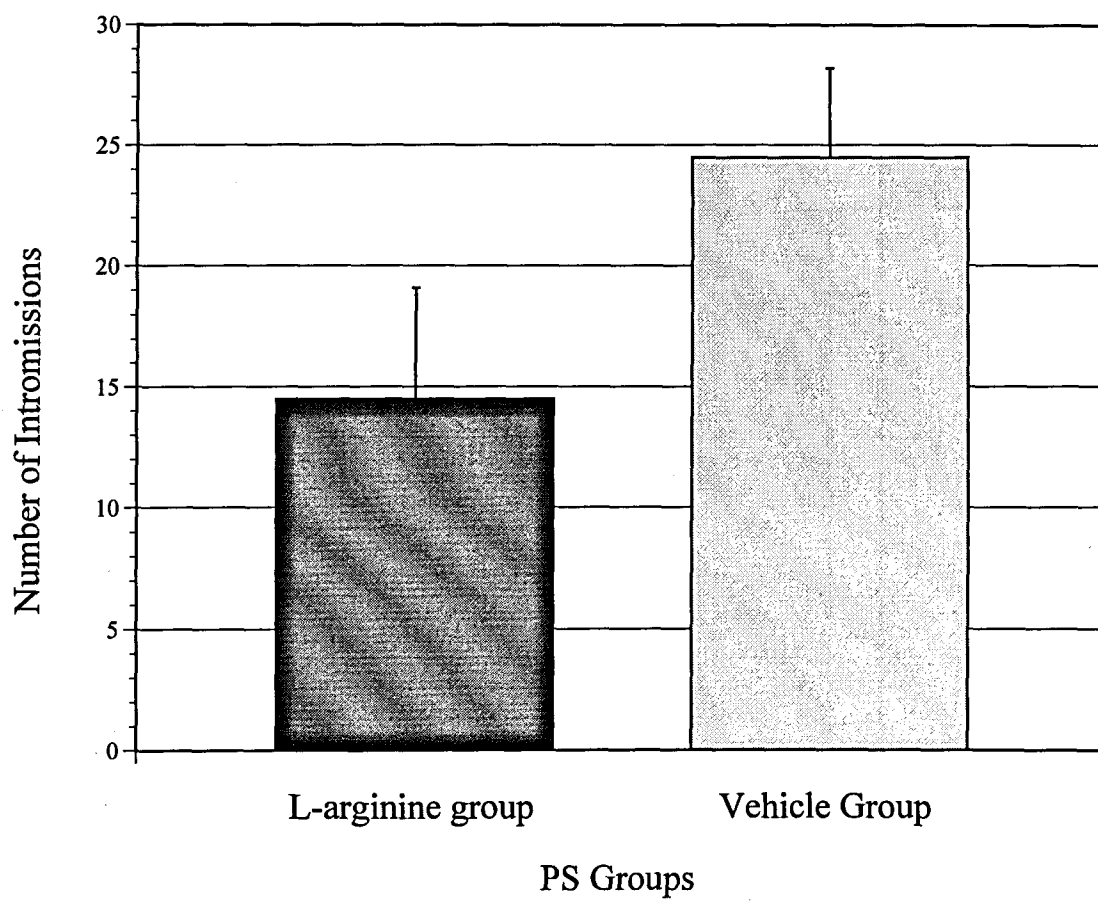
Control Animal

PS Animal

Stephen David Miller

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Education

Villanova University
BA, Psychology, May 1997
University of Richmond
MA, Psychology, August 1999

**Related
Experience**

Toxicology/pharmacology labs of Dr. Aron Lischman, Ph.D., Medical College of Virginia
Research Assistant: Ran animals through different behavioral and drug discrimination tests. Goal of research was determining effects of THC upon brain processes. Consequently, experience increased my knowledge of drug interaction and current research techniques. *(Summer 1998)*.

Neuroscience laboratories of Dr. Craig H. Kinsley, Ph.D., University of Richmond
As a graduate student, have had the opportunity to work on a number of studies. Consequently, have become proficient at perfusions, immunocytochemistry and Golgi staining, and computer enhanced tissue analysis (Neurolucida and Bioquant platforms). *(1997-present)*.

Actimed Laboratories, Mount Laurel, NJ
Production Technician: Manufactured components of a rate-limiting enzyme, cholesterol-monitoring device. Required knowledge and application of organic and inorganic chemistry. Increased my familiarity with chemistry, while working in a corporate setting. *(Summer 1997)*.

Neuroendocrinology laboratory of Dr. Ingeborg L. Ward, Ph.D., Villanova University
Assisted with longitudinal study using male rats to investigate the effects of prenatal exposure to alcohol on the development of adult sexual behaviors. Have learned to perform matings, behavioral observations, statistical analyses, tests, and surgeries, including castrations, ovarectomies, and implanting subcutaneous continual release hormonal capsules, that I helped design and create. *(1996-1997)*.

University Information Technologies, Villanova University
Computer Consultant/Technician: Assisted people with hardware and software problems, became completely familiar with the latest versions of word processing programs, spread sheet and statistical programs, and am conversant with several computer operating systems. *(1996-1997)*.

**Awards
And Grants**

<u>1998-1999</u>
  1999 Outstanding Graduate Student of the Year
  Research grant ($500)
  Travel grant to present research at Society for Neuroscience, Los Angeles ($775)
  Travel grant to present research at the New York Academy of Sciences, Charlottesville, VA ($350)
<u>1997-1998</u>
  Research grant ($1,450)
  Travel grant to present research at the International Society for Developmental Psychobiology, New Orleans, ($850)

Activities   Member of Psi Chi, National Honor Society in Psychology, University of Richmond *(1998-present)*

Member of Delta Kappa Epsilon, Sigma Phi Chapter, Villanova University *(1994-1997)*
President *(1997)*
Secretary *(1995-1996)*

Bibliography

Published Papers

Miller, S. D., Mueller, E., Gifford, G. W., & Kinsley, C. H. (1999). Prenatal stress-induced modifications of neuronal nitric oxide synthase in amygdala and medial preoptic area. *Annals of the New York Academy of Sciences, 877, p. 760-763.*

Published Abstracts

Kinsley, C. H., Madonia, L., Trainer, R., Gifford, G., Miller, S., Tureski, K. & Lambert, K.G. Motherhood enhances learning and memory: Accompanying alterations in neuronal and glial morphology. *Society for Neuroscience Abstracts, 24 (952).*

Gifford, G., Miller, S, Quadros, P. & Kinsley, C.H. Alterations in glial cells (astrocytes) may accompany changes in reproductive condition in the female rat. *Journal of the Virginia Academy of Science, 49* (2), p. 96.

Miller, S., Mueller, E. & Kinsley, C.H. The effects of prenatal stress on concentrations of brain/neuronal nitric oxide synthase (nNOS) in the preoptic area (POA) of male rats. *Journal of the Virginia Academy of Science, 49* (2), p. 99.

Quadros, P.S., Kishore, R., Trainer, R., Gifford, G., Miller, S. & Kinsley, C.H. Prenatal stress (PS)-induced alterations of medial preoptic area (mPOA) estrogen (E2) receptor concentration. *Developmental Psychobiology,* in press.

Convention/Meeting Abstracts

Aurentz, C.A., Felts, P.T., Lowry, C.A., Wartella, J.E., Miller, S.D., Amory, E.A., Gifford, G.W., Kinsley, C.H. & Lambert, K.G. Increased c-fos and tyrosine hydroxylase activity in the extended amygdala of rats displaying compulsive-like running. Paper presented at the annual meeting of the International Behavioral Neuroscience Society meeting, Nancy, France, June, 1999.

Lowry, C.A., Lambert, K.G., Aurentz, C.A., Felts, P.T., DeVries, A.C., Sundstrum, J., Amory, E.A., Wartella, J.E., Gifford, G.W., Miller, S.D. & Kinsley, C.H. An investigation of sex differences and behavioral responses to ecologically relevant stressors. Paper presented at the annual meeting of the International Behavioral Neuroscience Society meeting, Nancy, France, June, 1999.

Williams, J.J., Aurentz, C.A., Gifford, G.W., Wartella, J.E., Miller, S.D., Amory, E.A., Kinsley, C.H. & Lambert, K.G. Paper presented at the annual meeting of the International Behavioral Neuroscience Society meeting, Nancy, France, June, 1999.

Kinsley, C.H., Madonia, L., Trainer, R., Gifford, G., Miller, S., Tureski, K. & Lambert, K.G. Motherhood enhances learning and memory: Accompanying alterations in neuronal and glial morphology. Paper presented at the annual meeting of the Society for Neuroscience, Los Angeles, CA, November 1998.

Miller, S., Mueller, E., Gifford, G., & Kinsley, C.H. Prenatal stress-induced modifications of neuronal nitric oxide synthase (nNOS) in amygdala and medial preoptic area (mPOA) is associated with reductions in sexual and aggressive behavior in the rat. Paper presented at the New York Academy of Sciences meeting *"Advancing from the Ventral Striatum to the Extended Amygdala: Implications for Neuropsychiatry and Drug Abuse,"* University of Virginia, Charlottesville, VA, October, 1998.

Quadros, P.S., Kishore, R., Trainer, R., Gifford, G., Miller, S. & Kinsley, C.H. medial preoptic area (mPOA) estrogen (E2) receptor concentration is modified by prenatal stress (PS). Paper presented at the annual meeting of the International Behavioral Neuroscience Society meeting, Richmond, VA, June, 1998

Gifford, G., Miller, S., Quadros, P., Lambert, K.G., & Kinsley, C.H. Alterations in glial cells (astrocytes) may accompany changes in reproductive condition in the female rat. Paper presented at the annual meeting of the Virginia Academy of Sciences, May 1998.

Miller, S., Mueller, E. & Kinsley, C.H. The effects of prenatal stress on concentrations of brain/neuronal nitric oxide synthase (nNOS) in the preoptic area (POA) of male rats. Paper presented at the annual meeting of the Virginia Academy of Sciences, May 1998.

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