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Effects of Perinatal Cocaine Exposure on Sexual Differentiation in the Rat Brain

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EFFECTS OF PERINATAL COCAINE EXPOSURE ON SEXUAL DIFFERENTIATION IN THE RAT BRAIN.

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INTRODUCTION

A. Purpose. The purpose of this study was to test the hypothesis that perinatal exposure to cocaine (gestational day 15 through postnatal day 10) compromises the development of the medial preoptic nucleus - central part (MPNc), a nucleus involved in sexual differentiation of the brain. Other gestational parameters were also studied to ensure findings of this study were consistent with the literature in order to more confidently test a novel hypothesis. This hypothesis was tested by: (1) comparing the volume of the MPNc in male and female pups exposed to 0mg/kg, 7.5mg/kg, 15mg/kg, and 30mg/kg of cocaine in the womb; and by (2) measuring the gestational parameters of maternal weight gain, litter size, maternal weight gain/litter size, pup weight, male/female sex ratio, and gross birth defects.

This research is important not only for the insight gained into cocaine's effects upon the process of sexual differentiation in rodents, but also for what might be speculated concerning cocaine's effects upon the process of sexual differentiation in humans. Indeed, past research has shown similarities as well as differences in the process of sexual differentiation during development in rodents and primates (Manson, 1989). The most concrete difference to be

shown is the timing of the critical periods for morphogenesis of the reproductive tract and sexual differentiation of the central nervous system (CNS). In primates these events occur late in the first trimester while in rodents they occur in the perinatal period (Manson, 1989).

The gonadal hormones involved in morphogenesis of the reproductive tract and the CNS are identical for all mammalian species studied (Wilson and Lasznitzki, 1971; Gondos, 1980; Maclusky and Naftolin, 1981). Testosterone produced by the Leydig cells of the fetal testis promotes masculinization of both the male reproductive tract and the CNS (Langman, 1981). Therefore, if cocaine could be shown to impair this process of brain sexual differentiation in rodents, insights could be gained concerning cocaine's teratogenic potential in prenatally-exposed human fetuses.

The teratogenicity of cocaine upon the human fetus has become an issue of grave concern due to the increasing numbers of pregnant women who expose their offspring to cocaine prenatally. In 1986, the National Institute on Drug Abuse estimated that 3 million people in the United States abuse cocaine regularly (Adams et al., 1986). Use by pregnant women, which has been shown to have deleterious consequences for both mother and child, has been reported to be common in inner city communities (Zuckerman et al., 1989). As of 1990, it has been reported that between 10 and 20% of births at metropolitan and suburban hospitals are complicated by drug

abuse as indicated by the presence of cocaine metabolites in urine collected during the gestational period (Dow-Edwards et al., 1990). According to Dow-Edwards et al. (1990), the actual prevalence of cocaine use during pregnancy is likely to be even greater. If it could be shown, therefore, that cocaine impairs the development of the rat MPNc, insight could be gained into the behavioral consequences of perinatal cocaine exposure as well as the mechanism of brain sexual differentiation itself.

B. Review of Related Literature. Prenatal cocaine exposure has been shown to have many deleterious developmental effects. The first well-controlled clinical study of the developmental effects of prenatal cocaine exposure was done by Chasnoff et al. (1985). This study revealed that cocaine-influenced pregnancies were characterized by an increased incidence of abruptio placentae (miscarriage) as well as neurobehavioral abnormalities in the offspring. These infants prenatally exposed to cocaine were described as appearing "jittery" and as exhibiting decreased interactive behavior and poor organizational responses. A later study (Bingol et al., 1987) documented retardation of intrauterine growth, increased stillbirth, and bony skull defects in cocaine-exposed babies. Church et al. (1987) also reported an increase in cephalic hemorrhages in cocaine-exposed infants. Further studies performed by MacGregor et al. (1987) reported a significant decrease in birth weight and head circumference in infants exposed to cocaine in the womb. From all these studies one can suggest that prenatal cocaine exposure can induce a number of different developmental abnormalities.

A specific developmental abnormality of perinatal cocaine exposure, decreased head circumference, has been linked to brain damage. Studies of children with compromised head and/or somatic growth have documented neurobehavioral impairment (Villar et al., 1984). Other researchers have reported that babies with slow head growth in utero had

delayed cognitive index, motor performance, perceptual performance, and motor ability at three to seven years of age (Harvey et al., 1982). A later study (Hadeed and Siegel, 1989) confirmed that perinatally-exposed infants exhibited microcephaly when compared to non-exposed infants. Neurobehavioral developmental studies of rats exposed prenatally to cocaine revealed that treated pups showed decreased learning and memory capabilities coupled with an increase in locomotor activity (Spear et al., 1987). Hence, previous research has established a link between perinatal cocaine exposure and compromised brain development as evidenced by microcephaly.

One deleterious effect cocaine may have upon the developing brain is alteration of neurotransmitter concentrations. In adults, cocaine has been found to inhibit the presynaptic reuptake of the neurotransmitters norepinephrine, serotonin, and dopamine resulting in increased levels of these neurotransmitters in the synaptic cleft (Ritz et al., 1987). As a result, cocaine has been found to enhance the interaction of the neurotransmitters with both presynaptic and postsynaptic receptors by prolonging contact time (Bradford, 1986). In the case of chronic cocaine abuse, the nervous system responds to this persistent neurochemical stimulation with compensatory receptor adaptations (Gawin, 1991). Gawin and Ellinwood (1988) have hypothesized that cocaine abuse over long periods of time modifies the density

of the postsynaptic receptors. Specifically, cocaine-induced postsynaptic stimulation of norepinephrine receptors (Dackis and Gold, 1987) has been found to increase the density of both beta- and alpha-receptors (Banerjee et al., 1979; Chanda et al., 1979; Pert et al., 1979). In addition, norepinephrine-induced increases in cyclic-adenylate monophosphate (cAMP) are potentiated by chronic cocaine administration. Together, these results have been suggested to indicate postsynaptic receptor supersensitivity (Banerjee et al., 1979; Seidler, 1991).

An understanding of cocaine's effects upon catecholamine systems is important for interpreting the results of this project, in that catecholamines have been hypothesized to be responsible for the development of the brain regions they influence in adult life (Lauder and Krebs, 1986). In addition, it has been found that the MPNc is a region richly innervated by catecholamine-containing terminals (Simerly et al., 1986; Jacobson et al., 1989). Therefore, cocaine's effects upon catecholamines may result in changes in the development of the brain regions they influence, e.g. the MPNc.

Another possibility by which cocaine could affect the development of the MPNc is by alteration of the interactions between hormones and neurotransmitter systems. Preliminary studies have suggested that there is an interactive effect between neurotransmitters and hormones in mediating aspects of

sexual differentiation of the brain (Lauder and Krebs, 1986). The neurotransmitter norepinephrine, in particular, has been suggested to be primarily involved in androgen-dependent sexual differentiation of the brain (Raum and Swerdloff, 1981). While a single injection of testosterone propionate given to female rats within the first 9-10 days of postnatal life will permanently block the luteinizing hormone surge mechanisms and ovulation (Barraclough, 1961; Barraclough and Gorski, 1961; Diaz et al., 1989), norepinephrine stimulation prior to such administration was shown to reverse this effect (Raum and Swerdloff, 1981). The researchers found that beta-adrenergic receptor stimulation by norepinephrine prior to testosterone administration in four-day-old female rats prevented the development of anovulatory sterility in adulthood, i.e. had a feminizing effect that overrode the masculinizing effect of testosterone.

In a later study conducted by Jacobson et al. (1989) the medial preoptic area was found to be a region rich in catecholamine-containing terminals. In addition, these catecholamine innervations were found to be sexually dimorphic, with the female MPNc receiving a greater density of terminals than the male MPNc (Jacobson et al., 1989). This work would seem to imply that there is a sexually dimorphic innervation pattern of catecholaminergic stimulation that either enhances or causes sexual dimorphisms in MPNc volume. Therefore, if Raum and Swerdloff's research concerning

norepinephrine is correct, it would be reasonable to hypothesize that cocaine, which has been found to indirectly increase the number of norepinephrine postsynaptic receptors (Dackis and Gold, 1987), would interfere with androgen-dependent brain sexual differentiation.

Indeed, a recent study conducted by Chasnoff et al. (1988) supported the hypothesis that cocaine interferes with some aspects of somatic sexual differentiation. This study reported the existence of genitourinary tract anomalies in cocaine-exposed infants. Of the fifty cocaine-exposed infants, nine demonstrated anomalies, some infants exhibiting more than one malformation. Anomalies among the fifty infants included: female pseudohermaphroditism (4%), hydronephrosis (14%), ambiguous genitalia (4%), and anal atresia (4%). Ultrasound examination of one female infant revealed the absence of both the uterus and ovaries. In addition, the Center for Disease Control (1989) reported urogenital tract malformations in the offspring of women using cocaine during early pregnancy. Most recently, a study conducted by El-Bizri et al. (1991) confirmed cocaine's teratogenic potential on the genitourinary tract in rats. This study found a dose-dependent increase in such soft tissue malformations as enlarged bladders and hydronephrosis at increasing intraperitoneal dosages. In addition, perinatal cocaine exposure was also found to reduce sperm counts by 40% in male pups exposed to only 10 mg/kg of cocaine (McGivern et al.,

1989). Further, research done by Raum et al. (1990) has suggested that exposure to cocaine compromises sexual differentiation of the male brain by interfering with hypothalamic nuclear incorporation of testosterone and estradiol during the critical perinatal period for MPNc development. Thus, there is sufficient evidence to support the idea that perinatal cocaine exposure compromises both sexual differentiation of the brain as well as the genitourinary tract.

Brain sexual differentiation has been hypothesized to be controlled by the preoptic-anterior hypothalamic area (Gorski, 1974). Gorski et al. (1978) have recently discovered a hypothalamic nucleus, the medial preoptic nucleus - central part (MPNc), that is hypothesized to be involved in sexual differentiation of the brain. The volume and shape of this nucleus have been found to be sexually dimorphic in rats (Gorski et al., 1978), ferrets (Tobet et al., 1986), gerbils (Commins and Yahr, 1984), monkeys (Bubenik and Brown, 1973; Ayoub et al., 1983), and most recently in humans (Swaab and Fliers, 1985; Allen et al., 1989; LeVay, 1991). In rats, the MPNc has been found to be three to eight times larger in males than in females (Gorski et al., 1978; Gorski et al., 1980; Jacobson et al., 1981; Jacobson et al., 1985; Handa et al., 1986; Jacobson et al., 1989; Jarzab et al., 1990; Rhees et al., 1990). This morphological difference has been shown to be independent of the steroidal environment in the adult while

being profoundly influenced by the perinatal steroid environment (Gorski et al., 1978; Jacobson et al., 1980; Jacobson et al., 1981; Dohler et al., 1984A). Hence, since perinatal cocaine exposure has been found to influence the steroid environment of the developing fetus (Raum et al., 1990; Benton et al., 1991), morphological differences might be observed in the size of this nucleus.

Many different studies have implicated the medial preoptic area (MPOA), of which the MPNc is a part, as a critical brain structure for the expression of sexual behavior (Heimer and Larsson, 1967; Slimp et al., 1978; Gray and Brooks, 1984; Turkenburg et al. 1988). Lesions in this area have been found to impair masculine sexual behavior in both male (Heimer and Larsson, 1967) and female (Gray and Brooks, 1984) rats as well as male primates (Slimp et al., 1978). Lesions in the MPOA seem to promote feminine sexual responses, such as lordosis, in both male (Hennessey et al., 1986) and female (Powers and Valenstein, 1972; Turkenburg et al., 1988) rats. Electrical stimulation of the monkey MPOA evokes penile erection, ejaculation, mounting, and thrusting behaviors (Robinson and Mishkin, 1966). Similarly, changes in neuronal activity in the MPOA of the male monkey have been related to sexual activity (Maclean and Ploog, 1962; Robinson and Mishkin, 1966; Oomura et al., 1983). According to Gorski (1974), "It is generally accepted that neonatal androgen exposure in some way permanently masculinizes the preoptic-

anterior hypothalamic area," which he suggests regulates ovulation in females. While the MPOA is essential for cyclic gonadotropin regulation in rats (Gorski, 1968), it is not necessary for this function in primates, but still plays a modulatory role (Plant et al., 1979; Pohl and Knobil, 1982). Therefore, if cocaine could be found to interfere with the development of this area, resultant complications in reproductive behavior as well as gonadotropin regulation could be postulated.

Most interestingly, a recent study conducted by Raum et al. (1990) found that perinatal cocaine exposure inhibited uptake of sex steroids in the hypothalamus. Cocaine was found by Raum et al. (1990) to inhibit nuclear uptake of testosterone and estradiol in the hypothalamus of neonatal rats by approximately 50%. Further, this inhibition of hypothalamic nuclear uptake of sex hormones during the critical period for sexual differentiation has been postulated by Raum et al. (1990) to be responsible for demasculinization of adult male sex-related behaviors. Hence, if cocaine could be shown to influence the size of the MPNc, correlative evidence of a role for the MPNc in sexually differentiated behaviors would be obtained.

While the reproductive behavioral consequences of perinatal cocaine exposure have been studied (Abel et al., 1989; Raum et al., 1990), the possible morphological changes o specific brain regions have not. Therefore, it is the

purpose of this study to investigate the effects of perinatal cocaine exposure on the morphology of the MPNc. If it could be shown that this adrenergic uptake blocker impairs development and maturation of the MPNc, insight could be gained not only into the brain damage imposed upon the cocaine-exposed offspring, but also into the mechanism of brain sexual differentiation itself.

MATERIALS AND METHODS

A. Experimental Design. Thirty female Sprague-Dawley rats were housed with a lighting cycle of twelve hours light and twelve hours dark (lights on at 0700h). From these thirty rats, six experimental blocks were designated (see Table I). Each block consisted of five rats mated within a few days of each other. With the exception of the 30mg/kg rat, the other four pregnant rats in each group were randomly assigned, one to each treatment group. The 30mg/kg rat was mated first so that pair-feeding and pair-watering could be carried out (see below). These blocks were created allowing one dam from each treatment group to be evaluated in six consecutive time frames to rule out technique effects, resulting from increased experience on the part of the investigator.

Beginning on day 15 of pregnancy until parturition, the females were weighed on a pan-balance and subsequently injected subcutaneously with 0mg/kg (two control groups), 7.5mg/kg, 15mg/kg, or 30mg/kg of cocaine hydrochloride. A stock solution of cocaine was made by dissolving 30 mg of cocaine hydrochloride in 1 ml of sterile 0.9% (w/v) saline. This stock solution was diluted with saline and injected into the rats belonging to the 15 mg/kg and the 7.5 mg/kg treatment groups so that the final volume of each injection was

Table 1. Experimental Design.

	TREATMENT GROUP				
	NO PF/W	PF/W	7.5MG/KG	15MG/KG	30MG/KG
MALE	6*	6	6	6	6
FEMALE	6	6	6	6	6

*Number of pups analyzed in each treatment group.

equivalent to that administered to the 30 mg/kg treatment rat in each experimental block on the specified day of pregnancy.

For example, if a 15mg/kg rat weighed 200gm, and the 30mg/kg rat received a total injection volume of 0.20ml on this gestational day, 0.1ml of cocaine stock solution in addition to 0.1ml of saline would be drawn. The volume of injections ranged between 0.23ml-0.45ml. Control animals were injected with sterile saline at an equivalent volume as was injected into the 30 mg/kg rat on the specified day of pregnancy. All injections were given at 0800h.

Beginning also on day 15 of pregnancy until parturition, the pregnant females were pair-fed and pair-watered according to the 30mg/kg treatment group animals. Pair-feeding and pair-watering were employed to rule out a possible malnutrition effect confounding the results obtained for the cocaine-treated animals. This was accomplished by weighing the food and water consumed by the 30mg/kg treatment group animals on day 15 of gestation onward. Except for the first control group, this amount of food and water was given to the other three treatment animals in the experimental block on the same gestational day as the 30mg/kg treatment animal. The first control group animal in the block was neither pair-fed nor pair-watered in order to differentiate between effects of malnutrition and effects of cocaine exposure upon brain development. All 30mg/kg treatment animals were mated first so that the amount of food and water consumed on each

gestational day was established for the other four treatment animals in each experimental block.

At parturition, four male and four female pups from each litter were cross-fostered. Cross-fostering was accomplished by mating a surrogate dam at the same time as each treatment animal. At birth, the surrogate pups were removed and replaced with the eight treatment pups to rule out possible postgestational malnutrition and caretaking effects. These pups were weighed every day and continued to receive subcutaneous cocaine hydrochloride injections for 10 days post-gestation during their critical period of brain sexual differentiation. This time frame of cocaine injections (from day 15 of gestation until day 10 postpartum) was based upon previous studies on the developmental sensitivity (the period during which hormones have been found to influence the volume of the MPNc) of the MPNc to hormones. Developmental sensitivity has been shown to begin on day 18 of gestation (Rhees et al., 1990) and continue through days 7-10 postpartum (Dunlap et al., 1978). The concentration of the pup injections remained consistent with their parent's treatment group specifications. This continuation of maternal cocaine dosage was chosen because according to Dow-Edwards (1990), rat maternal and fetal plasma cocaine levels are essentially the same by fifteen minutes after administration to the mother.

B. Perfusions. On day ten of postnatal life one male and one female pup were obtained from each dam. These pups were anesthetized with either Methoxyflurane or ethyl-ether. Once deep anesthesia was achieved, an intracardiac injection of one hundred units of heparin in 0.10 ml was administered. One minute later, an intracardiac perfusion was performed by making an incision in the bottom of the left ventricle and inserting a canula gently upward into the ascending aorta. The first perfusate, 0.9% saline (w/v), was flushed through the rat's circulatory system until the rat had become exsanguinated. Two-hundred milliliters of the second perfusate, 10% formalin in 0.10 M sodium phosphate (pH 7.4), were subsequently flushed through the rat's circulatory system. The total perfusion time lasted approximately thirty minutes. Following this procedure, the pups were decapitated, their brains removed and stored the same solution of 10% formalin in 0.10 M sodium phosphate at 4°C. Brains were allowed to postfix in the 10% formalin in 0.10 M sodium phosphate for a minimum of two weeks. Following postfixation, the brains were transferred to a solution composed of 30% sucrose (w/v), 10% formalin in 0.10 M sodium phosphate for a minimum of four days, were subsequently embedded in 20% gelatin (w/v). The gelatin block was allowed to fix in the 30% sucrose, 10% formalin in 0.10 M sodium phosphate for a minimum of one week to ensure proper sectioning.

C. Sectioning and Staining. The brains were sectioned at a thickness of 60 um on a dry-ice freezing microtome. This thickness was chosen to ease the identification of the boundaries of the MPNc. According to studies done by Gorski et al. (1980), the boundaries of the MPNc of thinner sections become "much more difficult to recognize." In addition, analysis of thinner sections (6 um) has been found to underestimate MPNc volume (Allen et al., 1988). The volume of the rat MPNc was reported to be underestimated by approximately 60% when 6 um sections were employed over 60 um sections for analysis (Allen et al., 1988). Following sectioning, serial sections were mounted onto gelatin-coated slides and stained. Briefly, sections were dehydrated with alcohol, defatted with xylene, rehydrated with distilled water, stained with an aqueous solution of 0.1% thionin in 0.10 M sodium acetate buffer (Ph 5.2) for 3 to 4 minutes, differentiated with alcohol, and finally coverslipped with Permount (Gorski et al., 1978). Both thionin and Cresyl violet staining have been employed for processing MPNc tissue sections in earlier studies. Thionin staining was chosen for this project because studies conducted by Gorski et al. (1980) found that Cresyl violet failed to differentiate the MPNc from the surrounding medial preoptic area. Earlier studies done by Gorski et al. (1978) employing thionin staining found the MPNc to be "intensely stained" in comparison with the surrounding medial preoptic area. Since defining the boundaries of the

MPNc apart from the surrounding medial preoptic area was of critical importance in determining the volume of the MPNc, a stain was chosen that maximizes this contrast.

D. MPNc Volume and Brain Volume Determinations.

Following these histological procedures, the volumes of the MPNc were determined for five brains from each treatment group. This was accomplished by employing a series of standard tracing and computer procedures (Gorski et al., 1978; Gorski et al, 1980; Barron et al., 1988; Allen et al., 1989; Jarzab et al., 1990). MPNc sections were projected onto paper with the aid of a microprojector set at 25X magnification. For each section, the boundaries of the left and right MPNc were drawn, as defined by the structural criteria of Robert McGivern at the University of California Los Angeles School of Medicine (Neptune Soleimanzadeh, University of California Los Angeles, personal communication, 1992). These criteria included specifications concerning location landmarks, roughly the number of sections the MPNc can be found in, cellular density, cell size, and staining criteria. In terms of location landmarks, the MPNc can be easily identified as the darkest-staining area half-way between the closed anterior commissure and the optic chiasm. The MPNc can be found in roughly eight to twelve sections, depending upon the angle at which the brain was cut. The MPNc contains larger neurons with approximately 33% more cells/unit area than does the surrounding medial preoptic area. Lastly, the MPNc stains roughly as darkly as the suprachiasmatic nucleus. The MPNc drawings were done in a "blind" fashion to prevent experimental bias on the part of the investigator. Briefly,

the brains were coded so that the sex and treatment group of the pups were unknown at the time of measurement. These tracings of the MPNc were made from the microprojector onto paper and the resultant areas were calculated with the aid of the computer program BIOQUANT II (Bioquant Apple Program version 2.1; R & M Biometrics) with a digitalizing pad (Allen et al., 1989). The MPNc volumes were subsequently computed by summing all the MPNc areas and then multiplying this sum by the section thickness (60 um) (Barron et al., 1988; Allen et al., 1989).

A rough index corresponding to total brain volume was calculated by determining the area of the first brain section containing the suprachiasmatic nucleus (SCN) and multiplying this area by the section thickness (60 um). This measure was calculated to see if MPNc volume changed relative to total brain volume to ensure that any changes observed in the MPNc were due to volume changes in the nucleus itself and not merely resultant changes arising from overall changes in brain volume. Such an index has been demonstrated to represent an accurate assessment of overall brain size (Jacobson et al., 1980). Whereas Jacobson et al. (1980) employed the indexes of brain height and brain width from one representative section, this study employed the index of brain volume from one representative section. A volumetric index was chosen over a linear measure to allow a ratio of the (MPNc volume)/(brain volume index) to be calculated.

E. Statistical analyses. The effect of cocaine and sex upon the measured gestational parameters were determined by a two-way analysis of variance (ANOVA). When a significant F-value was obtained, means of the right MPNc volumes among the treatment groups were compared by the parametric Newman-Keuls Test (Zar, 1984). This parametric multiple comparison test was employed because the assumption of population normality had been previously accepted in the literature (Gorski et al., 1978; Gorski et al., 1980; Jacobson et al., 1981; Dohler et al., 1982; Commins and Yahr, 1984; Dohler et al., 1984A; Anderson et al., 1986; Handa et al., 1986; Barron et al., 1988; Allen et al., 1989; Cherry and Baum, 1990) and the assumption of homogeneity of variance in the present study was confirmed by the use of the Bartlett test (Zar, 1984).

Paired t Tests were performed for comparison of results obtained from the left and right sides of the brain. This statistical analysis was employed to determine whether the right and left nuclei differed significantly in terms of volume. While both right and left MPNc volumes were analyzed to determine if they differed significantly, only right MPNc volumes were utilized for all other MPNc analysis. This decision was made due to the fact that the left MPNc had tissue tears through the nucleus itself. Finally, linear regression analyses were performed to determine lines of "best fit" to express the relationship between cocaine dosage and MPNc volume.

RESULTS

A. Gestational Parameters.

1. Maternal Weight Gain. Treatment group and block were found to have a significant effect upon maternal weight gain ($p<0.01$). Non-pair-fed, non-pair-watered controls showed significantly greater maternal weight gains ($p<0.001$) than pair-fed, pair-watered controls or any of the three cocaine treatment groups. However, the three cocaine treatment groups and the pair-fed, pair-watered controls showed no statistically significant ($1.0>p>0.50$) differences in their maternal weight gains (see Fig. 1).

2. Litter Size. Treatment group, block, and the interaction between the treatment group and experimental block were all found not to have any effect ($1.0>p>0.50$) upon dam litter size (see Fig. 2). Litter sizes ranged from 10-20 pups in the two control groups and from 5-19 in the cocaine treated groups. Non-pair-fed, non-pair-watered controls had a mean litter size of 16.000; pair-fed, pair-watered controls had a mean litter size of 13.167; 7.5mg/kg treatment dams had a mean litter size of 15.000; 15 mg/kg treatment dams had a mean litter size of 15.5; and 30mg/kg treatment dams had a mean litter size of 12.571 pups.

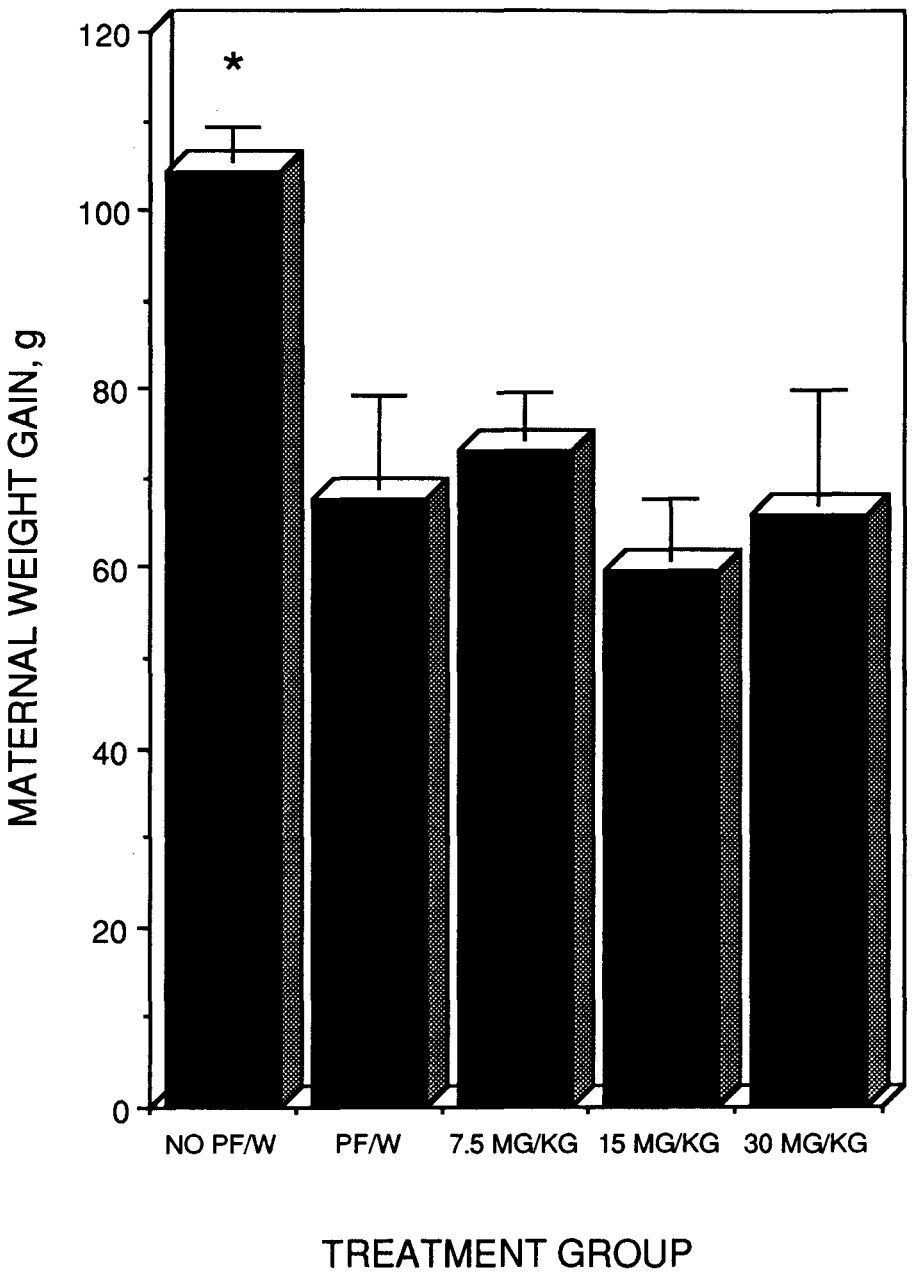

Fig. 1. Effect of treatment group on maternal weight gain. Bars represent the mean +/- S.E.M. of 6 replicates. Non-pair-fed controls gained significantly more weight than other treatment groups ($p<0.05$).

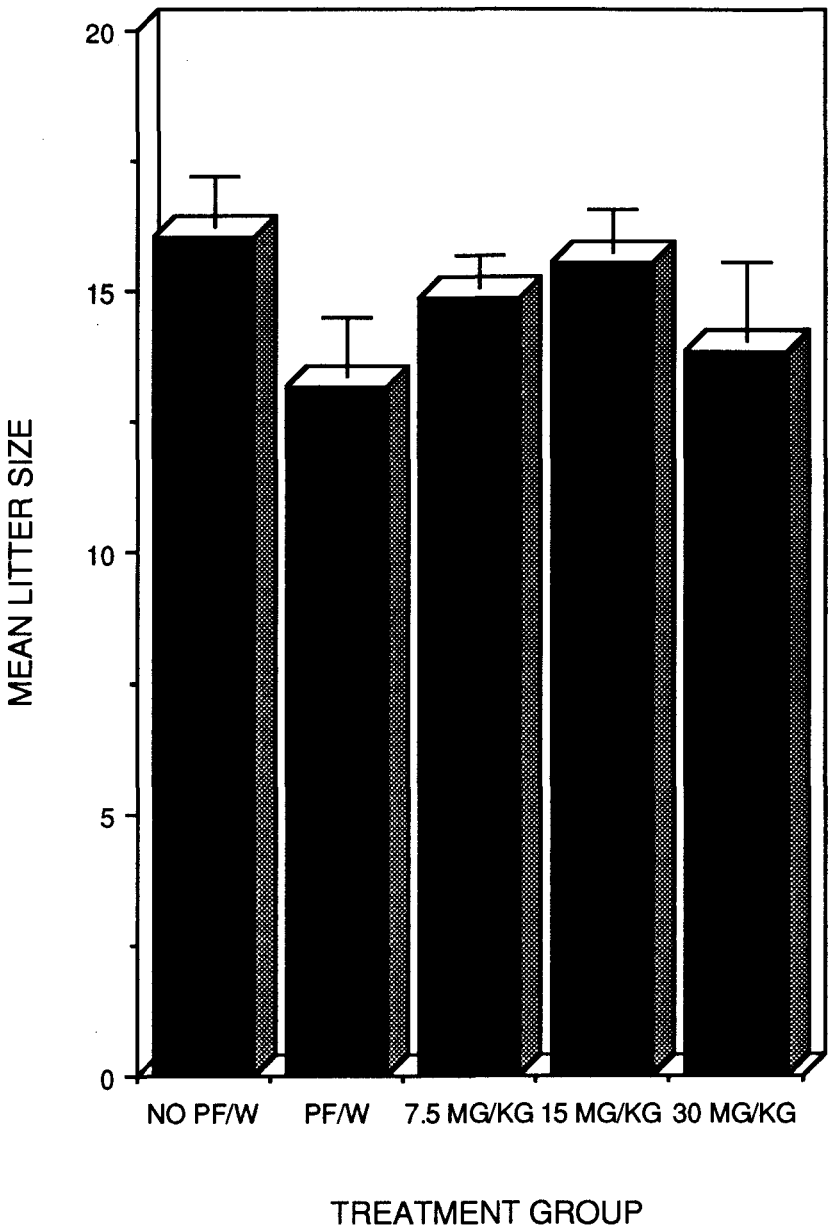

Fig. 2. Effect of cocaine on litter size. Bars represent the mean +/- S.E.M. of 6 replicates. Treatment group had no effect upon the size of the litter delivered by each dam.

3. Maternal Weight Gain Per Pup. Treatment group and experimental block were found to have a significant ($p<0.01$) effect upon maternal weight gain/litter size (see Fig. 3). When compared to the non-pair-fed, non-pair-watered controls, the cocaine-treated dams and the pair-fed, pair-watered dams exhibited a reduction in maternal weight gain (see Fig. 3). When compared to the pair-fed, pair-watered controls, none of the cocaine-treatment dams exhibited a significant ($1.0>p>0.15$) reduction in maternal weight gain (see Fig. 3).

4. Pup Weight. Treatment group and block were not found to have a significant effect ($0.75>p>0.15$) upon the pup weights as measured on day ten of postnatal life (see Fig. 4).

5. Male-Female Sex Ratio. Treatment group, experimental block, and the interaction between treatment group and block were all found not to affect the male-female sex ratio (total number of males/litter size) ($0.75>p>0.50$) (see Fig. 5).

6. Gross Birth Defects. No gross birth defects were observed in any of the pups (489 total) from any of the litters in the five treatment groups.

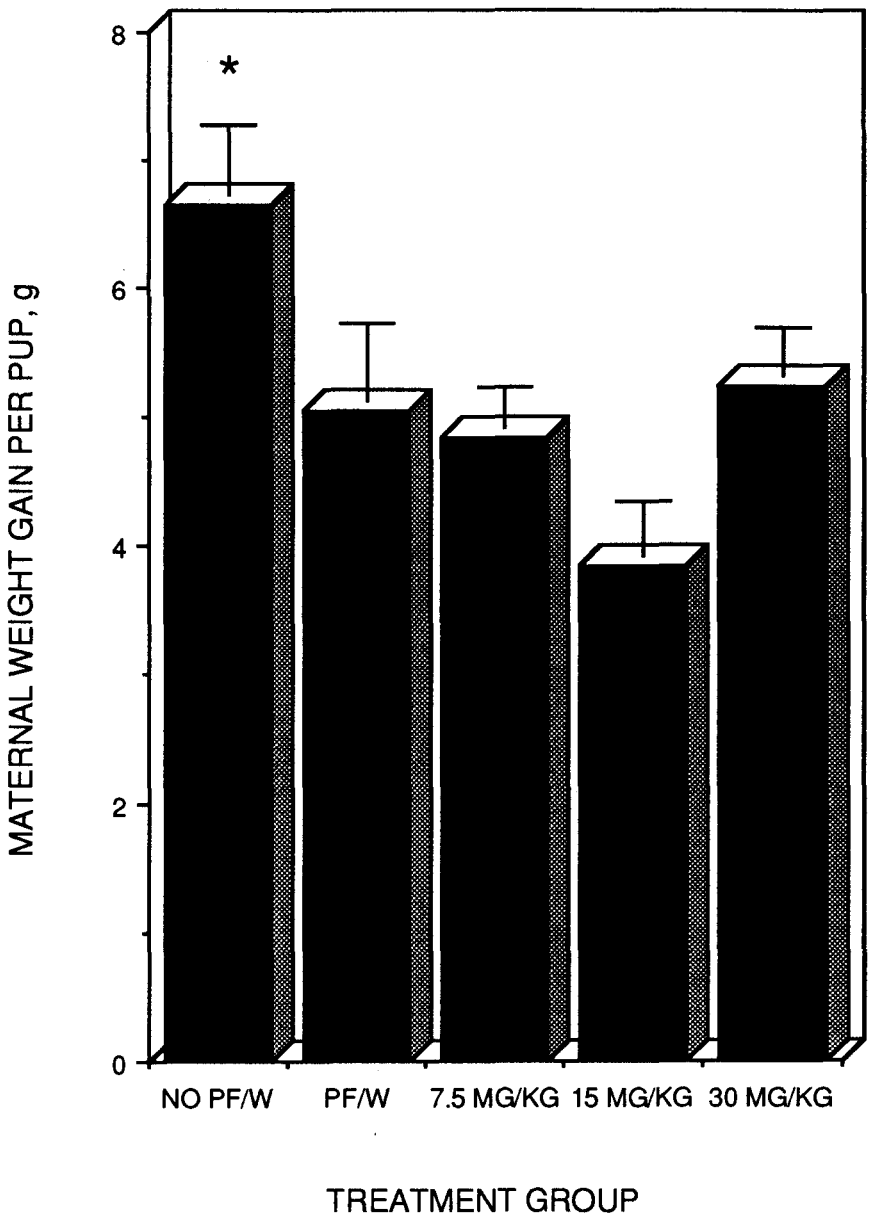

Fig. 3. Effect of cocaine on maternal weight gained per pup. Bars represent the mean +/- S.E.M. of 5-6 replicates. Only non-pair-fed controls were statistically different ($p<0.05$) from other treatment groups.

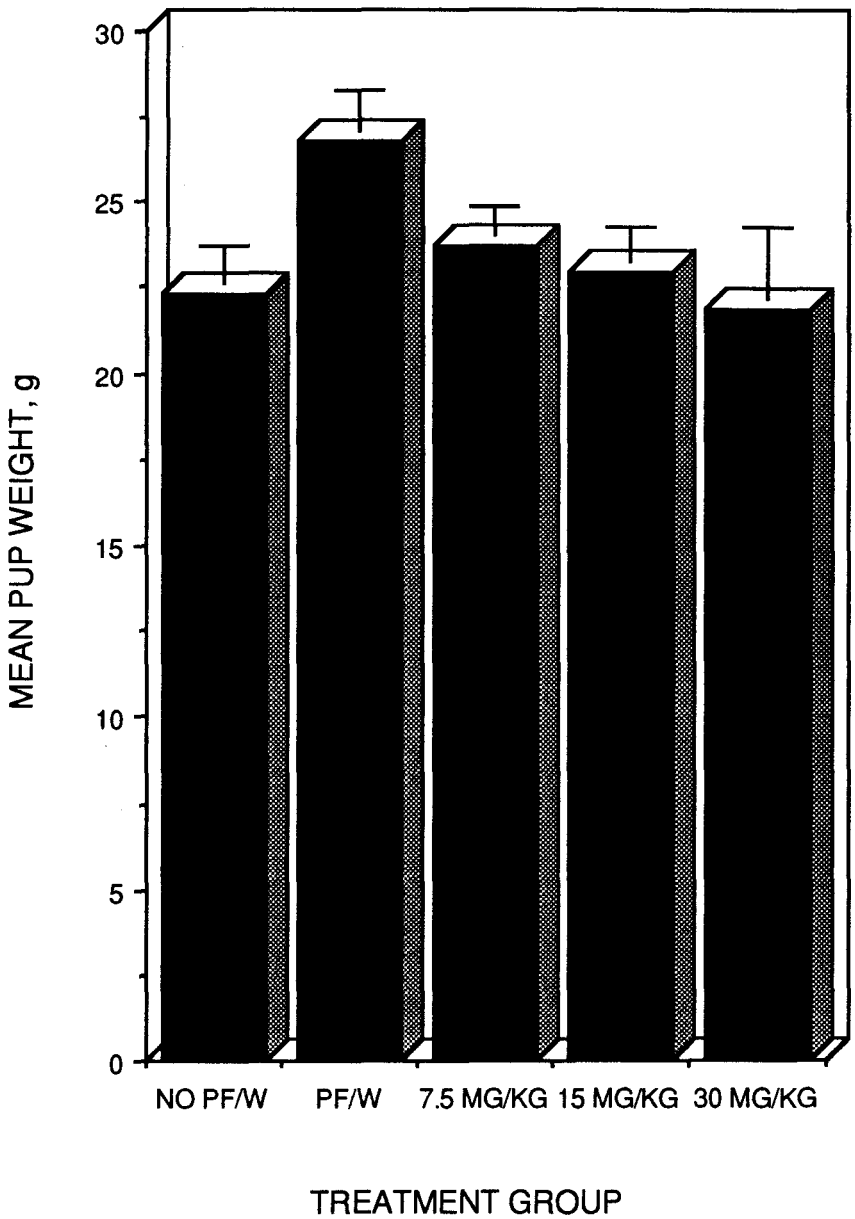

Fig. 4. Effect of cocaine on pup weight. Bars represent the mean +/- S.E.M. of 5-6 replicates. Treatment group had no effect (0.25>p>0.10) on the weight of the pups.

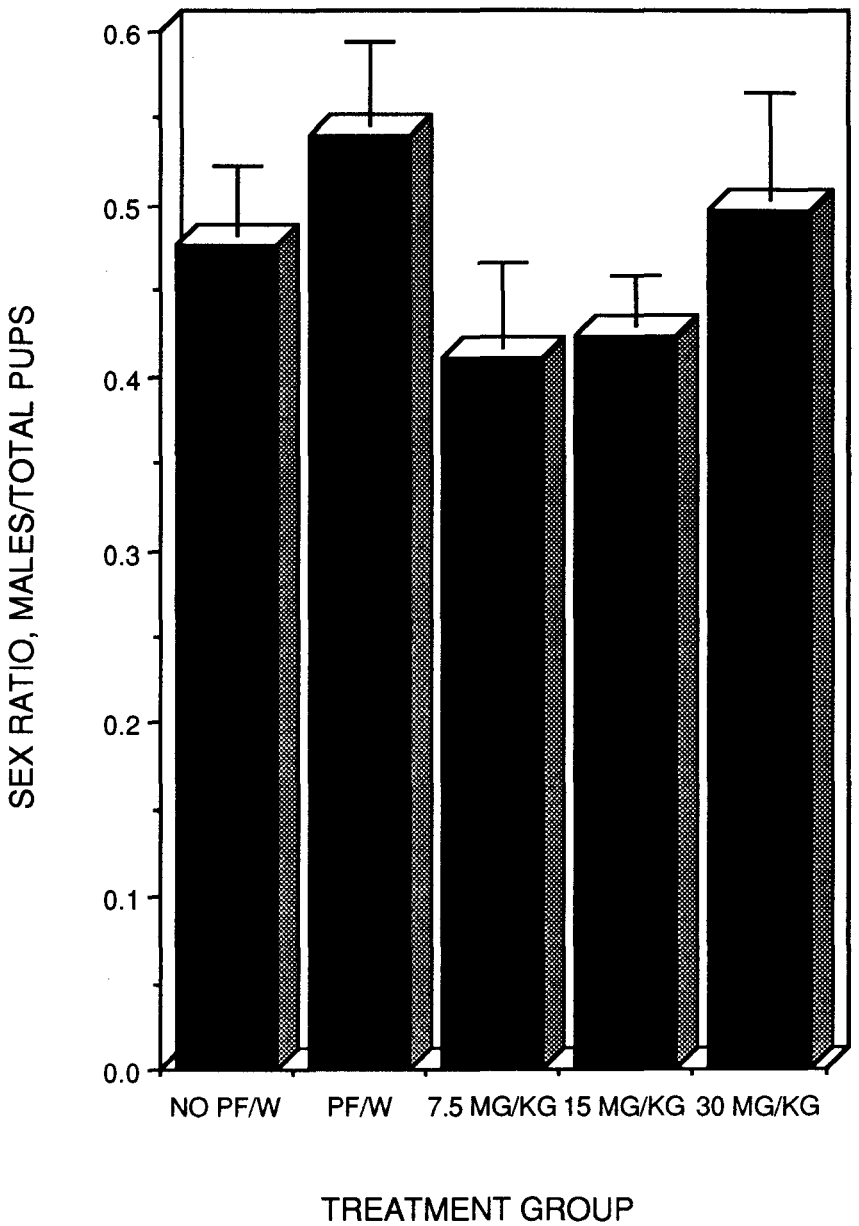

FIG. 5. Effect of cocaine upon the sex ratio (males/total pups). Bars represent the mean +/- S.E.M. of 6 replicates. Treatment group had no effect (0.75>p>0.50) upon the sex ratio.

B. MPNc Measurements.

1. MPNc Volumes. Photographs of representative sections containing the MPNc are shown for a control female, control male, and a 30 mg/kg treatment male (see Fig. 6-8). Treatment group, sex, and the treatment-sex interaction were all found to have a significant effect upon MPNc volume ($p<0.010$). One way ANOVAs revealed that cocaine was found to have a significant inhibitory effect ($p<0.001$) upon male MPNc volumes while not affecting the female MPNc volumes ($1.0>p>0.75$).

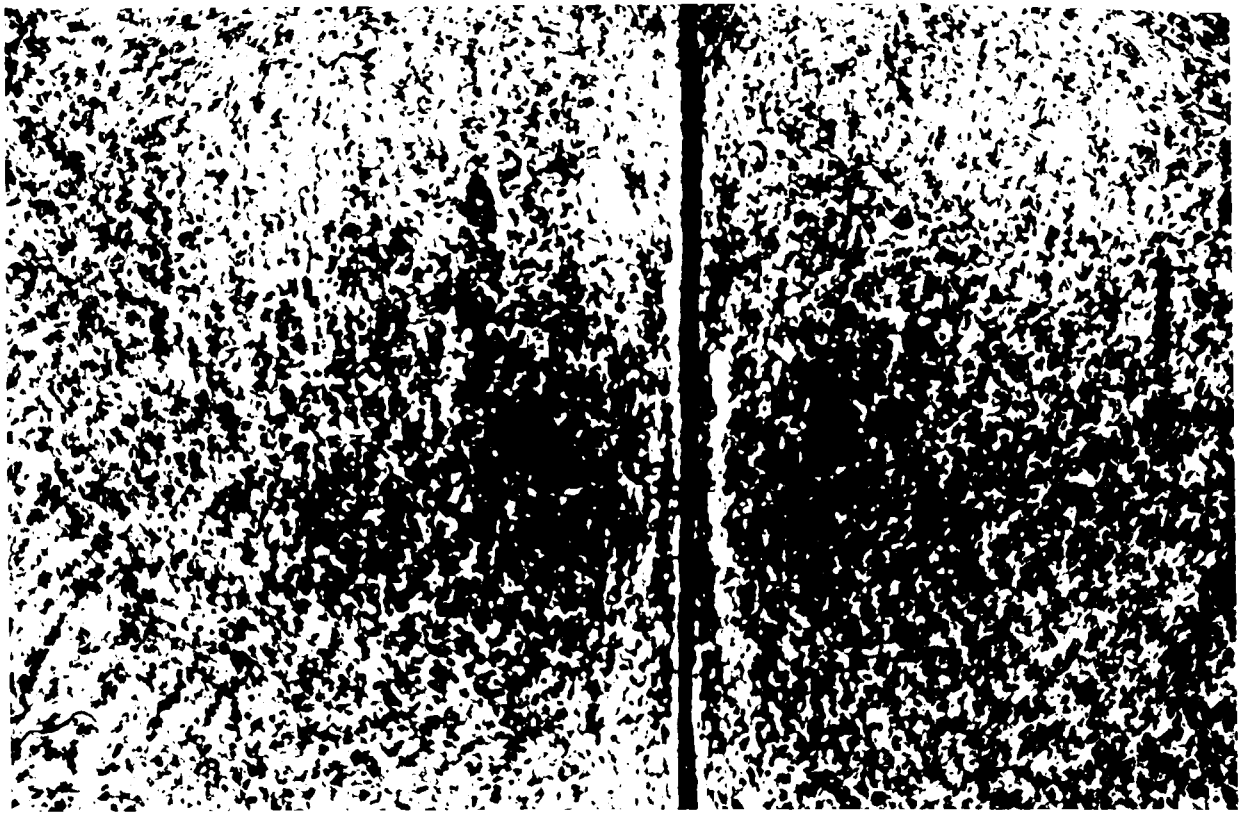

Fig. 6. Representative section through the MPNc of a control female. Magnification = 296X.

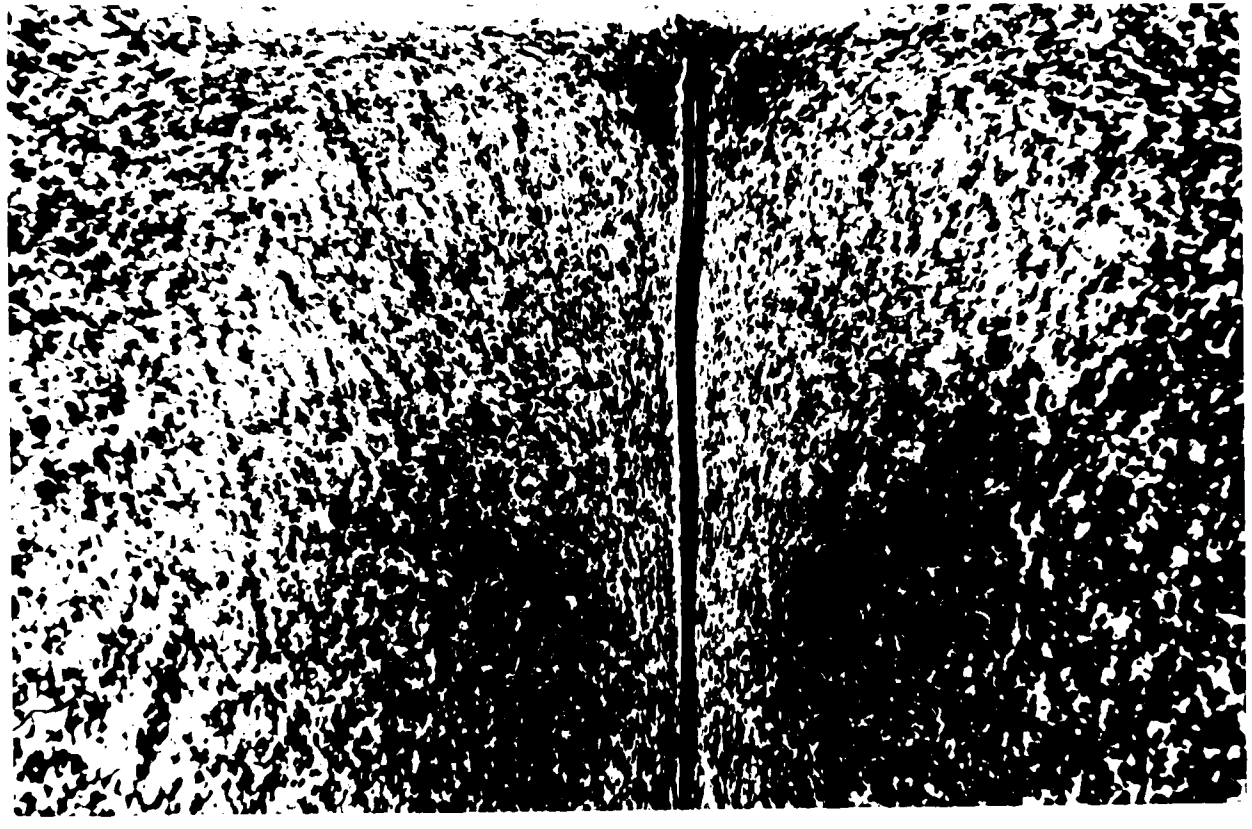

Fig. 7. Representative section through the MPNc of a control male. Magnification = 296X.

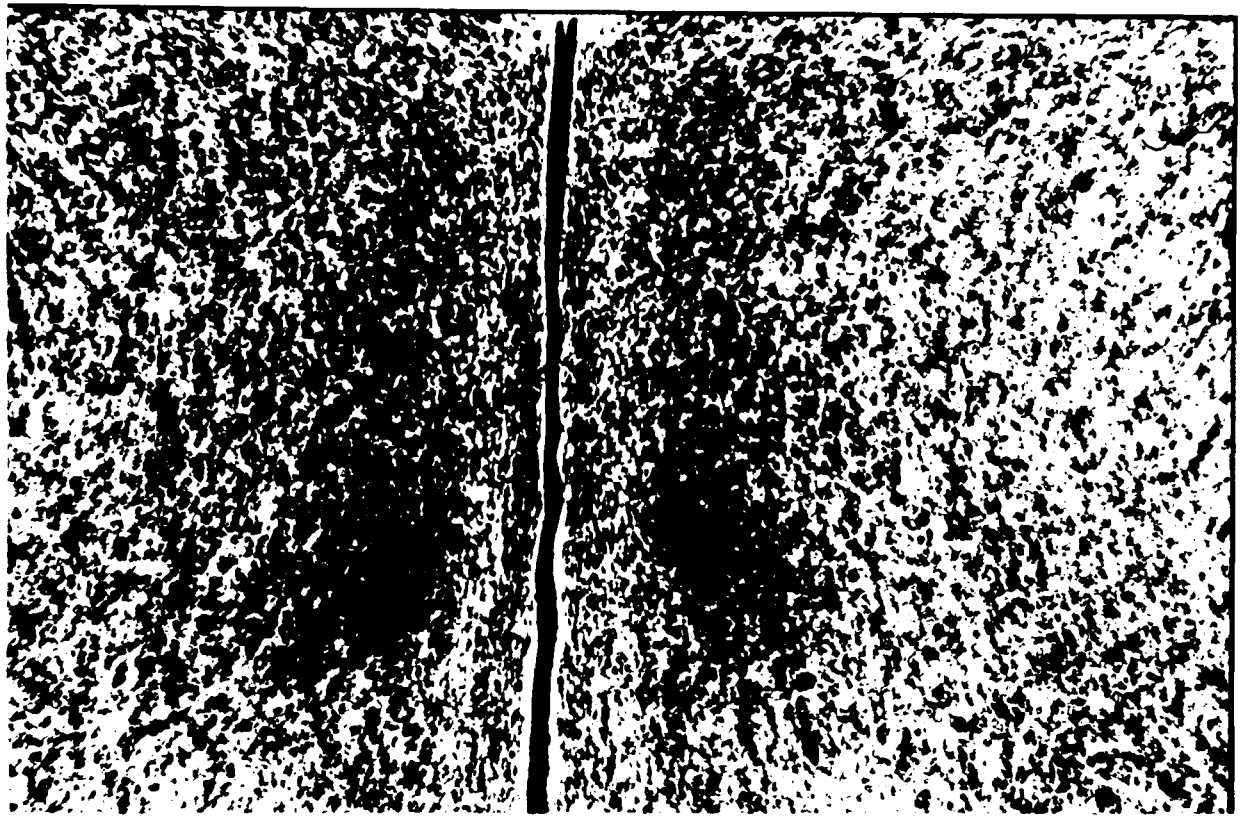

Fig. 8. Representative section through the MPNc of a male treated with 30 mg/kg cocaine. Magnification = 296X.

While the female MPNc volumes were statistically indistinguishable among all the treatment groups (see Fig. 9), a decrease in male MPNc volumes was observed in the cocaine-treated pups (see Fig. 10). This relationship between the dose of cocaine administered and MPNc volume resulted in a linear regression equation of $Y = -0.451X + 24.577$ with a squared multiple r equal to 0.422 when the cocaine doses were plotted in a linear fashion against the male MPNc volumes. When the logarithm of cocaine dosages were plotted against the male MPNc volumes, this relationship resulted in a linear regression equation of $Y = -9.269X + 26.557$ with a squared multiple r equal to 0.933 (see Fig. 11). These data would seem to indicate that this dose-response relationship best fits a logarithmic curve. However, the possibility of an "all-or-none" effect of cocaine upon male MPNc development still exists in that the volumes for the cocaine treatment groups were not found to differ significantly from one another ($0.75>p>.35$).

2. Index of Brain Volume. A rough measure of overall brain volume was calculated by computing an index of brain volume. This index of brain volume was computed by determining the area of the most rostral section of each brain that contained the suprachiasmatic nucleus (SCN) and then

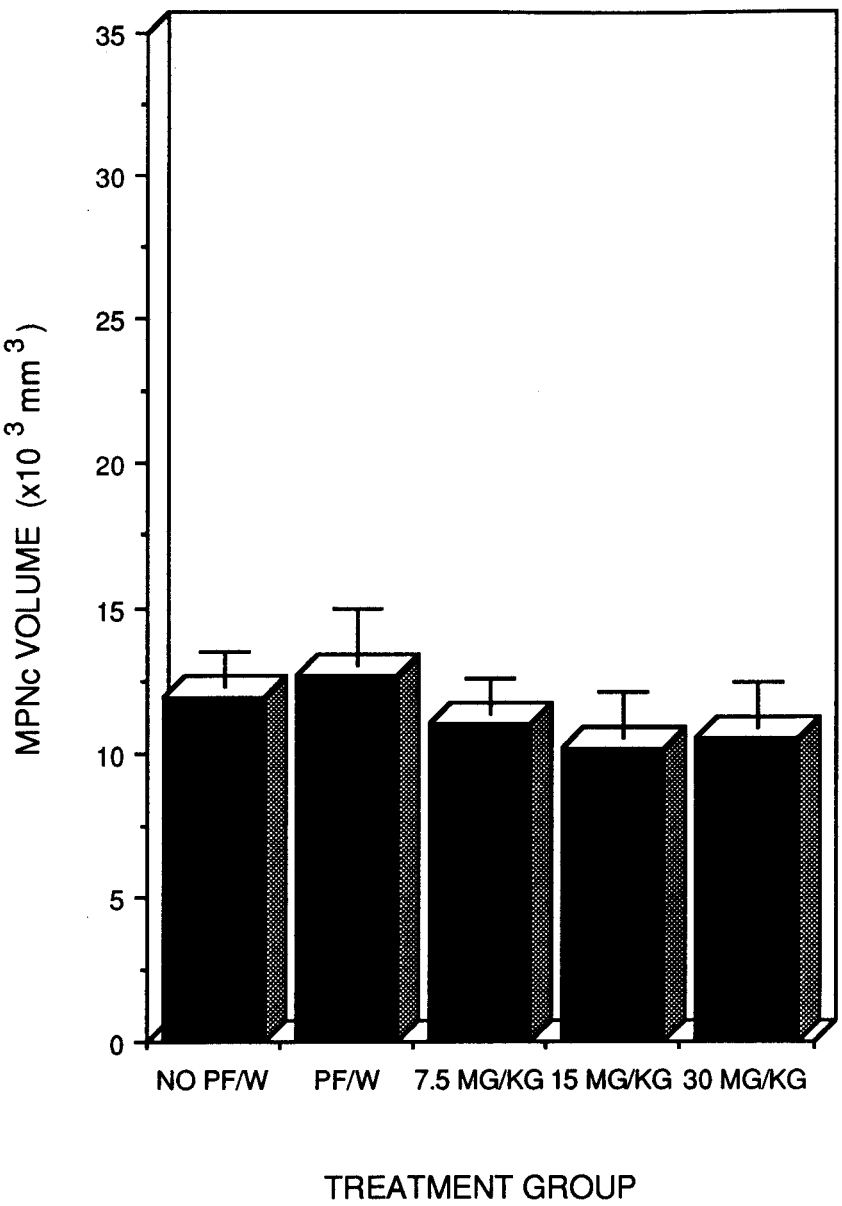

Fig. 9. Effect of cocaine upon right female MPNc volumes. Bars represeent the mean +/- S.E.M. of 5 replicates. Treatment group had no effect (1.0>p>0.75) upon female MPNc volumes.

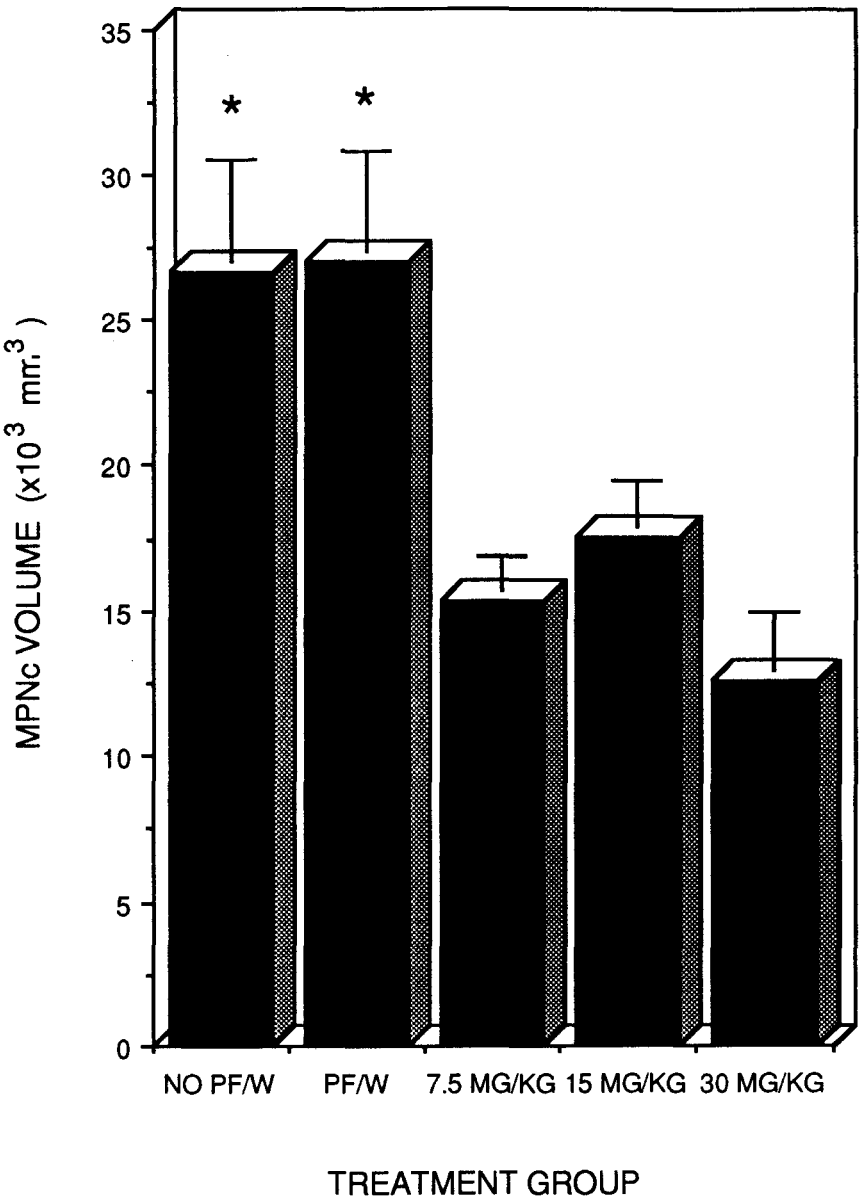

Fig. 10. Effect of cocaine upon male right MPNc volume. Bars represent the mean +/- S.E.M. of 5 replicates. Cocaine had a significant ($p<0.05$) inhibitory effect upon male MPNc volume.

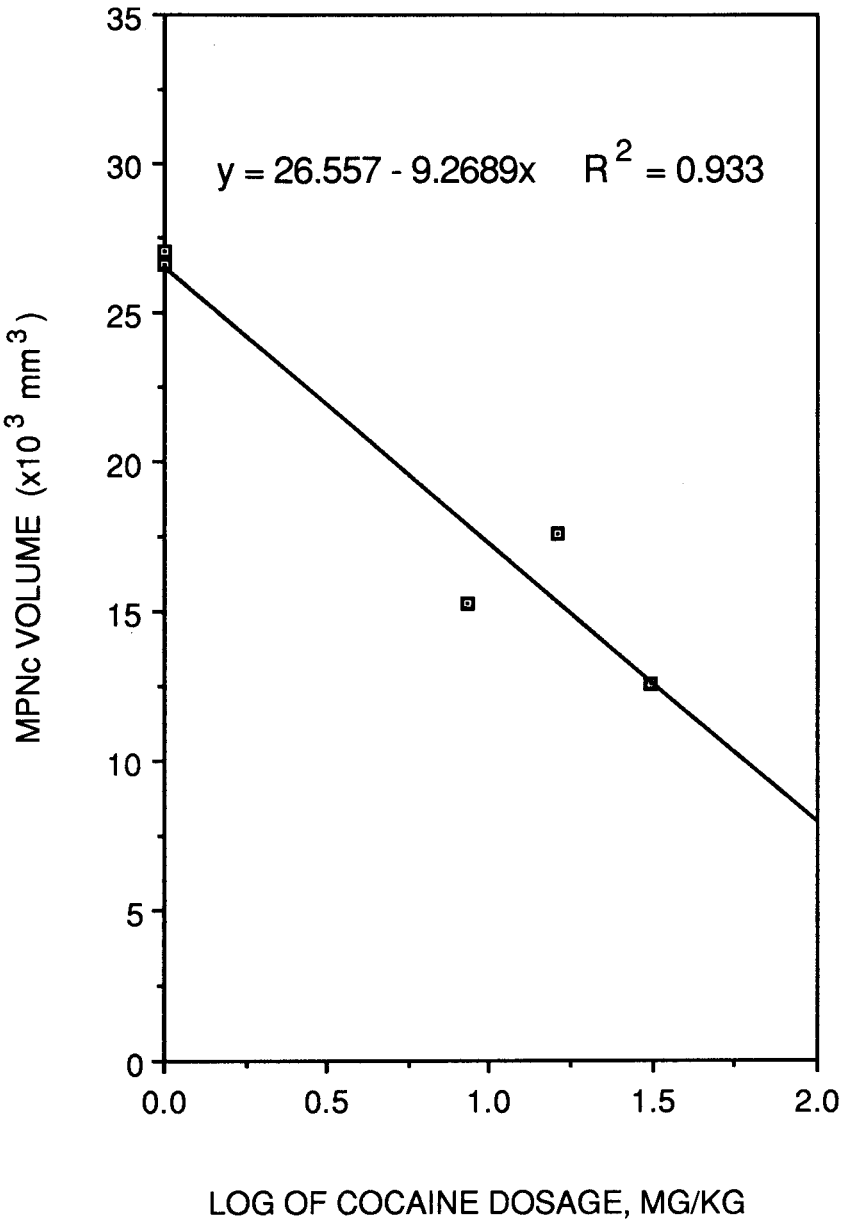

Fig. 11. Male MPNc volume versus logarithm of cocaine dose.

multiplying this area by the section thickness (60 um). These computed measures of rough brain volume were then analyzed by a two factor ANOVA. Treatment group ($0.75>p>0.50$), the sex ($1.0>p>0.75$) of the pup, and the treatment*sex interaction ($1.0<p<0.75$) were all found to have no effect upon the calculated measure of overall brain volume size (see Fig. 12).

3. (MPNc Volume)/(Brain Volume Index) Ratio. Once a rough measure of total brain volume was determined, a ratio of MPNc volume to "total" brain volume was calculated and then analyzed by a two factor ANOVA. This statistical test was performed to determine if the changes observed in MPNc volume were the result of overall changes in brain size or rather the result of changes in the nucleus itself. The two factor ANOVA revealed that there were indeed significant sex ($p<0.001$) and treatment group ($0.005>p>0.001$) effects when this ratio was employed. The treatment group*sex interaction did not significantly affect this ratio ($0.50>p>0.25$). Further, a Newman-Keuls posthoc test confirmed that there were no differences ($1.0>p>0.75$) among the various treatment groups in female (MPNc volume)/(brain volume) ratios (see Fig. 13) as was observed when the female MPNc volumes were compared. In addition, the cocaine-treated male (MPNc volume)/(brain volume) ratios were significantly ($p<0.10$) lower than the control male (MPNc volume)/(brain volume) ratios (see Fig.

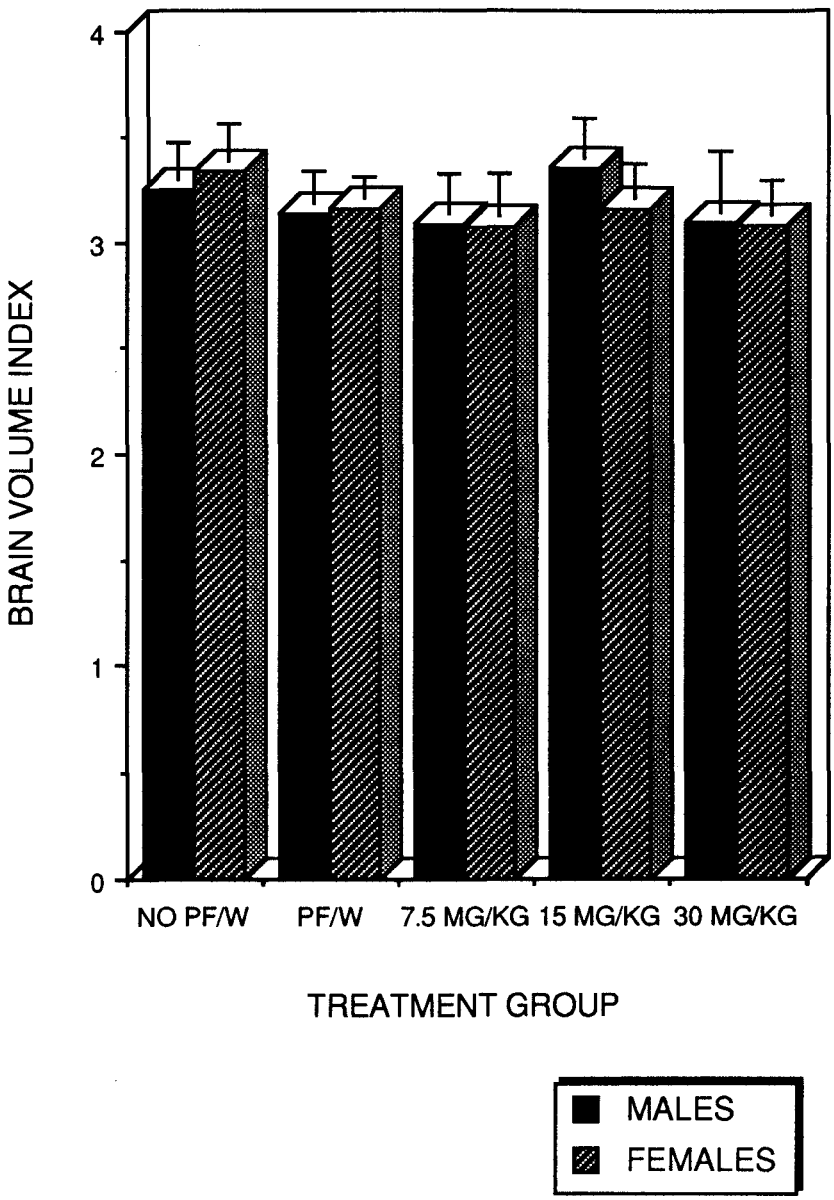

Fig. 12. Index of brain volume. Bars represent the mean +/- S.E.M of 5 replicates. Treatment group (0.75>p>0.50) and sex (1.0>p>0.75) had no effect upon the index of brain volume.

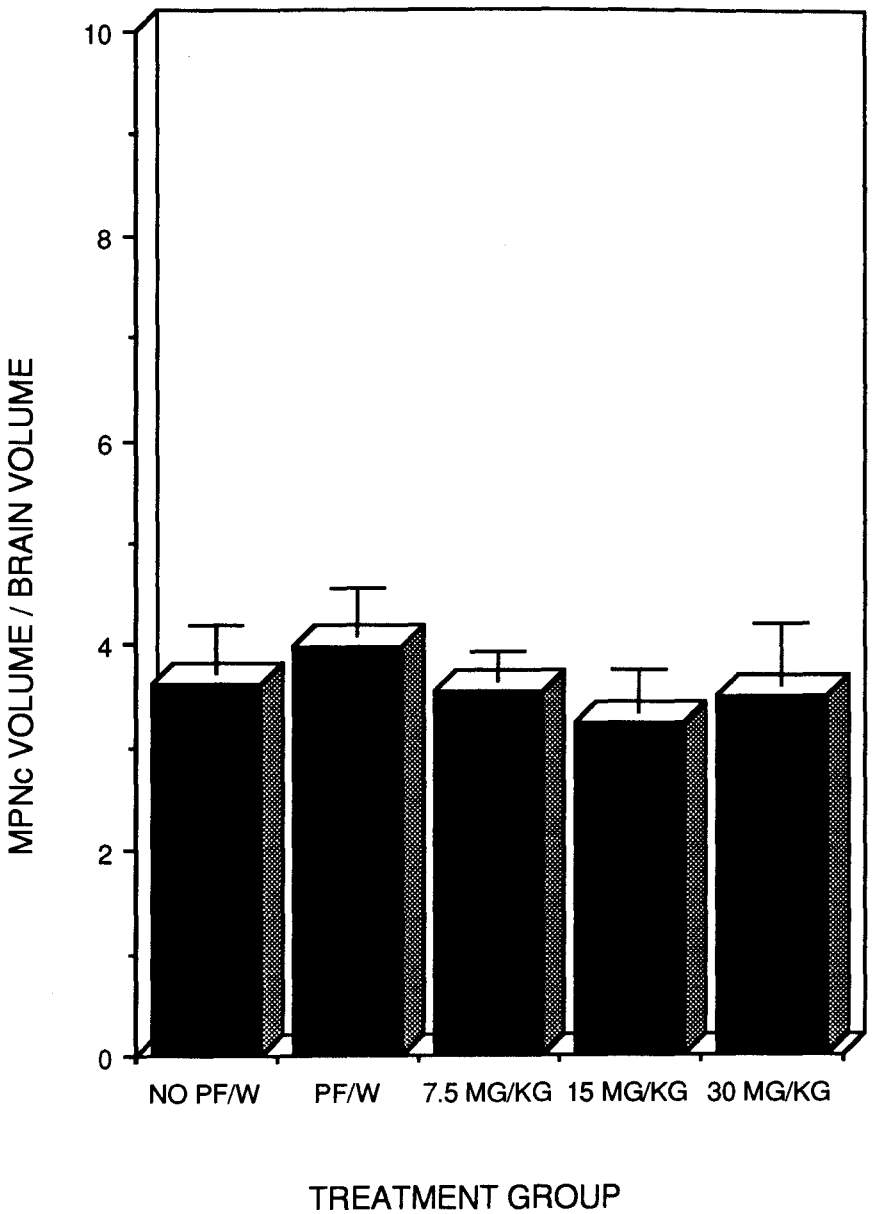

Fig. 13. Effect of cocaine upon female MPNc volume/brain volume. Bars represent the mean +/- S.E.M. of 5 replicates. Treatment group had no effect upon female MPNc volume/brain volume.

14). These results confirmed the above findings that differences observed between cocaine-treated and control-treated MPNc volumes were the result of changes in this nucleus itself and not merely resultant changes arising from overall changes in brain size.

With the confirmation of differences among the male treatment groups, a linear regression analysis was again employed to determine a line of "best fit" to express a possible relationship between the dose of cocaine administered and MPNc volume. This relationship resulted in a linear regression equation of $Y = -0.134X + 7.822$ with squared multiple r equal to 0.713 when the cocaine dosages were plotted in a linear fashion against the average MPNc volumes. When the logarithm of the cocaine dosages were employed, this relationship resulted in a linear regression equation of $Y = -2.787X + 8.435$ with a squared multiple r equal to 0.935 (see Fig. 15). Again, these data would seem to indicate and confirm that this dose-response relationship best fits a logarithmic curve.

Thus, these data indicated that perinatal cocaine exposure compromised the development of the male MPNc (while not affecting the female MPNc), possibly in a logarithmic, dose-dependent manner. Furthermore, this decrease in male MPNc volume was not due to an overall decrease in brain size, but rather was specific to the MPNc.

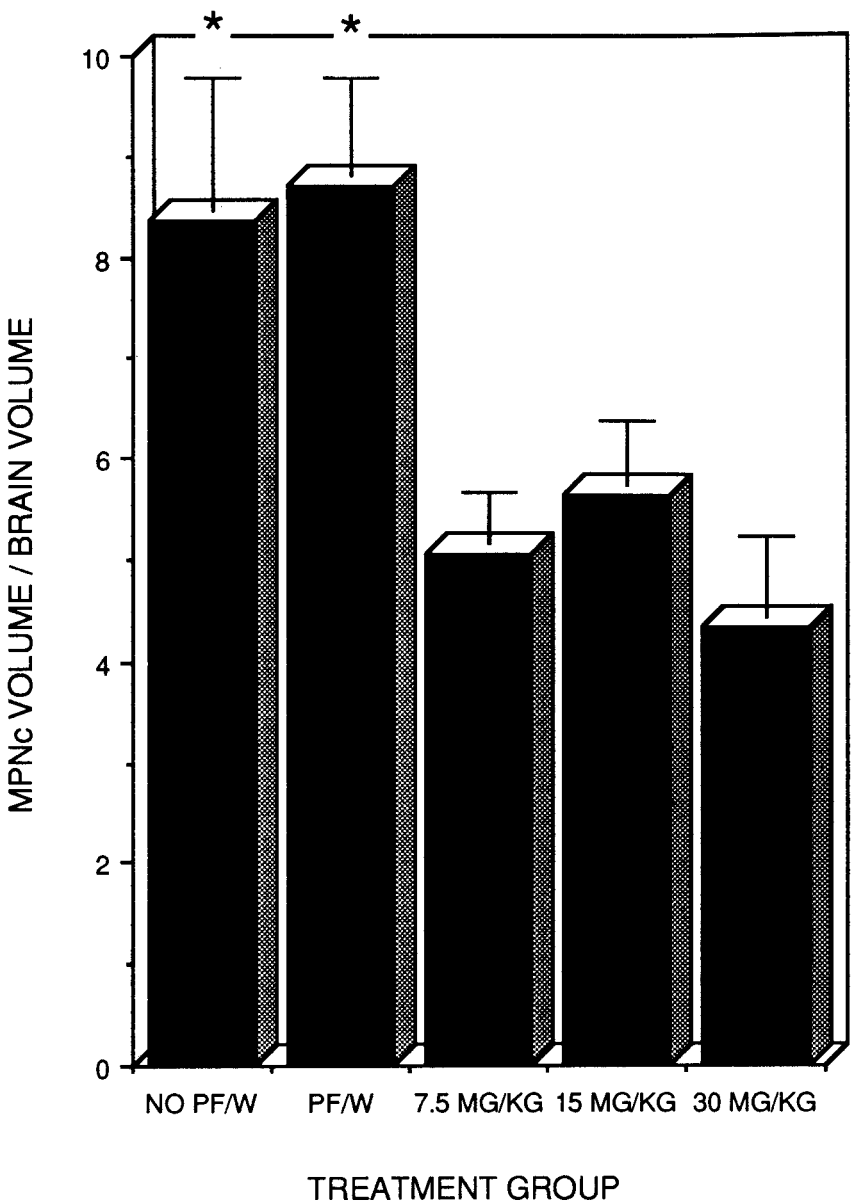

Fig. 14. Effect of cocaine upon male MPNc volume/brain volume. Bars represent the mean +/- S.E.M. of 5 replicates. Cocaine had a significant ($p<0.05$) inhibitory effect upon male MPNc volume/brain volume.

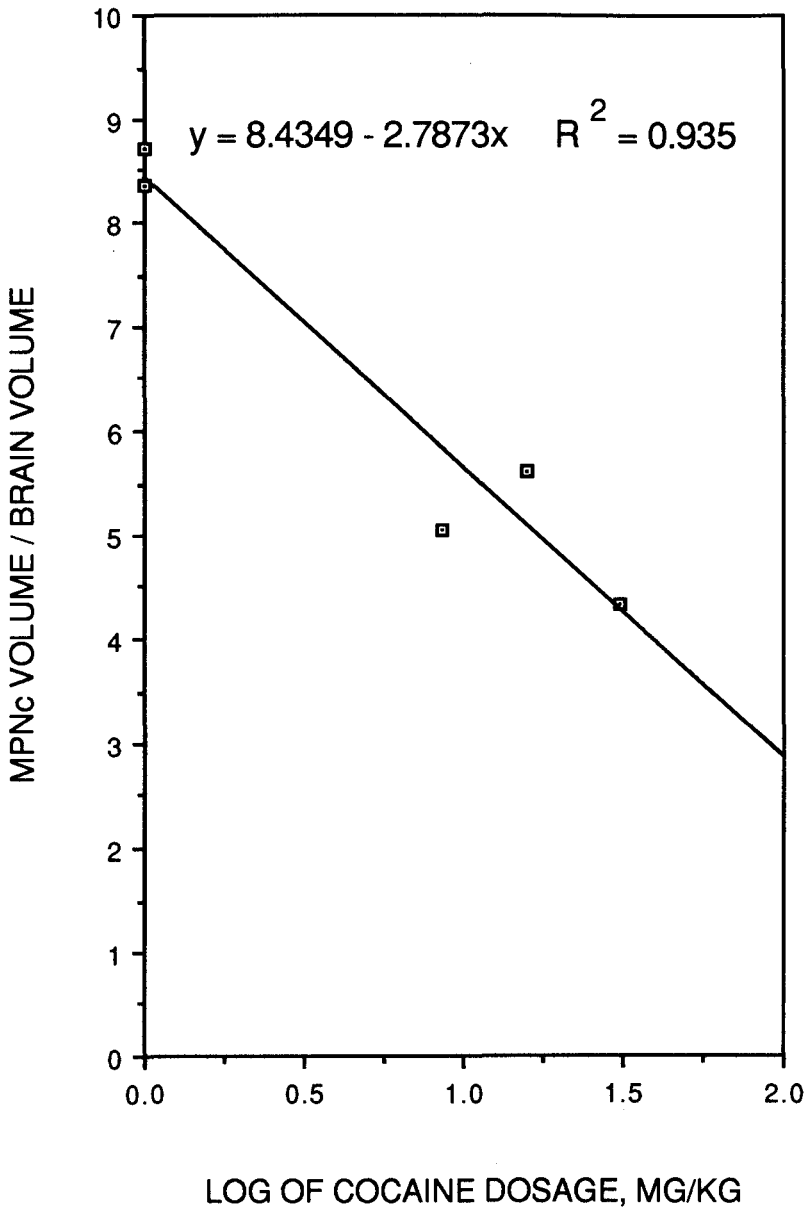

Fig. 15. Male MPNc volume/brain volume versus logarithm of cocaine dosages.

IV. DISCUSSION

The purpose of this project was to test the hypothesis that perinatal cocaine exposure (gestational day 15 through postnatal day 10) compromises the development of the medial preoptic nucleus - central part (MPNc), a nucleus involved in sexual differentiation of the brain. Other gestational parameters were also studied ensure findings of this project were in line with those of the literature in order to more confidently test a novel hypothesis. This hypothesis was tested by comparing volume of the MPNc in male and female pups exposed to 0mg/kg, 7.5mg/kg, 15mg/kg, and 30mg/kg of cocaine perinatally. In addition, an index of brain volume was obtained to ensure that any changes observed in MPNc volume were due to volume changes in the nucleus itself and not merely resultant changes arising from overall changes in brain volume. This index of brain volume was determined by measuring the area of the most rostral section containing the SCN and multiplying this area by the section thickness (60um). The ratio of (MPNc volume)/(brain volume index) was also calculated to allow comparison of adjusted MPNc volumes encompassing possible changes in overall brain volume. Gestational parameters such as maternal weight gain, litter

size, maternal weight gain per pup, pup weight, male/female sex ratio, and gross birth defects were studied to verify that the findings of this project were in line with those reported in the literature. This framework allowed for the more confident testing of a novel experimental measure, that being the effect of cocaine upon the development of the MPNc.

A. Potential Outcomes. Several different experimental outcomes of cocaine's effect on MPNc volume could have been postulated prior to the execution of this project.

First, some previous literature predicted that cocaine would have no effect upon the volume of the female MPNc, while significantly decreasing the volume of the male MPNc. Studies done by Raum et al. (1990) showed that cocaine decreased hypothalamic uptake of gonadal steroids by 50%. Earlier studies had found the volume of the MPNc to be profoundly influenced by the steroidal environment of the medial preoptic area from day eighteen (Dohler et al., 1984B; Rhees et al., 1990) of gestation through day ten of postnatal life (Dunlap et al., 1978; Jacobson et al., 1981). Since this was precisely the time frame that the pups were exposed to the drug, cocaine could have been postulated to inhibit the hypothalamic uptake of the steroids testosterone and estradiol in males, resulting in a volume reduction in the MPNc. According to this hypothesis, the female MPNc would not be affected, since females are not believed to be exposed to gonadal steroids during their critical period of brain sexual differentiation (Gorski, 1974). In fact, it has been accepted that masculinization of the brain occurs by this gonadal steroid exposure during the critical period (Gorski, 1974).

Second, it would also have been reasonable to hypothesize, previous to this study, that cocaine exposure could inhibit the development of not only the male MPNc, but

also the female MPNc to a much lesser extent. Although this hypothesis was less likely for the reason stated above, it was possible that the female hypothalamus has some gonadal steroid uptake, although to a much lesser extent than that of the male.

Third, it would have been possible to hypothesize that cocaine could have actually increased the volume of the MPNc. This result would have been possible if cocaine inhibited the synthesis of the neurotransmitter serotonin in the MPNc. Previous studies had shown that cocaine has a serotonin-depleting effect upon the brain and reduces serotonin synthesis from its precursor, tryptophan (Schubert et al., 1970; Knapp and Madell, 1972). Handa et al. (1986) reported an increase of MPNc volume in one-day-old female rats after prenatal inhibition of serotonin synthesis with para-chlorophenylalanine. In their discussion, the authors argued for a direct, steroid-independent effect of para-chlorophenylalanine on the development of the MPNc. If cocaine were to exert a similar serotonin-inhibiting action upon the MPNc, it might have been found to augment MPNc development in a steroid-independent manner.

Cocaine could also have been postulated to augment the volume of the MPNc if it could be shown that cocaine utilized a similar mechanistic pathway as the beta-2-receptor agonist, salbutamol. In a study conducted by Jarzab et al. (1990), salbutamol, a beta-2-receptor agonist, was found to increase

the volume of the MPNc in both male and female rats. This effect was particularly impressive in males because previous studies utilizing pre- and/or postnatal treatment of male rats with large amounts of gonadal steroids had been unable to increase the volume of the MPNc above normal (Dohler et al., 1984A). Thus, if cocaine acted in a similar fashion to salbutamol on the MPNc, then increases in both male and female MPNc could have been predicted.

Last, it was possible that cocaine would fail to influence the size of either the male or female MPNc with statistical significance. This possible outcome would have resulted if indeed cocaine had no effect upon the development of the MPNc. If this were actually the case, this study would raise questions concerning the precise role of the MPNc in brain sexual differentiation in rats due to the fact that cocaine had been previously shown to demasculinize adult male sex related behaviors (McGivern et al., 1989; Raum et al., 1990). This study was therefore imperative to ascertain not only the possible effects of cocaine on MPNc development, but also to further elucidate the pathway of brain sexual differentiation.

B. Gestational Effects. In this study, no effects of treatment group were found upon any of the gestational parameters (litter size, pup weight, male/female sex ratio, and gross birth defects) with the exception of maternal weight gain and maternal weight gain per pup. The lack of effect of cocaine upon these gestational parameters is consistent with numerous perinatal studies in which similar dosages of cocaine were administered (Fantel and MacPhail, 1982; Church et al., 1988; Abel et al., 1989; Fung et al., 1989; Hutchings et al., 1989; Spear et al., 1989; Dow-Edwards, 1990; Dow-Edwards et al., 1990; Henderson and McMillen, 1990; Raum et al., 1990; Sobrian et al., 1990; Vasa et al., 1990; El-Bizri et al., 1991).

In a study conducted by Abel et al. (1989) employing the same subcutaneous dosages used in this experiment, no significant differences in litter sizes were observed between the control- and cocaine-treated dams. These researchers also reported that this maternal cocaine dosage did not significantly affect the number of implants or fetal resorptions. The present results in which cocaine was found not to affect litter size are consistent with other perinatal cocaine studies in which similar dosages of cocaine were administered to gravid dams (Abel et al., 1989: Fung et al., 1989; Hutchings et al., 1989; Spear et al., 1989; Dow-Edwards et al., 1990; Raum et al., 1990; Sobrian et al., 1990; Henderson and McMillen, 1990; Vasa et al., 1990).

Treatment group was found, however, to have a significant effect upon maternal weight gain. The cocaine-treated dams and the pair-fed, pair-watered controls were found to gain significantly less weight than did the non-pair-fed, non-pair-watered controls. These results in which cocaine-treated dams were found to gain less weight than non-pair-fed, non-pair-watered controls are consistent with other perinatal cocaine studies (Abel et al., 1989; Fung et al., 1989; Hutchings et al., 1989; Dow-Edwards et al., 1990; Henderson and McMillen, 1990; Sobrian et al., 1990; Vasa et al., 1990). These results therefore reiterate the importance of pair-feeding and pair-watering in perinatal cocaine studies to remove possible maternal nutritional effects from confounding the data. These data would also suggest that most of the statistical differences observed in maternal weight gain were due to the appetite-suppressing effects of cocaine. Since cocaine-treatment was not found to significantly affect litter size or pup weights, it could be postulated from these findings that gestational cocaine use has deleterious consequences on maternal health apart from those on the developing fetus(es).

It is an interesting finding of this and other studies that while cocaine-exposed dams exhibited an anoretic reduction in weight gain, perinatally-exposed pups did not (Church et al., 1988; Abel et al., 1989; Spear et al., 1989; Dow-Edwards et al., 1990; Raum et al.. 1990; Vasa et al., 1990; El-Bizri et al., 1991). The lack of pair-feeding and

pair-watering in the newborn pups cannot account for this effect, since this would be expected to further increase the weight difference between the cocaine-injected and saline-injected pups. Indeed, this was not found to be the case. While this study did not investigate cocaine's effects upon ingestive behavior, it might be postulated that the discrepancy between the two cases may be due to an immaturity in the neural circuitry controlling ingestive behavior in the pups. The ventromedial hypothalamic nucleus (VMH) is a region implicated in the control of ingestive behavior (Hughes et al., 1987). Neuronal genesis with maturation of synaptic contacts in the VMH has been reported to occur between gestational day 14 and the early postnatal period (Lauder, 1983). Of note, Dow-Edwards et al. (1990) reported that in rats exposed to cocaine prenatally, glucose metabolism in the VMH was significantly decreased by 31%. This decrease in VMH glucose metabolism was greater than in any other selected structure in the brain. A reduction in glucose metabolism in the VMH during its period of development may thus result in a delayed maturity of the neural circuitry controlling ingestive behavior. Such a delay could explain why the cocaine-exposed pups failed to exhibit an anoretic decrease in weight gain.

In terms of cocaine's teratogenic potential upon the developing fetus, this study failed to find any observable gross birth defects among the cocaine-exposed infants. This finding is consistent with the literature (Fantel and

MacPhail, 1982; Dow-Edwards, 1988; Abel et al., 1989; Fung et al., 1989; Hutchings et al., 1989; Sobrian et al., 1990; Vasa et al., 1990; El-Bizri et al., 1991). Some studies employing dosages of 30 mg/kg of cocaine or less have reported a low incidence (one or two) gross birth defects (Henderson and McMillen, 1990; Raum et al., 1990). This discrepancy in the literature could be due to the rate of gross birth defects in the population perinatally exposed to cocaine. If this rate of gross birth defects was one pup out of 500 pups, then it would be just as likely to find one birth defect as it would be to find zero.

Summarizing this portion of the project, it was seen that the treatment group significantly affected the maternal weight gain in general as well as maternal weight gain per pup. The non-pair-fed, non-pair-watered controls were found to gain more weight than any of the cocaine-treated dams or the pair-fed, pair-watered controls. There were no significant effects of treatment group on litter size, pup weight, sex ratio, or occurrence of gross birth defects. These findings are all in corroboration of previous studies. In addition, these findings set up an experimental framework to test a significant novel hypothesis, namely, that cocaine would affect the development of the brain, in particular, the MPNc.

C. Neuroanatomical Effects.

The neuroanatomical measures studied in this project were a computed index brain volume, MPNc volume, and the MPNc volume/brain volume index. Cocaine treatment and sex of the pup were not found to have any effect upon the computed index of brain volume. This finding is in agreement with other rat studies which found no differences in brain size between control males and females after perfusion (Gorski et al., 1980; Jacobson et al., 1980; Robinson et al., 1986; Baron et al., 1988).

The MPNc volumes obtained in the measurement of control males and females were also found to be in agreement with those reported in the literature (Jacobson et al., 1980). Jacobson et al. (1980) found male MPNc volumes to average around 0.0260 mm^3, and this study found control MPNc volumes to average around 0.0255 mm^3 in ten day old pups. Likewise, Jacobson et al. (1980) found ten day old female MPNc volumes to average around 0.0120 mm^3, and this study determined control female MPNc volumes to average around 0.0123 mm^3.

While many findings in this study have repeated what has been reported in the literature, the novel finding of this project was that male MPNc volumes were reduced in pups perinatally-exposed to cocaine. Cocaine was found to decrease the volume of the male MPNc, while having no effect upon the female MPNc. A similar result was obtained in another study conducted by Barron et al. (1988) in which the effects of

prenatal alcohol exposure upon the MPNc of male and female rats were studied. These researchers found that both the volume and average cell size were markedly smaller in alcohol-exposed males relative to the control males. In contrast, prenatal alcohol exposure did not affect the MPNc volume or cell size in females. The authors postulated that this reduction in male MPNc volume was due to impairment of brain masculinization during the critical period of development for this nucleus. Since it had been demonstrated earlier that both chronic (Ylikahri et al., 1974; Gordon et al., 1976) and acute (Lester and Van Theil, 1977; Gordon et al., 1978) alcohol consumption lowers sex steroids in males, in particular testosterone synthesis in the testes (Cicero et al., 1980; Ficher and Levitt, 1980), the researchers postulated that alcohol decreased testosterone synthesis in the male fetal testes. This postulated decrease in fetal testosterone during the perinatal critical period had been previously shown to decrease the volume of the male MPNc (Gorski et al., 1978; Gorski et al., 1980; Jacobson et al., 1981; Dohler et al., 1982).

While alcohol has been postulated to reduce male MPNc volume indirectly by decreasing fetal testosterone synthesis in the testes (Barron et al., 1988), cocaine may be hypothesized to reduce male MPNc volume directly by inhibiting hypothalamic nuclear uptake of estradiol and testosterone. This hypothesis is supported by the work of Raum et al. (1990)

who found prenatal inhibition of hypothalamic sex steroid uptake by cocaine. In this study female pups delivered on day 22 of gestation by cesarean section were injected subcutaneously within 30 minutes of delivery with either saline or 10 mg/kg cocaine. Thirty minutes postinjection, all animals were injected intracerebroventricularly with 25 microCi of tritiated testosterone and were decapitated 60 minutes later. When the nuclear concentrations of testosterone and estradiol incorporated into the hypothalami were measured, it was discovered that cocaine inhibited the mean nuclear incorporation of testosterone and estradiol by 46% and 51% respectively.

In their discussion, Raum et al. (1990) postulated that their data indicate that prenatal exposure to cocaine will disrupt normal sexual differentiation of the male brain by interfering with nuclear incorporation of testosterone and estradiol during the critical perinatal period. The results obtained from this thesis support their hypothesis by providing neuroanatomical evidence for such a disruption in sexual differentiation of the male brain. Further, the results of this project provide a neuroanatomical basis to support reported resultant impairment of male reproductive behavior induced by perinatal exposure to cocaine (McGivern et al., 1989; Raum et al., 1990).

Just how cocaine is able to inhibit hypothalamic incorporation of estradiol and testosterone was recently

elucidated by Benton et al. (1991). These researchers found that beta-1-adrenergic stimulation by cocaine inhibited a critical step in neuronal sexual differentiation - estradiol nuclear receptor binding. Since beta-1-adrenergic stimulation was shown previously to inhibit nuclear accumulation of estradiol in hypothalamic nuclei (Raum et al., 1984), beta-adrenergic stimulation caused by cocaine (Seidler, 1991) was hypothesized to be responsible for this inhibited receptor binding. This hypothesized effect could be mediated by cyclic AMP (CAMP). Resultant phosphorylation of the estradiol receptor, due to increased CAMP levels, could be found to block the binding of the receptor to the nuclear DNA acceptor sites. If this were indeed the case, decreased estradiol nuclear receptor binding as well as increased CAMP levels would need to exist. This hypothesis was confirmed in that cocaine was indeed found to block estradiol nuclear receptor binding as well as stimulate CAMP levels (Benton et al., 1991).

Additional studies conducted by Petitti and Etgen have also provided evidence supporting the hypothesis that cocaine interferes with masculinization of the brain by noradrenergic, beta-1 receptor activated CAMP formation (Etgen and Petitti, 1987; Petitti and Etgen, 1990). These researchers found that beta-1-stimulated CAMP formation was reduced in the presence of estrogen in the preoptic area. Since estrogen binding in this area has been postulated to be responsible for

masculinization of the brain, substances found to decrease in this steroid's binding could be postulated to interfere with the sexual differentiation process.

This hypothesis of cocaine-induced stimulation of beta-1 receptors leading to the inhibition of male MPNc development is not contradictory to the study done by Jarzab et al. (1990) citing increases in MPNc volume after postnatal exposure to the beta-2-adrenergic agonist salbutamol. Beta-1 and beta-2 receptors, although structurally related, differ in their affinity to various adrenergic agonists (Stiles et al., 1984). According to Katzung (1989) norepinephrine has relatively no effect on beta-2 receptors while having a high affinity for beta-1-receptors. In addition, brain sexual differentiation has been postulated to result from noradrenergic-steroid interactions (Raum and Swerdloff, 1981; Nock and Feder, 1981). Hence, since salbutamol binds exclusively to beta-2-adrenergic receptors, it operates outside of this pathway. Another consideration is that cocaine is known the block the reuptake of norepinephrine (Ritz et al., 1987), leading to an increase in noradrenergic postsynaptic receptors (Banerjee et al., 1979; Chanda et al., 1979; Pert et al., 1979). This effect would seem to imply that cocaine alters the beta-1 postsynaptic receptor as evidenced by its supersensitivity (Banerjee et al., 1979; Seidler, 1991).

In terms of sexual differentiation, this work supports the role of the MPNc as one critical brain region in the

control of masculine sexual behavior. Since it was previously shown that cocaine inhibits masculine sexual behavior while not affecting female sexual behavior (McGivern et al., 1989; Raum et al., 1990), reductions in cocaine-exposed male MPNc volumes and not in female MPNc volumes lend anatomical support to MPNc's neuronal control of masculine sexual behavior. Further, in light of recent work conducted by Anderson et al. (1986) and Cherry and Baum (1990) correlating male MPNc volume to coital behavior in males, this work predicts dose-dependent reductions in perinatally-exposed males' capabilities to engage in copulatory behavior.

D. Summary. In summary, even though cocaine was found to have no effect upon the developing fetuses in terms of litter size, pup weight, sex ratio, and occurrence of gross birth defects, the same doses were found to have a significant effect upon male MPNc development, but not upon female MPNc development. Cocaine was found to induce a reduction in male MPNc volume. Further studies, with increased sample sizes, would need to be done to determine if indeed this reduction in male MPNc volume is dose-dependent. It is possible that cocaine reduces MPNc in an "all-or-none" fashion in that the MPNc volumes from the three cocaine treatment groups did not differ statistically from one another. Based upon related studies, this reduction in male MPNc volume may be due to decreased hypothalamic nuclear uptake of gonadal steroids due to beta-1-adrenergic stimulation induced by cocaine. The present work further provides an anatomical correlate, supporting a role for the differentiated MPNc in controlling male copulatory behavior. The behavioral implication of these results is that males perinatally exposed to cocaine during their critical period of MPNc differentiation may exhibit compromised coital capabilities as well as impaired gonadotropin regulation.

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