Prenatal Exposure to Drugs of Abuse: Methodological Considerations and Effects on Sexual Differentiation

Robert F. McGivern and Robert J. Handa

INTRODUCTION

Studies conducted over the past 25 years have revealed a biological pervasiveness to the neurobehavioral sexual differentiation process that extends far beyond behaviors associated with reproduction. This pervasiveness is now recognized to include sex differences in cognition and affect (Halpern 1992) as well as structural and biochemical differences in the central mechanisms involved in their expression (Arnold and Gorski 1984). In humans, sex differences have been observed in communication style, verbal and spatial skills, mathematical reasoning ability, and even play behavior in children (Beatty 1984; Benbow 1988; Nyborg 1984). Many of the nonlinguistic sex differences in humans have been observed inother mammals, attesting to the primary role of biology over cultural factors in the determination of such differences (Beatty 1979). These differences include juvenile play behavior (Meaney and Stewart 1981), pain sensitivity (Mogil et al. 1993; Pare 1969), social interaction (Kellogg et al. 1991), taste preferences (Valenstein et al. 1967), and spatial skills (Stewart et al. 1975). The reader is referred to a series of reviews by Beatty (1979, 1984, 1992) for references and discussion of nonreproductive sex differences in animals. Several reviews of nonreproductive sex differences in humans have addressed cognitive, emotional, and neuropsychological function (Beatty 1984; Halpern 1992; Levy and Heller 1992; Maccoby and Jacklin 1974).

While biology provides a template for the neurobehavioral development of sex-related behaviors across the phylogenetic scale, the flexibility of this template has increased with evolutionary pressure. Such flexibility has allowed adult mammalian behavior to reflect environmental influences during development to a degree not observed in the fixed action patterns seen in insects, fish, or reptiles. However, an untoward effect has been the increased susceptibility of the mammalian neurobehavioral sexual differentiation process to disruption by extrinsic factors such as drugs.

Animal models of prenatal or perinatal exposure to drugs of abuse have demonstrated a variety of subtle behavioral alterations in offspring in the absence of any clear teratological consequences related to physical development. These have included alterations in attention, learning, emotional reactivity, drug sensitivity, and sexual behavior (Gorski 1974; Meyer and Riley 1986b; Vorhees 1986). The most recent class of behaviors to come under scrutiny following prenatal drug exposure is that of nonreproductive sexually dimorphic behaviors.

Investigations into the mechanisms mediating these behavioral alterations have identified drug-induced disruptions of gonadal function or neuronal or glial development in sexually dimorphic brain regions. This finding has provided a basis to interpret the enduring alterations in the expression of these behaviors (Arnold and Gorski 1984; McGivern and Riley 1993; Ward 1992; Weinberg et al. 1991). This chapter provides a brief overview of basic mechanisms involved in sexual differentiation of the mammalian brain, followed by a consideration of selected methodological issues associated with studies of neurobehavioral sexual differentiation. The inclusion of sexually dimorphic behaviors as an area of study in the field of developmental neuroteratology has introduced methodological considerations specific to sex-related behaviors. Therefore, the authors have attempted to address some specific methodological considerations relevant to the area of animal models of sexual differentiation. Finally, a brief synopsis of findings concerning the effects of several major drugs of abuse on the sexual differentiation process is presented.

MECHANISMS OF SEXUAL DIFFERENTIATION

Normal sexual differentiation is determined by genetic and phenotypic factors acting during different stages of development (George and Wilson 1988). Genetic or chromosomal sex is determined at the time of fertilization by (as yet) unidentified factors associated with the X and Y chromosomes. Genetic activity related to the sex chromosomes subsequently differentiates the embryonic gonad into a testis or an ovary. Phenotypical sex is under the direct control of gonadal hormone secretion and other biochemical factors. Phenotypical expression relates to develop-ment of sex organs such as the penis or vagina as well as sex-specific regional differentiation of the central nervous system (CNS). Current evidence indicates that factors associated with phenotypical differentiation are also involved in the expression of affective and cognitive sex differ-ences in humans (Halpern 1992; Reinisch and Sanders 1992). Drugs generally influence the phenotypical aspects of the sexual differentiation process. The drugs that influence biochemical

factors critical to the sexual differentiation process, such as sex steroid hormones (McEwen 1988) or catecholamines (Mirmiran et al. 1988), are of most concern.

Broadly speaking, gonadal hormones serve to organize the brain during critical periods of development to respond with a masculine or feminine pattern to later activational effects of hormonal stimulation (Phoenix et al. 1959). Organizational effects of hormones are long-lasting and occur in both the brain and periphery through direct effects on genomic activity in the developing organism (McEwen 1992). By definition, organizational actions of hormones are restricted to critical periods of development to induce their long-lasting consequences. Regardless of the developmental timeframe of a given species to reach maturity, there is a good correlation between the timing of the critical period for sexual differentiation and the stage of biological development (Phoenix et al. 1959). Thus, prenatal exposure of either males or females to adequate levels of testosterone at the proper point in fetal development leads to later masculine sex behavior patterns (i.e., mounting or intromission). Such behavior is observed only in the presence of a receptive female and adequate circulating testosterone levels. Conversely, a lack of adequate hormonal exposure during a critical organizational period produces an adult who fails to respond to the same amount of testosterone with normal sex behavior.

Activational effects of a hormone occur rapidly in response to circulating levels of the hormone. The sensitivity of such effects to hormone stimulation is often determined by the prior exposure to testosterone or estrogen during critical organizational periods. However, the activational effects of gonadal hormones can also be influenced by environmentally induced alterations in the biochemical milieu at the time the hormone is present, such as those induced by stress or the estrual cycle (Becker and Cha 1989; Levine et al. 1989).

Some aspects of masculine sex behavior are organized by genomic actions of androgens such as testosterone or dihydrotestosterone acting directly through the androgen receptor. Other aspects of male sex behavior are organized by estrogens such as estradiol acting in specific brain regions such as the hypothalamus, preoptic area, or amygdala (McEwen 1992). Part of testosterone's role in the masculinization process is to serve as a substrate for estrogen. Estrogen in the male brain is derived primarily from the intracellular aromatization of testosterone by aromatase. Aromatase is a P450 enzyme which has a limited regional distribution in the brain to areas such as hypothalamus, amygdala, and cerebral cortex. Its activity in adulthood is sexually dimorphic, a

phenomenon determined by hormonal exposure during early development as well as genetics (Roselli and Resko 1993).

Females are protected from the masculinizing effects of ovarian estrogen by a plasma-binding protein that prevents estrogen from crossing the cell membrane. In the rat, this binding protein is a-fetoprotein; in the human, it is sex hormone binding globulin (SHBG). Both SHBG and a-fetoprotein remain elevated during the infantile period and decline significantly prior to puberty, allowing the onset of adult patterns of negative feedback control of gonadotropin secretion in females (Ojeda and Urbanski 1988). Mascu-linized behavior patterns have been observed in women prenatally exposed to high levels of adrenal androgens such androstenedione (Galatzer and Laron 1989) or to estrogens such as diethylstilbestrol (Hines and Shipley 1984) which are not bound by SHBG. Limited evidence also exists indi-cating that ovarian steroids may be important for complete feminization of the brain (Dohler et al. 1984; Fitch et al. 1991; Gerall and Dunlap 1971). In the rat model of sexual differentiation, it has generally been assumed that behavioral masculinization of females following exposure to drugs that activate the adrenal gland is due to the increased release of androstenedione (McGivern et al. 1984; Meyer and Riley 1986a), similar to that which takes place in humans (Casey et al. 1992). However, recent evidence indicates that androstenedione is not made in the adrenal gland of the rat (Fitch et al. 1992; van Weerden et al. 1992), indicating that other mechanisms are mediating such masculinization.

Sex steroids influence genomic activity through binding to the intracellular receptor. This is accomplished by the binding of the steroid-receptor complex to hormone response elements in deoxyribonucleic acid (DNA). Such genomic actions appear to be intimately connected with neuronal survival and growth (McEwen 1992). This process is also thought to permanently define a neuron's subsequent responsiveness to stimulation (Hasegawa and Sakuma 1993), as suggested by studies showing that sex steroids can produce long-term changes in estrogen stimulation in synapses and postsynaptic membranes in brain areas such as the hypothalamus (Hasegawa and Sakuma 1993; Naftolin et al. 1990).

The timing of any disruption in the steroid milieu during development is of particular importance in determining the long-term consequences on brain and behavior. The normal masculinization process in males is dependent in part upon surges of testosterone secretion during critical periods of development. In the rat, the critical period for sexual differentiation is considered to extend from approximately the last week of gestation through the first few days postnatally. During this period, there is a

normal surge of testosterone on days 18 and 19 of gestation (McGivern et al. 1988a; Weisz and Ward 1980), as well as a second surge at birth (Corbier et al. 1978; McGivern et al. 1993). If the amplitude or time course of either surge is altered, sexual differentiation of the male will be incomplete.

Corresponding surges of testosterone have been identified in the human male fetus and neonate. A prenatal surge of testosterone occurs around 16 to 20 weeks of gestation (Parker 1993). A postnatal testosterone surge has also been observed during the first few hours after birth (Corbier et al. 1990; Stahl et al. 1978). It is presumed that interference with either surge will lead to incomplete masculinization as occurs in the rat (Corbier et al. 1992; Roffi et al. 1987). No prenatal surge of estrogen has been observed in females, and the capacity to produce estrogen from exogenous precur-sors primarily evolves during the infantile period (Ojeda and Urbanski 1988).

Results from studies of the effects of prenatal stress or alcohol exposure provide good examples of the impact environmental stimuli can have on the sexual differentiation process. Exposing a pregnant rat to stress accelerates the testosterone surge in the male fetus to day 17, resulting in incomplete masculinization of sex behavior in the adult male offspring (Ward 1972). Alcohol exposure attenuates the prenatal testosterone surge (McGivern et al. 1988a) rather than shifting it, but the behavioral effects (McGivern and Riley 1993) on reproductive behaviors appear to be broadly similar to those of prenatal stress (Ward 1972, 1980).

Recent studies have also revealed notable effects of neurotransmitters on the process of sexual differentiation in the CNS. In addition to their neurotrophic actions early in brain development (Lauder and Krebs 1986), serotonin and norepinephrine (NE) have also been identified as playing a significant modulatory role on sex steroid actions during development (Beyer and Feder 1987; Handa et al. 1986; Jarzab et al. 1986; Raum and Swerdloff 1981; Raum et al. 1984). A novel interaction of the gamma aminobutyric acid (GABA) system with neurosteroids to influence social behavior has been observed by Kellogg and coworkers (1991) in the course of their studies of the effects of prenatal diazepam exposure. Specifically, they have identified an important developmental role for the GABA receptor in the adult modulation of social behavior by steroids. Such findings have led to an increased appreciation of alterna-tive mechanisms to explain alterations in sexual differentiation in the absence of any apparent change in steroid secretion or action (Beyer et al. 1992; Reisert and Pilgrim 1991).

METHODOLOGICAL CONSIDERATIONS

Stress

Stress interacts both behaviorally and physiologically with sex-related behaviors (Levine et al. 1989). Stress reactivity in the adult animal can be influenced by developmental exposure to stress (Levine et al. 1989) or to drugs such as ethanol (Taylor et al. 1982; Weinberg 1988). For these reasons, it is imperative to limit the influence of stress as much as possible when studying sex-related behaviors per se in prenatally drug-exposed animals. The subtlety of potential stress effects in development studies is highlighted by results from Meyer and colleagues (1992), who observed an effect of saline treatment of dams during pregnancy on the responsiveness of offspring to cocaine. These authors observed no effect of increasing doses of cocaine (1.25, 2.5, or 5.0 milligrams per kilogram (mg/kg) subcutaneously (SC)) on locomotor activity of 11-day-old males from dams injected twice daily with saline from days 11 to 20 of gestation, compared with a linear response in males from untreated dams. Females from saline-injected dams exhibited less locomotor activity following saline injections than untreated controls. Their locomotor response to cocaine was similar to all three doses and an increase over saline injection. In contrast, females from untreated dams were unresponsive to the lowest doses of cocaine and exhibited a decrease to the highest dose. These results point to the importance of including untreated controls in studies of drug effects on development.

One of the basic organizational actions of androgens is to desensitize the adult hypothalamus to the positive feedback effects of estrogen on luteinizing hormone (LH) secretion. Using this information, LH responsiveness to estrogen can be used to study masculinization of females or incomplete defeminization of males following prenatal exposure to a drug such as ethanol (Handa et al. 1985). In castrated female rats, a positive feedback response to estrogen occurred approximately 54 hours after a 2-microgram (µg) injection of estrogen benzoate at 10 a.m. (Handa et al. 1985). To study this phenomenon, the rat is often implanted with an indwelling jugular catheter 48 to 72 hours prior to the LH surge, so that hourly blood samples can be obtained before and during the LH surge.

A major consideration for this type of study is the experimental conditions under which a blood sample is obtained from the rat. A large stress-induced rise in glucocorticoids several hours before the surge can attenuate or eliminate the positive feedback response to LH. Therefore stress must minimized both during the time the catheter is hooked up, as

well as the during the blood sampling period, to obtain accurate reflections of hypothalamic-pituitary-gonadal (HPG) function in these animals.

The ability of glucocorticoids to suppress LH (Baldwin and Sawyer 1974; Olster and Ferin 1987; Ringstrom and Schwartz 1985) is also a major consideration. A reduction in LH or change in its pulsatile characteristics can result in significant decreases in sexual behavior. For the rat, a novel situation such as a test arena is a stressor and leads to a notable rise in glucocorticoids (Fitch et al. 1992). For this reason, male rats often exhibit little sex behavior in the presence of a receptive female during the first test session. Asymptotic levels of behavior are generally not reached until the second or third test session. Consequently, assessment of an animal's sex behavior potentials should be made on the basis of performance over multiple test sessions.

Even under optimized test conditions, alterations in sex behavior by prenatally drug-exposed animals may still reflect an increased responsiveness to environmental stress such as that reported consistently in animals prenatally exposed to alcohol (Taylor et al. 1982; Weinberg 1988, 1992). Thus, reductions in sex behavior of drug-exposed animals could reflect a secondary effect of increased stress reactivity rather than a basic dysregulation of the HPG. Optimally, all testing for sex behavior potentials in drug-exposed animals would include followup studies of stress hormone responsiveness as well as extensive habituation to the testing situation. Such baseline data obtained under minimal stress conditions can be used to assess the independent effect of the prenatal exposure regimen on stress-responsive systems that might be secondarily influencing sex-related behaviors.

Housing

Housing is also a major consideration in tests of sex behavior. The rodent is a social animal and normal behavior is best assessed in animals raised under group-housed conditions. Individual housing is a potent stressor (Valzelli 1981) that can result in significant changes in monoami-nergic systems (Segal et al. 1973) and opioid systems (Adler et al. 1975a, 1975b) after 3 to 4 weeks. Marked alterations in circadian patterns of hormone release and hypothalamic NE content are also apparent (Greco et al. 1992). These changes are presumed to underlie the alterations in normal sex behavior potentials observed in individually housed animals (Valzelli 1981). Therefore, animals used for assessment of alterations in sex-related behaviors following prenatal drug exposure should be group housed from weaning.

Gestational Period of Drug Exposure

While exposure to a drug throughout gestation can provide important preclinical information regarding its teratogenic potential for sex-related behaviors, such broad exposure periods are very restricted with respect to understanding potential mechanisms involved. The extended presence of ethanol prior to the differentiation of the HPG axis raises the possibility that effects of ethanol on sex-related behaviors result from an indirect effect on progenitor cells.

In the rat, the development of the pituitary gland begins about day 12 of gestation (Hebel and Stromberg 1986). The hypothalamus and gonads differentiate over the course of the following 3 days, and by day 15.5 the testes are capable of secreting testosterone in response to LH (Nemeskeri et al. 1984; Warren et al. 1984). Subsequently, as noted above, two surges of testosterone occur in the male that are important for complete neurobehavioral masculinization and defeminization. This series of events in the male is important to consider when designing experimental protocols to study the effects of a drug on sex-related behaviors or physiol-ogy. Generally, the timing of the drug exposure should be correlated with the gestational period of differentiation or secretion related to the structures considered to mediate potential alterations in behavior or biology. This period of exposure can then be contrasted with effects observed in animals exposed to ethanol before or after this period in development. For interpretive reasons, care should be taken to entirely include or exclude drug exposure during a testosterone surge. Thus, a commonly used regimen of exposure from days 12 through 18 should be avoided for the study of sex-related effects in males since the drug exposure period would end in the middle of the prenatal testosterone surge.

Hormone Levels and Blood Sampling

The pulsatile or circadian patterns of hormone secretion in plasma make single time point estimations of hormone levels of limited value, especially when small sample sizes are employed (e.g., N = 6 to 10), as is the norm in animal studies. This limitation can be easily illustrated with testosterone, which has a broad normal range for serum values as well as a marked circadian rhythm. Normal testosterone values in rat plasma can vary between 2 and 10 nanograms per milliliter (ng/mL). A significant difference ($p < 0.05$) in testosterone levels between two groups of males, although still within the normal range (e.g., 4.2 versus 3.5), can be difficult to interpret unless it can be associated with a clear alteration in sex behavior in the same animals. Since stress can also depress testosterone levels by inhibiting LH secretion, counterbalanced procedures between groups for obtaining blood samples are also important to consider when assessing data from single blood sample determinations.

Serial blood samples obtained from freely moving catheterized animals provide much more information than single point determinations with respect to hormonal secretory profiles and their relationship to function. When using this method is not possible, such as in the measurement of the fetal testosterone surge, the litter or a litter representative should be considered the unit of analysis rather than treating data from several individuals within the litter as statistically independent points.

Maturational and Physiological Status of the Animal

Several sex-related behavioral tests are influenced significantly by the age or physiological state of the animal. These include open-field behavior, wheel-running activity, taste preferences, and neuroendocrine stress responsiveness. Both open-field behavior and wheel-running activity of female rodents change during the estrous cycle (Beatty 1979), primarily through estrogen's actions on the nigrostriatal dopamine system (Castner et al. 1993). When comparing male and female postpubertal behavior on these type of tests, the stage of estrus should be determined by vaginal cytology. Alternatively, endogenous hormone levels can be removed and circulating levels clamped by implanting a hormone capsule or by hormone injection.

Age and sex differences in neuroendocrine responsiveness are also well documented (Brett et al. 1983; Critchlow et al. 1963). Estrogen (Burgess and Handa 1992; Phillips and Poolsanguan 1978) or the stage of estrus (Viau and Meany 1992) has been shown to influence the adrenocorticotropic hormone (ACTH) and adrenal steroid response to environmental

stress. In most cases, estrogen appears to enhance the response of the hypothalamic-pituitary-adrenal (HPA) axis (Burgess and Handa 1992; Phillips and Poolsanguan 1978; Viau and Meany 1992). In contrast, androgen appears to inhibit the HPA response to stress (Handa et al. 1994). Thus, response differences in neuroendocrine activity due to age and sex should be considered in any study of stress responsiveness of drug-exposed offspring.

Water consumption is also sexually dimorphic in the rat, a fact that is not generally recognized in psychobiological studies of taste preferences. Adult female rats consume approximately 15 percent more water per day than males when consumption is calculated on the basis of body weight (McGivern and Henschel 1990). This fact appears to relate to the sex difference in circulating levels of vasopressin (Crofton et al. 1985). The authors have conducted a series of studies (McGivern et al., in press-a) to further characterize this sex difference in the Sprague-Dawley rat. It was found that the sex difference is present at weaning, but tends to be masked by the marked drop in water consumption that occurs in both sexes from weaning through adulthood. At weaning, the water consumption range for male and female rats is approximately 24 to 30mL/day per kg -0.1 of body weight. A clear sex difference is always present by 60 days of age, when values drop to 16 to 20 mL/day for females and 12 to 16 mL/day for males. Daily consumption continues to decline until about 120 days of age when values are approximately 10to 12 mL/day/kg-1 for females and 8 to 10mL/day/kg-1 for males. This decrease in water intake appears to reflect the decrease with age in the percentage of body weight made up by water (Hays 1980).

Generally, studies looking at taste preferences in adult males and females, including the authors' studies (McGivern et al. 1984, 1987), have not examined daily water consumption. Therefore it is unknown how sex differences in consumption patterns relate to sex differences in taste preferences. However, the authors' data indicate that animals should be tested at ages that are within a few days of each other to determine sex-equivalent water consumption.

Anogenital (AG) distance at birth is a commonly used marker of masculinization, since it reflects the amount of androgenic stimulation during the prenatal period. Specifically, this region is stimulated by testosterone or dihydrotestosterone acting through the androgen receptor. However, AG distance, like internal organ size, is correlated with body size. This is exemplified by studies of AG distance in males prenatally exposed to alcohol. At birth, AG distance in alcohol-exposed males has been consistently found to be smaller compared with controls (Chen and

Smith 1979; McGivern 1987; McGivern et al. 1992; Rudeen et al. 1986; Udani et al. 1985). However, the difference is insignificant when corrected for body weight (McGivern 1987; McGivern et al. 1992). Therefore, in prenatal drug studies where birthweight is reduced, a correction for body weight or crown-to-rump length is essential for an accurate assessment of androgenization. Graham and Gandelman (1986) have found that body weight appears to be a somewhat better correction factor than body length, although either can be used.

Reproductive Versus Nonreproductive Sex Differences

The study of nonreproductive sex-related behaviors has a relatively recent history compared with the study of reproductive behaviors, with the result that much less is known regarding the biological substrates of nonreproductive compared with reproductive behaviors. However, it is becoming increasingly clear that while the neurobehavioral organization of the two classes of behavior are biologically related, their organization and expression can differ substantially. Knowing the performance of an animal with respect to a nonreproductive behavior does not necessarily predict its behavior potentials for reproductive behaviors. For instance, in male rats with normal testosterone levels prenatally exposed to cocaine, the authors observed significant reductions in adult scent marking, but found no changes in mounting, intromission, or ejaculation behavior of the same animals (Raum et al. 1990). Overall, little data have been collected regarding a systematic consideration of the relationship within animals between the expression of reproductive and nonreproductive behaviors in the rat. Until such data become available, interpretation of one class of behaviors appears to have very limited value with respect to other behavioral classes.

DRUGS OF ABUSE: EFFECTS ON SEXUAL DIFFERENTIATION

For the purposes of this chapter, the authors have provided only a brief consideration of the effects of drugs of abuse on the sexual differentiation process. A more extended consideration of the perinatal influence of drugs of abuse on neurobehavioral sexual differentiation is provided in any of several recent reviews (McGivern and Riley 1993; Segarra and McEwen 1992; Ward 1992; Weinberg et al. 1991).

Cocaine

A very limited amount of information is currently available regarding the influence of cocaine on the sexual differentiation process. Pharmacolog-

ically, the drug is well known to block reuptake of monoamines in adult animals and to release dopamine (Heikkila et al. 1975; Komiskey et al. 1977; Ritz et al. 1987; Ross and Renyi 1969). Prenatal exposure might be expected to have significant effects on the sexual differentiation process, since monoamines have been demonstrated to modulate steroid actions as well as to have neurotrophic actions early in development (Lauder and Krebs 1986). Fetal brain tyrosine hydroxylase activity is significantly increased by cocaine exposure (Akbari and Azmitia 1992; Meyer and Dupont 1993), consistent with the marked hyperactivity of the noradrenergic system observed in neonatal brains of males and females exposed to cocaine from days 8 to 20 of gestation (Seidler and Slotkin 1992). Little or no effect of prenatal cocaine exposure was observed on dopamine activity, although it altered dopamine and serotonin receptor number (Byrnes et al. 1993; Henderson et al. 1991; Scalzo et al. 1990).

Raum and colleagues (1984) observed that adrenergic stimulation of the neonatal brain inhibited hypothalamic nuclear incorporation of estrogen. The authors subsequently demonstrated that cocaine administered intracerebroventricularly to 4-day-old female rats significantly inhibited the incorporation of estradiol in the hypothalamus. Since estradiol is a critical factor in the masculinization of the brain, these results suggest that prenatal cocaine exposure might interfere with neurobehavioral masculinization in males.

To date, two published studies have directly addressed this question with conflicting behavioral results. The authors treated pregnant Sprague-Dawley dams with cocaine (10 mg/kg SC) twice a day during the last week of gestation and studied only male offspring (Raum et al. 1990). No effects of cocaine were observed on AG distance at birth. In adulthood, males prenatally exposed to cocaine were found to have increased latencies to initiate sexual behavior, but other aspects of masculine sex behavior were similar to controls. However, another testosterone-dependent behavior, territorial scent marking, was significantly reduced in these animals.

Some evidence for alterations of adult endocrine function was also detected. Plasma LH in cocaine-exposed males was significantly higher than controls, whereas testosterone levels were the same as controls, suggesting some measure of insensitivity to negative feedback by testosterone in these animals. Other endocrine measures were normal, as were sex organ weights, but sperm counts were significantly reduced. The reduction in scent marking in the face of normal circulating levels of testosterone and elevated LH suggests a relative CNS insensitivity to androgens in these animals compared with controls.

Vathy and colleagues (1993) treated dams of the same strain with the same dose regimen of cocaine from days 11 through 18 of pregnancy and observed a different pattern of results. Adult cocaine-exposed males were observed to have facilitated sexual activity patterns, as well as markedly reduced postejaculatory intervals. Conversely, the sexual behavior of cocaine-exposed females was significantly inhibited. Catecholamine levels in the preoptic area of cocaine-exposed males, but not females, were significantly higher than controls. The reasons for the different findings of the two studies are not immediately apparent, but could reflect differences in timing of gestational drug administration or differences in housing of the offspring. Animals in the Raum and colleagues (1990) study were group housed from weaning and during the several weeks of sex behavior testing, whereas the animals in the study by Vathy and colleagues (1993) were singly housed throughout from weaning. Thus housing conditions may have influenced the pattern of results observed. If so, the results imply that prenatal exposure to cocaine may significantly influence the way males and females respond to long-term environmental stress in adulthood. Clearly, additional studies are needed to assess the long-term effects of cocaine on sexual differentiation.

In a study examining locomotor and stereotypy responses to cocaine in animals prenatally exposed to cocaine, Peris and colleagues (1992) reported that prenatal cocaine exposure influences dopamine release from nigrostriatal terminals in a sex-dependent manner. Both males and females exhibited an increased sensitivity to cocaine compared with controls, but females prenatally exposed to cocaine also exhibited increased locomotor activity following saline injection. Sex differences were also detected in amphetamine-induced release of 3[H]-dopamine from striatal slices. In utero cocaine exposure increased amphetamine-stimulated release in females, but decreased release in males. However, since the stage of the estrual cycle was not reported, it not clear whether these effects might also reflect changes in hormonal status during the estrual cycle.

The effect of prenatal cocaine exposure on locomotor activity and acoustic startle response of the offspring appears to be sex related. Hughes and colleagues (1990) found that baseline locomotor activity was reduced in 21- to 22-day-old female rats exposed to cocaine (60 mg/kg) from days 8to 22 of gestation. Cocaine-exposed females were also observed to respond significantly less to an injection of amphetamine (Hughes and Dow-Edwards 1991). No effect was observed in the baseline activity or drug-related activity of cocaine-exposed males at this age

compared with controls. Females exposed to this same dose regimen exhibited a decreased acoustic startle response at 60 to 65 days of age compared with controls (Hughes and Dow-Edwards 1992), but no effect of the prenatal drug exposure was observed on the startle response of males. Jackson and colleagues (1992) reported that prenatal exposure to 15 mg/kg cocaine injected twice daily during the last week of gestation reduced striatal tyrosine hydroxylase immunoreactivity in 20-day-old females but not males. These results appear to be consistent with the effects on locomotor activity observed by Hughes and colleagues (1990, 1991).

Several other studies have noted sex-related differences in cocaine's effects on development which suggest that females may be more affected than males. Sex-related differences in regional brain glucose utilization were noted in adult animals injected with 50 mg/kg cocaine from days 1to 10 (Dow-Edwards et al. 1988) or 11 to 20 (Dow-Edwards et al. 1993) postnatally. Cocaine-exposed females exhibited increased glucose utilization in several cortical and limbic regions, whereas little change, or a decrease, was observed in males. In another study (Kunko et al. 1993), prepubertal females, but not males, were observed to exhibit greater sensitization to cocaine-induced stereotypy following prenatal cocaine exposure. Levin and Seidler (1993) recently reported that exposure to 30mg/kg cocaine from days 8 to 20 of gestation resulted in impaired radial arm performance in females, but not males.

Sex-related differences in cocaine toxicity have been reported in adult rats with respect to cardiovascular function (Morishima et al. 1993). The effect appears to be sex steroid-mediated since it is dependent upon the presence of ovaries (Morishima et al. 1993). However, it is not known at this time whether sex differences in toxicity extend to fetal development. The relatively larger number of toxic effects reported to date in females prenatally exposed to cocaine compared with males suggests that a sex difference in fetal toxicity could be present.

Another important issue to the overall toxicity of cocaine may be the dose of cocaine to which the animals has been exposed prenatally. Evidence from the authors' studies in which pregnant dams have been injected with one of three doses of cocaine during the last week of gestation suggests that the neurobehavioral effects of the drug on the offspring are not always linearly related to the dose of cocaine to which the animals was exposed prenatally. In a recent study involving adult males only, the authors observed a significant lack of sensitization to cocaine-induced stereotypy in adult males exposed prenatally to 3.0, 10.0, or 30.0 mg/kg twice daily for the last week of gestation (McGivern and Hutcheson

1993). In adulthood, the animals were administered 8 daily injections of cocaine (10 mg/kg SC) and their behavior was monitored in the open field for 60 minutes after injection. One week later the animals were again injected with cocaine to examine sensitization to the drug. Sensitization to cocaine was observed in controls, but not in animals prenatally exposed to cocaine. The lack of sensitization to cocaine-induced stereotypy extended to all animals exposed to cocaine, regardless of prenatal dose. A similar lack of sensitization to cocaine has been recently reported in mice prenatally exposed to cocaine (Byrnes et al. 1993).

However, differential effects of prenatal dose were noted for the behavioral responsiveness to cocaine with respect to locomotor activity and rearing behavior (McGivern and Hutcheson 1993). In this study, the authors noted an overall inverted U-shaped function with respect to cocaine responsiveness which was related to the prenatal dose of cocaine exposure. This response pattern is similar to the behavioral results for scent marking which the authors observed in a previous study in adult males prenatally exposed to the same three doses (Raum et al. 1990). A similar inverted U-shaped response to cocaine has been observed by Meyer and colleagues (1992) in 11-day-old males and females exposed to cocaine from gestational days 11 to 20. Since cocaine has pharmacologically relevant anesthetic properties at higher doses (Gifford and Johnson 1992), it may be producing developmental effects that differ from its classic action of reuptake blockade at lower doses.

Opiates

Data concerning the long-term effects of opiates on sexual differentiation are also limited. Prenatal exposure to morphine from day 5 to 14 (Vathy et al. 1983) or from days 11 to 18 (Vathy et al. 1985) reduced lordosis frequency in females. The effect was more pronounced in animals exposed from days 11 to 18 of gestation (40 to 57 percent) compared with those exposed from days 5 to 14 (20 percent). This difference suggests the importance of morphine exposure during the entire period of hypothalamic differentiation in producing this effect. Sexual behavior of males was unaltered, with the exception that morphine-exposed males had significantly shorter postejaculatory intervals (Vathy and Katay 1992; Vathy et al. 1985). Lordosis behavior was not examined in males.

Hypothalamic NE content was dramatically altered in these animals. The NE content in morphine-exposed females was elevated by 95 percent, while the content in males was reduced by 57 percent relative to controls (Vathy and Katay 1992). It should be noted that animals used in these studies were single housed from weaning, which may have contributed to

eliciting the differences in drug-exposed animals. No differences were observed in estrogen receptor regulation in the hypothalamic-preoptic region of morphine-exposed animals (Vathy et al. 1985).

Perinatal morphine exposure had no effect in golden hamsters on the ability of the female to display hormone-induced male or female sexual behavior patterns (Johnston et al. 1992). Morphine-exposed males exhibited normal masculine sex behavior patterns but significantly more lordosis behavior than controls, indicating incomplete defeminization.

Other evidence for a masculinizing effect of morphine on females has been obtained in the rat, in addition to the reduced lordotic potential observed by Vathy and coworkers (1983, 1985, 1992). Lapointe and Nosal (1982) reported increased AG distance in females at weaning after exposure to morphine from conception through postnatal day 16. Delayed vaginal opening was also reported in females exposed to morphine from days 5 to 12 of gestation (Litto et al. 1983). This latter effect contrasts with the effect of postnatal administration of morphine, which has been found to induce precocious puberty in females (Sonderegger et al. 1977).

Plasma levels of the androgens testosterone and androstenedione on day 20 of gestation were significantly reduced in males, but not females, exposed to methadone from days 14 through 19 of gestation (Singh et al. 1980). However, no evidence for a direct effect of the drug at the site of the testis, nor any effect of the drug on aromatization of testosterone to estrogen, was observed. The results of this study, as well as many of the above-cited studies on the prenatal effects of morphine, should be interpreted with caution since cessation of morphine treatment causes severe withdrawal in rodents, and is known to induce long-term effects on growth and differentiation. Sparber has provided evidence that many of the effects attributed to prenatal morphine exposure, both biochemical and behavioral, are a result of withdrawal rather than the direct effects of the drug (Lichtblau and Sparber 1984; Sparber 1986).

While the limited evidence to date is not conclusive with respect to the effects of opiates on the sexual differentiation process, circumstantial evidence strongly justifies further study. Perinatal opiate treatment decreases dendritic arborization in cortical neurons (Ricalde and Hammer 1990). Mu opioid receptors are present in the brain as early as day 14 of gestation (Bayon et al. 1979; Clendeninn et al. 1976). This class of opioid receptors binds opiates such as morphine, heroin, and methadone, and is distributed throughout brain regions integral to reward and the expression of sex behavior including the medial preoptic area (MPOA)

and the ventral tegmental area (VTA). Stimulation of mu receptors in adult animals inhibits luteinizing hormone-releasing hormone (LH-RH) release through an inhibitory influence on excitatory NE projection to the MPOA (Kalra and Kalra 1984) and inhibits female sexual behavior through an inhibition of NE release in the ventromedial hypothalamus of females (Vathy et al. 1991). Injection of beta-endorphin directly into the MPOA produces a cessation of copulation in male rats (Hughes et al. 1987).

Chronic morphine treatment during the last 2 weeks of gestation did not alter the number or functional efficacy of the mu receptors in striatal and cortical slices from fetal brain at 21 days of gestation (DeVries et al. 1991). However, electrically stimulated, calcium-dependent release of both dopamine and NE in morphine-exposed tissue was dramatically enhanced, indicating an excessive activation of signal transduction mechanisms regulating catecholamine release. These opioid-related actions on catecholamines, combined with evidence that opiate receptor systems modulate development of catecholaminergic systems (Seidler et al. 1982), suggest that morphine has a strong potential to alter the normal development of neurobehavioral sexual differentiation. Studies of the susceptibility of nonreproductive sex-related behaviors to disruption by prenatal opiate exposure will be important in assessing the overall impact of the drug on the sexual differentiation process.

Nicotine

Male offspring prenatally exposed to nicotine have been found to exhibit demasculinized behavior patterns in adulthood. Decreased mounting and intromission behavior in adult nicotine-exposed males has been reported (Segarra and Strand 1989), indicating incomplete masculinization of the brain in these animals. Bernardi and colleagues (1981) reported a decrease in the postejaculatory interval of males prenatally exposed to cigarette smoke, suggesting an increase in sexual drive. Evidence for feminization of the brain in males prenatally exposed to nicotine is provided by data indicating an increase in saccharin preference in these animals (Lichtensteiger and Schlumpf 1985).

Female sex behavior has not been found to be altered, but an increase in ovarian weight in females prenatally exposed to nicotine was observed (Segarra and McEwen 1992). Other sex-related nonreproductive behaviors have generally been found to be unaffected in females following perinatal nicotine exposure. These behaviors include saccharin preference (Lichtensteiger and Schlumpf 1985), salt preference (Segarra and McEwen 1992), radial arm-maze performance (Levin et al. 1993),

and open-field behavior (Peters and Tang 1982). An exception is active avoidance behavior, which was found to be improved in adult females exposed to nicotine throughout gestation (Genedani et al. 1983). Meyer and Carr (1987) found a consistent delay in vaginal opening in females exposed to a low or high dose of nicotine either prenatally or postnatally. In addition, elevations in peripubertal LH values were observed in nicotine-exposed males and females, suggestive of a relative insensitivity to steroid negative feedback.

In males exposed prenatally to nicotine, plasma testosterone levels have been reported to be significantly reduced in adulthood (Segarra and Strand 1989) and when measured on day 18 of gestation in male fetuses (Lichtensteiger et al. 1988), which appear to be consistent with the effects of the drug on masculine sex behavior. However, since the prenatal surge peaks on days 18 to 19 (McGivern et al. 1988a; Weisz and Ward 1980), additional time points during this period of gestation need to be measured to reach a more definitive assessment of nicotine's effect on fetal testosterone levels. AG distance at birth was also reported to be significantly smaller in nicotine-exposed males, but birthweight was significantly lower in these animals compared with controls, which likely accounted for the decrease in AG distance. Peters and Tang (1982) observed decreased birthweight in male, but not female, offspring from dams treated with 6 mg/kg of nicotine prior to and during gestation, which is in the same range as the animals from the study of Lichtensteiger and Schlumpf (1985). Segarra and Strand (1989) failed to find a difference in birthweights of either sex from dams treated with 0.25 mg/kg of nicotine twice daily from days 3 to 21 of gestation.

The mechanisms whereby nicotine might alter the sexual differentiation process are multiple. Nicotine receptors in the brain are most dense in the hypothalamus and preoptic area (Clarke et al. 1988) and cholinergic regula-tion in the preoptic area is modulated by gonadal steroids (Commins and Yahr 1984). Prenatal nicotine exposure has been reported to induce tran-sient increases in nicotinic receptors in fetal and postnatal brains of rats (Slotkin et al. 1987); however, long-lasting effects of prenatal nicotine exposure on the cholinergic system have not been studied. Nicotine treatment of the fetus is known to have long-term effects on catechol-aminergic function. Deficiencies in postnatal catecholamine activity have been observed (Navarro et al. 1988, 1990; Ribary and Lichtensteiger 1989; Seidler et al. 1992), in contrast with significant increases in catecholamine turnover observed in fetal brain (Lichtensteiger et al. 1988). Such increases could hypothetically result in a decrease in hypothalamic nuclear binding of estradiol and subsequent demasculinization of the male (Raum et al. 1984, 1990).

Nicotine administration elevates several pituitary hormones, including ACTH, LH, vasopressin, endorphins, and prolactin (Fuxe et al. 1989). Fetal adrenal function, as well as aromatase activity in fetal forebrain, is affected by prenatal nicotine exposure (von Zigler et al. 1991). Activation of the HPA axis in pregnant animals by stress or ACTH injection is known to demasculinize or feminize reproductive behavior of male offspring (Segarra and McEwen 1992); this mechanism may account for a significant portion of the long-term effects of prenatal nicotine exposure on sex-related behaviors.

Marijuana

The animal literature regarding the effects of perinatal exposure to marijuana is quite small, but demonstrates consistent demasculinizing effects of the drug on sexual differentiation of males. In male mice, exposure to Δ9-tetrahydrocannabinol (THC) or cannabinol during the last week of gestation reduced masculine sex behaviors in adulthood (Dalterio 1980; Dalterio and Bartke 1979). No other behavioral studies of sex-related behaviors have been conducted to the authors' knowledge. In light of the recent identification of the cannabinoid receptor and its wide-spread distribution in the CNS (Howlett et al. 1990), more studies of the effects of this drug appear warranted.

Walters and Carr (1986) observed long-term decreases in striatal tyrosine hydroxylase activity as well as decreases in dopaminergic autoreceptor binding in the cortex of rats exposed prenatally to crude marijuana extract. These results suggest a potential role for involvement of catecholamines in alterations of sex-related behaviors of cocaine-exposed offspring. Stronger evidence indicates a role for reduced action of androgens. Marijuana has been consistently observed to decrease testosterone in adults rats and humans (see Ward 1992 for review). Current evidence indicates that it has a similar effect in the fetus and neonate. Dalterio and Bartke (1981) found a decrease in testosterone and dihydrotestosterone in male mice on day 16 of gestation when exposed to THC or cannabinol (50 mg/kg) from days 12to 16 of gestation. AG distance was significantly increased in these animals, in spite of the fact that body weights were significantly smaller than controls and AG distance was not corrected. Given the fact that AG distance is dependent upon circulating androgen levels, these results appear inconsistent with an effect of the drug on AG distance which is mediated through a reduction in androgens.

However, the reduced androgen level in drug-exposed males is consistent with a significant reduction in testosterone and LH in male rats at birth following exposure to 6 mg/kg THC from days 14 to 19 of gestation (Ahluwalia et al. 1985). Abnormal prostate morphology and long-term deficits were observed in fertility of these rats, which was accompanied by lower prepubertal, but not postpubertal, plasma levels of LH and testosterone. Reductions in fertility have also been observed in mice exposed to THC postnatally (Dalterio 1980; Dalterio and Bartke 1979) and rat offspring of dams exposed to marijuana smoke during pregnancy (Freid and Charlebois 1979).

Alcohol

The literature regarding the effect of alcohol on neurobehavioral sexual differentiation is significantly greater than that for other drugs of abuse and has recently been extensively reviewed (McGivern and Riley 1993). Ethanol is well known to suppress HPG function in adults and this action provided the original basis to hypothesize effects of the drug on the sexual differentiation process (Chen and Smith 1979).

Reproductive and Maternal Behaviors. Several aspects of sexual behavior have been found to be altered in adult rodents prenatally exposed to alcohol. Chen and Smith (1979) reported the first study of sexual behavior in the fetal alcohol-exposed (FAE) male rat in which they observed poorer penile reflexes in FAE males compared with controls. Udani and colleagues (1985) reported a decrease in intromission behavior in FAE males in the presence of receptive females, suggesting incomplete masculinization. Hard and colleagues (1984) reported increased lordosis in FAE males primed with estrogen and progesterone, indicating incomplete defeminization in FAE males. However, others have not observed differences in FAE males with respect to masculinization (Dahlgren et al. 1989; Hard et al. 1984; McGivern and Handa, unpublished observations) or feminization (Dahlgren et al. 1989; McGivern and Handa, unpublished observations) of sexual behavior.

This inconsistency between studies may reflect the effect of prenatal alcohol exposure on the sensitivity of the HPA axis. Prenatal ethanol exposure has also been found to have long-term effects on the developingHPA axis. FAE female rats exhibit a greater response to stress in adulthood as measured by the release of corticosterone from the adrenal gland. This effect has been found with both prenatal (Taylor et al. 1982; Weinberg 1988, 1992) and postnatal alcohol exposure (Kelly et al. 1991). Stress responsiveness of FAE males was not found to be affected in these studies, but a recent study indicates that increased HPA activation in

response to stress can also be observed in adult FAE males (Weinberg 1992). Stress-induced suppression of reproductive behavior is well known (Sachs and Meisel 1988), an effect that is mediated by glucocorticoids (Baldwin and Sawyer 1974; Brann et al. 1990; Kononen et al. 1993; McGivern and Redei 1994). Thus it is possible that the decreases in sexual behavior observed by some investigators reflect a lack of habituation to the open-field testing situation, a situation well known to increase HPA activa-tion in the rat (Fitch et al. 1992). Alternatively, olfactory cues important to initiation of sexual behavior in the rat may be compromised due to the loss of mitral cells in the olfactory bulb following early alcohol exposure (Bonthius et al. 1992).

Fewer sex-related effects of prenatal alcohol exposure have been observed in FAE female offspring. However, a delay in the onset of sexual maturation in mice and rats, as measured by date of vaginal opening, has been consistently reported in FAE females (Boggan et al. 1979; Esquifino et al. 1986; Farry and Tittmar 1975; McGivern and Yellon 1992; McGivern et al. 1992). In a recent study, the date of vaginal opening in females exposed to ethanol during days 7 to 21 of gestation was compared with that of females exposed from days 14 to 21 of gestation (McGivern et al. 1992). An equal period of delay was observed in both groups. These results suggest that exposing the developing hypothalamus to ethanol during the last week of gestation is a more important factor in causing this delay than alcohol exposure to the developing ovary, which differentiates around day 12 of gestation. This suggestion is supported by the findings of Sonderegger and colleagues (1986), who observed no effect of prenatal ethanol exposure on days 1to7 or 8 to 14 of gestation on female reproductive function. However, females exposed to a much higher level of ethanol only on day 8 of gestation have been reported to be more sexually responsive to estrogen in adulthood (Minetti and Fulginiti 1991). Neither prenatal nor postnatal alcohol exposure has been found to alter fertility (Hard et al. 1985; Mitchell 1994; Sonderegger et al. 1986). However, the authors have found that prenatal exposure to alcohol during the last week of gestation accelerates the age-related loss in estrous cyclicity in females (McGivern et al. 1995). These results suggest that prenatal alcohol exposure can shorten the window of reproductive competence in the life of the female.

FAE females display deficits in maternal behavior as evidenced by taking longer to retrieve their pups and poorer nest building (Hard et al. 1985). Retrieval deficits can also be observed in virgin FAE rats presented repeatedly with pups from another mother (Barron and Riley 1985), indicating that alcohol has a disruptive effect on the organization of this behavior. However, the role played by increased stress responsiveness of

FAE females needs to be determined to better assess whether prenatal ethanol exposure directly influences the organization of maternal behavior.

Nonreproductive Behaviors. Several sex-related behaviors unrelated to reproduction are feminized in adult FAE males. Such behaviors include saccharin preference, maze performance, and juvenile play behavior, all of which are organizationally dependent upon testosterone for their sexually dimorphic expression. Normally adult female rats consume greater quantities of sweetened solutions such as saccharin than males (Valenstein et al. 1967). This behavior, like most sexually dimorphic behaviors, can be altered by changes in the steroid hormonal environment during either late prenatal or early postnatal life (Beatty 1979). Follow-ing both prenatal (McGivern et al. 1984) and neonatal (Barron et al., in press) alcohol exposure in rats, the normal sex difference in saccharin preference has been reported to be eliminated due to an increase in FAE males and a decrease in FAE females. However, in another study (McGivern et al. 1987), preference was increased in FAE males, but unchanged in FAE females. In mice, fetal alcohol exposure has been found to increase saccharin consumption by both sexes (Middaugh et al. 1993).

The literature on the effects of prenatal alcohol exposure on learning documents consistent decrements in learning abilities of alcohol-exposed animals (Meyer and Riley 1986b). However, in studies involving spatial mapping when both sexes have been tested, sex-dependent effects have also been observed in complex learning paradigms that involve spatial learning or spatial mapping. Males' learning ability in spatial tasks is generally found to be significantly better than females' (Beatty 1979, 1984, 1992). Prenatal alcohol exposure appears to influence adult per-formance of a spatial task in a sex-dependent manner. In the Lashley III maze, FAE males required more trials to learn the maze than controls, while the performance of FAE females improved to the level of control males (McGivern et al. 1984). Performance of FAE males in other com-plex mazes is also impaired (Blanchard et al. 1987; Zimmerberg et al. 1991).

Sex-dependent effects of prenatal alcohol exposure have also been observed in play behavior. Juvenile rats engage in a type of rough and tumble play that resembles wrestling. The degree to which this behavior is expressed is dependent upon perinatal testosterone levels; males typically engage in more of this play behavior than females (Meaney and Stewart 1981). FAE males have been found to display less of this aggressive behavior than controls, while FAE females display more (Meyer and Riley

1986a), again suggesting a partial masculinization of females and a demasculinization of males.

Social behavior in the rat is sexually dimorphic (Kellogg et al. 1991) when conspecific animals are paired in a familiar versus an unfamiliar environment. Males exhibit much less interaction with another male in an unfamiliar environment compared with one that is familiar. This difference is not observed in females. Kelly and Dillingham (1994) recently observed a loss of this sexual dimorphism in adult animals exposed to alcohol from postnatal days 4 to 12. This is a period in rat brain development that roughly corresponds to the third trimester in humans. Males exposed to alcohol during this period exhibited significant decreases in social behavior compared with controls, whereas a significant increase was observed in alcohol-exposed females. This feminization of male behavior and masculinization of female behavior by postnatal alcohol exposure was accompanied by reduced cell number in the amygdala region of males, but not females. However, alcohol-exposed females exhibited significant increases in dopamine metabolism in this region. The amygdala has been shown to play a critical role in the expression of social behavior (Meaney and McEwen 1986).

The authors originally proposed that masculinization of FAE females might be due to ethanol-induced activation of the HPA axis, resulting in excessive release of adrenal androgens such as androstenedione (McGivern et al. 1984). However, recent evidence indicates that androstenedione is not released in response to stress in the rat (Fitch et al. 1992), unlike the human, because it is not synthesized in significant quantities in the adrenal gland (van Weerden et al. 1992). Thus it seems unlikely that the mascu-linization observed in FAE females is related to excessive androgen production.

Other learning paradigms also reveal sex differences in sensitivity to alcohol's effects, but these differences appear to depend upon the period of exposure. While males appear more affected in some paradigms following prenatal alcohol exposure, the reverse appears to be true following postnatal alcohol exposure. When mice prenatally exposed to alcohol were trained to press a bar for reward in an operant learning task, the normal sex differences were eliminated and males appeared to be more affected than females (Gentry and Middaugh 1988). Control males responded at a significantly higher rate than control females under a fixed-ratio 5 (FR-5) schedule. This sex difference was absent in ethanol-exposed mice, primarily due to lower responding by the males. When the response rate by the same animals on the FR-5 schedule was compared with the rate of a differential reinforcement of other behavior (DRO)

schedule designed to produce low rates of responding, elevated responses were observed in ethanol-exposed animals of both sexes. The authors argue convincingly that these results may reflect a diminished efficacy of the reinforcer in ethanol-exposed animals (Gentry and Middaugh 1988).

In a passive avoidance paradigm, a simple learning task in which the subject must learn to inhibit its normally preferred response to avoid punishment, both sexes had difficulty with learning following prenatal alcohol exposure (Riley et al. 1979). However, following neonatal alcohol exposure, only females were impaired (Barron and Riley 1990). When spatial navigation was examined in a Morris water maze, the performance of adult females was impaired while adult male performance was unaffected following postnatal alcohol exposure (Kelly et al. 1988).

Fadem (1993) recently examined the behavioral and anatomical effects of postnatal alcohol exposure in the opossum, a species that emerges from the womb at a very immature stage of development. No effect of ethanol was observed in the reproductive behavior, anatomy, or physiology of either sex. However, some evidence was obtained for decreased fecundity in alcohol-exposed female opossums. In addition, ethanol exposure masculinized threat behavior and scent-marking behavior in females, while feminizing the expression of these behaviors in males. These findings indicate some generalization across species in the effects of ethanol on the sexual differentiation process.

Alterations in Neuroendocrine Function. Ethanol is well known to depress HPG function in both males and females (Purohit 1993) resulting in reduced testosterone levels in males. Ethanol also has been found to produce a marked depression in fetal and neonatal production of testosterone in males, an effect that is quite consistent with its behavioral effects. The prenatal testosterone surge on days 18 and 19 of gestation are greatly attenuated in FAE male fetuses (McGivern et al. 1988a). A less marked but significant attenuation has also been observed in the postnatal surge of males from dams consuming approximately 14g/kg/day of ethanol during the last week of gestation (McGivern et al. 1993). A decrease in testosterone levels of FAE males around the time of birth has been reported by others (Kelce et al. 1989; Rudeen et al. 1986), consistent with an attenuation of the postnatal testosterone surge. This depression of testosterone production appears to relate to a depression in 17-alpha-hydroxylase activity in neonatal testes from FAE males (Kelce et al. 1989, 1990), although changes in LH secretion or sensitivity to LH cannot be ruled out (McGivern et al. 1988a). Exposure to lower amounts of ethanol during this period does not appear to influence the postnatal rise in testosterone levels (Dahlgren et al. 1989). Aromatase activity in fetal and

neonatal brain is elevated by prenatal alcohol exposure in males, but not females (McGivern et al. 1988b). To some degree, this increase in enzymatic activity might be expected to limit the effect of a reduction in testosterone on the defeminization process by increasing the conversion of the available substrate.

Present evidence indicates that LH secretion is decreased in older adult FAE animals of both sexes. Basal LH secretion in adult castrated FAE males and females at 5 to 6 months of age was found to be reduced to nearly half the level of pair-fed controls (Handa et al. 1985). In addition, alterations in the amplitude and duration of pulsatile LH release were observed. This may reflect an accelerated rate of aging in FAE animals. Studies of reproductive function in females have established an age-related decline in circulating plasma LH levels (see Gerall and Givon 1992 for review). Plasma LH is 2 to 4 times less in old rats than young rats, with middle-aged rats exhibiting intermediate levels between the two. The authors have recently observed that FAE females enter anestraul sterility at a significantly earlier age than pair-fed (PF) or chow-fed (CF) females (McGivern et al. 1995, in press-b). Such results indicate that fetal alcohol exposure in females reduces the window of reproductive competency in the lifespan of the animal.

Both sexes have been reported to exhibit reduced sensitivity to sex steroid feedback in the brain (Handa et al. 1985; Jungkuntz-Burgett et al. 1990). Such effects may contribute significantly to the delay in puberty onset in FAE females as well as the demasculinized sex behavior of adult FAE males. However, corticosteroid levels were not measured in the studies cited above, leaving open the possibility that decreases in basal LH or in response to estrogen-induced positive feedback result in part from the increased stress responsiveness of FAE females.

Data concerning the effects of prenatal ethanol exposure on the adult male HPG axis are inconsistent. Reduced sex organ weights in FAE adult males, including testes, prostate, and seminal vesicles have been reported by Udani and colleagues (1985) in animals exposed to ethanol from day 12 through parturition. However, similar reductions were not observed in FAE males of the same strain exposed to ethanol during either the last 2 weeks of gestation or the last week alone (McGivern et al. 1992). In addition, males in this study were observed to have normal testosterone levels and normal sperm counts. Other studies have reported reduced plasma testosterone levels in adult FAE males (Dahlgren et al. 1989; Udani et al. 1985), although the measured plasma values were still in the normal male range. Given this fact, as well as the inconsistency between

studies with respect to male sex behavior, the significance of these reductions is not clear at this time.

A number of studies have reported that uncorrected AG distance in FAE males is reduced at birth (Chen and Smith 1979; Rudeen et al. 1986; Udani et al. 1985), which has been interpreted to indicate either reduced levels of testosterone or a decrease in 5-alpha reductase activity to convert testosterone to dihydrotestosterone. Significant reductions in AG distance of FAE males at birth have been observed (McGivern 1987; McGivern et al. 1992), but the results were no longer significant when AG distance was indexed to body weight. Thus, the authors believe that the reduction in AG distance primarily reflects an effect of prenatal ethanol exposure on somatic growth rather than a specific effect of the drug on peripheral androgen metabolism or sensitivity.

Neurotransmitter Function. Animal studies suggest that monoamine neurotransmitters such as NE and serotonin can act as modulators of neuroanatomical and behavioral sexual differentiation during prenatal development (Handa et al. 1986; Jarzab et al. 1986; Raum et al. 1984). Excessive NE activity is known to inhibit the actions of sex steroid hormones in areas of the brain such as the hypothalamus in the neonatal rat (Raum and Swerdloff 1981). Data from other studies indicate that both NE and serotonin play an important role in the structural development of the brain (Lauder and Krebs 1986; Mirmiran et al. 1988). Prenatal alcohol exposure has been shown to have long-term effects on neurotransmitters in the developing brain (Cooper and Rudeen 1988; Detering et al. 1980; Druse and Paul 1989; Druse et al. 1990; Rathbun and Druse 1985). Taken together, these data indicate that an interaction between monoamines and sex steroid hormones on brain development may be an important variable when considering the effects of prenatal alcohol exposure on sexual differentiation.

Functional consequences of catecholaminergic alterations in FAE animals that may be sex-related are suggested by findings from two recent studies. Becker and colleagues (1994) found that mice prenatally exposed to ethanol were more sensitive to the stimulation of locomotor activity by ethanol. This effect was more marked in FAE females than FAE males compared with controls. Both sexes were relatively more sensitive as adults to the antagonistic action of amphetamine on ethanol-induced stimulation of locomotor activity, a finding consistent with reduced monoaminergic function in the brain of FAE animals. Following ethanol administration (0.5 or 1.0 g/kg), Blanchard and colleagues (1993) measured dopamine release by microdialysis in the striatum and nucleus accumbens of FAE adult male and female rats. Dopamine release was

absent in FAE females at both doses, while release was evident in FAE males at the higher dose. In controls, release was observed at both doses. The decreased responsiveness in dopamine release in the accumbens appears consistent with the hypothesis of Gentry and Middaugh (1988) of reduced efficacy of reinforcers in FAE animals.

Neuroanatomical Changes. The preoptic area of the hypothalamus is known to play an important role in sex and maternal behaviors in rats (Numan 1988). Within this area is the sexually dimorphic nucleus of the preoptic area of the hypothalmus (SDN-POA), which is several fold larger in males than in females (Gorski et al. 1978). Following either prenatal or perinatal alcohol exposure, this nucleus has been found to be smaller in adult males, indicating a demasculinizing effect of alcohol during perinatal development (Barron et al. 1988; Rudeen et al. 1986).

The corpus callosum in rats has also been shown to be influenced by prenatal alcohol exposure in a sexually dimorphic manner. Typically, the corpus callosum is larger in males than females. However, data from a recent study suggest that prenatal alcohol exposure reduces or eliminates this sex difference (Zimmerberg and Scalzi 1989). A similar effect of prenatal alcohol exposure on cortical asymmetry has also been reported (Zimmerberg and Reuter 1989). In normal males, the right hemisphere of the cerebral cortex is thicker than the left cortex, whereas females show no such asymmetry. Following prenatal alcohol exposure, this cortical asymmetry appears reduced in males, again suggesting a demasculinizing influence of ethanol.

In the mouse, ethanol reduced the number of immunoreactive gonadotropin-releasing hormone (Gn-RH) neurons detectable at 18 days of gestation following exposure to a high dose of ethanol administered on day 8 of pregnancy (Scott et al. 1992). However, no effect of prenatal ethanol exposure was observed in the number of immunoreactive Gn-RH neurons in 44-day-old female FAE rats with delayed onset of puberty (McGivern and Yellon 1992). Subtle alterations were detected in the morphological characteristics of the neuronal processes of Gn-RH cells in these animals, but the significance is unclear at present. Differences in species, as well as prenatal timing and amount of exposure to alcohol, make comparisons to the results found in the mouse difficult. It remains to be determined whether the decreases in LH secretion of FAE animals reflect a functional deficit related to the Gn-RH neuron.

SUMMARY

The pattern of results from the studies reviewed above indicates that alcohol, morphine, nicotine, marijuana, and possibly cocaine can influence reproductive aspects of the neurobehavioral sexual differentiation process to varying degrees. However, with the exception of alcohol, little is currently known regarding the effects of these drugs on nonreproductive sex-related behaviors. Future studies are needed to define the extent of perinatal disruption induced by each drug on the nonreproductive aspect of the sexual differentiation process.

It is increasingly clear that the neurobehavioral development of reproductive and nonreproductive behaviors is not influenced to the same degree by alterations in the perinatal hormonal or monoaminergic environment, probably reflecting a fundamental underlying difference in the relative contributions of different brain areas to each behavior (Meaney and McEwen 1986). This fact points to the necessity of greater inclusion of sex-related behaviors in animal models used to assess the teratogenic potential of a given drug on the sexual differentiation process.

In light of recent demonstrations of regional structural sex differences in the human CNS (Allen et al. 1989, 1991; deLacoste-Utamsing and Holloway 1982; Hofman et al. 1988; Swaab and Hofman 1988) as well as reports of structural differences in male homosexuals (LeVay 1992; Swaab and Hofman 1990), there is an increasing interest in the contribution of prenatal drug exposure to homosexuality in humans. These findings appear to have led some investigators to interpret behavioral results from animal studies of prenatal drug exposure as being relevant to understanding the causes of homosexuality in humans (Dahlgren et al. 1991; Hard et al. 1984). However, while data from the animal models reviewed above can provide invaluable preclinical evidence to help understand the effects of perinatal drug exposure on brain development and the process of sexual differentiation, the authors believe that the results of these studies provide minimal useful information with respect the prenatal influence of these drugs on homosexual behavior in humans.

Animal models of homosexuality are inherently inadequate for several reasons. No adequate model exists for homosexual behavior in the rodent in the absence of pharmacological administration of steroids. Normal male rats that show low levels of masculine sex behavior in the presence of estrous females do not exhibit increased tendencies to mount other males nor to lordosis when mounted by another male. In fact, male preference behavior for an estrous female rat does not appear to be influenced by perinatal androgen exposure (Merkx 1984).

A second issue that cannot be addressed in an animal model is the fact that sexual orientation in humans is determined by an interaction between hormonal, environmental, and cultural factors (Money 1987). This problem, and others, with a developmental animal model of human homosexuality have been considered by Sachs and Meisel (1988), to which the reader is referred for a more extensive discussion.

Finally, in humans there is also the issue of gender identity, which refers to traits or conditions of maleness or femaleness. The degree to which gender identity in humans is causally linked to cultural or biological influences is an area of current debate (Gentile 1993; Unger and Crawford 1993), but such identity is clearly beyond the scope of animal modeling. Therefore, issues related to sexual orientation of humans and prenatal drug exposure likely await data from future human studies for further resolution.

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AUTHORS

Robert F. McGivern, Ph.D.
Professor
Department of Psychology
San Diego State University
6363 Alvarado Court
Suite 200 H
San Diego, CA 92120

Robert J. Handa, Ph.D.
Associate Professor
Department of Cell Biology, Neurobiology, and Anatomy
Loyola University Chicago
Stritch School of Medicine
Maywood, IL 60153

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