69

Masculinization and Defeminization in Altricial and Precocial Mammals: Comparative Aspects of Steroid Hormone Action

Kim Wallen
Department of Psychology and Yerkes Regional Primate Research Center
Emory University
Atlanta, Georgia

Michael J. Baum
Department of Biology
Boston University
Boston, Massachusetts

Altricial and precocial species follow different developmental trajectories possibly reflecting different reproductive strategies. This chapter describes evidence that this distinction may have heuristic value in understanding the nature of steroidal influences on masculinization and defeminization. Across both types of mammals, defeminization was found to utilize estrogenic metabolites of androgens. However, the evidence of this requirement was stronger in altricial than precocial species. Unambiguous evidence of defeminization by nonaromatizable androgens was found only in the precocial rhesus monkey. The role of aromatization in masculinization was less clear, with little evidence in any species that mounting potential differentiated under either androgenic or estrogenic influence. When all aspects of male sexual and social behavior were considered, altricial species relied more on aromatization for masculinization than did precocial species. No evidence was found in human males that the actions of estrogenic compounds were necessary for normal male sexual differentiation. These apparent differences between altricial and precocial species in the hormonal actions producing masculinization and defeminization might be an artifact of which species have become favored laboratory subjects. Alternatively, they may reflect a deeper organizing principle resulting from the different life strategies of altricial and precocial species. Resolving this issue awaits a broader comparative investigation of sexual differentiation than is currently available today.

Phoenix *et al.* (1959) closed their landmark paper proposing that steroid hormones organized the sexual characteristics of the developing nervous system with the following: "We are assuming that testosterone or some metabolite acts on these central nervous tissues in which patterns of sexual behavior are organized." This caution concerning whether testosterone (T) or its metabolites were the active agents in organizing the nervous system has assumed a central position in the 40 years of research following the paper's publication. While the Kansas group might have been cautious about which steroid was responsible for CNS

organization, they certainly did not foresee an era when the dominant view would be that estrogenic metabolites of androgens are the primary steroids organizing behavioral sexual differentiation in mammals. The discovery that neural tissues, particularly the hypothalamus, in some mammals contained the enzymes necessary to aromatize androgens to estrogen raised the possibility that high circulating levels of androgens might be regionally converted to estrogens, altering neural development. This notion, which came to be called the aromatization hypothesis, went from being counterintuitive to becoming a central tenet of sexual differentiation. However, its centrality may reflect an accident of the species that dominate laboratory studies of sexual differentiation. When sexual differentiation is explored from a comparative perspective, it is unclear that aromatization is involved in the sexual differentiation of all species. Furthermore, even for those species where aromatization has been demonstrated to be important it is not clear that it is required for all aspects of behavioral sexual differentiation.

This chapter describes evidence from a broad range of mammalian species of the involvement, or lack thereof, of estrogenic metabolites in behavioral sexual differentiation. In particular, we investigate the role of aromatized metabolites of androgens in the processes of masculinization and defeminization of behavior and explore the possibility that species differences in the role of estrogenic metabolites may reflect the relative completeness of sexual differentiation at birth.

I. BASIC PROCESSES OF BEHAVIORAL SEXUAL DIFFERENTIATION

Mammalian males and females have different sex chromosomes, and sexual differentiation results from a cascade of events that result from the expression of genes on these chromosomes, as well as autosomal genes (Swain and Lovell-Badge, 1999). Current evidence supports the view that products of the *Sry* gene on the Y chromosome interact with the X chromosome genes, *Sox9,* and autosomal genes to cause the undifferentiated fetal gonad to become a testicle instead of an ovary (Koopman, 1999). Gonadal differentiation then sets in motion a series of events in which testicular hormones direct the differentiation of masculine and suppress feminine characteristics. Although this developmental cascade from gene expression to gonadal differentiation, leading to hormone production, and finally to morphological and behavioral differentiation encompasses the principle pathway by which sexual differentiation occurs, evidence suggests that there may be nonhormonal ways in which the sex determining genes affect sexual differentiation (Arnold, 1996). For example, evidence from mice suggests that the *Sry* gene is transcribed in the developing male, but not female, brain raising the possibility of a direct effect of *Sry* transcripts on neural organization (Lahr *et al.,* 1995; Mayer *et al.,* 2000). While these findings are intriguing and demonstrate that a full description of the sexual differentiation process is likely to contain surprises, it is apparent that the actions of testicular hormones play a large and critical role in sexual differentiation. This role, specifically of steroid hormones, is the focus of this chapter. We first briefly describe the cascade of differentiating events that testicular hormones influence.

Mammalian sexual differentiation is biased in a female direction (Jost, 1970). By this we mean that morphogenic processes are geared to producing female endpoints in sexual differentiation more easily than they produce male endpoints. Some have thus referred to the female path of differentiation as the default path, meaning it is the pattern that most easily occurs. Unfortunately, others have equated default with passive or inactive and the term has become politicized and lost its original sense that masculine characteristics are imposed on an essentially female life-plan (Jost, 1970). This concept is valuable as it implies that the failure of a process necessary to produce a male trait leads to the creation of a female phenotypic trait instead. The converse is not true; that when a female process is blocked, a male characteristic arises. Thus, while there can be no doubt that female differentiation requires a suite of active morphogenic processes, it is also the case that male differentiation requires two specific processes that allow the male phenotype to alter what is essentially female-biased differentiation.

The nomenclature used to describe sexual differentiation has been historically quite confusing, using, often with little precision, terms such as feminization and demasculinization. It is now apparent that two processes are necessary to create a male; masculinization and defeminization. Masculinization imposes malelike

characters on the developing organism, whereas defeminization suppresses femalelike characteristics that would otherwise arise. These processes are involved whether the endpoints are anatomical or behavioral and can operate in concert or independently. The original notion for these processes came from anatomical investigations of sexual differentiation and is modeled after anatomical processes differentiating the primordial duct systems into male or female internal reproductive organs.

Prior to gonadal differentiation, males and females possess both Müllerian and Wolffian duct systems, which give rise, respectively, to the internal female and male nongonadal reproductive structures. These internal nongonadal reproductive structures arise from separate primordial structures, and thus errors in sexual differentiation allow either or both duct derivatives to exist concurrently, or derivatives from neither duct structure. This is in contrast to other reproductive structures, such as the gonad or the external genitalia, in which a single bipotential primordium differentiates into either a male or a female endpoint, and a failure of sexual differentiation results in an intersex form such as an ovotestis instead of either a testis or an ovary.

In normal female differentiation, the Müllerian ducts develop without any apparent hormonal input producing the uterus, fallopian tubes, and the distal portion of the vagina. The Wolffian ducts regress and disappear, again without any apparent morphogenic substance required for their demise, resulting in the presence of female internal reproductive organs. Male sexual differentiation requires both the suppression of the Müllerian ducts to prevent the development of female structures through anti-Müllerian hormone (AMH) produced by the testes and stimulation of the Wolffian ducts through testicular androgens. Thus male sexual differentiation of the internal nongonadal reproductive structures results from defeminization (suppression of Müllerian duct development) and masculinization (maintenance and differentiation of the Wolffian ducts). These two processes act throughout development to differentiate males from females.

A. Masculinization

This term refers to the production of male-typical characteristics. Anatomically, these would be the presence of penis, scrotum, testes, and internal Wolffian duct derivatives. Behaviorally, we limit our discussion to male-typical copulatory behavior and partner-preference, except in monkeys, where masculine patterns of juvenile behavior are described.

B. Defeminization

This term refers to the suppression of female-typical characteristics. Anatomically this results in the suppression of the development of uterus, fallopian tubes, portions of the vagina, and vaginal opening. Behaviorally, defeminization suppresses sexual receptivity, termed receptive defeminization. In monkeys, defeminization appears to suppress interest in sexual initiation, termed proceptive defeminization.

II. DEVELOPMENTAL STAGE AT BIRTH AND SEXUAL DIFFERENTIATION

Species vary widely in the extent of their development at birth. Following a distinction first developed in ornithology, species that are born with their eyes closed and lacking the capacity to function relatively independently at birth are termed altricial, and more completely developed offspring are termed precocial.

Many of the most often studied mammals are those where sexual differentiation is only partially completed at birth. In these species, a significant portion of the differentiating process occurs when the offspring is no longer attached to the maternal circulation. Typically, many neural systems in these species are not fully developed at birth and they are born with their eyes closed, poor motor coordination, and without the capacity for thermoregulation. Although the gonad and duct systems have differentiated *in utero*, the external genitalia have only begun to differentiate and distinguishing males from females typically requires measuring the distance from the penile/clitoral glans to the anus, ano-genital distance, which is longer in males than females.

Whether a species has altricial young follows no clear phylogeny. Species as diverse as rats and ferrets produce altricial young. While altricial offspring is a characteristic of many laboratory rodents, not all rodents are altricial, the guinea pig being the most widely studied

exception. It appears in some orders, such as the carnivores, that altricial offspring are the rule, with carnivores from ferrets to lions producing altricial young.

Altricial and precocial species probably reflect two different reproductive strategies that are expressed in different patterns of neural development (Gaillard *et al.,* 1997). In general, precocial species are characterized by a greater brain to body-weight ratio than are altricial species (Pagel and Harvey, 1989). In addition, a substantially greater portion of brain development occurs *in utero* in precocial than in altricial species. This difference in development may reflect a life-history difference in that altricial species produce young rapidly with short maturation times and rapid brain growth (Lewin, 1988). In contrast, precocial species develop more slowly, even though both species may reach maturity at comparable times (Tessitore and Brunjes, 1988).

In the sections that follow, we describe the characteristics of sexual differentiation in altricial and precocial species in order to investigate whether this developmental variable predicts the extent to which estrogens, or estrogenic metabolites affect sexual differentiation. Because sexual differentiation has been studied in many more altricial than precocial species, in the interests of space, we selected a limited number of atricial species.

III. ALTRICIAL SPECIES: RAT, MOUSE, AND FERRET

A. Rat

Gestation in the rat lasts approximately 22 days, with both males and females being exposed to surprisingly similar levels of circulating T beginning on embryonic day 16 (E16) (Weisz and Ward, 1980; Baum *et al.,* 1991). Only on E18 and E19 do male rat fetuses have significantly higher circulating levels of T than females. This difference presumably reflects the testicular secretion of T in the male. On E20, E21, and E22 T levels are again similar in the two sexes. Several lines of evidence (reviewed in Baum *et al.,* 1991) suggest that this T is of placental origin in both sexes. Testosterone levels in female fetuses were not influenced by the intrauterine proximity of male siblings (Baum *et al.,* 1991), regardless of whether data were computed over days E18–E19 (when males have significantly elevated T levels) or over days E17, E21, and E22 (when mean T levels were similar in male and female fetuses). Several studies (Corbier *et al.,* 1978; Slob *et al.,* 1980; Gogan *et al.,* 1981) showed that plasma T levels rise in male (but not in female) rats beginning 1–2 hr after birth, with levels declining again by 6 hr postpartum. This effect likely reflects increased testicular synthesis of T in response to a rise in luteinizing hormone (LH) combined with a postnatal delay in the expression of liver enzymes that metabolize circulating T (Baum *et al.,* 1988). Although there is much variability, average plasma levels of T remain significantly higher in male than in female rats over the first 10 postnatal days (Resko *et al.,* 1968; Pang *et al.,* 1979). Taken together, these endocrine data, along with numerous functional experiments (details later), suggest that brain and behavioral sexual differentiation in male rats extends over a 15-day period, beginning on E18 and ending by P10.

1. Defeminization of Receptive Responsiveness

a) Timing Some of the earliest studies of behavioral sexual differentiation (Barraclough and Gorski, 1962; Harris and Levine, 1965) showed that the later capacity of female rats to show lordosis in response to estradiol and progesterone treatment was greatly attenuated by administering testosterone proprionate (TP) between the day of birth and postnatal day 10 (P10). Conversely, castration anytime between P1 and P5 greatly enhanced the capacity of male rats to show lordosis behavior in adulthood after treatment with estradiol and progesterone (Grady *et al.,* 1965; Whalen and Edwards, 1967). Administration of TP to neonatally castrated male rats between P1 and P10 attenuated their later lordotic capacity whereas TP treatment on P13 and P14 had no such effect, suggesting that a critical period for defeminizing receptive sexual behavior ends around P10 (Beach *et al.,* 1969). The fetal age at which this process begins is less clearly apparent from the published literature. Several studies (Gerall and Ward, 1966; Ward, 1969; Ward and Renz, 1972) established that transplacental administration of TP to female rats between E16 and E21 reduced their later lordotic responsiveness to ovarian steroids whereas transplacental administration of the antiandrogen, cyproterone acetate, enhanced later lordotic responsiveness in male rats (Ward and Renz, 1972). As already explained, circulating T levels are significantly higher in male than in

female rat embryos only on E18–E19. It is noteworthy therefore that Hoepfner and Ward (1988) found that transplacental administration of TP reduced lordotic responsiveness in females when it was given on E17.5 and E18.5, but not on the two previous or two subsequent embryonic days. These workers also found that transplacental exposure to TP over any of these 2-day periods enhanced the later ability of a small (5 μg) dose of TP provided on P3 to defeminize later lordotic responsiveness (in the absence of prenatal TP treatments this low postnatal dose of TP failed to affect lordosis). Hoepfner and Ward (1988) suggested that in male rats receptive defeminization normally is initiated by the actions of T (or its neural metabolites) on E18–E19 and is then completed by subsequent actions of steroids secreted within hours after birth and then again over the first postnatal week of life. A similar case for a long-lasting period of steroid action has been made for the control of male-typical sexual behaviors (see later).

b) Role of Neural Aromatization Genes encoding estradiol receptors (ER-α and ER-β) are expressed in the hypothalamus and temporal lobes of both male and female rats beginning by E17 (Gerlach *et al.,* 1983). Likewise, expression of the *CYP19* gene encoding aromatase that coverts circulating T into estradiol is high in these same brain regions in both sexes beginning in fetal life. Indeed, the activity of aromatase is 5 to 10-fold higher during perinatal life than in adulthood in rats as in several other mammals (Naftolin *et al.,* 1975). These facts raised the possibility that the local conversion of T (of testicular origin) to estradiol in the developing brain is responsible for the defeminization of receptive capacity that normally occurs in male rats. The earliest experimental results pointing to such a conclusion showed that neonatal administration of estradiol benzoate (EB) reduced lordotic responsiveness in females (Levine and Mullins, 1964; Feder, 1967; Gerall, 1967) and in neonatally castrated male rats (Feder and Whalen, 1965). Subsequent studies have established beyond doubt that receptive defeminization depends on the perinatal aromatization of T in male rats. Thus neonatal administration of an aromatizing enzyme inhibitor, 1,4,5-androstatriene-3,17-dione (ATD), via subcutaneous (s.c.) silastic capsules to male rats significantly enhanced their adult capacity to show lordosis in response to ovarian hormones (McEwen *et al.,* 1977; Vreeburg *et al.,* 1977). These behavioral effects of neonatal ATD were correlated with the ability of the drug to inhibit the accumulation of ^{3}H-estradiol in 5-day-old female rat hypothalamic cell nuclei after an injection of ^{3}H-testosterone (Lieberburg *et al.,* 1977). More recently, Brand *et al.* (1991) compared the effects of prenatal, neonatal, and combined pre- and neonatal administration of ATD to male rats on their later display of lordosis behavior. When tested in adulthood while gonadally intact and treated with progesterone, all groups of ATD-treated males showed significantly higher lordosis quotients than perinatally untreated control males (Fig. 1A). Maximal lordotic responsiveness was seen in males that received neonatal ATD, suggesting that defeminization of receptive capacity occurs most strongly during the neonatal as opposed to prenatal period of brain sexual differentiation in males of this species. Perinatal treatment with ATD did not suppress serum T levels in males, suggesting that its behavioral effect was not the result of a functional castration (Vreeburg *et al.,* 1977; Brand *et al.,* 1991). Nor did ATD interfere with the binding of ^{3}H-DES (diethylstilbestrol) to hypothalamic cell nuclei, suggesting that the drug acts by blocking aromatization of T as opposed to acting as an ER antagonist. Indeed, neonatal administration to male rats of drugs that antagonize ER (e.g., MER-25; CI-628) duplicated the lordosis enhancing effects of neonatal ATD (Booth, 1977; McEwen *et al.,* 1977).

More recently, the conclusion that estradiol, formed in the male nervous system from circulating T, causes receptive defeminization has been further supported by the demonstration (McCarthy *et al.,* 1993) that intrahypothalamic infusion of oligodeoxynucleotide antisense to ER-α mRNA blocked the defeminizing action of TP given on P3 to female rats. By contrast, control females, which were TP treated on P3 after being infused with sense oligodeoxynucleotides, showed the expected reductions in later lordotic responsiveness to ovarian hormones. Further support for the view that ER activation is essential for receptive defeminization was provided by a study (Auger *et al.,* 2000) in which female rats were injected s.c. with TP and then given intrahypothalamic infusions of antisense oligodeoxynucleotides to the steroid receptor coactivator (SRC-1) on P1, P2, and P3. When tested for lordosis in adulthood, these females were significantly more responsive than

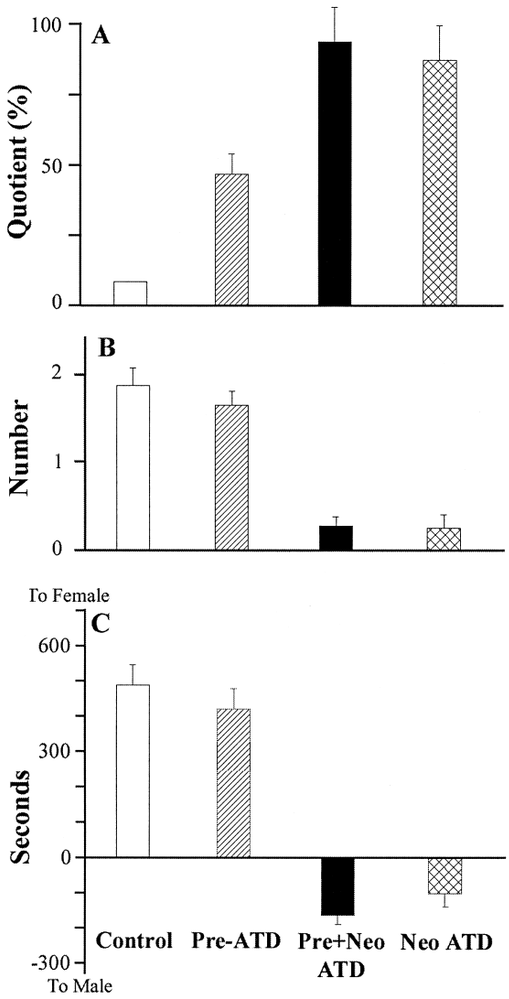

FIGURE 1 Effects of prenatal (pre-), neonatal (neo-), or combined prenatal and neonatal administration of the aromatase inhibitor, ATD, to male rats on their later ability to show lordosis behavior in response to mounts by a stimulus male (A), ejaculation in tests with an estrous female (B), and approach to either a stimulus female or male in a three-compartment test apparatus (C). All subjects were tested in adulthood while gonadally intact; plasma T levels did not differ among the four groups of males. All males received a s.c. injection of progesterone 4–7 hr prior to the test of lordosis behavior. Data are expressed as mean ± SEM and were adapted from Brand *et al.* (1991).

control females that received TP followed by scrambled oligodeoxynucleotides for SRC-1.

An obvious question raised by the observations showing that estrogenic metabolites of T are responsible for receptive defeminization in male rats is how females avoid being inadvertently defeminized by estrogens secreted neonatally from their own ovaries (Meijs-Roelofs *et al.,* 1973) or that leak across the placental barrier from the pregnant dam (Bridges, 1984). There are two possible answers to this question. First, any estradiol of maternal or neonatal ovarian origin is likely bound by high affinity plasma binding protein (α-fetoprotein of hepatic origin; Raynaud *et al.,* 1971) which sequesters estradiol in the circulation, thereby preventing it from entering neurons and being bound by ER. Males circumvent this protective barrier by secreting T from the testes, which enters the brain where it is converted locally to estradiol and is bound immediately by ER expressed in surrounding neurons. In rats of both sexes, the production of α-fetoprotein begins during fetal life and wanes by the second postnatal week. This protective mechanism against the potentially disruptive effects of estrogen on female brain development exists in rat and in other rodent species, but not in ferrets or other higher mammals (Baum *et al.,* 1982). A second explanation for the inability of any circulating estradiol of ovarian or maternal origin to defeminize the female rat brain is that the local, neural concentrations, and resultant ER occupation, needed to exert neural effects are never attained without local aromatization of circulating testosterone. This second mechanism probably applies to all species.

2. *Masculinization of Coital Behavior*

a) Timing After identifying and seeking out a sexually receptive female, male rats display a series of mounts, with or without penile intromission, which eventually lead to a prolonged intromission with ejaculation. Several studies (Beach, 1942; Emery and Sachs, 1975) have established that normal female rats, when ovariectomized in adulthood and given either EB or TP, will show high levels of mounting behavior toward stimulus females that are sexually receptive. Such female subjects occasionally display intromission-like behaviors (pelvic thrusts toward the receptive female followed by a rapid dismount) and even ejaculation-like responses (a malelike posturing over the female instead of a rapid dismount following a series of intromission-like behaviors). Beach *et al.* (1969) argued that the primary determinant of these sex differences in the expression of male-typical coital behaviors reflected the

presence of a penis in the male. Indeed, the capacity for intromission and ejaculation was reduced in males castrated immediately after birth as opposed to 7–15 days postnatally whereas mounting behavior was equivalent in these groups (Beach *et al.,* 1969). These group differences in intromissive and ejaculatory ability correlated perfectly with penile size. Several studies (Gerall and Ward, 1966; Sachs *et al.,* 1973) showed that extensive prenatal administration of TP, beginning as early as E14 followed by neonatal TP treatment, promoted phallic development in female rats so that after additional TP treatment in adulthood these females showed malelike patterns of coitus, including intromission and ejaculation. There is no consistent evidence that neonatal TP treatment of female rats, by itself, augments later mounting capacity (Whalen and Edwards, 1967; Pfaff and Zigmond, 1971). Instead, the available evidence suggests that T, which circulates prenatally in rats of both sexes, organizes the capacity to show this type of behavior in the presence of a sexually receptive female in males as well as in females. Several prenatal manipulations (administration of androgen receptor antagonists or of the aromatase blocker, ATD) caused significant reductions in the capacity of male as well as female rats to display mounting behavior (Stewart *et al.,* 1971; Booth, 1977). In the case of prenatal antiandrogen treatment, phallic development is greatly attenuated in males, with resulting deficits in intromission.

b) Role of Neural Aromatization There is consistent evidence that estradiol, formed neonatally in the male rat brain from circulating T, plays an essential role in establishing the capacity to ejaculate following a series of intromissions. Thus administration of the aromatase inhibitor, ATD, neonatally to male rats attenuated their later capacity to ejaculate in tests with receptive females (Stewart *et al.,* 1971; Booth, 1977; Bakker *et al.,* 1993). Combined pre- and neonatal exposure of male rats to ATD also attenuated males' later ejaculatory capacity whereas prenatal ATD alone had no such effect (Brand and Slob, 1991a; Houtsmuller *et al.,* 1994; Fig. 1B). Neonatal treatment with an antiestrogen had a similar disruptive effect on ejaculatory capacity in male rats (Booth, 1977). Whereas neonatal ATD treatments alone have never been reported to affect males' (or females') later mounting behavior, there are conflicting reports of deficient mounting capacity in male rats that received ATD prenatally. Thus in one study (Gladue and Clemens, 1980), prenatal ATD reduced males' mounting rates (mounts per min), though not the total number of mounts and intromissions displayed. In another study (Houtsmuller *et al.,* 1994), prenatal ATD failed to diminish males' mounting capacity. On balance, it appears that neural aromatization of T contributes little to the organization of mounting capacity, a finding that contrasts with the essential role of this process in the defeminization of receptive behavior.

Two studies (Stewart *et al.,* 1971; Clemens *et al.,* 1978) reported that prenatal administration of androgen receptor antagonists significantly reduced the capacity of both male and female rats to display mounting behavior in later life. These results imply that testosterone, either directly or after its 5α reduction to dihydrotestosterone (DHT), acts in the fetal nervous system to organize circuits controlling male-typical mounting behavior. However, in two more recent studies (Brand and Slob, 1991a; Dominguez *et al.,* 2001) prenatal treatment with the androgen receptor antagonist, flutamide, failed to reduce the capacity of either male or female rats to display mounting behavior in later life after gonadectomy and treatment with TP. In both instances the drug treatment had significantly reduced ano-genital distances in males (Dominguez *et al.,* 2001) and in females (Brand and Slob, 1991a), suggesting that the flutamide treatments used did, in fact, reach the developing fetuses. In the absence of consistent long-term behavioral effects of prenatal flutamide across studies, one is left to conclude that the capacity of both male and female rats to display male-typical mounting behavior is not organized by the fetal actions of testosterone (acting via androgen receptors) in this species.

3. Organization of Sexual Partner Preference

Identifying and seeking out an opposite-sex mate is as critical to reproduction in sexual species as is the capacity for displaying sex-specific patterns of copulation. It is surprising, therefore, that relatively little attention has been paid to the issue of how sex-specific neural mechanisms controlling mate selection are differentially organized in males and females (reviewed in Adkins-Regan, 1989). Some of the earliest studies in this field by Meyerson and co-workers (Meyerson

and Lindstrom, 1973; Hetta and Meyerson, 1978) showed that when given a choice to be in close proximity with sexually active male vs female stimulus animals (kept behind wire-mesh barriers at opposite ends of an open field), rats of both sexes preferred opposite-sex conspecifics. In addition, administering TP to female rats on P3 and again later in life caused them to approach an estrous female whereas neonatal castration of male rats led to a later preference for a sexually active male, provided they were treated with ovarian hormones at the time of testing. In a later experiment (Vega Matuszczyk *et al.,* 1988), neonatally castrated male rats, when treated in adulthood with ovarian steroids, preferred to approach a sexually active male whereas when they were given T neonatally after castration, they preferred to approach an estrous female in adult tests. Control males, which were castrated in adulthood, preferred the estrous female, regardless of the adult steroid treatment received. In a related study (Brand and Slob, 1991b), neonatally castrated male rats that received either oil vehicle or DHT immediately after castration and then tested in adulthood while receiving TP showed a lower preference to approach an estrous female (as opposed to an active male) than other neonatal castrates that received the aromatizable androgen, testosterone, immediately after surgery. These studies imply that T, perhaps acting via its estrogenic metabolite, acts neonatally in the male rat to defeminize responsiveness to male-derived cues that would otherwise attract males to other sexually active males. The hypothesis was confirmed in a series of studies by Slob and co-workers (Brand and Slob, 1991b; Bakker *et al.,* 1993, 1996) which showed that neonatal administration of the aromatase inhibitor, ATD, duplicated the long-lasting effects of neonatal castration on the partner preferences of male rats. When tested in adulthood while gonadally intact, males treated neonatally with ATD preferred to approach a tethered male stimulus (and showed lordosis in response to mounts received from that stimulus male; Fig. 1C). When castrated in adulthood and treated with EB, male rats that received ATD neonatally, like control females, preferred to approach a sexually active male whereas control males preferred an estrous female (Bakker *et al.,* 1996). In contrast, male rats that were castrated at birth (Brand and Slob, 1991b) and treated with T in adulthood or that received ATD neonatally and then castrated in adulthood (Bakker *et al.,* 1993) prior to receiving the combination of EB and dihydrotestosterone propionate (DHTP) in adulthood both preferred to approach an estrous female. The persistence of female-oriented approach behaviors in these groups of males suggests that the neural circuits controlling this motivational state begin to be organized in males during prenatal life. Unfortunately, data supporting this view have not been forthcoming. As far we are aware, nobody has assessed the ability of combined pre- and early postnatal testosterone treatment to create a completely male-typical profile of sex partner preference in female rats. Transplacental administration of antiandrogenic drugs including cyproterone acetate (Vega Matuszczyk and Larsson, 1995) and flutamide (Dominguez *et al.,* 2002) to male rats failed to disrupt their later preference to approach an estrous female as opposed to a sexually active male, even though the treatments caused significant reductions in ano-genital distances (an indication that the antiandrogens administered transplacentally actually reached the fetuses). There is conflicting evidence about the ability of prenatal inhibition of estrogen synthesis or action to affect males' later preference for an estrous female. Thus prenatal administration of the aromatase inhibitor, ATD, to male rats failed to disrupt their later preference for an estrous female as opposed to a sexually active male (Brand *et al.,* 1991; Fig. 1C; Dominguez *et al.,* 2002). By contrast, transplacental administration of the antiestrogen CI-628 to male rats caused a significant reduction in their female-oriented behaviors in adult tests (Vega Matuszczyk and Larsson, 1995).

To summarize, evidence in the rat suggests that neonatal actions of estradiol, formed in the male brain from circulating T, defeminize neural mechanisms that control sexual orientation toward other males. Further support for this view stems from a study (Bakker *et al.,* 1996) that compared the ability of odors from soiled bedding to augment Fos immunoreactivity (Fos-IR, a marker of neuronal activation) in the vomeronasal projection circuit of rats. Thus male odors stimulated neuronal Fos-IR at several levels of this circuit, including the bed nucleus of the stria terminalis (BNST) and medial preoptic area (mPOA) in gonadectomized female, but not in male subjects, that received EB at the time of testing. A female-typical profile of neuronal Fos-IR was seen in male rats that received ATD neonatally. Exposing adult male and female rats to estrous female odors

stimulated equivalent neuronal Fos-IR in both the BNST and mPOA, and a similar result was obtained in males treated neonatally with ATD. This implies, again, that prenatal T exposure may masculinize motivational systems in both sexes. More recently, Dominquez *et al.* (2002) found that prenatal exposure to the androgen receptor antagonist, flutamide (but not prenatal ATD), attenuated later neuronal Fos responses to estrous odors in both male and female subjects. However, this effect on odor responsiveness was not associated with significant reductions in the preference of these animals to approach an estrous female as opposed to a sexually active male, suggesting that nonolfactory cues whose processing is equivalent in the two sexes is responsible for the persistent female-oriented behavior that has been seen in so many different rat experiments.

B. Mouse

Gestation in mice lasts approximately 19 days. Although fewer systematic data are available and those that do exist may not apply to all strains of inbred mouse, it seems very likely that plasma T levels are significantly higher in male than in female mice beginning as early as E14 (Pointis *et al.,* 1980; vom Saal and Bronson, 1980). In male mice as in several other mammalian species there is a dramatic rise in circulating T within 2 hr after parturition; these levels then drop off again almost immediately, falling to the low levels which characterize females continuously after birth (Motelica-Heino *et al.,* 1988). Between P1 and P5 plasma T levels are significantly higher in male than in female mice (Pang and Tang, 1984). Plasma T levels were elevated on E17 in female fetuses located between two males (vom Saal and Bronson, 1980), and variations in plasma T depending on the presence of male siblings have been associated with variations in several phenotypic behavioral, neuroendocrine, and genital characteristics of female mice (reviewed in vom Saal *et al.,* 1990). Taken together, these data suggest that the critical period of brain and behavioral sexual differentiation in male mice extends over a 10-day period.

1. Defeminization of Receptive Responsiveness

a) Timing Female mice that were injected s.c. With TP within 24 hr after birth showed much lower levels of sexual receptivity after adult treatment with ovarian hormones than other groups of females that were either given oil vehicle at birth or were treated with TP on P10 (Edwards and Burge, 1971). Similar results were obtained in two additional studies by these investigators (Edwards and Thompson, 1970; Edwards, 1971). Nobody has assessed the ability of prenatal T administration to affect later receptivity in female mice. Thus although the critical period for receptive defeminization in males certainly includes the period between birth and P10, it may also extend to earlier fetal ages (as in the rat).

b) Role of Neural Aromatization The capacity for aromatization of androgen to estrogen has been demonstrated in the hypothalamus and amygdala of male mice, both fetally and shortly after birth (Compaan *et al.,* 1994). Furthermore, ER-α and androgen receptor (AR) are both expressed in the hypothalamus of fetal male and female mice (Wieland *et al.,* 1978). The earliest indication that neural aromatization of T contributes to receptive defeminization came from the observation (Edwards and Thompson, 1970) that a neonatal injection of EB was as effective as neonatal TP in reducing females' later lordotic responsiveness to ovarian hormones. To our knowledge, no studies have been carried out using male mice in which aromatase inhibitor drugs have been administered perinatally prior to assessing later receptive responsiveness to ovarian hormones. However, analysis of the behavioral potential of mice which bear either spontaneously mutations in the genome, or more recently in which the genome has been experimentally manipulated so as to "knock out" specific genes that encode the receptors for estradiol (ER-α and ER-β) has been illuminating. In the case of the spontaneously occurring *Tfm* mutation, the X chromosome gene encoding the AR undergoes a single base deletion in the N-terminal region. This results in androgen insensitivity so that the internal and external genital organs of Tfm males remain femalelike (reviewed in Olsen, 1992). Male mice carrying the *Tfm* mutation do not show lordosis in adulthood in response to ovarian steroid treatment (Olsen, 1992), presumably because defeminization of this capacity normally occurs during perinatal life in response to the action of estradiol, formed in the nervous system from circulating T and acting on estradiol receptors (which are normally expressed in *Tfm* animals). An alternative approach to

this issue would be to examine the ability of ovarian hormones to activate receptive behavior in male mice in which the gene encoding ER-α has been knocked out (ER-αKO). However, female ER-αKO mice fail to show lordosis behavior in response to ovarian steroids (Rissman *et al.,* 1997; Ogawa *et al.,* 1998b). This result simply reflects the essential role of ER-α in mediating the activational effect of estradiol on the neural circuits controlling lordosis. As a result, it is impossible to know whether the inability of male ER-αKO mice to show lordosis in response to adult treatment with ovarian steroids reflects their inability to respond to the activational effects of estradiol or the nonimportance of perinatal estradiol actions in the defeminization of receptive behavioral capacity. Recently, the *cyp*19 gene encoding aromatase P450 enzyme that converts androgen to estrogen has been experimentally knocked out in mice (Fisher *et al.,* 1998). These ARKO mice provide an ideal opportunity to analyze the role of perinatal aromatization in behavioral sexual differentiation, to the extent that these animals express ER-α. Thus by administering estradiol to adult ARKO males one can assess the consequences of the absence of estradiol production earlier in life. Such data are currently awaited. In summary, although the data establishing an obligatory role for estradiol in receptive defeminization of male mice are less complete than those for the rat, it seems likely that this process is also required in the mouse.

The importance of the evolution of *CYP*19/aromatase as a means of providing high concentrations of estradiol to specific targets in brain during embryonic development is revealed by a study (Mahendroo *et al.,* 1997) that analyzed the phenotype of transgenic mice in which the gene encoding 5α-reductase Type 1 was knocked out. Females that were homozygous for the null mutation of the 5α-reductase Type 1 gene became pregnant, but then experienced substantially more fetal resorption and loss than wild-type controls beginning on E11, which is the time when placental androgen production begins. These investigators found that administering either antiestrogenic or aromatase inhibitor drugs to 5α-reductase Type 1 knock-out females prevented this fetal death whereas administering estradiol to pregnant wild-type females promoted fetal wastage. They suggested that a primary role of 5α-reductase Type 1 is to convert T to its 5α-reduced metabolite, DHT, thereby limiting the amount of circulating T available as substrate to aromatase, and the resultant formation of estradiol. Estradiol plays an essential role in aspects of male-typical brain sexual differentiation, including the defeminization of circuits controlling receptive sexual behavior. It appears, however, that the developing embryo is especially sensitive to toxic effects of the levels of circulating estradiol which otherwise are needed in order to achieve the localized, neural actions required for male-typical brain development. The fetal expression of *CRP*19 in specific forebrain regions and the resultant provision of high levels of estradiol to particular sites in the male's brain coupled with increased expression of 5α-reductase Type 1 in the placenta and decidua of the pregnant mother act in consort to provide the necessary estradiol to target sites in brain while limiting the toxic side effects of widespread estradiol action in nonneural tissues.

2. *Masculinization of Coital Behavior*

a) Timing Several studies (Edwards, 1971; Edwards and Burge, 1971; Wersinger *et al.,* 1997) suggest that both male and female mice show high levels of male-typical mounting behavior in adult tests given after gonadectomy and T treatment. This result is comparable to that obtained in rats and raises the question of whether prenatal exposure to T, either of placental origin and/or from adjacent male siblings *in utero,* promotes the masculinization of neural systems that control male-typical sexual behavior. To date, nobody has systematically tested this hypothesis either by administering T to female mice transplacentally or (more importantly) by giving drug treatments that block the prenatal actions of T or estradiol. There are several reports (Vale *et al.,* 1973; Manning and McGill, 1974; Gandelman and Kozak, 1988) that neonatal administration of TP to female mice enhanced their later capacity to show male-typical sexual behaviors, including intromission- and ejaculation-like behaviors. These results parallel those obtained in the rat, and suggest that the process of masculinization may be completed in males by the immediate postnatal surge in T production and/or by the subsequent early postnatal action of T in males.

b) Role of Neural Aromatization In studies using gonadectomized, T-treated subjects, males with a homozygous null mutation of ER-α showed significant

reductions in the capacity to achieve ejaculation with an estrous female (Wersinger *et al.,* 1997; Ogawa *et al.,* 1998), although up to 50% of these males mounted (and occasionally intromitted) with a stimulus female. Ovariectomized, T-treated ER-αKO females also showed significantly less mounting and pelvic thrusting behavior than wild-type control females in tests with estrous females. An analysis of the behavioral effects of knocking out the ER-β gene (Ogawa *et al.,* 1999) revealed no disruptive effect on the capacity of male mice to display mating behavior. Combined knock outs of ER-β and ER-α in the same male mice led to even greater deficits in mounting and intromission behaviors than those seen in ER-αKO males (Ogawa *et al.,* 2000). These latter animals were tested while gonadally intact, and definitive characterization of their behavioral phenotype awaits assessment after gonadectomy and T replacement. It seems unlikely, however, that these deficiencies in the display of male-typical mating behavior by mice with null mutations of either one or both of the estradiol receptor genes reflects a disruption of perinatal organization of the neural circuits that control these behaviors. The administration of the dopamine receptor agonist, apomorphine, to ER-αKO mice that had been gonadectomized and treated with TP caused males to show all aspects of mating behavior, including ejaculation, while females showed mounts and pelvic thrusting in tests with stimulus females. An initial study of the phenotype of *CRP*19KO male mice (Honda *et al.,* 1998) showed that mounting behavior was significantly reduced, though not eliminated, in tests with estrous females. These results suggest that the perinatal actions of estradiol in the nervous system, acting via ER-α, may influence the circuits that control sexual arousal and/or partner identification, but not those circuits that control coitus per se.

There is some evidence suggesting that AR activation (by either T or DHT) contributes to the differentiation of neural circuits controlling male-typical sexual behavior in male mice (Olsen, 1992). *Tfm*/Y mutant male and *Tfm*/Ta carrier female mice, along with wild-type controls of both sexes, were gonadectomized in adulthood and then given a sequence of steroid treatments (TP, EB, EB + DHT, and oil vehicle) followed by tests of male-typical sexual behavior with estrous females. Only three of eight *Tfm* males, compared with eight of eight wild-type control males and seven of eight of each of the female groups ever displayed mounting behavior while receiving EB or EB + DHT. The high level of mounting performance shown by wild-type controls of each sex when treated with EB suggests that this steroid was adequate to activate the neural circuits controlling this behavior. Scordalakes and Rissman (2000) observed no significant deficits in the ability of *Tfm* males to display mounting or pelvic thrusting behaviors after castration and treatment with EB. Thus it is questionable whether AR activation by T or DHT is essential for the perinatal organization of the neural circuits controlling mounting behavior. More information is needed about sexual partner preference and olfactory responsiveness of *Tfm* males, as well as the ability of apomorphine to activate mounting and other male-typical sexual behaviors in these animals. In the mouse as in the rat, it remains to be determined whether perinatal activation of either neural AR or ER is required for the differentiation of circuits controlling mounting and intromissive behaviors, although neonatal activation of ER may be required for the development of ejaculatory capacity.

3. Organization of Sexual Partner Preference

Male and female mice identify and are motivated to approach opposite sex conspecifics in response to urinary odors (Vandenbergh, 1994). Very few studies have analyzed the contribution of perinatal steroid hormone actions to the differentiation of heterosexual preference shown in response to conspecific odors alone or toward opposite-sex animals. In one such study (Wersinger *et al.,* 1997), ER-αKO male mice, which were castrated and treated with T, showed no preference for an estrous female over a stud male while both stimulus animals were tethered in opposite ends of a test apparatus. Wild-type males strongly preferred to approach estrous females. In a subsequent study (Wersinger and Rissman, 2000) castrated, T-treated wild-type male mice preferred to approach and sniff an estrous female as opposed to either a stud male or an anestrous female. ER-αKO males showed no such preference. Interestingly, ER-αKO and wild-type males showed similar Fos responses to estrous odors in the accessory olfactory bulb and in the medial amygdala as well as hypothalamic regions that receive olfactory inputs. This suggested that the detection and initial neural processing of pheromones is not sexually differentiated whereas

the response to such odors is masculinized perinatally via the action of estradiol, formed from circulating T and acting on ER-α expressed perinatally in the nervous system. This motivational deficit in odor responsiveness may account for the deficits observed in coital behaviors displayed by ER-αKO male and female mice (Wersinger *et al.,* 1997; Ogawa *et al.,* 1998a,b) when they were tested in a small compartment with an estrous female. In the future it will be essential to conduct similar analyses of sex partner preference and motivation in *Tfm* as well as mice of other genotypes (*CRY*19 knock-outs) relevant to brain and behavioral sexual differentiation.

C. Ferret

The ferret is a carnivore in which gestation lasts approximately 41 days, with testicular steroidogenesis beginning in the male around day E26. Ferrets are born in an altricial state, with eye opening delayed until after P23. Circulating T levels are consistently higher in male than in female fetuses between E28 and E38 (Krohmer and Baum, 1989). Subsequently, a sharp rise in plasma T occurs in males, but not in females, within 2 hr after birth (Erskine *et al.,* 1988), and males again have significantly higher circulating T over the first 3 postnatal weeks with a peak occurring at P15 (Erskine and Baum, 1982). Plasma estradiol levels are elevated in both sexes over the last 10 days of gestation (Erskine and Baum, 1984), a finding that is surprising in so far as ferret blood lacks any of the estrogen binding capacity present perinatally in rat and mouse (Baum *et al.,* 1982). Taken together, these endocrine data suggest that T is elevated perinatally so that it is available for organizational brain actions in male ferrets over approximately 32 days.

1. Defeminization of Receptive Responsiveness

In contrast to all other nonprimate mammalian species for which data are available, there is no evidence of receptive defeminization in the ferret. When in estrous, female ferrets display a limp, acceptance posture with tail deviation in response to the neck grip of a male. This behavior is fully elicited in ovariectomized female ferrets by administering estradiol alone; progesterone is not required (Baum, 1979). Following gonadectomy and treatment with EB in adulthood, male and female ferrets showed equivalent levels of receptive behavior (both qualitatively and quantitatively) in response to a stud male's neck grip (Baum, 1976; see Fig. 2). This capacity to show full receptive responsiveness to estradiol after castration in adulthood implies that the neural mechanisms controlling this behavior are not susceptible to defeminizing actions of perinatal T exposure. Indeed, when groups of female ferrets were administered T prenatally, at birth, and again over P5–P20 at doses that mimicked endogenous levels of circulating T characteristic of males (Baum *et al.,* 1990b), there was no evidence of any reduction in acceptance behavior shown after adult treatment with EB and tests with a stud male. In this way the male ferret resembles the male rhesus monkey where castrated and estrogen treated males are as likely to accept a mount from a stud male as are similarly treated females (Pomerantz *et al.,* 1985).

2. Masculinization of Coital Behavior

a) Timing Several lines of evidence suggest that steroid-mediated masculinization of coital capacity in male ferrets begins shortly after the onset of testicular steroidogenesis (circa E25) and ends around P20. Evidence that the critical period for masculinization ends by P20 stems from experiments (Baum and Erskine, 1984) showing that castration of male ferrets on P5, but not on P20, reduced later capacity to display neck grip, mount, and pelvic thrusting behaviors in adulthood. Also, administering T to females between P5 and P20 masculinized aspects of their later coital capacity whereas no such long-term effects were seen in females given T between P20 and P35. Administering T transplacentally to female ferrets, by itself, failed to augment later mating capacity (Tobet and Baum, 1987). At first blush this implies that embryonic exposure of the developing male ferret brain to T contributes nothing to coital masculinization. Additional results (Baum *et al.,* 1990a) suggest, however, that prenatal T initiates a developmental process in the embryonic male's nervous system that is completed by the postnatal actions of T. Thus female ferrets that received T transplacentally (E27–E38) and then again immediately after birth and over P5–P20 showed much higher levels of masculine coital behavior in adult tests than another group of females that received no prenatal T together with the same postnatal T treatments. These results

FIGURE 2 A male ferret that was castrated in adulthood and treated daily with s.c. injections of estradiol benzoate in oil vehicle shows a femalelike receptive posture (including tail deviation) in response to a neck grip and mount by a stud male. Photo by Lex Doff.

point to an extended perinatal period during which T (or its metabolite, estradiol) acts to masculinize coital capacity.

The observation (Tobet and Baum, 1987) that females treated prenatally with T failed to show male-typical levels of masculine sexual behavior obscured the fact that this prenatal steroid treatment markedly enhanced the ability of T, when present over the first 3 weeks of life, to augment masculine mating potential. This "sensitization–completion" sequence of steroid actions on the process of behavioral masculinization is reminiscent of a similar sequential phenomenon that occurs when steroid hormones defeminize receptive behavior in the rat (Hoepfner and Ward, 1988). In both instances the fact that T must act over such an extended perinatal period in order to complete this aspect of brain sexual differentiation provides an opportunity for natural variations in the timing and intensity of T action to lead to variations in males' capacity to display male-typical behaviors (e.g., neck grip behavior; preference to approach a female vs male stimulus; see later).

b) Role of Neural Aromatization Results of a series of studies (Baum *et al.,* 1983; Tobet and Baum, 1987) suggest that estrogenic metabolites of circulating T initiate the process of behavioral masculinization during the last quarter of the 41-day gestation whereas T itself, acting via AR, completes this process on or shortly before P20. Thus transplacental administration of the aromatase-inhibiting drug, ATD, combined with maternal ovariectomy, caused significant deficits in the capacity of male ferrets to show masculine coital behaviors when tested later in life. No such effect was seen in other males that received the antiandrogen, flutamide, transplacentally (and in whom genital development was significantly disrupted). Attempts to reverse the behavioral deficits caused by ATD were not successful when a low replacement dose of EB was given to pregnant ferrets. Administration of a higher dose of EB to pregnant ferrets that had been ovariectomized and treated with ATD usually led to death or resorption of the fetuses (an effect that is reminiscent of the fate of embryos in pregnant mice bearing a null mutation of the 5α-reductase Type 1 gene and in which treatment

with an antiestrogen rescued the fetuses). One male fetus that survived after being delivered from a mother that received ATD plus a high dose of EB showed a very high level of masculine coital responsiveness that was equivalent to that of control males (Tobet and Baum, 1987). These results suggest that estradiol is required in relatively high concentrations in specific brain regions of fetal male ferrets in order to initiate the process of coital masculinization. The systemic concentrations of estradiol necessary to attain the required neural concentrations of this steroid turn out to be toxic to fetal development for a variety of reasons, already described. The presence of aromatase in the fetal brain ensures that local production of sufficient amounts of estradiol from circulating T will be produced to masculinize the brain safely.

Whereas fetal actions of estradiol are needed to initiate masculinization in male ferrets, the completion of this process after birth seems to depend on T itself, presumably acting via neural ARs. Supportive evidence includes the observation that administering ATD to male ferrets over P5–P20 failed to disrupt later coital performance (Baum *et al.,* 1983), and that neither estradiol nor DHT masculinized coital behavior of female ferrets as effectively as T itself given over P5–P20 (Baum *et al.,* 1982). It is also possible that T contributes to the masculinization of males' coital behavior via an indirect mechanism. Moore and Morelli (1979) showed that male rat pups receive significantly more anogenital licking from their mother than female littermates do. They suggested (Moore and Samonte, 1986) that this results from the actions of an androgen-dependent preputial gland pheromone that is excreted in the urine of male pups. Mothers are attracted to this odor and lick the anogenital region of males preferentially in order to gain access to it. These workers also found that the male offspring of anosmic mothers, which displayed significantly less anogenital licking toward males than control mothers, showed deficient mounting and intromissive behaviors when tested in adulthood. Ferret mothers, like rat dams, showed significantly more anogenital licking directed toward neonatal male as opposed to female ferret kits (Baum *et al.,* 1996). The peak in this sex difference in the amount of anogenital licking received occurred on P15, which is when plasma T levels were highest in neonatal male ferrets (Erskine and Baum, 1982). More research is needed to assess the relative contribution of direct neural actions of T as opposed to indirect, mother-mediated contributions of T in the male ferret and rat to the masculinization of coital potential.

3. Organization of Sexual Partner Preference

Stockman *et al.* (1985) found that ovariectomized female ferrets strongly preferred to approach and interact with a stud male as opposed to another estrous female in T-maze tests of sexual partner preference. This preference of females for an opposite-sex stimulus contrasted with that of castrated male ferrets that were tested while receiving TP. Such males showed an equal preference to approach the estrous female and the stud male, perhaps because both sexual and aggressive motivational states were expressed during these tests. Indeed, when castrated males were given EB instead of TP they preferred to approach the estrous female with whom they achieved neck grips and mounts. Other evidence (Carroll *et al.,* 1988) suggests that in adult male ferrets, as in numerous other nonprimate mammals, the central aromatization of T to estradiol contributes to the activation of masculine coital behavior. A more recent study (Chang *et al.,* 2000) showed that castrated male ferrets treated with TP strongly preferred to investigate wood blocks previously soiled by an estrous female as opposed to a stud male whereas ovariectomized female ferrets treated with EB expressed the opposite preference. In another study (Kelliher and Baum, 2002), gonadectomized male and female ferrets that received TP and EB, respectively, were tested in an airtight Y-maze for their preference to approach odors from a stud male versus an estrous female. Prior to receiving coital experience, males and females both preferred to approach odors from an opposite sex ferret. When subjects were allowed to see and hear (in addition to smell) the stimulus subjects, females continued to prefer the male stimuli. Male subjects, however, preferred to approach the goal boxes containing a stud male and an estrous female on an equal number of trials—a result resembling that of Stockman *et al.* (1985) in which castrated males treated with TP showed an ambivalent preference for these two biologically relevant stimuli. The male ferrets tested in both of these studies had not had any coital experience. After receiving coital experience, castrated, TP-treated males (like ovariectomized, EB-treated females) showed a strong

preference to approach the odors, sight, and sounds of opposite-as opposed to same-sex stimulus ferrets (Kelliher and Baum, 2001).

Heterosexual partner preference in ferrets depends critically on the sense of smell. When male and female ferrets were made anosmic by the infusion of dental impression material into the nasal sinuses, heterosexual partner preferences were eliminated in Y-maze tests (Kelliher and Baum, 2001). This was true even when subjects were able to see, hear, and interact physically with the stimulus ferrets in the goal boxes of the Y-maze. When placed in a small chamber with opposite-sex breeding ferrets, anosmic male and female subjects showed normal mating behaviors, suggesting that it is the appetitive as opposed to the consummatory aspects of sexual behavior that rely on the sense of smell.

The preference of male ferrets to seek out an estrous female is organized by the perinatal actions of T. Thus female ferrets that received transplacental TP over E27–E38, within minutes after birth, and again over postnatal days 5–20 showed a partner preference profile in later T-maze tests while receiving EB that was identical to that of control males that had been castrated on P20 (Baum *et al.,* 1990a; Fig. 3). Females that received TP only at birth, from P5 to P20, or only prenatally, showed adult profiles of sex partner preference intermediate to those of control males and females. In male ferrets, as in males of several other mammalian species (reviewed in Baum *et al.,* 1990b; Tobet and Fox, 1992), there is a sexually dimorphic cluster of neurons in the medial preoptic area/anterior hypothalamus (mPOA/AH) that is either absent (as in the ferret) or smaller (as in rat, gerbil, guinea pig) in females. The existence of this sexually dimorphic male nucleus (MN) of the POA/AH in ferrets depends on the organizing action of estradiol, formed from circulating T, during the last quarter of gestation (Tobet *et al.,* 1986). Placement of excitotoxic lesions in the MN-POA/AH caused adult male ferrets (that were castrated and treated with EB) to prefer to approach another male as opposed to an estrous female in T-maze tests (Paredes and Baum, 1995). Females that received such lesions continued to prefer a stud male. These results suggest that the male-typical profile of sex partner preference depends on the presence of the sexually dimorphic cluster of neurons in the MN-POA/AH. Females or males bearing destructive lesions of this part of the POA/AH show a female-typical profile of sex partner preference.

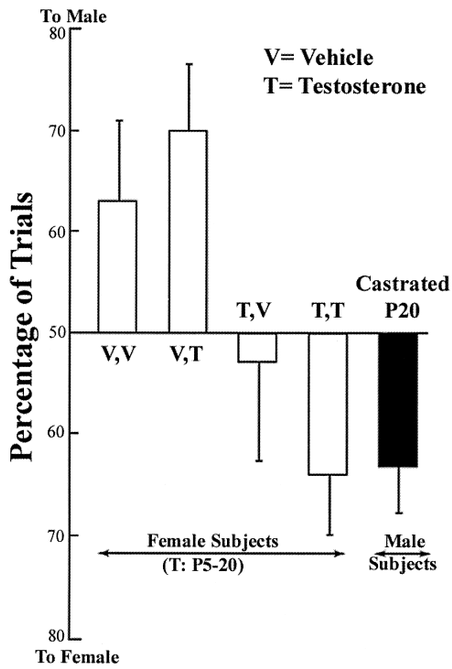

FIGURE 3 Prenatal, + immediate postpartum + neonatal treatment with testosterone masculinized sexual partner preference in genetic female ferrets. Groups of female ferrets were treated with vehicle prenatally and within minutes after birth (V,V), with vehicle prenatally followed by testosterone within minutes after birth (V,T), with testosterone prenatally followed by vehicle within minutes after birth (T,V), or with testosterone prenatally and within minutes after birth (T,T). The ovaries of all female subjects were removed on postnatal day (P)5, and they received testosterone between P5 and P20. Male subjects received vehicle prenatally and within minutes after birth whereupon they were castrated on P20. In adulthood all subjects were treated with estradiol benzoate and tested in a T-maze for their preference to approach and interact with an estrous female (to female) versus a stud male (to male). Data are expressed as mean ± SEM and are adapted from Baum *et al.* (1990a).

As already explained, olfactory cues are the critical determinants of ferret sex partner selection and preference. Therefore, it stands to reason that sex differences in the detection and processing of odors from conspecifics may underlie sex differences in partner preference. The immediate early gene, c-*fos,* is expressed

in neurons that are activated by sensory inputs associated with mating, including olfactory cues (Baum and Everitt, 1992). Using the presence in neuronal nuclei of immunoreactivity for Fos protein (Fos-IR) as a marker of activation, the responsiveness of the male and female ferret nervous system to odors from estrous females and from breeding males has been compared (Kelliher *et al.,* 1998). Gonadectomized ferrets of both sexes that either received TP injections or oil vehicle showed significant increments in the number of Fos-IR granule cells in the main olfactory bulb (MOB) but not in the accessory olfactory bulb (AOB), when they were killed 1.5 hr after being placed onto soiled bedding from an estrous female. In both sexes, TP-treated subjects showed significantly more odor-induced Fos-IR MOB granule cells than oil-treated animals, suggesting that T somehow sensitized the initial segments of the MOB detection system to activation by the social odors. Although exposure to peppermint odor also augmented Fos-IR in the MOB of both sexes, TP treatment failed to enhance this effect. This result shows that the steroidal modulation of processing by the MOB is limited to those odors that are biologically relevant to the organism.

Odors from estrous bedding caused significant increments in Fos-IR in the medial amygdaloid nucleus in both gonadectomized female and male ferrets, regardless of whether they received TP or oil vehicle (Kelliher *et al.,* 1998; Fig. 4). This odor stimulus significantly augmented neuronal Fos-IR in the mPOA/AH of ovariectomized females, with the effect being greatest in TP-treated animals (Fig. 4). No such stimulation in Fos-IR was seen in male subjects exposed to this same odor stimulus, regardless of whether they received TP or oil vehicle. A similar set of results was obtained in gonadectomized, TP-treated ferrets that were exposed to odors emitted from soiled bedding of breeding male ferrets (Kelliher *et al.,* 1998). Again, the Fos responses in the MOB and medial amygdala were equivalent in males and females; however, only females had significant increments in Fos-IR in the hypothalamus in response to male odors. These hypothalamic responses were localized in females to the mPOA/AH and the ventrolateral portion of the ventromedial nucleus (VLH). This latter region was not activated by estrous odors, raising the possibility that females' behavioral responses to male

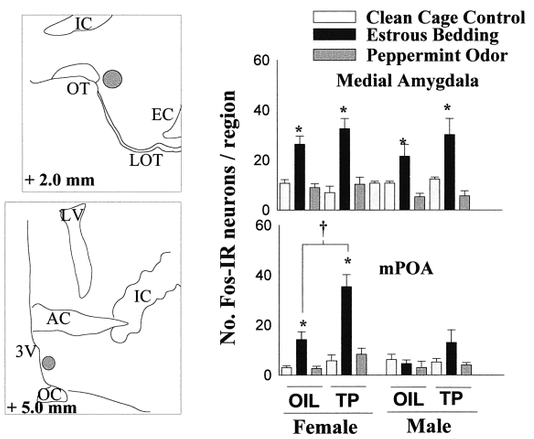

FIGURE 4 Effect of exposing groups of gonadectomized male and female ferrets (treated either with testosterone propionate, TP, or oil vehicle) to odors in soiled bedding from an estrous female ferret, peppermint odor, or the odors of a clean test cage on the induction of neuronal Fos-IR in the medial amygdala and medial preoptic area (mPOA). Data are expressed as mean ± SEM; $*p < .05$ comparisons with clean cage control values; $+p < .05$ comparison with oil-treated females exposed to estrous bedding. Data are adapted from Kelliher *et al.* (1998).

odors depend importantly on the activation of VLH neurons. Indeed, in the ferret as in numerous other mammalian species, VLH neurons have been shown to express ER-α (Tobet *et al.,* 1993). More research is needed to determine whether females' heterosexual partner preference, which is estrogen dependent, depends on the function of these neurons.

The absence of odor-induced Fos responses in the hypothalamus of male ferrets cannot be taken as evidence that these odors failed to activate neurons in this region. Indeed, an early study by Pfaff and Pfaffmann (1969) showed that odors from estrous female rats augmented the electrical activity of mitral cells in the MOB and of cells in the male's mPOA/AH. Instead, the different profiles of odor-induced Fos seen in the female versus male ferret hypothalamus in response to the same social odors suggests that their processing between the medial amygdala and mPOA/AH and/or VLH differs in some significant manner. In so far as opposite-sex odors play a central role in mate recognition and heterosexual partner preference in the ferret, these sex differences in hypothalamic processing of social odors may be critical

to the preference of males for female odors and vice versa.

IV. PRECOCIAL SPECIES: GUINEA PIG, PIG, MONKEY, AND HUMAN

A. Guinea Pig

The guinea pig was the first species where androgenic influences on behavioral sexual differentiation were unequivocally demonstrated (Phoenix *et al.,* 1959). This pioneering work built on the work of Vera Dantchakoff (1937), who argued that androgens injected directly into the developing fetus masculinized the behavior of female offspring. Dantchakoff was apparently unaware that adult female guinea pigs typically display mounting during estrus (Young *et al.,* 1939; Young and Rundlett, 1939) and thus it was unclear whether the mounting shown by prenatally androgenized females in adulthood resulted from their prenatal treatment or was female-typical mounting. Phoenix and colleagues resolved this issue, but the mounting behavior of female guinea pigs has made it difficult to assess the masculinizing and defeminizing effects of different prenatal treatments.

Female guinea pigs mount when they come into spontaneous estrus or after ovariectomy when treated with a sequential estradiol and progesterone treatment (Young *et al.,* 1939; Young and Rundlett, 1939). Female guinea pigs will also show increased mounting in response to exogenous TP treatment, but they require a longer period of androgenic stimulation to significantly increase mounting (Phoenix *et al.,* 1959; Goldfoot and van der Werff ten Bosch, 1975). In contrast, castrated adult male guinea pigs do not display mounting to a sequential estradiol and progesterone treatment (Phoenix *et al.,* 1959), but will show increased mounting to either TP or DHTP treatment (Alsum and Goy, 1974; Goldfoot and van der Werff ten Bosch, 1975). Similarly, castrated males display increased mounting to TP when aromatization is concurrently blocked with ATD (Roy and Goy, 1988). Female mounting is not activated by either DHTP (Goldfoot and van der Werff ten Bosch, 1975) or by TP when ATD blocks aromatization (Roy and Goy, 1988). Thus in guinea pigs the sex difference in mounting is in the hormonal regimen that induces mounting, and not the behavior itself. This sex difference in the hormones that activate mounting appears to be unique to guinea pigs and has led to research results that are difficult to compare to the more commonly studied altricial species. However, some similarities and some marked differences appear to exist between precocial guinea pigs and the altricial species previously discussed.

Guinea pig gestation lasts approximately 68 days (Goy *et al.,* 1957), with gonadal differentiation occurring between E22 and E26 (Price *et al.,* 1963; Black and Christensen, 1969). The fetal testes secrete elevated levels of T starting around E22 (Price *et al.,* 1963). Fetal males have higher circulating levels of T at E35 than do fetal females (Buhl *et al.,* 1979; Rigaudiere, 1979) with highest levels from E28 to E36 and decreasing until E52 when T levels increase in male, but not female fetuses, where androgen levels are low throughout fetal life (Rigaudiere, 1979). Fetal testes, *in vitro,* produce elevated androstenedione and testosterone from days E30 to E65 (Sholl and Goy, 1978). In contrast, fetal ovaries were not found to synthesize T or DHT and synthesized about 16% of the androstenedione produced by fetal testes (Sholl and Goy, 1978). Aromatization of androstenedione was significantly higher in fetal ovaries than testes and higher at days E30 and E50 than it was at days E60 through E65 (Sholl and Goy, 1978). Thus it appears likely that males experience higher prenatal levels of T than do females from E30 through the end of gestation and that little, if any of the aromatizable testicular androgen is aromatized in the testes to estrogenic metabolites. Thus it appears, in the guinea pig, that testicular hormones would be available to sexually differentiate males over the last two-thirds of pregnancy.

1. Defeminization of Receptive Responsiveness

a) Timing Phoenix *et al.* (1959) treated pregnant female guinea pigs with daily injections of 20 mg of TP starting on E25. Female offspring were born with masculinized external genitalia and with both Wolffian and Müllerian duct derivatives internally, as well as ovaries. The androgenized females, termed pseudohermaphrodites, when ovariectomized as adults and treated with a sequential estradiol and progesterone treatment, which reliably induced sexual receptivity in control females, were found to be receptively

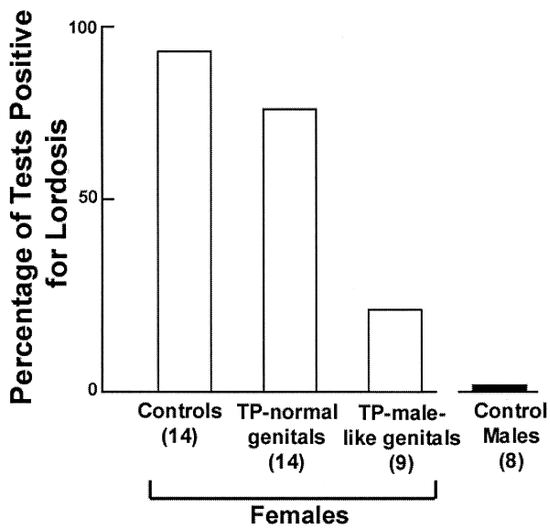

FIGURE 5 Prenatal exposure to testosterone propionate (TP) decreased sexually receptive lordosis behavior in female guinea pigs. Lordosis behavior, elicited by manual palpation of the flanks, was studied in female guinea pigs that received transplacental TP. Mothers of controls received no treatments. Subjects were gonadectomized in adulthood, later injected with estradiol benzoate followed 36 hr later with progesterone, and given tests for lordosis behavior over the next 12 hr. Data shown are means; *n* shown in parentheses. Data are adapted from Phoenix *et al.* (1959).

defeminized (Fig. 5). Subsequent studies demonstrated that the TP treatment needed to occur between E20 and E65, in order to produce receptive defeminization, with the most complete defeminization occurring with treatments between E30 and E55 (Goy *et al.,* 1964). Treatments of adults with TP had no effect on the induction of adult sexual receptivity with ovarian hormones (Phoenix *et al.,* 1959).

b) Role of Neural Aromatization Table 1 summarizes the various prenatal treatments given to genetic females and provides some support for the notion that estrogen or estrogenic metabolites of androgens are necessary to produce receptive defeminization. Prenatal treatment with DHTP (Goldfoot and van der Werff ten Bosch, 1975), or the androgen receptor blocker, flutamide (Thornton *et al.,* 1991) did not defeminize females to the activation of sexual receptivity by estradiol and progesterone, suggesting that aromatization of prenatal androgens is necessary for receptive defeminization. However, several pieces of evidence contradict this view (Table 1), making it impossible to firmly conclude what role aromatization plays in guinea pig receptive defeminization. First, females treated with estradiol prenatally not only display lordosis following adult sequential EB and P treatment, but actually show significantly longer heat duration, suggesting increased sensitivity to estradiol's activational effects (Hines and Goy, 1985). In contrast, prenatal treatment with the synthetic estrogen, diethylstilbestrol propionate (DESP) significantly defeminized female offspring (Hines and Goy, 1985; Hines *et al.,* 1987). Furthermore, prenatal DESP treatment increased the size of the sexually dimorphic area of the guinea pig hypothalamus (Hines *et al.,* 1987), which has previously been shown to be increased by prenatal androgen treatment (Byne and Bleier, 1987). Lesions of similar anatomical areas in the rat (Hennessey *et al.,* 1986) and ferret (Cherry and Baum, 1990) eliminate defeminization of behavior in normal males, suggesting that this area may be the anatomical site of behavioral defeminization. These results with DESP are consistent with the notion that estrogens produce receptive defeminization.

In males, prenatal treatment with ATD had contradictory effects on the receptive responding of treated males. One study reported no increase (Roy, 1992) while another reported increased receptive responding in prenatally ATD-treated males (Choate and Resko, 1994). Reconciling these two finding is difficult. Both studies used dosages of ATD that have been reported to block neural aromatization, and the Choate and Resko (1994) study demonstrated a 71% decrease in fetal brain aromatization when the dose they used was administered to the mother. Thus it seems likely that both studies produced an effective blockade of aromatization. Possibly the difference lies in the amounts of estradiol used to test for male receptive responding. Roy (1992) used 5 μg of estradiol, whereas Choate and Resko (1994) used 10 μg of estradiol. This latter dose is three times that typically used to activate females sexual receptivity, suggesting that prenatal ATD treatment may have blocked defeminization but that ATD-males are not as sensitive to estradiol as are normal females. In this view, the males capacity to display lordosis was only evident when tested with a substantially larger amount of estradiol than that used by

TABLE 1
Relationships between Prenatal Hormonal Conditions and Female- and Male-Typical Adult Sexual Behavior in Guinea Pigs[a]

	Female-typical behavior			*Male-typical behavior*		
	Lordosis		*Mounting*	*Mounting*		*Intromission*
Hormonal condition	*Display[b]*	*Heat duration*	*Sequential E2 & P[c]*	*Daily TP[c]*	*Daily DHTP[c]*	*Daily TP[c]*
Normal males[d,e]	No	—	No	<7 days	Yes	Yes
Normal females[d,e]	Yes	>6 hr	Yes	>21 days	No	Very rare
			Female experimental treatments			
Prenatal TP[d,e]	No	Shorter	Yes	7 days	No	Yes
Prenatal DHTP[f]	Yes	—	—	14 days	No	Yes
Prenatal estradiol[g]	Yes	>10 hr	Yes	—	—	—
Prenatal DESP[g,h]	Reduced	<6 hr	Yes/No[i]	Yes	—	—
Prenatal tamoxifen[h]	Yes	>6 hr	Yes	No	—	—
Prenatal ATD[j]	Yes	>6 hr	No	Reduced	No[k]	—
Prenatal flutamide[l]	Yes	>6 hr	—	—	—	—
Neonatal estradiol[m]	Yes	>6 hr	Reduced	Yes	—	—
			Male experimental treatments			
Prenatal flutamide[l]	No	—	—	7 days	—	Infrequent
Prenatal ATD[j,n]	No/Yes[o]	—	No	Yes[p]	Yes[k]	—
Prenatal CA[q]	No	—	No	21 days	—	No

[a] Abbreviations: E2, estradiol; P, progesterone; TP, testosterone propionate; DHTP, dihydrotestosterone propionate; DESP, diethylstilbestrol propionate; ATD, 1,4,5-androstatriene-3,17-dione; CA, cyproterone acetate,—, not tested or not applicable.

[b] Whether or not a sequential E2 and P treatment significantly increased the percentage of subjects displaying lordosis.

[c] Whether or not hormone administration significantly increased behavior over no hormone condition. The number of days to significantly increase behavior if reported.

[d] Phoenix *et al.* (1959).

[e] Goy *et al.* (1964).

[f] Goldfoot and van den Werff ten Bosch (1975).

[g] Hines and Goy (1985).

[h] Hines *et al.* (1987).

[i] Hines and Goy (1985) reported no significant increase in mounting following E2 and P treatment, while Hines *et al.* (1987) did. The difference may reflect that DESP-treated females display very high levels of mounting without any hormonal treatment.

[j] Roy (1992).

[k] Roy (1992) tested subjects with both TP and with concurrent TP and ATD in adulthood, blocking aromatization and presumably leaving only 5 α-reduced metabolites. Prenatally ATD treated males, but not ATD-treated females mounted to this treatment.

[l] Thornton *et al.* (1991).

[m] Feder and Goy (1983).

[n] Choate and Resko (1994).

[o] Roy (1992) found no evidence of lordosis in prenatally ATD treated males, wehreas Choate and Resko (1994) reported significantly more prenatally ATD treated than control males displayed lordosis.

[p] Roy (1992) does not provide weekly mounting data, but the prenatally ATD-treated males displayed the highest rate of mounting of all groups during TP treatment.

[q] Goldfoot *et al.* (1971).

Roy (1992). The resolution of this issue requires further investigation, but these studies provide some evidence that receptive defeminization in male guinea pigs results from the estrogenic metabolites of testicular androgens.

2. *Female-Typical Mounting*

Mounting in female guinea pigs is activated by the same sequential estradiol and progesterone treatments that also activate female sexual receptivity. In contrast, males do not show estrogen activation of mounting

(Goy *et al.,* 1964). Prenatal treatment with TP, which produces receptive defeminization, does not diminish estrogen-activated mounting (Goy *et al.,* 1964). In fact, some evidence suggests that estrogens or estrogenic metabolites of androgens may organize the potential to display estrogen-activated mounting. However, the evidence is not consistent, with studies suggesting that estrogens both increase and decrease the likelihood of displaying adult estrogen-activated mounting (Table 1). Inhibiting aromatization prenatally in either male or female fetuses eliminates estrogen-activated mounting in adulthood in both sexes (Roy, 1992), suggesting that estrogenic metabolites organize this adult activational sensitivity. However, DESP reduced estrogen-activated mounting in adulthood in one study (Hines and Goy, 1985), but had no effect on estrogen-activated mounting in another study (Hines *et al.,* 1987). Prenatal administration of estradiol had no detectable effect on estrogen-activated mounting. Interestingly, treatment of newborn females with 200 μg of estradiol for the first 15 days of life significantly reduced estrogen-activated mounting in adulthood (Feder and Goy, 1983), suggesting a partial defeminization by early postnatal estrogen. Unfortunately, the only study to create DHTP pseudohermaphrodites did not assess adult estrogen-activated mounting, thus the specific effect of estrogenic metabolites on this behavioral endpoint remains unclear (Goldfoot and van der Werff ten Bosch, 1975). The data in females, however, seem most consistent with the notion that estrogens organize an adult sensitivity to estrogen activation of mounting.

The situation in males remains completely unresolved, as no prenatal treatment has been found that augments adult male estrogen-activated mounting. Whether this reflects the paucity of studies varying prenatal hormones in males, or a nonsteroidally organized difference between males and females, remains to be resolved.

3. Masculinization of Coital Behavior

Mounting in adult male guinea pigs is activated in adulthood by either TP or DHTP (Alsum and Goy, 1974; Goldfoot and van der Werff ten Bosch, 1975). Female mounting can be activated by TP, but a longer period of testosterone stimulation is required before mounting increases significantly (Phoenix *et al.,* 1959; Goldfoot and van der Werff ten Bosch, 1975). In addition, normal females do not show any activation of adult mounting with DHTP (Goldfoot and van der Werff ten Bosch, 1975). Thus in guinea pigs, the sex difference in mounting is defined by the amount and type of hormonal stimulation that is required to activate adult mounting, and not in the form of the behavior itself.

a) Role of Aromatization in Male-Typical Mounting Prenatal treatment with TP (Phoenix *et al.,* 1959; Goldfoot and van der Werff ten Bosch, 1975), or DHTP (Goldfoot and van der Werff ten Bosch, 1975), significantly increased the sensitivity of androgenized females to TP in adulthood and increased the display of intromission patterns (Goldfoot and van der Werff ten Bosch, 1975). These prenatal treatments, however, had no effect on adult female mounting in response to DHTP, suggesting that this characteristic of guinea pig males does not stem from exposure to prenatal androgens.

There is some evidence that estrogens or estrogenic metabolites of androgens may influence TP-activated mounting in females. Prenatal treatment of females with ATD reduced adult TP-activated mounting below that of control females (Roy, 1992). Although Roy (1992) did not test ATD-treated females for DHTP-activated mounting, he did test them with concurrent TP and ATD treatment, which eliminates the aromatized metabolites of TP, making TP treatment more like DHTP. Male, but not female guinea pigs, mount when treated with this combined TP and ATD treatment (Roy and Goy, 1988). Prenatally ATD-treated females did not show increased adult mounting to a combined TP and ATD treatment (Roy and Goy, 1988). Interestingly, prenatally TP-treated females displayed increased mounting to the combined TP and ATD treatment, suggesting that estrogenic metabolites of T were not necessary to activate adult mounting. This stands in contrast with the two studies finding that DHTP could not activate adult female mounting (Goldfoot and van der Werff ten Bosch, 1975; Goldfoot, 1979).

Studies using estrogen receptor blockers provide additional support that TP-activated mounting in adult females may depend on the presence of estrogenic compounds prenatally. Treatment of fetal females with the estrogen receptor blocker, tamoxifen, eliminated TP-activated mounting in adulthood (Hines *et al.,* 1987). Together, the various studies of prenatal hormonal manipulations in females suggest that aromatization, or estrogenic compounds themselves, may be necessary

for female mounting, whether mounting is activated by sequential estradiol and progesterone or by TP.

The picture in males is quite different, with no evidence supporting the idea that aromatization is necessary for androgen-activated mounting in males. Prenatal ATD treatment had no effect on adult TP- or concurrent TP and ATD-activated mounting, supporting a lack of involvement of aromatization in the organization of male mounting. Unfortunately, the data do not provide conclusive evidence for the involvement of androgens in the organization of male coital behavior. Prenatal flutamide had no detectable effect on TP-activated mounting (Roy, 1992) and prenatal cyproterone acetate treatment only decreased the sensitivity to adult TP (Goldfoot *et al.,* 1971). Possibly, as was concluded for the rat, the capacity to activate mounting with androgen is not organized by prenatal steroids. This conclusion, however, awaits studies that suppress or eliminate endogenous prenatal androgen stimulation, which better test the hypothesis that endogenous androgens of testicular origin organize male copulatory potential.

B. Pig

The pig is an ungulate in which gestation lasts approximately 115 days, with offspring being born in a precocial state (Graves, 1984). Both plasma and amniotic fluid levels of T are significantly higher in males than in females beginning around E30, the time when the internal and external genitalia differentiate in the male (Ford *et al.,* 1980). Although this sex difference in circulating T persists throughout gestation, it is much smaller from E50 until parturition than between E30 and E50. Males and females have similar, increasing plasma levels of estrone across gestation (Ford, 1990). Immediately after birth there is a sharp elevation of plasma T levels only in males. This elevation lasts for approximately 2 weeks whereupon T levels drop again for approximately 3 months. There is a pubertal rise in plasma T in males beginning around postnatal month 4. Several lines of evidence (summarized later) suggest that brain and behavioral sexual differentiation in the male pig is completed by this pubertal rise in T. Plasma levels of estradiol also begin rising to adult levels in males beginning at 4 months of age (Ford, 1983a), whereas circulating estradiol remains low in prepubertal female pigs (Elsaesser *et al.,* 1982). As will be documented, both receptive defeminization and establishment of the male-typical profile of sexual partner preference appear to depend on the actions of estradiol that is derived from the plasma and/or is formed in the male's brain from circulating T over an extended 3–4 month peripubertal period.

1. Defeminization of Receptive Responsiveness

a) Timing Estrous female pigs show an immobile receptive posture in response to mounts by or even the mere presence of a male. Full receptive responsiveness can be induced in ovariectomized adult sows with estradiol alone (Signoret, 1970). As in numerous other infraprimate mammals, administration of estradiol to male pigs that were castrated postnatally failed to stimulate femalelike immobilization posturing in response to the presence of a boar. By contrast, males that were castrated within a few days after birth later showed high levels of receptive behavior when given estradiol and tested in adulthood (Diehl *et al.,* 1972; Ford and Schanbacher, 1977). Given the long (115 day) gestation period in this species, it is surprising that receptive defeminization does not occur in males in response to fetal actions of T. Indeed, two studies suggest that receptive defeminization is only completed in males 4–6 months after birth, at the time of puberty onset. In one study (Ford, 1982), male pigs that were castrated within days after birth, or at 2 or 4 months postnatally, showed femalelike levels of immobilization behavior when given estradiol and tested in adulthood. By contrast, males castrated 8 months postnatally showed very low levels of receptivity. Support for the view that receptive defeminization occurs in male pigs several months after birth is provided from a study (Ford and Christenson, 1987) in which TP was administered to groups of female pigs at different perinatal ages. Administration of TP between E29 and E35 or E39 and E45 in doses that caused genital virilization failed to cause later receptive defeminization, assessed after 10 months of age. Other groups of females that received one of these prenatal TP regimens plus TP over postnatal months 4–6 were also not defeminized. By contrast, males that were castrated at birth and given TP replacement over postnatal months 2–6 were as defeminized as other males that were castrated at 8 months. There are at least two possible explanations for this surprising failure of extended TP treatment to defeminize females (in contrast to its action in neonatally castrated males). First, in the

pig, as in the rat (Hoepfner and Ward, 1988) and ferret (Baum *et al.,* 1990a), elevated levels of T during gestation may sensitize the male to the defeminizing actions of T at the age of puberty. Second, to the extent that the defeminizing actions of T in the male pig depend on the aromatization of T to estradiol (details later), there may be deficits in some aspect of estradiol availability or action during the first several postnatal months.

b) Role of Neural Aromatization Administration of EB over postnatal months 3–5.5 to male pigs that had been castrated at 19–22 days of age and to females that had been ovariectomized at 3 months strongly defeminized receptive responsiveness to a boar, as assessed after a single injection of EB at 6.5 months of age (Adkins-Regan *et al.,* 1989). This defeminizing effect of EB treatment in females contrasts with the absence of such an effect in females treated with TP over a similar postnatal period (Ford and Christenson, 1987). To our knowledge, nobody has assessed the ability of postnatal aromatase inhibitors to duplicate the effects of castration of males over postnatal months 0–4. However, the ability of prepubertal EB to defeminize receptivity in both sexes raises the possibility that estradiol is required in male pigs for receptive defeminization just as it is in rats and other rodents. Thus even though the gestational life span and length of the prepubertal period of these species differs considerably, the requirement for postnatal estradiol (derived either from neural aromatization of T or possibly in pigs from the circulation) for defeminizing receptive capacity is similar.

2. Masculinization of Coital Behavior

a) Timing Beginning around 1 month postnatally, mounting of pen mates has been observed more frequently in male than in female pigs. This increased mounting behavior in males wanes around 2–3 months of age and then reemerges at the age of puberty. Despite this suggestion of a sex dimorphism in mounting capacity, there are no convincing data suggesting that these male-typical coital behaviors are permanently organized by the perinatal actions of T in the male. Thus both male and female postpubertally gonadectomized pigs given TP for an extended period showed equivalent levels of mounting behavior in tests with estrous females (Ford, 1990). This outcome is like that reported in the guinea pig and is consistent with results obtained in rat and mouse for which questions remain about the role of perinatal steroid action in the organization of male-typical mounting behavior. In the case of the guinea pig, activation of mounting by the nonaromatizable androgen, DHT, distinguished males from females. Additional studies are needed in the pig that assess the relative ability of different types and amounts of androgens to augment mounting, intromission, and ejaculation in the two sexes following castration. Likewise, data are needed to determine whether perinatal aromatization of T plays any role in the process of coital masculinization in this species.

3. Organization of Sexual Partner Preference

Signoret (1967) first used a T-maze and operant procedures to compare the motivation of sows to approach a boar versus an ovariectomized female. After estrogen priming, ovariectomized sows strongly preferred to spend time in the vicinity of the male. More recently, Ford (1983b) used the same method to compare the motivation of groups of male pigs that had been castrated within a few days after birth as opposed to 4 or 8 months postnatally. In response to adult treatment with EB, males castrated at birth, like females that had been ovariectomized at 8 months of age, strongly preferred to spend time in the vicinity of the stimulus boar whereas no such preference was seen in males that had been castrated at 4 or 8 months of age. This suggests that the defeminizing actions of postnatal steroid exposure on males' sex partner preferences occur somewhat earlier than the effects of these steroids on receptive responsiveness (see earlier for a discussion of immobilization responses in male pigs castrated at different postnatal ages). Administration of EB over postnatal months 3–5.5 to male pigs that had been castrated at 19–22 days of age and to females that had been ovariectomized at 3 months strongly defeminized the motivation of both sexes to spend time in the vicinity of a stimulus boar, as assessed after a single injection of EB at 6.5 months of age (Adkins-Regan *et al.,* 1989). Males that were castrated 4 months postnatally and then tested 9 days or 2.5 months later spent time near the stimulus male that was intermediate to values shown by control males and females that had been gonadectomized at 3 or 5.5 months, respectively, and did not receive EB immediately after gonadectomy. These results

suggest that estradiol promotes the male-typical organization of brain mechanisms controlling sex partner preference by acting as late as 3 months postnatally. As Adkins-Regan *et al.* (1989) pointed out, this prolonged postnatal sensitivity to organizing effects of estradiol is very surprising in that pigs, like other ungulates, are born in a precocial state compared to many other altricial species (e.g., rat, ferret) in which behavioral sexual differentiation is completed 10–20 days postnatally as well as another precocial species (i.e., the guinea pig), in which behavioral sexual differentiation is completed prior to birth (68-day gestation). Adkins-Regan *et al.* also note that the sexual differentiation of male-typical preference profiles in male pigs is more clear-cut than the defeminization of the capacity to display receptive sexual behavior in response to adult estrogen. A similar conclusion can be drawn from the results of studies using ferrets and rhesus monkeys (see other sections), although in pig studies (Ford, 1983b; Signoret, 1970) this conclusion is tempered somewhat by the use of stimulus sows that were ovariectomized and given no estrogen replacement.

Signoret (1970) showed that the odor of the androgenic steroid, androstenone, which is secreted by the testes, taken up by the submaxillary salivary glands and excreted in the boar's saliva, attracts the estrous sow and even promotes sexual receptivity. Spraying androstenone onto a female sow is routinely used to identify estrous animals for the purpose of artificial insemination (Reed *et al.,* 1974). Dorries and co-workers reported that the ability to detect androstenone is sexually differentiated in pigs, raising the possibility that sex differences in sex partner preferences may actually reflect a sex difference in the ability to detect and/or process this odor stimulus. In an initial study (Dorries, 1991), pregnant sows were able to use androstenone to identify a food reward whereas gonadally intact, adult males were unable to use this odor at the concentration presented, which was somewhat above the concentration normally present in boars' saliva. A subsequent study (Dorries *et al.,* 1995) compared the ability of gonadally intact, nonestrous sows and adult males to use decreasing concentrations of androstenone as a discriminative stimulus in an operant task for sucrose water reward. Females reliably detected significantly lower concentrations of androstenone than did intact males (Fig. 6A) whereas no such sex difference were found in pigs' ability to use geraniol (a floral odor) as a discriminative stimulus (Fig. 6B). A group of neonatally castrated male pigs showed a discrimination threshold for androstenone which fell in between the two extremes for gonadally intact males and females (data not shown in Fig. 6). The sex difference in pigs' ability to detect androstenone persisted in animals whose vomeronasal organ was occluded with surgical cement (Dorries *et al.,* 1997), suggesting that it is the main olfactory epithelium, as opposed to the VNO neuroepithelium, that detects androstenone. In the pig, as in

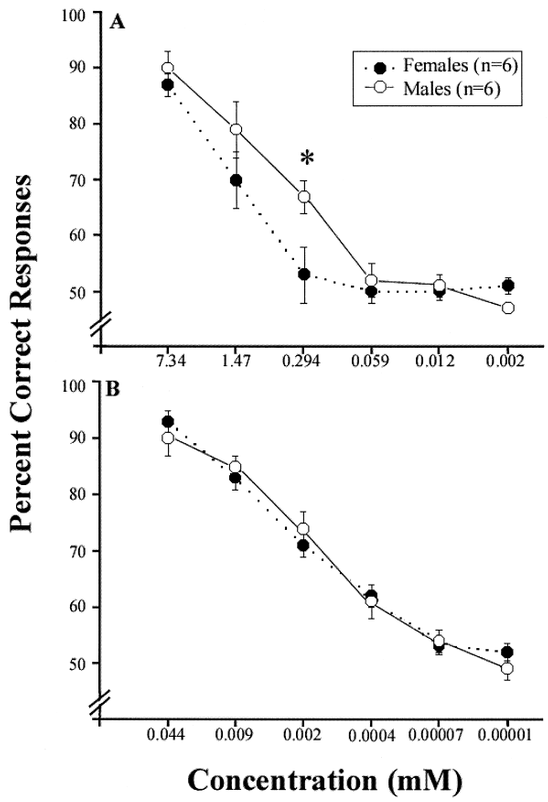

FIGURE 6 Ability of gonadally intact, adult male and female pigs to use increasing dilutions of either the male pheromone, androstenone (A), or the nonbiological odor, geraniol (B), as a discriminative stimulus in an operant task to obtain a sweet sucrose water reward. The performance of males fell to chance (50% correct responses) when they were required to use a 0.294 m*M* solution of androstenone whereas females were able to use this dilution to perform the task successfully ($*p < .05$). Data are expressed as mean ± SEM and were adapted from Dorries *et al.* (1995).

the ferret, understanding how males and females use odors differently to determine the sex of conspecifics may hold the key to the neural basis of mate recognition and heterosexual partner preference.

C. Monkey

In the many different species of monkey there are only a few where anything is known about their sexual differentiation processes. Thus this review concentrates on macaques, primarily the rhesus monkey (referred to as "monkey" hereafter), since this is the species for which the most is known. Monkeys have an approximately 168 day gestation with the testes differentiating between E38 and E40 (Resko, 1985). Fetal testes become steroidogenically active around day E40 and secrete androgens throughout gestation with peak levels at days E40–E75 then declining for the rest of gestation with another apparent increase around day E140 (Resko, 1985). Throughout the prenatal period males experience significantly higher levels of T, though there are not apparent differences between the sexes in either DHT or androstendione (Resko and Ellinwood, 1981; Resko, 1985). The fetal ovaries are apparently quiescent at this time since females show significantly elevated LH levels in comparison to males and LH levels are suppressed by exogenous T in fetally ovariectomized females (Ellinwood *et al.,* 1982). Thus fetal males are exposed to elevated levels of T from their own testes and females are exposed to lower, but quantifiable T levels, presumably of maternal origin, since the fetal ovary is inactive during this time.

Testicular activity falls on the day of birth and then increases, remaining at postpubertal levels for the first 2–3 months of life (Mann *et al.,* 1984). Suppression of this neonatal T secretion appears to influence the timing of puberty (Mann *et al.,* 1993, 1998), but has no striking effects on either juvenile (Wallen *et al.,* 1995) or adult male behavior (Eisler *et al.,* 1993). Thus it appears that the principle hormonal influences on monkey sexual differentiation occur during the prenatal period.

The monkey has been of particular interest in investigations of behavioral sexual differentiation because is shows both sexually differentiated patterns of hormonally activated adult behavior, and in sexually differentiated patterns of juvenile behavior that require no hormonal activation (Young *et al.,* 1964; Lovejoy and Wallen, 1988; reviewed in Wallen, 1996). These sex differences in juvenile behavior are not limited to rhesus macaques as similar behavioral differences have been reported for the closely related Japanese macaque (Eaton *et al.,* 1985, 1986, 1990). Thus in the monkey it is possible to identify patterns of behavior that are modified by prenatal hormonal conditions without the confound of concurrent activational effects of hormones. Two juvenile behaviors, mounting and rough and tumble play (rough play) have been consistently found to differ between males and females, with males displaying greater frequencies of both throughout the juvenile period (Wallen, 1996). The capacity to mount, both during the juvenile period and in adulthood, does not depend on concurrent hormonal stimulation. However, high frequencies of adult mounting in a sexual context require activation like the male guinea pig, with either TP or DHTP (Phoenix *et al.,* 1973; Phoenix, 1974). Females do not display increased mounting to TP as adults (Pomerantz *et al.,* 1986). Thus in monkeys, masculinization of behavior is measured both by increased juvenile patterns of mounting and rough play and by increased adult androgen activated mounting by either TP or DHTP.

Assessing defeminization in monkeys is more problematic in that female monkeys do not display any behavior analogous to lordosis in rodents, or the immobility posture in pigs (Wallen, 1990). In fact, it appears that receptivity, in the sense used in studies of rodents, is not under hormonal regulation in monkeys (Johnson and Phoenix, 1976; Wallen and Goy, 1977). Thus in the monkey, a better measure of female sexual behavior is her propensity to initiate sexual activity activated by ovarian hormones (Wallen *et al.,* 1984; Wallen, 1990). Sexual initiation by females is overwhelmingly directed toward males and reflects a sexual preference, similar to those described earlier in this chapter for rats, ferrets, and pigs. Thus in the monkey, this attraction to males and initiation of sexual behavior (proceptivity, Beach, 1976) is used to assess defeminization in males and not the willingness of a male or female to accept a male mount.

1. Defeminization of Female Sexual Initiation: Lack of Involvement of Aromatization

Male monkeys either castrated neonatally or as adults do not attempt to initiate sexual activity with stud males

when treated with estradiol as adults, unlike ovariectomized, estrogen-treated females (Thornton and Goy, 1986). Prenatal treatment with either TP or DHTP defeminizes this behavior as both TP and DHTP pseudohermaphrodites displayed decreased sexual initiation in comparison to control females (Pomerantz *et al.,* 1985; Thornton and Goy, 1986). Thus, unlike all of the species previously discussed, there is no evidence in the monkey that androgen is not responsible for proceptive defeminization. This may reflect a difference in behavioral systems, receptivity may have different differentiating influences than does proceptivity. In light of the pronounced effects that estrogens have on partner preference in the rat and ferret, it seems unlikely that this finding reflects a difference in the hormones differentiating these two behavioral systems. It is more likely that this reflects a characteristic of monkeys, and possibly other primates, where defeminization is androgen regulated and does not require neural aromatization to be effective. The definitive resolution to this issue will require studies that directly manipulate prenatal androgens as well as prenatal aromatization.

We have started such studies by exposing fetal males to flutamide (Herman *et al.,* 2000), from either E35, or E40 through E75 (early gestation treatment), or from E110 or E115 through E150 (late gestation treatment). The early gestation treatment significantly altered the genitalia of five of the seven flutamide treated males. Figure 7 illustrates one of the most extensively modified males (males whose mothers received similar amounts of flutamide, but later in gestation had completely normal male genitalia). The determination of whether this treatment blocked defeminization in any of these males awaits studies with exogenous hormone treatments in adulthood to determine whether estrogens will activate sexual initiation with adult males as they do in adult females.

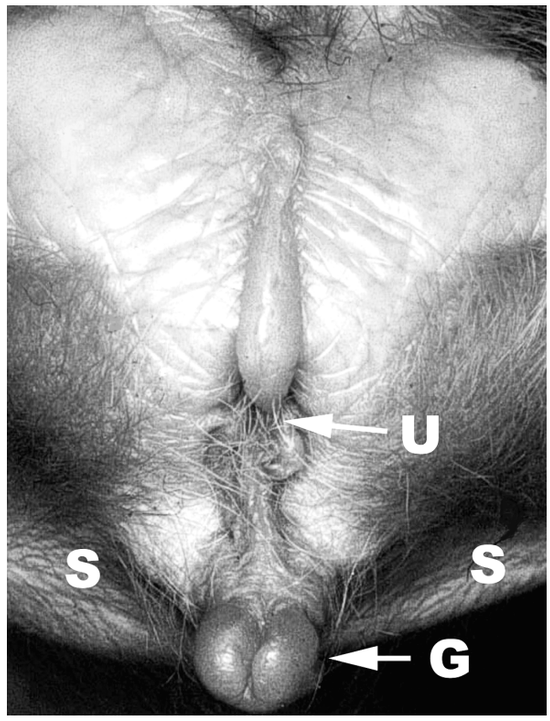

FIGURE 7 Posterior view of a genetic male whose mother was treated with 30 mg/kg of flutamide from E40–E75 of a 170 day gestation. The anus is at the top of the figure and the scrotum is at the bottom. Unlike an unexposed male, the scrotum (S) is rostral, instead of caudal, to the penile shaft. The prominent glans penis (G) is separate from the urethral opening (U), which is a female-typical configuration. (Photo by K. Wallen.)

2. *Masculinization of Juvenile Behavior*

Prenatal treatment with either TP or DHTP masculinized the juvenile behavior of genetic females (Goy, 1978, 1981). Androgenized females displayed elevated levels of rough play and mounting throughout their first 3 years of life. Japanese macaques treated with TP from approximately E40–E100 of gestation showed masculinization of mounting, but not play behavior during the first 2 years of life (Eaton *et al.,* 1990). The smaller effect seen in Japanese macaques may reflect the lower doses of TP used in this study, or the shorter period of TP treatment than in the rhesus studies.

It has been found that when a fetal female is exposed to androgen during gestation it influences the extent to which juvenile behavior is modified (Goy, 1981; Goy *et al.,* 1988). Twenty-five daily treatments from E40 to E65 extensively masculinized the genitalia of female offspring, but only masculinized their mounting behavior. Treatments of the same length starting at E115 produced no genital masculinization, but masculinized both mounting and rough play (Goy *et al.,* 1988). This study demonstrated that the masculinizing effects of androgen on genital differentiation could be separated from the masculinizing effects on juvenile behavior. Unfortunately, these late-treated

androgenized females were never studied as adults so we do not know whether this treatment which masculinized the female's juvenile behavior would have also defeminized their adult behavior toward males.

While both aromatizable and nonaromatizable androgens masculinize juvenile behavior, there is some evidence suggesting that estrogens can also masculinize juvenile behavior. Goy and Deputte (1996) treated pregnant females with 100 μg per day of DESP, a dose 33 times that shown to masculinize and defeminize aspects of the behavior of genetic female guinea pigs, for more than 100 days of gestation. These DESP females had apparently normal genitalia, but displayed increased levels of juvenile mounting and rough play. Another group of females received DESP treatments timed similarly to the late gestation androgen treatments described earlier. These short DESP females showed no evidence of behavioral masculinization, in contrast to the effects of short TP treatments. These DESP females were only studied for their first year of life, when they were still in the presence of their mothers and when the full expression of juvenile sex differences had yet to be realized (Goy and Wallen, 1979). Thus, it is hard from these data to determine whether the masculinization produced by long treatments with large amounts of DESP reflect an involvement of estrogens in masculinization, or a pharmacological effect. Our own studies of prenatal flutamide treatment have not yet found evidence that the masculinization of juvenile behavior was prevented by either early or late flutamide treatment. In fact, we have preliminary evidence that late flutamide treated males display significantly higher levels of juvenile play and mounting than do control males. We think it unlikely that these data are truly evidence that androgens late in gestation suppress the masculinization of male juvenile behavior. Instead we think it more likely that these results reflect an increased testicular output during the flutamide block due to the suppression of negative feedback, as had previously been demonstrated in rats and humans (Sodersten *et al.,* 1975; Viguier-Martinez *et al.,* 1983; Giusti *et al.,* 1995). If this is the case, these preliminary findings might provide support for an effect of estrogen on masculinization of juvenile behavior, since AR would be blocked by the flutamide, but ER would not, and the increased testicular activity might elevate estrogen levels enough to produce effects similar to those reported for DESP. Whether or not estrogens or estrogenic metabolites play any role in the normal course of behavioral differentiation remains an open question.

3. Masculinization of Adult Coital Behavior

Copulatory behavior in castrated male rhesus monkeys, like castrated male guinea pigs, is reinstated by either TP or DHTP treatment (Phoenix, 1974), but not by estradiol treatment (Phoenix and Chambers, 1982; Michael *et al.,* 1990). Similarly, castrated male cynomolgus monkeys respond to DHTP replacement therapy with increased ejaculation (Michael *et al.,* 1986, 1987). However, it has been suggested that DHTP, in cynomolgus monkeys, is less effective than TP in reinstating behavior since given the same amounts of TP or DHTP males displayed higher copulatory behavior on TP than DHTP treatment (Michael *et al.,* 1986, 1987). In addition, there is contradictory evidence in cynomolgus monkeys that blocking aromatization decreases testosterone activation of copulatory behavior. Castrated male cynomolgus monkeys were treated with TP and either concurrent fadrozole, an aromatase inhibitor that eliminated more than 98% of the neural aromatization of T, or a control treatment. Fadrozole treatment reduced, but did not eliminate, male copulatory behavior (Zumpe *et al.,* 1993). Concurrent estradiol with the fadrozole had mixed effects, reversing the copulatory decline in three males, but decreasing it even further in three others (Zumpe *et al.,* 1993). Later studies produced similarly contradictory findings in that the small reduction in male copulatory behavior seen during fadrozole treatment was further reduced by the addition of exogenous estradiol (Zumpe *et al.,* 1996). Thus as in the rhesus, aromatization does not appear to be obligatory for the activation of male copulatory behavior. However, it is still possible that aromatization plays some facilitating role in male copulatory behavior.

Prenatal treatments with TP and DHTP have had mixed effects on adult copulatory behavior. However, the interpretation of these results is complicated by the different rearing conditions employed in the studies. The earliest androgenized female rhesus monkeys were reared under restricted social conditions that produce very poor copulatory behavior in the control males (Goy and Wallen, 1979). When androgenized females reared under these socially restrictive conditions were tested for male copulatory behavior as adults,

exogenous TP treatment did not increase copulation with sexually receptive females (Eaton and Goy, 1973; Phoenix and Chambers, 1982). Inexplicably, the most extensive test of these androgenized females reared under socially restricted conditions (Phoenix and Chambers, 1982) used wild-caught adult males as the comparison group instead of socially restricted reared males who would have shown similarly poor adult copulatory behavior to that reported for the androgenized females (Goy and Wallen, 1979). Although these findings have been interpreted as showing that male monkey copulatory behavior is not organized by prenatal androgens (Phoenix and Chambers, 1982), a more conservative interpretation would be that the rearing history of these subjects had a greater influence on their adult sexual behavior than did their prenatal hormonal environment (Wallen, 1996).

More recent studies of prenatally androgenized females used a mother–peer rearing system that produces robust sex differences in juvenile behavior and sexually competent adult males and females (Goy *et al.,* 1974; Wallen *et al.,* 1977). When tested at 8 years of age with sexually receptive females, both TP and DHTP prenatally androgenized females displayed increased mounting when treated with exogenous TP (Pomerantz *et al.,* 1986). In contrast, ovariectomized females showed no increases in mounting during adult TP treatment, in fact they were never observed to mount on any test. Although mounting was increased, it was not at the level typically seen under these testing conditions in intact adult males, nor did the androgenized females show intromission or ejaculatory patterns, even after 12 weeks of TP stimulation (Pomerantz *et al.,* 1986). Thus the copulatory behavior of these pseudohermaphrodites is more masculinized than is that of control females, but is less masculinized than that seen in control males. This moderate level of masculinization of copulatory behavior may reflect the timing and duration of the prenatal treatments, but it may also reflect the fact that these androgenized females had a markedly different life-history exposure to androgen than do normal males. These androgenized females did not experience the neonatal elevated T typical of normal males (Mann *et al.,* 1984). Whereas this neonatal T was not found to affect sexually dimorphic patterns of juvenile behavior, its elimination did result in a later puberty and a lower sex drive in these males as adults (Eisler *et al.,* 1993; Mann *et al.,* 1993, 1998). Thus the lowered responsiveness of androgenized females to the activating effects of adult TP treatment could reflect a developmental effect of neonatal T. Similarly, these androgenized females were not exposed to any TP until 8 years of age, almost 5 years later than a male would typically be exposed to pubertal elevations of androgen. Whether there is pubertal period of sensitivity to androgens that affects postpubertal sensitivity to T is not known, but the possibility remains that that full activation of adult male copulatory behavior by T requires not only prenatal organization of neural systems, but also additional neonatal and pubertal T exposure to produce maximal sensitivity. A similar possibility has been reported in human hypogonadal males who were exposed to T for the first time as older adults. Although there were increases in erections and sexual functioning, these changes required months of T therapy, even though T levels increased to within the normal range within 48 hr after the start of T therapy (Burris *et al.,* 1992). In contrast, normal men reported decreased sexual interest after 2 weeks of testicular suppression with a GnRH antagonist (Bagatell *et al.,* 1994). While the role that androgen, during the life span, plays in determining sensitivity and responsiveness for androgen-activation of male copulatory behavior cannot be currently resolved, it is interesting that it did not matter whether the prenatal androgen given to females was aromatizable or not for it to masculinize adult copulatory potential.

Very little is known about the effects of removing endogenous prenatal androgens on the adult copulatory behavior. Our current studies of the effects of prenatal flutamide either early or late in gestation are not yet at the stage to provide a definitive answer. We do know, however, that our prenatal flutamide male with the least masculinized genitalia (Fig. 7), has gone through puberty and has been observed to ejaculate with a female, even though he cannot achieve vaginal penetration. Thus his motivational systems appear to be completely masculinized, even though his genitalia are not.

D. Human

Humans, like other primates, are precocial mammals with the majority of sexual differentiation occurring prenatally, with an extensive postnatal period in which social and environmental influences affect the degree of

behavioral differentiation between males and females. What we know about the hormonal influences on sexual differentiation comes from "accidents of nature," in which specific genetic anomalies alter normal patterns of hormone secretion, as in congenital adrenal hyperplasia (CAH) where an enzymatic defect cause the fetal adrenal to secrete elevated levels of androgen, or change the capacity to respond to endogenous hormones as in androgen insensitivity, where individuals lack the androgen receptor. Thus, unlike the species described earlier in this chapter, little can be said about the specific timing of events crucial to masculinization and defeminization, except that, as in the monkey, the critical events are prenatal. Unlike other species, whether one is considered to be a male or a female has a tremendous impact on development. Thus the perception by others of one's sex becomes as important as is the complement of sex chromosomes one carries. For humans we will focus more on the contribution of prenatal hormones to gender development rather than on masculinization and defeminization per se. There is little agreement among researchers about which patterns of behavior would reflect either. There are no human analogues to lordosis, and comparing male and female tendencies to initiate sexual interactions does not provide a clear-cut sex difference. Although one could argue that the male behavioral role in heterosexual intercourse is analogous to male mounting in other species, no study has actually empirically characterized this seeming sex difference. Thus in humans we are left with a different set of measures than we have encountered in other animals; measures that have more to do with gender perception and sexual orientation than with specific behavioral patterns. Here we will focus on gender differentiation and the role that steroid hormones play in this process.

Gender differentiation in humans, as in animals, is initiated by the sex chromosomes, which determine gonadal sex. If an individual develops fully functional testes and is capable of responding to testicular secretions, they develop male genitalia and are sex assigned as males at birth. If an individual develops ovaries, nonfunctional testes, does not develop a gonad at all (pure gonadal dysgenesis (PGD); Raboch *et al.,* 1987), is incapable of responding to testicular secretions (androgen insensitivity; Money *et al.,* 1984), or does not produce the necessary type of androgen (5α-reductase deficiency; Imperato-McGinley *et al.,* 1974; Peterson *et al.,* 1977), femalelike or ambiguous genitalia develop and the individual is assigned as a female. If a genetic female is exposed to androgens during sexual differentiation, she develops virilized genitalia and is assigned as a male at birth if virilization is extensive (Money and Norman, 1987) or as a female if she is less virilized (Money *et al.,* 1984). Thus in humans, genital differentiation determines sex assignment with important consequences for theories of gender development.

1. Differentiation of Gender Identity

Two psychological characteristics of gender development have been described. One is an internal conviction about one's gender that has been termed the *core gender identity* (gender identity, Stoller, 1968). The second is based on the constellation of sex-typed behaviors individuals exhibit in accordance with their gender and which differ between males and females. Taken together these behaviors have been termed *gender role* (Stoller, 1968). Both of these psychological traits are thought to develop during the first 2–4 years of life. Thus human gender is initially based on three phenotypic traits: the externally observable genital sex, the externally observable gender role, and an internal perception, gender identity.

While the degree of prenatal exposure to androgens produces genital sex, the factors influencing gender role and gender identity are poorly understood. This is partly because many behaviors constitute gender role and they differ in the degree to which they are modifiable by experience. For example, clothing styles, adornment, and hairstyles are all used to distinguish males from females but are also all easily changeable and vary across cultures and between generations. In contrast, other patterns of gender-role behavior such as activity level (Maccoby and Jacklin, 1974; O'Brien and Huston, 1985), spatial ability (Newcombe *et al.,* 1983), and toy choice (O'Brien and Huston, 1985; Caldera *et al.,* 1989; Berenbaum and Hines, 1992) are less variable and may constitute a core of gender role behavior that is less socially labile. However, there is no agreement on which behaviors are the best measure of gender role. Gender identity, it has been argued, develops during the first several years of life through socialization processes and then becomes fixed (Hampson, 1965). Genetic sex and genital sex are seen as irrelevant as only unambiguous

TABLE 2
Relationship between Genital Sex, Sex of Rearing, and Gender Identity in Humans

Genetic sex	*Genital sex*	*Sex of rearing*	*Gender identity*	*Clinical description*	*Reference*
XY	Male	Male	Male	Male	Common definition
XY	Male	Male	Female	Transsexual male	Stoller (1968)
XY	Female[a]	Female	Male	Androgen insensitivity (partial)	Gooren and Cohen-Kettenis (1991)
XY	Female	Female	Female	Androgen insensitivity or PGD	Money *et al.,* 1984
XX	Female	Female	Female	Female	Common definition
XX	Female	Female	Male	Transsexual female	DSM-IIIR
XX	Male[b]	Female	Female	Congenital–adrenal hyperplasia	Money *et al.* (1984)
XX	Male	Male	Male	Congenital–adrenal hyperplasia	Money and Norman (1987)

[a] Genitalia were actually ambiguous, which was not discovered more than 20 years after birth.
[b] Genitalia are masculinized, but not to the extent that sex was not assigned as female at birth.

sex of rearing determines gender identity. In this view, gender role is the behavioral manifestation of one's internal perception of their gender.

This view has dominated thinking about gender development to such a degree that the interactionist alternative, that male gender identity is strongly influenced by prenatal androgens that organize behavioral predispositions that are realized through the interactions with the social environment (Diamond, 1965, 1976), is only recently becoming more widely considered (Zucker and Green, 1991; Zucker, 1999). Because we cannot ethically experiment on humans, we have to rely on informative experiments in nature to provide insight into the process of humans' sexual differentiation. There is now a reasonable body of evidence to argue against socialization as the only or primary influence on gender identity. Table 2 illustrates eight gender phenotypes generated from all possible combinations of genetic sex, genital sex, and gender identity. Two are "normal" males and females, and the other six reflect some discordance between two of the phenotypic factors, all of which occur in humans. Sex of rearing has been added to allow comparison between this variable and adult gender identity.

In seven of eight cases, sex of rearing is concordant with genital sex, as would be expected. If sex of rearing determines gender identity, then genital sex should also be concordant. This is true for four of the seven cases, but not true for the other three. These cases contradict a socialization explanation for the development of gender identity. They would be consistent with an organizational explanation if androgens independently affect genital and neural differentiation, as suggested in monkey studies, and there are neural mechanisms underlying gender identity.

In addition, there are two cases in Table 2 in which the genitals are femalelike, but actually ambiguous (partial androgen insensitivity and CAH girls raised as females). These cases, like the early cases of human 5α-reductase deficient males (Imperato-McGinley *et al.,* 1974), are both sex assigned as females, yet these genetic females develop female gender identities, whereas genetic males develop male gender identities. While the argument has been made that these cases are actually not reared as either males or females, but ambiguously (Money, 1976; Herdt and Davidson, 1988), this clearly cannot account for two different gender identity outcomes. An explanation consistent with all eight cases is that male gender identity, like male genital sex, results from the actions of testicular steroids. In the absence of androgenic influence, a female gender identity develops independent of sex of rearing, while a male gender identity develops in the presence of adequate levels of androgens. Crucial to this model is the notion that genital differentiation and gender identity development occur at different times and possibly have different sensitivities. Although this argument is consistent with all of the cases, it is somewhat circular, because unlike genital sex, which is open for inspection at birth, we do not know what gender identity a newborn is fated to develop. There is, however, significant evidence that prenatal androgens affect the predisposition

to develop a male gender identity, even when reared as a female. The signature case of a normally differentiated boy who was reared as a girl when his penis was accidentally damaged beyond repair and was described as developing well as a girl (Money and Ehrhardt, 1972), has, as an adult, expressed a fully masculine gender identity and lives as a heterosexual male (Diamond and Sigmundson, 1997).

2. *Role of Aromatization in Masculinization and Gender Identity*

There are now several reports of human males who either have a point mutation in the estrogen-receptor gene (Smith *et al.,* 1994) or lack the aromatase enzyme (Morishima *et al.,* 1995). While the psychological and behavioral descriptions of these males is minimal in all reported cases the males are described as having a male identity and heterosexual orientation. In contrast to the lack of effect of a null estrogen-receptor gene on male gender development, missing or nonfunctional androgen receptors [androgen insensitivity syndrome (AIS); De Bellis *et al.,* 1994] profoundly affect male gender development resulting in phenotypic females, even though the individual has a Y chromosome and testes. These individuals are reared as females, have a female gender identity, and the few reports of their adult behavior describe them as heterosexual women (Money *et al.,* 1984; Wisniewski *et al.,* 2000). Thus there is currently no evidence that suggest that the development of a male gender identity, or a heterosexual orientation requires estrogens or estrogenic metabolites of androgens.

In females, the most extensive data on possible estrogenic influences on sexual differentiation comes from studies of females exposed to DES during fetal development. It has been suggested that DES exposed females have a higher incidence of bisexuality and homosexuality than would be expected by chance (Ehrhardt *et al.,* 1985; Meyer-Bahlburg and Ehrhardt, 1986). Whether this is replicated in other studies and whether this is evidence of defeminization or masculinization of behavior remains to be determined.

V. SUMMARY AND CONCLUSIONS

We have attempted in this chapter to describe evidence from a representative sample of altricial and precocial species of the role that androgens and estrogens play in masculinization and defeminization. For altricial species, the evidence of estrogen or estrogenic compound involvement in defeminization is very convincing and consistent. We suspect that the same could be said of other altricial species we did not include. For precocial species, the data are mixed, with the guinea pig showing evidence that DES, but not estradiol could defeminize females and mixed evidence that ATD could block defeminization in males. The pig also provided some evidence that estrogen, in this case prepubertally, defeminized females, but the aromatizable androgen, TP, could not duplicate this effect. Thus it is not possible to determine whether the effects of estrogen reflect a potential that is not actually involved in the natural process of defeminizing boars.

In contrast to these species, the monkey provided clear evidence that proceptive defeminization did not require aromatization as prenatal DHTP treatment was as effective as TP in defeminizing the behavior of females. In humans, there were no behavioral studies comparable to those in other mammalian species and the psychological endpoints were quite different. However, with these limitations, there was no evidence in males that estrogens or estrogenic metabolites were required for normal masculine behavior. Prenatal DES exposure to females had some effect on sexual orientation, but it is unclear whether this reflects masculinization, defeminization, both, or neither.

It is apparent from these data that it appears that in altricial species aromatization of androgens is obligatory to produce defeminization, but the case for a role of aromatization in precocial species is decidedly mixed. Thus it seems unlikely that a role for aromatization in defeminization is a mammalian characteristic, but may reflect one of two hormone action phenotypes that can achieve the same end. Whether the weaker evidence for an effect of aromatization on defeminization in precocial species reflects a characteristic of these species, or is a chance finding reflecting the accidental establishment of dominant laboratory species awaits a more systematic investigation of this issue.

The case for masculinization of coital behavior seems somewhat clearer. There appears to be no evidence in any species that the capacity to mount requires estrogenic input for its differentiation. In fact there is only

weak support for the idea that steroids organize mounting potential during development. The principle problem is that studies that have blocked either endogenous androgen or aromatization in males have not eliminated mounting, but only reduced it. This seems to be the case whether or not the male is from an altricial or a precocial species. These findings, however, may not reflect a lack of involvement of endogenous androgens or their estrogenic metabolites in the differentiation of mounting, but reflect instead the difficulty of blocking one aspect of endogenous hormone action without producing a compensatory change in another aspect. Thus prenatal or neonatal treatment of males with flutamide when their testes are active, may block their androgen receptors, which could prevent masculinization, but at the same time this treatment blocks negative feedback increasing testicular activity and producing a suite of elevated testicular products that could affect sexual differentiation. This same treatment to females may be quite effective since the ovary may be inactive and the hormones to be blocked are of placental origin or from neighboring siblings.

While the altricial/precocial distinction did not bring as much clarity to this literature as we had hoped, one aspect stands out. In general, altricial species appear to rely on aromatization more extensively for male sexual differentiation than do precocial species. Clearly the capacity to aromatize androgens is necessary for female gonadal function in all mammalian species, but it appears that in those species born only partially differentiated that estrogenic metabolites of androgens have assumed a more prominent role in male sexual differentiation than they have in precocial species. Both estrogens and androgens affect neural development, but they appear to have qualitatively different effects (Lustig, 1994). Possibly, the dependence on either androgenic or estrogenic compounds for sexual differentiation reflects the different developmental life histories of altricial and precocial species. Resolution of this question awaits detailed studies of sexual differentiation in a much wider range of species than are currently available.

Acknowledgments

We thank Molly Embree, Franklynn Graves, and Rebecca Herman for assistance with this review. Preparation was supported in part by NIH Grants K02-MH01062 (K.W.) and HD R01-HD 2121094 (M.J.B). The research on the developmental effects of prenatal flutamide in rhesus monkeys was supported by NIH Grant R01-MH50268 (K.W.) and NCRR Grant RR-00165 to the Yerkes Regional Primate Research Center, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

References

Adkins-Regan, E. (1989). Sex hormones and sexual orientation in animals. *Psychobiol.,* **16**, 335–347.

Adkins-Regan, E., Orgeur, P., and Signoret, J. P. (1989). Sexual differentiation of reproductive behavior in pigs: Defeminizing effects of prepubertal estradiol. *Horm. Behav.* **23**, 290–303.

Alsum, P., and Goy, R. W. (1974). Actions of esters of testosterone, dihydrotestosterone, or estradiol on sexual behavior in castrated male guinea pigs. *Horm. Behav.* **5**, 207–217.

Arnold, A. P. (1996). Genetically triggered sexual differentiation of brain and behavior. *Horm. Behav.* **30**, 495–505.

Auger, A. P., Tetel, M. J., and McCarthy, M. M. (2000). Steroid receptor coactivator-1 (src-1) mediates the development of sex-specific brain morphology and behavior. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7551–7555.

Bagatell, C. J., Heiman, J. R., Rivier, J. E., and Bremner, W. J. (1994). Effects of endogenous testosterone and estradiol on sexual behavior in normal young men. *J. Clin. Endocrinol. Metab.* **78**, 711–716.

Bakker, J., van Ophemert, J., and Slob, A. K. (1993). Organization of partner preference and sexual behavior and its nocturnal rhythmicity in male rats. *Behav. Neurosci.* **107**, 1049–1058.

Bakker, J., van Ophemert, J., and Slob, A. K. (1996). Sexual differentiation of odor and partner preference in the rat. *Physiol. Behav.* **60**, 489–494.

Barraclough, C. A., and Gorski, R. A. (1962). Studies on mating behavior in the androgen-sterilized female rat in relation to the hpothalamic regulation of sexual behavior. *Endocrinology (Baltimore)* **25**, 175–182.

Baum, M. J. (1976). Effects of testosterone propionate administered perinatally on sexual behavior of female ferrets. *J. Comp. Physiol. Psychol.* **90**, 399–410.

Baum, M. J. (1979). Differentiation of coital behavior in mammals: A comparative analysis. *Neurosci. Biobehav. Rev.* **3**, 265–284.

Baum, M. J., and Erskine, M. S. (1984). Effect of neonatal gonadectomy and administration of testosterone on coital masculinization in the ferret. *Endocrinology (Baltimore)* **115**, 2440–2444.

Baum, M. J., and Everitt, B. J. (1992). Increased expression of c-fos in the medial preoptic area after mating in male rats: Role

of afferent inputs from the medial amygdala and midbrain central tegmental field. *Neuroscience* **50**, 627–646.

Baum, M. J., Gallagher, C. A., Martin, J. T., and Damassa, D. A. (1982). Effects of testosterone, dihydrotestosterone, or estradiol administered neonatally on sexual behavior of female ferrets. *Endocrinology (Baltimore)* **111**, 773–780.

Baum, M. J., Canick, J. A., Erskine, M. S., Gallagher, C. A., and Shim, J. H. (1983). Normal differentiation of masculine sexual behavior in male ferrets despite neonatal inhibition of brain aromatase or 5-alpha-reductase activity. *Neuroendocrinology* **36**, 277–284.

Baum, M. J., Brand, T., Ooms, M., Vreeburg, J. T., and Slob, A. K. (1988). Immediate postnatal rise in whole body androgen content in male rats: Correlation with increased testicular content and reduced body clearance of testosterone. *Biol. Reprod.* **38**, 980–986.

Baum, M. J., Erskine, M. S., Kornberg, E., and Weaver, C. E. (1990a). Prenatal and neonatal testosterone exposure interact to affect differentiation of sexual behavior and partner preference in female ferrets. *Behav. Neurosci.* **104**, 183–198.

Baum, M. J., Carroll, R. S., Cherry, J. A., and Tobet, S. A. (1990b). Steroidal control of behavioural, neuroendocrine and brain sexual differentiation: Studies in a carnivore, the ferret. *J. Neuroendocrinol.* **2**, 401–418.

Baum, M. J., Woutersen, P. J., and Slob, A. K. (1991). Sex difference in whole-body androgen content in rats on fetal days 18 and 19 without evidence that androgen passes from males to females. *Biol. Reprod.* **44**, 747–751.

Baum, M. J., Bressler, S. C., Daum, M. C., Veiga, C. A., and McNamee, C. S. (1996). Ferret mothers provide more anogenital licking to male offspring: Possible contribution to psychosexual differentiation. *Physiol. Behav.* **60**, 353–359.

Beach, F. A. (1942). Male and female mating behavior in prepuberally castrated female rats treated androgens. *Endocrinology (Baltimore)* **31**, 373–378.

Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm. Behav.* **7**, 105–138.

Beach, F. A., Noble, R. G., and Orndoff, R. K. (1969). Effects of perinatal androgen treatment on responses of male rats to gonadal hormones in adulthood. *J. Comp. Physiol. Psychol.* **68**, 490–497.

Berenbaum, S. A., and Hines, M. (1992). Early androgens are related to childhood sex-typed toy preferences. *Psychol. Sci.* **3**, 203–206.

Black, V. H., and Christensen, A. K. (1969). Differentiation of interstitial and sertoli cells in fetal guinea pig testes. *Am. J. Anat.* **124**, 211–238.

Booth, J. E. (1977). Sexual behavior of male rats injected with anti-estrogen mer-25 during infancy. *Physiol. Behav.* **19**, 35–39.

Brand, T., and Slob, A. K. (1991a). Perinatal flutamide and mounting and lordosis behavior in adult female wistar and sprague-dawley rats. *Behav. Brain Res.* **44**, 43–51.

Brand, T., and Slob, A. K. (1991b). Neonatal organization of adult partner preference behavior in male rats. *Physiol. Behav.* **49**, 107–111.

Brand, T., Kroonen, J., Mos, J., and Slob, A. K. (1991). Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations. *Horm. Behav.* **25**, 323–341.

Bridges, R. S. (1984). A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. *Endocrinology (Baltimore)* **114**, 930–940.

Buhl, A. E., Pasztor, L. M., and Resko, J. A. (1979). Sex steroids in guinea pig fetuses after sexual differentiation of the gonads. *Biol. Reprod.* **21**, 905–908.

Burris, A. S., Banks, S. M., Carter, C. S., Davidson, J. M., and Sherins, R. J. (1992). A long-term, prospective study of the physiologic and behavioral effects of hormone replacement in untreated hypogonadal men. *J. Androl.* **13**, 297–304.

Byne, W., and Bleier, R. (1987). Medial preoptic sexual dimorphisms in the guinea pig. I. An investigation of their hormonal dependence. *J. Neurosci.* **7**, 2688–2696.

Caldera, Y. M., Huston, A. C., and O'Brien, M. (1989). Social interactions and play patterns of parents and toddlers with feminine, masculine, and neutral toys. *Child Dev.* **60**, 70–76.

Carroll, R. S., Weaver, C. E., and Baum, M. J. (1988). Evidence implicating aromatization of testosterone in the regulation of male ferret sexual behavior. *Physiol. Behav.* **42**, 457–460.

Chang, Y. M., Kelliher, K. R., and Baum, M. J. (2000). Steroidal modulation of scent investigation and marking behaviors in male and female ferrets. *J. Comp. Psychol.* **114**, 401–407.

Cherry, J. A., and Baum, M. J. (1990). Effects of lesions of a sexually dimorphic nucleus in the preoptic/anterior hypothalamic area on the expression of androgen- and estrogen-dependent sexual behaviors in male ferrets. *Brain Res.* **522**, 191-203.

Choate, J. V., and Resko, J. A. (1994). Prenatal inhibition of aromatase activity affects luteinizing hormone feedback mechanisms and reproductive behaviors of adult guinea pigs. *Biol. Reprod.* **51**, 1273–1278.

Clemens, L. G., Gladue, B. A., and Coniglio, L. P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Horm. Behav.* **10**, 40–53.

Compaan, J. C., Hutchison, J. B., Wozniak, A., de Ruiter, A. J., and Koolhaas, J. M. (1994). Brain aromatase activity and plasma testosterone levels are elevated in aggressive male mice

during early ontogeny. *Brain Res. Dev. Brain Res.* **82**, 185–192.

Corbier, P., Kerdelhue, B., Picon, R., and Roffi, J. (1978). Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. *Endocrinology (Baltimore)* **103**, 1985–1991.

Dantchakoff, V. (1937). Realisation du sexe a volonte. *Biol. Bull. (Woods Hole, Mass.)* **3**, 1–53.

De Bellis, A., Quigley, C. A., Marschke, K. B., el-Awady, M. K., Lane, M. V., Smith, E. P., Sar, M., Wilson, E. M., and French, F. S. (1994). Characterization of mutant androgen receptors causing partial androgen insensitivity syndrome. *J. Clin. Endocrinol. Metab.* **78**, 513–522.

Diamond, M. (1965). A critical evaluation of the ontogeny of human sexual behavior. *Q. Rev. Biol.* **40**, 117–175.

Diamond, M. (1976). Human sexual development: Biological foundations for social development. *In* "Human Sexuality in Four Perspectives" (F. A. Beach, ed.), pp. 22–61. Johns Hopkins Press, Baltimore, MD.

Diamond, M., and Sigmundson, H. K. (1997). Sex reassignment at birth. Long-term review and clinical implications. *Arch. Pediatr. Adolesc. Med.* **151**, 298–304.

Diehl, J. F., Godke, R. A., and Day, B. N. (1972). Induction of behavioral estrus in barrows with estradiol benzoate. *J. Anim. Sci.* **35**, 1117–1118.

Dominguez, E., Portillo, W., Baum, M. J., Bakker, J., and Paredes, R. G. (2002). Effect of prenatal androgen receptor antagonist or aromatase inhibitor on the differentiation of neuronal fos responses to estrous female pheromones in the rat accessory olfactory system. *Physiol. Behav.* (in press).

Dorries, K. M. (1991). Sex difference in olfactory sensitivity to the boar chemosignal, androstenone, in the domestic pig. *Anim. Behav.* **42**, 403–411.

Dorries, K. M., Adkins-Regan, E., and Halpern, B. P. (1995). Olfactory sensitivity to the pheromone, androstenone, is sexually dimorphic in the pig. *Physiol. Behav.* **57**, 255–259.

Dorries, K. M., Adkins-Regan, E., and Halpern, B. P. (1997). Sensitivity and behavioral responses to the pheromone androstenone are not mediated by the vomeronasal organ in domestic pigs. *Brain, Behav. Evol.* **49**, 53–62.

Eaton, G. G., and Goy, R. W. (1973). Effects of testosterone treatment in adulthood on sexual behavior of female pseudohermaphrodite rhesus monkeys. *Nature (London), New Biol.* **242**, 119–120.

Eaton, G. G., Johnson, D. F., Glick, B. B., and Worlein, J. M. (1985). Development in Japanese macaques (macaca fuscata): Sexually dimorphic behavior during the first year of life. *Primates* **26**, 238–247.

Eaton, G. G., Johnson, D. F., Glick, B. B., and Worlein, J. M. (1986). Japanese macaques (macaca fuscata) social development: Sex differences in juvenile behavior. *Primates* **27**, 141–150.

Eaton, G. G., Worlein, J. M., and Glick, B. B. (1990). Sex differences in japanese macaques (macaca fuscata): Effects of prenatal testosterone on juvenile social behavior. *Horm. Behav.* **24**, 270–283.

Edwards, D. A. (1971). Neonatal administration of androstenedione, testosterone or testosterone propionate: Effects on ovulation, sexual receptivity and aggressive behavior in female mice. *Physiol. Behav.* **6**, 223–228.

Edwards, D. A., and Burge, K. G. (1971). Early androgen treatment and male and female sexual behavior in mice. *Horm. Behav.* **2**, 49–58.

Edwards, D. A., and Thompson, M. L. (1970). Neonatal androgenization and estrogenization and the hormonal induction of sexual receptivity in rats. *Physiol. Behav.* **5**, 1115–1119.

Ehrhardt, A. A., Meyer-Bahlburg, H. F. L., Rosen, L. R., Feldman, J. F., Veridiano, N. P., Zimmerman, I., and McEwen, B. S. (1985). Sexual orientation after prenatal exposure to exogenous estrogen. *Arch. Sex. Behav.* **14**, 57–77.

Eisler, J. A., Tannenbaum, P. L., Mann, D. R., and Wallen, K. (1993). Neonatal testicular suppression with a gnrh agonist in rhesus monkeys: Effects on adult endocrine function and behavior. *Horm. Behav.* **27**, 551–567.

Ellinwood, W., Baughman, W., and Resko, J. (1982). The effects of gonadectomy and testosterone treatment in luteinizing hormone secretion in fetal rhesus monkeys. *Endocrinology (Baltimore)* **110**, 183–189.

Elsaesser, F., Stickney, K., and Foxcroft, G. (1982). A comparison of metabolic clearance rates of oestradiol-17 beta in immature and peripubertal female pigs and possible implications for the onset of puberty. *Acta Endocrinol. (Copenhagen)* **100**, 606-612.

Emery, D. E., and Sachs, B. D. (1975). Ejaculatory pattern in female rats without androgen treatment. *Science* **190**, 484–486.

Erskine, M. S., and Baum, M. J. (1982). Plasma concentrations of testosterone and dihydrotestosterone during perinatal development in male and female ferrets. *Endocrinology (Baltimore)* **111**, 767–772.

Erskine, M. S., and Baum, M. J. (1984). Plasma concentrations of oestradiol and oestrone during perinatal development in male and female ferrets. *J. Endocrinol.* **100**, 161–166.

Erskine, M. S., Tobet, S. A., and Baum, M. J. (1988). Effect of birth on plasma testosterone, brain aromatase activity, and hypothalamic estradiol in male and female ferrets. *Endocrinology (Baltimore)* **122**, 524–530.

Feder, H. H. (1967). Specificity of testosterone and estradiol in the differentiating neonatal rat. *Anat. Rec.* **157**, 79–86.

Feder, H. H., and Goy, R. W. (1983). Effects of neonatal estrogen treatment of female guinea pigs on mounting behavior in adulthood. *Horm. Behav.* **17**, 284–291.

Feder, H. H., and Whalen, R. E. (1965). Feminine behavior in neonatally castrated and estrogen-treated male rats. *Science* **147**, 306–307.

Fisher, C. R., Graves, K. H., Parlow, A. F., and Simpson, E. R. (1998). Characterization of mice deficient in aromatase (arko) because of targeted disruption of the cyp19 gene. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6965–6970.

Ford, J. J. (1982). Testicular control of defeminization in male pigs. *Biol. Reprod.* **27**, 425–430.

Ford, J. J. (1983a). Serum estrogen concentrations during postnatal development in male pigs. *Proc. Soc. Exp. Biol. Med.* **174**, 160–164.

Ford, J. J. (1983b). Postnatal differentiation of sexual preference in male pigs. *Horm. Behav.* **17**, 152–162.

Ford, J. J. (1990). Differentiation of sexual behavior in pigs. *J. Reprod. Fertil., Suppl.* **40**, 311–321.

Ford, J. J., and Christenson, R. K. (1987). Influences of pre- and postnatal testosterone treatment on defeminization of sexual receptivity in pigs. *Biol. Reprod.* **36**, 581–587.

Ford, J. J., and Schanbacher, B. D. (1977). Luteinizing hormone secretion and female lordosis behavior in male pigs. *Endocrinology (Baltimore)* **100**, 1033–1038.

Ford, J. J., Christenson, R. K., and Maurer, R. R. (1980). Serum testosterone concentrations in embryonic and fetal pigs during sexual differentiation. *Biol. Reprod.* **23**, 583–587.

Gaillard, J. M., Pontier, D., Allaine, D., Loison, A., Herve, J. C., and Heizmann, A. (1997). Variation in growth form and precocity at birth in eutherian mammals. *Proc. R. Soc. London, Ser. B* **264**, 859–868.

Gandelman, R., and Kozak, M. (1988). Neonatal testosterone administration, but not in utero contiguity to males, augments the display of male sexual behavior by testosterone-treated adult female mice. *Physiol. Behav.* **42**, 453–456.

Gerall, A. A. (1967). Effects of early postnatal androgen and estrogen injections on the estrous activity cycles and mating behavior of rats. *Anat. Rec.* **157**, 97–104.

Gerall, A. A., and Ward, I. L. (1966). Effects of prenatal exogenous androgen on the sexual behavior of the female albino rat. *J. Comp. Physiol. Psychol.* **62**, 370–375.

Gerlach, J. L., McEwen, B. S., Toran-Allerand, C. D., and Friedman, W. J. (1983). Perinatal development of estrogen receptors in mouse brain assessed by radioautography, nuclear isolation and receptor assay. *Brain Res.* **313**, 7–18.

Giusti, M., Falivene, M. R., Carraro, A., Cuttica, C. M., Valenti, S., and Giordano, G. (1995). The effect of non-steroidal antiandrogen flutamide on luteinizing hormone pulsatile secretion in male-to-female transsexual subjects. *J. Endocrinol. Invest.* **18**, 420–426.

Gladue, B. A., and Clemens, L. G. (1980). Masculinization diminished by disruption of prenatal estrogen biosynthesis in male rats. *Physiol. Behav.* **25**, 589–593.

Gogan, F., Slama, A., Bizzini-Koutznetzova, B., Dray, F., and Kordon, C. (1981). Importance of perinatal testosterone in sexual differentiation in the male rat. *J. Endocrinol.* **91**, 75–79.

Goldfoot, D. A. (1979). Sex-specific, behavior-specific actions of dihydrotestosterone: Activation of aggression, but not mounting in ovariectomized guinea pigs. *J. Comp. Physiol. Psychol.* **13**, 241–255.

Goldfoot, D. A., and van der Werff ten Bosch, J. J. (1975). Mounting behavior of female guinea pigs after prenatal and adult administration of the propionates of testosterone, dihydrotestosterone, and androstanediol. *Horm. Behav.* **6**, 139–148.

Goldfoot, D. A., Resko, J. A., and Goy, R. W. (1971). Induction of target organ insensitivity to testosterone in the male guinea-pig with cyproterone. *J. Endocrinol.* **50**, 423–429.

Gooren, L., and Cohen-Kettenis, P. T. (1991). Development of male gender identity/role and a sexual orientation towards women in a 46,xy subject with an incomplete form of the androgen insensitivity syndrome. *Arch. Sex. Behav.* **20**, 459–470.

Goy, R. W. (1978). Development of play and mounting behavior in female rhesus virilized prenatally with esters of testosterone or dihydrotestosterone. *Recent Adv. Primatol.* **1**, 449–462.

Goy, R. W. (1981). Differentiation of male social traits in female rhesus macaques by prenatal treatments with androgens: Variation in the type of androgen, duration, and timing of treatment. *In* "Fetal Endocrinology" (M. J. Novy and J. A. Resko, eds.), pp. 319–339. Academic Press, New York.

Goy, R. W., and Deputte, B. L. (1996). The effects of diethylstilbestrol (des) before birth on the development of masculine behavior in juvenile female rhesus monkeys. *Horm. Behav.* **30**, 379–86, published erratum: *Ibid.* **32**(1), (1997) 69.

Goy, R. W., and Wallen, K. (1979). Experiential variables influencing play, foot-clasp mounting and adult sexual competence in male rhesus monkeys. *Psychoneuroendocrinology* **4**, 1–12.

Goy, R. W., Hoar, R. M., and Young, W. C. (1957). Length of gestation in the guinea pig with data on the frequency and time of abortion and stillbirth. *Anat. Rec.* **128**, 747–758.

Goy, R. W., Bridson, W. E., and Young, W. C. (1964). Period of maximal susceptibility of the prenatal female guinea pig to masculinizing actions of testosterone propionate. *J. Comp. Physiol. Psychol.* **57**, 166–174.

Goy, R. W., Wallen, K., and Goldfoot, D. A. (1974). Social factors affecting the development of mounting behavior in male rhesus monkeys. *Adv. Behav. Biol.* **11**, 223–247.

Goy, R. W., Bercovitch, F. B., and McBrair, M. C. (1988). Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. *Horm. Behav.* **22**, 552–571.

Grady, K. L., Phoenix, C. H., and Young, W. C. (1965). Role of the developing rat testis in differentiation of the neural tissues mediating mating behavior. *J. Comp. Physiol. Psychol.* **59**, 176–182.

Graves, H. B. (1984). Behavior and ecology of wild and feral swine *(sus scrofa)*. *J. Anim. Sci.* **58**, 482–489.

Hampson, J. L. (1965). Determinants of psychosexual orientation. *In* "Sex and Behavior" (F. A. Beach, ed.), pp. 108–132. Krieger Publ. Co., Huntington, NY.

Harris, G. W., and Levine, S. (1965). Sexual differentiation of the brain and its experimental control. *J. Physiol. (London)* **181**, 379–400.

Hennessey, A. C., Wallen, K., and Edwards, D. A. (1986). Preoptic lesions increase the display of lordosis by male rats. *Brain Res.* **370**, 21–28.

Herdt, G. H., and Davidson, J. (1988). The sambia "turnimman": Sociocultural and clinical aspects of gender formation in male pseudohermaphrodites with 5-alpha-reductase deficiency in papua new guinea. *Arch. Sex. Behav.* **17**, 33–56.

Herman, R. A., Jones, B., Mann, D. R., and Wallen, K. (2000). Timing of prenatal androgen exposure: Anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Horm. Behav.* **38**, 52–66.

Hetta, J., and Meyerson, B. J. (1978). Sex-specific orientation in the male rat. A methodological study. *Acta. Physiol. Scand., Suppl.* **453**, 5–27.

Hines, M., and Goy, R. W. (1985). Estrogens before birth and development of sex-related reproductive traits in the female guinea pig. *Horm. Behav.* **19**, 331–347.

Hines, M., Alsum, P., Roy, M., Gorski, R. A., and Goy, R. W. (1987). Estrogenic contributions to sexual differentiation in the female guinea pig: Influences of diethylstilbestrol and tamoxifen on neural, behavioral, and ovarian development. *Horm. Behav.* **21**, 402–417.

Hoepfner, B. A., and Ward, I. L. (1988). Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. *Behav. Neurosci.* **102**, 61–65.

Honda, S., Harada, N., Ito, S., Takagi, Y., and Maeda, S. (1998). Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp19 gene. *Biochem. Biophys. Res. Commun.* **252**, 445–449.

Houtsmuller, E. J., Brand, T., de Jonge, F. H., Joosten, R. N., van de Poll, N. E., and Slob, A. K. (1994). Sdn-poa volume, sexual behavior, and partner preference of male rats affected by perinatal treatment with atd. *Physiol. Behav.* **56**, 535–541.

Imperato-McGinley, J., Guerrero, L., Gautier, T., and Peterson, R. E. (1974). Steroid 5-alpha-reductase deficiency in man: An inherited form of male pseudohermaphroditism. *Science* **186**, 1213–1215.

Johnson, D. F., and Phoenix, C. H. (1976). Hormonal control of female sexual attractiveness, proceptivity, and receptivity in rhesus monkeys. *J. Comp. Physiol. Psychol.* **90**, 473–483.

Jost, A. (1970). Hormonal factors in the sex differentiation of the mammalian foetus. *Philos. Trans. R. Soc. London, Ser. B* **259**, 119–130.

Kelliher, K. R., and Baum, M. J. (2001). Nares occlusion eliminates heterosexual partner selection without disrupting coitus in ferrets of both sexes. *J. Neurosci.* **21**, 5832–5840.

Kelliher, K. R., and Baum, M. J. (2002). Effect of sex steroids and coital experience on ferrets' preference for the smell, sight, and sound of conspecifics. *Physiol. Behav.,* in press.

Kelliher, K. R., Chang, Y. M., Wersinger, S. R., and Baum, M. J. (1998). Sex difference and testosterone modulation of pheromone-induced neuronal fos in the ferret's main olfactory bulb and hypothalamus. *Biol. Reprod.* **59**, 1454–1463.

Koopman, P. (1999). Sry and sox9: Mammalian testis-determining genes. *Cell Mol. Life Sci.* **55**, 839–856.

Krohmer, R. W., and Baum, M. J. (1989). Effect of sex, intrauterine position and androgen manipulation on the development of brain aromatase activity in fetal ferrets. *J. Neuroendocrinol.* **1**, 265–271.

Lahr, G., Maxson, S. C., Mayer, A., Just, W., Pilgrim, C., and Reisert, I. (1995). Transcription of the y chromosomal gene, sry, in adult mouse brain. *Brain Res. Mol. Brain Res.* **33**, 179–182.

Levine, S., and Mullins, R. (1964). Estrogen administered neonatally affects adult sexual behavior in male and female rats. *Science* **144**, 185–187.

Lewin, R. (1988). Living in the fast track makes for small brains. *Science* **242**, 513–514.

Lieberburg, I., Wallach, G., and McEwen, B. S. (1977). The effects of an inhibitor of aromatization (1,4,6-androstatriene-3,17-dione) and an anti-estrogen (cl-628) on in vivo formed testosterone metabolites recovered from neonatal rat brain tissues and purified cell nuclei. Implications for sexual differentiation of the rat brain. *Brain Res.* **128**, 176–181.

Lovejoy, J., and Wallen, K. (1988). Sexually dimorphic behavior in group-housed rhesus monkeys *(macaca mulatta)* at 1 year of age. *Psychobiology* **16**, 348–356.

Lustig, R. H. (1994). Sex hormone modulation of neural development in vitro. *Horm. Behav.* **28**, 383–395.

Maccoby, E. E., and Jacklin, C. N. (1974). "The Psychology of Sex Differences." Stanford University Press, Standford, CA.

Mahendroo, M. S., Cala, K. M., Landrum, D. P., and Russell, D. W. (1997). Fetal death in mice lacking 5-alpha-reductase type 1 caused by estrogen excess. *Mol. Endocrinol.* **11**, 917–927.

Mann, D. R., Davis-DaSilva, M., Wallen, K., Coan, P., Evans, D. E., and Collins, D. C. (1984). Blockade of neonatal activation of the pituitary-testicular axis with continuous administration of a gonadotropin-releasing hormone agonist in male rhesus monkeys. *J. Clin. Endocrinol. Metab.* **59**, 207–211.

Mann, D. R., Akinbami, M. A., Gould, K. G., Paul, K., and Wallen, K. (1998). Sexual maturation in male rhesus monkeys: Importance of neonatal testosterone exposure and social rank. *J. Endocrinol.* **156**, 493–501.

Mann, D. R., Akinbami, M. A., Gould, K. G., Tanner, J. M., and Wallen, K. (1993). Neonatal treatment of male monkeys with a gonadotropin-releasing hormone agonist alters differentiation of central nervous system centers that regulate sexual and skeletal development. *J. Clin. Endocrinol. Metab.* **76**, 1319–1324.

Manning, A., and McGill, T. E. (1974). Neonatal androgen and sexual behavior in female house mice. *Horm. Behav.* **5**, 19–31.

Mayer, A., Mosler, G., Just, W., Pilgrim, C., and Reisert, I. (2000). Developmental profile of sry transcripts in mouse brain. *Neurogenetics* **3**, 25–30.

McCarthy, M. M., Schlenker, E. H., and Pfaff, D. W. (1993). Enduring consequences of neonatal treatment with antisense oligodeoxynucleotides to estrogen receptor messenger ribonucleic acid on sexual differentiation of rat brain. *Endocrinology (Baltimore)* **133**, 433–439.

McEwen, B. S., Lieberburg, I., Chaptal, C., and Krey, L. C. (1977). Aromatization: Important for sexual differentiation of the neonatal rat brain. *Horm. Behav.* **9**, 249–263.

Meijs-Roelofs, H. M., Uilenbroek, J. T., de Jong, F. H., and Welschen, R. (1973). Plasma oestradiol-17beta and its relationship to serum follicle-stimulating hormone in immature female rats. *J. Endocrinol.* **59**, 295–304.

Meyer-Bahlburg, H. F. L., and Ehrhardt, A. A. (1986). Prenatal diethylstilbestrol exposure: Behavioral consequences in humans. *Monogr. Neural Sci.* **12**, 90–95.

Meyerson, B. J., and Lindstrom, L. H. (1973). Sexual motivation in the female rat. A methodological study applied to the investigation of the effect of estradiol benzoate. *Acta Physiol. Scand., Suppl.* **389**, 1–80.

Michael, R. P., Zumpe, D., and Bonsall, R. W. (1986). Comparison of the effects of testosterone and dihydrotestosterone on the behavior of male cynomolgus monkeys (macaca fascicularis). *Physiol. Behav.* **36**, 349–355.

Michael, R. P., Bonsall, R. W., and Zumpe, D. (1987). Testosterone and its metabolites in male cynomolgus monkeys (macaca fascicularis): Behavior and biochemistry. *Physiol. Behav.* **40**, 527–537.

Michael, R. P., Zumpe, D., and Bonsall, R. W. (1990). Estradiol administration and the sexual activity of castrated male rhesus monkeys (macaca mulatta). *Horm. Behav.* **24**, 71–88.

Money, J. (1976). Gender identity and hermaproditism. *Science* **191**, 872.

Money, J., and Ehrhardt, A. E. (1972). "Man & Women Boy & Girl." Johns Hopkins Press, Baltimore, MD.

Money, J., and Norman, B. F. (1987). Gender identity and gender transposition: Longitudinal outcome study of 24 male hermaphrodites assigned as boys. *J. Sex Marital Ther.* **13**, 75–92.

Money, J., Schwartz, M., and Lewis, V. G. (1984). Adult erotosexual status and fetal hormonal masculinization and demasculinization: 46,xx congenital virilizing adrenal hyperplasia and 46,xy androgen-insensitivity syndrome compared. *Psychoneuroendocrinology* **9**, 405–414.

Moore, C. L., and Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. *J. Comp. Physiol. Psychol.* **93**, 677–684.

Moore, C. L., and Samonte, B. R. (1986). Preputial glands of infant rats provide chemosignals for maternal discrimination of sex. *J. Comp. Psychol.* **100**, 76–80.

Morishima, A., Grumbach, M. M., Simpson, E. R., Fisher, C., and Qin, K. (1995). Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J. Clin. Endocrinol. Metab.* **80**, 3689–3698.

Motelica-Heino, I., Castanier, M., Corbier, P., Edwards, D. A., and Roffi, J. (1988). Testosterone levels in plasma and testes of neonatal mice. *J. Steroid Biochem.* **31**, 283–286.

Naftolin, F., Ryan, K. J., Davies, I. J., Reddy, V. V., Flores, F., Petro, Z., Kuhn, M., White, R. J., Takaoka, Y., and Wolin, L. (1975). The formation of estrogens by central neuroendocrine tissues. *Recent Prog. Horm. Res.* **31**, 295–319.

Newcombe, N., Bandura, M. M., and Taylor, D. G. (1983). Sex differences in spatial ability and spatial activities. *Sex Roles* **9**, 377–386.

O'Brien, M., and Huston, A. C. (1985). Activity level and sex-stereotyped toy choice in toddler boys and girls. *J. Genet. Psychol.* **146**, 527–533.

Ogawa, S., Washburn, T. F., Taylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1998a). Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. *Endocrinology (Baltimore)* **139**, 5058–5069.

Ogawa, S., Eng, V., Taylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1998b). Roles of estrogen receptor-alpha gene

expression in reproduction-related behaviors in female mice. *Endocrinology (Baltimore)* **139**, 5070–5081.

Ogawa, S., Chan, J., Chester, A. E., Gustafsson, J. A., Korach, K. S., and Pfaff, D. W. (1999). Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaerko) male and female mice. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12887–12892.

Ogawa, S., Chester, A. E., Hewitt, S. C., Walker, V. R., Gustafsson, J. A., Smithies, O., Korach, K. S., and Pfaff, D. W. (2000). From the cover: Abolition of male sexual behaviors in mice lacking estrogen receptors alpha and beta (alpha beta erko). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 14737–14741.

Olsen, K. (1992). Genetic influences on sexual behavior differentiation. *In* "Handbook of Behavioral Neurobiology, Sexual Differentiation" (A. A. Gerall, H. Moltz, and I. L. Ward, eds.), Vol. 11, pp. 1–40. Plenum Press, New York.

Pagel, M. D., and Harvey, P. H. (1989). Taxonomic differences in the scaling of brain on body weight among mammals. *Science* **244**, 1589–1593.

Pang, S. F., and Tang, F. (1984). Sex differences in the serum concentrations of testosterone in mice and hamsters during their critical periods of neural sexual differentiation. *J. Endocrinol.* **100**, 7–11.

Pang, S. F., Caggiula, A. R., Gay, V. L., Goodman, R. L., and Pang, C. S. (1979). Serum concentrations of testosterone, oestrogens, luteinizing hormone and follicle-stimulating hormone in male and female rats during the critical period of neural sexual differentiation. *J. Endocrinol.* **80**, 103–110.

Paredes, R. G., and Baum, M. J. (1995). Altered sexual partner preference in male ferrets given excitotoxic lesions of the preoptic area/anterior hypothalamus. *J. Neurosci.* **15**, 6619–6630.

Peterson, R. E., Imperato-McGinley, J., Gautier, T., and Sturla, E. (1977). Male pseudohermaphroditism due to steroid 5-alpha-reductase deficiency. *Am. J. Med.* **62**, 170–191.

Pfaff, D. W., and Pfaffmann, C. (1969). Olfactory and hormonal influences on the basal forebrain of the male rat. *Brain Res.* **15**, 137–156.

Pfaff, D. W., and Zigmond, R. E. (1971). Neonatal androgen effects on sexual and non-sexual behavior of adult rats tested under various hormone regimes. *Neuroendocrinology* **7**, 129–145.

Phoenix, C. H. (1974). Effects of dihydrotestosterone on sexual behavior of castrated male rhesus monkeys. *Physiol. Behav.* **12**, 1045–1055.

Phoenix, C. H., and Chambers, K. C. (1982). Sexual behavior in adult gonadectomized female pseudohermaphrodite, female, and male rhesus macaques (macaca mulatta) treated with estradiol benzoate and testosterone propionate. *J. Comp. Physiol. Psychol.* **96**, 823–833.

Phoenix, C. H., Goy, R. W., Gerall, A. A., and Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology (Baltimore)* **65**, 369–382.

Phoenix, C. H., Slob, A. K., and Goy, R. W. (1973). Effects of castration and replacement therapy on sexual behavior of adult male rhesuses. *J. Comp. Physiol. Psychol.* **84**, 472–481.

Pointis, G., Latreille, M. T., and Cedard, L. (1980). Gonadopituitary relationships in the fetal mouse at various times during sexual differentiation. *J. Endocrinol.* **86**, 483–488.

Pomerantz, S. M., Roy, M. M., Thornton, J. E., and Goy, R. W. (1985). Expression of adult female patterns of sexual behavior by male, female, and pseudohermaphroditic female rhesus monkeys. *Biol. Reprod.* **33**, 878–889.

Pomerantz, S. M., Goy, R. W., and Roy, M. M. (1986). Expression of male-typical behavior in adult female pseudohermaphroditic rhesus: Comparisons with normal males and neonatally gonadectomized males and females. *Horm. Behav.* **20**, 483–500.

Price, D., Ortiz, E., and Zaaijer, J. J. P. (1963). Secretion of androgenic hormone by testes and adrenal glands of fetal guinea pigs. *Am. Zool.* **3**, 553–554.

Raboch, J., Kobilkova, J., Horejsi, J., and Starka, L. (1987). Sexual development and life of women with gonadal dysgenesis. *J. Sex Marital Ther.* **13**, 117–127.

Raynaud, J. P., Mercier-Bodard, C., and Baulieu, E. E. (1971). Rat estradiol binding plasma protein (ebp). *Steroids* **18**, 767–788.

Reed, H. D., Melrose, D. R., and Patterson, R. L. S. (1974). Androgen steroids as an aid to the detection of oestrus in pig artificial insemination. *Br. Vet. J.* **130**, 61–67.

Resko, J. A., and Ellinwood, W. (1981). Testicular hormone production in fetal rhesus macaques. *Contrib. Gynecol. Obstet.* **7**, 253–267.

Resko, J. A. (1985). Gonadal hormones during sexual differentiation in vertebrates. *In* "Handbook of Behavioral Neurobiology" (N. Adler, D. Pfaff, and R. W. Goy, eds.), Vol. 7, pp. 21–42. Plenum Press, New York.

Resko, J. A., Feder, H. H., and Goy, R. W. (1968). Androgen concentrations in plasma and testis of developing rats. *J. Endocrinol.* **40**, 485–491.

Rigaudiere, N. (1979). The androgens in the guinea-pig foetus throughout the embryonic development. *Acta Endocrinol. (Copenhagen)* **92**, 174–186.

Rissman, E. F., Early, A. H., Taylor, J. A., Korach, K. S., and Lubahn, D. B. (1997). Estrogen receptors are essential for female sexual receptivity. *Endocrinology (Baltimore)* **138**, 507–510.

Roy, M. M., and Goy, R. (1988). Sex differences on the inhibition by atd of testosterone-activated mounting behavior in guinea pigs. *Horm. Behav.* **22**, 315–323.

Roy, M. M. (1992). Effects of prenatal testosterone and atd on reproductive behavior in guinea pigs. *Physiol. Behav.* **51**, 105–109.

Sachs, B. D., Pollak, E. K., Krieger, M. S., and Barfield, R. J. (1973). Sexual behavior: Normal male patterning in androgenized female rats. *Science* **181**, 770–772.

Scordalakes, E., and Rissman, E. R. (2000). The role of estrogen and androgen receptors in the activation of masculine sexual behavior in c57/bl6 mice. *Trabajos del Instituto Cajal, Tomo LXXVII,* 117–118.

Sholl, S. A., and Goy, R. W. (1978). Androgen and estrogen synthesis in the fetal guinea pig gonad. *Biol. Reprod.* **18**, 160–169.

Signoret, J. P. (1967). Attraction de la femelle en oestrus par le male chez les porcins. *Rev. Comp. Anim.* **4**, 10–22.

Signoret, J. P. (1970). Reproductive behavior of pigs. *J. Reprod. Fertil., Suppl.* **11**, 105.

Slob, A. K., Ooms, M. P., and Vreeburg, J. T. (1980). Prenatal and early postnatal sex differences in plasma and gonadal testosterone and plasma luteinizing hormone in female and male rats. *J. Endocrinol.* **87**, 81–87.

Smith, E. P., Boyd, J., Frank, G. R., Takahashi, H., Cohen, R. M., Specker, B., Williams, T. C., Lubahn, D. B., and Korach, K. S. (1994). Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N. Engl. J. Med.* **331**, 1056–1061.

Sodersten, P., Gray, G., Damassa, D. A., Smith, E. R., and Davidson, J. M. (1975). Effects of a non-steroidal antiandrogen on sexual behavior and pituitary-gonadal function in the male rat. *Endocrinology (Baltimore)* **97**, 1468–1475.

Stewart, J., Pottier, J., and Kaczender-Henrik (1971). Male copulatory behavior in the female rat after perinatal treatment with an antiandrogenic steroid. *Horm. Behav.* **2**, 247–254.

Stockman, E. R., Callaghan, R. S., and Baum, M. J. (1985). Effects of neonatal castration and testosterone treatment on sexual partner preference in the ferret. *Physiol. Behav.* **34**, 409–414.

Stoller, R. J. (1968). "Sex and Gender." Science House, New York.

Swain, A., and Lovell-Badge, R. (1999). Mammalian sex determination: A molecular drama. *Genes Dev.* **13**, 755–767.

Tessitore, C., and Brunjes, P. C. (1988). A comparative study of myelination in precocial and altricial murid rodents. *Brain Res.* **471**, 139–147.

Thornton, J. E., and Goy, R. W. (1986). Female-typical sexual behavior of rhesus and defeminization by androgens given prenatally. *Horm. Behav.* **20**, 129–147.

Thornton, J. E., Irving, S., and Goy, R. W. (1991). Effects of prenatal antiandrogen treatment on masculinization and defeminization of guinea pigs. *Physiol. Behav.* **50**, 471–475.

Tobet, S. A., and Baum, M. J. (1987). Role for prenatal estrogen in the development of masculine sexual behavior in the male ferret. *Horm. Behav.* **21**, 419–429.

Tobet, S. A., and Fox, T. O. (1992). Sex differences in neuronal morphology influenced hormonally throughout life. *In* "Handbook of Behavioral Neurobiology" (A. A. Gerall, H. Moltz, and I. L. Ward, eds.), Vol. 11, pp. 41–83. Plenum Press, New York.

Tobet, S. A., Zahniser, D. J., and Baum, M. J. (1986). Differentiation in male ferrets of a sexually dimorphic nucleus of the preoptic/anterior hypothalamic area requires prenatal estrogen. *Neuroendocrinology* **44**, 299–308.

Tobet, S. A., Chickering, T. W., Fox, T. O., and Baum, M. J. (1993). Sex and regional differences in intracellular localization of estrogen receptor immunoreactivity in adult ferret forebrain. *Neuroendocrinology* **58**, 316–324.

Vale, J. R., Ray, D., and Vale, C. A. (1973). The interaction of genotype and exogenous neonatal androgen and estrogen: Sex behavior in female mice. *Dev. Psychobiol.* **6**, 319–327.

Vandenbergh, J. G. (1994). Pheromones and mammalian reproduction. *In* "The Physiology of Reproduction" (B. Knobil and J. D. Neill, eds.), 2nd ed., pp. 343–359. Raven Press, New York.

Vega Matuszczyk, J. V., Fernandez-Guasti, A., and Larsson, K. (1988). Sexual orientation, proceptivity, and receptivity in the male rat as a function of neonatal hormonal manipulation. *Horm. Behav.* **22**, 362–378.

Vega Matuszczyk, J. V., and Larsson, K. (1995). Sexual preference and feminine and masculine sexual behavior of male rats prenatally exposed to antiandrogen or antiestrogen. *Horm. Behav.* **29**, 191–206.

Viguier-Martinez, M. C., Hochereau de Reviers, M. T., Barenton, B., and Perreau, C. (1983). Endocrinological and histological changes induced by flutamide treatment on the hypothalamo-hypophyseal testicular axis of the adult male rat and their incidences on fertility. *Acta Endocrinol. (Copenhagen)* **104**, 246–252.

vom Saal, F. S., and Bronson, F. H. (1980). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science* **208**, 597–599.

vom Saal, F. S., Quadagno, D. M., Even, M. D., Keisler, L. W., Keisler, D. H., and Khan, S. (1990). Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. *Biol. Reprod.* **43**, 751–761.

Vreeburg, J. T., van der Vaart, P. D., and van der Schoot, P. (1977). Prevention of central defeminization but not masculinization in male rats by inhibition neonatally of oestrogen biosynthesis. *J. Endocrinol.* **74**, 375–382.

Wallen, K. (1990). Desire and ability: Hormones and the regulation of female sexual behavior. *Neurosci. Biobehav. Rev.* **14**, 233–241.

Wallen, K. (1996). Nature needs nurture: The interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. *Horm. Behav.* **30**, 364–378.

Wallen, K., and Goy, R. W. (1977). Effects of estradiol benzoate, estrone, and propionates of testosterone or dihydrotestosterone on sexual and related behaviors of ovariectomized rhesus monkeys. *Horm. Behav.* **9**, 228–248.

Wallen, K., Bielert, C., and Slimp, J. (1977). Foot clasp mounting in the prepubertal rhesus monkey: Social and hormonal influences. *In* "Primate Bio-social Development" (S. Chevalier-Skolnikoff and F. E. Poirier, eds.), pp. 439–461. Garland Publishing, New York.

Wallen, K., Winston, L. A., Gaventa, S., Davis-DaSilva, M., and Collins, D. C. (1984). Periovulatory changes in female sexual behavior and patterns of ovarian steroid secretion in group-living rhesus monkeys. *Horm. Behav.* **18**, 431–450.

Wallen, K., Maestripieri, D., and Mann, D. R. (1995). Effects of neonatal testicular suppression with a gnrh antagonist on social behavior in group-living juvenile rhesus monkeys. *Horm. Behav.* **29**, 322–337.

Ward, I. L. (1969). Differential effect of pre- and postnatal androgen on the sexual behavior of intact and spayed female rats. *Horm. Behav.* **1**, 25–36.

Ward, I. L., and Renz, F. J. (1972). Consequences of perinatal hormone manipulation on the adult sexual behavior of female rats. *J. Comp. Physiol. Psychol.* **78**, 349–355.

Weisz, J., and Ward, I. L. (1980). Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology (Baltimore)* **106**, 306–316.

Wersinger, S. R., and Rissman, E. F. (2000). Oestrogen receptor alpha is essential for female-directed chemo-investigatory behavior but is not required for the pheromone-induced luteinizing hormone surge in male mice. *J. Neuroendocrinol.* **12**, 103–110.

Wersinger, S. R., Sannen, K., Villalba, C., Lubahn, D. B., Rissman, E. F., and De Vries, G. J. (1997). Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. *Horm. Behav.* **32**, 176–183.

Whalen, R. E., and Edwards, D. A. (1967). Hormonal determinants of the development of masculine and feminine behavior in male and female rats. *Anat. Rec.* **157**, 173–180.

Wieland, S. J., Fox, T. O., and Savakis, C. (1978). DNA-binding of androgen and estrogen receptors from mouse brain: Behavior of residual androgen receptor from tfm mutant. *Brain Res.* **140**, 159–164.

Wisniewski, A. B., Migeon, C. J., Meyer-Bahlburg, H. F., Gearhart, J. P., Berkovitz, G. D., Brown, T. R., and Money, J. (2000). Complete androgen insensitivity syndrome: Long-term medical, surgical, and psychosexual outcome. *J. Clin. Endocrinol. Metab.* **85**, 2664–2669.

Young, W. C., and Rundlett, B. (1939). The hormonal induction of homosexual behavior in the spayed female guinea pig. *Psychosom. Med.* **1**, 449–460.

Young, W. C., Dempsey, E. W., Hagquist, C. W., and Boling, J. L. (1939). Sexual behavior and sexual receptivity in the female guinea pig. *J. Comp. Psychol.* **27**, 49–68.

Young, W. C., Goy, R. W., and Phoenix, C. H. (1964). Hormones and sexual behavior. *Science* **143**, 212–218.

Zucker, K. J. (1999). Intersexuality and gender identity differentiation. *Annu. Rev. Sex Res.* **10**, 1–69.

Zucker, K. J., and Green, R. (1991). Gender identity disorders. *In* "Child and Adolescent Psychiatry: A Comprehensive Textbook" (M. Lewis, ed.), pp. 604–613. Williams & Wilkins, Baltimore, MD.

Zumpe, D., Bonsall, R. W., and Michael, R. P. (1993). Effects of the nonsteroidal aromatase inhibitor, fadrozole, on the sexual behavior of male cynomolgus monkeys (macaca fascicularis). *Horm. Behav.* **27**, 200–215.

Zumpe, D., Clancy, A. N., Bonsall, R. W., and Michael, R. P. (1996). Behavioral responses to depo-provera, fadrozole, and estradiol in castrated, testosterone-treated cynomolgus monkeys (macaca fascicularis): The involvement of progestin receptors. *Physiol. Behav.* **60**, 531–540.