Published in final edited form as:
Brain Res Rev. 2008 March ; 57(2): 321–331.

A role for the androgen receptor in the sexual differentiation of the olfactory system in mice

Cristian Bodo
Graduate Program in Neuroscience, University of Virginia, Charlottesville, VA 22908

Abstract

Olfactory signals play a central role in the identification of a mating partner in rodents, and the behavioral response to these cues varies markedly between the sexes. As several other sexually dimorphic traits, this response is thought to differentiate as a result of exposure of the developing individual to gonadal steroids, but both the identity of the specific steroid signal and the neural structures targeted for differentiation on this particular case are largely unknown. The present review summarizes results obtained in our lab using genetic males affected by the testicular feminization syndrome (Tfm) as experimental model, and that led to the identification of a role for non-aromatized gonadal steroids acting through the androgen receptor (AR) in the differentiation of olfactory cues processing in mice. The existing literature about AR-mediated sexual differentiation of the CNS in animal models is discussed, along with potential targets for the action of non-aromatized gonadal steroids in either one of the subsystems that detect and process olfactory information in rodents.

Keywords

Sexual orientation; testicular feminization; androgen insensitivity syndrome; olfactory system; sexual differentiation; androgen receptor

Introduction

Many sexual dimorphisms in mammals are the result of the differential exposure to steroid hormones secreted by the gonads during development (Jost, 1972; Jost, 1978; Jost, et al., 1973; Morris, et al., 2004). Included in this general model are sexually dimorphic behaviors related to reproduction, as well as non-reproductive behaviors. This was first demonstrated empirically by the classic experiments conducted by Phoenix and colleagues (Phoenix, et al., 1959), in which administration of elevated doses of testosterone to pregnant guinea pigs induced the display of typically masculine stereotyped behavior in their female offspring, as well as a reduced ability to exhibit lordosis later in life. Conversely, males castrated immediately after birth and therefore deprived of the source of differentiating steroids exhibited reduced masculine behavior and increased levels of sexual receptivity. These initial findings were later extended to other mammalian species, with the studies yielding similar results (Baum, et al., 1990; Beach, et al., 1969; Grady, et al., 1965; Whalen, 1964; Whalen and Edwards, 1966).

Correspondence: C. Bodo, 1300 Jefferson Park Avenue, room 1229, Jordan Hall, University of Virginia, Charlottesville, VA 22908, FAX 434 243-8433, phone: (434) 982-4742, email: ccb6j@virginia.edu.

The endogenous steroid signal responsible for this differentiation was initially assumed to be testosterone, since this is the main steroid secreted by the testes (George, et al., 1978; Wilson and Lasnitzki, 1971). However, exogenous administration of estradiol to gonadectomized individuals during the perinatal period readily mimicked the effects of testosterone in the differentiation of reproductive behaviors (Feder and Whalen, 1964; Levine and Mullins, 1964). This initially puzzling observation was explained by the characterization of the estrogen synthase (aromatase) enzyme in the central nervous system, responsible for catalyzing the conversion of testosterone to estradiol (Ryan, et al., 1972). This led to the formulation of the "aromatization hypothesis", which states that gonadal testosterone exerts its differentiating effects on the central nervous system of males by neural conversion to estradiol in areas expressing aromatase, and that this locally-synthesized estradiol binds to estrogen receptors present in the same areas as a first step in the organization of those circuits towards a masculine phenotype (Baum, 2003; Naftolin, 1994). The distribution pattern of aromatase expression in the brain (Lauber and Lichtensteiger, 1994; Roselli and Resko, 1993), the variation in the levels of expression during the lifespan with a peak matching the perinatal period in males (MacLusky, et al., 1985; Tobet, et al., 1985) and the disruptive effect that both aromatase inhibitors and anti-estrogens have in the masculinization of reproductive behavior (McEwen, et al., 1977; Vega Matuszczyk and Larsson, 1995; Vreeburg, et al., 1977) are all in good agreement with this hypothesis.

But despite the bulk of empirical support for this mechanism, the androgen receptor (AR) is also abundantly expressed in the same hypothalamic areas that are thought to undergo this estrogen-mediated differentiation (Attardi and Ohno, 1976; Fox, 1975; Kato, 1976; McAbee and DonCarlos, 1998; Vito, et al., 1979; Simerly, et al., 1990) and also exhibits some sexual dimorphism in its expression (Shah, et al., 2004). AR function is essential for the sexual differentiation of the male genitalia (Goldstein and Wilson, 1975) and the sexually dimorphic groups of motoneurons in the spinal cord that inervate the muscles attached to the penis and that are collectively known as the spinal nucleus of the bulbocavernosus (SNB) (Breedlove and Arnold, 1983a; Breedlove and Arnold, 1983b; Breedlove, et al., 1982; Freeman, et al., 1996) and dorsolateral nucleus (DLN) (Grisham, et al., 1992). Furthermore, some recent studies have uncovered that androgen action influences the morphology of several sexually dimorphic structures in the rodent brain, such as the posterodorsal medial amygdala, the suprachiasmatic nucleus (Morris, et al., 2005), the arcuate nucleus (Ciofi, et al., 2007), the ventromedial hypothalamus (Dugger, et al., 2007), the anteroventral periventricular nucleus (Lund, et al., 2000), the locus coeruleus (Garcia-Falgueras, et al., 2005), the bed nucleus of the accessory olfactory tract (Collado, et al., 1992) and the posteromedial bed nucleus of the stria terminalis (Durazzo, et al., 2007). This raises in turn the question of whether its interaction with non-aromatized ligands plays a role on the process of differentiation of sex-specific behavioral patterns (Sato, et al., 2004).

The initial studies on the role of gonadal steroids in the differentiation of the central nervous system focused specifically on mating behavior, but we now know that the range of behavioral traits affected by a differential exposure to these compounds is much wider (Casto, et al., 2003; Juarez, et al., 1998; Motelica-Heino, et al., 1993; Smith, et al., 1998). In particular, the ability to identify and exhibit a preference for an apropriate mating partner has also been studied. These behaviors collective refered to as "social preferences" are necessary for successful reproduction (Lumia, et al., 1987; Murphy and Schneider, 1970) and vary dramatically according to the sex of the individual. Perhaps because of the particular ecological niche that they occupy, rodent species rely heavily on olfactory cues derived from conspecifics for this particular function (Johnston, 1998). Therefore, neural circuits associated with the detection and processing of olfactory signals are good targets for sexual differentiation. Like many other terrestrial vertebrates, rodents exhibit two paralell olfactory systems that differ from each other both morphologically and functionally. Known as the main olfactory system

(MOS) and the accesory olfactory system (AOS), they are associated with the main olfactory epithelium of the nasal cavity and the vomeronasal organ, respectively (Raisman, 1972; Scalia and Winans, 1975). In addition to olfactory receptors located in two different anatomical structures, the MOS and AOS also have segregated projection pathways to morphologically distinct areas of the olfactory bulbs, and from there to adjacent terminal fields in the limbic system (Halpern, 1987). Of these two systems, the accesory or vomeronasal system has traditionally been associated with the detection of pheromonal cues used for intra-species chemical communication and regulation of reproductive function (Powers and Winans, 1975; Wysocki, et al., 1982; Lepri and Wysocki, 1987). The VNS projects to the hypothalamus, including the medial preoptic area (Dong, et al., 2001; Gu, et al., 2003; Simerly and Swanson, 1988). Sex differences in the VNS are pronounced: The vomeronasal organ itself exhibits a higher overall and neuroepithelial volume, as well as a higher number of bipolar neurons in males compared to females (Segovia and Guillamon, 1982). In turn, the accesory olfactory bulb (AOB), which receives direct projections from the vomeronasal receptor neurons (Barber and Raisman, 1974), is larger in males than in females in terms of total volume, individual volume of each layer and several other morphometric variables (Caminero, et al., 1991; Segovia, et al., 1984; Valencia, et al., 1986). Finally, sexual dimorphisms have been described in areas that receive either direct or indirect projections from the AOB, including the bed nucleus of the accesory olfactory tract (BAOT) (Collado, et al., 1990), the medial division and encapsulated region of the bed nucleus of the stria terminalis (BNST) (del Abril, et al., 1987; Guillamon, et al., 1988; Hines, et al., 1992), posteromedial cortical and medial nuclei of the amygdala (Hines et al., 1992; Vinader-Caerols, et al., 1998) and medial preoptic area (Gorski, et al., 1978). All of these regions have been described to express AR in several rodent species, including rats (Sar and Stumpf, 1977; Handa, et al., 1986; Handa, et al., 1987; Sar, et al., 1990; Simerly et al., 1990; Menard and Harlan, 1993; Clancy, et al., 1992), mice (Lu, et al., 1998; Apostolinas, et al., 1999; Shah et al., 2004) and hamsters (Chen and Tu, 1992; Meek, et al., 1997). One of the studies performed in rats (Simerly et al., 1990) describes moderate expression of AR mRNA in both the main and accesory olfactory bulbs, as well as in the anterior olfactory nucleus. These data suggest that androgens secreted by the gonads during development may act directly through the AR to differentiate one or more components of the rodent olfactory pathway, which in turn could translate into a sexually dimorphic behavioral response.

Animals affected by the Tfm mutation as experimental models

To study the role of the androgen receptor in the sexual differentiation of brain circuits, we have adopted as our experimental model male mice carrying the testicular feminization mutation (Tfm). This syndrome was first described in humans (Morris, 1953) and later in mice (Lyon and Hawkes, 1970), rats (Stanley, et al., 1973), cattle (Nes, 1966) and chimpanzees (Eil, 1980). In all cases, the individuals affected are insensitive to androgens, and as a consequence the genetic males exhibit feminine external genitalia (Bardin, et al., 1973). These individuals present instead a blind vagina, a clitoris, developed mammary glands and a feminine number and location of nipples, with small testes present in an inguinal or abdominal position (Shapiro, et al., 1980; Stanley et al., 1973).

The Tfm syndrome is caused by several different naturally occurring mutations in the AR gene, whose common denominator is that they render the receptor non-functional (McPhaul, 2002a). Since the AR gene is located on the X chromosome, and males carrying a mutant allele are sterile, female homozygotes for the mutation have never been found in nature. The severity of androgen insensitivity is variable and directly related to extent of the disruption of receptor function (Lee and Chang, 2003; McPhaul, 2002b). Analysis of the mutation on the AR locus of the Tfm rat in the Stanley-Gumbreck strain showed that the disruption is caused by a single amino acid substitution on a position that is highly conserved within the nuclear receptor family

(Yarbrough, et al., 1990). In contrast, the mutant sequence in the Tfm mouse presents a single-base deletion in the N-terminal region that causes a shift in the reading frame, generates a premature stop codon and thus causes an early termination of transcription and the production of a truncated receptor (Charest, et al., 1991; Gaspar, et al., 1991; He, et al., 1990). In both cases, androgen binding in brain tissue from Tfm animals has been shown to be only 10-20% compared with WT individuals, indicating that there is an important reduction in the number of functional receptors (Attardi, et al., 1976; Fox, 1975; MacLusky, et al., 1988). However, the small number of functional ARs present in Tfm rats appear to be indistinguishable from those of their WT counterparts (Wieland, et al., 1978). In contrast, the mutant protein in Tfm mice is considerably smaller, confirming what was predicted from the particular characteristics of the mutation and suggesting that in this case there is a qualitative deficit in addition to the quantitative deficit exhibited by both species (Young, et al., 1989). The biochemical evidence thus shows that Tfm rats retain some residual degree of responsiveness to androgens conferred by the few functional androgen receptors present.

Male Tfm rats have been described as completely asexual (Shapiro, et al., 1976), exhibiting neither mounting behavior when injected with testosterone nor lordosis when administered estradiol in combination with progesterone. However, only a year later, Beach and Buehler (Beach and Buehler, 1977) reported that almost all the mutant, gonadally intact subjects in their study exhibited mounting, with some individuals even exhibiting a characteristic behavioral pattern associated with ejaculation. Another report in Tfm rats (Shapiro et al., 1980) also stated that they of exhibited male-like mating behavior when treated with supraphysiological doses of gonadal steroids, and suggested that brain masculinization is independent of androgen action via the AR. The data on the ability of Tfm rats to exhibit feminine behavior has been much more consistent, with all the studies reporting that the lordotic coefficient was never significantly different from that of WT males, regardless of the hormone treatment (Beach and Buehler, 1977; Olsen, 1979; Olsen and Whalen, 1981; Shapiro et al., 1976). The relevance of the behavioral masculinization in Tfm rats has nevertheless been challenged (Bardin and Catterall, 1981) based on the data (discussed above) suggesting that Tfm rats may not be completely unresponsive to androgens. On the other hand, the AR signal appears to be entirely suppressed in mice, making them a more robust experimental model to study the role of androgen-mediated sexual differentiation of brain circuits.

AR-mediated differentiation of sexually dimorphic traits in mice

The first report of reproductive behavior in Tfm mice was included in the original work describing the syndrome on this species (Lyon and Hawkes, 1970). They were described as asexual, based entirely on anecdotic observations from gonadally-intact individuals, without any specific quantitative data. Four years later, Ohno and colleagues (Ohno, et al., 1974) reported that many of their Tfm subjects were capable of exhibiting male copulatory behavior, although in this study the Tfm males were heterozygotes obtained from a cross with a strain carrying the *sex-reversed* mutation, casting doubts on their complete unresponsiveness to androgens. A more detailed analysis was undertaken by Olsen (Olsen, 1993), who examined Tfm mice with intact gonads as well as gonadectomized and treated with testosterone, dihydrotestosterone or estradiol. The results confirmed that gonadally intact Tfm mice do not show masculine behavior spontaneously. However, hormone replacement was effective in eliciting some degree of masculine behavior in these animals, with 37% of the Tfm mice displaying mounting and thrusting after estrogen stimulation. Unfortunately, no data on specific behavioral patterns were reported in the study, and therefore it is not possible to assess whether the behavior exhibited by the androgen-insensitive individuals differed from their littermates with a functional AR.

When we tested Tfm subjects for masculine coital behavior after gonadectomy and estrogen replacement, we did not find significant differences between WT male littermates, both in terms of percentage of individuals exhibiting the behavior and in the values of the specific variables displayed during the sexual behavior tests. Moreover, WT female littermates exhibited similar levels of masculine behavior towards the stimulus animals (Bodo and Rissman, 2007). We concluded from this study that mice subjected to gonadectomy and estradiol treatment did not display any sex differences in the ability to mount exhibit pelvic thrusts with a sexually receptive female, however, some features of the temporal patterning of the behavior are dimorphic. This observation has been reported by other groups studying laboratory mice (see Table 1). Moreover, these behaviors do not require the activation of the AR for its differentiation at any point during development. An androgen receptor knockout mouse has been developed (Sato et al., 2004) and these mice have been reported to have deficiencies in their male sexual behavior. Interestingly, when 17β–estradiol was used as the activational hormone of choice in the sexual behavior test, a partial rescue of the mating phenotype was achieved, a result that is similar to what Olsen had previously reported using Tfm mice (Olsen, 1993). However, since the percentage of individuals exhibiting the behavior was approximately 50% compared with that of the WT group, the authors interpreted their results as evidence of an involvement of AR in the differentiation of the trait. A possible explanation for the discrepancy between our results and theirs may be differences in the doses of estradiol used. The estradiol-releasing pellet used by Sato and colleagues yield lower levels of estradiol in plasma (50–60 pg/ml)(Granholm, et al., 2002) compared to those attained with the implants used in our study (80-90 pg/ml), (Wersinger, et al., 1997). It may be necessary to use supraphysiological plasma levels of estradiol in order to elicit a high enough concentration in the brain to stimulate male sexual behavior. Confirmation of this hypothesis requires dose-response studies in AR knockouts and Tfm mice.

In contrast with mating, when we tested our subjects for their olfactory preferences, males exhibited a clear preference for female-soiled bedding, whereas females preferred to spend more time investigating male odors (Bodo and Rissman, 2007). Interestingly, castrated and E2-treated Tfm males exhibited a female-like preference, spending more time sniffing male-soiled bedding (Fig 1). In addition, when a group of identically treated WT males were offered their own soiled bedding versus bedding soiled by a different male, they showed a clear preference to investigate the latter stimulus, indicating that the comparatively small amount of time spent investigating male-soiled bedding during regular tests by this group cannot be attributed to simple habituation to a familiar odor (Bodo and Rissman, unpublished results).

It has been previously shown in both rats (Bakker, et al., 1996) and mice (Halem, et al., 1999) that chemosignals present in male-soiled bedding are capable of eliciting neuronal activation in several brain areas in females, including the bed nucleus of the stria terminalis (BNST), medial preoptic area (mPOA) and medial amygdala (MeA), while males are unresponsive to the same chemical stimulus. We replicated these original results using gonadectomized, E2-implanted subjects, and found that two of the three areas (BNST and mPOA) exhibited increased numbers of c-fos-expressing neurons in WT females and Tfm males, but not in WT males (Fig 2). Finally, when actual partner preference was tested experimentally using a Y maze, Tfm males again failed to exhibit a clear preference to spend time in the vicinity of a receptive female as opposed to a male. The behavior of females was the same, whereas a preference for females was clearly marked in their WT male littermates. As in the other studies described all mice were gonadectomized and treated with E2 to activate the behavior.

In order to confirm the hypothesis that AR is involved in the normal differentiation of these traits, we administered dihydrotestosterone to WT females on postnatal day one (PN1) and then tested their preference for soiled bedding after they reached sexual maturity, as well as

their pattern of neural activation in response to male-derived olfactory cues. Again all mice were gonadectomized and treated with E2, and the results were in agreement with the hypothesis that AR mediates sexual differentiation of this set of traits. DHT-injected females exhibited a marked preference for female-soiled bedding and a masculinized pattern of fos immunoreactivity in the same areas where we had previously characterized an abnormal response in our Tfm subjects (Bodo & Rissman, unpublished results).

General Conclusions

Considered together, the results described above show that the normal differentiation of the neural circuitry involved in recognizing and processing olfactory signals derived from conspecifics in mice is controlled by non-aromatized steroids interacting with the androgen receptor, and that this process takes place during the early neonatal period. Moreover, the differentiation of these traits is a prerequisite in male mice to making an appropriate choice of partner according to their genetic sex.

It is difficult with the data available so far to speculate on which specific component of the neural pathway that process the olfactory signal is targeted for differentiation by androgens in the male. The accessory (vomeronasal) olfactory system is a good candidate, since it is specialized in recognizing non-volatile cues used for chemical communication that are likely to be present in soiled bedding (Keverne, 2004). Moreover, the two hypothalamic areas where an abnormal c-Fos response was identified in Tfm individuals are central projections of the vomeronasal system. Genetic ablation of the TRPc2 ion channel, expressed specifically in the vomeronasal organ and required for transduction of the pheromonal signals, led to a gender-blind phenotype, with males initiating courtship with both females and other males, and failing to display territorial aggression towards the latter (Leypold, et al., 2002; Stowers, et al., 2002). There is a wealth of evidence, so far restricted mainly to the rat, pointing to specific sexual dimorphisms in several neural structures belonging to the VNS, and it has been shown empirically that gonadal steroids play a key role in the establishment of these differences during development. For instance, sex differences in the vomeronasal organ itself are abolished in males castrated immediately after birth or when females received exogenous androgens during the same developmental period (Segovia and Guillamon, 1982). These experimental manipulations had a similar effect on the morphology of the AOB (Segovia et al., 1984; Segovia, et al., 1986; Valencia et al., 1986), BAOT (Collado, et al., 1998; Collado, et al., 1993), BNST (Chung, et al., 2000; del Abril et al., 1987; Guillamon et al., 1988), MeA (Mizukami, et al., 1983) and sexually dimorphic nucleus of the POA (Rhees, et al., 1990a; Rhees, et al., 1990b). It is tempting to speculate that one or more of these morphological dimorphisms may underlie the differential response to olfactory cues determined by the organizational action of gonadal steroids. Already some evidence has been advanced on this direction, with one study linking reversal of the sex-specific morphology of the AOB as a result of experimental manipulation, with the expression of maternal behavior in male rats (Segovia, et al., 1996).

But it is important to remember that the original dual olfactory hypothesis, which postulated segregated, non-overlapping patterns of projections for the two olfactory systems, has been revised in recent years, with at least one study showing convergence of both pathway at the level of individual neurons in the hamster amygdala (Licht and Meredith, 1987). Moreover, recent studies have called into question the exclusive role of the vomeronasal system in the detection of socially relevant olfactory cues. Schaefer and colleagues (Schaefer, et al., 2002) showed that glomerular activation in the male olfactory bulb is sensitive to subtle variations in the MHC odortype of the individual providing the stimulus, a trait known to be relevant for mate choice in rodents (Penn and Potts, 1998). Removal of the vomeronasal organ, on the other hand, does not seem to affect the ability of mice to discriminate between these odortypes

(Wysocki, et al., 2004). 2-Heptanone, a well-known mouse pheromone, elicits responses in both the main and accessory olfactory bulbs (Xu, et al., 2005). In addition, genetic manipulations to selectively disrupt the function of the main olfactory epithelium, result in the loss of olfactory investigation and discrimination between different types of urine, as well as deficits in mating and aggressive behavior (Ma, et al., 2002; Mandiyan, et al., 2005). Similar results have been recently reported using intranasal irrigation of zinc sulfate (Keller, et al., 2006a; Keller, et al., 2006b), whereas surgical removal of the VNO did not affect olfactory discrimination, at least in females (Keller, et al., 2006c). Additional data has been collected in other species pointing to the same direction, including the opossum (Shapiro, et al., 1996), the pig (Dorries, et al., 1997) and the ferret (Kelliher, et al., 1998). Taken together, these data strongly argue for an important role of the main olfactory system in the detection of chemical cues known to be important for intraspecies communication.

In order to discriminate between these two olfactory systems, it would be useful to collect additional information on morphological and/or biochemical dimorphisms that may exist in the olfactory pathways of other mammalian species, since at present this has only been studied with detail in rats (reviewed in Guillamon and Segovia, 1997). Furthermore, it is necessary to establish more firmly the functional significance of these dimorphisms, particularly since some studies have failed to find a link with dimorphic patterns of neural activation or behavioral responses to olfactory stimuli (Bressler and Baum, 1996; Paredes, et al., 1998). Finally, there is still a great gap in our knowledge of steroid receptor expression in neural structures during development, since the organizational actions of gonadal steroids are exerted in the immature brain whose characteristics may vary from those exhibited by adult animals. There is evidence suggesting that the levels of expression of steroid receptors may be considerable higher during development, and they may be present in certain areas that do not express them during adulthood (Karolczak and Beyer, 1998; Raab, et al., 1999). Such information may provide the key to explain, for instance, the sexual dimorphisms identified at the level of overall morphology (Segovia and Guillamon, 1982), pheromone receptors expression (Alekseyenko, et al., 2006) and functional response (Halem et al., 1999; Halem, et al., 2001) in the rodent VNO, even though this structure does not appear to express any receptor for gonadal steroids in the adult (Alekseyenko et al., 2006).

The available data are sufficient to postulate that non-aromatized steroids provide a signal for the differentiation of at least certain aspects of the olfactory processing system in male mice. Additional evidence of a direct involvement of the AR in the differentiation of play behavior (Casto et al., 2003), coital behavior (Brand and Slob, 1991; Casto et al., 2003; Clemens, et al., 1978), sexual receptivity (Gladue and Clemens, 1978) and even HPA axis function (Seale, et al., 2005) has been advanced, as well as in the remodeling of sexually dimorphic brain nuclei (Dugger et al., 2007; Durazzo et al., 2007; Goto, et al., 2005) and aromatase activity (Beyer and Hutchison, 1997). The picture that emerges is thus one of aromatized and non-aromatized compounds acting via their specific receptors and complementing each other in the overall process of gonadal steroids-mediated brain differentiation.

It is yet unclear why two different signals instead of a single one have to be used to achieve this result, and that is without taking into account gonad-independent mechanisms of brain differentiation that also appear to be acting in parallel, as increasing evidence collected from mammals seems to indicate (Arnold, et al., 2003; Gatewood, et al., 2006). Finally, and perhaps linked with this latter issue, there is the question of why there is so much variation in the relative contribution of each specific signaling pathway (via ER or AR), even between closely related species. In rats, the model in which sexual differentiation of the brain by steroids has been studied more extensively, convincing evidence has been presented supporting a role of aromatized steroids in the differentiation of partner preference in males (Brand, et al., 1991; Vega Matuszczyk and Larsson, 1995) as well as in some of the morphological dimorphisms

characterized in the olfactory pathway in this species (Collado et al., 1993; Perez-Laso, et al., 1997). On the other hand, the differentiation of coital behavior seems to be at least partially AR-dependent in this case (Brand and Slob, 1991; Clemens et al., 1978). If it is so easy to switch the identity of the differentiating signal for a given trait in a relatively brief period of evolutionary divergence, why are both of them still functional among the different taxa? To find an answer to this will require studying these processes in a much wider range of species. Hopefully a phylogenetic approach will reveal a logical pattern behind what appears as random variation based on our present state of knowledge.

Acknowledgements

The author would like to thank Dr. Emilie Rissman for her useful comments on the manuscript. The work described from the Rissman Lab was supported by NIH grant R01 MH057759.

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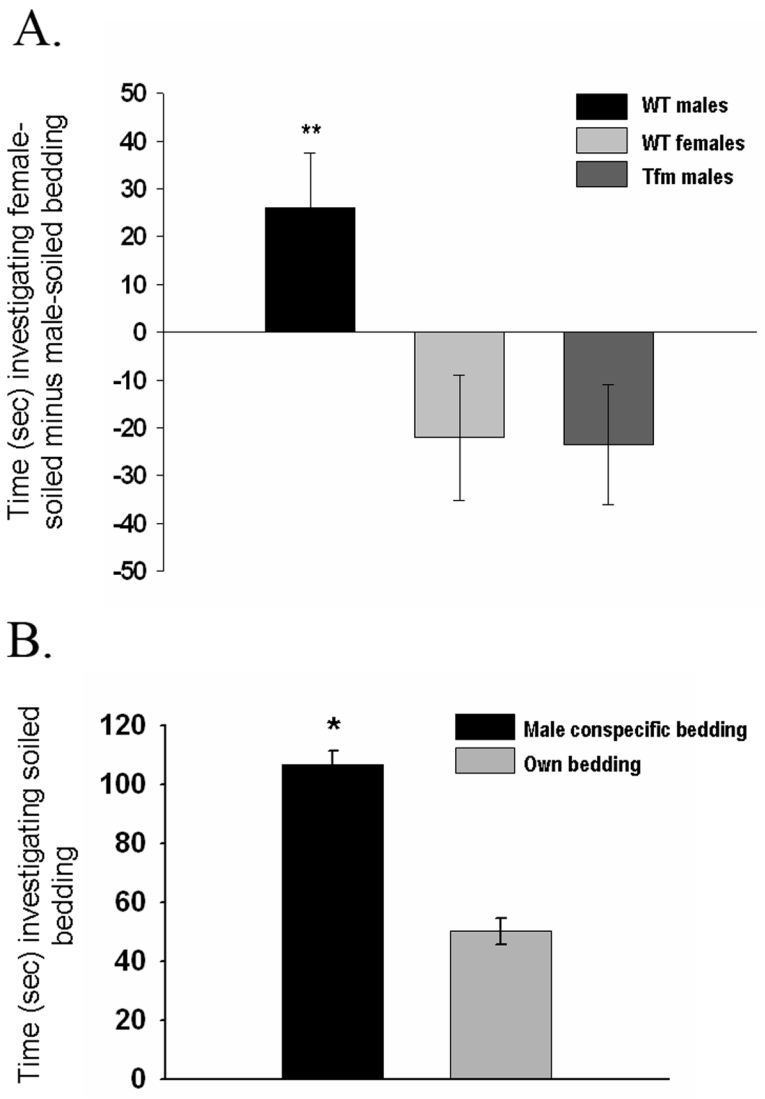

Fig. 1.

(A) Mean (± SEM) time spent sniffing soiled bedding from hormone-primed females minus time spent sniffing male-soiled bedding during the olfactory preference test. When time spent sniffing clean bedding was compared between the groups, no significant differences were found. WT males n=12, WT females n=9, Tfm males n=10. *Significantly different from the other two groups ($p<0.01$). Originally reported in (Bodo and Rissman, 2007)

(B) Mean (± SEM) time spent by gonadectimized, E2-treated adult males (n=4) sniffing soiled bedding from an unfamiliar male versus own bedding ($p<0.001$)

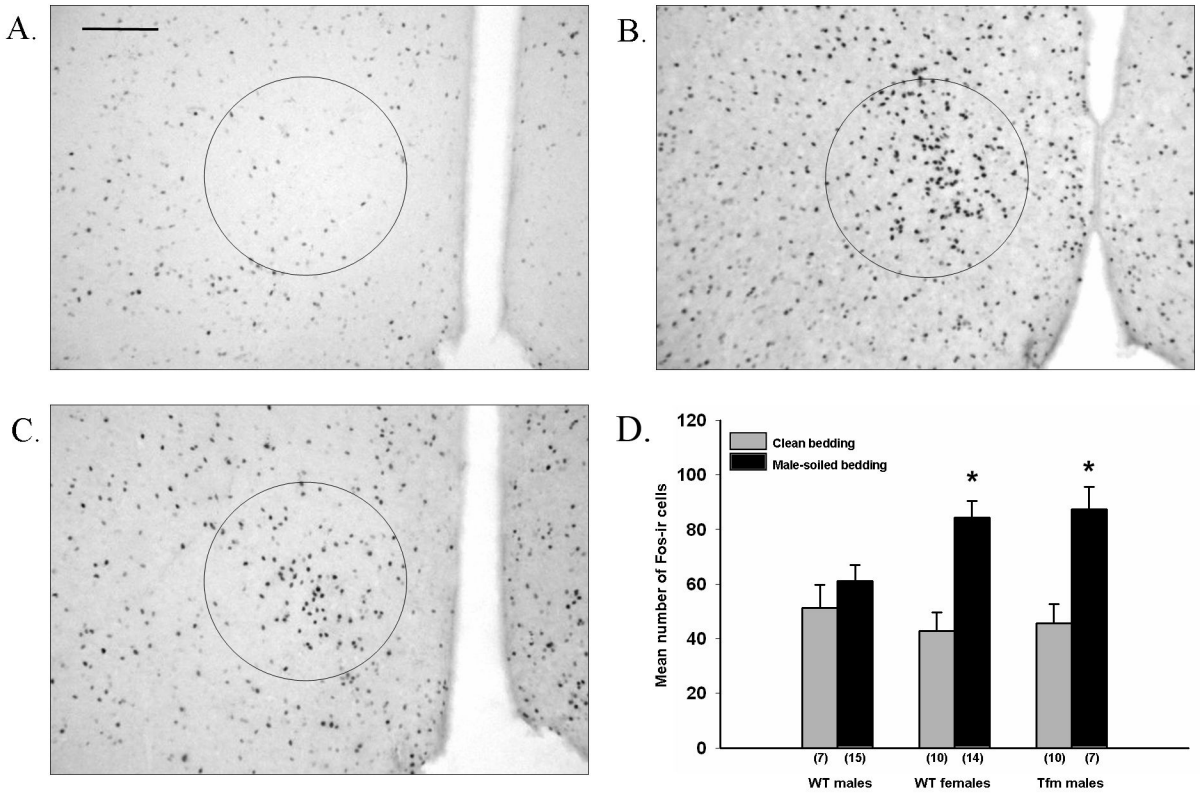

Fig. 2.

(A-C) Representative photomicrographs of c-Fos-ir neurons in the MPOA of Wild-type males (A), Wild-type females (B) and Tfm males (C) after exposure to male-soiled bedding. The circle indicates the area sampled on each section. 3v, third ventricle. Scale bar: 100 μm. (D) Mean (± SEM) number of c-Fos-ir cells present in the MPOA of mice after exposure to either clean or male-soiled bedding. The number of individuals in each group is shown in parentheses below the bars *Significantly different from subjects of the same experimental group exposed to clean bedding ($P < 0.05$). Originally reported in (Bodo and Rissman, 2007)

Table 1

Original reports describing the occurrence of high levels of masculine mating behavior (mounting with pelvic thrusting) in female rats and mice.

Reference	Species	Comparison with male group in the same study
(Beach, 1942)	Rat	no
(Beach and Rasquin, 1942)	Rat	no
(Whalen, et al., 1969)	Rat	no
(Pfaff, 1970)	Rat	yes
(Edwards and Burge, 1971)	Mouse	yes
(Coniglio and Clemens, 1972)	Rat	no
(Sodersten, 1972)	Rat	no
(Baum, et al., 1974)	Rat	no
(Emery and Sachs, 1975)	Rat	no
(Gladue, 1984)	Rat	no
(de Jonge, et al., 1986)	Rat	no
(Boehm and Aron, 1988)	Rat	no
(Oboh, et al., 1995)	Rat	yes
(Wersinger et al., 1997)	Mouse	yes
(Fang and Clemens, 1999a)	Rat	no
(Fang and Clemens, 1999b)	Rat	no
(Afonso and Pfaus, 2006)	Rat	no
(Bodo and Rissman, 2007)	Mouse	yes
(Jyotika, et al., 2007)	Mouse	yes