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Of mice and rats: key species variations in the sexual differentiation of brain and behavior

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Abstract

Mice and rats are important mammalian models in biomedical research. In contrast to other biomedical fields, work on sexual differentiation of brain and behavior has traditionally utilized comparative animal models. As mice are gaining in popularity, it is essential to acknowledge the differences between these two rodents. Here we review neural and behavioral sexual dimorphisms in rats and mice, which highlight species differences and experimental gaps in the literature, that are needed for direct species comparisons. Moving forward, investigators must answer fundamental questions about their chosen organism, and attend to both species and strain differences as they select the optimal animal models for their research questions.

Keywords

hypothalamus; calbindind28k; progestin receptor; estrogen receptor; nitric oxide; sexual dimorphism

Introduction

One distinct advantage of a comparative approach to neuroendocrine research is that information obtained from different model organisms can be used to determine general rules that may apply across many vertebrates. In behavioral neuroendocrinology, the goal is to establish both structural similarities and differences in the brain, and to relate these to function. Researchers have used a broad arsenal of species, but when it comes to rodents, the rat has been the traditional animal of choice. Laboratory rats display a variety of well-characterized behaviors and are practical to work with; they are docile, breed readily, have relatively large brains, enough blood for multiple hormone assays, and are easily maintained. Nonetheless, work with mice is on the rise for a number of reasons, particularly the availability of genetic models and tools.

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Where there is commonality, it is helpful to discuss research results from rats and mice (often referred to as “rodents”) together. Work done previously on rats is useful to guide and inform mouse studies. However, data from rats may not always generalize to mice. For example, the role of gonadal steroid hormones in the regulation of mounting behavior seems to differ between rats and mice, as well as between inbred mouse strains [38]. Additionally, the role of androgen versus estrogen receptors in brain sexual differentiation also varies between species. There are now enough critical data on sexual differentiation in the mouse that it is useful to compare this body of work with rat studies.

A few caveats need to be made as we describe and interpret the data within this review. First, sex differences are often examined in gonad-intact adult animals. This is not a problem if the goal is to identify sex differences *per se* and not to study the development of these differences. However, data from animals of different sexes, tested with different levels of gonadal hormones in circulation cannot be used to address questions on sexual differentiation. Second, hundreds of inbred rat and mouse strains exist and there are neural and behavioral differences between and within strains [135]. It is clear that similar studies conducted in different rat or mouse strains may have different outcomes, and we need to be cautious about general conclusions based on only one or two strains. Strain differences can be used to the experimenters’ advantage when examining genetic factors that influence behavior. In addition, for specific behaviors, some strains are more useful than others. For example, in mice, DBA/2 is an aggressive strain while C57BL/6 is not [116]. If expression of a gene is hypothesized to reduce resident-intruder aggression, it might be best to knock it down in a relatively unaggressive strain, such as C57BL/6. However, if the gene is predicted to increase aggression, the more aggressive DBA/2 strain might be a better choice. Therefore, the conclusion that a gene does or does not affect a particular behavior might vary depending on the inbred strain employed.

Another source of variation is that individual investigators working with mice often do their own colony maintenance using different breeding protocols. Consequently, a C57BL/6J mouse in one investigator’s lab may be genetically different from a C57BL/6J purchased from Jackson Laboratories [61]. Additionally, different commercial breeding houses produce their own lineages of rats and mice that, while derived from the same ancestral strains (i.e. C57BL/6), have been maintained in closed colonies for multiple generations. Gene mutations and changes in copy numbers between lineages have been observed [61] and may affect brain and behavior [206]. Due to the early development of inbred strains of mice and their current widespread use in molecular genetics, there is substantially more information concerning strain differences in mice than rats on numerous, specific levels [36,91,128]. Even so, both strain and species differences will be considered in this review.

Finally, much of the mouse data has been generated with gene-disrupted, KD, or knockout (KO) models. If we are concerned with the developmental process of sexual differentiation, and are examining endpoints at or shortly after birth, the fact that the targeted genes are not functional during development helps in determining the developmental function of the knocked out gene. Alternatively, if we are assessing developmental effects on adult behaviors, and these behaviors require adult expression of the same gene that has been disrupted, we cannot learn much about the developmental role of the gene. A related issue pertinent to work done with knockout and transgenic mice, is that newly generated models are often tested before they are completely backcrossed (at least 10 generations) into a more uniform genetic background. Many reports of phenotypic effects of gene knockouts have been based on work done in mice from mixed genetic backgrounds. Once a disrupted gene is moved into a more uniform background, previously reported differences might be diminished, amplified, or no longer exist because of allelic changes in behavior modifying genes. These issues may make it difficult for laboratories to replicate each other’s work, or

even lead to failures to replicate within a lab as the KO mouse is bred into a pure background.

While keeping these confounding factors in mind, this review will first consider sexual differentiation of neuroanatomical markers in the hypothalamus that provide examples of how sex differences in mice differ from rats. Next we will discuss sexual differentiation of three behaviors: partner preference, masculine sexual behavior, and female sexual behavior. Certainly there are many more sexually dimorphic behaviors in rodents (and other animals); however, the paucity of mouse data prevents comparing mice with rats for many behavioral tasks. We have selected these three particular behaviors, because there are a sufficient number of mouse studies from which to draw information.

Sex and Species Differences in Neuroanatomical Markers in the Hypothalamus

A major contributing factor in sexual differentiation of the rodent brain is the gonadal secretion of testosterone during a specific critical period in male development. In certain tissues, including the brain, testosterone is converted to estradiol by the enzyme aromatase. Brain sexual dimorphisms and concordant sex differences in physiology and behavior arise primarily due to these critical period influences. The neonatal critical period for development of rat brain begins before birth ending by postnatal day 10 (PN10) [76,243]. Exposure to testosterone or estradiol masculinizes and defeminizes aspects of brain morphology and results in sex-specific behaviors (reviewed in [18]). Whether the critical period for mouse brain sexual differentiation is comparable to that of the rat is discussed in later sections.

Calbindin in the Preoptic Area (POA)

Calbindin, a 28kD protein with a Ca^{2+} binding domain, was discovered in peripheral tissues (e.g., intestine and kidney), but has emerged as an interesting molecular marker in brain. For now, ‘marker’ may be the best term, as the exact function(s) of calbindin remain unclear. Calbindin is important for Purkinje cell physiology (reviewed in [42]), and *in vivo* rat studies have demonstrated that calbindin plays a role in neuroprotection following insult to the hippocampus [168,187], substantia nigra [96,240] and cortex [242]. In mice, knockout studies have not supported the hypothesis that calbindin plays a significant role in preventing neuronal damage following insult [2,16,68,106,188], but it is possible that other redundant calcium binding proteins in the brain compensate for the loss of calbindin expression. In some studies calbindin is referred to as a phenotypic marker for GABAergic neurons in telencephalic regions ([220] monkey; [104] rat); whereas in other studies it is referred to it as a “*biomarker*” for sexual dimorphism in the hypothalamus [56]. Sex differences (male > female) in the levels of both calbindin immunoreactivity and mRNA expression in the preoptic area (POA) were first identified by western and northern blots [117,118,208]. These findings were further refined to show a specific sex difference in calbindin expression in the neonatal rat preoptic area/anterior hypothalamus (POA/AH; [31,161,163,186,197]) with the highest level of calbindin expression in the classically identified (i.e., based on Nissl stained cellular architecture) sexually dimorphic nucleus (SDN).

From the perspective of comparing rats and mice in the context of sex differences, calbindin is an interesting protein. In rats, the levels of immunoreactive calbindin in the SDN are low at birth and increase as the nucleus emerges and differentiates postnatally [197]. In mice, however, calbindin expression is high in the POA prenatally (Tobet and Rissman et al., unpublished observation), and sex differences (male > female) only become apparent as the

levels of immunoreactive calbindin fall postnatally, becoming highly restricted to the potential homolog of the adult rat SDN [37,56]. On the day of birth (PN0), a sex difference is apparent in the number of calbindin immunoreactive cells in mice [56]. However, in adulthood the sex difference is related to the position of calbindin immunoreactive cells in males compared to female, and not to the total number of calbindin immunopositive cells in this region [37].

Studies on the hormone dependence of calbindin expression in rats are somewhat conflicting. Dissections of both prepubertal and adult hypothalamic tissue followed by western blot analysis showed both estrogen and androgen dependent increases in expression [209], but immunocytochemistry (ICC), only revealed an estradiol driven increase in calbindin within the prepubertal rat SDN [197]. The difference in results between these two rat studies may indicate the existence of a within-strain difference in terms of how gonadal hormones regulate calbindin. However, another complication is the fact that the suprachiasmatic nucleus (SCN), which contains high levels of calbindin, was contained in the dissections for the Western analysis in rats. In general, both of these studies demonstrated that calbindin expression in rat hypothalamus is dependent upon estrogens, and it is unlikely that calbindin expression selectively in the SDN is regulated by androgens.

In mice, it is clear that the pattern of calbindin immunoreactivity is gonad dependent, as the arrangement, but not the total number, of these cells is altered when the *steroidogenic factor-1* gene (*SF-1*) is disrupted [37]. In SF-1 knockout mice (SF-1 KO), the gonads and adrenals do not form and these mice provide a model of gonadal hormone independent sexual differentiation [37,77,123]. SF-1 KO genetic males have immunoreactive calbindin cells scattered within the POA/AH similar to both knockout and wildtype (WT) females [37]. Furthermore, evidence from examination of the POA/AH of testicular feminization (Tfm) mutants, which lack a functional androgen receptor (AR) gene, and also of estrogen receptor α knockout (ERαKO) mice (both on the C57BL/6J background) suggest that the sex difference in calbindin is caused by activation of androgen receptors rather than estrogen receptors ([56]; J. H. Hall, T. L. Dellovade, E. F. Rissman and S. A. Tobet, unpublished data). Moreover, as with Tfm mutants, females exposed to the non-aromatizable androgen dihydrotestosterone (DHT) at birth show male-typical numbers and patterns of calbindin immunoreactive cells [26].

Estrogen Receptor β in the Anteroventral Periventricular Preoptic Area (AVPV)

The anteroventral periventricular nucleus (AVPV) in the POA lies adjacent medially and ventral to the medial POA (mPOA). The AVPV is well characterized as sexually dimorphic in rats [216] and mice [67] with females in both cases having greater volume and number of cells than males. There is a significant difference in the expression pattern of estrogen receptor β (ERβ) in the AVPV of rats as compared to mice. ERβ [143] is one of two identified classical nuclear steroid receptors that binds estradiol and impacts both transcription in the nucleus, and induces signal transduction at extra-nuclear sites (reviewed in [138]). In adult rats, ERβ in the AVPV is both highly expressed [195] and sexually dimorphic (female > male) [154], whereas in adult mice the levels of ERβ expression in the AVPV are low [139] to undetectable [40]. In the female rat AVPV 18% of ERβ positive cells co-localize with TH [154]. The cells expressing both ERβ and TH in the female rat AVPV may play a role in the modulation of the luteinizing hormone (LH) surge that occurs during the proestrus phase of the estrous cycle [154,198]. Comparable studies have not been done in mice.

The expression of ERβ in the rat AVPV during development has not been as clearly defined as in the adult, although a role for ERβ in development has been indicated [160]. There are conflicting studies that describe the presence [154] or absence [165] of ERβ in the AVPV at

postnatal day 7 (PN7). One possible explanation for this discrepancy could be due to strain differences in the rats used in these studies (Sprague-Dawley versus Fisher 344, respectively). Heterogeneity of ERβ expression in the AVPV between rat strains may be less surprising in the context of the regional differences (and lack of sex differences) found for AVPV structure in general in other mammalian species including rabbits [24], ferrets [20], and sheep [201].

In contrast to rats, no expression of ERβ in the AVPV has been observed during development in mice (authors unpublished observation [238]. However, modulation of ERβ activity appears to impact the development of the AVPV in mice, as seen through increased TH-immunoreactive neurons in the AVPV of ERβ knockout (ERβKO) males as compared to females, possibly through extra-nuclear activation [27,111]. In both rats and mice ERα plays a necessary role in development of the AVPV [27,199,229] and it is likely that a combination of effects, activated by both ERα and ERβ, leads to the normal phenotype. It is possible that development of the AVPV in both rats and mice follow similar patterns, being dependent on both receptors, with the impact of ERβ occurring indirectly through projections from other brain regions. In reference to ERβ, the endpoint of development of rats versus mice is different. Rats have a clearly defined sex difference in levels of ERβ expression in the AVPV, while expression of ERβ in the mouse AVPV is largely undetectable.

Progestin Receptor in the POA and Ventromedial Nucleus of the Hypothalamus (VMH)

One of the clearest examples of a transcriptional event mediated directly by the estrogen receptor is the induction of progestin receptors (PRs). PRs are important for the display of sexual receptivity in adult female rodents. In both rats and mice, there is a striking sex difference in the number of PR immunoreactive cells in the neonatal mPOA that is dependent upon gonadal steroid hormone induction (reviewed [223]). Estradiol, aromatized from circulating testosterone, induces large areas positive for PR immunoreactivity in the POA of perinatal male rats, whereas PR immunoreactivity is virtually absent in females at this stage [224,225]. This proved to be similar in C57BL/6J mice [225]. The similarity in PR distribution between rats and mice extends to the more rostral region of the AVPV [175,223], but not to the more caudal region of the ventrolateral quadrant of the ventromedial nucleus of the hypothalamus (VMH; In the VMH, a sex difference in the total area of PR immunoreactivity was seen in neonatal mice (male > female as for the rostral regions) [225], In rats, however, this sex difference was not found (male = female for total area of PR immunoreactivity at P7) [174].

Neuronal Nitric Oxide Signaling Throughout the Hypothalamus

Nitric oxide (NO) is a signaling molecule that is synthesized through the conversion of L-arginine to L-citrulline by nitric oxide synthases (NOS) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen. Neuronal NOS (nNOS) is differentially distributed in a number of brain regions across vertebrate phylogeny. Reports relating NO to the reproductive axis date back to the early 1990's when NO was found to facilitate sexual behaviors in female rats [124]. Sex hormones were found to influence NOS activity in a number of tissues in guinea pigs [230], and a sex difference (female > male) was reported in the ability of estradiol to induce NADPH-diaphorase activity (an indicator for nNOS) in the VMH [149]. A number of reports over the years have focused on the ability of gonadal steroids to regulate NOS expression and/or NO production in regions of the brain that are important for neuroendocrine function (e.g., hypothalamus). While there is agreement that sex steroids can influence expression of NOS protein, diaphorase activity, or NO production, there are fewer studies that show sex differences *per se*. However, sex differences have been found in three hypothalamic regions: the POA, AVPV, and VMH.

Studies of the POA in rats and mice show high levels of immunoreactive nNOS (reviewed [95,157,190], suggesting that NO is abundant. In gonad-intact, wildtype adult rats (~ 2 months age), obtained by breeding Tfm and Wistar strains, males have higher numbers of immunoreactive nNOS (ir-nNOS) cells than estrus females [127]. In the absence of fully functional androgen receptors there are fewer numbers of ir-nNOS containing cells (in Tfm males) suggesting that the sex difference in wildtype rats is due to androgen induction of nNOS in males [127]. By contrast, gonadectomized juvenile Sprague–Dawley female rats have more nNOS mRNA containing cells than males [97]. Moreover, estradiol followed by progesterone treatment decreases the number of nNOS mRNA containing cells in female rats, but has no effect in males [97]. Moreover, estradiol followed by progesterone treatment decreases the number of nNOS mRNA containing cells in female rats, but has no effect in males [97]. In sum, androgens appear to upregulate nNOS expression in the rat POA, whereas exposure to ovarian steroids leads to a reduction in expression levels.

Sex differences in nNOS expression and activity have been found in the POA of several mouse strains, and studies have identified a role for gonadal steroids in nNOS regulation. D2C6F1 hybrid adult males have more ir-nNOS containing cells in the mPOA than females at every stage of the estrous cycle except estrus [196]. In another strain (Thy1-YFP), sex differences in ir-nNOS are noted as early as PN0 in the POA/AH (males > females) [56]. Interestingly, testosterone appears to reduce the levels of ir-nNOS labeling in males. When intact adult C57BL/6 males and females were testosterone treated, males showed a greater number of ir-nNOS cells than females [190]. This action occurred through both ERα and AR activation, with ERα inducing an increased number of ir-nNOS cells and total area, and AR reducing the total ir-nNOS cell number. A second study (mice on a C57BL/6 background) also identified a sex difference in ir-nNOS area in the caudal POA/AH (male > female) in both WT and SF-1 knockout mice [37]. The fact that the difference was noted in the agonadal SF-1 knockout mice indicates that the maintenance of this sex differences is gonad-independent. Thus, while both rats and mice express ir-nNOS in the POA, the steroidal modulation of this expression appears to be opposite. In rats, androgens increase nNOS levels through the AR, while estrogens decrease expression. In mice the opposite is true, with estrogens acting through ERα to increase ir-nNOS levels, while activation of the AR decreases expression.

There are few studies examining sex differences in nNOS in the AVPV in mice or rats. In mice, (C57BL/6 background) more ir-nNOS is present in males than females at birth (predominantly in fibers; [56]) and in adulthood (a mixture of fibers and cell bodies; [37]). The only experiment showing nNOS in the region of AVPV in male and female rats suggested that cells containing nNOS mRNA lie outside the defined nucleus. Interestingly, in the region surrounding the nucleus, juvenile gonadectomized females had more nNOS mRNA containing cells than gonadectomized males (Sprague-Dawley strain; [97]).

The VMH has been characterized for a number of sexual dimorphisms [78], and, with regard to sex differences in nNOS, it provides the additional complication of potential strain differences within both rats and mice. For WT litter mates from a Wistar-Tfm cross, ir–nNOS cell numbers in the VMH were greater in intact adult males than females [127]. However, in Sprague-Dawley rats no sex difference was observed for NADPH-diaphorase positive cells in the VMH [149]. In mice (a cross between DBA/2 and C57BL/6J), males had a larger number of ir-nNOS cells in the VMH than females sampled on several different cycle days [196]. However, in another study using mice on a C57BL/6 background treated with testosterone propionate for 7 days prior to sacrifice, more cells were found in the most ventrolateral portion of the VMH of adult females than equivalently treated males [37]. These findings in adults were consistent with C57BL/6 mice examined at birth in which more ir-nNOS was found in females than males [56]. The differences in results may be due

to the strains used in these studies, or alternatively, caused by differences in experimental conditions, like testosterone treatment, the laboratory conditions, the assays used, or the developmental stage of the animal. Regardless of the reason for the discrepancies, they make it difficult to identify clear differences in NO signaling pathways between rats and mice.

Summary

Examination of neuroanatomical markers within the hypothalamus of rats and mice, demonstrate some species differences. Developmental expression of calbindin in the POA differs between the two species, and the development of sexual dimorphism in this nucleus is dependent upon different hormones. ERβ expression in the AVPV is virtually absent in mice, yet the differentiation of the AVPV is altered in the ERβ KO mouse suggesting that differentiation of this nucleus may rely on input from other, ERβ expressing cells outside of the AVPV. The sexual dimorphism in progesterone receptor expression is similar between rats and mice, but mice have an additional sex difference in the VMH that is not seen in rats. Finally, the more generalized dimorphism in nNOS expression throughout the hypothalamus is difficult to contrast in these species, since there appear to be strain differences within species.

Sex and Species Differences in Partner Preference Behavior

The hypothalamus is one of the major brain areas responsible for sex-specific behaviors, but inputs from the olfactory system to the hypothalamus are also very important to the display of these behaviors. Many of the sex differences within the olfactory system of rats and mice have been presumed to be the same, therefore there are gaps in the literature that make direct comparisons of rats and mice difficult. Nonetheless, species differences in olfactory regulated behaviors, including partner preferences, and the hormonal influences over these behaviors suggest that olfactory systems may develop differently across rodent species.

Sex Differences in the Rodent Olfactory System

The rodent olfactory system has been divided into at least two, functionally distinct pathways: the main olfactory system (including the olfactory epithelium and main olfactory bulb {MOB}) and the accessory olfactory system (including the vomeronasal organ {VNO} and the accessory olfactory bulb {AOB}). The main olfactory system is thought to be devoted to detection of volatile odorants such as those from food, predators, and potential mates [66]. The accessory olfactory system is used to detect non-volatile odorants that influence reproductive and aggressive behaviors [105], and also to aid in recognition of conspecifics [122]. While the separation of the two systems has provided a useful heuristic model, in reality there is overlap in function [101,102]. For many years the accessory olfactory pathway was believed to exert more influence on sexually dimorphic behaviors than the MOB; and sex differences in this pathway have been more extensively studied in this context.

In rats, most of the studies of sexual dimorphism in the accessory pathway have been structural rather than functional. In Wistar rats, the VNO [192,194], AOB [193], bed nucleus of the accessory olfactory tract (BAOT) [44], medial amygdala (MeA) [140], and medial posterior nucleus of the bed nucleus of the stria terminalis (BNST) [193] are all larger in males than in females. One exception is the medial anterior region of the BNST which is larger in females than males [53]. In contrast to rats, the focus in mice has been on sex differences in the activation of the accessory pathway as shown by immediate early gene activation (Fos) in specific olfactory processing regions, including the VNO, the AOB, and downstream nuclei. There are sex differences in VNO Fos responses to both male [80] and female bedding [81] and the response of the AOB to volatile odors is sexually dimorphic,

with opposite-sex odors activating the accessory olfactory bulb of both males and females and same-sex odors having no effect [126].

Sex Differences in Partner Preferences

While it remains to be determined whether rats and mice have the same structural dimorphisms within the accessory olfactory pathway, the function of these regions has been examined by assessment of partner preferences. Partner preference has been studied a number of ways, including measuring the amount of time spent associating with a male versus a female or samples of their soiled bedding, assessing hormonal responses such as luteinizing hormone (LH) surges to conspecific odorants, and/or analysis of neuronal responses such as Fos (as previously discussed). The large variety of methods used has led to a rather confusing body of literature.

In 1978, Hetta and Meyerson showed that sexually experienced male Sprague-Dawley and Wistar rats display a female-oriented partner preference when choosing between a male and an estrus female separated by a wire mesh [89]. When given full access to the female, complete olfactory bulb removal in male rats abolishes the preference for an estrus female over a non-receptive female [59]. However, lesions of the POA, which results in cessation of copulation in males, do not eliminate partner preferences [60]. Similarly, female rats maintain a preference for sexually active males over castrated males, even after receiving bilateral midbrain lesions that eliminate their display of other sexual behaviors [167]. When POA lesions were combined with olfactory bulb deafferentation (removal of olfactory inputs from the olfactory epithelium to the olfactory bulbs), male partner preferences were abolished [60]. Thus, in rats, preferences for the opposite sex are independent of the ability to mate, and olfactory inputs through the olfactory bulbs are necessary to maintain partner preferences.

Male mice are also attracted to urine from gonad-intact females in behavioral tests, but show less investigatory interest when presented with urine from gonadectomized females [98]. In the same way, female mice sniff urine from gonad-intact males more frequently than urine from castrated males, suggesting a preference for these odors [191]. In C57BL/6 female mice with complete VNO ablation, preference for male volatile odors persist even though the ability to discriminate between non-volatile odors is decreased [103]. On the other hand, male C57BL/6 mice without a VNO can distinguish between volatile urinary odors from estrus females and intact males, as well as between non-volatile odors from estrus versus ovariectomized (OVX) females and from intact versus castrated males [156]. These findings suggest that both olfactory systems regulate preference for male urine in female mice, while in male mice the VNO is not required for sex discrimination. Moreover, these results strengthen the argument that sex differences within the accessory olfactory pathway may be responsible for behavioral sex differences in mice.

The Critical Period in Development of Partner Preferences: Do Species Differences Exist?

In rats, early studies sought to discover whether the critical period for partner preference is the same as that of male sexual behavior. In the first such study, Davis *et al.* [51] treated male rats of the CD strain with the aromatase inhibitor androstatriene-3, 17-dione (ATD) from PN1-10. Neonatal ATD treatment did not affect male preferences for females, suggesting that the critical period for male partner preference does not coincide with that of masculine sexual behavior. However, subsequent studies reported different results. When male rats were castrated neonatally, and tested as adults, they showed a decreased preference for an estrus female as compared to control males, even after testosterone treatment [33]. Moreover, when ATD was administered prenatally (from E11 to birth) and/or neonatally (PN0-10), there was a decline in male preference behaviors [35]. When repeatedly tested

with an estrus female and an intact male, neonatally ATD-treated males showed significantly lower preference scores for an estrus female, whereas prenatally treated males scored only slightly lower than controls. Few reciprocal studies have been reported in female rats, but when given androgens on PN0, testosterone but not DHT promoted female preferences for estrus females over gonad-intact males [33]. Taken together, these results suggest that the critical period for partner preference in rats appears to coincide with the prenatal critical period for sexual behaviors. While experiments similar to these have yet to be conducted in normal mice, a discussion of similar work in KO mice follows.

Estrogens During the Critical Period

To ascertain whether aromatization of androgens to estrogens is essential for sexual differentiation of partner preference, Matuszczyk and Larrson [221] treated pregnant Wistar rats with either the anti-estrogen nitromifene citrate (CI628) or the anti-androgen cyproterone acetate (CA) and examined the behavior of their male offspring. As gonad-intact adults, all males showed a partner preference for a female after sexual experience. However, after castration and testosterone treatment, preference for a female persisted only in vehicle and anti-androgen treated animals, while males given anti-estrogen during gestation showed a reduced preference. These results are perplexing; it is not clear why castrated and testosterone treated males would not behave like gonad-intact males. Differences in circulating hormone levels and/or repeated testing may account for this discrepancy; but those data have not been provided. Additional evidence for the role of estrogens exists, though. Female rats treated with estradiol or testosterone as pups had male-like partner preference when given access to tethered stimulus animals [33,87]. Thus, in male rats, estrogens likely act during development to differentiate partner preferences, but it is not clear how normal female partner preferences are established.

The majority of data on sexual differentiation of partner preference in mice have been collected using knockout mouse models. Male ERαKO mice (backcrossed into C57BL/6) showed no partner preference and spent little time investigating stimulus males or females. However, when gonad-intact adult males of both WT and ERαKO genotypes were exposed to female-soiled bedding they exhibit both an LH surge and Fos responses in the POA [232], indicating that their olfactory systems were capable of responding to female odors at a neural but not a behavioral level. Neural integration of olfaction may involve the organization of dopamine inputs or activation of dopamine release, because ERαKO male and female mice treated with a dopamine agonist displayed sex-typical partner preferences [233]. The lack of ERα during development was not an impediment, as adult ERαKO mice could regain sex-typical preference behaviors. This argues against a critical role for ERα in the development of this behavior.

Other evidence for a role of estrogens in organization of partner preference comes from studies of aromatase knockout (ArKO) mice (on C57BL/6 background). Male ArKO mice have no preference for female over male volatile odors [10], and, while treatment with estradiol in adulthood restored coital ability in ArKO male mice, it did not stimulate olfactory investigation of volatile body odors [12]. Perhaps hormonal treatment in adulthood was not sufficient to reinstate male odor preference behaviors because the olfactory pathways have been organized in the absence of prenatal estrogens. Together with the data on ERαKO mice these studies raise the possibility that estrogens are acting developmentally via an ER other than ERα.

Organization of female partner preferences in rats does not require estrogens, however, there are data to suggest this is the case in mice. ArKO female mice treated with testosterone as adults spend significantly less time sniffing both volatile and non-volatile odors from either males or females as compared to heterozygous or WT females. Adult treatment with

estradiol recovers female-typical investigation of non-volatile odors, but not of volatile odors [9]. In addition, gonadectomized and estradiol-treated ArKO female mice show normal Fos responses to male odors in several regions along the olfactory input pathway. The only area in which ArKO females fail to demonstrate a Fos response to male odors is the VMH; this area might be organized by prenatal and/or pubertal estradiol [171]. Thus, in mice, estrogens may be involved in the development of female olfactory circuits.

Androgens During the Critical Period are Important for Mouse, but not Rat Partner Preferences

Most of the data collected in rats indicate that developmental estrogens are required for male partner preference behavior. However, a role for androgen signaling has not been completely ruled out. Male rats treated prenatally with the AR antagonist flutamide had normal partner preference for females over males, but theirl Fos responses elicited by exposure to estrus bedding were eliminated [55]. Tfm male rats had partner preferences similar to WT males when choosing between an estrus and a non-estrus female [82], but there are no data on Fos responses from these experiments. In addition, females treated prenatally with an anti-androgen or neonatally with DHT showed a normal preference for an intact male [34]. Taken together, these results suggest that androgens do not influence preference behaviors in rats, but may still play a role in the organization of olfactory pathways.

In mice, a role of androgen signaling in partner preference behaviors is more compelling. The developing brains of female mice lacking alpha fetoprotein (AFP-KO), an estrogen binding protein present in the developing fetus, are exposed to free estradiol. Using adult, gonadectomized and estradiol treated AFP-KO mice (CD-1 background), Bakker *et al.* [13], showed that exposure to estrogens during development did not change female preference for either male volatile odors or male soiled bedding. Moreover, AFP-KO females on a C57BL/6 background, showed a preference for male odors that was not observed in C57BL/6 control females.

Another line of evidence for a role for androgens comes from Tfm mice tested with soiled bedding from estrus females and sexually active males. The Tfm mice, like WT females, show a slight preference for male-soiled over female-soiled bedding, while WT males show a strong preference for female bedding. Using awake stimulus animals in a Y-maze apparatus, Tfm mice behaved more like WT females than WT males and expressed no preference for either stimulus mouse [25]. Moreover, when DHT was administered on PN0 to C56BL/6J females, it masculinized their partner preference and Fos responses in the medial POA and BNST. In contrast, estradiol given to female pups from PN0-2 did not affect either measure [26]. Taken together, these findings suggest that androgens acting through the AR organize male-typical partner preference in mice.

One recent study using the AR^{NesCre} mouse (a conditional knockout designed to lack AR only in neurons) is in seeming opposition to the data collected using Tfm mice. When tested with gonads intact, males with the neural-KO males showed a normal preference for estrus female odors and when presented with male-soiled, female-soiled or clean bedding, they displayed normal Fos responses to female-soiled bedding in the MeA and POA [176]. There are several potential reasons for these differences including differences in hormone levels at the time of the tests. The data collected with Tfm mutants and reviewed above used low levels of estradiol to produce comparable hormone levels between test subjects, while the work with the conditional KO mice used gonad-intact animals. In addition, it is not clear that these mice represent a complete neural knockout of AR, since astrocytes [121] also express AR in rat brain, finally it is also possible that the presence or absence of AR in non-neural tissues causes behavioral changes.

Summary

Partner preference behaviors have been examined in both rats and mice using a variety of techniques (reviewed in Tables 1 and 2). In rats, sexual dimorphisms within the accessory olfactory pathway have been well characterized, and partner preference behaviors persist without MOB inputs. The critical period for development of partner preference behaviors has been well defined in rats, and estrogens during development are required for organizing the olfactory pathways involved in partner preference. In mice, functional dimorphisms exist throughout the olfactory pathway, but structural dimorphisms are not as well defined. Additionally, both the main and accessory pathways are necessary for the display of female partner preferences, while in male mice the VNO is not required for sex discrimination. The critical period in mice has not been separately determined, but, in contrast to rats, it appears that both estrogens and androgens organize partner preference behaviors.

Despite the body of work on partner preferences in rats and mice, there are still several basic questions that remain to be answered. First, in rats, it is necessary to examine whether the structural sexual dimorphisms in the olfactory pathways have functional correlates in the display of preference behavior. It is no longer sufficient to assume that the Fos studies in mice correspond with findings in rats, particularly since there are differences in which olfactory pathways are utilized for partner preference. Second, the critical period for development of partner preference needs to be defined in mice. Third, the role of estrogens and androgens in development of preferences needs to be investigated more completely, particularly in mice.

Masculine Sexual Behavior

Not surprisingly, much of the social behavior that rodents engage in is related to reproduction, such as maintaining a breeding territory, seeking mates, mating, and caring for young. All these behaviors are sexually dimorphic, but the sexual differentiation of copulatory behavior is the best characterized of all rodent social behaviors. This is likely due to several factors, including that the behaviors are stereotyped, stable, and quantifiable. In a landmark study of sexual differentiation, pregnant female guinea pigs received androgen injections. Females born to testosterone-injected dams had masculinized external genitalia, and after adult treatment with additional testosterone, they displayed more male-like mounting and thrusting behaviors as compared with females born to control dams. Despite these prenatal treatments, females did not equal males in the numbers of mounting events [170].

Subsequent studies, done in rats, sought to determine how androgens organized these behaviors. Interestingly, female Sprague-Dawley rats, like guinea pigs, treated in adulthood only with testosterone proprionate (TP) showed low levels of mounting and thrusting behaviors [189]. This strain has been especially valuable for studies of sexual differentiation, because hormonal manipulations of females during development can produce readily discernible changes in the expression of male-like behaviors in adulthood. In contrast, female Long Evans rats can display male-like mounting and thrusting at high levels when they receive adult hormone treatment [1,62]. Strain differences are present in mice also, but female mice of several strains display high levels of mounting and thrusting male-like sexual behavior when tested as adults [38,54,234]

Androgens and the Organization of Masculine Sexual Behaviors (MSB)

Androgen Levels during Development—Studies in rats have pursued the “critical period” for androgen exposure by measuring hormone concentrations in male and female rat pups and embryos [4,136,200,228,231]. Male rats have elevated testosterone concentrations

relative to females just before birth (E18, 19, and 20), and again after birth for at least 3 hours and up to 5 days. These findings, along with the ease and precision of giving hormones postnatally, have led to an extensive focus on administration of hormones or drugs that interfere with hormone exposure during the first 5 days (or less) after birth.

The neonatal patterns for androgen secretion in the male mouse may be similar to the rat. Male embryos of the CF1 strain have higher testosterone levels in plasma on E17 than females [222]. In C57BL/6J mice, testosterone concentrations increase an average of 4-fold in male plasma during the first 2 hours after birth and then drop to low levels, comparable to females, for the next 24 hours [46,144]. In addition, when three postnatal days (PN0, 2, and 4) were compared in ICR mice, at all time points males had 2–3 times higher concentrations of testosterone in plasma than females [155]. These are the only studies we are aware of that have measured androgen levels during neonatal mouse development. There remains a need for a thorough investigation of steroid hormone levels in both mouse embryos and pups, along with direct strain comparisons.

Androgen Exposure and Male Sexual Behavior in Females—In 1973, Sachs and colleagues [184] found that female Long-Evans rats can perform male sexual behavior, and do so in a manner closest to normal males when both pre- and postnatal testosterone is provided. When testosterone was given both before and after birth, the majority of adult females displayed the complete ejaculatory reflex and the patterning of their mounting and thrusting behavior was similar to control males, with the exception of longer latencies to display the ejaculatory reflex. However, in a separate study OVX adults received long-term treatment with estradiol produced ejaculatory reflexes similar to males. In fact, post-ejaculatory mounting and intromissive behaviors resumed more rapidly in these females than in intact adult males [62]. These results demonstrate that no extra perinatal androgen exposure is needed to elicit adult masculine behaviors in female Long-Evans rats as long as high levels of estradiol are provided chronically to the adults prior to and during testing.

Comparable hormone treatment studies have been performed in mice of various strains, with varying results. In C57BL/6 by DBA/2 hybrid females, a single injection of testosterone on PN0 did not increase the display of masculine sexual behaviors in ovary-intact adult females [125]. However, when early androgen-treated females also received testosterone at the time of the test, ejaculatory responses were noted in most animals (13 out of 16). When compared with normal males, the duration of the ejaculations were shorter, and the latencies longer, in the neonatal androgen-treated females. Interestingly, these females exhibit much longer intra-ejaculation “refractory” intervals than males. This result leads to the hypothesis that neonatal androgens in mice is to organize the ejaculatory reflex.

Inbred strains of mice have also been tested. BALB/c female pups that received testosterone on PN0 did not show any higher levels of masculine sexual behaviors in adulthood as compared with control females, the vast majority of which displayed mounting and thrusting [90]. Additional work in other strains reported that neonatal testosterone injections had little impact on male sex behaviors. In Swiss-Webster female mice, an injection of testosterone or oil on PN0 or on PN10 and additional testosterone in adulthood lead to mounting, but the majority (68–90%) of the control-injected females likewise displayed these behaviors. Mount frequencies were equivalent in all groups and, in all animals, mounting behavior increased with testosterone dose at the time of the test [58]. To directly compare strain differences, females from three strains (A strain, BALB/c, and C57BL/6) were injected on PN3 with oil, testosterone or estradiol and, as ovary-intact adults tested, with receptive females. In general, strain differences accounted for more of the variability than hormone treatments and C57BL/6 females given neonatal testosterone displayed the most male-typical behavior. Neonatal estradiol treatment was less effective in promoting male-like

sexual behavior than was testosterone [218]. These data suggest that, in contrast to rats, female mice of several strains are able to display masculine sexual behaviors in adulthood without any extra treatment with testosterone or estradiol during development. However, in some strains, perinatal testosterone enhanced adult behaviors.

In mice, intra-uterine position has provided an additional, indirect way to investigate prenatal hormone exposure in females. These studies classify female embryos into three groups: (0M) positioned *in utero* with no male sibling beside them; (1M) positioned *in utero* beside one male sibling; and (2M) positioned *in utero* between two males. When ovariectomized, testosterone treated 0M and 2M females of the CF-1 strain were compared, the 2M females displayed more mounting than 0M females at 5 months of age. However, by 21 months of age all females showed large amounts of masculine sexual behavior [178]. 2M animals are exposed to more androgens from neighboring males than are 0M animals [222], [163]), therefore androgen exposure *in utero* may impact male sexual behavior in female mice.

Disruption of Androgen Exposure and Sexual Behavior in Males—The reciprocal study to giving excess testosterone to females is to castrate males shortly after birth and examine their masculine sexual behaviors in adulthood after testosterone administration. Castration of rats during the critical period reduces adult male sexual behavior [22]. Combined castration and postnatal testosterone administration in male rat pups demonstrated that T replacement compensates for gonad removal. Male rats exposed to flutamide, both pre- and postnatally, had reduced intromissions and ejaculations as adults but mounting behavior was similar to control males and control females [55]. One problem with flutamide administration, and neonatal castration to a lesser extent, is that complete differentiation of peripheral androgen-target tissues, such as the phallus and scent marking glands can be disrupted, particularly when treatment is given *in utero*. This may affect the males' abilities to display intromission and ejaculation. This issue has been addressed in work with non-human primates that have gestational durations long enough to administer flutamide selectively when the brain, but not the periphery, is undergoing sexual differentiation [226].

Studies in which male mice were deprived of gonadal hormones during various perinatal periods. In hybrid (129/ReWI by C57BL/6JWe) male mice, castration on PN25 followed by testosterone replacement did not reduce sexual behavior, while castration on PN0 significantly inhibited behavior [173]. However, when an anti-gonadotropin antiserum was given on PN0, PN2, and PN4 to reduce steroid hormone levels (passive immunization), adult penis size was reduced with no effect on sexual behavior. In addition, neonatal (PN3) testosterone treatment did not enhance male sexual behavior in gonad-intact C57BL/6 males, but it did increase the number of BALB/c males displaying sexual behavior [219]. In a similar study, a single testosterone injection on PN4 failed to affect adult masculine sexual behaviors in BALB/c, C57BL/6Fa or hybrid (C57BL/6Fa by DBA/2J) males, while a small enhancement was noted in DBA males [17]. Therefore, strain differences in mice appear to influence how androgens affect sexual behaviors in males, but there are still few data that directly addresses the role of androgens in inbred strains of mice.

Another way to specifically block the actions of AR is to use spontaneous mutants with reduced AR function (testicular feminized or Tfm) or engineered AR knockout mice. Mutations of the AR gene are fairly common, and the type and location of the mutation dictates the amount of AR function [137]. In rats, the mutation in the AR gene is a single amino acid substitution in the steroid-binding domain resulting in an AR protein that has a reduced ability to bind ligand [241]. On the other hand, the Tfm mouse mutant has a single base deletion in the open reading frame of the N-terminal domain which results in a frame

shift. Transcription is disrupted, less mRNA is present, and the resulting AR protein is truncated and unstable [41]. Tfm rats and mice both have a female external appearance, but it is possible that some of their differences in behavior are related to the degree of their AR mutation.

Tfm rats either tested with gonads intact, or castrated as adults and treated with estradiol or T, displayed mounting and thrusting behavior similar to WT males [82,151]. However, intromission and ejaculations were compromised; only about half of the Tfm rats displayed intromissions and one third showed an ejaculatory reflex in sexual behavior tests. Moreover, the latencies to display these behaviors were longer in Tfm than WT males [82]. Frequencies of intromission and ejaculations were reduced in Tfm rats; however, numbers of mounts were increased relative to WT males [82]. These final data suggest that the sexual motivation in the Tfm males is reduced as compared with WT males. Overall, work with Tfm rats tends to support a role for AR in sexual motivation and expression of intromissions and ejaculations.

Early studies in adult Tfm mice tested without androgen or estrogen replacement described their behavior as “asexual” meaning they did not display male- or female-typical sexual behaviors [148]. Gonad-intact Tfm rats can display low levels of male sexual behavior. This discrepancy may be caused by the differences in circulating hormones in Tfm mice and rats. Tfm rats, like humans, have elevated T levels in adulthood whereas Tfm mice have low androgen concentrations [151]. When Tfm mice were tested after castration and hormone replacement (testosterone, DHT or estradiol), estradiol-implants restored mounting and thrusting behavior in close to one half of Tfm males [25,151]. Comparable to Tfm rats, hormone treated Tfm mice have frequencies of mounts with thrusts that are similar to female controls and increased when compared with WT males [25]. More recently, several engineered AR knockout mice have been used, and in these mice masculine sexual behaviors were also severely compromised [176,185]. Thus, sexual behavior is greatly reduced in engineered mouse AR mutants and the spontaneous mutants (rats and mice). The largest difference between Tfm rats and mice is in the absence of ejaculatory behavior in mice, and its retention in a subpopulation of Tfm rats.

Estrogens and the Organization of Masculine Sexual Behaviors

The aromatization hypothesis predicts that the important steroid for sexual differentiation is estradiol [133,145]. Masculinization of sexual behavior is partially blocked by aromatase inhibitors administrated either prenatally or just after birth in Wistar rats [71,94]. Likewise, various estrogen receptor antagonists can have a similar effect [70,141]. Strain differences in response to ATD do exist, however. Sprague-Dawley males, exposed to the aromatase inhibitor ATD both during gestation and every other day after birth for a total of 6 treatments, did not demonstrate any differences in masculine sexual behavior as compared with control males [55]. Thus, while estrogen may be important for the development of MSB, in rats it is not sufficient in every strain.

Data on the role of ERs in sexual differentiation of the mouse brain come primarily from knockout animals, and the findings have been taken as support for the role of estrogens during development. Several groups of researchers working with ERαKO mice noted reduced masculine sexual behaviors [146,234]. Particularly when the mice were first phenotyped and still in a mixed genetic background (C57BL/J and SV129), different levels of mounting behavior were reported by different groups. To test the hypothesis that genetic heterogeneity leads to more sexual behavior in male ERαKO mice, mice in the C57BL/J6 strain were crossed twice into one of three other backgrounds: A/J, BALB/c or DBA/2J. Genetically the resulting F2 mice were 25% C57BL/6 and 75% new strain [54]. Nearly 40% of ERαKO males crossed into DBA or BALB/c displayed intromissions and one ERαKO in

the mixed DBA background sired a litter. Given the work in the B6D2F1 hybrid mouse [159] it is interesting to speculate that these animals are less dependent on ER than other strains for control of male sexual behavior, and perhaps this is why the ERα disruption lead to a less severe phenotype. Thus, earlier reports of more or less male sexual behavior in ERαKO mice were likely due to their mixed (C57BL and SV129) genetic background. In addition, when the general dopamine agonist, apomorphine, was given systemically to ERαKO males they were able to display ejaculations [233]. This result suggests that ERα is not an absolute developmental requirement for masculine sexual behaviors in the mouse. It also suggests that in adults a functional ERα may be important for dopamine release related to sexual behaviors.

When studying the developmental effects of estrogens, the ArKO mouse has a major advantage over ERαKO animals since estrogens can be administered at any time point and the mice have all the ERs needed to respond. In the first published study on masculine sexual behaviors in the ArKO male, long latencies to mount and reduced frequencies of mounting behavior were reported, similar to data collected in ERαKO mice [92,181]. Yet, when ArKO males were maintained in long-term pairs with fertile females, half of them were able to sire litters [181]. Moreover, when adult ArKO males were treated with estradiol many aspects of their sexual behaviors were similar to those in WT males including intromission frequencies and latencies to mount, intromit, and ejaculate [12] suggesting an activational, but no organizational, requirement for estradiol. Corresponding developmental studies were also done, and adult ArKO males that were administered estradiol shortly after birth displayed mounting behavior. Fertility was also recovered in some as adults, even when additional estradiol was not provided [215].

The opposite KO to ArKO males are AFP-KO female mice, which are exposed to higher titers of free estradiol than normal controls. Thus, female AFP-KOs can be used to determine if exposure to maternal estradiol *in utero* masculinizes adult behaviors. When adult females, in the out-bred CD-1 background, were OVX and treated with estradiol implants, the AFP-KO females displayed more mounting than WT females. Thus, exposure to estradiol during development did indeed masculinize their behavior. Treatment of pregnant dams with an aromatase inhibitor blocked the effect of gene disruption in their AFP-KO offspring [14]. The AFP-KO data show nicely that exposure to estrogen *in utero* enhances masculine sexual behaviors in females, but taken together with other data, discussed in the previous section, it is clear that masculine sexual behaviors are not especially sex dependent in mice. In sum, these KO studies suggest a small role for estradiol and ERα during neonatal development, and a larger role for ERα in activation of the behavior; however, the degree of variability between individuals is great. More normative data collected from normal mice and estrogen agonist versus antagonist administration both before and after birth would be immensely useful.

Summary

The data reviewed here are also presented in Tables 3 and 4. In rats, clear strain differences in females' capacity for masculine sexual behavior present researchers the opportunity to select the best animal model for particular questions. Long-Evans adult females given continued high levels of testosterone or estradiol performed all aspects of masculine sexual behavior, including the ejaculation reflex. Even when levels of testosterone and/or estradiol are low, appropriately tested females can display mounts and deep intromission-like thrusts. Studies in this strain might reveal endocrine and genetic pathways that are similar between the sexes and this should inform data on sex differences. Conversely, one might consider the strain of choice for sexual differentiation work as the Sprague-Dawley rat, since adult females require prenatal treatment with estradiol or testosterone to perform male-like sexual behavior in adulthood. In mice, females of many inbred strains are capable of displaying

masculine sexual behavior and neonatal hormone injections are not necessary if adults receive adequate testosterone.

Despite females' ability to mount and thrust as do males, the timing of these behaviors during the mating test is different in male versus female mice and rats. When given equivalent doses of testosterone, adult female mice begin to mount and thrust faster than males and display these behaviors with a higher frequency [234]. In addition, the ejaculatory reflex is rarely expressed by females of either species. Perinatal androgen exposure may be related to these variations in masculine sexual behavior, particularly the expression of the ejaculatory reflex in mice. Many questions remain, including do females normally display mounts and thrusts, and if so, under what type of conditions? These behaviors are likely to have different signaling purposes in females than in males. In fact, mounting and thrusting are routinely noted in gonad-intact females paired with receptive female partners [90]. More recently mounting and thrusting has been reported in adult female mice housed with their juvenile offspring [49].

The role of perinatal estradiol in the sexual differentiation of the rat brain has been well established, but in mice the evidence is inconclusive. Sexual motivation in mice may require neonatal estradiol, as the response of the dopamine system to contact with a female appears to be compromised in ERαKO males [38,233]. The absolute necessity of ER activation during the neonatal period in male mice awaits future studies that limit the use of estradiol, anti-estrogens, or siRNA to specific periods during development.

Female Sex Behavior: Receptivity and Lordosis

Similar to male sexual behavior, female sexual behavior has been extensively studied to the point where striking differences between rats and mice have been revealed. To gauge receptivity in female rodents when mounted by a male, the most frequently used measure is the stereotypical lordosis reflex [21,166]. Sexually experienced female rats and mice in the estrus phase of their reproductive cycle assume the lordosis posture if mounted by a male [131]. Diestrus and OVX females with low levels of ovarian hormones rarely display lordosis and actively reject mount attempts [28,131,134]. The arched-backed dorsiflexion and raised head is easily recognizable in rat lordosis. Receptive rats also display precopulatory proceptive behaviors such as hopping, darting, and ear-wiggling to solicit male coital behaviors. In sexually naive OVX rats, exogenous estradiol and progesterone replacement is sufficient to quickly establish lordosis equal to that of ovary-intact females [69].

Female sexual behavior in mice differs from rats in several important aspects, including their response to activational hormones. Receptive mice stand in lordosis when mounted, but the dorsiflexion does not appear to be as extreme and is sometimes hard to detect. Unlike rats, mice do not exhibit the precopulatory proceptive behaviors of hopping, darting and ear-wiggling. Additionally, C57BL/6 mice are less sensitive than rats to exogenous hormone replacement, because hormone primed sexually naive OVX mice initially show only low levels of lordosis. With continued sexual experience over subsequent trials, mice predictably demonstrate a gradual enhancement of receptivity [9,54,75,110,114,213]. Sexual experience appears to be an additional, necessary factor for maximal receptivity in the mouse, but there are strain differences in the initial receptivity of sexually naive females [75,213]. However, doses and timing of exogenous estradiol and progesterone replacement used in mice are examples of methods that have been derived empirically in rats. Perhaps these methods are not optimized for expression of receptivity in mice, and it remains to be determined if the requirement of sexual experience in the mouse is an artifact of suboptimal hormone replacement or a true difference between mice and rats.

Organization of Lordosis

Neonatal Androgens and the Reduction of Female Receptivity—Treatment of neonatal female rats with androgens has long-lasting behavioral effects on receptivity. Neonatal TP injection blocks normal estrus cycles and receptivity in adulthood [15,63,85]. However, the deficits in androgenized female receptivity are not the consequence of abnormal estrus cycles as adults, because ovariectomy and exogenous estradiol and progesterone replacement cannot restore receptivity [15,50,69,73,85,236]. The ability of exogenous androgens to permanently affect lordosis depends upon the timing of administration. Females injected with TP between PN0-5 have minimal lordosis quotients, but females treated after PN19 are highly receptive and no different than controls [15,50,63,69,73,85,236].

Even though the ovaries during perinatal development do not secrete appreciable hormones, prenatal exposure to androgens produced by male siblings *in utero* may affect normal females and be a source of natural variation of receptivity within a population. Studies that use intrauterine position as a variable offer indirect support of this hypothesis (reviewed in [183]). Female rats that develop in litters with more males or downstream in the direction of uterine blood flow from adjacent males are less receptive as adults than females not so positioned [88,93], although not all studies have replicated this finding [214]. Additionally, prenatal treatment of pregnant females with the anti-androgen flutamide increased lordosis in both PN0 androgenized and normal female offspring [72,73]. Most experiments support the conclusion that only aromatizable androgens are effective at defeminization of lordosis [237]. These experiments and others led to the *defeminization* theory of sex differences in behavior. This theory posits that normal neonatal exposure to androgens reduces feminine behaviors in males through irreversible developmental processes acting on neural substrates, one of those is likely aromatase enzyme.

Neonatal hormone administration to female mice has also revealed the defeminizing properties of androgens. Subcutaneous injection of TP on PN2 to A strain and BALB/c mice, reduces the number of times adult ovary-intact females receive mounts with an intromission as compared to same strain controls [217]. Like rats, adult hormone replacement does not eliminate long-lasting effects of neonatal androgens. Exogenous estradiol and progesterone replacement to adult OVX mice injected with TP on PN0 produces lordosis quotients as low as males castrated in adulthood. However, females injected with TP on PN9 are highly receptive and have lordosis quotients equivalent to PN0 oil injected control females, demonstrating that androgens need to be administered within the first few days after birth to defeminize lordosis [57,58]. Additionally, perinatal exposure to the non-aromatizable metabolite DHT does not reduce lordosis in female mice, suggesting that AR activation is not sufficient for blocking the organization of female receptivity [26,185]. To date, no major species differences have been directly demonstrated for defeminization by androgens between rats and mice. One could argue, however, that more data are needed in mice to determine whether there are subtle, yet important, differences in the critical period for androgen action.

Hormone Manipulations in Males—During the perinatal period, male rats are exposed to more testosterone than females at two points in time: around embryonic day 18 (E18) and postnatally (PN0) [47,200,231]. The presence of greater levels of testosterone during this time period in males, and the lower levels in females, fits well with the role of androgens in the process of defeminization. However, male rats and mice are able to display lordosis under certain conditions [111,114,153,202]. Wistar males can show lordosis with intact gonads, and both Wister and Sprague-Dawley males can display the behavior after pulsed estradiol and progesterone priming [153,205,206]. Nonetheless, in most strains of rats and

mice, unmanipulated males do not display female sexual receptivity [76,142,244]. Removal of endogenous gonadal androgens early in development by neonatal castration greatly reduces sex differences in lordosis.

Similar to experiments of androgenized females, the timing of castration is critical. Neonatal castration of rats between PN0 and PN5, followed by administration of estradiol and progesterone in adulthood, produces lordosis quotients in males that are similar to females [64,65,73,76,86,203,236]. In contrast, males castrated after PN7 show low levels of lordosis [64,65,76,86,203,244]. Furthermore, PN0 castrated rats given exogenous androgen replacement starting the day of castration, were less receptive than castrates that received androgens after PN10 [73,86,203,236]. Thus, androgens must be present before PN10 to efficiently defeminize rat female sex behavior, and, in general, the earlier rats are castrated the more receptivity is increased [45]. The temporal sensitivity of removing or blocking perinatal androgens in males to increase lordosis corresponds well with the effective time for androgenization to decrease lordosis in females. Unfortunately, gonadectomy of mice on the day of birth is technically difficult and experiments to support species differences and similarities with rats are lacking.

Aromatization and Estrogen Receptors

Estrogens and Defeminization—Defeminization of receptivity is typically attributed to estradiol produced after the local aromatization of testosterone in brain. Injections of estradiol benzoate to female and neonatal castrated rats during the critical period demonstrate that estradiol is just as effective and probably more effective than androgens at reducing adult expression of lordosis [63,64,69,160,[162],189,203,235,236]. As is the case with androgens, estradiol has no defeminizing effects when administered late in development (i.e. after P19; [63]). Neonatal administration of the antiestrogen ethamoxytriphetol (MER25), or aromatase inhibitors, block defeminization, providing good evidence that androgens must be converted to estradiol to affect lordosis [6,29,203,204]. Additionally, Tfm male rats, with a mutant androgen receptor and high levels of circulating testosterone [172], do not display lordosis, suggesting that aromatization of androgens and signaling through the estrogen receptor may be sufficient for defeminization [150]. Modulation of NMDA receptor activity in the medial basal hypothalamus during development, may be a crucial mechanism by which estradiol mediates defeminization of lordosis [189]. Thus, the evidence to support the aromatization hypothesis for defeminization is extensive in the rat.

Several studies support the aromatization hypothesis in mice, including experiments showing low receptivity in adult females that were given exogenous estradiol as neonates [152,218]. A good example of the potential of perinatal estradiol to reduce receptivity in mice has been demonstrated in α-fetoprotein KO (AFP-KO) mice. Female AFP-KOs are not protected from perinatal estradiol and show no lordosis [14]. Using an aromatase inhibitor to block synthesis of estradiol rescues the expression of lordosis in the knockouts. Interestingly, male aromatase knockout mice (ArKO) in the C57BL/6 background display low levels of lordosis similar to their WT male littermates [114]. This is a surprising finding; however, ArKO male mice are exposed to androgens and some androgenic metabolites may bind to an ER and reduce feminization [84,115]. Despite the data from ArKO male mice, it is clear that perinatal estradiol can reduce receptivity in both mice and rats.

Estrogens and Feminization—In light of the ability of perinatal estradiol to disrupt the development of receptivity, the dogma has been that feminine neural development occurs via a “default” mechanism without any need for facilitation by hormones. Yet, early studies of rats with ovarian tissue or low levels of exogenous estradiol during late postnatal

development suggested enhanced receptivity (reviewed in [5]. Compared to controls, female rats neonatally administered the anti-estrogen tamoxifen, perform less lordosis as adults, which could be taken to suggest that small amounts of neonatal estrogens are needed for *feminization* of lordosis behavior [83]. This interpretation was muddled by the fact that tamoxifen can also have ER-agonist properties in some tissues and perhaps *defeminize* lordosis. Based on these rat studies alone, evidence for estradiol promoting neural development in favor of high receptivity remains weak.

Studies in the mouse provide plausible support for a role of perinatal estrogens in the feminization of this species. ArKO females display less lordosis than their WT female littermates [9,11]. As the authors pointed out, it is unlikely that prenatal and early neonatal ovarian secretions were responsible for estrogenic feminization, since the ovaries cannot produce estradiol until PN7 and α-fetoprotein initially protects the brain from all sources of estradiol. In fact, an exogenous source of environmental estrogens may be responsible for the observed differences. ArKO females placed on a phytoestrogen free diet were no different than WT in their ability to acquire lordosis behavior [114]. Moreover, ArKO females given a phytoestrogen rich diet displayed significantly less lordosis than WT females on the same diet, and all females on the phytoestrogen-free diet. Thus, it is possible that ArKO females are more sensitive to defeminizing phytoestrogens, perhaps through enhanced activation of estrogen receptor-β [114]. Based on these results, a model has been proposed in which prenatal estrogens defeminize, while postnatal estrogens, including those experienced during puberty, feminize development of lordotic potential [5].

Which Estrogen Receptor(s)?—Given that androgens promote defeminization by acting through estrogenic metabolites, the question of which ER is critical has been addressed in both rats and mice. Antisense-RNA to ERα administered on PN2 blocked a reduction of lordosis behavior in concurrently androgenized female rats [132]. More recent studies using pharmacological approaches have yielded conflicting results on the ability of ERα to affect receptivity. Wistar female rats subcutaneously injected with 5μg of ZK 281471 an ERα-agonist, starting on PN0 and repeated every other day for 12 days displayed a sharp reduction in the lordosis quotient compared to controls [160]. However, injections of ERα-agonist PPT to Long-Evans female rat pups on the first four days after birth resulted in high lordosis quotient scores, whereas estradiol benzoate treated females had low scores [162]. The contradictory results of the two ERα-agonist experiments could be explained by strain, experimental design, and agonist differences. In contrast, experiments examining the role of ERβ in the rat are in agreement. Neonatal injections of the selective ERβ-agonists ZK 281738 or DPN to female rats do not reduce lordosis [160,162].

Most of the work in mice examining ER effects on lordosis has used gene knockouts. Even though ERαKO mice are unreceptive [180], the fact that ERα is necessary for the activation of lordosis in adulthood precludes the use of knockouts to study ERα's role in developmental defeminization [112,179]. Pharmacological evidence suggests that ERα is not important for sexual differentiation of mouse lordosis. Female C57BL/6J mice given PPT (ERα-agonist) during the first 3 days of life do not differ from controls in the acquisition of lordosis [112]. In contrast to the caveats of ERαKO mice, use of ERβKOs is much more promising because selectively activating or blocking ERβ in the adult animal has no major effects on receptivity in either rats or mice [110,130]. Interestingly, the lack of a functional *ERβ* gene in ERβKO mice facilitates the acquisition of lordosis in estrogen plus progesterone primed males castrated as adults compared to their WT littermates [111]. Furthermore, ovary-intact ERβKO mice can display lordosis at times in the ovarian cycle when WT females are unreceptive [110,147]. Pharmacologically, neonatal DPN (ERβ-agonist) injections given to normal WT C57BL/6 female mice reduce lordosis quotients as

effectively as estradiol, providing further proof that ERβ effects on receptivity are organizational and not activational [112].

Results are mixed between rats and mice when manipulating ERα and ERβ during perinatal development, and highlight a species difference. There is only evidence for the ability of ERα to mediate perinatal estradiol influences on receptivity in the rat, whereas in the mouse there is evidence for ERβ. To date, much less is known about ERβ and the role it plays in discreet or overlapping functions with ERα. The finding that ERβKO male mice are incompletely defeminized as adults suggests this ER has a role in behavioral defeminization.

Summary

General principles of organization of receptivity mostly are in agreement between rats and mice (see Tables 5 and 6). The critical period for organization of receptivity in both rats and mice likely begins after E15 and ends before P10 in rats, but the data for a definitive critical period in mice are still scarce (reviewed in [18]). Although it is clear that testosterone secretion by the testes during early development is responsible for reduced receptivity, animals exposed to perinatal androgens can still display lordosis; however, it takes a much higher dose of estradiol in adulthood to induce lordosis [76,202]. Therefore, it does not appear that developmental androgen exposure permanently abolishes the neural circuitry to express the lordosis reflex. Rather, early exposure to high levels of androgens, or estrogens, decreases the sensitivity of the adult brain to ovarian hormones to stimulate lordosis [52].

A few notable differences between rats and mice in the development of receptivity include the necessity of sex experience in at least certain strains of mice, versus sufficiency of activational hormones for lordosis in rats. Additionally, there is a potential rat versus mouse difference in the importance of specific estrogen receptor isoforms on defeminization. One gap in understanding the development of sex differences in behavior is discovery of mechanisms downstream of hormone signaling that set the feminization and/or defeminization processes in motion. The genetic tools available to the mouse may make these animals particularly suited for these sorts of experiments. As always, a strong foundation is needed in WT control mice to place the work in KO and other mutants into context.

Concluding Summary

Studies of sexually dimorphic brain regions and behaviors may be more advanced in rats, but work in mice has been progressing. Findings with KO mice of mixed genetic background, when compared directly to discoveries in rats, can create some confusion. This review highlights some of the apparent species differences as well as similarities. The gonadal steroid modulation and developmental progression of the calbindin-ir sub-region of the POA, and the role and distribution of the ERβ in the AVPV are divergent in rats and mice. Sexual dimorphisms in PR are similar in one region (POA), but divergent in another (VMH), while the literature on nNOS is too complex at this point to make useful general conclusions. Partner preference behaviors likely require organizational actions of ERα in rats and in mice, but AR may play an added role in mice. Male sexual behaviors are less sexually dimorphic in mice than in Sprague-Dawley rats, and thus assumptions based on information from rats should be carefully considered. The importance of organizational/developmental hormones on sexual differentiation of male sex behaviors could be a species-specific phenomenon. On the other hand, lordosis is sexually dimorphic in mice and rats and estradiol during development is likely responsible for defeminization of this behavior in both species. Interestingly, the roles of ERα versus ERβ for defeminization of receptivity could be species specific. Studies conducted in the same laboratory, with both species,

employing the same treatments and timing would be useful for the resolution of this interesting issue.

For animal models in general, it is important to understand the relationship of the molecular mechanisms in the model species to the target species; frequently humans. For the choice of rats versus mice, there are clear differences between them as reviewed above. For some of these characteristics there are comparisons that can be made with humans. For example, in humans, calbindin is expressed in both the developing [108] and adult POA [107]. The ontogeny of calbindin expression in the human POA may be more similar to the pattern found in mice than to rats, with greater expression levels occurring during development [108] and becoming more restricted in adulthood [107]. As there is more than one potential homolog of the rodent SDN-POA in humans, it is unclear if the expression of calbindin in the rodent is analogous to events occurring during the development of the human POA. Although mice might be appropriate for modeling calbindin in the human POA, ERβ expression in primates appears to occur in a region that might be homologous to the rat AVPV perhaps making it the more attractive rodent for studying ERβ at this location [79,109]. In humans, no sex difference in expression pattern was detected by immunohistochemistry [109], although, due to limitations of post-mortem tissue examined in this study and questions regarding the specificity of the antibody used, these findings are inconclusive.

On the behavioral side, it is always difficult to compare rats and mice with humans, but it can still be relevant. Difficulties have been reviewed by others [19], but it is important to note that the behaviors measured in animals are rarely the same as in humans. Lordosis and mounting/thrusting behaviors are stereotypical mating postures in rodents. In humans, the sexual postures *per se* are not sexually dimorphic and it is probably motivation, desire, and other psychological aspects of these behaviors, not the mating stances that are sexually dimorphic. The second hurdle for translation of these behaviors to humans is their dependence on species typical hormone levels. In humans, the expression of sexual behaviors is far less dependent on activational hormones [158]. Partner preference behaviors in animals have been taken as indications of gender identification and/or sexual orientation [19], but even this analogy is complex. Residing next to a conspecific behind a mesh barrier, or sniffing bedding soiled by a male versus a female, is not necessarily an indication of a sexual partner preference.

One of the positive aspects of the analogy between gender identification and mating/preference behaviors is that human data collected from clinical populations with various neuroendocrine disorders of sexual differentiation can be compared to experimental animal data [43,74]. These “experiments of nature,” range from increased fetal androgen exposure (congenital adrenal hyperplasia) to inability to respond to androgens (complete and partial androgen insensitivity mutations) or estrogens (aromatase enzyme or ER mutations). Recent data from rhesus monkeys, exposed to non-aromatizable androgens (females) or anti-androgens (males) during development at gestational ages that do not coincide with genital differentiation, argue that AR activation *in utero* can produce a suite of male-typical behaviors even in individuals without a male-like phallic structure [227]. These studies, along with other data, suggest that brain differentiation of male-like behaviors in humans may rely on androgen receptor mechanisms. Our review of the rat and mouse literature suggests that the AR may be more critical for partner preference behavior in mice than in rats. Moreover, some strains of mice might be more appropriate for these models than others.

In summary, behavioral neuroendocrinologists have frequently utilized comparative approaches, and mice and rats are important components of these studies. A practical outline

of the research strategy for studying sexual differentiation in rodents [23] notes key guidelines that include establishment of sex differences in uniform endocrine environments, a critical step often missed by investigators new to the field. In genetically modified animals, investigators' results should always be interpreted in the context of backcross generation [169]. The proper use of engineered mice, along with other model organisms including, but not limited to, rats, will undoubtedly enrich the field.

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Table 1

Summary of major studies on sexual differentiation of partner preference behavior in the rat.

Sex	Treatment Period	Hormone/ Treatment	Strain	Preference Task	Observed Effects	Refs.
male	E12-PN12; adult	AR antagonist; Gdx + E2 and P, followed by T	Sprague-Dawley	Non-volatile	Preference for Female	[55]
male	Tfm		Sprague-Dawley	Non-volatile	Preference for Female	[82]
female	PN0	E2	Long-Evans	Non-volatile	Preference for Female	[87]
male	E10-19	Anti-estrogen or Anti-androgen; Sexual experience	Wistar	Volatile	Preference for Female after anti androgen and sexual experience	[221]
male	PN0-PN21; adult	Aromatase inhibitor; Gdx + E	Wistar	Volatile and Non-volatile	Volatile preference for Female, Non-volatile preference for Male	[7]
male	PN0-PN21; adult	Aromatase inhibitor; Gdx + DHT, E2, or combined E/DHT	Wistar	Non-volatile	DHT: No preference E2: Preference for Male E2/DHT: ↓ Preference for Female	[8]
male	E11-PN0, E11-PN10, PN0-PN10	Aromatase inhibitor	Wistar	Volatile and Non-volatile	↓ Preference for Female	[35, 94]
male	PN0; adult	Gdx; T	Wistar	Non-volatile	↓ Preference for Female	[33]

PN0= the day of birth; PN= postnatal day; E12=embryonic day 12; Tfm= testicular feminization mutant; AR= androgen receptor; Gdx= gonadectomized; E2= estrogen, P= progesterone; T= testosterone; DHT= dihydrotestosterone. Mutant strains were considered to be under a constant treatment and thus are indicated in the treatment/period column; blank squares in the hormone/treatment column indicate that the test animals were gonad-intact. Volatile tasks were tests without direct contact with another rat, either by physical separation or exposure to urinary volatiles only. Non-volatile tasks were tests in which the subject had direct contact with another, usually tethered, rat or bedding.

Table 2

Summary of major studies on sexual differentiation of partner preference behavior in the mouse.

Sex	Treatment Period / KO	Hormone/ Treatment	Strain	Preference Task	Observed Effects	Refs.
male			CF1	Non-volatile	Preference for Female	[98]
male	AR^{NesCre}		C57/BL6 and 129SvEv	Volatile	Preference for Female	[176]
female	PN0; Adult	DHT or E2 treatment; Gdx + E2	C57BL/6	Volatile and Non-volatile	DHT: Preference for Female	[26]
male	ERαKO		C57BL/6	Volatile and Non-volatile	Volatile preference for Female, No Non-volatile preference	[232]
male	ArKO	Gdx + T	C57BL/6	Volatile and Non-volatile	No volatile preference, Non-volatile preference for Female	[10]
male	Tfm	Gdx + E2	C57BL/6J	Volatile and Non-volatile	Volatile preference for Female, ↓ non-volatile preference for Female	[25]
male	PEG3-KO	Sexual experience	C57BL/6J	Volatile and Non-volatile	No non-volatile preference, ↑ volatile preference for Female	[210, 211]
male	GPR54 KO	Intact or Gdx + T	129S1/SvImJ	Volatile	No preference	[100]
male	Trpc2 KO		129SV/C57BL/6	Volatile	No preference	[119, 207]
male	ArKO	Gdx or intact + E2 and/or DHT	C57BL/6	Volatile	No preference	[12]
female	ArKO	Gdx + T or E2	C57BL/6	Volatile and Non-volatile	T: No preference, E: Non-volatile preference for Male	[9]
female	AFP-KO	Gdx + E2	CD1 and C57BL/6	Volatile and Non-volatile	Preference for Male	[13]

PN0= day of birth; AR^{NesCre}=Conditional Androgen Receptor Knockout; ERαKO= estrogen receptor α knockout; ArKO= aromatase knockout; Tfm= testicular feminization mutant; Peg3-KO= paternally expressed gene 3 knockout; GPR54 KO= G-protein coupled receptor 54 (Kisspeptin Receptor) knockout; ArKO= aromatase knockout; AFP-KO= α fetoprotein knockout; Gdx= gonadectomized; E2=

estrogen,; T= testosterone; DHT= dihydrotestosterone.. Mutant strains were considered to be under a constant treatment and thus are indicated in the treatment/period column; blank squares in the hormone/treatment column indicate that the test animals were gonad-intact. Volatile tasks were tests without direct contact with another rat, either by physical separation or exposure to urinary volatiles only. Non-volatile tasks were tests in which the subject had direct contact with another, usually tethered, rat or bedding.

Table 3

Summary of major studies on sexual differentiation of male sexual behavior in the rat.

Sex	Treatment Period	Hormone/ Treatment	Strain	Observed Effects	Refs.
male	PN0	Gdx + T or DHT with estrogen	Sprague-Dawley	—	[30]
male	Tfm		Stanley-Gumbreck	—	[150]
male	E12-22 and PND2,4,6,8,10, 12	Aromatase inhibitor	Sprague-Dawley	—	[55]
male	E10-19	Anti-estrogen	Wistar	Small ↓	[221]
male	E17,18,19	Anti-androgen	Long-Evans	↓ ejaculation	[164]
male	PN0-9	Anti-estrogen		↓ ejaculation	[204]
male	E11-21	Anti-androgen	Sprague-Dawley	↓	[39]
male	PN0	Gdx	Sherman	↓	[182]
male	PN0	COX inhibitor	Sprague-Dawley	↓	[3]
male	PN0	Anti-estrogen	Wistar	↓	[48]
female	E11-21 & PN0-9	Anti-androgen	Sprague-Dawley	↓	[32]
female	PN0	Androgen	Long-Evans	↑	[212]
female	E18	DHT	Long-Evans	↑	[164]
female	PN0	PGE2	Sprague-Dawley	↑	[3]

PN0=the day of birth; PN=postnatal day; E12= embryonic day 12; Tfm= testicular feminization mutant; Gdx= gonadectomized; T= testosterone; DHT= dihydrotestosterone; E= estrogen; PGE2=prostaglandin E2. Mutant strains were considered to be under a constant treatment as indicated in the treatment period column; blank squares in the hormone/treatment column indicate that the test animals were gonad-intact.. ↓ indicates a decrease in behaviors, ↑ an increase, and — indicates no change.

Table 4

Summary of major studies on sexual differentiation of male sexual behavior in the mouse.

Sex	Treatment Period / KO	Hormone/ Treatment	Strain	Observed effects	Refs.
male	*Hpg* PN1 or 2	Androgen	C3H/HeHx101H	—	[120]
male	PN0	Androgen	Balb/c	—	[90]
female	PN0,7, and 14	Estrogen	SV129 and C57BL/6J	—	[239]
female	PN0	Androgen	Balb/c	—	[90]
male	ARKO		CD-1 and C57BL/6J	↓	[185]
male	$AR^{Nes/Cre}$		129SvEv and C57BL/6	↓	[176]
male	PN1	Gdx	129/ReWi by C57BL/6jWe	↓	[173]
male	ArKO		SV129 and C57BL/6	↓	[129, 181]
male	ERαKO		SV129 and C57BL/6J	↓	[234]
female	*in utero*	2M	CD-1	↑	[178]

PN0=the day of birth; PN=postnatal day; E12= embryonic day 12; *Hpg*= hypogonadal mutant; ARKO= androgen receptor knockout; $AR^{Nes/Cre}$= conditional androgen receptor knockout; ArKO= aromatase knockout; T= testosterone; E= estrogen; Gdx= gonadectomized; 2M= female between 2 males *in utero*. Mutant strains were considered to be under a constant treatment as indicated in the treatment period column; blank squares in the hormone/treatment column indicate that the test animals were gonad-intact. ↓ indicates a decrease in behaviors, ↑ an increase, and — indicates no change.

Table 5

Summary of major studies on the sexual differentiation of lordosis behavior in the rat.

Sex	Treatment Period	Hormone/ Treatment	Strain	Observed Effects	Refs.
female	PN0	Gdx	Sprague-Dawley, Wistar	—	[203,236]
female	PN0-3	ERα-agonist	Long-Evans	—	[162]
female	PN0-3, PN0-11*	ERβ-agonist	Long-Evans, Wistar	—	[160,162]
female	PN2*	ERα RNAi intrahypothalamic	Sprague-Dawley	RNAi — Cont. ↓	[132]
female		Intrauterine position	Wistar	↓	[88]
Female	PN0	GAD RNAi intrahypothalamic + oil or Androgen	Sprague-Dawley	↓	[50]
female	PN0, PN3-5*	Androgen or Estrogen	Holtzman, Listar, Long-Evans, Sprague-Dawley, Wistar	↓	[15,50,63, 69,85,160, 162,189, 235,236]
female	PN0-11*	ERα-agonist	Wistar	↓	[160]
female	PN0-2	NMDA	Sprague-Dawley	↓	[189]
female	E10-22 PN0	Anti-androgen + oil or Androgen	Long-Evans	↑	[72,73]
male	PN7, adult	Gdx	Holtzman, Sprague-Dawley, Wistar,	—	[64,65,76, 86,203, 244]
male	PN0 PN0-9, PN0-59*	Gdx + Androgen or Estrogen	Holtzman, Long-Evans, Sherman, Sprague-Dawley, Wistar,	T — E2 — DHTB ↑	[45,73,86, 203,236]
male	E10-22 PN0	Anti-androgen Gdx + oil or Androgen	Long-Evans	↑	[72,73]

Sex	Treatment Period	Hormone/ Treatment	Strain	Observed Effects	Refs.
male	E19-PN4*	5α-reductase inhibitor	Wistar	↑	[177]
male	PN0, PN5	Gdx	Holtzman, Long-Evans, Sherman, Sprague-Dawley, Wistar	↑	[45,64,65, 73,76,86, 203,236]
male	PN0 PN0-9	Gdx Androgen or Estrogen + Anti-estrogen	Wistar	↑	[29,203, 204]
male	PN0-14, PN5-14	Anti-aromatase	Wistar	↑	[6]
male	PN0 PN10-19, PN13-59*	Gdx + Androgen	Wistar, Holtzman	↑	[86,203]

Adult treatment is gonadectomy (Gdx) with E2 or E2+P replacement unless otherwise stated. PN0 is the day of birth.

*Indicates that it was unclear in some reports whether the authors define the day of birth as PN0 or PN1 and we assumed the latter, thus the true ages may be a day older than indicated in this table. .

↓ indicates a decrease in lordosis, ↑ an increase, and — indicates no change. Where multiple citations and/or multiple developmental time-points were investigated with the same treatment and achieved the same result, the earliest and latest time-points are separated by a comma. PN= postnatal day; E=embryonic day; Tfm= testicular feminization mutant; AR= androgen receptor; Gdx= gonadectomized; E2= estrogen, P= progesterone; T= testosterone; DHT= dihydrotestosterone; NMDA= N-methyl-D-aspartic acid; RNAi= RNA interference (anti-sense); GAD= glutamic acid decarboxylase

Table 6

Summary of major studies on the sexual differentiation of lordosis behavior in the mouse.

Sex	Treatment Period/ KO	Hormone/ Treatment	Strain	Observed Effects	Refs.
female	PN0-2	ERα-agonist	C57BL/6J	—	[112]
female	AFP-KO E12-21	Anti-aromatase	CD1	—	[14]
female	ARKO; E14-19 & PN0-8	Vehicle or DHT	C57BL/6J	—	[185]
female	ArKO; E1-Adult	Low Phytoestrogen Diet	C57BL/6	—	[114]
female	ERβKO		C57BL/6J	—	[110]
female	PN9	Androgen	Swiss-Webster	—	[58]
male	PN29	Gdx	Swiss-Webster	—	[58]
male	ARKO		C57BL/6J	—	[185]
male	ArKO; E1-Adult	Low Phytoestrogen Diet	C57BL/6	—	[114]
male	BaxKO		C57BL/6	—	[99]
female	PN2*	Androgen or Estrogen	C57BL/6	T — E2 ↓	[218]
female	E1-21	2M Intrauterine Position	CF-1	↓	[178]
female	PN0 PN0-2	Androgen or Estrogen	Swiss-Webster, C57BL/6J	↓	[57,58, 112]
female	PN0-2	ERβ-agonist	C57BL/6J	↓	[112]
female	PN2*	Androgen or Estrogen	A, BALB/c	↓	[218]
female	AFP-KO		CD1, C57BL/6J	↓	[14]
female	ArKO		C57BL/6	↓	[9]
female	ArKO; E1-Adult	High Phytoestrogen Diet	C57BL/6	↓	[114]
female	BaxKO		C57BL/6	↓	[99]
female	D5KO		C57×129 hybrid	↓	[113]

Sex	Treatment Period/ KO	Hormone/ Treatment	Strain	Observed Effects	Refs.
female	ERαKO ERαβKO		129xC57, C57xDBA, C57BL/6J	↓	[54,110, 180]
male	ArKO; E1-Adult	High Phytoestrogen Diet	C57BL/6	↓ trend	[114]
male	ERβKO		C57BL/6J	↑	[111]

Adult treatment is gonadectomy with E or E+P replacement unless otherwise stated. PN0 is the day of birth.

* Indicates that it was unclear in some reports whether the authors define the day of birth as PN0 or PN1 and we assumed the latter, thus the true ages may be a day older than indicated in this table. .

↓ indicates a decrease in lordosis, ↑ an increase, and — indicates no change. ArKO= aromatase knockout; AFP-KO= α fetoprotein knockout; ARKO= androgen receptor knockout; ERαKO= estrogen receptor-α knockout; ERβKO= estrogen receptor-β knockout; ERαβKO= estrogen receptor-α/β double knockout; D5KO= dopamine receptor-5 knockout

BaxKO= BCL2-associated X protein.