HHS Public Access

Author manuscript

Eur J Neurosci. Author manuscript; available in PMC 2017 March 15.

Published in final edited form as:

Eur J Neurosci. 2010 December ; 32(12): 2096–2104. doi:10.1111/j.1460-9568.2010.07511.x.

Organized for sex – steroid hormones and the developing hypothalamus

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Abstract

Steroid hormones of gonadal origin act on the neonatal brain, particularly the hypothalamus, to produce sex differences that underlie copulatory behavior. Neuroanatomical sex differences include regional volume, cell number, connectivity, morphology, physiology, neurotransmitter phenotype and molecular signaling, all of which are determined by the action of steroid hormones, particularly by estradiol in males, and are established by diverse downstream effects. Sex differences in distinct hypothalamic regions can be organized by the same steroid hormone, but the direction of a sex difference is often specific to one region or cell type, illustrating the wide range of effects that steroid hormones have on the developing brain. Substantial progress has been made in elucidating the downstream mechanisms through which gonadal hormones sexually differentiate the brain, but gaps remain in establishing the precise relationship between changes in neuronal morphology and behavior. A complete understanding of sexual differentiation will require integrating the diverse mechanisms across multiple brain regions into a functional network that regulates behavioral output.

Keywords

estradiol; hypothalamus; sex differences; sexual behavior; steroids

The brain is a major target of steroid hormones

Behavioral differences between males and females result from the potent ability of three families of steroid hormones to sexually differentiate the nervous system: estrogens, androgens and progestins. It has long been established that the brain is a target organ for steroid hormones, as estrogens, androgens, progestins (as well as adrenal steroids) all bind in the brain with high affinity (Stumpf & Sar, 1976). Gonadal steroids each bind to their own cognate receptors, which are members of a nuclear receptor superfamily and which act in their classical capacity as transcription factors after dimerizing, translocating to the nucleus and binding to appropriate hormone response elements on DNA (King & Greene, 1984; O'Malley & Tsai, 1992). In addition to this classical mode of steroid hormone action, steroid hormone receptors can regulate gene transcription independently of hormone response elements by associating with co-factors such as c-fos, which themselves bind to specific

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sequences of DNA on gene promoters (Paech *et al.*, 1997; Uht *et al.*, 1997). Steroid hormones also rapidly activate kinases, proteases and other molecules that initiate signaling cascades and lead to gene transcription without hormone receptors ever acting directly on DNA (Gu *et al.*, 1996; Zhou *et al.*, 1996; Watters *et al.*, 1997; Bi *et al.*, 2001; Abraham *et al.*, 2004; Zadran *et al.*, 2009). Lastly, hormones can act within seconds to change cell physiology (Kelly *et al.*, 1976) and these rapid effects are now largely attributed to their action on transmembrane receptors (Towle & Sze, 1983; Mermelstein & Micevych, 2008), although some of the receptors mediating these effects are still being characterized (Toran-Allerand, 2005). These multiple modes of steroid hormone action converge on the developing nervous system to produce sex differences in the brain and in behavior.

Steroids exert organizational and activational effects on the brain

As so elegantly demonstrated in the seminal study by Phoenix *et al.* (1959), sex differences in reproductive behavior are achieved via a combination of organizational and activational effects of hormones. Hormones first act on the developing brain during a perinatal sensitive period to shape subsequent responsivity to the adult hormonal profiles that mediate reproductive physiology and behavior. Organizational effects are such that the developmental hormonal milieu both potentiates as well as limits the brain's subsequent response to adult hormone exposure. In this way a brain organized as male during development has a greatly reduced ability to produce female sexual behavior in adulthood, even when receiving female-typical hormone replacement. Thus, the organizational effects of hormones ensure that the brain of an animal is appropriately matched to the hormonal signals it will receive in adulthood. Additionally, sensory circuits in the nervous system are differentiated such that they become limited in their ability to respond to sexually relevant stimuli, such as olfactory signals.

In the rodent, the organizing hormonal signal is the perinatal androgen surge from the testes, which begins prenatally (at approximately embryonic day 18), peaks on the day of birth and rapidly declines within hours (Konkle & McCarthy, in press; Fig. 1). Emerging evidence from hamster studies also implicates an additional peripubertal organizing period in further priming the brain to respond to activational steroid hormones in adulthood (reviewed in Schulz *et al.*, 2009), although much of this work remains to be replicated in other rodent species.

Masculinization, defeminization and feminization are distinct processes of sexual differentiation

Sexual differentiation of the brain involves three processes: masculinization, defeminization and feminization. Masculinization and defeminization are active but distinct processes in the male brain, both of which rely on gonadal hormone action during the perinatal period. Masculinization is the process through which the brain becomes capable of producing male sexual behavior, which includes sexual motivation as well as the copulatory behavior itself, consisting of mounting, erection, intromitting and ejaculating. Defeminization is the process through which the brain loses the ability to produce female-typical sex behavior. Feminization is a default program that proceeds in the absence of organizing steroid

hormone action, but is nonetheless an active process. Feminization results in a brain that mediates female sexual responding, which in the rodent consists of a combination of proceptive solicitous behavior and the receptive posture called lordosis. Although the process is poorly understood, feminization, too, is probably dependent upon estrogens of ovarian origin, possibly acting outside the critical period for masculinization (Bakker & Baum, 2008). As the female brain is never exposed to the masculinizing organizational effects of androgens, it is inherenlty kept incapable of producing behavioral responsivity to the activational effects of androgens in adulthood.

Masculinization and defeminization of the hypothalamus are both driven by gonadal hormones, but are distinguishable from one another, in that they are differentially sensitive to neonatal hormone levels (Debold & Whalen, 1975), occur across slightly different developmental time courses (Wallen & Baum, 2002) and involve different brain regions. Masculinization and defeminization of a single brain region can also be controlled by different underlying mechanisms, as our laboratory has shown to be the case in the preoptic area (Todd *et al.*, 2005). Therefore, it is possible for a single adult male to display either male or female copulatory behaviors given the appropriate activational hormones if the mechanisms underlying brain defeminization are interfered with developmentally while those underlying masculinization are left undisturbed. The organizational role of each class of gonadal hormones on these different processes of sex differentiation will be explored subsequently in this review, with a focus on the mechanisms through which gonadal hormones shape the morphology of the developing brain and adult sexual behavior.

Androgens in the brain are converted to estrogens

The organizing effects of steroid hormones are derived from the testicular androgen surge, yet androgens acting on androgen receptors do not alone organize the rodent brain to produce sex-specific copulatory behavior. Rather, in the late 1970s, it became apparent that neonatal estradiol could masculinize the rodent brain as easily as testosterone. The Aromatization Hypothesis reconciled the potent ability of estrogens to masculinize the brain with the established fact that the perinatal androgen surge from the testes was necessary for masculinization to occur. Testosterone is enzymatically converted by cytochrome p450 aromatase into estradiol in the brain, which then acts to produce a complex mosaic of sexually differentiated brain regions. The aromatase enzyme is highly expressed in the brains of both males and females, especially in sexually differentiated areas such as the hypothalamus, and especially during the perinatal androgen surge (George & Ojeda, 1982; Roselli & Resko, 1993; Lauber & Lichtensteiger, 1994). Preventing aromatase activity in the neonate blocks the masculinizing effect of androgens on partner preference (Bakker *et al.*, 1993a, 2002) and impairs several aspects of copulatory behavior, including intromission latency and ejaculation (Bakker *et al.*, 2004).

Once aromatized from testosterone, estradiol acts in the brain on several different estrogen receptors (ERs): ERα, once thought to be the only estrogen receptor, the more recently characterized ERβ, and the G-protein-coupled receptors ER-X and GPR30. The effects of ERα and ERβ can occur either in the nucleus in the ‘classical’ fashion or at the membrane (Mermelstein & Micevych, 2008), ER-X appears to act also largely at the membrane and to

activate intracellular signaling cascades (Toran-Allerand *et al.*, 2002; Qiu *et al.*, 2003), whereas GPR30 is most highly expressed on the endoplasmic reticulum and acts to alter intracellular calcium signaling (Revankar *et al.*, 2005). Interestingly, recent knockout mouse models show that ERα is more closely coupled with masculinization and ERβ with defeminization (Kudwa *et al.*, 2006).

The developing female brain is protected from the masculinizing effects of maternal estrogens by α-fetoprotein, a plasma protein which has a high affinity for estradiol and sequesters the hormone in the bloodstream (McEwen *et al.*, 1975). α-Fetoprotein does not bind androgens, and therefore in males testosterone synthesized in the testes gains access to the brain and is locally aromatized to estradiol. As a result, males experience significantly higher estradiol levels in sexually differentiating brain regions than do females (Amateau *et al.*, 2004). Although there is debate over whether α-fetoprotein acts solely to sequester estradiol or also to selectively transport it into the female brain to produce behavioral feminization (Toran-Allerand, 1980, 1987; Bakker & Baum, 2008), female mice with a mutated and therefore dysfunctional form of α-fetoprotein have masculinized sexual behavior and masculine neurochemistry, confirming that α-fetoprotein protects the female brain from masculinization and defeminization (Bakker *et al.*, 2006). Interestingly, α-fetoprotein levels drop significantly in the early postnatal period (Raynaud, 1973; Meijs-Roelofs & Kramer, 1979; Andrews *et al.*, 1982), so α-fetoprotein may first protect the female brain from masculinization and defeminization during the sensitive period for these processes, and then its absence subsequently permits estrogens of ovarian origin to participate in brain feminization.

Sex differences in the brain take many forms

Sex differences in the brain can be broadly categorized as structural, neurochemical or molecular. Structural sex differences range from macroscopic (e.g. differences in brain region area, volume, cell number or projection density) to the microscopic (e.g. differences in cell size, neurite complexity or morphology, dendritic length and spine number). Neurochemical sex differences have been established in neurotransmitter, enzyme or local hormone levels. Molecular sex differences occur in signaling cascade activation, gene expression and epigenetic modifications. Each of these types of sex differences can be found in the hypothalamus and all are regulated by the organizing effects of estradiol (McCarthy *et al.*, 2009; Fig. 2).

At the broadest level, neonatal estradiol exposure produces sex differences in cell number and in the size or volume of certain regions of the hypothalamus. The two best characterized are the sexually dimorphic nucleus of the preoptic area (SDN-POA), which is larger in males, and the anteroventral periventricular nucleus (AVPV), which is larger in females. These two are a perfect point–counterpoint in that they demonstrate the specificity of estradiol's actions, either promoting or preventing cell death depending on the brain region (Arai *et al.*, 1996).

The SDN-POA is over five times larger in males than females because it contains many more cells (Gorski *et al.*, 1978). Although we still lack a precise understanding of the role of

the SDN in sexual behavior, the profound sex difference in cell number in the SDN-POA is regulated by neonatal estrogen exposure (Rhees *et al.*, 1990). Estradiol prevents cell death in the SDN of males (Arai *et al.*, 1996; Davis *et al.*, 1996) through NMDA receptor activation and a classic downstream expression of the anti-apoptotic protein bcl-2 (Hsu *et al.*, 2001, 2005). In the case of the AVPV, which is involved in producing the luteinizing hormone (LH) surge necessary for ovulation, a single neonatal dose of aromatizable androgen to a female is sufficient to reduce the number of dopaminergic cells to that of a male (Simerly *et al.*, 1985a) and to prevent the ability to produce a preovulatory LH surge (Simerly *et al.*, 1985b). Correspondingly, treating males with an ER antagonist produces cell death in dopaminergic neurons, via downstream upregulation of caspases (Waters & Simerly, 2009). In addition to a sex difference in the number of dopaminergic cells, there are also twice as many estrogen-sensitive GABAergic / glutamatergic cells in the female AVPV (Ottem *et al.*, 2004), and death of these cells is mediated by tumor necrosis factor alpha, which is higher in males (Krishnan *et al.*, 2009). The pro-apoptotic protein Bax also contributes to cell death in GABAergic cells, but does not regulate the sex difference in dopaminergic cell number (Forger *et al.*, 2004). There is yet another sex difference in the AVPV – females have significantly more kisspeptin-positive cells, which are a distinct population from the dopaminergic AVPV neurons (Kauffman *et al.*, 2007). Estradiol also regulates this sex difference, decreasing the number of kisspeptin-positive cells in the AVPV, which are involved in producing the LH surge that is critical for ovulation (Homma *et al.*, 2009). Therefore, estradiol in the AVPV regulates cell death of multiple cell types in the male and appears to do so through different signaling cascades in each instance (Krishnan *et al.*, 2009). Surprisingly, while the sex differences in both of these nuclei is established via cell death during perinatal development, the dimorphism is further reinforced by hormonally regulated neurogenesis in the peripubertal organizational period (Ahmed *et al.*, 2008).

Sex differences in cell morphology and synaptic patterning are regulated by a variety of molecular mechanisms

Many subregions of the hypothalamus contain neurons with sex differences in cell morphology and synaptic connectivity, including differences in neurite or dendritic morphology, dendritic spine number or density, and synapse number or type. Each of these microscopic morphological sex differences is shaped by early estradiol exposure and regulated by distinct molecular underpinnings, sometimes even in the same brain region. The specific mechanisms of cellular sex differentiation have been best characterized in three hypothalamic areas: the arcuate nucleus (ARC), the preoptic area (POA) and the medial basal hypothalamus (MBH) (Fig. 2).

Estradiol in the ARC regulates sex differences in physiology

The ARC of the hypothalamus is located around the third ventricle, near the median eminence. Neurons in the ARC regulate the anterior pituitary release of hormones, including GnRH and prolactin, and control the LH surge (Micevych *et al.*, 2009), lactation (Smith & Grove, 2002) and stress-induced suppression of reproduction (Dobson *et al.*, 2003), as well as controlling non-reproductive functions such as appetite regulation and growth hormone

release (reviewed in Bluet-Pajot *et al.*, 1998; Bouret & Simerly, 2006). There are robust sex differences in cell morphology in the ARC. At the ultrastructural level, males have more axosomatic synapses, which are probably GABA-ergic, and females have a higher density of axodendritic spine synapses, which are glutamatergic (Matsumoto & Arai, 1980; Mong *et al.*, 1999). Glia in the ARC are also dimorphic, with males having more complex, stellate astrocytes than females (Mong *et al.*, 1996, 2001; Mong & McCarthy, 1999). These sex differences in cell morphology and synaptic patterning in the ARC are all regulated by estradiol – neonatal castration of males feminizes synapse type (Matsumoto & Arai, 1980; Mong *et al.*, 2001) and spine density (Mong *et al.*, 1999), while treating females neonatally with steroid hormones masculinizes each of these morphological parameters (Mong & McCarthy, 1999; Mong *et al.*, 1999).

The mechanisms through which estradiol inhibits spine formation in the male ARC have yet to be described, but the sex differences in glial morphology in the ARC are dependent upon GABA signaling. Activating $GABA_A$ receptors with muscimol induces stellate astrocytic morphology, and reducing the synthesis of the major GABA synthesizing enzyme, GAD, prevents this morphological change (Mong *et al.*, 2001). As estrogen receptors have not been detected in the astrocyte population in the ARC and GAD is expressed only by neurons, it is concluded that estradiol acts first on GABAergic neurons, which then release GABA onto the astrocytes and induce morphological changes. It remains unclear whether hormone-induced glial differentiation then leads to down-regulated spine density in neighboring neurons, or whether estradiol regulates dendritic morphology via entirely independent mechanisms.

Glial morphology and neuron–glia interactions play an integral role in neuroendocrine processes in adulthood (Garcia-Segura *et al.*, 2008). Rapid, hormonally regulated morphological changes in specialized glia of the median eminence are necessary to allow GnRH neuron terminals physical access to the hypophyseal portal capillaries to produce the release of GnRH (Prevot *et al.*, 1999). Similarly, astrocytes in the rostral POA vary in process number and surface area over the estrous cycle of adult females (Cashion *et al.*, 2003), as do both neurons and glia in the ARC (Witkin *et al.*, 1991; Garcia-Segura *et al.*, 1994). This morphological change regulates afferent synaptic connectivity and subsequent hormone release. It is crucial, then, that the effects of gonadal hormones on glia are well characterized in order to gain a comprehensive understanding of how hormones organize sex-specific reproductive behaviors.

Estradiol acting on the POA contributes to behavioral masculinization

The medial preoptic area is critical for the expression of male sexual behavior (Larsson & Heimer, 1964) and receives input from most major hypothalamic regions and limbic areas, such as the amygdala, bed nucleus of the stria terminalis and septum, as well as sensory areas conveying information about sexually relevant stimuli (Simerly & Swanson, 1986), and sends reciprocal projections to other hypothalamic and extrahypothalamic regions involved in motivated behavior and motor regions involved in the expression of sexual behavior (Simerly & Swanson, 1988). Robust sex differences in neuronal morphology and connectivity as well as glial morphology in the POA are programmed by steroid hormone

exposure during the critical period. Given its central role in the control of reproductive behavior, its not surprising there is a sex difference in synaptic connectivity in the POA (Raisman & Field, 1973). In males, POA neurons have over twice the number of dendritic spines as females, with neonatal estradiol exposure being sufficient to masculinize spine number in females (Amateau & McCarthy, 2002a, 2004). Astrocytes of the POA are also sexually dimorphic, with males having longer and more numerous processes as a result of estradiol action during the neonatal period (Amateau & McCarthy, 2002b).

Downstream of estradiol in the POA is prostaglandin E_2 (PGE_2), a lipid molecule which is synthesized following the estradiol-mediated upregulation of cyclooxygenase-2 (COX-2), the major enzyme responsible for prostanoid synthesis in the brain. PGE_2 is necessary and sufficient to masculinize morphology in the POA (Amateau & McCarthy, 2002a). This PGE_2-induced masculinization of the POA is behaviorally relevant, as administration of PGE_2 to neonatal females allows for adult masculine copulatory behavior, whereas treating males with a COX-2 inhibitor significantly reduces these behaviors, even when estradiol is co-administered (Amateau & McCarthy, 2004; Wright & McCarthy, 2009). Glutamate and protein kinase A (PKA) are the downstream effectors of PGE_2 in the POA, with antagonism of the AMPA-kainate receptor, the metabotropic glutamate receptors and PKA all preventing PGE_2-induced increases in spine number as well as masculine sexual behavior (Amateau & McCarthy, 2002a; Wright & McCarthy, 2009).

One fascinating aspect of the PGE_2-induced sexual differentiation of the POA is that it specifically influences behavioral masculinization, while having no detectable effect on defeminization. This is evidenced by the fact that females masculinized with PGE_2 neonatally are nonetheless capable of female sexual behavior in adulthood when treated with estrogens and progestins (Todd *et al.*, 2005); in other words, they are masculinized, but not defeminized. An additional behavioral assay for PGE_2-induced defeminization in the POA is maternal behavior, a female-typical behavior that is also mediated by the POA (Numan *et al.*, 1985; Numan, 1986). Indeed, neonatal PGE_2 administration to females does not impair maternal behavior (Todd *et al.*, 2005), indicating that even in the same brain region masculinized by estradiol-driven prostaglandin signaling, there is no effect on defeminization of reproductive behavior.

Estradiol acting on the MBH contributes to behavioral defeminization

Immediately adjacent to the POA is the medial basal hypothalamus, which includes the ventromedial nucleus (VMN). The VMN is the critical node in the female sexual behavior circuit; stimulation of the VMN facilitates receptivity and lordosis (Pfaff & Sakuma, 1979b), while lesions of the VMN abolish lordosis responding (Pfaff & Sakuma, 1979a). Synaptic patterning and neuronal morphology in the VMN are sexually dimorphic, with the male VMN having more synapses on dendritic shafts and spines (Matsumoto & Arai, 1986), and a more highly branched dendritic morphology (Mong *et al.*, 1999). As with the POA, the sex difference in synaptic patterning is hormonally regulated; testosterone masculinizes this synaptic patterning in females (Matsumoto & Arai, 1986) and increases branch number (Mong *et al.*, 1999), whereas aromatase inhibitors prevent the masculine pattern (Lewis *et al.*, 1995). However, in contrast to the POA, the sex differences in spine number and

dendritic branching in the MBH are not mediated by PGE_2 (Todd *et al.*, 2005), nor are they accompanied by changes in glial morphology, as is the case in both the POA and the ARC (Mong *et al.*, 1999). Steroid-induced differentiation of the MBH does have one commonality with that of the POA, however – glutamate. Antagonism of the AMPA- and NMDA-type glutamate receptors prevents estradiol-induced spinogenesis (Todd *et al.*, 2007). Interestingly, estradiol in this brain region acts presynaptically, by increasing glutamate release which binds to post-synaptic receptors activating mitogen-activated protein kinase and leading to spine formation (Schwarz *et al.*, 2008). Moreover, glutamate receptor activation is critical to behavioral defeminization – NMDA receptor antagonism during the period of sexual differentiation prevents the steroid hormone-induced defeminization of adult sexual behavior (Schwarz & McCarthy, 2008).

An additional component of estradiol-induced defeminization may involve the suppression of genes important to normal female brain development. In the MBH there are at least two closely related molecules expressed at two-fold or higher magnitude in females compared with males – focal adhesion kinase (FAK) and paxillin (Speert *et al.*, 2007). Both of these molecules are important components of the focal adhesion complex, which negatively regulates cell motility and neurite outgrowth (Brown & Turner, 2004; Rico *et al.*, 2004; Robles & Gomez, 2006). In the developing MBH, estradiol down-regulates FAK within 6 h and paxillin within 48 h while also increasing neurite outgrowth in hypothalamic cultures (Speert *et al.*, 2007). As males have longer dendrites with more spines in the MBH, it follows that FAK and paxillin may negatively regulate neurite outgrowth in females, and that estradiol down-regulates this negative regulator of neurite extension in males. It is an intriguing possibility that FAK and paxillin are two molecular components of active feminization, and that estradiol acts to ‘override’ this signal and result in a defeminized neuronal phenotype.

Androgens contribute to behavioral masculinization via extrahypothalamic targets

Androgens also contribute to the sexual differentiation of brain and behavior, largely via effects on extrahypothalamic targets (Fig. 3). Behaviorally, androgens alone cannot support masculinization, with aromatase knockout males or males given aromatase inhibitors showing disrupted adult partner preference (Bakker *et al.*, 1993a,b), and increased intromission and ejaculation latencies (Bakker *et al.*, 2004). Males receiving aromatase inhibitors prenatally or just after birth also show enhanced lordosis and proceptive behavior (Whalen & Olsen, 1981; Dominguez-Salazar *et al.*, 2002), implying androgens do not support behavioral feminization. These studies are confounded in that androgen receptor expression is regulated by estradiol (McAbee & Doncarlos, 1999; Shah *et al.*, 2004; Juntti *et al.*, 2010), and thus aromatase inhibition may also be altering androgen receptor levels. As normal behavioral masculinization and defeminization are estrogen-dependent, blocking estrogen synthesis may lead to compensatory mechanisms that interfere with normal developmental actions of androgens.

Both rats and mice with the testicular feminization mutation (Tfm), which have a non-functioning androgen receptor, show normal partner and odor preference, mounting and thrusting (Olsen, 1979; Bodo & Rissman, 2007), and castrated Tfm male rats show more female sex behavior than intact Tfm males (Olsen, 1979). Tfm studies thus also implicate estrogens, and not androgens, as the driving force behind behavioral masculinization and defeminization. Tfm males do, however, show subtle changes in hypothalamic morphology, including decreased soma size in the SDN-POA (Morris *et al.*, 2005), and decreased volume of the ventromedial hypothalamus (Dugger *et al.*, 2007). These brain regions are implicated in the expression of masculine and feminine sexual behavior, respectively, although the specific consequences of the morphological changes in Tfm rats for behavior have not been determined. Further complicating the matter is that aromatase activity is lower in Tfm males (Roselli *et al.*, 1987), such that decreased estrogen levels may be responsible for the observed feminization. In fact, normal males treated with non-aromatizable androgens show corresponding decreases in aromatase mRNA expression in the hypothalamus, implicating androgens in the regulation of local estrogen synthesis (Abdelgadir *et al.*, 1994).

Androgen receptor knockout mice, unlike Tfm males, show copulatory deficits, including reductions in mounting, intromission and ejaculation even when administered androgens or estrogens in adulthood (Sato *et al.*, 2004; Raskin *et al.*, 2009; Juntti *et al.*, 2010). The fact that knockout animals do not have androgen receptors either developmentally or in adulthood precludes the ability to determine whether developmental and / or adult androgen receptor function is necessary to produce masculinized and defeminized behavior. Detailed anatomical study of androgen receptor knockouts has yet to be done, and will be helpful in determining whether androgens play a role in masculinizing the hypothalamus.

Androgens have been more firmly established in the regulation of sex differentiation of extrahypothalamic brain regions involved in copulatory behavior. The spinal nucleus of the bulbocavernosus (SNB), a motor nucleus involved in the production of penile reflexes in males, is highly androgen-dependent (Breedlove & Arnold, 1983a,b). Androgens save SNB motoneurons from cell death (Nordeen *et al.*, 1985) and support masculine patterns of dendritic arborization (Goldstein *et al.*, 1990), and anti-androgen-treated or Tfm male rats have a feminized SNB (Breedlove & Arnold, 1981, 1983a). Additionally, the bed nucleus of the stria terminalis, which is involved in the production of copulatory behavior (Emery & Sachs, 1976), shows regional sex differences in volume (Guillamon *et al.*, 1988), and is partially feminized in Tfm males (Durazzo *et al.*, 2007). Overall, evidence from multiple experimental models and various brain regions converges to suggest that androgens play a role in the sexual differentiation of copulatory behavior, largely via effects on extrahypothalamic targets.

Progestins support behavioral masculinization and defeminization

Progestins also contribute to the sexual differentiation of the hypothalamus and sexual behavior, although they have received considerably less attention than the other gonadal hormones. Progesterone levels in the neonate are not different in males and females (Weisz & Ward, 1980), but there are significant sex differences in progesterone receptor (PR) expression in the developing hypothalamus. Males have higher PR in the medial preoptic

nucleus, periventricular nucleus, AVPV and arcuate nucleus (Quadros *et al.*, 2002a,c, 2007). Sex differences in PR are age-dependent, with males having higher preoptic PR expression from embryonic day 18 until postnatal day 10, and females having a later surge in PR expression around postnatal day 10 which attenuates the sex difference (Quadros *et al.*, 2002a). This sex difference in PR is regulated by estrogens, with the delayed production of estradiol in the female resulting in delayed PR up-regulation relative to the earlier androgen surge in males (Quadros *et al.*, 2002a).

Progesterone signaling during the neonatal period regulates hypothalamic development as well as adult male sexual behavior (Fig. 3). Antagonizing progesterone action prevents the androgen-induced masculinization of the SDN-POA (Quadros *et al.*, 2002b), implicating progesterone as a mediator of this masculinization. Progesterone receptor antagonism during development also negatively impacts masculine sexual behavior (Lonstein *et al.*, 2001) and attenuates normal defeminization (Weinstein *et al.*, 1992). PR knockouts, in contrast, have decreased mount latencies, although this effect may also be activational in nature (Schneider *et al.*, 2005). Despite this suggestive data, research has yet to show the means through which progesterone acts to sexually differentiate the developing brain. Moreover, PR antagonism studies used RU-486, a compound which also antagonizes the glucocorticoid receptor, and thus these experiments are far from conclusive. The mechanisms through which progesterone contributes to sexual differentiation is an area ripe for discovery; such research could greatly enhance our understanding of whether the different gonadal hormones act independently or cross talk to produce and maintain behavioral sex differences.

Gonadal hormones and hypothalamic sex differences –concepts and conclusions

This review has focused on the mechanisms through which gonadal hormones organize the rodent hypothalamus to produce masculine or feminine sexual behavior. We as a field have an understanding of several well-characterized sex differences in the hypothalamus, how these sex differences are achieved mechanistically, as well a broad understanding of whether these sex differences are necessary for the production of adult sexual behavior. Although advances are being made in elucidating several unique mechanisms of gonadal hormone action on the brain, there remains a gap between directly associating changes in the neuronal substrate with changes in behavior. Moreover, an integrated conceptualization of hormonally induced sexual differentiation has not been realized. It is unknown whether each cellular mechanism is independent of others or whether there is cross talk between signaling cascades and brain regions that alters the outcome in subtle ways not yet detected? The influence of estradiol on the developing rodent brain is pervasive, having many more effects than those reviewed here and often involving modulation of the disparate molecular signaling cascades that govern normal brain development. Yet reconciling the potent ability of estradiol to regulate the development of the male rodent brain (and the avian brain) with the lack of estradiol-driven masculinization in primates remains a challenge. Generating a cohesive picture of gonadal hormone action on the developing brain and then causally connecting that to adult physiology and behavior as well as generalizing these mechanisms across mammalian and even vertebrate phylogeny is a fundamental future goal. Recent data

suggest that rapid and / or membrane effects of gonadal hormones contribute to the sexual differentiation of the hypothalamus (Speert *et al.*, 2007; Schwarz *et al.*, 2008), but future research will surely continue to characterize these non-classical actions. Future research will also continue to explore the extent to which the classical effects of gonadal hormones on gene transcription can be dampened, enhanced or maintained long-term via both classic protein-synthesis-mediated permanent changes and enduring imprints imposed via epigenetic changes to the genome. Recent work has also implicated direct effects of sex chromosome complement on sexual differentiation of the brain, which are independent of hormonally mediated organization (Arnold & Chen, 2009). Future research will thus better characterize how chromosomal sex and gonadal sex interact to produce sexually differentiated brain and behavior.

Abbreviations

ARC	arcuate nucleus
AVPV	anteroventral periventricular nucleus
COX-2	cyclooxygenase-2
ER	estrogen receptor
FAK	focal adhesion kinase
LH	luteinizing hormone
MBH	medial basal hypothalamus
PGE_2	prostaglandin E_2
PKA	protein kinase A
POA	preoptic area
PR	progesterone receptor
SDN-POA	sexually dimorphic nucleus of the preoptic area
SNB	spinal nucleus of the bulbocavernosus
Tfm	testicular feminization mutation
VMN	ventromedial nucleus

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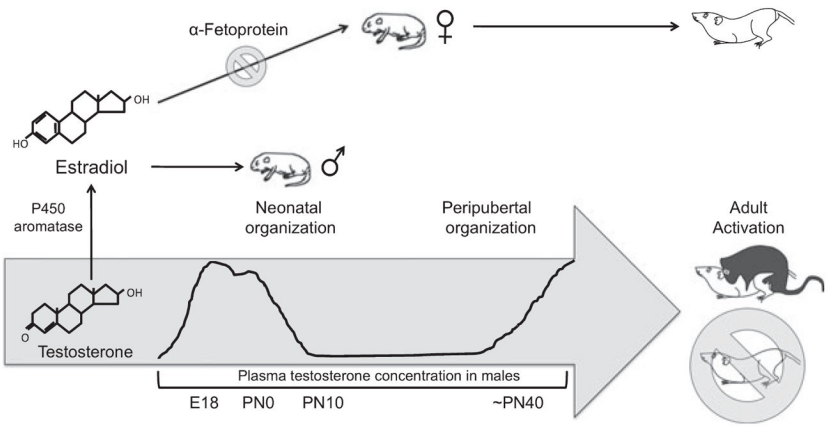

Fig. 1.

The sensitive period for the organizational effects of steroids on the brain. In males, plasma testosterone levels rapidly increase late during the embryonic period and peak on postnatal day (PN) 0. Once in the brain, testosterone is converted by the aromatase enzyme into estradiol, which acts to masculinize and defeminize the hypothalamus / preoptic area. A second organizational period occurs peripubertally, when steroid hormones begin to increase. The developing female brain is protected from the masculniizing effects of the maternal dam's estradiol by α-fetoprotein, a circulating steroid binding globulin that sequesters estradiol in the bloodstream of the fetus. In adulthood, the differentiated hypothalamus / preoptic area are subject to the activational effects of gonadal hormones, resulting in sex-specific copulatory behavior.

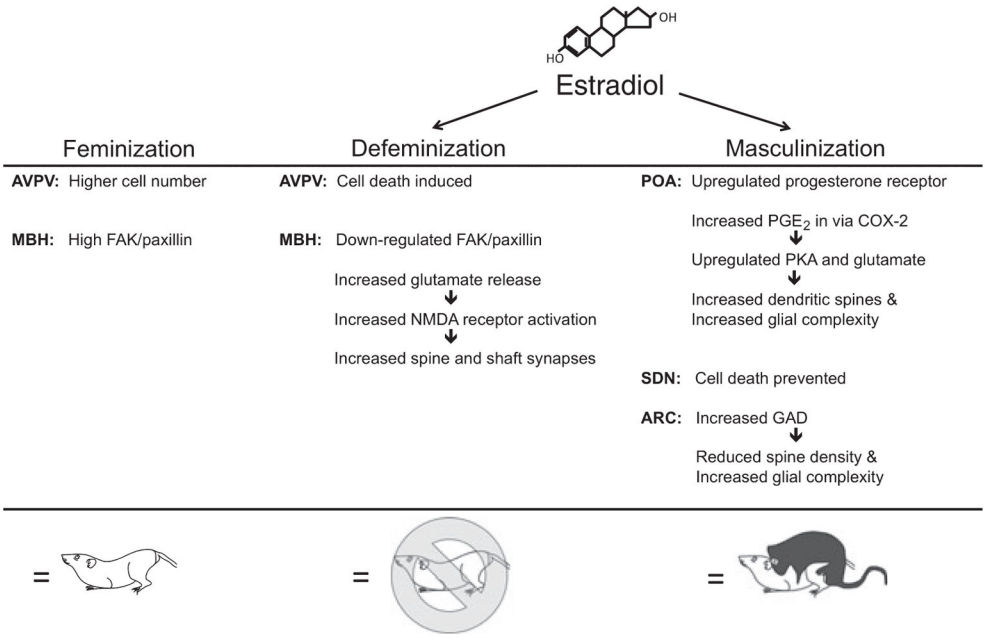

Fig. 2.

Estradiol is responsible for both masculinizing and defeminizing the hypothalamus / preoptic area. Estradiol induces a host of molecular and morphological changes in a regionally specific manner. Feminization of the brain is the default program, and prepares the brain for female sexual behavior in adulthood. For abbreviations, see text.

Evidence from several experimental models demonstrate the role of steroid hormones in sexually differentiating the brain and reproductive behavior

	Effect on masculinization or defeminization:	Model:
Estrogens	SDN and AVPV cell number not masculinized	ER antagonist
	Decreased glial complexity and increased spines in ARC	Non-aromatizable androgen treatment
	Decreased spine density in VMN	Aromatase antagonist
	Decreased male copulatory behavior, partner preference	ERKO; ArKO; Aromatase inhibition
	Increased lordosis behavior	ERKO; ArKO; Aromatase inhibition
Androgens	Decreased SDN soma size	Tfm
	Decreased vlVMH volume	Tfm
	Feminized BNST	Tfm
	Feminized SNB and penile reflexes	Tfm; Androgen receptor antagonist
	Partner and odor preference not masculinized	Tfm; ArKO; aromatase inhibition
	Mounting behavior not masculinized	Tfm; ArKO; aromatase inhibition
	Impaired copulatory behavior (could be activational)	Androgen receptor KO
Progestins	SDN cell number not fully masculinized	PR antagonist
	Decreased male copulatory behavior	PR antagonist
	Increased lordosis behavior	PR antagonist
	Decreased mount latency (could be activational)	PRKO

Fig. 3.

Estrogens, androgens and progestins all contribute to sexual differentiation. Evidence from multiple pharmacological and transgenic experimental models implicate each type of gonadal hormone in sexual differentiation of the brain and reproductive behavior. For abbreviations, see text.