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Estrogenic regulation of social behavior and sexually dimorphic brain formation

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Estrogenic Regulation of Social Behavior and Sexually Dimorphic Brain Formation

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[*:] All four authors equally contributed to this article. They also dedicate this article to their long-term collaborator and friend Dr. Sergei Musatov, who was tragically killed in May, 27, 2015 in New York City.

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Highlights

- Gonadal hormones affect the morphology of sexually dimorphic nuclei (SDNs) of the brain.
- The principal nucleus of the bed nucleus of the stria terminals (BNSTp) is a male-dominant SDN.
- The BNSTp contains not only a male-dominant subregion but also a female-dominant subregion.

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- ERα and ERβ may have opposing effects on the estrogenic regulation of sex-typical social behaviors.
- Both genomic and non-genomic signaling by estrogens regulate social recognition and learning.
- Spinogenesis may be a critical mechanism by which estrogens, via ERs, regulates social behaviors.

Abstract

It has long been known that the estrogen, 17β-estradiol (17β-E), plays a central role for female reproductive physiology and behavior. Numerous studies have established the neurochemical and molecular basis of estrogenic induction of female sexual behavior, i.e., lordosis, in animal models. In addition, 17β-E also regulates male-type sexual and aggressive behavior. In males, testosterone secreted from the testes is irreversibly aromatized to 17β-E in the brain. We discuss the contribution of two nuclear receptor isoforms, estrogen receptor (ER)α and ERβ to the estrogenic regulation of sexually dimorphic brain formation and sex-typical expression of these social behaviors. Furthermore, 17β-E is a key player for social behaviors such as social investigation, preference, recognition and memory as well as anxiety-related behaviors in social contexts. Recent studies also demonstrated that not only nuclear receptor-mediated genomic signaling but also membrane receptor-mediated non-genomic actions of 17β-E may underlie the regulation of these behaviors. Finally, we will discuss how rapidly developing research tools and ideas allow us to investigate estrogenic action by emphasizing behavioral neural networks.

Keywords: 17β-estradiol; genomic action; membrane-mediated non-genomic action; estrogen receptor α; estrogen receptor β; hypothalamus; hippocampus; sexual behavior; aggressive behavior; parental behavior; sexually dimorphic nuclei; testosterone; dendritic spines; social behavioral networks; social recognition; social learning

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1. Introduction

1.1 Genomic vs. non-genomic action of 17β-estradiol

The estrogen, 17β-E is a steroid hormone that signals via both non-genomic and genomic pathways in the central nervous system (CNS) in order to drive behavior. Considering that non-genomic signaling is often defined "in opposition" to genomic signaling, it is important to first define genomic signaling. 17β-E is a ligand for the nuclear receptors, ERα and ERβ which act as ligand-activated transcription factors. As transcription factors, they bind to estrogen response elements (ERE), a cognate enhancer element and regulate transcription from target genes. This "genomic" slow mode of signaling requires intracellular receptors and is thought to occur in all cells that express ERα or ERβ (Mangelsdorf et al., 1995; Nilsson et al., 2001). On the other hand, non-genomic signaling is fast signaling mostly initiated at the plasma membrane by a putative membrane estrogen receptor (mER) that binds 17β-E (Vasudevan and Pfaff, 2007). This results in the activation of kinases (Qiu et al., 2003) and calcium flux (Temple and Wray, 2005) within cells (Figure 1). Non-genomic signaling has been shown to be important in cardiovascular physiology (Feldman and Limbird, 2017) and in pathologies such as breast cancer where non-genomic signaling is an important pathway by which cancerous cells acquire resistance to endocrine therapy (Anbalagan et al., 2012; Le Romancer et al., 2010). In the last decade or so, the coupling of these pathways i.e. non-genomic signaling to genomic transcription has led to the idea of an integrated signaling pathway that starts at the membrane and culminates in the nucleus (Figure 1) (Micevych and Dewing, 2011) (Vasudevan et al., 2005).

1.2 The distribution of the intracellular ERα and ERβ in the brain

17β-E binds to intracellular ERα and ERβ with similar affinity. Though the distribution of the two receptor isoforms partly overlaps, some brain areas that are known to be part of the social behavioral network (Nelson and Trainor, 2007; Newman, 1999) express either ERα or ERβ more strongly (Mitra et al., 2003; Nomura et al., 2003; Shughrue et al., 1997). For instance, ERα, but not ERβ, is highly expressed in the ventromedial nucleus of the hypothalamus (VMH), which plays a central role for the induction of female sexual behavior via genomic action of 17β-E as we discuss extensively in Section 3. On the other hand, ERβ is more abundant than ERα in the brain areas such as the paraventricular nucleus of the hypothalamus (PVN) and the dorsal raphe nucleus of the midbrain (DRN). A number of hypothalamic and limbic brain regions such as the medial preoptic area (mPOA), the medial amygdala (MeA), the bed

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nucleus stria terminalis (BNST) and the lateral septum (LS) are known to express both ERα and ERβ. Although some studies have reported that there are neurons which express both ERα and ERβ (Greco et al., 2001; Shughrue et al., 1998) in rat brains, it is largely unknown how much ERα and ERβ are co-expressed at the level of an individual cell, partly due to a lack of effective antibody for ERβ detection (Snyder et al., 2010). It is expected that the recently developed transgenic mice tagged for ERβ (Milner et al., 2010; Oyola et al., 2017; Sagoshi et al., 2018; Zuloaga et al., 2014) will provide more detailed information on this matter and contribute to a better understanding of the respective roles of ERα and ERβ in the estrogenic regulation of social behavior.

1.3 The membrane ER(mER) in the hypothalamus and hippocampus

Unlike classical genomic signaling that is initiated by the intracellular ERα or ERβ, non-classical signaling is generally initiated by a mER but the identity of a unique mER is still controversial. ERα, including variants of ERα that lack some portions of the N-terminal domain has been shown at the membrane in hypothalamic cell lines (Dominguez et al., 2013) and in neurons (Dominguez and Micevych, 2010) though the mechanism of attachment to the plasma membrane for nuclear receptors has been disputed (Rainville et al., 2015). In the hippocampus, ERα and ERβ are linked to mGluR2/3 via the adaptor, caveolin 3, leading to $G\alpha_{i/o}$ signaling within cells. ERα can also be linked to mGluR1 via caveolin 1 (Boulware et al., 2007), resulting in $G_{\alpha q}$ signaling within cells. To our knowledge, ERβ signaling via glutamate receptors in the hypothalamus remains unexplored. Hence, ERα or ERβ serve not only as an intracellular receptor mediating classical genomic signaling but may also play a dual role as a receptor at the plasma membrane to mediate rapid non-genomic signaling. For a discussion on the various proteins that may function as mERs, the reader is referred to {Rainville et al., 2015)

A novel G-protein coupled receptor (GPCR) called GPR30 or GPER1 was shown to bind 17β-E at high affinity and activate $G\alpha_s$-coupled signaling in breast cancer cells in 2005 (Thomas et al., 2005). GPR30 is widely distributed in the CNS and is present in the amygdala, hypothalamus and hippocampus (Brailoiu et al., 2007). 17β-E binding to GPER1 can activate protein kinase A (PKA) and phosphoinositide-3-kinase (PI3K) pathways, as well as increase ERK activity via the activation of epidermal growth factor receptor (EGFR) (Prossnitz and Barton, 2009; Prossnitz and Maggiolini, 2009). In addition, GPER1 activation induces calcium increases which appear to be ryanodine receptor dependent in breast cancer cells (Ariazi et al., 2010). In the brain, GPER1 is implicated in reproductive behavior, social cognition,

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spatial memory, and neuroprotection (Alexander et al., 2016; Hadjimarkou and Vasudevan, 2018), suggesting that rapid, non-genomic signaling influences these behaviors.

This review is based on a symposium presented by the authors at the 2018 annual conference of the International Behavioral Neuroscience Society. The basis of the sex-specific social behaviors described in this review is the development of a sexually dimorphic brain, which is itself regulated by local production of steroid hormones during critical periods of development (Section 2). Our objectives in Sections 3 and 4 are to focus on both genomic and non-genomic signaling pathways that underlie 17β-E-mediated social behaviors such as sex and aggression as well as underlying state anxiety with particular emphasis on the contribution of the various receptors.

2. Role of sex steroids in the sexually dimorphic formation of brain structures underlying the control of social behavior

In the sexually differentiated brain, there are sex differences in the tissue structures. Nuclei exhibiting morphological sex differences are called sexually dimorphic nuclei, in which sex difference in the volume and the morphology and number of neurons and glial cells, synaptic number, etc. are seen. Sexually dimorphic nuclei are considered the structural basis for generating sex differences in brain functions. Estrogens not only modulate the neural control of social behavior but also play an essential role in the sexually dimorphic formation of brain structures to construct neural circuits that control sex-specific physiological phenomenon, including social behavior. Though sex is also genetically determined, in this section, we focus specifically on the role of sex steroids, 17β-E and testicular testosterone which not only acts directly but also acts as 17β-E after aromatization in the brain, in the formation of sex differences in volume and neuron number of the sexually dimorphic nuclei found in the mPOA and BNST that control social behavior. Additionally, a recently identified sexually dimorphic cell group is introduced. The roles of sex steroids in the formation of this sexually dimorphic cell group and a possible role of this cell group in social behavior will be discussed.

2.1 Contribution of sex steroids to the sexually dimorphic formation of brain structures

It has long been known that testosterone secreted from developing testes plays an important role in brain sexual differentiation. In 1959, Phoenix and colleagues reported on the sexual behavior of guinea pigs that were born from mothers injected with testosterone during pregnancy (Phoenix et al., 1959). According to their report, females exposed to exogenous testosterone in the embryonic period had

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greatly reduced performance to display female sexual behavior in adulthood. In contrast, these females displayed male sexual behavior when they were treated with exogenous testosterone in adulthood. Male littermates exposed to exogenous testosterone showed male sexual behavior in adulthood as normal males that secreted testosterone from the testes during the embryonic period displayed male sexual behavior. These results demonstrate that the action of testicular testosterone in the embryonic period causes masculinization and defeminization of sexual behavior, and the effects of testosterone persist into adulthood. The concept proposed by Phoenix and colleagues has been supported by many studies. Additionally, in rodents, it has been revealed that 17β-E, which is locally synthesized in the brain from testicular testosterone by aromatase in the perinatal period, affects the brain to masculinize and defeminize sexual behavioral patterns (MacLusky et al., 1979; MacLusky and Naftolin, 1981; McEwen et al., 1977). Injection of an anti-androgen drug or an aromatase inhibitor into pregnant rats enhances the activity of female sexual behavior in offspring in both sexes in adulthood (Clemens and Gladue, 1978; Gladue and Clemens, 1978, 1982). In contrast, neonatal testosterone treatment decreases the activity of female sexual behavior in rats of both sexes (Gladue and Clemens, 1982). This effect of testosterone can be mimicked by estrogens, because the activity of female sexual behavior in female rats and neonatally castrated male rats is reduced by β-estradiol-3-benzoate (EB) injection in the postnatal period (Brown-Grant, 1975; Feder and Whalen, 1965; Whalen and Nadler, 1963). Thus, the perinatal period is the critical period for the sexual differentiation of the brain in rodents. However, currently it is considered that the perinatal period is not the sole stage but the initial stage of sex steroid-dependent sexual differentiation of the brain in rodents (Juraska et al., 2013; Schulz et al., 2009). In the classic view of brain sexual differentiation, 17β-E affects the brain during the perinatal period to masculinize and defeminize the brain. However, in addition to this action, 17β-E plays a role in feminizing the brain during the peripubertal period. Female aromatase knockout mice showed low levels of the activity of lordosis, a female sexual behavior, even after being treated with 17β-E and progesterone at adulthood (Bakker et al., 2002). The decreased lordotic activity in female aromatase knockout mice is recovered by injection of EB in the prepubertal period (Brock et al., 2011). Thus, the neural system involved in the regulation of female sexual behavior is defeminized in males by 17β-E originating from testicular testosterone during the perinatal period and conversely feminized in females by 17β-E that is synthesized from the ovary during the prepubertal period. In addition to the effects of sex steroids, it has been demonstrated that genes on the sex chromosomes directly influence the sexual differentiation of the brain; these mechanisms and models to study them are reviewed in Arnold, 2014; Cox et al., 2014; McCarthy and Arnold, 2011.

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Testosterone secreted from the testes in the perinatal period is a key molecule in the formation of sexually dimorphic nuclei (Figure 2). The sexually dimorphic nucleus of the preoptic area (SDN-POA) and the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) in males are larger in volume and contain greater number of neurons than those in females of rats and mice (del Abril et al., 1987; Forger et al., 2004; Gorski et al., 1978; Gorski et al., 1980; Hines et al., 1992) (Figure 3). The SDN-POA and BNSTp exist in other species (Campi et al., 2013; Moe et al., 2016b), suggesting that these sexually dimorphic nuclei are evolutionally conserved across species. Intriguingly, the magnitude of sex difference in SDN-POA volume appears to be correlated with social organization: this sex difference is more salient in polygamous species than in monogamous species (Campi et al., 2013). The volume and neuron numbers of the SDN-POA and BNSTp in adult females are increased by administration of testosterone during the postnatal period, whereas those in adult males are decreased by neonatal orchidectomy (Chung et al., 2000; Gorski et al., 1978; Guillamon et al., 1988). In contrast, the anteroventral periventricular nucleus (AVPV) is a sexually dimorphic nucleus that is larger in size and has higher cell density in females than in males (Bleier et al., 1982; Sumida et al., 1993) (Figure 3). The volume of the AVPV in female rats is reduced by postnatal treatment with testosterone (Ito et al., 1986). Because the effect of testosterone on sexually dimorphic nuclei can be mimicked by 17β-E (Hisasue et al., 2010; Patchev et al., 2004), testicular testosterone during the perinatal period is thought to act on sexually dimorphic nuclei after it is converted to 17β-E by aromatase.

As mentioned above, testosterone secreted from the testis in the perinatal period is important for brain sex differentiation in rodents. However, this testosterone action is not enough to complete the processes of brain sexual differentiation. Besides perinatal testicular testosterone, gonadal hormones secreted in the peripubertal period have been shown to influence the sexual differentiation of the brain (Juraska et al., 2013; Schulz et al., 2009) (Figure 2). During puberty, gonads become mature, and thereby the secretion of testicular testosterone and ovarian 17β-E increases. The brain affected by testicular testosterone in the perinatal period is again affected by testicular testosterone in the pubertal period, so that the brain is masculinized and defeminized. Estrogens secreted from the ovaries during puberty act to feminize and demasculinize the brain. New cells generated during puberty are added to the AVPV, SDN-POA, and MeA, a sexually dimorphic nucleus having larger volume and greater number

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of spines in males (Mizukami et al., 1983; Nishizuka and Arai, 1981a, b), and that the numbers of new cells in the AVPV of female rats and in the SDN-POA and MeA of male rats are greater than those in the opposite sex (Ahmed et al., 2008). This finding indicates that cell generation during puberty significantly contribute to the formation of the sexually dimorphic nuclei. In addition, gonadectomy before puberty inhibits the increase in the number of new cells and the volume of the female AVPV and the male SDN-POA, and new cell numbers of the male MeA (Ahmed et al., 2008). These findings indicate that gonadal hormones during puberty also influence the formation of the sexually dimorphic nuclei.

Although the mechanisms of sex steroid actions on the formation of sexually dimorphic nuclei are not well understood, there are several studies that help to understand the mechanisms, in particular the contribution of the ERs and the AR. The volume and neuron numbers of the BNSTp in male mice are decreased by deletion of the ERα gene and become similar to those in female mice (Tsukahara et al., 2011). In addition, the volume and neuron numbers of the AVPV in ERα knockout male mice become similar to those in female mice compared with wild-type male mice (Kanaya et al., 2014). In contrast, there is no significant effect of lacking the ERβ gene on the sexually dimorphic formation of the BNSTp and AVPV (Kanaya et al., 2014; Tsukahara et al., 2011). However, there are reports showing that administration of an ERβ agonist, as well as an ERα agonist, into postnatal females has a pharmacological effect to increase the number of BNSTp neurons in mice (Hisasue et al., 2010) and decrease the number of AVPV neurons in rats (Patchev et al., 2004). Deletion of the aromatase gene, as well as the ERα gene, induces abnormal formation of the morphology of the BNSTp and AVPV in male mice but not in female mice (Kanaya et al., 2014; Tsukahara et al., 2011). Additionally, formation of male BNSTp is disrupted in rats that exhibit testicular feminization associated with mutation of the androgen receptor (AR) gene (Allieri et al., 2013; Durazzo et al., 2007) and in AR knockout mice (Kanaya et al., 2014), although the AVPV in the male brain develops normally without functional AR (Kanaya et al., 2014; Simerly et al., 1997). Based on these studies with gene knockout mice, it is likely that testicular testosterone promotes organization of the male AVPV through mainly binding to ERα after aromatization. The AVPV of mice expresses the mRNAs of ERα and aromatase during the perinatal period, and it expresses the mRNA of ERα but not aromatase in the peripubertal period (Kanaya et al., 2018; Kanaya et al., 2014), suggesting that the male AVPV is organized by the actions of aromatized testosterone via ERα during the perinatal period. Regarding the BNSTp, the formation of this nucleus in male mice requires not only aromatized testosterone actions via ERα but also the actions of androgens binding to AR. In the BNSTp of mice, the mRNAs of ERα and aromatase were expressed in the perinatal

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and peripubertal periods, and the mRNA of AR was expressed in the postnatal and peripubertal periods (Kanaya et al., 2018; Kanaya et al., 2014).

Sickel and McCarthy reported that the SDN-POA of rats contains neurons expressing calbindin-D28K (CALB), a calcium binding protein (Sickel and McCarthy, 2000). The cluster of CALB-D28K neurons in the male SDN-POA is larger than that in the female SDN-POA of rats and mice (Orikasa and Sakuma, 2010; Sickel and McCarthy, 2000). CALB-D28K is therefore used as a marker to identify a sexually dimorphic nucleus that is included in the SDN-POA and was termed the CALB-SDN (Figure 3). Like the SDN-POA, the BNSTp contains many CALB-D28K neurons, and the number of CALB-D28K neurons in the BNSTp is larger in males than in females (Gilmore et al., 2012; Morishita et al., 2017). Hereafter the BNSTp identified by CALB-D28K is termed CALB-BNSTp (Figure 3). Thus, CALB-D28K is useful to determine not only the CALB-SDN but also the CALB-BNSTp, although the physiological roles of CALB-D28K in the CALB-SDN and CALB-BNSTp require further investigation. The volume of the CALB-SDN and CALB-BNSTp are increased in adult female mice by postnatal treatment with testosterone propionate and EB, but not dihydrotestosterone, a non-aromatizable testosterone (Morishita et al., 2017; Orikasa and Sakuma, 2010), suggesting that postnatal testicular testosterone is aromatized and then acts via binding to ER for masculinization of the CALB-SDN and CALB-BNSTp.

The mechanisms by which the CALB-SDN and CALB-BNSTp are masculinized after testicular testosterone during the perinatal period acts there are largely unclear. Control of cell number by apoptosis and actions of sex steroids in apoptosis is known to be critical for the formation of sexually dimorphic nuclei (Tsukahara, 2009). However, sex differences in the number of CALB-D28K neurons in the CALB-SDN and CALB-BNSTp are not eliminated by the deletion of proapoptotic Bax gene (Gilmore et al., 2012), suggesting that apoptotic control of cell number contributes less to the formation of CALB-SDN and CALB-BNSTp. On the other hand, the formation of sex difference in the number of CALB-D28K neurons may involve the epigenetic control of cell phenotype, because neonatal treatment of a DNA methyltransferase inhibitor increases CALB-D28K neuron number in both sexes (Mosley et al., 2017). Additionally, it was reported that the volume of the BNSTp in male mice and neonatally testosterone-treated female mice is reduced by a histone deacetylase inhibitor (Murray et al., 2009), suggested that a disruption in histone deacetylation induces long-term alterations in gene expression that suppress the masculinizing actions of testosterone in the BNSTp.

In the CALB-SDN and CALB-BNSTp, the sex differences in the volume and numbers of CALB-D28K neurons emerge before puberty (Morishita et al., 2017; Wittmann and McLennan, 2013a, b). The sex

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differences become prominent in adulthood with increasing volume and neuron numbers in male mice and decreasing volume and neuron numbers in female mice, excepting CALB-SDN volume in females (Morishita et al., 2017; Wittmann and McLennan, 2013a, b). In addition to cell generation, cell loss during the pubertal period is also involved in the organization of sexually dimorphic nuclei. The volume and calbinidn-D28K neuron numbers of the CALB-BNSTp and CALB-SDN in adult male mice are decreased by prepubertal orchidectomy, although those in adult female mice are not affected by prepubertal ovariectomy (Morishita et al., 2017). Additionally, in the CALB-BNSTp of prepubertally orchidectomized male mice, it was revealed that the decreased volume and calbinidin-D28K neuron number by prepubertal orchidectomy are recovered by testosterone replacement in the peripubertal period (Kanaya et al., 2018). These findings indicate that testicular hormones in the peripubertal period act in remodeling the brain after it develops with the organizational effect of testicular testosterone during the perinatal period.

2.2 Morphology, sexual differentiation, and functions of a novel sexual dimorphic cell group

A number of sexually dimorphic nuclei have been identified in the brains of many species. However, potential still exists to uncover novel sexually dimorphic brain structures. Even if sexually dimorphic nuclei have been identified, the details of the morphological features and physiological functions of the sexually dimorphic nuclei are not well understood. As mentioned in the above section, the BNSTp identified by Nissl staining and the CALB-BNSTp identified by CALB-D28K are sexually dimorphic nuclei that contain more neurons and are larger in volume in male mice than in female mice. When the CALB-BNSTp is compared with the BNSTp, it can be found that the ventral part of the BNSTp does not express CALB-D28K (Morishita et al., 2017). It has been reported that p21 protein (Cdc42/Rac)-activated kinase 3 (Pak3) is abundantly expressed in the BNSTp of mice and can be used as another marker for BNSTp (Morishita et al., 2017). According to this study, a ventral part of the BNSTp expresses Pak3 but not CALB-D28K. The ventral part of the BNSTp (hereafter BNSTpv) in female mice was larger in volume than the BNSTpv of male mice, although the volume of BNSTp identified by Pak3 and CALB-BNSTp identified by CALB-D28K was larger in males than in females (Morishita et al., 2017). Thus, the BNSTp showing male-dominant morphological sex difference is composed not only of male-dominant sexually dimorphic cell group, CALB-BNSTp, but also of a newly found female-dominant sexually dimorphic cell group, BNSTpv (Figure 3).

Gonadal hormones play an essential role in the sexually dimorphic formation of the BNSTpv as well as other sexually dimorphic nuclei. The volume and neuron numbers of the BNSTpv in male mice

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are increased by neonatal orchidectomy. In contrast, subcutaneous injection of testosterone, 17β-E, or dihydrotestosterone into postnatal female mice reduce the volume and neuron numbers of the BNSTpv in adulthood (Morishita et al., 2017). These findings suggest that testicular testosterone in the postnatal period acts to defeminize the BNSTpv through binding to AR directly and through binding to ER after aromatization. Furthermore, the sexually dimorphic formation of the BNSTpv appears to be affected by gonadal hormones during puberty. The volume and neuron numbers of the BNSTpv in adult female mice are decreased by prepubertal ovariectomy (Morishita et al., 2017), suggesting that pubertal ovarian hormones affect the BNSTpv so that the BNSTpv are reorganized for feminization. The effects of prepubertal ovariectomy on the BNSTpv may be due to a lack of estrogens during puberty, because treatment with 17β-E during puberty can rescue the effects of prepubertal ovariectomy (unpublished data). In male mice, the volume and neuron numbers of the BNSTpv in adulthood are not affected by prepubertal orchidectomy, although neonatal orchidectomy significantly affects the formation of the BNSTpv (Morishita et al., 2017). Taken together, the sexually dimorphic formation of the BNSTpv requires the actions of testicular testosterone in the postnatal period and of ovarian 17β-E in the pubertal period.

As mentioned before, the BNSTpv of female mice is larger in volume and contains more neuron numbers compared to the BNSTpv of male mice. Additionally, the BNSTp may exhibit a female-dominant sex difference in ERα expression, because the dorsal hypothalamic area corresponding to the BNSTpv has more cells expressing ERα and shows a higher expression level of ERα in female mice than in male mice (Moe et al., 2016a). Physiological functions of the BNSTpv are largely unknown. However, because of the female-dominant sex differences in neuron numbers and ERα expression, the BNSTpv may be involved in female-typical behaviors that require 17β-E binding to ERα. In sexually receptive females displaying lordosis, the expression of c-Fos, a neuronal activity maker, increases or decreases in several brain regions regulating the behavior (Coolen et al., 1996; Pfaus et al., 1996). However, c-Fos expression does not change in the BNSTpv of females displaying lordosis (Moe et al., 2016a), suggesting that the BNSTpv of female mice contributes less to sexual behavior. The BNSTpv is a part of the bed nucleus of the stria terminalis (BNST) and is located dorsal to the mPOA. The mPOA and BNST play key roles in maternal behavior (Numan et al., 1988; Tsuneoka et al., 2013). 17β-E signaling via ERα is an important component of the pathways that stimulate maternal behavior (Bridges, 2015; Pfaff et al., 2011). Therefore, it was examined whether the BNSTpv in female mice is involved in maternal behavior. The expression of c-Fos in the BNSTpv was analyzed in virgin female mice and primiparous mothers after

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they were exposed to pups in their home cages (Moe et al., 2016a). In this analysis, primiparous mothers exhibited higher performance of maternal behavior than did virgin female mice. In the mPOA and BNST of female mice, exposure to pups and subsequent display of maternal behavior generally increase c-Fos expression (Tsuneoka et al., 2013). However, c-Fos expression in the BNSTpv of primiparous mothers was lower than that of virgin female mice. This suggests that there is a neuronal population, the activity of which decreases with increasing maternal behavior performance. The BNSTpv may contain neurons that play an inhibitory role in maternal behavior, although clarification of the physiological functions of the BNSTpv requires further investigation.

3. Estrogenic regulation of sex-typical social behavior

Social behaviors are behaviors that are influenced by and can influence behavior by other members of the same species. In this sense, both sex and aggression are social behaviors that are sexually dimorphic; however, both are typically considered separate from other social behaviors such as social recognition or pair bonding. We discuss the contribution of signaling by estrogens to these behaviors in rodents in this section, followed by a section of estrogenic regulation of social recognition and social learning.

3.1 Female sexual behavior

A neural "bioassay" of activational estrogenic action in the adult brain is the 17β-E-induced classical reproductive behavior, lordosis, in female rodents (Pfaff, 1999). In rats and mice, 17β-E signaling in the ventromedial hypothalamus (VMH), particularly in the ventrolateral part (vlVMH), is necessary for lordosis behavior (Davis et al., 1979; Pfaff and Sakuma, 1979). The time-dependent transcriptional upregulation of progesterone receptor (PR) in the VMH by 17β-E (Parsons et al., 1982a; Parsons et al., 1982b) is representative of the genomic signaling pathway that is required for lordosis behavior. Knockout mice of ERα, during gonadally intact as well as subsequent to priming with EB plus progesterone after ovariectomy, are not sexually receptive at all (Ogawa et al., 1998; Ogawa et al., 1996) (Rissman et al., 1997). It is reported that ERα knockout female mice do not show any proceptive still posture or receptive lordosis posture in response to attempted mounts by sexually active male mice and rather show very strong rejection responses, such as kicking, fleeing, and upright posture (Ogawa et al., 1998). In ERα knockout mice, as expected, PR induction by EB priming was greatly reduced in the vlVMH. These findings in knockout mouse model are consistent with pharmacological studies reporting facilitation of lordosis behavior in female rats by either subcutaneous or intracerebroventricular

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injections of ERα specific agonist, propyl-pyrazole triol (Dominguez-Ordonez et al., 2016; Mazzucco et al., 2008). All these studies do not provide further information about responsible brain site(s) of ERα-mediated estrogenic regulation on female sexual behavior. Studies using brain site-specific gene knockdown methods revealed that reduction of the levels of ERα in the VMH, but not in the adjacent ERα-rich arcuate nucleus, by siRNA abolishes both proceptive still posture and lordosis behavior (Musatov et al., 2006). These ERα knockdown mice show very similar behavioral responses (eg., kicking and fleeing, etc.) in response to sexually active male mice as those seen in ERα knockout mice (Musatov et al., 2006). In both cycling and EB -primed ovariectomized mice treated with siRNA for ERα knockdown, PR was not expressed in EGFP, the reporter protein for siRNA transduction, positive cells. Therefore, it is concluded that 17β-E via ERα-mediated transcription of the PR in the VMH is a critical genomic signaling pathway for this behavior.

The function of intracellular ERβ in the estrogenic regulation of lordosis behavior may be very different from that of ERα. Unlike ERα, only a small number of ERβ immunopositive cells are found in the limited area of the vlVMH (Mitra et al., 2003; Nomura et al., 2003; Shughrue et al., 1997). Not surprisingly, cycling ERβ knockout mice show equivalent levels of lordosis behavior as found in wild-type (βWT) mice on the day of behavioral estrus (Ogawa et al., 1999). However, the same study also reported that ERβ knockout mice unusually maintained high levels of receptivity on the day after behavioral estrus. This phenomenon was also reported in EB-primed ovariectomized βERKO mice tested at 30hr after progesterone treatment (Sano et al., 2018). Therefore, it is hypothesized that ERα in the VMH is absolutely necessary for the induction of lordosis behavior in female mice whereas ERβ, expressed in brain regions other than the VMH (Alves et al., 2000; Helena et al., 2009; Nomura et al., 2005), may also be involved in the regulation of lordosis. A recent study using siRNA has investigated possible involvement of ERβ–mediated action in one of the ERβ-rich brain regions (Sano et al., 2018). Female mice treated with siRNA to site-specifically knockdown ERβ expression in the dorsal raphe nucleus (DRN) were ovariectomized and injected with EB (48hr before the first test) and progesterone. They were tested for lordosis behavior on twice consecutive days: the first test on the day mimicking behavioral estrus (*i.e.,* 4-6 hr after progesterone treatment) and the second test on the day with the hormonal condition mimicking the day after behavioral estrus (*i.e.,* 30 hr after progesterone treatment). It was found that ERβ knockdown mice showed equivalent levels of lordosis behavior as control mice in the first test. However, unlike control mice, levels of lordosis behavior did not decline in ERβ knockdown mice in the second test. Therefore, it is concluded that ERβ in the DRN may be involved in the inhibitory

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regulation of sexual behavior specifically on the day after behavioral estrus in cycling female mice. Although the exact mechanism of this ERβ-mediated action has not been specified, it is hypothesized that the serotonergic system may be involved. Previous studies have shown that a large proportion of ERβ expressing neurons in the DRN are serotonergic (Nomura et al., 2005) and send projections to the hypothalamic regions (Lu et al., 2001) where activation of 5HT-1A receptors inhibit sexual behavior (Snoeren et al., 2014, Uphouse and Caldarola-Pastiszka, 1993). Furthermore, activation of ERβ by 17β-E or ERβ specific agonists, diarylpropionitrile (DPN) in ovariectomized female rats is reported to enhance mRNA expression of tryptophan hydroxylase 2 (TPH 2) in the DRN (Donner and Handa, 2009). Therefore, it is possible that ERβ in the DRN may be mediating the inhibition of female sexual behavior by up-regulating the activity of the serotonergic system within the DRN that further activates 5HT-1A receptors expressed in hypothalamic regions.

In addition to genomic action, a number of studies have reported that non-genomic signaling in the arcuate nucleus of the hypothalamus (ARH) is also important for the initial inhibition of lordosis, which precedes the later activation by 17β-E that is due to genomic signaling. Experiments from the Micevych laboratory demonstrated rapid signaling by the ERα at the plasma membrane in ARH neurons initiates a PKC cascade that leads to μ-opioid receptor (MOR) internalization and activation. In the ARH, MOR internalization is inhibited by inhibitors to the metabotropic glutamate receptor (mGluR1) and caveolin-1 which demonstrates that, similar to the hippocampus, ERα is linked to mGluR1 via caveolin-1 (Dewing et al., 2007). Hence, preliminary nongenomic signaling in the ARH precedes genomic signaling in the VMH to facilitate lordosis behavior and can be thought of as a single integrated signaling pathway from membrane to nucleus. Though it is possible that both pathways operate independently, it was initially investigated if non-genomic signaling could influence genomic signaling i.e. transcription in neuroblastoma cells. Using a consensus ERE, it was demonstrated that a membrane limited 17β-E conjugate (E2-BSA) that initiates non-genomic signaling can potentiate transcription that is driven by 17β-E bound to ERα (Vasudevan et al., 2001). In this integrated signaling pathway, potentiation was dependent on a number of kinases i.e. PKA, ERK and PI3K, whose activity can be increased rapidly by E2-BSA. What is the molecular mechanism that couples non-genomic kinase activation to increased transcription? In a neuroblastoma cell line, overexpressed ERα mutants that could not be phosphorylated on kinase- specific sites (phospho-mutants) failed to show E2-BSA mediated transcriptional potentiation compared to wildtype ERα, suggesting that phosphorylation of the ERα is critical to couple the non-genomic signaling initiated at the plasma membrane to transcription in the

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nucleus (Clark et al., 2014) (Figure 1). Similarly, in vivo, E2-BSA could potentiate lordosis that is driven by a brief pulse of 17β-E in female ovariectomized rats (Kow and Pfaff, 2004). Importantly, E2-BSA alone could not induce lordosis or transcription, suggesting that for outputs that are critically dependent on estrogens, coupled signaling is important. Recently, this paradigm of discontinuous treatment of estrogens where the first pulse of E2-BSA is followed by 17β-E potentiated the expression of the progesterone receptor in VMH neurons (Sa et al., 2014), required for lordosis.

What is the role of GPER1 in lordosis? Though GPER1 is expressed in the VMH and can activate PKA pathways that have been shown to potentiate lordosis, GPER1 activation alone did not facilitate lordosis in rats (Lebesgue et al., 2009). However, in ovariectomized mice, GPER1 activation by the use of a selective GPER1 agonist, G-1, followed by progesterone in a classic priming paradigm could facilitate lordosis, similar to EB administration. Specificity was demonstrated by the use of a specific GPER1 antagonist, G-15 that could block the G-1 facilitation of lordosis. Moreover, G-15 also decreased but not completely abolished the increase in lordosis seen with EB (Anchan et al., 2014), suggesting that GPER1 contributes, but is not the sole mediator of lordosis. It is not clear if this is a genuine species difference; both rats and mice express GPER1 in high levels in the hypothalamus (Hazell et al., 2009). In the adult hamster, GPER1 expression, as measured by RT-PCR, was higher in females in the hypothalamus than in males while GPER1 expression was higher in the amygdala of adult males compared to females (Canonaco et al., 2008). This suggests that GPER1 expression is variable and could be sexually dimorphic depending on the species. It is worth noting that GPER1 activation in rats primed with a low dose of EB could also facilitate lordosis, by releasing the initial inhibition that was mediated by activated MOR in the ARH (Long et al., 2014). The long-time scales used in all these experiments that measure lordosis behavior do not allow us to infer if GPER1 action is solely non-genomic or if activation of GPER1 regulates transcription i.e. integrated signaling which in turn facilitates lordosis.

What are the molecular mechanisms by which EB activates lordosis? One mechanism that occurs in both the ARH and VMH is the increase in dendritic spines and presumably synaptic plasticity (Christensen et al., 2011). The increase in dendritic spines is itself a process that depends on both non-genomic and genomic signaling by estrogens. In the ARH, mGluR1-ERα rapid signaling leads to ERK and PKC activation which, within 1 hour, activate LIMK, a kinase that phosphorylates cofilin. Phosphorylated cofilin that is inactive allows for the establishment of new spines (Meng et al., 2002). Administration of EB to ovariectomized rats results in immature, less stable filopodial spines within 4 hours which finally culminate in more stable, mushroom spines at 48 hours in the ARH. In the VMH, EB administration

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increases spines (Calizo and Flanagan-Cato, 2000) though the role of non-genomic signaling in this nucleus is not known. The role of GPER1 in spinogenesis in both these nuclei are not known but GPER1-mediated spinogenesis occurs in the hippocampus within time frames that imply non-genomic signaling without transcription is sufficient for this phenomenon (Refer to Section 4 for details). Unpublished data by the Vasudevan laboratory shows that GPER1 contributes to increased spinogenesis mediated by EB in the VMH and also increases dendritic arbor in the VMH. Similar to EB-mediated increases in the diameter of the spine head on pyramidal neurons in the female mouse hippocampus that is important for spatial memory (Li et al., 2004), GPER1 signaling in the hypothalamus may also change dendritic spine shape from immature filapodial spines to mushroom shaped spines that drive lordosis. However, this remains to be shown experimentally. This may provide an explanation for the puzzling observation that VMH neurons that showed increased spine density on EB administration did not express ERα (Calizo and Flanagan-Cato, 2000). 17β-E could also increase immature spines rapidly via non-genomic signaling pathways such that PI3K so that neurons can respond with greater efficiency to a subsequent stimulus and stabilize the nascent spines. Though this two-step wiring has been shown to occur in cortical neurons, it is unknown if this occurs in the hypothalamus (Srivastava et al., 2008).

3.2 Male sexual and aggressive behavior

Studies with knockout mice revealed that ERα and ERβ may also be involved in the expression of male social behaviors such as sex and aggression. Male sexual behavior such as ultrasonic vocalization, mounts, intromissions and ejaculations, were greatly reduced or completely abolished in the single ERα knockout (Ogawa et al., 1997; Ogawa et al., 1998b) (Wersinger et al., 1997), double ERα and ERβ knockout (Ogawa et al., 2000), and neuron specific ERα knockout mice (Sano et al., 2013b). In contrast, disruption of ERβ did not affect male sexual behavior in both gonadally intact and EB-treated mice (Nomura et al., 2006; Ogawa et al., 1999). Therefore, activation of functional ERα, but not ERβ, by 17β-E as the aromatization product of testosterone is necessary for the expression of male sexual behaviors.

Male aggressive behaviors, tested in a resident-intruder paradigm toward an olfactory bulbectomized male intruder mouse, were also completely abolished in ERα knockout, ERα and ERβ double knockout and neuron-specific ERα knockout (Nomura et al., 2006; Ogawa et al., 1999; Ogawa et al., 2000; Ogawa et al., 1998b; Sano et al., 2013b) mice. Unlike for sexual behavior, aggressive behavior was potentiated in ERβ knockout mice in an age- and experience-dependent manner. Increase in the levels of aggression by ERβ deletion was most clearly shown in mice during the adolescent period. Male

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aggressive behavior first appears at the time of puberty onset around 5-6weeks of age (Tsuda et al., 2011). ERβ knockout mice were more aggressive than βWT mice at 5 weeks of age (Nomura et al., 2002; Tsuda et al., 2014). Once they were sexually mature, genotype differences in the levels of aggression were no longer obvious unless mice were treated with EB or were instigated prior to the aggression test. EB treatment induced significantly higher levels of aggression in gonadectomized ERβ knockout mice than in WT mice (Nomura et al., 2006). Gonadally intact ERβ knockout mice were more easily irritated by exposure to an opponent male mouse (unpublished observations). Collectively, these findings suggest that ERα activation is necessary for the induction of male aggressive behavior whereas ERβ activation may inhibit aggressive behavior induced by ERα and/or the AR.

It is obvious that classical studies using global knockout mouse models have certain limitations to specify the time (during development vs in adults) and brain site(s) responsible for behavioral effects induced by disruption of each isoform of the ER. Among a number of regions constituting the neural network for social behaviors, responsible site(s) for the estrogenic regulation of sexual and aggressive behaviors have been determined by site-specific ER gene silencing. Sexual behavior was reduced by ERα silencing in the VMH or in the mPOA, whereas aggressive behavior was reduced by ERα silencing in the VMH only (Sano et al., 2013a). Considering the fact that all mice were tested as gonadally intact animals, these findings suggest that ERα activation in the targeted brain area at the time of behavioral testing in adults is essential for full expression of male-type social behaviors. Therefore, it was concluded that ERα activation in the VMH is necessary for both sexual and aggressive behavior in male mice. However, it is not yet determined whether the same neurons are responsible for the regulation of both behaviors. Optogenetic stimulation of ERα expressing neurons in the VMH revealed neuronal populations which were activated during sexual behaviors, but inhibited during aggressive behaviors study (Lee et al., 2014; Lin et al., 2011).

Silencing of ERα in the MeA, the brain region implicated in social information processing and estrogenic regulation of male sexual behavior (Huddleston et al., 2006; Wood et al., 2001), has no effect on the expression of sexual and aggressive behaviors (Sano et al., 2013a). A recent study using MeA-specific ERα suppression with antisense also showed that sexual behavior was not affected in gonadally intact male rats (Paisley et al., 2012). As for the ERβ, knockdown in the MeA as well as mPOA has no apparent effect on sexual and aggressive behaviors (Nakata et al., 2016). This study, however, has reported that ERβ knockdown in the MeA disrupts sexual preference for receptive females over non-receptive females in male mice.

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So far, these studies manipulated site-specific activation of ER by 17β-E only in adulthood. A recent study has reported that site-specific manipulation of ERα expression in sexually immature male mice has different effects (Sano et al., 2016) than in adult mice. Pre-pubertal (21days old) silencing of ERα in the MeA significantly reduces both sexual and aggressive behavior in adulthood. The siRNA used in this study irreversibly knocked down ERα expression in transfected cells. However, the same treatment in the adult does not affect male-type sexual and aggressive behavior (Sano et al., 2013a). Therefore, it is concluded that ERα activation in the MeA during pubertal period is crucial for male mice to fully express their male-type social behaviors in adulthood. Furthermore, pre-pubertal knockdown of ERα in male mice reduces the number of neurons in the MeA to the levels of female mice (Sano et al., 2016). It is expected that future studies will elucidate the exact mechanisms of ERα action that results in full masculinization of neural networks for male-type social behavior, during the pubertal period.

4. Estrogenic regulation of social recognition and social learning.

4.1 Social recognition

Social recognition allows members of a group to distinguish conspecifics on the basis of various characteristics. Through social recognition animals can assign others to specific categories (e.g. sex, reproductive status, familiarity, kinship, hierarchical status, health, specific individual) and utilize this information to adaptively adjust their behavior to their social context. For example, on the bases of social recognition processes a male may attempt to mate with an estrous female, whereas he may be aggressive towards unfamiliar male intruders to his territory. The most commonly studied form of social recognition in the laboratory is familiarity recognition (hereinafter referred to as “social recognition”). Although there exist a few different ways of assessing familiarity recognition, in all of them an animal’s social investigative responses towards familiar or unfamiliar conspecifics are observed. When an unfamiliar conspecific is investigated more than a familiar conspecific, either within a choice test or upon subsequent social exposures, it is inferred that the familiar conspecific has been recognized (reviewed and discussed in (Choleris et al., 2009; Engelmann et al., 1995; Gheusi et al., 1994; Johnston, 2003).

The involvement of estrogens in social recognition has been repeatedly described (reviewed in (Ervin et al., 2015a; Lymer et al., 2018). Female social recognition skills vary with the estrous cycle (Sanchez-Andrade and Kendrick, 2011), are reduced by ovariectomy, and can be restored with estrogens alone and with estrogens and progesterone replacement (Hlinak, 1993; Spiteri and Agmo, 2009; Tang et

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al., 2005). Investigations with mice with the gene for specific ERs specifically "knocked out", showed that ERα is necessary for social recognition in males and females (Choleris et al., 2003; Choleris et al., 2006; Imwalle et al., 2002; Sanchez-Andrade and Kendrick, 2011), whereas ERβ seems less or not involved, affecting behavior in the social recognition test in ways that appear not directly related to social recognition (e.g. by generally regulating social investigation or social anxiety) (Choleris et al., 2003; Choleris et al., 2006; Sanchez-Andrade and Kendrick, 2011); discussed in (Ervin et al., 2015a). The investigations with ER knockout mice, thus, implicated ERα in social recognition. Whether or not this function of ERα is due to organizational or activational effects of estrogens has not been investigated in the knockout mice. More recently, studies with hormonal and/or ER-specific pharmacological manipulations investigated the activational very rapid (likely non-genomic) mechanisms underlying estrogens' facilitation of social recognition in adults. In a "difficult" task (with reduced opportunities to investigate ovariectomized social stimuli during a training phase) where ovariectomized female mice typically showed no social recognition, a single subcutaneous injection with 17β-E was capable of restoring social recognition within 40 minutes of treatment (Phan et al., 2012). Using the same difficult task and timing of administration, both a selective ERα and a GPER agonist, but not an ERβ agonist, were also capable of restoring social recognition in ovariectomized mice, suggesting ER-specific regulation of social recognition *via* rapid, non-genomic, mechanisms (Gabor et al., 2015; Phan et al., 2012).

Subsequent studies have investigated the brain regions involved in estrogenic mediation of social recognition in adults. Long-term inhibition of the expression of the ERα gene in the medial posterodorsal amygdala (but not in the VMH) with shRNA blocked social recognition in female rats (Spiteri et al., 2010). Similarly, administration of 17β-E, and agonists for ERα, ERβ, and GPER into the MeA all facilitated or enhanced social recognition in ovariectomized mice within 40 min of treatment (Lymer et al., 2018), suggesting that all three ERs in the MeA are capable of mediating social recognition *via* rapid non-genomic mechanisms. Intriguingly, when the same treatments were administered in the dorsal hippocampus, 17β-E and ERα and GPER agonists each rapidly facilitated social recognition in ovariectomized mice, whereas an ERβ agonist did not facilitate social recognition, not even at doses that facilitated spatial recognition (Lymer et al., 2017; Phan et al., 2015). Thus, different brain regions can mediate estrogenic rapid facilitation of social recognition, with some region- and ER- specificity. In particular, the involvement of ERβ seems different in the dorsal hippocampus (not involved) and the MeA (involved).

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Systemic and dorsal hippocampal treatments with 17β-E and the ERα and GPER agonists also rapidly increased the number of dendritic spines in the CA1 subregion (Gabor et al., 2015; Phan et al., 2012; Phan et al., 2011; Phan et al., 2015), and decreased AMPA activity in dorsal hippocampal CA1 neurons of ovariectomized mice (Phan et al., 2015), suggesting that rapid induction of initially silent, immature, dendritic spines may mediate estrogenic rapid enhancement of social as well as other types of recognition in the dorsal hippocampus. The possible downstream mechanisms of these effects are only beginning to be investigated. Recently, with infusions of membrane impermeable BSA-conjugated 17β-E it has been shown that in the dorsal hippocampus rapid facilitation of social recognition by 17β-E in adult ovariectomized mice is due to membrane-initiated mechanisms (Kunn et al., 2017). In addition, it was found that in the dorsal hippocampus the extracellular signal-regulated kinase (ERK), and the phosphoinositide 3-kinase (PI3K)/Akt (a.k.a. protein kinase B or PKB), both mediate 17β-E and ERα facilitatory effects. Conversely, the PI3K, but not ERK, pathway mediates GPER facilitated social recognition (Sheppard et al., 2017a; Sheppard et al., 2017b); reviewed in (Paletta et al., 2018). It was also found that the nonapeptide oxytocin, which is necessary for social recognition in males and females (Ferguson et al., 2001; Ferguson et al., 2000) (Choleris et al., 2003; Choleris et al., 2006), may be downstream of rapid estrogenic enhancement of social recognition. Infusion of 17β-E in the oxytocin-producing paraventricular nucleus of the hypothalamus rapidly restored social recognition in adult ovariectomized mice in the difficult task and this effect could be blocked by infusion of a subeffective dose (i.e. that *per se* would not impair social recognition) of an oxytocin receptor antagonist in the MeA (Paletta et al., 2017) reviewed in (Paletta et al., 2018). The specific ERs mediating this estrogen/oxytocin interplay in the regulation of social recognition are currently unknown. Both ERβ and GPER are expressed in the paraventricular nucleus of the hypothalamus (Hazell et al., 2009; Mitra et al., 2003) and ERβ on oxytocinergic neurons in the other main nucleus of oxytocin production, the supraoptic nucleus, can stimulate the release of oxytocin within 5 min of estradiol application (Wang et al., 1995). Thus, both ERβ and GPER are well placed as possible mediators of the rapid estrogen/oxytocin interplay in the facilitation of social recognition. Overall, these recent findings show that estrogens can rapidly facilitate social recognition in adult females *via* the activation of specific cell kinase responses, as well as by acting upon oxytocin, possibly by inducing its release from cellular storage. Whether the same cell signaling pathways mediate the estrogenic rapid enhancement of social recognition in regions other than the dorsal hippocampus remains to be investigated.

4.2 Social Learning

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Social learning is "learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products" (such as odor cues; (Box, 1984; Galef, 1988; Heyes, 1994). By learning from others, animals can reduce the need for learning through personal experience that typically carries some degree of risk. For example, in order to learn whether or not an unknown food item is safe to eat, an animal needs to ingest it, wait for physiological responses (e.g. sickness), then either develop aversion to and avoidance of it or incorporate it in its diet. Through social learning these steps can be skipped. If a conspecific that has ingested a novel food safely returns to the group, others can acquire information about that food and safely consume it when they encounter it at a later time (Galef, 1989; Galef and Wigmore, 1983). The social transmission of food preferences (STFP) has been described in a number of animal species and has been extensively researched in the laboratory (reviewed in (Choleris et al., 2009) (Ervin et al., 2015a; Matta et al., 2016a), making it an excellent model for the investigation of the neurobiological bases of social learning.

A role for estrogens and their receptors in social learning in the STFP has been shown, *via* both delayed and rapid mechanisms. Although historically most studies have focused on one sex (mostly males), the few that compared the sexes often find a female advantage in social learning (reviewed in (Ervin et al., 2015a; Matta et al., 2016a) (Choleris and Kavaliers, 1999), suggesting a facilitatory role for female hormones. Similarly, an enhancing effect of pregnancy in the STFP in rats (Fleming et al., 1994) and gerbils (Choleris et al., 2012) and fluctuations in STFP performance over the estrous cycle in mice (Choleris et al., 2011), all point at a role for estrogens and/or progestins. Systemic administration of EB or an ERβ agonist 48 or 72 hr before testing prolonged a socially acquired food preference in adult ovariectomized mice, whereas an ERα agonist blocked it (Clipperton et al., 2008), suggesting opposing roles for ERα and ERβ, ERβ facilitating and ERα inhibiting social learning *via* delayed, likely genomic, mechanisms. Estrogens facilitate social learning in the STFP also *via* rapid mechanisms. Intriguingly, the rapid effects do not fully overlap with the delayed effects. Subcutaneous treatment with 17β-E restored the social acquisition of a food preference within 45 minutes of administration, under conditions where ovariectomized adult mice would not normally show social learning (Ervin et al., 2015b). The administration of a GPER agonist similarly rapidly facilitated social learning, whereas ERα and ERβ agonists neither rapidly facilitated nor blocked social learning in the STFP in ovariectomized adult mice (Ervin et al., 2015b). Thus, different ERs are involved in estrogenic facilitation of social learning, ERβ *via* delayed mechanisms and GPER *via* rapid mechanisms.

The brain regions involved in the estrogenic enhancement of social learning remain unknown.

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The dorsal hippocampus and the basolateral amygdala appeared strong candidate because both of these regions are involved in social learning and express the ERs (Carballo-Marquez et al., 2009; Hazell et al., 2009; Matta et al., 2017; Mitra et al., 2003; Winocur, 1990). However, infusions of a range of doses of 17β-E in these regions did not facilitate the STFP (Ervin et al., 2014) reviewed in (Ervin et al., 2015a), not even doses that in the dorsal hippocampus had a strong facilitatory effect on social recognition (Phan et al., 2015). Thus, it seems likely the rapid facilitation of social learning by estrogens is secondary to their action on other neurochemical systems in the brain. For example, clear sex differences are being found in the involvement of dorsal hippocampal dopamine receptors in the STFP (Matta et al., 2017; Matta et al., 2016b) reviewed in (Paletta et al., 2018), suggesting estrogens may promote social learning by rapidly enhancing the activity of dopamine or other neurochemical systems known to mediate the STFP (e.g. acetylcholine, (Carballo-Marquez et al., 2009) (Gold et al., 2011), possibly by acting on the synthetizing neurons that project to brain regions involved in social learning.

4.3 Estrogens facilitate different types of social cognition differently

Studies reviewed in the two parts above show a clear facilitation of social cognition by estrogens in adult females. The mechanisms of this facilitation are, however, different for social recognition and social learning and can also be different for rapid and delayed mechanisms. Social recognition is rapidly facilitated by ERα and GPER, whereas ERβ so far has been shown to facilitate it only in the MeA. Conversely, social learning is enhanced by ERβ (delayed effect, not rapid) or GPER (rapid effect) and is either blocked (delayed effect) or unaffected (rapid effect) by ERα. The brain regions involved also appear to be different for estrogenic facilitation of social recognition and social learning. The dorsal hippocampus mediates the rapid facilitation of social recognition but not of social learning by estrogens (reviewed above and in (Paletta et al., 2018). Thus, even though we often talk about social cognition collectively, the two types of social cognition most studied, learning *about* others (social recognition) and learning *from* others (social learning), though both facilitated by estrogens, are showing intriguingly different underlying mechanisms, both in terms of the ERs involved and whether the effects are delayed or rapid. Future research will need to further clarify overlapping and non-overlapping mechanisms of estrogenic facilitation of different types of social cognition. Recent findings are showing that estrogens can facilitate social cognition through an interplay with other neurochemical systems in the brain (e.g. estradiol/oxytocin and social recognition). Thus, future research should investigate the implications for social cognition of the rapid interactions of estrogens with other neurobiological systems.

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5. Estrogenic regulation of anxiety-related behavior

5.1 GPER1 activation leads to different outcomes in males and female mice

A number of the behaviors described in Sections 3 and 4 can be influenced by state anxiety. State anxiety is the unpleasant arousal in the face of impending dangers, as opposed to trait anxiety which is a stable inheritable difference in the ability to respond to environmental circumstances. One possibility is that rapid actions of estrogens modulate state anxiety which in turn can fine tune social behaviors that are driven by genomic signaling over longer time scales. Similar to other studies described in this review where GPER1 activation facilitates social cognition in female mice over shorter time scales that are commensurate with non-genomic signaling, GPER1 activation in castrated male mice was shown to be anxiolytic in males but not in females. In the elevated plus maze, G-1 administration to gonadectomized male mice increased time and distance in the open arms as compared to control within 30 minutes of administration, suggesting that GPER1 agonism is sufficient to change state anxiety (Hart et al., 2014). In male mice, G-1 administration increased the phosphorylation of ERα at an ERK site i.e. serine at position 118 in the ventral hippocampus within 30 minutes though ERK activation itself could not be detected in the hippocampus in male mice at this time point (Hart et al., 2014). In female mice, ERK activation by G-1 could be detected within 30 minutes in the dorsal, but not the ventral hippocampus (Hart et al., 2014). The phosphorylation of ERα in the male mouse ventral hippocampus by GPER1, presumably activated by G-1, is one of the first examples of ERα-GPER1 crosstalk.

In contrast to these studies where the phenotype i.e. state anxiety was studied within 30 minutes, G-1 administration to ovariectomized female mice and intact male mice increased anxiety in the elevated plus maze and the open field test, with males showing more sensitivity to G-1 administration than females (Kastenberger et al., 2012). Consistent with this data, GPER1 knockout mice showed reduced anxiety, with the effects being more pronounced in males than females (Kastenberger and Schwarzer, 2014). The differences in this data compared to the previous study (Hart et al., 2014) could be due to differing doses or time frames; the expression of GPER1 as measured by in situ hybridization of RNA does not show sexual dimorphism in either rats or mice (Hazell et al., 2009). Nevertheless, these studies suggest that males may be more sensitive to the rapid effects of GPER1 activation, at least in the ventral hippocampus than females; this may affect state anxiety and consequently, behaviors that depend on state anxiety. Consistent with this idea, in the ventral hippocampus, phosphorylated ERK is higher in males than females, 60 minutes after fear conditioning

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(Gresack et al., 2009). It is also possible that ERK signaling is less important in the hippocampus in males rather than in females. For example, estrogens do not increase the level of activated ERK in males in the dorsal hippocampus, as compared to females and activated ERK does not seem to be required for object memory consolidation (Koss et al., 2017). Conversely, the G-1 mediated upregulation of activated ERK in the dorsal hippocampus in females may be important in the GPER1 facilitation of social but not object memory, as shown by the Choleris Lab (Section 4). Also, GPER1 acts to increase memory consolidation in female mice via the JNK pathway, not the ERK pathway (Kim et al., 2016); it remains to be seen if this pathway is important in the ventral hippocampus of males to decrease anxiety. These differences in signaling pathways and receptor activation between sexes underline the importance of context for hippocampal-dependent tasks.

6. Is estrogenic regulation of sex-typical social behaviors species-specific?

6.1 Animal models of sex behavior, aggression and social cognition

Animal models have been utilized to study social behaviours for several reasons: a) as a model for steroid hormone action in the brain, b) as a model of sexual differentiation if the behaviour is sex typical c) for the sake of animal behaviour itself and d) for a minority of researchers, as a model for the comparable human behaviour. Rats and mice are the most common mammalian models. They are easily maintained, have stereotypical behaviours that are responsive to steroid hormones, and can be genetically manipulated. Rats, mice and hamsters are common models to study the hormonal control of lordosis behavior and show optimal proceptive and receptive aspects of lordosis when both estrogens and progesterone are present (Pfaff, 1980; Steel, 1983). Though the neuroendocrine circuitry and in particular reward mechanisms of lordosis appear to be conserved across mammalian species, there are differences between reflex and spontaneous ovulators. For example, the rabbit shows reduced but not absent lordosis after ovariectomy and does not show the stimulatory effect of progesterone (Beyer et al., 2007). In another reflex ovulator, the ferret, the serotonin receptor 5-HT1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) facilitates lordosis in EB primed ovariectomized females in contrast to the inhibition seen in the female rat (Paredes et al., 1994). Over the last two decades, the use of conditioning and preference paradigms in rodents has tested facets of appetitive sexual behaviour that have human equivalents (Pfaus et al., 2003). However, in the human female, sexual motivation is thought to be adrenal androgen driven as evidenced by the off-label use of testosterone for disorders of sexual desire in women. However, recently, estrogens-only therapies for postmenopausal women have been shown to increase desire; this is similar to the estrogenic regulation of sexual motivation in female

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rodents (Cappelletti and Wallen, 2016). Interestingly, polymorphisms in the ERα and an ERα upregulated gene, the oxytocin receptor are correlated with increased sexual motivation and arousal (Armeni et al., 2017).

Species differences in brain expression of the nonapeptide systems oxytocin and vasopressin have been shown in monogamous and polygamous vole species and they appear related to the capability of developing pair bonds after mating in monogamous species (reviewed in Gobrogge & Wang, 2015). Because pair bonding in monogamous species requires the cognitive skill of individual recognition (Zheng et al., 2013), similar species differences in the neurobiological mechanisms underlying social recognition may be hypothesized. To be best of our knowledge, the regulation of social recognition by estrogens has so far only been shown in rats and mice, with no species differences having been demonstrated. Whether estrogenic regulation of social recognition is similar in species with different social systems remains to be investigated. ERα involvement in social behavior has been hypothesized in monogamous and polygamous *Peromyscus* species on the basis of brain receptor distribution (Cushing, 2016). Medial amygdala ERα regulation of prosocial behavior in male monogamous and polygamous voles has been shown (Cushing et al., 2008). Intriguingly, specific polymorphisms in the ERα and ERβ genes are associated with enhanced social memory in women (Karlsson et al., 2016). These findings suggest estrogenic regulation of social recognition may be similar across species with different social and mating systems. Similarly, social learning has been shown in multiple species, from invertebrates to vertebrates (reviewed in (Ervin et al., 2015a; Matta et al., 2016a). Whether or not estrogens similarly regulate it in different species is, to the best of our knowledge, currently unknown, but it certainly deserves further investigation.

Future perspectives

Several outstanding questions remain, despite the progress made in understanding the contribution of the receptors in classical estrogenic-driven sex-specific behaviors, such as lordosis or aggression. For example, the role of ERβ, particularly in the developmental period remains under-explored, though this will be certainly facilitated by recently developed ERβ-*cre* mouse where *cre*-recombinase is expressed in ERβ neurons and by the availability of a mouse where ERβ expression is tagged by a fluorescent reporter. Similarly, whether organizational effects contribute to the estrogenic regulation of social cognition remains to be investigated. Secondly, in nuclei where both ERα and ERβ are equally expressed, their relative contribution to different behaviors is not clear even though recent

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investigations on social recognition and social learning are showing that ER-specific and brain region-specific mechanisms may be at play. Thirdly, in stereotypical behaviors which have several steps in a sequence, the relative contribution of non-genomic signaling to genomic signaling to these different steps is still being investigated. The role of ERα phosphorylation or other modifications, including DNA methylation, that are driven by non-genomic signaling could be important in both organizational and activational effects of estrogens that drive social behaviors. This has been underexplored in vivo. Lastly, not much is known about crosstalk between the different ERs and estrogens and other neurochemical systems in different brain regions which almost certainly modulates the final output.

Declaration:

The authors wish to declare that they do not have any conflicts of interest.

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Legends

Figure 1: Non-genomic signaling can potentiate the effects of genomic signaling. In this model, initial nongenomic signaling from a mER protein by estrogens (E2-BSA or the natural ligand, 17β-E) at the plasma membrane can activate several kinases and calcium. The identity of the mER is debated with GPER1, ERα and ERβ all seen as major candidates. ER?? phosphorylation, denoted in this figure by P, presumably as a result of kinase activation is important to potentiate transcription (genomic signaling) from genes that have EREs (Clark et al., 2014) in neuroblastoma cells. It is important to note that there is no strict demarcation between genomic and non-genomic signaling and such an integrated signaling pathway can conceivably increase spine density (S) and regulate social behaviors. Apart from integrated signaling, non-genomic signaling alone has been implicated in spinogenesis in the female mouse dorsal hippocampus (Section 4.1) though the molecular mechanisms remain to be elucidated.

Figure 2: Roles of gonadal sex steroids in the sexual differentiation of the brain in rodent models. Testosterone (T) secreted from the testes in the perinatal and peripubertal periods acts to masculinize and defeminize the sexually undifferentiated brain. T affects the brain via the androgen receptor. In addition, T affects the brain via the estrogen receptor after conversion to estradiol (17β-E), although the roles of 17β-E during the peripubertal period in organization of the male brain remains to be fully uncovered. 17β−E secreted from the ovaries in the peripubertal period acts to organize the female brain. In addition to sex steroids, sex chromosome genes expressed in the brain also play an important role in the sexual differentiation of the brain.

Figure 3: Location and sex difference in sexually dimorphic nuclei in the BNST and mPOA of mice. The rostral part of the mPOA contains the AVPV, which is larger in size and has more neurons in females than in males (Bleier et al., 1982; Kanaya et al., 2014; Sumida et al., 1993). The BNST and the caudal part of the mPOA have a cluster of neurons expressing calbindin-D28K. Here the clusters in the BNST and mPOA are termed CALB-BNSTp and CALB-SDN, respectively. The CALB-BNSTp and CALB-SDN in male mice are consisted of more calbindin-D28K neurons and larger in size compared to those in female mice (Gilmore et al., 2012; Morishita et al., 2017; Orikasa and Sakuma, 2010). The CALB-BNSTp is a part of the BNSTp that can be identified by Nissl staining and shows male-dominant sex differences in the volume and neuron number (Forger et al., 2004; Tsukahara et al., 2011). Although the BNSTp itself exhibits male-dominant sex differences in the morphology, the ventral part of the BNSTp (BNSTpv) is larger in size and contains greater number of non-calbindin-D28K neurons in female mice than in male

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mice (Morishita et al., 2017).

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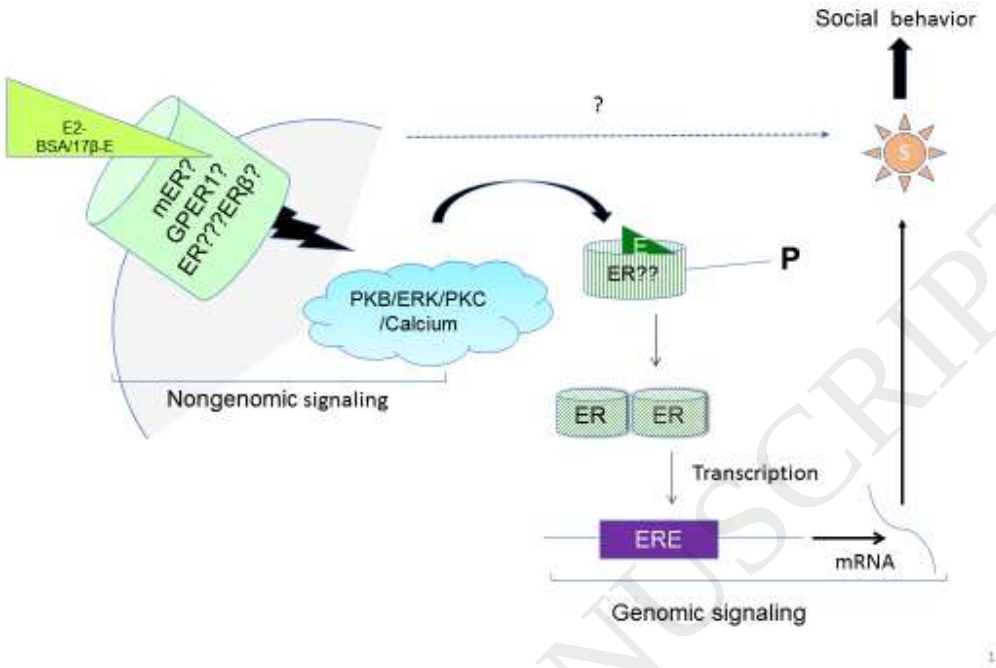

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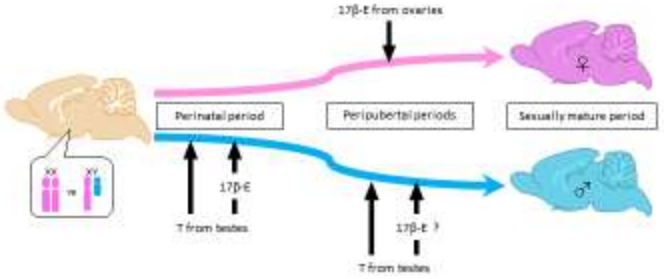

Fig. 2

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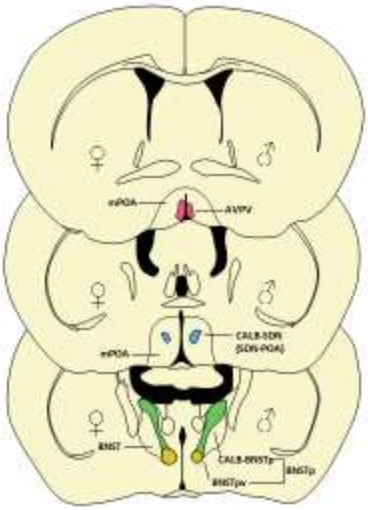

Fig. 3

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