Neural, Hormonal and Experiential Control of Sex-Typical Expression of Social Behavior

Sonoko OGAWA1,*, Mumeko C. TSUDA1, Kazuhiro SANO1, Shinji TSUKAHARA2, and Sergei MUSATOV3

1Laboratory of Behavioral Neuroendocrinology, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan
2Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan
3Laboratory of Molecular Biology, Weill Cornell University Medical College, New York 10065, U.S.A.

Received July 14, 2015; final version accepted July 22, 2015

Expression of social behaviors is regulated by various neuroendocrine and neurochemical factors. Among them, estradiol is known to have a profound influence on female sexual behavior as well as various types of social interactive behaviors, through its binding to two types of estrogen receptors, ERα or ERβ. Since male gonadal hormone, testosterone, is aromatized to estradiol in neuronal cells in the brain, ERs are also essential for the regulation of male-type social behavior and the development of their neural network. In this article, we discuss how each type of ER plays a role in the expression of sex-typical social behavior in males and females by focusing on both organizational and activational action of estradiol. For this purpose we overview behavioral and neuroanatomical studies reported in knockout as well as brain site-specific knockdown models of ER genes. We also discuss how early life experiences may affect subsequent expression of social and socio-emotional behavior.

KEYWORDS: estrogen receptors, sexual differentiation, knockout mice, RNAi, maternal separation

1. Introduction

Gonadal steroids are essential for the regulation of social and emotional behavior in both males and females. Particularly, 17β-estradiol (E2) not only plays a central role for the induction of female type sexual behavior (i.e., lordosis), but also contributes greatly to the expression of male-type sexual and aggressive behavior. In males, testosterone secreted from the testes is irreversibly aromatized to E2 after being taken up by neuronal cells in the brain. In recent studies, E2 has also been implicated in the regulation of various social interactive behaviors, including social investigation, preference, recognition and memory as well as emotional and anxiety-related behaviors in social contexts, i.e., socio-emotional behavior.

In the brain, E2 binds to its intracellular estrogen receptors (ERs), which act as ligand-activated transcriptional factors and regulate expression of a number of genes responsible for various brain functions and behaviors. There are two types of ER, ERα and ERβ, which may represent two gene-duplication products. The distribution of ERα and ERβ partly overlaps but there are a number of brain areas in which ERα or ERβ is more predominant. The differential brain localization may contribute to differences in their role in the regulation of social behavior by E2. In this article, we discuss these issues by summarizing our knowledge obtained from behavioral and neuroanatomical analysis of knockout mice of either ERα (αERKO) or ERβ (βERKO) as well as brain site-specific knockdown of the ER gene.

One of the prominent features of gonadal steroid hormones is their life-long action on the brain and behavior. They work on (1) sexually-undifferentiated brains to permanently and irreversibly structure male- or female-type brains, i.e., organizational action, and (2) sexually differentiated brains later in life, mainly in adults, to induce neurochemical, physiological, and behavioral effects, i.e., activational action. For organizational action, most of the studies have focused on the perinatal period, when high levels of testosterone secreted from testes induce brain masculinization/defeminization in males. However, studies from the last 10 years revealed that organizational action of gonadal steroids could also occur at puberty. Therefore, here we discuss the potential roles of the two types of ERs in organizational and activational action of gonadal steroids.

The actual expression of sex-typical social and socio-emotional behavior is modulated by environmental and experiential influences, especially those during neonatal period. Maternal separation (MS) is one of the most widely used experimental models for an adverse neonatal experience. We will discuss some of our recent findings on MS effects in both male and female mice.
2. Roles of Estrogen Receptors, ERα and ERβ, in the Regulation of Sex-Typical Social Behavior

E2 binds to ERα and ERβ with similar affinity. However, until ERβ was first described in 1996 [1], only one type of ER, now named ERα, was known. Our behavioral analysis using knockout mouse models were started in 1994 with αERKO mice (described as ERKO mice; [2]) and presented first in abstract form in 1995 [3] followed by original articles shortly thereafter [4–6]. We found that not only female reproductive behavior but also male sexual and aggressive behaviors were greatly affected by a lack of functional ERα proteins. After the first ERβ knockout mouse line (βERKO mice) was described in 1998 [7], we started analyses of both sexes of βERKO mice [8] and found very different behavioral characteristics from those found in αERKO mice. These findings led us to hypothesize that activation of ERα and ERβ may be involved in the final expression of social behavior, however, the brain site(s), neurochemical processes, etc. may be different. Here, we describe differential roles played by ERα and ERβ and possible brain mechanisms underlying their actions for the regulation of social behaviors in females and males.

2.1 Female social behavior

It is well established that E2 facilitates female sexual behavior either directly or through a time-dependent induction of progesterone receptors (PRs), via genomic action. Both cycling and E2 (17β-estradiol-3-benzoate) plus progesterone primed ovariectomized (OVX) αERKO mice were not receptive at all [4,9]. Detailed analysis of behavioral interactions between a female and a sexually active male mouse revealed that αERKO female mice did not show any proceptive still posture or receptive lordosis posture in response to attempted mounts by male mice [9]. Rather they showed very strong rejection responses, such as kicking, fleeing, and upright posture. As expected, PR induction in E2-primed OVX females was greatly reduced in the ventromedial nucleus of hypothalamus (VMN), which is known to play a central role in the expression of lordosis. There were only a few PR immunopositive cells in the ventrolateral part of the VMN (vlVMN) in αERKO mice, which were assumed to be due to ERβ mediated action of E2, although involvement of splicing variants could not be completely excluded [10].

To further specify the site of E2 action, we performed a series of gene silencing experiments with small hairpin RNA (shRNA) delivered by adeno-associated virus (AAV) to site-specifically knockdown ERα in C57BL/6J and Swiss-Webster mice [11]. When ERα was almost completely knocked down in the vlVMN but not in the adjacent ERα-rich arcuate nucleus, both proceptive still posture and lordosis behavior were abolished. Indeed ERα knockdown (αERKD) mice showed very similar behavioral responses (e.g., kicking and fleeing, etc.) to sexually active male mice as those seen in αERKO mice [4, 9]. We also found in both cycling and E2-primed OVX mice that PR was not expressed in ERα knocked down cells, which were identified by detecting EGFP inserted in the AAV vectors.

In contrast to αERKO mice, cycling βERKO mice showed equivalent levels of lordosis behavior as found in wild-type (βWT) mice, on the day of behavioral estrus [8]. The vlVMN is known to be a predominantly ERα-rich brain area and only a small number of ERβ immunopositive cells are found in the restricted area [12–14]. Therefore, our findings in βERKO mice were somewhat expected. However, we also noticed that βERKO mice maintained high levels of lordosis quotient (LQ, number of lordosis/number of male mounts X 100) on the day after behavioral estrus. We later confirmed this phenomenon in E2-primed OVX C57BL/6J mice by testing them 6 hr and 30 hr (i.e., next day of the first test) after progesterone treatment [15]. Our findings in knockout mice suggest that ERα in the VMN is absolutely necessary for the induction of lordosis behavior in female mice whereas ERβ, expressed in brain regions other than the VMN, may be involved in the regulation of lordosis behavior by controlling the exact timing, levels, etc. The mechanisms of ERβ-mediated action of E2 in the modulatory regulation of female sexual behavior needs to be investigated with AAV mediated RNAi methods in ERβ-rich brain regions [15]. These include midbrain dorsal raphe nuclei (DRN) and locus coeruleus (LC), where ERβ is expressed either in serotonergic or noradrenergic neurons and might be involved in PR induction by E2 [16–18].

In addition to facilitation of female sexual behavior, ERα activation is responsible for the regulation of home-cage activity. E2, at very low doses (as low as 16 ng/day) compared to those needed for induction of lordosis behavior, facilitated running wheel activity in βERKO as well as αWT and βWT mice but failed to do so in αERKO mice [19].

One of the brain sites responsible for this ERα mediated regulation of home cage activity is the vlVMN, since silencing of ERα in this region with shRNA almost completely abolished facilitatory action of E2 [20]. In this study, it was also reported that the same treatment induced metabolic abnormalities, similar to those reported in αERKO mice.

In our earlier studies with knockout mice, it was found that lack of ERα, but not ERβ, influenced parental behavior [9], which is more prominently controlled by the preoptic area. A number of recent studies revealed that sub-regions in this area are differentially involved in the regulation of different aspects of parental behaviors [21]. Therefore, it is necessary to carefully dissect out the roles of ERα and ERβ for the expression of parental behaviors, including nursing, retrieving, grooming and maternal aggression, in future studies.

Finally, it is worth discussing the roles of ERs in more complicated social interactive behaviors. A number of studies reported that oxytocin and oxytocin receptor knockout mice were impaired in social recognition. They failed to show...
habitation (a gradual decrease of investigation time) to a repeated presentation of the same opponent mouse and dishabituation (i.e., a restoration of investigation time) to one-time presentation of an unfamiliar opponent mouse in an habituation–dishabituation paradigm [22]. In a discrimination paradigm, they showed longer social sniffing to an unfamiliar opponent than to a familiar opponent [23]. In the paraventricular nucleus of the hypothalamus (PVN), oxytocin-synthesizing neurons express ERβ and E2-inducible increase of oxytocin mRNA levels is abolished in βERKO (male) mice [24]. In the medial amygdala (MA) where both ERα and ERβ are expressed, it is hypothesized that they are regulating the function of oxytocin receptors [25]. Based on these facts, both αERKO and βERKO female mice along with oxytocin knockout mice were tested in habituation–dishabituation and discrimination paradigms against female opponent mice, each presented in a clear perforated plexiglass cylinder placed in their home cage. We found all three types of knockout mice were impaired in social recognition [26, 27]. However, we also found that βERKO mice showed much less habituation compared to αERKO mice and maintained a persistently high level of social investigation during repeated presentation sessions of opponent mice. Similar behavioral characteristics (i.e., hyper-reactivity to social stimuli), were detected during a number of different behavioral tests in both sexes of βERKO mice [28–30]. It is known that anxiety levels measured in non-social context, such as light-dark transition, elevated plus maze and open field tests, are regulated by E2 presumably acting on ERβ [7, 28, 31]. A number of recent studies reported that ERβ-mediated E2 action could be modulating functions of oxytocin neurons in the PVN and/or serotonergic neurons in the DRN [32–35]. Therefore, it is reasonable to hypothesize that E2 action via ERβ could also play a role in the control of anxiety levels in social context.

2.2 Male social behavior

Behavioral studies using knockout mouse models revealed that lack of functional ERα and/or ERβ affected social behaviors in male mice as well. Sexual behaviors in αERKO mice were reduced compared to WT control mice [6, 36]. αERKO mice showed only mounting and mounts occasionally, and hardly exhibited intromissions and ejaculations. Furthermore, double knockout for both ERα and ERβ (αβERKO) [37] and neuron-specific ERα knockout mice [38] failed to show any sexual behaviors, including ultrasonic vocalization. In contrast, sexual behaviors of βERKO mice were not different from those of WT mice, in both gonadally intact and E2-treated conditions [8, 39]. These findings suggest that activation of functional ERα, but not ERβ, by E2 as aromatization product of testosterone is necessary for the expression of male sexual behaviors.

Findings in male aggressive behaviors, tested in a resident-intruder paradigm toward an olfactory bulbectomized male intruder, were somewhat different from those in sexual behaviors. In all αERKO, αβERKO, and neuron-specific αERKO mice, aggressive behaviors were almost completely abolished [6, 36–38]. Testosterone replacement also failed to induce aggressive behavior in castrated αERKO mice [36]. In contrast, aggressive behavior was rather potentiated in βERKO mice, compared to WT in an age- and experience-dependent manner. Although there was no genotype difference in the levels of aggression in gonadally intact adult mice, E2 treatment induced higher levels of aggression in βERKO than in WT mice [39]. Gonadally intact adult βERKO mice also showed significantly higher levels of aggression than WT after instigation procedure suggesting that βERKO mice were more easily irritated by exposure to an opponent male mouse (unpublished observations). However, the most striking effect of ERβ deletion was found during the adolescent period. Male aggressive behavior first appears at the time of puberty onset around 5–6 weeks of age [40]. βERKO mice were significantly more aggressive than WT mice when they were tested at 5 weeks of age [29, 41]. These findings collectively suggest that ERα activation is critical for induction of male aggressive behavior whereas ERβ activation may inhibit aggressive behavior induced by ERα and/or androgen receptor (AR) mediated actions of male gonadal steroids.

Knockout mouse models do not provide sufficient information on the exact brain site(s) of hormone action. It is known that ERα and ERβ are expressed in a number of regions constituting the neural network for social behaviors in mice and rats [42, 43]. As a natural next step, we have performed ER gene silencing experiments to determine the most critical site(s) for both sexual and aggressive behaviors. Since both types of behaviors are greatly reduced in αERKO mice, we first aimed to knockdown ERα gene in either of three regions, i.e., medial preoptic area (MPOA), VMN, and MA. ERα silencing in the MPOA greatly reduced the expression of sexual, but not aggressive behaviors, whereas silencing in the VMN reduced both sexual and aggressive behaviors [44]. It should be noted that all mice (ICR/Jcl) were treated as gonadally intact. Therefore, our 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. As for the role of ERα activation in the MPOA for the induction of sexual behavior, we further investigated possible downstream transcriptional products of ERα-mediated genomic action. A number of studies have shown that E2 up-regulates neuronal nitric oxide synthase (nNOS) in an ERα-dependent manner [45, 46] in the MPOA, and local administration of nNOS substrate in the MPOA facilitates male sexual behaviors [47, 48]. Consistent with these findings, we found that there was a parallel decrease of nNOS- and ERα-immunoreactive cells in the MPOA of αERKD male mice [44]. In the VMN, since both sexual and aggressive behaviors were affected by ERα-silencing, we could not determine whether both types of behaviors are similarly or differentially regulated by ERα-mediated E2 action in this brain region. A recent optogenetic study [49, 50] reported that there are VMN neuron populations which were activated during sexual behaviors, but inhibited during aggressive behaviors.
In contrast, silencing of ERα in the MA had no effect on the expression of sexual and aggressive behaviors [44]. Since MA is implicated in male sexual behavior, our results were somewhat unexpected ones. It was reported that local administration of E2 in the MA restored sexual behavior in castrated male hamsters [51] and that of aromatase inhibitor reduced sexual behavior in gonadally intact male rats [52]. However, a recent study using MA-specific ERα suppression with antisense reported that sexual behavior was not affected in gonadally intact male rats [53]. Furthermore, considering the fact that MA is one of the ERβ-rich brain areas, it is worth investigating effects of site-specific ERβ knockdown in the MA in the expression of sexual behavior. Likewise, ERβ may be responsible for the regulation of aggressive behavior by E2 in the MA — even though neuronal activation as measured by Fos induction was reported in the MA during aggressive encounter [54, 55], ERα knockdown failed to alter the levels of aggressive behavior in our study.

2.3 Sexual differentiation of neural network for social behavior

E2, as an aromatization metabolite of testosterone, may regulate the expression of male-type social behavior not only through activational action (which was the target of ERα silencing study mentioned above), but also through organizational action. It is well established that perinatal testosterone surge in males masculinizes/defeminizes the undifferentiated brain. Numerous studies, including the most convincing reports in aromatase knockout (AromKO) mice [56, 57], have supported the notion that aromatization of testosterone is critical for this sexual differentiation process in rodents [e.g., 58, 59]. Relative contribution of ERα and ERβ for organization action of E2 for the establishment of the neural network for sex-typical social behaviors is not completely understood. We performed neuroanatomical analysis for sexually dimorphic brain areas, such as the principal nucleus of bed nucleus of the stria terminalis (pBNST) and anteroventral periventricular nucleus (AVPV) using knockout mice for aromatase, ERα, and ERβ. In both male-dominant (male > female) pBNST and female-dominant (female > male) AVPV, AromKO and αERKO male mice had female-type morphology whereas βERKO male mice had male-type morphology [60, 61]. Therefore, ERα, but not ERβ, mediated action of E2 is required for both masculinization of pBNST and defeminization of AVPV.

In addition to perinatal period, it is now well accepted that gonadal steroids may exert organizational action in peripubertal period [62]. Unlike in a number of sexually dimorphic nuclei, such as pBNST, AVPV, and preoptic area, where perinatal testosterone surge is required for masculinization, sex differences in the morphology of posteroventral medial amygdala can be altered by testosterone manipulation in adults [63]. Moreover, it is reported in both hamsters [64] and mice (unpublished data) that prepubertal, but not postpubertal, castration greatly reduces the degree of restoration of sexual and aggressive behavior by testosterone treatment in adults. Considering these findings, we have hypothesized that ERα and/or ERβ may also be involved in peripubertal organizational action of testosterone in various brain regions in the social behavioral network. This is now being investigated using site-specific knockdown of ERs in prepubertal male mice [e.g., 65].

3. Influence of Early Life Experiences on Social and Socio-Emotional Behaviors

We have discussed regulatory brain mechanisms of social behaviors by gonadal steroid hormones, mainly focusing on sex-typical sexual and aggressive behaviors. The aim of this section is to discuss how sex-typical social and socio-emotional behaviors may be modified by environmental and experiential factors during early life. We describe some of our recent findings with a maternal separation (MS) paradigm in both male and female mice.

3.1 Effects of MS on female behavior

We have been using a MS paradigm in which pups were removed from their home cage as a group and kept at 36°C separated from dam for 3 hr each day during the dark phase from postnatal day (PND) 1 to 14. Although it was assumed that a number of stereotypical social behaviors such as sexual, aggressive and parental might be affected, we also aimed to examine the impact of early life experiences on more complicated social interactive, emotional and anxiety-related behaviors required for the formation and maintenance of social relationships. For this purpose, we developed a couple of new behavioral setups to measure social interactive and socio-emotional behaviors of mice taking their innate traits into account [66].

In the social investigation test, we measured behavioral responses of female mice toward an unfamiliar stimulus mouse, which was placed in a clear Plexiglas cylinder (with small holes in the bottom 3 cm) and presented in their home cage. C57BL/6J female mice in the MS group responded very differently from those in the non-separated control mice. In the MS group, the duration of social sniffing was reduced and the number of stretched approaches was increased, suggesting that MS suppressed social interest and potentiated social anxiety. We also established a social preference test to let female mice choose to stay with one of two types of stimulus mice presented in cages connected with tunnels to their own home cage. We assessed changes of social preference (female vs male) patterns of female mice over a period of 5 days, and found that unlike control females, MS females had a distinctive preference for female stimuli and avoidance of male stimuli. Furthermore, social stimulus exposure potentiated neuronal activity, measured as FosB expression, in the PVN, MA and central amygdala in MS females compared to control mice. These findings
collectively suggest that experiences in early neonatal life may have a profound effect on various aspects of socio-emotional behaviors, such as sexual preference, anxiety, and social interest.

3.2 Effects of MS on male behavior

Neonatal MS experience also influenced subsequent expression of male social behaviors. Unlike in female mice, however, MS did not change the levels of social anxiety or interest assessed against same sex opponent mice in the social investigation test [40]. Instead, MS greatly affected male aggressive behavior in pubertal period [29, 40]. In the control group, about half of the mice showed the first aggression at 6 weeks of age and by the 8th week more than 90% mice showed aggression. On the other hand, in the MS group, only 30% of mice showed aggression in 6 week of age, and 50% of mice still failed to show any aggression at the 9th week, when the final assessment of aggressive behavior was made. There was a rapid increase of serum testosterone levels from 4 weeks to 6 weeks of age in the control group, which may support the onset of aggression, whereas no such changes were seen in the MS group [40]. Moreover, MS increased the number of oxytocin immunopositive cells and decreased vasopressin positive cells in the PVN. These findings demonstrate that neonatal MS experience could greatly impair the neuroendocrine system, involved in the development of male-type social behaviors. It should be noted, however, that in recent years, a large number of studies have been reported on the consequence of MS treatment in various species or lines, using many different MS protocols and/or behavioral testing paradigms. Findings from these studies are often inconsistent and neuroendocrine mechanisms of MS effects are not completely understood [67, 68].

4. Closing Remarks

In this article, we reviewed findings on neural, hormonal and experiential control for sex-typical social behaviors. During the last two decades, thanks to a rapid progress in various research techniques, our knowledge of brain mechanisms of social behavior has been greatly advanced. However, we still have not understood completely how hormones act on the development of neural network, which in turn supports adaptive expression of social behavior throughout life. We need to further study brain mechanisms of time-, sex-, and brain site-specific action of gonadal steroid hormones and establish “a comprehensive brain map of hormonal action for the regulation of social behaviors.”

Acknowledgements

This work was supported by KAKEN #23240057 and #15H05724 to SO. Disclosure of Summary: The authors have nothing to disclose.

REFERENCES

[1] Kuiper GG, Enmark E, Pelto-Huikko M, Nilssson S, Gustafsson J-A (1996) Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930.
[2] Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA 90:11162–11166.
[3] Ogawa S, Lubahn DB, Korach KS, Pfaff DW (1995) Behavioral characteristics of transgenic estrogen receptor knockout male mice: sexual, aggressive and open-field behaviors. Abstract for the Annual Meeting of the Endocrine Society, pp 133.
[4] Ogawa S, Taylor JA, Lubahn DB, Korach KS, Pfaff DW (1996) Reversal of sex roles in genetic female mice by disruption of estrogen receptor gene. Neuroendocrinology 64:467–470.
[5] Ogawa S, Gordan JD, Taylor J, Lubahn D, Korach K, Pfaff DW (1996) Reproductive functions illustrating direct and indirect effects of genes on behavior. Horm Behav 30:487–494.
[6] Ogawa S, Lubahn DB, Korach KS, Pfaff DW (1997) Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci USA 94:1476–1481.
[7] Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson J-A, Smithies O (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci USA 95:15677–15682.
[8] Ogawa S, Chan J, Chester AE, Gustafsson J-A, Korach KS, Pfaff DW (1999) Survival of reproductive behaviors in estrogen receptor β gene-deficient (βERKO) male and female mice. Proc Natl Acad Sci USA 96:12887–12892.
[9] Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW (1998) Roles of estrogen receptor-α gene expression in reproduction-related behaviors in female mice. Endocrinology 139:5070–5081.
[10] Couse JF, Curtis SW, Washburn TF, Lindzey J, Golding TS, Lubahn DB, Smithies O, Korach KS (1995) Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. Mol Endocrinol 9:1441–1454.
[11] Musatov S, Chen W, Pfaff DW, Kaplitt MG, Ogawa S (2006) RNAi-mediated silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. Proc Natl Acad Sci USA 103:10456–10460.
[12] Shughrue PJ, Lane MV, Merchenthaler I (1997) Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. J Comp Neurol 388:507–525.
[13] Nomura M, Korach KS, Pfaff DW, Ogawa S (2003) Estrogen receptor β (ER/β) protein levels in neurons depend on estrogen receptor α (ERα) gene expression and on its ligand in a brain region-specific manner. Mol Brain Res 110:7–14.
Neural, Hormonal and Experiential Control of Sex-Typical Expression of Social Behavior

42] Newman SW (1999) The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. Ann N Y Acad Sci 877:242–257.

43] Nelson RJ, Trainor BC (2007) Neural mechanisms of aggression. Nat Rev Neurosci 8:536–546.

44] Sano K, Tsuda MC, Musatov S, Sakamoto T, Ogawa S (2013) Differential effects of site-specific knockdown of estrogen receptor α in the medial amygdala, medial pre-optic area, and ventromedial nucleus of the hypothalamus on sexual and aggressive behavior of male mice. Eur J Neurosci 37:1308–1319.

45] Putnam SK, Sato S, Riolo JV, Hull EM (2005) Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. Horm Behav 47:513–522.

46] Scordalakes EM, Shetty SJ, Rissman EF (2002) Roles of estrogen receptor α and androgen receptor in the regulation of neuronal nitric oxide synthase. J Comp Neurol 453:336–344.

47] Benelli A, Bertolini A, Poggioli R, Cavazzuti E, Calza L, Giardino L, Arletti R (1995) Nitric oxide is involved in male sexual behavior of rats. Eur J Pharmacol 294:505–510.

48] Sato Y, Horita H, Kurohata T, Adachi H, Tsukamoto T (1998) Effect of the nitric oxide level in the medial preoptic area on male copulatory behavior in rats. Am J Physiol 274:R243–R247.

49] Lin D, Boyle MP, Dollar P, Lee H, Lein ES, Perona P, Anderson DJ (2011) Functional identification of an aggression locus in the mouse hypothalamus. Nature 470:221–226.

50] Lee H, Kim D-W, Remedios R, Anthony TE, Chang A, Madisen L, Zeng H, Anderson DJ (2014) Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus. Nature 509:627–632.

51] Wood RI (1996) Functions of the steroid-responsive neural network in the control of male hamster sexual behavior. Trends Endocrinol Metab 7:338–344.

52] Huddleston GG, Paisley JC, Clancy AN (2006) Effects of estrogen in the male rat medial amygdala: infusion of an aromatase inhibitor lowers mating and bovine serum albumin-conjugated estradiol implants do not promote mating. Neuroendocrinology 83:106–116.

53] Paisley JC, Huddleston GG, Carruth LL, Petrilis A, Grober MS, Clancy AN (2012) Sexual responses of the male rat medial preoptic area to estrogen I: site specific suppression of estrogen receptor α. Horm Behav 62:50–57.

54] Kollack-Walker S, Newman SW (1995) Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. Neuroscience 66:721–736.

55] Veening JG, Coolen LM, de Jong TR, Joosten HW, de Boer SF, Koolhaas JM, Olivier B (2005) Do similar neural systems subserve aggressive and sexual behaviour in male rats? Insights from c-Fos and pharmacological studies. Eur J Pharmacol 526:226–239.

56] Toda K, Okada T, Takeda K, Akira S, Saibara T, Shiraishi M, Onishi S, Shizuta Y (2001) Oestrogen at the neonatal stage is critical for the reproductive ability of male mice as revealed by supplementation with 17beta-oestradiol to aromatase gene (Cyp19) knockout mice. J Endocrinol 168:455–463.

57] Toda K, Saibara T, Okada T, Onishi S, Shizuta Y (2001) A loss of aggressive behaviour and its reinstatement by oestrogen in mice lacking the aromatase gene (Cyp19). J Endocrinol 168:217–220.

58] McCarthy MM, Arnold AP (2011) Reframing sexual differentiation of the brain. Nat Neurosci 14:677–683.

59] Wallen K (2009) The Organizational Hypothesis: Reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). Horm Behav 55:561–565.

60] Kanaya M, Tsuda MC, Sagoshi S, Nagata K, Morimoto C, Thu CKT, Toda K, Kato S, Ogawa S, Tsukahara S (2014) Regional difference in sex steroid action on formation of morphological sex differences in the anteroventral periventricular nucleus and principal nucleus of the bed nucleus of the stria terminalis. PLoS ONE 9:e112616.

61] Tsukahara S, Tsuda MC, Kurihara R, Kato Y, Kuroda Y, Nakata M, Xiao K, Nagata K, Toda K, Ogawa S (2011) Effects of aromatase or estrogen receptor gene deletion on masculinization of the principal nucleus of the bed nucleus of the stria terminalis of mice. Neuroendocrinology 94:137–147.

62] Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040–1047.

63] Morris JA, Jordan CL, Breedlove SM (2004) Sexual differentiation of the vertebrate nervous system. Nat Neurosci 7:1034–1039.

64] Romeo RD, Richardson HN, Sisk CL (2002) Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. Neurosci Biobehav Rev 26:381–391.

65] Ogawa, S, Sano, Nakata K, M, Musatov, S, Tsukahara, S (2014) Activation of estrogen receptor α in the medial amygdala during pubertal period is essential for the full expression of male-type social behavior in mice. Abstract for the International Congress of Neuroendocrinology, 2014.

66] Tsuda MC, Ogawa S (2012) Long-lasting consequences of neonatal maternal separation on social behaviors in ovariecctomized female mice. PLoS ONE 7:e33028.

67] Tsuda MC, Ogawa S (2011) Behavioral neuroendocrine studies. Tsukuba Psychol Res 41:1–10.

68] Veenema AH (2012) Toward understanding how early-life social experiences alter oxytocin- and vasopressin-regulated social behaviors. Horm Behav 61:304–312.