



ELSEVIER



Frontiers in Neuroendocrinology xxx (2007) xxx–xxx

Frontiers in
Neuroendocrinologywww.elsevier.com/locate/yfrne

Review

Role for estradiol in female-typical brain and behavioral sexual differentiation

Julie Bakker^{a,*}, Michael J. Baum^b^a Center for Cellular & Molecular Neurobiology, University of Liège, Belgium^b Department of Biology, Boston University, USA

Abstract

The importance of estrogens in controlling brain and behavioral sexual differentiation in female rodents is an unresolved issue in the field of behavioral neuroendocrinology. Whereas, the current dogma states that the female brain develops independently of estradiol, many studies have hinted at possible roles of estrogen in female sexual differentiation. Accordingly, it has been proposed that α -fetoprotein, a fetal plasma protein that binds estrogens with high affinity, has more than a neuroprotective role and specifically delivers estrogens to target brain cells to ensure female differentiation. Here, we review new results obtained in aromatase and α -fetoprotein knockout mice showing that estrogens can have both feminizing and defeminizing effects on the developing neural mechanisms that control sexual behavior. We propose that the defeminizing action of estradiol normally occurs prenatally in males and is avoided in fetal females because of the protective actions of α -fetoprotein, whereas the feminizing action of estradiol normally occurs postnatally in genetic females.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Sexual differentiation; Brain; Estrogens; Aromatase; α -Fetoprotein; Sexual behavior

1. Introduction

The classic view of sexual differentiation in mammalian species holds that sex differences in the brain develop under the influence of testosterone and/or estradiol derived from neural aromatization of testosterone: the brain develops as male in the presence of these steroid hormones, and as female in their absence. In agreement with this view, it has been proposed by McEwen et al. [64] that the female rodent brain needs to be protected from estrogens produced by the placenta or male siblings, and that α -fetoprotein (AFP)—an important fetal plasma protein present in many developing vertebrate species and produced transiently in great quantities by the hepatocytes of the fetal liver [3,94]—is the most likely candidate to achieve this protection because of its estrogen-binding capacity. However, the idea that the female brain develops in the absence

of estrogens as well as the role of AFP in protecting the brain against the differentiating action of estrogens have been challenged. First, there is evidence that the normal development of the female brain might actually require the presence of estrogens (e.g. [29,35]). Second, the presence of AFP within neurons in the absence of any evidence for local AFP synthesis suggests that AFP is transported from the periphery into the brain. It was therefore proposed by Toran-Allerand [99] that AFP acts as a carrier, which actively transports estrogens into target brain cells and, by doing so, has an active role in the development of the female brain. Thus, two clearly opposing views exist on the function of AFP in the sexual differentiation of the rodent brain, as well as on the role of estrogens in the development of the female brain. In this review, we re-examine the role of perinatal estrogens and consequently the role of AFP in the development of the female brain by discussing results obtained in two different knockout mouse models, i.e. the aromatase knockout (ArKO) mouse [44] and the AFP-KO mouse [34]. Behavioral evidence from these mouse models suggests that estrogens can have

* Corresponding author. Fax: +32 4 366 5971.
E-mail address: jbakker@ulg.ac.be (J. Bakker).

both feminizing and defeminizing effects on the developing brain mechanisms that control sexual behavior. We therefore suggest here that the defeminizing action of estradiol normally occurs prenatally in males and is avoided in fetal females because of the protective actions of AFP. Furthermore, the feminizing action of estradiol normally occurs in genetic females between birth and the age of puberty, when the ovaries start to produce estrogens and AFP no longer plays a significant role.

2. Classical theory of brain and behavioral sexual differentiation

In male mammals, the presence of the Sry gene on the Y-chromosome causes the undifferentiated gonads to develop into testes instead of ovaries [50]. Testosterone secreted by the testicular Leydig cells promotes the development of the Wolffian ducts into the internal male genital structures whereas anti-Müllerian hormone secreted by testicular Sertoli cells causes regression of the female-typical Müllerian ducts. The penis and scrotum develop under the influence of dihydrotestosterone which is formed from testosterone by the enzyme, type II 5 α -reductase. In normal female differentiation, the Müllerian ducts develop without any apparent hormonal input into the uterus, the fallopian tubes, and the distal portion of the vagina. The Wolffian ducts regress and disappear in the absence of any androgenic stimulation. Phoenix and co-workers [75] provided the first evidence that the capacity to display sex-specific behaviors in adulthood (and by inference, the sexual differentiation of the brain) follows the same pattern as that of the genitals. Thus female guinea pigs treated with testosterone propionate *in utero* showed elevated levels of male-typical mounting behavior together with reduced levels of female-typical lordosis behavior in adulthood [75]. Supportive evidence for a role of perinatal testosterone in the development of the male brain came from subsequent studies by Feder and Whalen [31], and Grady et al. [40], and many others (reviewed in [10]) showing that removal of testosterone by neonatal castration reduced males' later capacity to show male sexual behaviors while enhancing their ability to show female sexual behaviors. Additional evidence suggested that testosterone secreted by the testes acts perinatally, either directly via androgen receptors or after being aromatized into estradiol and stimulating estradiol receptors ([58,68]) to masculinize (enhance male-typical sexual responses) and/or defeminize (suppress female-typical responses) the neural substrate that controls sexual behavior (Fig. 1). The results of these early studies also implied that the neural mechanisms which control later female-typical sexual behavior normally develop perinatally in females "by default", i.e. without the need for any sex steroid stimulation. Consistent with this view is the observation that the female ovaries are quiescent during perinatal development, i.e. the rodent ovary does not secrete significant amounts of estradiol before postnatal day 7 [53]. Accordingly, the fetal rodent ovary does not



Fig. 1. Sexual differentiation of the brain. In male rodents, testosterone (T) secreted by the testes enters the brain where it is aromatized to estradiol (E), which subsequently binds to the estradiol receptor to promote gene expression that masculinize and defeminize the neural mechanisms controlling sexual behavior.

seem to express at least 3 of the enzymes (P450scc, P450c17, and p450arom) that are necessary for estrogen production whereas these enzymes are present in the fetal rodent testis [41]. Finally, any estrogens secreted by the mother during gestation are thought to be not available to the fetal (male or female) rodent brain because they are bound with high affinity and capacity to α -fetoprotein (AFP), a plasma glycoprotein produced in high quantities by the fetal liver [3,64,94].

3. Role of estradiol in female-typical brain and behavioral sexual differentiation

Whereas, the concept of the male sexual differentiation of the brain depending on the presence of testosterone and/or estradiol has been based on the results of a large number of studies (reviewed in [10,18,27,38,39,78]), the concept of the female sexual differentiation of the brain proceeding in the absence of these hormones has been primarily based on assumptions. For example, the finding that neonatally castrated male rats show lordosis behavior when primed with ovarian hormones in adulthood certainly suggests that gonadal hormones may not be necessary to develop the potential to show lordosis behavior in adulthood; however, it does not prove that it is the case. In fact, several studies have suggested that ovarian secretions are necessary for a normal development of the female brain, thereby challenging the concept of a default developmental program for the female brain. However, it has been proven difficult to provide solid experimental evidence of a

role for ovarian hormones in the development of the female brain, mainly because of technical difficulties in manipulating estrogen levels (or neural actions) during early development in females. Here, we will give a short overview of some of these studies.

3.1. *The role of the ovary in female sexual differentiation*

In a first approach to investigate the role of ovarian hormones in female sexual differentiation, ovariectomy was used as method to clear the developing female of circulating estrogens. Thus, early studies by Jost [45] and Pfeiffer [74] showed that fetal or neonatal gonadectomy did not interfere with the female differentiation of the genitals thereby setting the basis for the concept of a default developmental program in the female. Estrogen levels are shown to be very high during fetal development [104] as well as during early postnatal life [65] in females. It was thus assumed that ovariectomy would render the developing female free from circulating estrogens. However, the ovaries are probably not the primary source of these estrogens since they do not secrete any detectable levels of estrogens before day 7 after birth [53]. Thus any estrogens circulating in the fetal and newborn female rodent must be derived from extra-ovarian sources, such as the adrenal glands [36], the mother [116] or male siblings that are adjacent to the female *in utero* [104]. Therefore, there is little reason to believe that fetal or neonatal ovariectomy would actually render the female fetus free from estrogens and thus that the subjects in the Jost and Pfeiffer studies were not exposed to any estrogens.

In a second approach to address the role of the ovary in sexual differentiation, female rats were ovariectomized on the day of birth and subsequently re-implanted (or not) with ovaries until the age of puberty in order to determine whether ovaries are needed to be present to develop the potential to show lordosis behavior in adulthood. Thus, both Lisk [56] and Gerall et al. [35] reported that female rats which were ovariectomized on the day of birth had lower lordosis quotients after adult treatment with estradiol and progesterone than females which either kept their ovaries [56] or were ovariectomized at birth and subsequently implanted with ovaries from the day of birth until day 60 [35]. In addition, neonatally castrated male rats implanted with ovaries at several different periods between birth and day 60 showed higher lordosis quotients than neonatally castrated males which were not given ovarian implants [29,35]. In another study [16], the possession of ovaries beyond the age of puberty attenuated the ability of an injection of testosterone propionate on postnatal day 4 to reduce later lordotic responses of female rats to ovarian hormones. Also, subcutaneous administration of a silastic capsule containing estradiol over postnatal days 30–40 enhanced their later lordotic responsiveness in both male and female rats that were gonadectomized on day one after birth whereas estradiol

treatment over postnatal days 10–20 was less effective in feminizing this capacity [93]. These early behavioral results thus suggest that exposure to a (low) level of estrogenic stimulation over a postnatal interval between birth and the age of puberty facilitates the later capacity to display female sexual behavior; however, they do not provide incontrovertible evidence that estradiol normally contributes to the development of female sexual behavior in female mammals. First, the effects of neonatal ovariectomy on the potential to show lordosis behavior later in life were only transient since any differences in female sexual behavior disappeared following repeated testing [35]. Second, the study by Whalen and Edwards [113] actually showed no effect of neonatal ovariectomy on later lordosis behavior. Male and female rats which were gonadectomized on the day of birth, showed equivalent levels of receptivity as gonadally intact female rats. Finally, it was never experimentally established that these ovarian implants actually secreted estradiol.

Therefore, in a third approach to investigate the potential role for estrogens in the development of the female brain, estrogen action was inhibited pharmacologically by either treating newborn female rats with the estrogen receptor antagonist tamoxifen [26] or administering the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD; [12]) to female ferrets during embryonic development. Dohler et al. [26] showed that neonatal treatment of female rats with tamoxifen decreased their later capacity to show lordosis behavior whereas concurrent neonatal administration of a low dose of estradiol benzoate prevented this effect. Likewise, Baum and Tobet [12] found that female ferrets which were treated prenatally with ATD and then treated in adulthood with a low or moderate dose of estradiol benzoate displayed decreased acceptance quotients when paired with a stimulus male. However, these later two studies also did not provide conclusive evidence of a role of estrogen in female development. First, in addition to its anti-estrogenic actions, tamoxifen can exert estrogen-like agonist actions in the brain [61]. Therefore, the observed reduction in lordosis behavior induced by administering tamoxifen neonatally to female rats [26] may actually have resulted from a partial defeminization of the brain by the estradiol-like actions of tamoxifen on neural estradiol receptors. Finally, prenatally ATD-treated female ferrets and control females displayed equivalent, high, acceptance quotients when tested after receiving a high dose of estradiol benzoate in adulthood [12] again indicating that ATD-treated females were capable of displaying full-blown receptive behavior.

Thus, there are certainly indications for a role of estrogens in the development of the female brain; however, it has been difficult to provide conclusive evidence for such a role. As a result, the hypothesis languished since the mid-1980s due to the absence of a suitable animal model in which rigorously to assess the possible contribution of estradiol to the development of the female brain.

3.2. New approach to investigate the role of estrogens in the development of the female brain

The introduction of the aromatase knockout (ArKO) mouse [33,44,97], which is deficient in aromatase activity due to a targeted mutation in the *Cyp19* gene, has provided a new model in which to study the role of estradiol in the development of the brain and behavior. The ArKO model provides unique research opportunities in that it allows the study of animals that are devoid of endogenous estrogen production but whose genetic deficiency can be corrected at the phenotypic level by simply administering exogenous estrogens since they have functional estrogen receptors. This model is therefore more amenable to the experimental testing of the effects of estrogen on the development of the brain than the models in which estrogen action was inhibited pharmacologically [12,26] or those in which the estradiol receptor has been disrupted (ERKO). In the latter case, the phenotypic correction is difficult if not impossible. Thus by administering estradiol to adult ArKO mice, one can assess the consequences of the absence of estradiol biosynthesis and cellular action earlier in life and thus distinguish between organizational and activational effects of estradiol on the brain and behavior. This makes the ArKO mouse an excellent model to readdress the question whether the normal female-typical differentiation of the brain and behavior requires perinatal exposure to estrogens.

3.2.1. Reduced female sexual behavior in female ArKO mice

In a first experiment, we determined whether lordosis behavior was affected in female ArKO mice [8]. If the female brain really develops by default, i.e. in the absence of any estrogens, lordosis behavior of female ArKO mice should be indistinguishable of that of normal, wild-type (WT) female mice. Thus, female mice of three genotypes, i.e. wild-type (WT), heterozygous (HET), and homozygous ArKO, were ovariectomized in adulthood and subsequently implanted with a silastic capsule containing crystalline estradiol (diluted 1:1 with cholesterol). All females were injected subcutaneously with 500 μ g progesterone 2–4 h before each lordosis test with a sexually active male. We found that the display of lordosis in response to the mounts of the stimulus male was severely impaired in ArKO females (Fig. 2). The same hormone treatment was, however, effective in inducing lordosis behavior in WT and HET females. In contrast with previous studies [12,35], the reduction in lordosis behavior observed in ArKO females did not disappear with repeated testing or prolonged (>6 weeks) of estradiol treatment in adulthood suggesting permanent effects of early estradiol deprivation on the later potential to show female sexual behavior.

The absence of lordosis behavior in ArKO females could have been caused by a partial defeminization of their brains due to the presence of phytoestrogens in the food. In contrast with endogenous estrogens, phytoestrogens are generally non-steroidal in nature and have lower affinities for

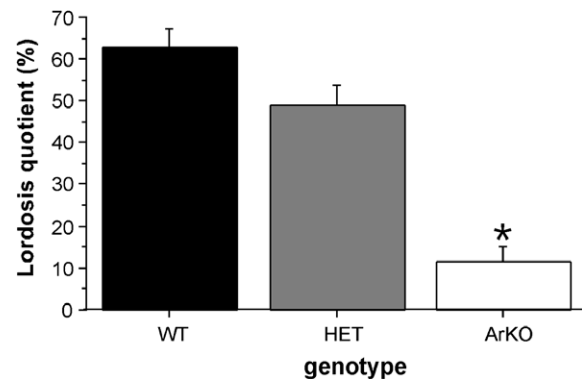


Fig. 2. Lordosis quotients of female wild-type (WT), heterozygous (HET), and aromatase knockout (ArKO) mice. All females were ovariectomized in adulthood and subsequently treated with estradiol and progesterone prior to each behavioral test with a sexually active male. * $p < 0.05$ compare to WT and HET females. Data shown are means (\pm SEM) of a total of five tests.

estrogen-binding plasma proteins such as AFP [69]. They may thus evade the protective actions of AFP and freely enter the brain where they could interfere with brain sexual differentiation [55,85,114]. This suggests that phytoestrogens could be a potential source of estrogen action in the ArKO female brain, in particular in the absence of any local competition with endogenous estrogens for binding to neural estradiol receptors. Furthermore, Kudwa et al. [51] showed that ArKO females whose mothers were fed a phytoestrogen-free diet showed higher lordosis quotients (equivalent to those of WT females) than ArKO females whose mothers were fed a phytoestrogen-rich diet suggesting that estrogens present in diet can attenuate the display of female sexual behavior in ArKO mice. Furthermore, Kudwa et al. [51] proposed that these phytoestrogens probably act via the ER β receptor since male ER β KO mice were capable of showing lordosis behavior in adulthood following castration and subsequent treatment with estradiol and progesterone [51], and phytoestrogens have been shown to preferentially bind to the ER β receptor [52]. However, it is unlikely that the effects observed in our ArKO mice [8] relate to an estrogenic action by phytoestrogens since (i) our mice were fed a mouse chow (UAR 03, Epinay sur Orge, France) that does not appear to have any biologically active estrogens, as revealed by its lack of effect on uterine growth and on the growth of estrogen-dependent cell lines (M. Huard, UAR, personal communication), and (ii) we recently observed very low, almost non-detectable, progesterone receptor (PR) levels in the medial preoptic (MPN) and ventromedial nuclei (VMN) of gonadally intact ArKO mice of both sexes at several postnatal ages (days 10, 20, 30, and 40; Fig. 4; unpublished results). The latter animals were derived of mothers fed the same diet as in our previous study [8]. The results on PR expression provide the best evidence for the lack of any significant estrogenic action in the ArKO brain during development since previous studies by Wagner and colleagues [80–82,105,106] have clearly

shown that the sex difference in PR levels in the MPN and VMN, with fetal and neonatal males showing a higher PR expression than females, depends on the production of estradiol acting via the ER α receptor in the developing male brain. For instance, maternal administration of the aromatase inhibitor ATD significantly decreased hypothalamic PR expression in male rat fetuses [82], whereas 3 days of administering testosterone propionate or the synthetic estrogen DES (but not dihydrotestosterone) to pregnant female rats over embryonic days 19–22 stimulated hypothalamic PR levels in female offspring when they were killed on E22 [82]. The sex difference in PR expression in the MPN, which also exists in mice, is abolished in transgenic mice lacking the functional ER α gene [105]. In the rat, the female-typical profile of PR expression develops after birth. Thus ovariectomy on postnatal day 4 prevented the female-typical increase in PR levels observed between day 10 and 28 in the MPN [80]. Interestingly, preliminary results from our laboratory also suggest a role for postnatal estrogens in the development of PR in the female mouse. We observed a similar increase in PR levels in WT female mice between days 10 and 30 after birth, whereas this increase was completely absent in ArKO females, confirming the lack of any estrogen action in this mouse model.

It seems unlikely that the deficit in lordosis behavior can be attributed to excessive androgen action during development leading to a masculinization and defeminization of the brain in ArKO females. Previous work on another strain of ArKO mice [33] has indicated that ArKO mice of both sexes are exposed to increased plasma levels of testosterone during adulthood. These increased levels of androgens probably result from the interruption of the steroid feedback on the gonadotropin secretion, which is known to be mediated by estrogens, as suggested by the increased levels of circulating luteinizing hormone and follicle-stimulating hormone in these mice. Alternatively, the increase in plasma testosterone could be caused, at least in part, by the accumulation of the androgenic substrate, which can no longer be transformed into an estrogen by the ovaries because of the disruption of the aromatase gene. Because the fetal and neonatal ovaries are not very active [53,108,109], it is unlikely that this increase in androgen levels actually takes place during early development, but since no data are available to evaluate this question, it could be speculated that increased levels of androgens contribute to the development of the behavioral phenotype of ArKO mice. Thus to determine whether the absence of lordosis behavior in ArKO females did not result from masculinization and defeminization of their brains by excessive androgen action during development, new groups of female WT, HET, and ArKO mice were tested for their potential to show male-typical sexual behavior with an estrous female. Female subjects were ovariectomized and implanted subcutaneously with a silastic capsule containing crystalline testosterone. Following several (>3) weeks of testosterone treatment, female ArKO mice showed no

mounting behavior whereas WT and, to a lesser extent, HET females readily displayed mounting and intromission-like behaviors (Fig. 3). The addition of estradiol (5 μ g/mouse/day) to the testosterone treatment stimulated a little mounting and intromission-like behavior in ArKO females, but not to the levels shown by WT and HET females (Fig. 3). Such an outcome would not have occurred had ArKO females been exposed to high levels of testosterone perinatally capable of masculinizing their coital capacity. These results also suggest that estrogens organize mounting behavior in mice to some extent since ArKO females showed no or very little mounting behavior and HET females showed intermediate levels of male sexual behavior, thereby indicating a dose-dependent effect of estrogens on this behavior. By contrast, results obtained in male ArKO mice strongly suggest that male sexual behavior is organized by androgens and not estrogens since almost normal levels of male sexual behavior could be induced in male ArKO mice when treated in adulthood with estrogens in combination with dihydrotestosterone [9]. In addition, work from the group by Sato et al. [86] using androgen receptor knockout mice (ARKO), also provided evidence of the differentiation of male sex behavior by androgens. Male ARKO showed very little mounting behavior, whereas female mice treated with DHT perinatally showed high levels of mounting behavior, but this effect of perinatal DHT treatment was not observed in female ARKO mice [86]. Finally, to make matters even more confusing, recent data obtained in mice carrying the testicular feminization (*Tfm*) mutation of the androgen receptor suggest that male sexual behavior is not organized in the mouse since estrogen-treated male and female mice including *Tfm* mice, showed equivalent, high levels of mounting behavior [17]. Perhaps the sex difference in mounting behavior lies in the sensitivity to adult steroid treatment, i.e. larger doses of testosterone or estradiol are

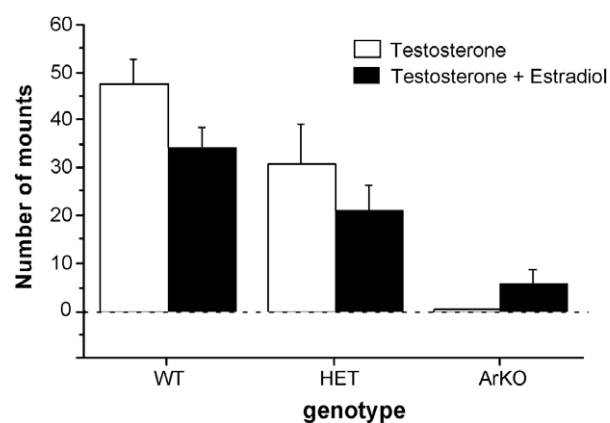


Fig. 3. Mounting behavior of female wild-type (WT), heterozygous (HET), and aromatase knockout (ArKO) mice. All females were ovariectomized in adulthood and tested once for mounting behavior with an estrous female when treated with testosterone and then once more when treated with both testosterone and estradiol (5 μ g/mouse/day). * p < 0.05 compared to WT and HET females, # p < 0.05 compared to WT females. Data shown are means \pm SEM.

needed to stimulate mounting behavior in female compared to male mice. In the Bodo and Rissman study [17], mice were tested using adult estradiol treatment by silastic implant (5 mm of 17 β -estradiol diluted 1:1 with cholesterol). This treatment has been shown to lead to very high levels of estradiol [110] and has been used to induce lordosis behavior in female mice in our laboratory (e.g. [8,46,47]). Thus, this treatment did not allow for the detection of any possible genotype differences in sensitivity to estradiol in activating male sexual behavior. Clearly more studies are needed to determine whether male sexual behavior is organized or not by steroids in the mouse and if so, by which steroids. It is possible that in mice, as in ferrets [95], fetal exposure to estrogens followed by neonatal exposure to testosterone is required for complete masculinization of male-typical mating behavior. It should be noted that even though ArKO male mice readily mounted and intromitted an estrous female when treated with estrogens and DHT in adulthood [9], they rarely showed any ejaculatory behavior, suggesting a possible contribution of estrogens to the organization of male sexual behavior.

Taken together, the results obtained in female ArKO mice [8] are best explained by assigning an active role for estradiol in females in the development of female sexual behavior. At present, these results provide the best available evidence affirming a role of estradiol in female brain sexual differentiation.

3.2.2. Neural mechanisms potentially affected in female ArKO mice

The reduction in lordosis behavior observed in female ArKO mice [8] may reflect deficits in (i) the neural circuitry regulating the lordosis reflex and/or (ii) the neural mechanisms regulating sexual motivation and sexual partner preference. Classically, studies of female sexual behavior have concentrated on the neural and hormonal control of lordosis behavior in the female rat (for a detailed description, see [73]). Briefly, male mounting stimulates pressure receptors on the flanks, posterior rump, tail base, and perineum of the female. Axons of these receptors form a sensory nerve that projects to the dorsal root of the ganglion of the spinal cord. Then, the signal is transmitted to the reticular formation in the brain stem and the midbrain central gray area. When the female is in estrus, i.e. when estradiol concentrations are high, several brain regions including the ventrolateral portion of the VMN (a brain region rich in estrogen receptors) and the MPN, activate via the midbrain central gray, medullary reticular formation, and medial geniculate body, the spinal motoneurons innervating the back muscles critical to the display of lordosis [23]. Thus, the VMN plays a critical role in integrating hormonal and sensory information necessary for the display of lordosis in female rodents. Accordingly, lesions of the VMN, or destruction of its afferent and efferent fibers, typically reduce the frequency of lordosis behavior in female rats [20,60,72] and hamsters [59], whereas implants of estradiol into the VMN induce lordosis behavior in ovariectomized

female rats [84]. Our preliminary results (unpublished) indicated that gonadally intact ArKO females, which were not supplemented with any estrogens, show much lower levels of PR in the VMN than WT females. Whether the estradiol treatment used to induce female sexual receptivity in our previous study [8] also normalized PR expression in the VMN of ArKO females has not yet been investigated. However, preliminary results from Kudwa et al. (Kudwa, Schank, Honda, and Rissman, SBN abstracts, 2001) suggest that adult treatment with estradiol failed to induce normal, WT female, levels of PR expression in the VMN of ArKO females indicating a contribution of postnatal estradiol to the development of PR receptors, as was already suggested by the work of Quadros et al. [80]. Further studies are needed to determine whether the hormonal–neural circuitry of lordosis behavior has not been feminized in ArKO females and thus whether there is an active contribution of postnatal estradiol to the development of this circuit.

In contrast with the neural and hormonal control of lordosis behavior, very few studies have concentrated on other aspects of female sexual behavior, including proceptivity (i.e. sexual motivation) and sexual partner preference. Nevertheless, the ability to seek out and identify potential mates is as critical to female sexual behavior as is the capacity to display the lordosis reflex when mounted by a male. Rodent species use primarily odors to identify individuals of their own species and accordingly, release to their environment a wide variety of volatile and non-volatile odors via extraorbital lacrimal glands [49], skin glands, urine, and feces. Two different olfactory systems have evolved to detect these odors. It is generally thought that the main olfactory system is used to detect a wide variety of volatile odorants derived from food and potential predators, among many sources, whereas the accessory olfactory system evolved to detect and process a subset of non-volatile odors that influence a variety of reproductive and aggressive behaviors in mammalian species [32,48]. However, recent evidence points to an important role for the main olfactory system in detecting and processing olfactory cues used for mate recognition [46,47,89]. In female mice, olfactory cues have been shown to facilitate the display of lordosis behavior. Peripherally induced anosmia by intranasal application of zinc sulfate solution attenuated lordosis behavior in hormone-primed female mice [30,46] whereas removal of the vomeronasal organ actually completely abolished lordosis behavior in estrogen and progesterone-treated female mice [47] thereby emphasizing the importance of the accessory olfactory system in the control of female sexual behavior. Thus the reduction in lordosis behavior observed in female ArKO mice may reflect deficits in the detection and processing of olfactory cues. For instance, it is possible that ArKO female mice did not recognize the stimulus male on the basis of his odors and as a result did not become sexually receptive. If so, then this would suggest a role for estrogens in the activation and/organization of olfactory function. Indeed, sex differences

have been reported in olfactory sensitivity with females being better able than males to detect male-derived odors. For instance, sows are significantly better than boars at using decreasing concentrations of the volatile male pig pheromone, androstenone, as a discriminative stimulus in operant tests for a sucrose award [28]. These sex differences may not only be restricted to the detection of opposite-sex odors, but may also involve same-sex odors. Using habituation/dishabituation tests to determine odor attraction thresholds, female mice responded more reliably than male mice to low concentrations of volatile urinary odors from either sex [11]. The greater olfactory sensitivity observed in female mice probably reflects perinatal actions of gonadal hormones since these sex differences were already observed in long-term gonadectomized mice suggesting that gonadal hormones are not necessary to activate this behavior [11]. Furthermore, Dorries et al. [28] showed that the olfactory performance of neonatally castrated male pigs falls between those of sows and boars indicating a contribution of perinatal androgens and/or estrogens in the ability to detect androstenone. Thus, we hypothesized that olfactory investigation of conspecific odors is affected in ArKO females due to them being deprived of estrogens during perinatal development. Indeed, in our initial study ([8]; Fig. 4) we observed that female ArKO mice displayed reduced levels of olfactory investigation directed towards volatile odors emitted from either estrous female or male conspecifics when provided with a choice between both odor sources in a Y-maze [8]. Female subjects were ovariectomized in adulthood and treated over a prolonged (>3 weeks) period of time with estradiol indicating that the reduction in olfactory investigation could not be corrected by supplementing ArKO females with estradiol (Fig. 4). By contrast, no differences were observed between ArKO and WT females in olfactory investigation of soiled bedding

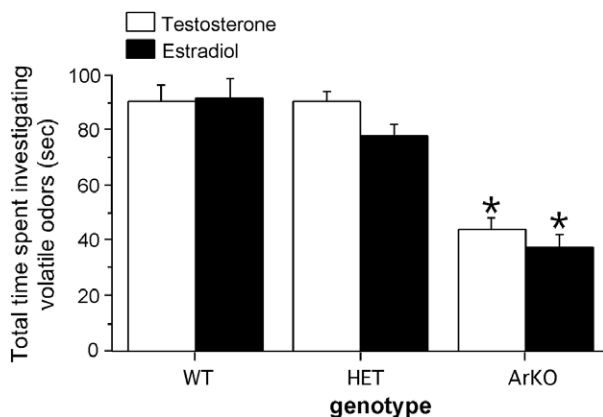


Fig. 4. Total time spent investigating volatile odors by female wild-type (WT), heterozygous (HET), and aromatase knockout (ArKO) mice when given the choice between volatile body odors from an intact male versus those from an estrous female in a Y-maze. All female subjects were ovariectomized in adulthood and first tested for their odor preferences when receiving testosterone and then when receiving estradiol. * $p < 0.05$ compared to WT and HET females. Data shown are means \pm SEM of two successive tests.

which contains primarily non-volatile odors and are thought to be detected and processed by the accessory olfactory system. These results thus suggest in particular, deficits in main olfactory function in ArKO females. Therefore, in a follow-up study [76], we determined whether this reduction in olfactory investigation reflect deficits in the ability of the main olfactory system to detect and/or discriminate volatile odors derived from male as opposed to female conspecifics. Using habituation/dishabituation tests, we found that gonadectomized ArKO and WT mice, which were tested without any sex hormone replacement, reliably distinguished between undiluted volatile urinary odors of either adult males or estrous females versus deionized water as well as between these two urinary odors themselves (Fig. 5). Thus, the previously observed deficits [8] in the preference of female ArKO mice to approach volatile body odors from conspecifics of either sex cannot be attributed to an inability of ArKO mice to detect or discriminate volatile urinary odors from males versus females.

Next, we compared the ability of ArKO and WT mice to detect decreasing concentrations of either male or female urinary odors [76]. We found a clear-cut sex difference in urinary odor attraction thresholds among WT mice: WT females responded to higher urine dilutions than male mice, thereby confirming previous results obtained by Baum and Keverne [11]. Interestingly, male ArKO mice resembled WT females in their ability to detect lower concentrations of urinary odors, raising the possibility that the observed sex difference among WT mice in urine attraction thresholds results from the perinatal actions of estrogen in the male nervous system. Female ArKO mice failed to respond to some of the female urine dilutions, suggesting olfactory-perceptual deficits. Therefore, the ability of ArKO and WT mice to discriminate low concentrations

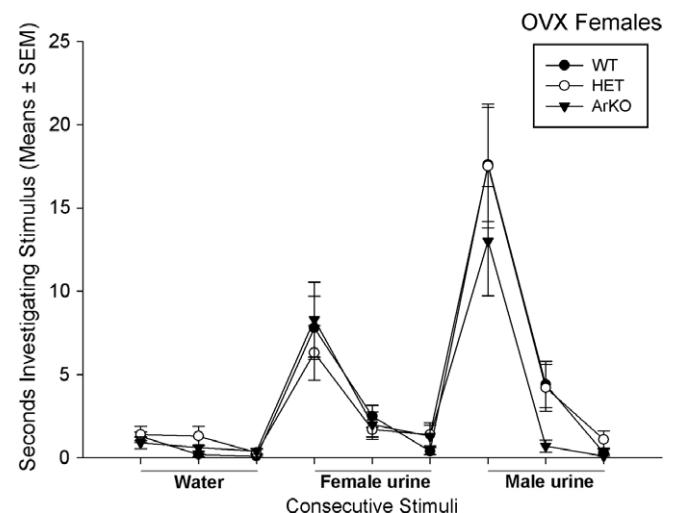


Fig. 5. Time spent by ovariectomized female wild-type (WT), heterozygous (HET), and aromatase knockout (ArKO) mice investigating deionized water or volatile urinary stimuli. * $p < 0.05$ between the time spent investigating the third presentation of a particular stimulus and the first presentation of the subsequent stimulus.

of different volatile urinary odors was also assessed using a food-motivated operant (olfactometer) task [112]. All mice were gonadectomized in adulthood and subsequently treated with a low (1 μg) dose of estradiol benzoate. All groups of animals, regardless the sex or the genotype, eventually learned to distinguish between intact male urine and estrous female urine. Among the four groups of subjects, WT females performed the worst and ArKO females the best in discriminating several pairs of urinary and non-biological (amyl versus butyl acetate) odors. This was most apparent when animals had to discriminate volatile urinary odors from ovariectomized female mice treated with estradiol sequenced with progesterone versus estradiol alone (Fig. 6): ArKO females quickly learned this discrimination whereas WT females and males as well as ArKO males failed to do so. Thus these results suggest that the weak performance of ArKO females in the habituation/dishabituation tests of odor detection [76] cannot be attributed to deficits in the function of the main olfactory system *per se*. It is possible that the previously observed [8] decrease in olfactory investigation of volatile body odors in Y-maze tests actually reflected an increased olfactory sensitivity in ArKO females, i.e. less proximal investigation of the source of urinary odors was needed for ArKO females to distinguish the different odors presented in the two arms of the Y-maze. The superior olfactory performance of ArKO females over WT females may have resulted from an increased estradiol-induced olfactory neurogenesis in these females.

Taken together, the deficits observed in lordosis behavior of female ArKO mice [8] are not due to deficits in their capacity to recognize the stimulus male on the basis of his

odors. There are certainly indices of a reduced motivation in ArKO females to investigate conspecific odors [8,76], but when appropriately motivated (by food-depriving them for instance), they are capable and even better than WT females in discriminating between different pairs of biological and non-biological odors [112]. Thus, the neural systems underlying the reduced display of lordosis behavior in ArKO females do most likely not include the olfactory systems.

4. Role of α -fetoprotein in the sexual differentiation of the rodent brain

The role of α -fetoprotein (AFP) in brain sexual differentiation has been another topic of debate in the field of behavioral neuroendocrinology during the 1970–1980s [26,64,99]. AFP was discovered about half a century ago to be the major serum fetal protein in mammals [1,15]. AFP is produced in great quantities during fetal life by the endodermal cells of the visceral yolk sac, by the hepatocytes, and in lesser amounts, by the gastrointestinal tract [3,90,94]. The protein produced by the embryo is transferred into the maternal blood circulation, and levels of AFP in the maternal serum are commonly used as a diagnostic marker to reveal developmental anomalies of the fetus [19,42,70]. Abnormally high levels of AFP in the maternal serum indicate an elevated risk of neural tube defects of the fetus such as spina bifida or anencephaly [54], whereas abnormally low levels indicates an elevated risk of Down's syndrome [22]. The synthesis of AFP decreases rapidly after birth and only trace amounts are detected in adults [3,83].

Until recently, the physiological function of AFP during embryonic development remained largely unidentified. The observation that AFP is able to bind estrogens with high affinity in rats and mice has led to the suggestion that AFP may play a role in the sexual differentiation of the brain, in particularly in protecting the female brain from excessive exposure to estrogens that are circulating in high concentrations during the critical period of sexual differentiation ([64,83]; Fig. 7a). However, in addition to binding estradiol, AFP, like albumin, is able to bind other steroids as well as endogenous and exogenous substances such as fatty acids, bilirubin, and various pharmaceutical agents, suggesting that AFP may play a transportation role in general (for review see [37]). In fact, an intracellular pool of AFP of unknown function has been detected in the brain cytosol during fetal life in various vertebrate species, including rats, mice, sheep, pig, and humans (reviewed in [99]). AFP is present within neurons of both sexes at all stages of development of the central nervous system from the postmitotic neuroblast to more differentiated neurons. However AFP is not present in the adult mouse brain underlying that AFP may have important functions within the developing central nervous system. Furthermore, although some reports exist of local synthesis (e.g. [2,57], there is no convincing evidence that intraneuronal AFP is

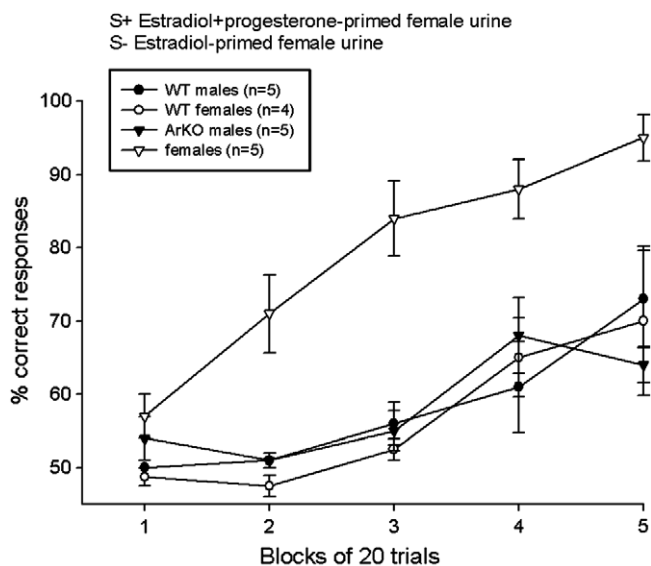


Fig. 6. Ability of wild-type (WT) and aromatase knockout (ArKO) male and female mice to learn to discriminate between two female urine stimuli in an olfactometer test. S+ = rewarded stimulus; S- = non-rewarded stimulus. Data expressed as means \pm SEM. * p < 0.05 compared to WT males and females, and ArKO males.

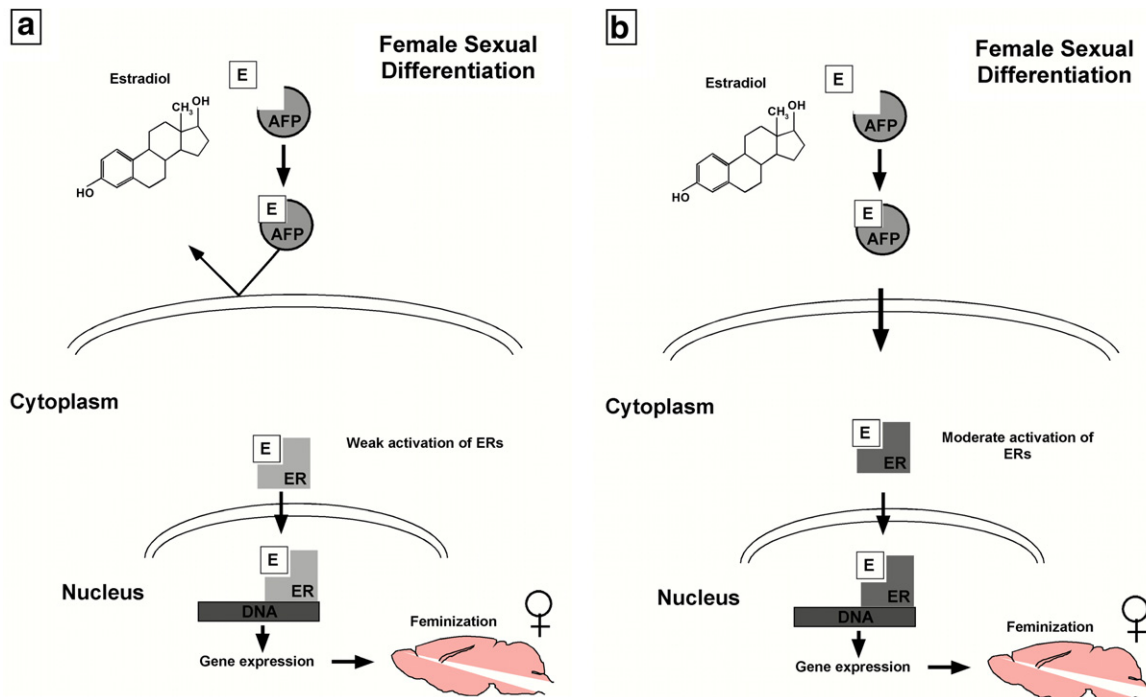


Fig. 7. Two competing hypotheses on the role of α -fetoprotein (AFP), a fetal plasma protein that binds estrogens with high affinity, in female sexual differentiation: (a) AFP may serve to keep estrogens from entering the brain (hypothesis proposed by [64]) and (b) AFP may deliver estrogens to specific brain regions to promote feminization of the neural mechanisms controlling female sexual behavior (hypothesis proposed by [99]).

synthesized locally since no messenger RNA for AFP could be detected in the fetal, newborn, or adult mouse brain [88] suggesting that the observed high levels of AFP immunoreactivity in the brain must be derived from external sources, perhaps by receptor-mediated endocytosis [99,100]. These latter observations suggest that AFP may have more than the attributed “protective” role [64] and may serve as a transport carrier for estrogens into the brain [99]; Fig. 7b). In particular, the discrete intracytoplasmic localization of rodent AFP suggests its possible active involvement in estrogen-sensitive neurons during the critical period of sexual differentiation. Since there is a difference of several orders of magnitude between the affinity constants for estradiol binding by AFP ($K_D 10^{-8}$ M; [87]) and by the estrogen receptor ($K_D 10^{-11}$ M) the subsequent intracytoplasmic dissociation of the AFP/estradiol complex in estrogen-receptor containing neurons could liberate the steroid and lead directly to receptor binding. By doing so, intraneuronal AFP could thus provide target neurons of both sexes with low levels of estrogen and thus serve as an intracellular reservoir of estrogen (Fig. 7b). It should be noted, however, that although a widespread intraneuronal localization of immunoreactive AFP is observed in numerous brain regions in rodents of both sexes, estrogen-receptor containing regions of the brain, such as the diagonal band of Broca, the medial preoptic area, the arcuate, and the ventral premammillary nuclei of the hypothalamus, and the medial and cortical amygdaloid nuclei are characterized by a complete absence of AFP-immunoreactivity [98]. This latter observation clearly questions the pos-

sible role of AFP as transporter of estrogens to estrogen-sensitive neural targets during the sexual differentiation of the brain. While the estrogen-binding capacity of the rodent AFP emphasizes its potential importance for brain sexual differentiation, its possible role with respect to its capacity to bind substances other than estrogens, such as teratogens and polyunsaturated free fatty acids such as arachidonic, docosahexaenoic, and docosatetraenoic acids, of which their importance has been shown with regard to neural development in mammals, including humans [21,77], should not be overlooked, in particular since these ligands are bound to AFP in all species [103]. Taken together, clearly opposing views exist on the function of AFP during development and in particular during sexual differentiation of the brain (Fig. 7). These different hypotheses have not been experimentally tested due to the absence of a suitable animal model. Some indirect evidence for a protective role of AFP comes from a study [62] showing that the addition of neonatal serum to [3 H] estradiol strongly reduced its uptake into the brain of adult female rats. Furthermore, the study by Mizejewski and Vonnegut [67] in which neonatal male and female mice were injected intracranially with anti-AFP immunoglobulin, showed an androgenization of the female mouse, suggesting a protective role of AFP. However, these mice also had gross neurological lesions, i.e. external hydrocephaly, as a result of the intracranial injection with anti-AFP thereby making it difficult to interpret the data.

The recent introduction of an AFP-KO mouse [34] has now made it possible to test these opposing hypotheses

and thus to determine the function of AFP in brain sexual differentiation.

4.1. The α -fetoprotein knockout mouse

4.1.1. α -Fetoprotein knockout mice are infertile

Afp knockout mice were generated by replacing an *Afp* genomic fragment extending from exon 1 to intron 3 (AFP-KO1), or extending from exon 2 to intron 3 (AFP-KO2), by a IRES-*LacZ-neo* selection cassette [34]. The AFP-KO1 allele was generated in two different genetic backgrounds, the outbred *CD-1* and the inbred *C57Bl/6j* strain, whereas the AFP-KO2 allele was generated in only the *CD-1* background. Both invalidations gave rise to viable homozygous animals. AFP-KO animals are apparently normal with males being fertile, but females are not, owing to a complete absence of ovulation [34]. AFP-KO ovaries contain follicles at different stages of maturation, including the last Graafian follicle stage, but no corpora lutea, indicative of ovulation, could be detected, which is in accordance with the low levels of progesterone in the serum. Reciprocal ovarian transplantation experiments demonstrated that AFP-KO ovaries are functional: AFP-KO ovaries transplanted in normal mice were able to ovulate and the transplanted females generated pups from the mutated parental oocytes. By contrast, WT ovaries implanted into female AFP-KO mice did not show any ovulation. However, ovulation in AFP-KO females could be induced by injecting gonadotropins indicating that their ovaries are responsive to any signals from the hypothalamus–pituitary. These results thus suggest that the infertility observed in AFP-KO females is not due to any defects in the ovaries, but is most likely caused by a deficit in hypothalamic–pituitary–gonadal (HPG) axis. Indeed, microarray studies [24] showed that several genes implicated in female fertility, such as *Egr1*, *Cish2*, *Ptprf*, *Psa*, and *Tkt*, were down-regulated in the pituitary of AFP-KO females. Furthermore, several genes implicated in the GnRH pathway, such as the GnRH receptor gene, and several genes activated by the GnRH receptor (*cFos*, *Egr2*, *Tgfb li4*, and *Ptp4a1*) are down-regulated in female AFP-KO mice [24]. In the hypothalamus, the gene encoding the hypothalamic GnRH decapeptide is itself down-regulated, suggesting a dysfunction of the GnRH pathway in AFP-KO females.

4.1.2. Brain and behavior of AFP knockout mice are defeminized

In order to determine whether their infertility reflected anomalies in the sexual differentiation of the brain, we first tested female AFP-KO mice for their ability to show lordosis behavior when paired with a sexually active male [6]. Groups of female WT, HET, and AFP-KO mice were ovariectomized in adulthood and subsequently implanted with a silastic capsule containing crystalline estradiol (diluted 1:1 with cholesterol). All female subjects received 500 μ g of progesterone subcutaneously 2–4 h before testing. AFP-KO females never showed any lordosis behavior

in any of the three behavioral tests, whereas WT and HET females showed substantial levels of lordosis behavior (Fig. 8a). Very similar results were obtained in all AFP-KO mouse models generated [6]. These results suggest that AFP-KO females have lost their ability to show lordosis behavior. As discussed previously, olfaction plays a pivotal role in the expression of lordosis behavior in female mice [30,46,47]. The complete absence of lordosis behavior in female AFP-KO mice may be explained by a reduced motivation to investigate male-derived odors. Therefore we investigated odor preferences in AFP-KO mice of the two different background strains [7]. AFP-KO females always preferred to investigate male over female odors when given the choice between these two odor stimuli in a Y-maze, and thus remained very female-like in this regard. The absence of lordosis behavior in these females can thus not be explained by a reduced motivation of AFP-KO females to investigate male-derived odors.

We also determined whether AFP-KO females have an increased capacity to display male-typical sexual behaviors. Normal female mice can show substantial levels of mounting and intromission-like behaviors when ovariectomized in adulthood and treated with high doses of either testosterone or estradiol [8,111] suggesting that this behavior is less sexually differentiated than the ability to show lordosis behavior. Nevertheless, mount frequencies were higher in AFP-KO females compared to WT females and this effect was already visible in HET females ([6]; Fig. 8b). The latter have probably been exposed to increased concentrations of estrogens due to having decreased levels of AFP [34]. The binding affinity of estradiol for AFP is lower than for its own receptor stressing the importance of having excess levels of AFP during fetal development [5]. These results also point to a role of fetal estrogens in the organization of male sexual behavior in the mouse and thus further support the notion of a possible synergistic role of estradiol and androgen receptor activation in masculinization of mating behavior capacity ([9,86,95]; see discussion in previous section).

4.1.3. Reduced female sexual behavior in female *ArKO* mice

This behavioral phenotype in AFP-KO females was associated with neurochemical changes [6]. The sexually dimorphic expression of tyrosine hydroxylase (TH) and vasopressin (VP) were used as indices of brain sexual differentiation. The number of TH-expressing neurons in the anteroventral preoptic area (AVPv) is sexually dimorphic with females having greater numbers than males [91,92,96]. The AVPv plays a critical role in female reproductive function by transducing hormonal feedback controlling hypothalamic GnRH release and consequent pituitary luteinizing hormone secretion, as well as that it is needed for hormonally induced ovulation [115]. By contrast, males show a denser expression of VP particularly in the lateral septum than females [25]. We confirmed the sex differences in the number TH-expressing neurons in the AVPv as well as in VP expression in the lateral septum.

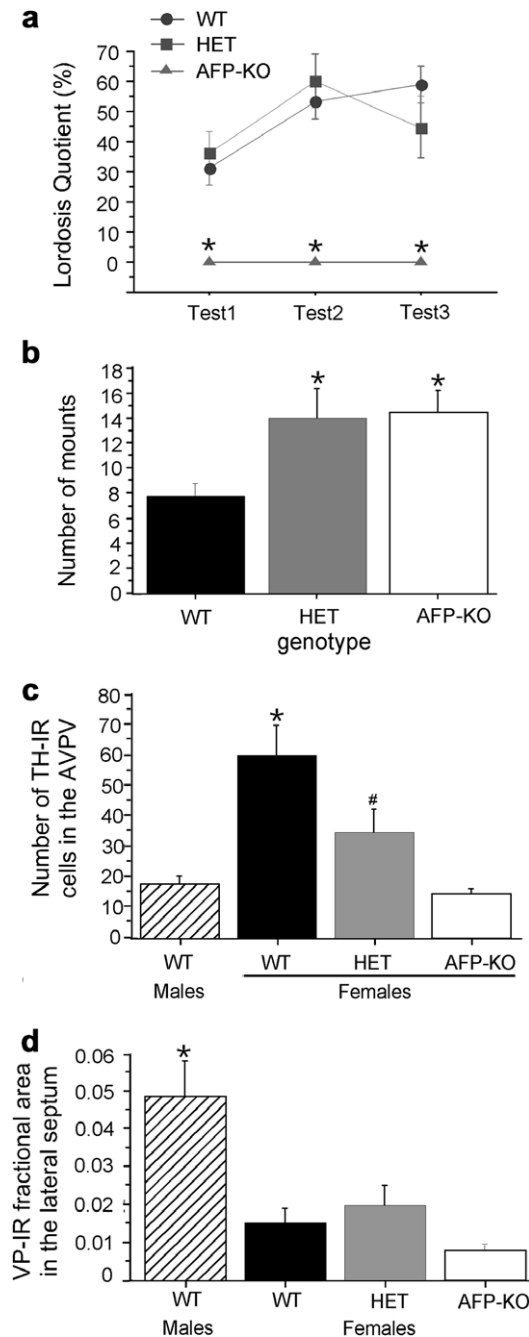


Fig. 8. Behavioral and neurochemical phenotype of female α -fetoprotein knockout (AFP-KO) mice. (a) Lordosis quotients of female wild-type (WT), heterozygous (HET), and AFP-KO mice. All female subjects were ovariectomized in adulthood and subsequently tested with estradiol and progesterone prior to each behavioral test. * $p < 0.05$ compared to WT and HET females. (b) Mounting behavior of female WT, HET, and AFP-KO females when paired with an estrous female. Female subjects were ovariectomized in adulthood and subsequently treated with estradiol. * $p < 0.05$ compared to WT females. (c) Numbers of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the anteroventral preoptic area (AVPV). Female subjects were ovariectomized in adulthood and treated with estradiol. * $p < 0.05$ compared to WT males, and HET and AFP-KO females, # $p < 0.05$ compared to WT males and females and AFP-KO females. (d) Brain vasopressin expression assessed by the fractional areas covered by vasopressin-IR structures in the posterior lateral septum. Female subjects were ovariectomized in adulthood and treated with estradiol. * $p < 0.05$ compared to all female groups.

Female AFP-KO mice had decreased, male-like numbers of tyrosine hydroxylase (TH) neurons in the anteroventral preoptic area (AVPV; Fig. 8c) which may be related to their infertility [24,34]. By contrast, female AFP-KO mice showed female-typical densities of VP-immunoreactive fibers in the lateral septum (Fig. 8d). The latter result was rather surprising in light of earlier observations showing an important role for perinatal estrogens in the sexual differentiation of the vasopressin system in the rat [43]. It is possible, however, that the development of sex differences in the vasopressin system relies more on the perinatal action of androgens than on that of estrogens in the mouse, an idea supported by recent findings in male ArKO mice ([79]; unpublished results). Furthermore, sex chromosomes may also contribute directly to the development of the sexually dimorphic vasopressin system in mice [25]. Indeed, XY males and XY female mice (i.e. females with a deletion of the Sry gene) are more masculine than XX mice with regard to the density of vasopressin-expressing fibers in the lateral septum [25]. So perhaps there is an interaction between estrogens and sex chromosomes in the sexual differentiation of this neuropeptide system.

4.1.4. AFP protects the developing female brain from estrogens

Our observations of a complete absence of female sexual behavior and male-like numbers of TH neurons in the AVPV in female AFP-KO mice did not allow us to discriminate between the two opposing theories about the role of AFP in brain sexual differentiation (Fig. 7). Both hypotheses would predict that male AFP-KO mice would be more or less normal but that the females would be affected. It could be argued that the brains of AFP-KO females were defeminized because they were no longer protected from the estrogens produced by their mother or male siblings. This argument would support the hypothesis that AFP serves primarily to protect the female brain from excessive exposure to estrogens [64]. By contrast, it could also be argued that the brains of AFP-KO females were not feminized because they were lacking the AFP to transport small quantities of estrogens to target brain areas and thus to promote the differentiation of these structures in a female direction. This argument would favor the hypothesis that AFP is needed to transport estrogens into the brain and thus has an active role in female sexual differentiation [99].

To discriminate between these competing hypotheses, we blocked estrogen production during prenatal development by treating pregnant female mice heterozygous for the *Afp* mutation with the aromatase inhibitor ATD [6]. If AFP protects the female brain from being exposed to estrogens, AFP-KO offspring of ATD-treated mothers should not be defeminized, as the ATD treatment would prevent the formation, during prenatal development, of high defeminizing levels of estradiol. However, if AFP actually acts as an estrogen carrier and is thus necessary for the feminizing the female brain, then the AFP-KO offspring of ATD-treated mothers should not show normal

lordosis behavior because of a lack of feminization by estrogens (absence of the steroid and its carrier).

Prenatal treatment with ATD completely rescued lordosis behavior in AFP-KO females (Fig. 9a). Furthermore, such ATD-treated AFP-KO females had female-like numbers of TH neurons in the AVPv (Fig. 9b). Accordingly, the fertility of AFP-KO females was also restored as well as gene expression in the HPG [24]. These results clearly demonstrate that AFP serves to protect the female brain from becoming masculinized and defeminized by estrogens circulating during embryonic development [6].

These findings do not explain, however, why AFP is found inside neurons without being locally produced [88]. Little or no intraneuronal AFP is found in limbic, hypothalamic, and amygdaloid areas, whereas large amounts are present in adjacent regions [98]. This could indicate that AFP protects from estrogens those brain regions involved in reproductive function, such as the hypothalamus, but

may deliver estrogens to other brain regions, and thereby may influence the sexual differentiation of various functions, including non-reproductive behaviors such as learning and memory capacity [63]. However, it should be noted that the mechanisms controlling the differentiation of sex differences in learning and memory capacity remain to be elucidated. Perhaps a closer look at the neurons that contain AFP will suggest a function for this protein in these cells. In addition, it will be interesting to determine whether non-reproductive behaviors have been altered in AFP-KO mice and if so, whether this can be corrected or not by blocking estrogen synthesis during fetal development.

Whether AFP plays a similar protective role in the sexual differentiation of the human brain is unclear. There are diverging views in the literature as to whether human AFP has any estrogen-binding capacities. Nunez et al. [71] reported a low-binding affinity of human serum for steroid hormones during embryonic and early postnatal development. By contrast, both Uriel et al. [101] and Arnon et al. [4] showed that human AFP could actually bind estrogens. In either case, human AFP-derived peptides are able to display some anti-estrogenic activity [14,66,102]. These AFP-derived peptides are currently under investigation as potential agents for treating estrogen-dependent breast cancers and other tumors [13,66]. Finally, it is not known if a protection from maternal estrogens is actually required since the reigning idea is that in humans, androgens, not estrogens, are the primary cause of brain masculinization (reviewed in [107]). Furthermore, primates do not undergo receptive defeminization, i.e. males will accept repeated mounting by other males by displaying the posture normally associated with female receptivity. As a result, primates may not need a protective role of AFP against a defeminizing action of fetal estradiol exposure [10].

5. Conclusions and future directions

The results obtained in AFP-KO mice show that the principal action of prenatal estrogen exposure, regardless of whether it occurs in female or male mice, is to defeminize and, to some extent, masculinize brain and behavior. Furthermore, AFP, which binds estradiol circulating in the female fetus with affinity and capacity, protects the developing brain from a male-typical organization by this steroid. So at first glance, these findings are at odds with the results [8] obtained in female ArKO mice implying an active contribution of estrogens to the development of the female brain. However, such a dual role of estradiol in brain sexual differentiation was earlier suggested by Dohler et al. [26]. Based on their results, they proposed the “progressive hypothesis of brain sexual differentiation” which asserts that (i) the embryonic brain is not differentiated in either males or females, and (ii) under the influence of moderate levels of estrogen, female-typical neural and behavioral traits develop whereas under the influence of high levels of estrogen, male-typical neural and behavioral

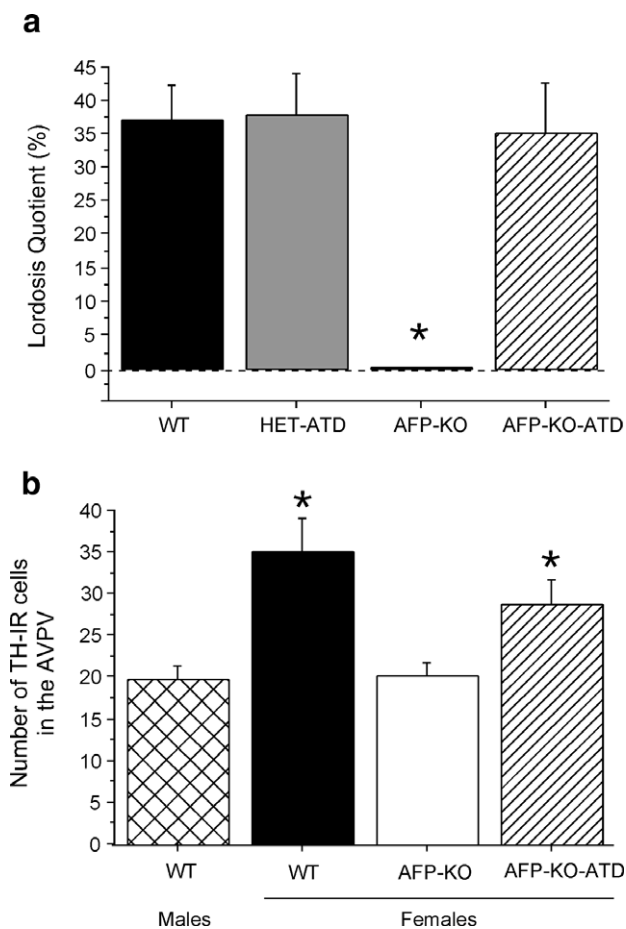


Fig. 9. Female phenotype of AFP-KO females is rescued by prenatal treatment with the aromatase inhibitor ATD. (a) Lordosis quotients of wild-type (WT), AFP-KO, and heterozygous (HET-ATD) and AFP-KO females treated prenatally with ATD (AFP-KO-ATD). All female subjects were ovariectomized in adulthood and treated subsequently with estradiol and progesterone. * $p < 0.05$ compared to WT, HET-ATD, and AFP-KO-ATD females. (b) Numbers of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the anteroventral preoptic region (AVPv). Female subjects were ovariectomized in adulthood and treated with estradiol. * $p < 0.05$ compared to WT males and AFP-KO females.

traits develop. However, we now know that any prenatal estrogen action is blocked by AFP in the developing female, thus if estrogens normally contributes to the development of the female brain, they most likely act postnatally when the amount of AFP has decreased substantially and AFP no longer plays a protective role. AFP levels are the highest at birth, after which they decrease by about 50% during the first 24 h. Only trace levels (about 0.01% of fetal levels) are detected at 3 weeks of age in rats [83]. Thus presumably when the ovaries start to secrete estrogens at day 7 after birth [53], AFP no longer plays a significant role. Consistent with a postnatal role of estradiol in feminization of the brain is the observation by Steward and Cygan [93] of an enhanced of female receptivity in neonatally gonadectomized male and female rats treated with estradiol over postnatal days 30–40. We propose thus that the defeminizing action of estradiol normally occurs prenatally in males and is avoided in fetal females because of the protective actions of AFP. We further propose that the feminizing action of estradiol normally occurs in genetic females between birth and the age of puberty (postnatal days 40–50). Accordingly, several studies in which estrogen exposure was manipulated in both sexes during the perinatal developmental period have suggested that different critical periods exist for male- and female-typical organization of the brain [80,82]. Thus, prenatal exposure to estrogens induces the male-typical pattern of PR expression in the MPN and VMN, whereas postnatal exposure to estrogens leads to the female pattern of PR expression in these brain regions. Future studies should take advantage of the ArKO mouse model to determine the contribution of postnatal estradiol to the development of female sexual behavior. If normal levels of lordosis behavior can be induced in female ArKO mice by treating them with estradiol postnatally, then these results would provide the best evidence for a normal role of postnatal estradiol in promoting female-typical brain and behavioral sexual differentiation.

Acknowledgments

This work was supported by NICHD Grant No. HD044897 to M.J.B. and J.B., two grants from the Fonds National de la Recherche Scientifique (No. 1.5.082.04 and 1.5.104.06), and one grant from the University of Liège (No. C-06/89), to J.B. J. Bakker is a research associate from the Fonds National de la Recherche Scientifique.

References

- [1] G.I. Abelev, S.D. Perova, N.I. Khramkova, Z.A. Postnikova, I.S. Irlin, Production of embryonal alpha-globulin by transplantable mouse hepatomas, *Transplantation* 1 (1963) 174–180.
- [2] M. Ali, K. Mujoo, M.K. Sahib, Synthesis and secretion of alpha-fetoprotein and albumin by newborn rat brain cells in culture, *Brain Res.* 282 (1982) 47–55.
- [3] G.K. Andrews, M. Dziadek, T. Tamaoki, Expression and methylation of the mouse alpha-fetoprotein gene in embryonic, adult, and neoplastic tissues, *J. Biol. Chem.* 257 (1982) 5148–5153.
- [4] R. Arnon, E. Teicher, M. Bustin, M. Sela, Preparation of antisera to alpha-fetoprotein making use of estradiol affinity column, *FEBS Lett.* 32 (1973) 335–338.
- [5] C. Aussel, R. Masseyeff, Rat alpha-fetoprotein-estrogen interaction, *J. Steroid Biochem.* 9 (1978) 547–551.
- [6] J. Bakker, C. De Mees, Q. Douhard, J. Balthazart, P. Gabant, J. Szpirer, C. Szpirer, Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens, *Nat. Neurosci.* 9 (2006) 220–226.
- [7] J. Bakker, C. De Mees, J. Szpirer, C. Szpirer, J. Balthazart, Exposure to oestrogen prenatally does not interfere with the normal female-typical development of odour preferences, *J. Neuroendocrinol.* 19 (2007) 329–334.
- [8] J. Bakker, S. Honda, N. Harada, J. Balthazart, The aromatase knock-out mouse provides new evidence that estradiol is required during development in the female for the expression of sociosexual behaviors in adulthood, *J. Neurosci.* 22 (2002) 9104–9112.
- [9] J. Bakker, S. Honda, N. Harada, J. Balthazart, Restoration of male sexual behavior by adult exogenous estrogens in male aromatase knockout mice, *Horm. Behav.* 46 (2004) 1–10.
- [10] M.J. Baum, Differentiation of coital behavior in mammals: a comparative analysis, *Neurosci. Biobehav. Rev.* 3 (1979) 265–284.
- [11] M.J. Baum, E.B. Keverne, Sex difference in attraction thresholds for volatile odors from male and estrous female mouse urine, *Horm. Behav.* 41 (2002) 213–219.
- [12] M.J. Baum, S.A. Tobet, Effect of prenatal exposure to aromatase inhibitor, testosterone, or antiandrogen on the development of feminine sexual behavior in ferrets of both sexes, *Physiol. Behav.* 37 (1986) 111–118.
- [13] J.A. Bennett, L. DeFrest, I. Anaka, H. Saadati, S. Balulad, H.I. Jacobson, T.T. Andersen, AFPep: an anti-breast cancer peptide that is orally active, *Breast Cancer Res. Treat.* 98 (2006) 133–141.
- [14] J.A. Bennett, F.B. Mesfin, T.T. Andersen, J.F. Gierthy, H.I. Jacobson, A peptide derived from alpha-fetoprotein prevents the growth of estrogen-dependent human breast cancers sensitive and resistant to tamoxifen, *Proc. Natl. Acad. Sci. USA* 99 (2002) 2211–2215.
- [15] C.G. Bergstrand, B. Czar, Demonstration of a new protein fraction in serum from the human fetus, *Scand. J. Clin. Lab. Invest.* 8 (1956) 174.
- [16] D. Blizard, C. Deneff, Neonatal androgen effects on open-field activity and sexual behavior in the female rat: the modifying influence of ovarian secretions during development, *Physiol. Behav.* 11 (1973) 65–69.
- [17] C. Bodo, E.F. Rissman, Androgen receptor is essential for sexual differentiation of responses to olfactory cues in mice, *Eur. J. Neurosci.* 25 (2007) 2182–2190.
- [18] J.E. Booth, Sexual differentiation of the brain, in: C.A. Finn (Ed.), *Oxford Reviews of Reproductive*, vol. I, Clarendon Press, Oxford, 1979, pp. 58–158, Ref Type: Serial (Book, Monograph).
- [19] P. Brownbill, D. Edwards, C. Jones, D. Mahendran, D. Owen, C. Sibley, R. Johnson, P. Swanson, D.M. Nelson, Mechanisms of alphafetoprotein transfer in the perfused human placental cotyledon from uncomplicated pregnancy, *J. Clin. Invest.* 96 (1995) 2220–2226.
- [20] A.S. Clark, J.K. Pfeifle, D.A. Edwards, Ventromedial hypothalamic damage and sexual proceptivity in female rats, *Physiol. Behav.* 27 (1981) 597–602.
- [21] M.A. Crawford, A.J. Sinclair, Nutritional influences in the evolution of the mammalian brain, in: *CIBA Foundation Symposium*, Churchill London, Malnutrition and the Developing Brain, 1972, pp. 267–287.
- [22] H.S. Cuckle, N.J. Wald, R.H. Lindenbaum, Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome, *Lancet* 1 (1984) 926–929.
- [23] D. Daniels, R.R. Miselis, L.M. Flanagan-Cato, Central neuronal circuit innervating the lordosis-producing muscles defined by transneuronal transport of pseudorabies virus, *J. Neurosci.* 19 (1999) 2823–2833.

- [24] C. De Mees, J.F. Laes, J. Bakker, J. Smits, B. Hennuy, P. Van Vooren, P. Gabant, J. Szpirer, C. Szpirer, Alpha-fetoprotein controls female fertility and prenatal development of the gonadotropin-releasing hormone pathway through an antiestrogenic action, *Mol. Cell. Biol.* 26 (2006) 2012–2018.
- [25] G.J. De Vries, E.F. Rissman, R.B. Simerly, L.Y. Yang, E.M. Scordalakes, C.J. Auger, A. Swain, R. Lovell-Badge, P.S. Burgoyne, A.P. Arnold, A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits, *J. Neurosci.* 22 (2002) 9005–9014.
- [26] K.D. Dohler, J.L. Hancke, S.S. Srivastava, C. Hofmann, J.E. Shryne, R.A. Gorski, Participation of estrogens in female sexual differentiation of the brain; neuroanatomical, neuroendocrine and behavioral evidence, in: G.J. De Vries, J.P.C. De Bruin, H.B.M. Uylings, M.A. Corner (Eds.), *Progress in Brain Research*, Sex differences in the brain, the relation between structure and function, 61, Elsevier, Amsterdam, 1984, pp. 99–117, Ref Type: Serial (Book, Monograph).
- [27] G. Dorner, Sexual differentiation of the brain, *Vitam. Horm.* 38 (1981) 325–381, Ref Type: Serial (Book, Monograph).
- [28] K.M. Dorries, E. Adkins-Regan, B.P. Halpern, Olfactory sensitivity to the pheromone, androstenone, is sexually dimorphic in the pig, *Physiol. Behav.* 57 (1995) 255–259.
- [29] J.L. Dunlap, A.A. Gerall, L.D. McLean, Enhancement of female receptivity in neonatally castrated males by prepubertal ovarian transplants, *Physiol. Behav.* 10 (1973) 701–705.
- [30] D.A. Edwards, K.G. Burge, Olfactory control of the sexual behavior of male and female mice, *Physiol. Behav.* 11 (1973) 867–872.
- [31] H.H. Feder, R.E. Whalen, Feminine behavior in neonatally castrated and estrogen-treated male rats, *Science* 147 (1965) 306–307.
- [32] S. Firestein, How the olfactory system makes sense of scents, *Nature* 413 (2001) 211–218.
- [33] C.R. Fisher, K.H. Graves, A.F. Parlow, E.R. Simpson, Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene, *Proc. Natl. Acad. Sci. USA* 95 (1998) 6965–6970.
- [34] P. Gabant, L. Forrester, J. Nichols, T. Van Reeth, C. De Mees, B. Pajack, A. Watt, J. Smits, H. Alexandre, C. Szpirer, J. Szpirer, Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility, *Proc. Natl. Acad. Sci. USA* 99 (2002) 12865–12870.
- [35] A.A. Gerall, J.L. Dunlap, S.E. Hendricks, Effect of ovarian secretions on female behavioral potentiality in the rat, *J. Comp. Physiol. Psychol.* 82 (1973) 449–465.
- [36] G. Gibori, R. Sridaran, Sites of androgen and estradiol production in the second half of pregnancy in the rat, *Biol. Reprod.* 24 (1981) 249–256.
- [37] J.R. Gillespie, V.N. Uversky, Structure and function of alpha-fetoprotein: a biophysical overview, *Biochim. Biophys. Acta* 1480 (2000) 41–56.
- [38] R.A. Gorski, C.D. Jacobson, Sexual differentiation of the brain, in: S.E. Kogan, E.S.E. Hafez (Eds.), *Pediatric Andrology*, Martinus Nijhoff, The Hague, The Netherlands, 1981, pp. 109–134, Ref Type: Serial (Book, Monograph).
- [39] R.W. Goy, B.S. McEwen, *Sexual Differentiation of the Brain*, Massachusetts Institute of Technology Press, Cambridge MA, USA, 1980, Ref Type: Serial (Book, Monograph).
- [40] K.L. Grady, C.H. Phoenix, W.C. Young, Role of the developing rat testis in differentiation of the neural tissues mediating mating behavior, *J. Comp. Physiol. Psychol.* 59 (1965) 176–182.
- [41] T.L. Greco, A.H. Payne, Ontogeny of expression of the genes for steroidogenic enzymes P450 side-chain cleavage, 3 beta-hydroxysteroid dehydrogenase, P450 17 alpha-hydroxylase/C17-20 lyase, and P450 aromatase in fetal mouse gonads, *Endocrinology* 135 (1994) 262–268.
- [42] J.E. Haddow, J.N. Macri, M. Munson, The amnion regulates movement of fetally derived alpha-fetoprotein into maternal blood, *J. Lab. Clin. Med.* 94 (1979) 344–347.
- [43] T.M. Han, G.J. De Vries, Organizational effects of testosterone, estradiol, and dihydrotestosterone on vasopressin mRNA expression in the bed nucleus of the stria terminalis, *J. Neurobiol.* 54 (2003) 502–510.
- [44] S. Honda, N. Harada, S. Ito, Y. Takagi, S. Maeda, Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp19 gene, *Biochem. Biophys. Res. Commun.* 252 (1998) 445–449.
- [45] A. Jost, Sur le contrôle hormonal de différenciation sexuelle du lapin, *Arch. Anat. Microscop. Morphol. Exp.* 39 (1950) 577–598.
- [46] M. Keller, Q. Douhard, M.J. Baum, J. Bakker, Destruction of the main olfactory epithelium reduces female sexual behavior and olfactory investigation in female mice, *Chem. Senses* 31 (2006) 315–323.
- [47] M. Keller, S. Pierman, Q. Douhard, M.J. Baum, J. Bakker, The vomeronasal organ is required for the expression of lordosis behaviour, but not sex discrimination in female mice, *Eur. J. Neurosci.* 23 (2006) 521–530.
- [48] E.B. Keverne, The vomeronasal organ, *Science* 286 (1999) 716–720.
- [49] H. Kimoto, S. Haga, K. Sato, K. Touhara, Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons, *Nature* 437 (2005) 898–901.
- [50] P. Koopman, A. Munsterberg, B. Capel, N. Vivian, R. Lovell-Badge, Expression of a candidate sex-determining gene during mouse testis differentiation, *Nature* 348 (1990) 450–452.
- [51] A.E. Kudwa, W.C. Boon, E.R. Simpson, R.J. Handa, E.F. Rissman, Dietary phytoestrogens dampen female sexual behavior in mice with a disrupted aromatase enzyme gene, *Behav. Neurosci.* 121 (2007) 356–361.
- [52] G.G. Kuiper, J.G. Lemmen, B. Carlsson, J.C. Corton, S.H. Safe, P.T. van der Saag, B.B. van der, J.A. Gustafsson, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, *Endocrinology* 139 (1998) 4252–4263.
- [53] S.A. Lamprecht, F. Kohen, J. Ausher, U. Zor, H.R. Lindner, Hormonal stimulation of oestradiol-17 beta release from the rat ovary during early postnatal development, *J. Endocrinol.* 68 (1976) 343–344.
- [54] P.C. Leighton, M.J. Kitau, T. Chard, Y.B. Gordon, A.E. Leek, Levels of alpha-fetoprotein in maternal blood as a screening test for fetal neural-tube defect, *Lancet* 2 (1975) 1012–1015.
- [55] E.D. Lephart, T.W. West, K.S. Weber, R.W. Rhees, K.D. Setchell, H. Adlercreutz, T.D. Lund, Neurobehavioral effects of dietary soy phytoestrogens, *Neurotoxicol. Teratol.* 24 (2002) 5–16.
- [56] R.D. Lisk, Progesterone: biphasic effects on the lordosis response in adult or neonatally gonadectomized rats, *Neuroendocrinology* 5 (1969) 149–160.
- [57] A. Mackiewicz, J. Breborowicz, The in vitro production of alpha-fetoprotein variants by human fetal organs, *Oncodev. Biol. Med.* 1 (1980) 251–261.
- [58] N.J. MacLusky, F. Naftolin, Sexual differentiation of the central nervous system, *Science* 211 (1981) 1294–1302.
- [59] C.W. Malsbury, L.M. Kow, D.W. Pfaff, Effects of medial hypothalamic lesions on the lordosis response and other behaviors in female golden hamsters, *Physiol. Behav.* 19 (1977) 223–237.
- [60] D. Mathews, D.A. Edwards, The ventromedial nucleus of the hypothalamus and the hormonal arousal of sexual behaviors in the female rat, *Horm. Behav.* 8 (1977) 40–51.
- [61] G.A. Mathews, E.A. Brenowitz, A.P. Arnold, Paradoxical hypermasculinization of the zebra finch song system by an antiestrogen, *Horm. Behav.* 22 (1988) 540–551.
- [62] A.L. McCall, S.J. Han, W.R. Millington, M.J. Baum, Non-saturable transport of [³H]oestradiol across the blood-brain barrier in female rats is reduced by neonatal serum, *J. Reprod. Fertil.* 61 (1981) 103–108.
- [63] M.M. McCarthy, A.T. Konkle, When is a sex difference not a sex difference? *Front. Neuroendocrinol.* 26 (2005) 85–102.
- [64] B.S. McEwen, L. Plapinger, C. Chaptal, J. Gerlach, G. Wallach, Role of fetoneonatal estrogen binding proteins in the associations of

- estrogen with neonatal brain cell nuclear receptors, *Brain Res.* 96 (1975) 400–406.
- [65] H.M. Meijs-Roelofs, J.T. Uilenbroek, F.H. de Jong, R. Welschen, Plasma oestradiol-17beta and its relationship to serum follicle-stimulating hormone in immature female rats, *J. Endocrinol.* 59 (1973) 295–304.
- [66] G.J. Mizejewski, M. Muehlemann, M. Dauphinee, Update of alpha fetoprotein growth-inhibitory peptides as biotherapeutic agents for tumor growth and metastasis, *Chemotherapy* 52 (2006) 83–90.
- [67] G.J. Mizejewski, M. Vonnegut, Characteristics of the androgenization produced in mice by neonatal exposure to alpha-fetoprotein antibodies, *Teratology* 25 (1982) 351–360.
- [68] F. Naftolin, K.J. Ryan, I.J. Davies, V.V. Reddy, F. Flores, Z. Petro, M. Kuhn, R.J. White, Y. Takaoka, L. Wolin, The formation of estrogens by central neuroendocrine tissues, *Recent Prog. Horm. Res.* 31 (1975) 295–319.
- [69] S.C. Nagel, F.S. vom Saal, W.V. Welshons, Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum, *J. Steroid Biochem. Mol. Biol.* 69 (1999) 343–357.
- [70] D. Newby, G. Dalgliesh, F. Lyall, D.A. Aitken, Alphafetoprotein and alphafetoprotein receptor expression in the normal human placenta at term, *Placenta* 26 (2005) 190–200.
- [71] E.A. Nunez, C. Benassayag, L. Savu, G. Vallette, M.F. Jayle, Serum binding of some steroid hormones during development in different animal species, discussion of the biological significance of this binding, *Ann. Biol. Anim. Biochem. Biophys.* 16 (1976) 491–501.
- [72] D.W. Pfaff, Y. Sakuma, Deficit in the lordosis reflex of female rats caused by lesions in the ventromedial nucleus of the hypothalamus, *J. Physiol.* 288 (1979) 203–210.
- [73] D.W. Pfaff, S. Schwartz-Giblin, M.M. McCarthy, L.-M. Kow, Cellular and molecular mechanisms of female reproductive behaviors, in: E. Knobil, J. Neill (Eds.), *The Physiology of Reproduction*, vol. 2, Raven Press, New York, 1994, pp. 107–220, Ref Type: Serial (Book, Monograph).
- [74] C.A. Pfeiffer, Sexual differences in the hypophyses and their determination by the gonads, *Am. J. Anat.* 58 (1936) 195–226.
- [75] C.H. Phoenix, R.W. Goy, A.A. Gerall, W.C. Young, Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig, *Endocrinology* 65 (1959) 369–382.
- [76] S. Pierman, Q. Douhard, J. Balthazart, M.J. Baum, J. Bakker, Attraction thresholds and sex discrimination of urinary odors in male and female aromatase knockout (ArKO) mice, *Horm. Behav.* 49 (2006) 96–104.
- [77] A. Pineiro, A.M. Olivito, J. Uriel, Fixation of polyunsaturated fatty acids by alpha fetoprotein and serum albumin in rats. Comparison with the accumulation of these acids in developing rat brain, *C. R. Seances Acad. Sci. D* 289 (1979) 1053–1056.
- [78] L. Plapinger, B.S. McEwen, Ontogeny of estradiol-binding sites in rat brain. I. Appearance of presumptive adult receptors in cytosol and nuclei, *Endocrinology* 93 (1973) 1119–1128.
- [79] L. Plumari, C. Viglietti-Panzica, F. Allieri, S. Honda, N. Harada, P. Absil, J. Balthazart, G.C. Panzica, Changes in the arginine-vasopressin immunoreactive systems in male mice lacking a functional aromatase gene, *J. Neuroendocrinol.* 14 (2002) 971–978.
- [80] P.S. Quadros, A.Y. Goldstein, G.J. De Vries, C.K. Wagner, Regulation of sex differences in progesterone receptor expression in the medial preoptic nucleus of postnatal rats, *J. Neuroendocrinol.* 14 (2002) 761–767.
- [81] P.S. Quadros, V. Lopez, G.J. De Vries, W.C. Chung, C.K. Wagner, Progesterone receptors and the sexual differentiation of the medial preoptic nucleus, *J. Neurobiol.* 51 (2002) 24–32.
- [82] P.S. Quadros, J.L. Pfau, A.Y. Goldstein, G.J. De Vries, C.K. Wagner, Sex differences in progesterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation, *Endocrinology* 143 (2002) 3727–3739.
- [83] J.P. Raynaud, Influence of rat estradiol binding plasma protein (EBP) on uterotrophic activity, *Steroids* 21 (1973) 249–258.
- [84] B.S. Rubin, R.J. Barfield, Priming of estrous responsiveness by implants of 17 beta-estradiol in the ventromedial hypothalamic nucleus of female rats, *Endocrinology* 106 (1980) 504–509.
- [85] B.S. Rubin, J.R. Lenkowski, C.M. Schaeberle, L.N. Vandenberg, P.M. Ronsheim, A.M. Soto, Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A, *Endocrinology* 147 (2006) 3681–3691.
- [86] T. Sato, T. Matsumoto, H. Kawano, T. Watanabe, Y. Uematsu, K. Sekine, T. Fukuda, K. Aihara, A. Krust, T. Yamada, Y. Nakamichi, Y. Yamamoto, T. Nakamura, K. Yoshimura, T. Yoshizawa, D. Metzger, P. Chambon, S. Kato, Brain masculinization requires androgen receptor function, *Proc. Natl. Acad. Sci. USA* 101 (2004) 1673–1678.
- [87] L. Savu, C. Benassayag, G. Vallette, N. Christeff, E. Nunez, Mouse alpha 1-fetoprotein and albumin. A comparison of their binding properties with estrogen and fatty acid ligands, *J. Biol. Chem.* 256 (1981) 9414–9418.
- [88] B.S. Schachter, C.D. Toran-Allerand, Intraneuronal alpha-fetoprotein and albumin are not synthesized locally in developing brain, *Brain Res.* 281 (1982) 93–98.
- [89] M.L. Schaefer, D.A. Young, D. Restrepo, Olfactory fingerprints for major histocompatibility complex-determined body odors, *J. Neurosci.* 21 (2001) 2481–2487.
- [90] S. Sell, F.F. Becker, Alpha-fetoprotein, *J. Natl. Cancer Inst.* 60 (1978) 19–26.
- [91] R.B. Simerly, L.W. Swanson, R.J. Handa, R.A. Gorski, Influence of perinatal androgen on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral periventricular nucleus of the rat, *Neuroendocrinology* 40 (1985) 501–510.
- [92] R.B. Simerly, M.C. Zee, J.W. Pendleton, D.B. Lubahn, K.S. Korach, Estrogen receptor-dependent sexual differentiation of dopaminergic neurons in the preoptic region of the mouse, *Proc. Natl. Acad. Sci. USA* 94 (1997) 14077–14082.
- [93] J. Steward, D. Cygan, Ovarian hormones act early in development to feminize adult open-field behavior in the rat, *Horm. Behav.* 14 (1980) 20–32.
- [94] S.M. Tilghman, A. Belayew, Transcriptional control of the murine albumin/alpha-fetoprotein locus during development, *Proc. Natl. Acad. Sci. USA* 79 (1982) 5254–5257.
- [95] S.A. Tobet, M.J. Baum, Role for prenatal estrogen in the development of masculine sexual behavior in the male ferret, *Horm. Behav.* 21 (1987) 419–429.
- [96] S.A. Tobet, I.K. Hanna, Ontogeny of sex differences in the mammalian hypothalamus and preoptic area, *Cell. Mol. Neurobiol.* 17 (1997) 565–601.
- [97] K. Toda, K. Takeda, T. Okada, S. Akira, T. Saibara, T. Kaname, K. Yamamura, S. Onishi, Y. Shizuta, Targeted disruption of the aromatase P450 gene (Cyp19) in mice and their ovarian and uterine responses to 17beta-oestradiol, *J. Endocrinol.* 170 (2001) 99–111.
- [98] C.D. Toran-Allerand, Regional differences in intraneuronal localization of alpha-fetoprotein in developing mouse brain, *Brain Res.* 281 (1982) 213–217.
- [99] C.D. Toran-Allerand, On the genesis of sexual differentiation of the central nervous system: morphogenetic consequences of steroidal exposure and possible role of alpha-fetoprotein, in: G.J. De Vries, J.P.C. De Bruin, H.B.M. Uylings, M.A. Corner (Eds.), *Progress in Brain Research, Sex differences in the brain, the relation between structure and function*, 61, Elsevier, Amsterdam, 1984, pp. 63–98, Ref Type: Serial (Book, Monograph).
- [100] C.D. Toran-Allerand, Neuronal uptake of alpha-fetoprotein (AFP) synthesized and secreted by hepatocytes in liver/brain co-cultures, *Neurosci. Lett.* 83 (1987) 35–40.
- [101] J. Uriel, B. de Nechaud, M. Dupiers, Estrogen-binding properties of rat, mouse and man fetospecific serum proteins. Demonstration by

- immuno-autoradiographic methods, *Biochem. Biophys. Res. Commun.* 46 (1972) 1175–1180.
- [102] D. Vakharia, G.J. Mizejewski, Human alpha-fetoprotein peptides bind estrogen receptor and estradiol, and suppress breast cancer, *Breast Cancer Res. Treat.* 63 (2000) 41–52.
- [103] G. Vallette, C. Benassayag, L. Savu, J. Delorme, E.A. Nunez, J. Doumas, G. Maume, B.F. Maume, The serum competitor of oestrogen-rat alpha 1-foetoprotein interactions. Identification as a mixture of non-esterified fatty acids, *Biochem. J.* 187 (1980) 851–856.
- [104] F.S. vom Saal, W.M. Grant, C.W. McMullen, K.S. Laves, High fetal estrogen concentrations: correlation with increased adult sexual activity and decreased aggression in male mice, *Science* 220 (1983) 1306–1309.
- [105] C.K. Wagner, J.L. Pfau, G.J. De Vries, I.J. Merchenthaler, Sex differences in progesterone receptor immunoreactivity in neonatal mouse brain depend on estrogen receptor alpha expression, *J. Neurobiol.* 47 (2001) 176–182.
- [106] C.K. Wagner, J. Xu, J.L. Pfau, P.S. Quadros, G.J. De Vries, A.P. Arnold, Neonatal mice possessing an Sry transgene show a masculinized pattern of progesterone receptor expression in the brain independent of sex chromosome status, *Endocrinology* 145 (2004) 1046–1049.
- [107] K. Wallen, Hormonal influences on sexually differentiated behavior in nonhuman primates, *Front. Neuroendocrinol.* 26 (2005) 7–26.
- [108] J.P. Weniger, Estrogen production by fetal rat gonads, *J. Steroid Biochem. Mol. Biol.* 44 (1993) 459–462.
- [109] J.P. Weniger, A. Zeis, J. Chouraqui, Estrogen production by fetal and infantile rat ovaries, *Reprod. Nutr. Dev.* 33 (1993) 129–136.
- [110] S.R. Wersinger, D.J. Haisenleder, D.B. Lubahn, E.F. Rissman, Steroid feedback on gonadotropin release and pituitary gonadotropin subunit mRNA in mice lacking a functional estrogen receptor alpha, *Endocrine* 11 (1999) 137–143.
- [111] S.R. Wersinger, K. Sannen, C. Villalba, D.B. Lubahn, E.F. Rissman, G.J. De Vries, Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene, *Horm. Behav.* 32 (1997) 176–183.
- [112] D.W. Wesson, M. Keller, Q. Douhard, M.J. Baum, J. Bakker, Enhanced urinary odor discrimination in female aromatase knockout (ArKO) mice, *Horm. Behav.* 49 (2006) 580–586.
- [113] R.E. Whalen, D.A. Edwards, Hormonal determinants of the development of masculine and feminine behavior in male and female rats, *Anat. Rec.* 157 (1967) 173–180.
- [114] P.L. Whitten, H.B. Patisaul, L.J. Young, Neurobehavioral actions of coumestrol and related isoflavonoids in rodents, *Neurotoxicol. Teratol.* 24 (2002) 47–54.
- [115] S.J. Wiegand, E. Terasawa, Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat, *Neuroendocrinology* 34 (1982) 395–404.
- [116] J.A. Witcher, L.G. Clemens, A prenatal source for defeminization of female rats is the maternal ovary, *Horm. Behav.* 21 (1987) 36–43.