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The α-fetoprotein knock-out mouse model suggests that parental behavior is sexually differentiated under the influence of prenatal estradiol

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ABSTRACT

In rodent species, sexual differentiation of the brain for many reproductive processes depends largely on estradiol. This was recently confirmed again by using the α-fetoprotein knockout (AFP-KO) mouse model, which lacks the protective actions of α-fetoprotein against maternal estradiol and as a result represents a good model to determine the contribution of prenatal estradiol to the sexual differentiation of the brain and behavior. Female AFP-KO mice were defeminized and masculinized with regard to their neuroendocrine responses as well as sexual behavior. Since parental behavior is also strongly sexually differentiated in mice, we used the AFP-KO mouse model here to ask whether parental responses are differentiated prenatally under the influence of estradiol. It was found that AFP-KO females showed longer latencies to retrieve pups to the nest and also exhibited lower levels of crouching over the pups in the nest in comparison to WT females. In fact, they resembled males (WT and AFP-KO). Other measures of maternal behavior, for example the incidence of infanticide, tended to be higher in AFP-KO females than in WT females but this increase failed to reach statistical significance. The deficits observed in parental behavior of AFP-KO females could not be explained by any changes in olfactory function, novelty recognition or anxiety. Thus our results suggest that prenatal estradiol defeminizes the parental brain in mice.

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 (see Bakker and Baum, 2008; McCarthy, 2008 for recent reviews). The recent introduction of the α-fetoprotein knockout (AFP-KO) mouse (Gabant et al, 2002), which lacks the neuroprotective effects of α-fetoprotein against maternal estrogens during the prenatal period, has provided a new model in which to study the role of estradiol in the development of the brain and behavior. Thus it has been shown that the principal action of prenatal estrogen exposure is to defeminize and, to some extent, masculinize the brain and behavior (Bakker and Baum, 2008; Bakker et al., 2006).

The role of prenatal estrogens in the sexual differentiation of reproductive behavior, using AFP-KO mice, has been extensively explored in the context of sexual behavior (Bakker and Baum, 2008; Bakker et al., 2006), but at present other reproductive behaviors that are also essential to ensure successful reproduction, namely parental behavior, have not been analyzed in this mouse model. Most mammalian species exhibit strong sex differences in their parental behavior towards offspring. For example, in adulthood, laboratory mice display pronounced sex differences in their responses towards pups so that almost all virgin females are spontaneously maternal (Gandelman, 1972; Gandelman et al., 1970, 1971a,b; Lonstein and de Vries, 2000b; Noirot, 1964; Rosenson, 1975; Svare, 1979) whereas males are usually infanticidal (Beilharz, 1975; Gandelman, 1972, 1973b; Lonstein and de Vries, 2000b; Perrigo et al., 1989; Rosenson, 1975; Svare and Mann, 1981; Svare et al., 1977; vom Saal and Howard, 1982).

In mice, parental responses are most likely sexually differentiated before puberty as sex differences in parental behavior are not under the control of circulating gonadal hormones. For example, the increase in infanticide observed in peripubertal male mice does not appear to be due to the sudden rise in circulating testosterone at puberty

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(Gandelman, 1973a,b; Svare et al., 1978). Furthermore the change in parental responsiveness that occurs when females age is not governed by gonadal activity because ovariectomy just before weaning has no effect on later parental responsiveness (Gandelman, 1973b). Finally, in many strains of mice, removal of circulating pituitary and gonadal hormones does not affect maternal responsiveness of adult virgin females (Davis and Gandelman, 1972, Gandelman, 1973a,b; Lonstein and de Vries, 2000b). By contrast, exposure to gonadal hormones during the perinatal period affects later responses of virgin adult mice toward pups; for example, female mice exposed to testosterone neonatally are often infanticidal (Gandelman, 1973a,b). These data demonstrate that the expression of parental responses in adult mice are not under the influence of circulating hormones in adulthood but are most likely affected by the organizational effects of hormones during development.

Therefore, in the present study, we determined whether prenatal exposure to estradiol affects parental behavior in adulthood by characterizing the parental behavior of male and female, parentally naïve, gonadectomized AFP-KO and WT mice. Furthermore, to ensure that any potential deficit in parental behavior was not related to changes in other behaviors, all mice were also tested for their olfactory function using olfactory habituation/dishabituation tests, their state of anxiety using the elevated plus maze test and their novel object recognition.

Material and methods

Animals

Heterozygous male and female mice of the CD1 (also known as Swiss–Webster) strain were bred to generate wild-type (WT) and homozygous-null AFP-KO offspring. Mice were genotyped by PCR analysis of tail DNA (for more detailed description, see Bakker et al., 2006). Groups were as follows: WT females ($n=7$), AFP-KO females ($n=8$), WT males ($n=10$), and AFP-KO males ($n=8$). All animals were 10 weeks of age at the beginning of the experiment. None of the animals had previously been exposed to pups (except to their siblings during development). Prior to testing animals were individually housed in a normal light–dark cycle (12L/12D, lights on at 08:00 h) and allowed access to food and tap water ad libitum. For behavioral testing animals were tested once on each test in the order described below; usually tests were performed at 48 h intervals. All experiments were conducted in accordance with the guidelines of the National Institutes of Health and were approved by the Ethical Committee for Animal Use at the University of Liège.

It should be noted that homozygous AFP-KO females are sterile and do not show any sexual behavior (Bakker et al., 2006) and as a consequence do not undergo pregnancy and parturition. Thus, it is not possible to test maternal responses in these animals during the post-partum period. However, as mentioned in the Introduction, circulating hormones in adult mice do not exert a major control on parental responses as adult mice can exhibit parental responsiveness without any hormonal priming (Lévy and Keller, 2009; Lévy et al., 2004; Lonstein and de Vries, 2000b; Weber and Olsson, 2008). Therefore all aspects of parental behavior can be observed in virgin animals when exposed to pups. Obviously, lactation and suckling cannot be observed however, since virgin females do not produce any colostrum and milk.

Gonadectomy

To avoid any possible confounding effects of differences in levels of circulating gonadal steroids between the sexes or genotypes on the expression of parental behaviors, all animals were gonadectomized 3 weeks prior to testing. All animals were gonadectomized under general anesthesia induced by an intraperitoneal injection of a mixture of ketamine (80 mg/kg per mouse) and medetomidine (Domitor, Pfizer, 1 mg/kg per mouse). Mice received atipamezole (antisedan, Pfizer, 4 mg/kg per mouse) subcutaneously at the end of the surgery to antagonize medetomidine-induced effects, thus accelerating their recovery.

Observation and quantification of parental behavior

Parental behavior was assessed during a 30 min test based on the classical pup retrieval experiment previously described (Rosenblatt, 1967; Brown et al., 1996). Briefly, parentally naive virgin mice were housed individually and provided with cotton for nesting 1 day prior to pup exposure. As nesting material would have obscured direct observation of parental behavior, most of the nesting material was removed on the day of testing so that observers were able to quantify crouching and licking behavior when the animals were in the nest with the pups. At the beginning of the test, three foster pups (between 3 and 5 days of age) were placed in the home cage. One pup was placed in each of the three corners that did not contain the nest. According to the literature (Svare and Broida, 1982; Svare et al., 1984; vom Saal, 1985) neither the relatedness (e.g. mouse strain) nor sex or age (1 to 5 days) of the pups has a significant influence on a female's or male's initial tendency to show retrieval or infanticide. Therefore we used Balb/c mouse pups which were more abundantly available at the animal facility. The latency to retrieve each pup, the number of pups retrieved, the relative time spent crouching in the nest over the pups, the time spent self-grooming and the time spent off the nest exploring the cage were determined over 30 min. Retrieval was defined as the animal picking up a pup by the mouth and transporting it to the nest. If the animal picked up and dropped the same pup more than once on the way to the nest, the retrieval was not scored until the pup was in the nest. If all three pups were not retrieved by an animal at the time infanticidal behavior occurred the test was immediately stopped and a maximal latency (30 min) was attributed. Animals always retrieved the pups in the nest, thus suggesting that the previous removal of some part of the nest material did not disturb the animals. Crouching was defined as the animal arching and assuming the nursing posture over all three pups in the nest. Finally, animals that started to bite pups, producing repeated series of rough-handling calls, during the test were scored "infanticidal". In that case, the test was stopped immediately for ethical considerations (Elwood, 1991) and pups were either placed back with their mother or, if injured, killed immediately by cervical dislocation. Since some animals were infanticidal, different test durations were obtained between animals. Therefore, all measures are expressed as behavioral frequencies (% of time engaged in one activity over the duration of the observation). In addition, it should be noted that as the relative time spent crouching over the pups and the time spent licking them were determined (and only occurred) once the pups were retrieved into the nest, these percentages were also based on different test durations since differences in the latency to retrieve pups in the nest were observed between groups. Thus if pups were retrieved quickly (as was the case in WT females), the subject had more time to display crouching than if the retrieval was slow.

Olfactory habituation–dishabituation test

Since disruption of olfactory function leads to a disruption of maternal behavior and even to infanticide in virgin female mice (Lévy et al., 2004; Lévy and Keller, 2009), we assessed olfactory discrimination abilities of all animals through habituation/dishabituation tests, using volatile urinary odors derived from male and estrous female mice as olfactory stimuli. Animals were tested in their home cage as described previously (Pierman et al, 2006; Keller et al., 2006a, b). Briefly, the steel cage top containing food and water was removed and replaced with a clean top. Odor stimuli were presented by applying 10 µl of urine onto a piece of filter paper glued to a plastic

weighing boat (4.3×4.3 cm), which was then placed in the food hopper so that volatile odors from the stimulus were available at body level. Subjects were unable to make physical contact with the filter paper using either their snout or paws. Each test consisted of a sequence of three water presentations followed by three odor presentations of two different urinary odors, namely intact male and estrous female urine. The duration of investigation of the odor stimuli was recorded and any significant increase of olfactory investigation (dishabituation) when being exposed to the odorant stimulus was considered as the subject detecting the odor.

Elevated plus maze

Given that pups represent a potentially neophobic stimulation in the home-cage environment, we also determined whether the potential behavioral differences observed during the interactions with the pups might be due to any differences in subjects' state of anxiety. Therefore, animals were tested for differences in anxiety-like behavior using the elevated plus maze. Tests were conducted as previously described (Dalla et al, 2005; Keller et al., 2006a,b). Briefly, the elevated plus maze consisted of four arms (each arm 30 cm long×15 cm high×8 cm wide), two open and two closed arms formed a cross, which were raised 80 cm above the floor. At the beginning of the test, each mouse was placed in the center, and subsequently the time spent in the center, open, and closed arms was recorded for 5 min. Animals were tested individually in random order under normal white light. Between tests, the maze was cleaned with 70% ethanol to eliminate any odors.

Novel object recognition test

Given that pups represent a novel stimulus in the home-cage environment, animals were subject to a novel object recognition test to determine whether there were differences in novel object exploration. This was done as previously described (Bruel-Jungerman et al., 2005; Ennaceur and Meliani, 1992). Briefly, two identical novel objects 2–3 cm in height and 1–2 cm in diameter were introduced into the mouse home cage. Objects were placed equidistance apart, in opposite corners of the cage and were secured to the floor with tape. Behavior was observed for 5 min after the objects were introduced and, 15 min later, for another 5 min, immediately after one of the now familiar objects was replaced with a distinct novel object of similar size. The identity of the objects used on the first and second observations was counterbalanced. Exploration was recorded when the animal's nose was within 1 cm of an object and was oriented towards that object. Objects were cleaned thoroughly with ethanol between trials to ensure the absence of olfactory cues. Mice that failed to reach the criteria of a minimum total object exploration time of 20 s for each presentation were excluded from the statistical analysis (two WT males and one WT female removed). Measurement of the time spent exploring the novel object was expressed as a percentage of the exploration time of the novel object related to the total exploration time for both objects (preference index: time investigating the novel object/total time of investigation×100).

Statistics

Latencies to retrieve pups and behavior expressed toward pups were analyzed using non-parametrical statistics given the fact that some of these data did not follow a normal distribution. Between groups comparisons were performed using Kruskal–Wallis tests and these tests were followed by Mann–Whitney *U* post-hoc tests if overall significant differences were observed (Siegel, 1956). All other tests (habituation/dishabituation, elevated plus maze and novel object recognition) were analyzed using analysis of variance (ANOVA). When appropriate, all ANOVAs were followed by Fisher PLSD post hoc comparisons. Only significant ($p<0.05$) effects detected by the ANOVAs are mentioned in detail in the Results.

Results

Parental behavior

Maternal behavior was clearly affected in AFP-KO females as many of their behaviors expressed toward pups resembled those expressed by males (both AFP-KO and WT); by contrast WT females were immediately maternal, retrieving their pups rapidly to the nest (<2.5 min) and in general showing significantly higher levels of care toward pups.

Latencies to retrieve the pups were higher in AFP-KO females and males than in WT females (Table 1; for the clarity of the results only the latencies to retrieve the first and the last (third) pups are shown): Kruskal–Wallis analysis revealed between group differences for both the first ($H_{(3,\ N=33)}=11.60$; $p=0.0089$) and the last pup ($H_{(3,\ N=33)}=10.99$; $p=0.0118$). Mann–Whitney post-hoc tests revealed differences between WT females and both groups of males for the latency to retrieve the first pup (WT F vs. WT M: $U=8.50$, $z=-2.586$, $p=0.0096$; WT F vs. KO M: $U=0.00$, $z=-3.246$, $p=0.0003$) and differences between WT females and all other groups to retrieve the third pup (WT F vs. KO F: $U=10.00$, $z=-2.083$, $p=0.0372$; WT F vs. WT M: $U=8.00$, $z=-2.637$, $p=0.0068$; WT F vs. KO M: $U=3.00$, $z=-2.898$, $p=0.0028$). In addition, WT females retrieved more pups in comparison to AFP-KO female mice and males (both WT and AFP-KO), but differences did not reach statistical significance.

When analyzing parental behavior performed once pups were retrieved into the nest, AFP-KO females like WT and AFP-KO males spent significantly less time crouching over the pups in a "nursing" posture in comparison to WT females. This was confirmed by a Kruskal–Wallis analysis ($H_{(3,\ N=33)}=14.265$; $p=0.0026$) and Mann–Whitney *U* comparisons (Fig. 1; WT F vs. KO F: $U=1.00$, $z=3.124$, $p=0.0018$; WT F vs. WT M: $U=4.00$, $z=3.025$, $p=0.0025$; WT F vs. KO M: $U=1.00$, $z=3.124$, $p=0.0018$). In addition, AFP-KO females appeared to spend less time licking the pups in comparison to WT females (Fig. 1), however this effect did not reach statistical significance.

When analyzing any parental behaviors which are not pup-directed, such as time spent out of the nest and self-grooming, AFP-KO females still differed from WT females and appeared to be very similar to males (Fig. 1). Thus, with regard to the relative time spent out of the nest, a Kruskal–Wallis analysis revealed between group differences ($H_{(3,\ N=33)}=14.681$; $p=0.0021$). Mann–Whitney *U* post-hoc tests revealed that AFP-KO females and both male groups spent more time out of the nest in comparison to WT females (WT F vs. AFP-KO F: $U=2.50$, $z=-2.951$, $p=0.0031$; WT F vs. WT M: $U=2.00$, $z=-3.220$, $p=0.0013$; WT F vs. AFP-KO M: $U=0.00$, $z=-3.124$, $p=0.0012$). Regarding the time spent self-grooming, again between group differences were found ($H_{(3,\ N=33)}=8.362$; $p=0.0039$), in particularly between WT females and both male groups (WT F vs. WT M: $U=10.50$, $z=-2.391$, $p=0.0168$; WT F vs. AFP-KO M: $U=7.50$, $z=-2.372$, $p=0.0139$) which spent significantly more time self-grooming than females.

Table 1
Latencies (min ± SEM) to retrieve the first and the last (third) pup and mean number of pups retrieved. *$p<0.05$ compared to WT females.

Groups	Latency to retrieve 1st pup	Latency to retrieve 3rd pup	Number of pups retrieved
WT females	0.56 ± 0.18	2.19 ± 0.52	3.00 ± 0
AFP-KO females	7.37 ± 4.33	9.32 ± 4.04*	2.62 ± 0.37
WT males	5.67 ± 2.92*	14.38 ± 3.97*	2.50 ± 0.31
AFP-KO males	6.39 ± 3.41*	12.15 ± 4.06*	2.50 ± 0.38

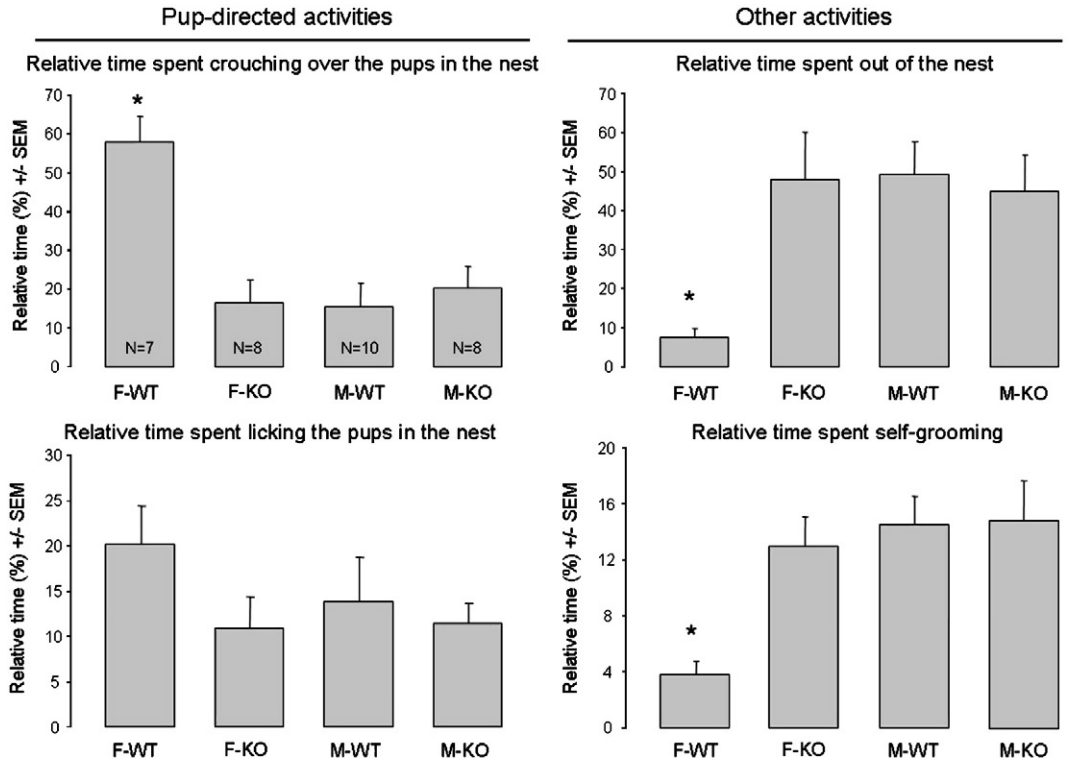

Fig. 1. Relative time spent by male (M) and female (F) mice of both WT and AFP-KO genotypes in activities directed (time spent crouching over pups and licking) or not (time spent out of the nest or self-grooming) towards pups. *$p<0.05$ compared to F-KO, M-WT, and M-KO.

Finally, analysis of infanticidal behavior revealed that 3 out of 8 (37.5%) AFP-KO females attacked pups during testing compared to 0/7 (0%) WT females, 1/10 (10%) WT males and 1/8 (12.5%) AFP-KO males (Fig. 2).

Other behaviors

The deficits in maternal care observed in AFP-KO females were not due to any disruption in their ability to detect and discriminate between odors since olfactory habituation/dishabituation tests showed normal responses towards urinary odor cues in AFP-KO females (Fig. 3). Indeed, a repeated measures ANOVA with sex and genotype as independent factors and trial as a repeated factor (the dependent measure being the time spent sniffing the odors) revealed a significant effect of the different trials ($F_{(8,232)}=35.21$; $p<0.001$). Therefore, subsequent repeated measures ANOVAs were conducted with sex and genotype as independent factors and the last presentation of water versus the first presentation of male urine or the last presentation of male urine versus the first presentation of female urine as repeated factors (the dependent measure being again the time spent sniffing the urinary odors). These analyses revealed that all animals showed a clear and significant dishabituation response to the first presentation of male urine versus the last presentation of water ($F_{(1,29)}=91.23$; $p<0.001$). Similar results were obtained when comparing the first presentation of female urine versus the last presentation of male urine ($F_{(1,29)}=77.92$; $p<0.001$). In addition, a significant effect of sex ($F_{(1,29)}=13.82$; $p<0.001$) and a significant sex×trial interaction ($F_{(1,29)}=22.13$; $p<0.001$) was observed with males (both WT and AFP-KO) investigating female urine for a longer period of time than females (both WT and AFP-KO; Fig. 3).

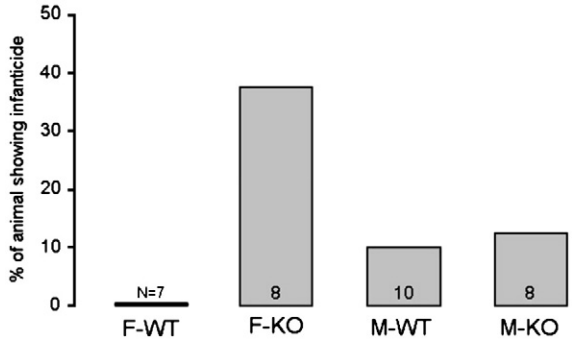

Fig. 2. Proportion of animals attacking pups in each group during the 30 min observation test.

Likewise, the deficits in maternal care of AFP-KO females were not due to a higher state of anxiety in these females since no differences were observed in the elevated plus maze between mice according to genotype or sex (Fig. 4). Indeed, ANOVA with sex and genotype as independent factors and the time spent investigating each arm of the maze (open or closed) as repeated factor revealed only a main effect of the time spent investigating the arms of the maze. In fact, all groups spent significantly more time in the closed arms of the maze than in the open arms ($F_{(1,29)}=16.16$; $p<0.001$); no significant interactions were detected.

Finally, there were no significant differences between groups in object recognition and exploration (Fig. 5). ANOVA with sex and genotype as independent factors and time spent investigating the object as a dependent factor revealed no differences between groups with all groups investigating the novel object nearly twice as much as the familiar object (between 62% and 69% of the total time of investigation was dedicated to the novel object in the various groups).

Discussion

The present study is consistent with previous results showing that the principal action of prenatal estradiol is to defeminize and masculinize the brain and behavior (Bakker et al., 2006) and now

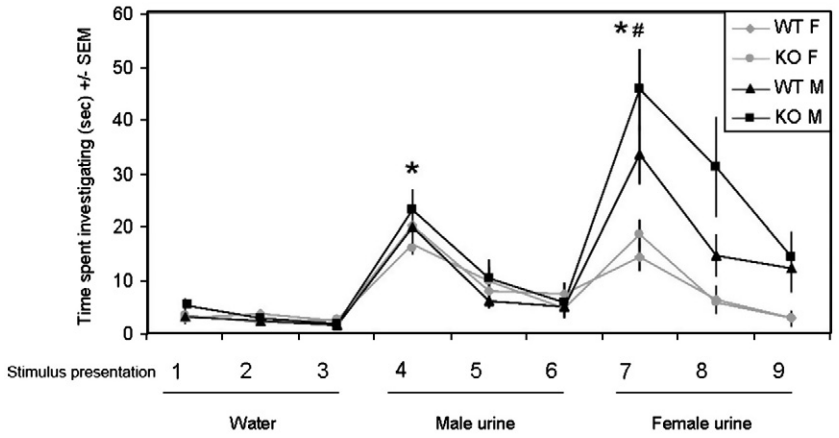

Fig. 3. Mean (±SEM) time spent by AFP-KO and WT mice of both sexes investigating deionized water or volatile urinary stimuli. *$p<0.05$ post-hoc comparisons between the time spent investigating the third presentation of a particular stimulus and the first presentation of the next stimulus. #$p<0.05$ post-hoc comparisons between sexes.

extends this conclusion to another behavior that is important for reproductive success, i.e. parental care. Indeed, our data demonstrate that AFP-KO females show very little maternal behavior and actually do not differ from male mice, whereas WT females show substantial levels of maternal behavior, spending a long time crouching over the pups. These findings suggest that parental behavior (like sexual behavior) differentiates sexually under the influence of prenatal estradiol. This interpretation should however be taken with some caution since not all behaviors were affected in AFP-KO females. For example, no statistical significant differences were observed in the number of pups retrieved into the nest or in the percentage of time spent licking the pups.

Few studies have examined the influence of prenatal gonadal hormones in generating sex differences in adult parental behavior. In addition to the present results obtained in mice, adult female rats prenatally exposed to testosterone show masculinized (i.e. reduced) parental responsiveness (Ichikawa and Fujii, 1982; Juarez et al., 1998). Prenatally testosterone-treated female rats showed less time crouching over pups in adulthood in comparison with vehicle-treated females in tests similar to those used in the present study (Juarez et al., 1998). Such masculinizing effects of prenatal testosterone have also been observed in prairie voles (Lonstein and de Vries, 2000a). Interestingly, postnatal treatment with testosterone has little or no effect on parental behavior in rats or prairie voles (Bridges et al., 1973; Leboucher, 1989; Lonstein and de Vries, 2000a; Quadagno et al., 1973, 1974; Rosenberg et al., 1971), suggesting that a sensitive period may exist for the differentiation of parental behavior during the prenatal period. Whether the masculinizing effect of prenatal testosterone is mediated through its androgenic or estrogenic (via aromatization) metabolites has not been explored, but it has been shown that exposing female mice in utero (between days 14 and 18 of gestation) to low doses of the estrogenic chemical bisphenol-A induces a reduction in maternal behavior in female offspring (Palanza et al., 2002). This latter finding supports our data and suggests that parental behavior is differentiated by an estrogenic rather than an androgenic mechanism during the prenatal period.

In the future, the prenatal role of estradiol in the differentiation of the parental brain should be further confirmed by blocking estrogen production during prenatal development by treating AFP-KO females in utero with an aromatase inhibitor such as 1,4,6-androstatriene-3,17-dione (ATD). This treatment has been previously performed in the context of sexual behavior and resulted in a complete reversal of the behavioral phenotype of AFP-KO females. Indeed, once in adulthood, ATD treated AFP-KO females showed levels of lordosis behavior similar to those observed in WT females (Bakker et al., 2006) and were fertile. Future work will thus aim to investigate the effect of prenatal ATD treatment on parental behavior.

In addition to their lack of maternal behavior, female mice perinatally exposed to testosterone are often infanticidal in adulthood when primed with testosterone (Gandelman, 1972). Interestingly, AFP-KO females showed the highest percentage of infanticide behavior in the present study suggesting that prenatal estradiol is important for parental behavior. However it should be noted that the level of infanticide was generally low in the present study and this

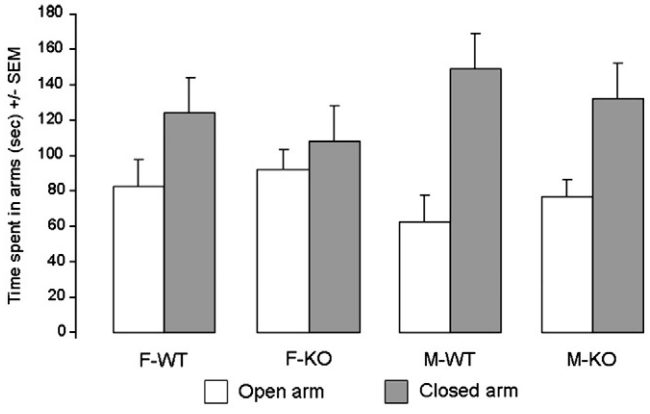

Fig. 4. Mean (±SEM) total time spent in the open and closed arms in the elevated plus maze test. ANOVA with sex and genotype as independent factors and the time spent investigating the arms of the maze as repeated factors indicated a general effect of time spent investigating each arm (open/closed) but no interactions between factors.

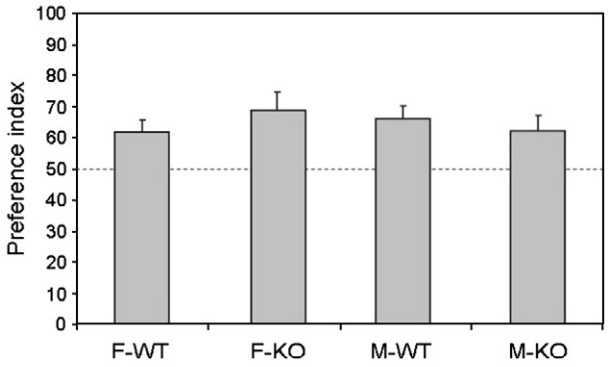

Fig. 5. Novel object recognition test. Time spent investigating the novel object is expressed as a preference index (time investigating the novel object/total time of investigation).

may have been due to gonadectomy or limited exposure to pups. Indeed, infanticide in males is greatly influenced by circulating gonadal hormones (Svare et al., 1977) since males that are castrated during adulthood showed lower levels of infanticide than gonadally intact males (Gandelman and vom Saal, 1975). In females, an increase in infanticide can be induced by a treatment of estradiol and dihydrotestosterone but this increase is reversed after the hormonal treatment is terminated (Davis and Gandelman, 1972; Gandelman, 1972; Gandelman and vom Saal, 1975; Svare, 1979).

The changes observed in parental behavior in AFP-KO females appear to be specific to this behavior as they cannot be explained by a deficit in olfactory perception, novelty detection or anxiety. Using habituation/dishabituation tests of olfactory discrimination, we confirmed that AFP-KO and WT mice of both sexes were able to discriminate between male and estrous female urine. Rather remarkably, AFP-KO females remained very female-like in their odor preference: they resembled WT females, i.e. spent less time investigating female urine compared to male mice (WT and AFP-KO). The present study is in line with earlier results obtained in our lab of normal male-directed odor preference in AFP-KO females (Bakker et al., 2007). These results suggest thus that not all aspects of reproductively relevant behaviors are defeminized and masculinized in AFP-KO females. Our observation of normal olfactory abilities in AFP-KO mice confirm our results obtained in aromatase knockout (ArKO) mice (Pierman et al., 2006) suggesting that estradiol is not directly involved in the development of olfactory discrimination abilities. Most importantly, these findings suggest that the differences observed in parental behavior are not the consequence of any olfactory deficit. This is important because parental responsiveness in mice is under the control of sensory cues; disruption of olfactory cues leads to a disruption in maternal behavior and even to infanticide in virgin female mice (Gandelman et al., 1971a,b; Lévy et al., 2004; Lévy and Keller, 2009). For example, anosmia produced by nasal irrigation of zinc sulfate or depletion of noradrenaline within the main olfactory bulb results in the majority of the females killing their offspring (Dickinson and Keverne, 1988; Seegal and Denenberg, 1974).

In addition, olfaction may be involved in discriminating pups from other conspecifics and can be readily related to sex differences in parental or infanticidal behaviors. Olfactory cues from pups usually stimulate various brain structures across the main and accessory olfactory systems that are sexually dimorphic (Baum, 2009; Insel et al., 1991; Swann and Fiber, 1997) and involved in the expression of parental behavior (Fleming and Rosenblatt, 1974a,b; Fleming et al., 1980; Segovia and Guillamon, 1993). For example one olfactory structure, namely the medial nucleus of the amygdala, appears to be larger in male than female rats (Hines et al., 1992) and this larger size seems to be correlated with the greater reluctance of males to act parentally. Interestingly some sex differences in the function and characteristics of more central regions of the olfactory systems (e.g., amygdala, medial preoptic area), are under the control of perinatal estradiol (Keller et al., 2009; Pierman et al., 2008; Wesson et al., 2006). Therefore, it would be interesting to analyze the effects of prenatal estradiol exposure on the processing of olfactory information related to the expression of maternal behavior.

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