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Aggressive Behaviors in Adult SF-1 Knockout Mice That Are Not Exposed to Gonadal Steroids During Development

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Abstract

Sex hormones are a major factor responsible for the development of sex differences. Steroidogenic factor 1 (SF-1) is a key regulator of gonadal and adrenal development, and SF-1 knockout mice (SF-1 KO) are born without gonads and adrenal glands. Consequently, these mice are not exposed to gonadal sex steroids. SF-1 KO pups die shortly after birth due to adrenal deficiency. In the present study, SF-1 KO mice were rescued by neonatal corticosteroid injections followed by adrenal transplantations on day 7–8 postnatally. Control mice received corticosteroid injections and were gonadectomized prior to puberty. Mice were observed interacting with ovariectomized hormone primed females and gonad-intact males. In the absence of sex steroid replacement, adult SF-1 KO mice were significantly more aggressive than control mice in tests with stimulus females. After testosterone treatment, control males displayed significantly more aggression towards male intruders than control female mice, or male and female SF-1 KO mice, suggesting a developmental role of gonadal hormones in the expression of aggressive behavior and affirming SF-1 KO mice as a behavioral model to investigate affects of fetal gonad deficiency.

Keywords

sexual differentiation; VMH; steroidogenic factor 1; sex steroids; aggression

Steroidogenic factor 1 (SF-1), officially designated NR5A, was discovered as a regulator of the cytochrome P450 steroidogenic enzymes (Lala, Rice, & Parker, 1992; Morohashi et al., 1994). SF-1 gene expression is restricted to few locations (i.e., gonads, adrenals, pituitary, and the ventromedial nucleus of the hypothalamus [VMH]; Parker et al., 2002). It is an essential factor for gonadal and adrenal development as SF-1 knockout (KO) mice are born without gonads and adrenal glands and have male to female sex reversal (Ingraham et al., 1994; Luo, Ikeda, & Parker, 1994). The structure of the VMH (Dellovade et al., 2000; Ikeda, Luo, Abbud, Nilson, & Parker, 1995; Shinoda et al., 1995) and gene expression in pituitary gonadotropes

is also markedly altered in SF-1 KO mice (Zhao et al., 2001). Adrenal transplantation rescues these mice so that they can survive to adulthood, and transplanted adrenal glands appear to function normally as corticosterone levels are indistinguishable between adult WT and KO mice (Majdic et al., 2002). With highly selective alterations in VMH organization and lack of developmental exposure to gonadal steroid hormones, SF-1 KO mice provide a potentially important tool for delineating the roles of gonadal hormones and the VMH in a variety of sex-dependent aspects of physiology and behavior.

In the context of behaviors, male mice castrated on the day of birth display less intermale aggressive behavior than males castrated at PND 10 when treated with androgens in adult life, suggesting that the first 10 days of postnatal development are critical for the development of the capacity for intermale aggression (Edwards, 1971; Motelica-Heino, Edwards, & Roffi, 1993). Thus, gonadal steroids influence aggressive behavior and neonatal exposure to testosterone is necessary for normal expression of intermale aggression in mice (Bakker et al., 2006; Gatewood et al., 2006; MacLusky & Naftolin, 1981).

The VMH may be involved in defensive responses as well as in reproductive and social agonistic behaviors (Canteras, 2002). In rats, VMH lesions increased the spontaneous and shock induced aggression, but decreased dominance in food competition tests (Colpaert, 1975; Grossman, 1972). C-fos expression in male rats showed strong and specific neuronal activation in cells of the VMH after aggressive encounters, but not after male sex behavior tests (Veening & Coolen, 1998), suggesting that the VMH may be primarily involved in the expression of defensive behavior and not in spontaneous aggression. Given the lack of gonadal steroid hormone exposure during development and known alterations in the cytoarchitecture of the VMH, three possible outcomes for behavioral phenotypes of SF-1 KO mice raised to adulthood were predicted. First, if sex differences exist in WT mice and not SF-1 KO mice, these differences are likely dependent on developmental gonadal steroid exposure. Second, if SF-1 KO mice exhibit similar sex differences to gonadectomized wild-type (WT) mice, these differences are likely developmental-gonad independent. Finally, if SF-1 KO mice of both sexes behave differently than WT mice, then these differences may be due to alterations in the VMH. Therefore, aggressive and social behaviors were tested in the absence of adult hormonal stimulation to determine if genotypic differences might either be hormone-independent or due to alterations in the VMH. This was followed by examination of aggressive behaviors after hormone stimulation in adulthood to ask if a testosterone-activated sex-dependent behavior was retained or absent in animals that lacked exposure to endogenous gonadal steroids throughout development. (Bakker et al., 2006; Gatewood et al., 2006; MacLusky & Naftolin, 1981).

Materials and Method

Animals

All experiments were done according to the EU Directive 86/609/EEC (1986) and Guide for the Care and Use of Laboratory Animals (1996) guidelines and were approved by Veterinary Commission of Slovenia. Mice with the disrupted SF-1 allele were backcrossed for more than 10 generations to C57BL/6J mice to produce a congenic line. All mice were housed under standard laboratory conditions at constant temperature (21 °C) in 13:11 light/dark cycle (lights off at 1600) with food (Altromin mouse chow, 4% fat, Lage, Germany) and water ad libitum and wood chips for bedding.

Mice that were heterozygous for the disrupted SF-1 allele were mated to produce homozygous SF-1 KO and WT offspring. The general scheme for animal treatments is presented in Figure 1. All newborn pups were injected daily for 6 to 7 days with 50 µl of a corticosteroid cocktail in corn oil (s.c. 0.4 mg/ml hydrocortisone, 400 ng/ml dexamethasone, and 500 ng/ml

fludrocortisone acetate; all from Sigma, Steinheim, Germany). Mice were genotyped by PCR assay of tail DNA on PND 6 or 7 as previously described (Luo et al., 1994). Thus all pups, including control mice, received daily corticosterone treatment until genotyping was completed on PND 7 and 8. Female WT littermates or female pups from other C57BL/6J litters born within 3 days were used as a source of adrenal transplants. After donor sacrifice, the adrenal glands were excised and washed in cold 0.01M PBS. A small transdermal puncture was made in the subaxillary region of SF-1 KO pups using a 20g needle and two adrenal glands were placed subcutaneously (Majdic et al., 2002). SF-1 KO pups were injected on the day of surgery (PND 7 or 8) and thereafter on PND 9, 12, and 16 with corticosteroid cocktail and after that received no additional treatment. After weaning at 21 days, SF-1 KO mice were housed individually in polycarbonate cages (35 × 15 × 13 cm) until behavioral tests. Ovariectomized (OVX) and castrated (CAS) C57BL/6J mice were used as controls and housed under the same conditions as SF-1 KO mice. Control mice were gonadectomized prior to puberty between PND 21 and 25. For each test, 4 WT mice and 6 or 7 SF-1 KO mice of both sexes were observed.

For stimulus animals, 10 OVX females and 8 gonadal intact, sexually experienced males (C57BL/6J) were used in total. Stimulus females had been mated once prior to ovariectomy, and received estradiol benzoate (EB; Sigma; 1 µg/0.15 ml s.c.) 48 hr prior to testing followed by progesterone (P; Sigma; 1.6 mg/0.2 ml s.c.) approximately 4 to 9 hr prior to testing. The doses were tested before the study to confirm the ability to induce lordosis behavior. Stimulus female mice were group and stimulus males were individually housed throughout the testing period.

Surgery and Hormone Administration

For surgery, the anesthesia was Ketamine (25 mg/ml; Vetoquinol Biowet, Gorzowie, Poland), Xylazine (2.5 mg/ml; Chanelle Pharmaceuticals Ltd., Loughrea, Ireland) and Acepromazine (0.5 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA), given 5 µl/g body weight i.m. In control WT mice, surgery was performed before puberty, between PND 21 and 25. Testes removal was made after bilateral incisions, while females were ovariectomized through a single medial incision. For two days after surgery, control WT mice were housed in pairs and then housed individually.

For hormone treatments, testosterone propionate (TP; Sigma), EB or P were dissolved in ethanol and mixed with corn oil, previously filtered through a 0.2 µm nylon membrane. For examining behavior under the influence of TP, we tested SF-1 KO and WT control mice that received daily TP injections (100 µg/0.05 ml s.c.) for 7 days prior to testing and throughout the testing period (Halem, Baum, & Cherry, 2001).

Behavioral Tests

Behavioral testing began when animals were between 10 and 14 weeks of age. All behavioral tests were performed in a clear glass aquarium (26 × 42 cm) containing wood chips bedding. A standard cage top with a water bottle and food in the hopper was used. Subjects were placed in the test boxes for 4 to 6 hr to habituate before a stimulus mouse was added. Tests were conducted during the first 6 hr of the dark period of the LD cycle under red light illumination. Each test lasted for 45 min, with the exception of tests that included excess aggression. Tests ended earlier if any mouse was considered to be unreasonably stressed.

Tests were divided into two sets (see Figure 2). In the first set of tests SF-1 KO and control mice were tested without hormone treatment and in the second set the same subjects were treated with TP. In each set subjects were tested 8 times; 4 times with stimulus females and 4 times with stimulus males. Typically on each test day the subject interacted with both a male and a female stimulus mouse, with at least a 30 min break in between; the order of testing with

male or female stimulus mice was random. Over the course of testing, subjects were introduced to different stimulus females and males each time. Each animal received no more than two tests per day and was tested over a period of 2 to 4 weeks (depending on treatment). Each mouse was therefore tested 16 times altogether in two sets of tests. Repeated tests were performed on each mouse to maximize their use and take advantage of the power of repeated measures analysis of variance (ANOVA). All SF-1 KO and WT animals were tested in the same order (first without hormones followed by tests with TP treated mice) so if test order had an influence on behavior it should have been equivalent over the four groups of subjects.

For each test, behaviors of both the test subject and the stimulus mice were scored. Initiation of a particular type of behavior was scored as one event, and total number of events during the 45-min test was recorded (yielding data on latency and frequency for each behavior). All behaviors were scored during the test by the same observer (NG). Two general types of behaviors were recorded; aggressive and chemo-investigatory. Aggressive data included: frequencies and latencies of bites and attacks and investigatory behaviors included numbers of contacts and anogenital sniffs.

Statistics

Frequency and latency data were analyzed with NCSS 2001 software (Version 2005, Number cruncher statistical systems, Kaysville, Utah), repeated measures ANOVA; genotype and sex were the independent factors and test trial was the within-subjects repeated measures factor. Latency data were normalized by calculating log 10 for each value prior to conducting statistical analyses. Differences between groups were examined with Fisher's LSD multiple-comparison tests that we selected due to the relatively small number of animals examined. Because six tests with stimulus males and two with stimulus females with TP treated mice ended early (due to excessive aggression), in all cases only the data from the first 15 min of the tests were used. All statistical analyses were conducted using repeated measures ANOVA. However, given the absence of statistically significant interaction involving the trials variable, for presentation purposes the data in figures and tables are presented as $M \pm SEM$, calculated from all individual tests taken together.

Results

Aggression Is Displayed by SF-1 KO Mice Without Sex Steroid Hormone Treatment

In the first set of tests, prior to hormone replacement, SF-1 KO mice of both sexes displayed striking spontaneous aggression directed toward receptive stimulus females. SF-1 KO mice displayed 4 to 10-fold more bites (Figure 3a), $F(1, 87) = 8.16, p < .05$, and attacks (Figure 3b), $F(1, 87) = 6.63, p < .01$ toward females than did WT mice. Also latencies to first attack (18.7 ± 2.6 min. in SF-1 KO female, 19.1 ± 2.6 min. in SF-1 KO male, 43 ± 1.5 min. in WT female, 33.5 ± 3.2 min. WT male) and bite (24.1 ± 2.6 min. in SF-1 KO female, 23.7 ± 2.7 min. in SF-1 KO male, 43.8 ± 1.3 min. in WT female, 35.3 ± 3.0 min. in WT male) were shorter in KO mice compared to WT mice, $F(1, 87) = 10.32$ for attack and 8.87 for bite, $p < .01$. However, sex differences in aggressive behavior were not observed. When SF-1 KO mice were tested with stimulus male mice, they also displayed more attacks than WT mice (14.3 ± 5.5 in KO male, 17.3 ± 4.8 in KO female, 8.0 ± 5.1 in WT male and 0.6 ± 0.1 in WT female mice), although the difference was not significant. The behavior of the intruder stimulus male or female mice did not differ among the four groups of tested mice. No aggressive behavior was displayed by stimulus mice of either sex, and the number of anogenital sniffs and contacts initiated by stimulus mice was similar in tests with mice from all four experimental groups (data not shown).

TP Treatment Induced a Sex Difference in Aggression in WT Mice, but Not SF-1 KO Mice

Treatment with TP induced intermale aggressive behavior in WT male control mice (see Figure 4). When the stimulus animal was a gonad-intact male, interactions between genotype and sex of the test animal were highly significant for attacks, $F(1, 79) = 17.63, p < .001$, and bites, $F(1, 79) = 17.85, p < .001$. WT male mice showed 30-fold more bites than WT female mice or KO mice of either sex. However, when the stimulus animals were receptive females, aggressive behavior was not observed in any of the four groups of tested mice.

As aggression in SF-1 KO mice was greatly diminished after TP treatment, we paid particular attention to whether this could be due to increased socialization of tested mice caused by repeated testing. For measures of aggression, there were no indications during the initial four observations with stimulus females that would suggest diminished aggression during testing (see Figure 5), although there was a statistically significant difference in number of bites, $F(1, 103) = 14.98, p < .001$ and number of attacks, $F(1, 103) = 36.7, p < .01$, between the first four and final four tests with stimulus females (the first four without sex steroid hormone replacement and next four with TP treatment). This suggests that this diminished aggression was due to the effect of TP treatment and not due to repeated testing.

Genotype Differences in Investigatory Behaviors Noted Without Sex Steroid Hormone Replacement

Differences between genotype were found when evaluating investigatory behavior by test mice toward stimulus mice. When tested with stimulus females, SF-1 KO mice initiated and received fewer olfactory contacts, $F(1, 87) = 8.98, p < .01$ and fewer anogenital sniffs, $F(1, 87) = 20.91, p < .001$, than did WT mice (see Table 1). When the stimulus mice were male, experimental mice initiated few contacts or anogenital sniffing and there were no differences among the four groups of mice that were tested.

Adult TP Treatment Produced a Sex Difference in Investigation in WT Mice

In tests with TP treated mice, an interaction between sex and genotype was noted in anogenital sniffs and contacts by stimulus mice of both sexes. WT male mice received fewer anogenital sniffs and contacts from stimulus males, $F(1, 79) = 2.05, p < .05$, and females, $F(1, 79) = 9.89, p < .01$, as compared to KO mice and WT females (see Table 2). When evaluating social behavior initiated by test animals toward stimulus animals no sex differences were found within groups, but differences between genotype emerged in the opposite direction as in tests with hormonally naïve animals. TP treatment increased anogenital sniffing in KO mice of both sexes which displayed more anogenital sniffing of stimulus females than was demonstrated by WT mice of both sexes, $F(1, 79) = 21.49, p < .001$ (Table 2).

Discussion

Numerous studies over many years have demonstrated that sex differences in behavior are often due to the developmental actions of gonadal steroid hormones (Beatty, 1984). In particular, intermale aggressive behavior is strongly influenced by exposure to sex steroids and neonatal exposure to testosterone is thought to be necessary for the development of this behavioral pattern. Neonatal injections of testosterone induce aggressive behavior in female mice (vom Saal, 1979; Whitsett, Bronson, Peters, & Hamilton, 1972), and for aromatase disrupted (ArKO) mice, males show adult aggressive behavior following neonatal estrogen replacement (Toda, Saibara, Okada, Onishi, & Shizuta, 2001). Therefore, early postnatal and not necessarily prenatal exposure to sex steroids is sufficient for development of intermale aggressive behavior in adult life. In the present study, we observed strong aggressive behavior after adult TP treatment only in WT males, as predicted if the SF-1 KO mouse provides a model for gonadal hormone dependence of the differentiation of behavior. Thus far, the major models

for selectively removing fetal gonadal steroids have been pharmacological. The SF-1 KO mouse therefore provides a novel genetic model of fetal gonadectomy.

The absence of intermale aggression in SF-1 KO male mice could be explained by the absence of perinatal gonadal steroids acting at androgen receptors or estrogen receptors (α or β). Loss of intermale aggression was also reported in ER α KO that rarely display aggressive behavior against bulbectomized WT males (Ogawa, Lubahn, Korach, & Pfaff, 1997; Ogawa et al., 1998), but this is not the case in ER β KO (Krege et al., 1998). Finally, androgen receptor dysfunction as in testicularly feminized mice (Tfm) had no effect in tests of intermale aggression (Scordalakes & Rissman, 2004). Taken together, these data suggest that neonatal aromatization of T is a major factor responsible for development of adult intermale aggression. Immunoreactive aromatase in the preoptic area and amygdala is not affected in SF-1 KO mice (Shinoda et al., 1995), suggesting that SF-1 KO mice may have normal aromatase function. Without fetal gonads, however, there is no circulating testosterone that could be transformed into estradiol-17 β in the brain during sexual differentiation. In the adult TP treated condition, it is likely that SF-1 KO mice produced significant estradiol in brain. In perinatal SF-1 KO mice, ER α (Dellovade et al., 2000) and ER β (Wolfe et al., 2005) are both present in hypothalamus, although for ER β in the preoptic area expression may more closely track a female selective pattern (Wolfe et al., 2005). It is not known whether ER α and β and AR are present at normal levels in adult SF-1 KO mice. Responsiveness of these mice to TP treatment suggests that at least some AR or ERs are present to produce effects in the brain/behavior of adult SF-1 KO mice.

In the current study SF-1 KO mice were aggressive toward stimulus females in the absence of any endogenous sex steroids developmentally and in adulthood. It is possible that the absence of gonadal steroids contributed to the alteration in adult behavior, and this might be a plausible explanation as TP treatment diminished spontaneous aggression in SF-1 KO mice and overall induced more female-like behavior, more consistent with the external phenotype of these mice. This is interesting as it suggests that SF-1 KO mice are still responsive to testosterone or its aromatized product estradiol, even though they were never exposed to sex steroid hormones until their first experimental treatment in adult life. Responsiveness to TP treatment suggests that steroid receptors are present and therefore, fetal/neonatal exposure to gonadal steroids is not needed for induction of receptor expression as suggested previously (Paden, Gerlach, & McEwen, 1985). Aggression toward females has been observed in other sex-steroid related knockout models. ArKO, estrogen receptor α deficient (ER α KO), and double knockouts for both estrogen receptor α and β (ER $\alpha\beta$ KO) male mice showed aggression against females during mating tests (Matsumoto, Honda, & Harada, 2003; Nomura et al., 2002; Ogawa et al., 1998; Scordalakes & Rissman, 2003), although in these studies subjects received sex steroid hormones prior to testing. Studies with aromatase (Bakker, 2003; Bakker, Honda, Harada, & Balthazart, 2002; Wesson, Keller, Douhard, Baum, & Bakker, 2006) and estrogen receptor α (Dominguez-Salazar, Bateman, & Rissman, 2004; Rissman, Wersinger, Fugger, & Foster, 1999) knockout mice provide evidence of altered olfactory function in tests with soiled bedding that might partially explain aggressive behavior observed in these mice models. Further experiments will be needed to specifically test olfactory function in SF-1 KO mice, although preliminary results from olfactory preference testing showed that SF-1 KO mice can distinguish between clean and female- or male-soiled bedding (N. Grgurevic and G. Majdic, personal communication, June, 2007), suggesting that at least to some extent the olfactory system in SF-1 KO mice functions normally.

Another factor that may have contributed to increased aggressive behavior in the absence of gonadal steroids is the reorganization of the VMH in SF-1 KO mice (Davis et al., 2004; Dellovade et al., 2000; Majdic et al., 2002). The VMH has been implicated as a part of a circuitry that regulates various behaviors including aggressive, sexual and feeding behavior,

and locomotor activity (Canteras, Simerly, & Swanson, 1994). The abnormalities in VMH organization that are present in SF-1 KO mice might impact the function of key cell types that are not in the correct adult position (e.g., estrogen receptor α containing neurons relocated medially; Dellovade et al., 2000) as well as neuronal connections with other regions, particularly to nuclei of the amygdala involved in emotional responses and aggressive behaviors. Moreover, the dorsomedial part of the VMH is one of three cell groups that play critical roles in the expression of defensive behavior (Canteras, 2002; Swanson, 2000). Earlier studies showed that rats with VMH lesions were more sensitive to electric shock and showed increased aggression after footshock (Grossman, 1972) in comparison to controls. Male rats with VMH lesions also showed increased incidence of agonistic behavior. However, they did not actively initiate fights and did not show aggression toward female partners (Colpaert, 1975). This is in contrast with the results of the current study where SF-1 KO mice actively initiated aggressive behavior toward stimulus females in the absence of sex steroid hormones, but displayed no aggression when treated with TP. This difference might be species specific, as most studies with VMH lesions have been done in rats. Alternatively, cellular rearrangement and subsequent altered wiring in the region of the VMH as opposed to a deletion (as with lesions) might result in circuitry causing dysregulation of aggressive behavior.

Social behaviors are also thought to be dependent on steroid hormone exposure during fetal or postnatal life. For example, ArKO mice exhibit disrupted sociosexual behaviors (Bakker et al., 2002). Testosterone treated ArKO mice showed less investigative olfactory behavior in comparison to WT mice. In the current study, hormonally naïve SF-1 KO mice displayed fewer contacts toward stimulus females, however, in contrast to ArKO mice, after TP treatment SF-1 KO mice displayed increases in anogenital sniffing toward stimulus females, and displayed significantly more of this behavior than WT control mice. This difference could arise due to the fact that ArKO mice still produce steroid hormones, and perhaps in the absence of estradiol, testosterone may drive androgen receptor dependent organizational effects that might impair the development of social behaviors in ArKO mice (Bodo & Rissman, 2007). Such an effect of testosterone would be absent in SF-1 KO mice that do not produce endogenous gonadal steroid hormones from early fetal life.

Social behaviors are also thought to be dependent on steroid hormone exposure during the pubertal period (Primus & Kellogg, 1990; Schulz & Sisk, 2006; Shrenker, Maxson, & Ginsburg, 1985). In the current study, the control mice were gonadectomized prior to puberty so that all mice were without hormonal stimulation during this potential second critical period. The lack of pubertal exposure to testosterone, however, did not prevent the development of intermale aggression in the WT males once given replacement TP in adulthood. Likewise GPR54-KO mice that do not enter spontaneous puberty display normal male and female sexual behavior when treated as adults with the appropriate steroids (Kauffman et al., 2007). Thus mice, unlike hamsters, may not require pubertal steroid exposure for normal adult social behaviors. Although they may have shown less aggression than mice gonadectomized after puberty (Shrenker et al., 1985), we did not test this. Other work (Motelica-Heino et al., 1993) suggested that the perinatal dependence of intermale aggression on gonadal steroids in mice may be fully realized by PND 6.

Although SF-1 KO mice are born without gonads and are not exposed to endogenous steroid hormones from adrenal glands or gonads, SF-1 KO mice received three additional corticosteroid injections after adrenal transplantation and WT mice did not. Many studies have shown that exposure to glucocorticoids in neonatal life could have long term consequences for aspects of behavior in rodents. The most commonly observed effects, however, have been differences in learning, social recognition and spatial memory (Kamphuis et al., 2004; Pavlovskaja-Teglia, Stodulski, Svendsen, Dalton, & Hau, 1995; Roskoden, Linke, & Schwegler, 2005). Of direct relevance to the current study, neonatal treatment with glucocorticoids may

increase submissiveness in mice (Leshner & Schwartz, 1977). This stands in direct contrast to the current results, in which an increase in aggression was seen. It is also important to note that most studies examining glucocorticoid effects on neonatal brain development used much higher doses than ours (Leshner & Schwartz, 1977; Pavlovska-Teglia et al., 1995; Roskoden et al., 2005).

In summary, we set out to examine sex differences that exist in WT mice and not SF-1 KO mice. We found a sex difference in aggressive behavior that was present in WT, but not SF-1 KO mice suggesting likely developmental dependence on gonadal steroid exposure. More important, the loss of the ability for adult TP treatment to drive intermale aggression in SF-1 KO males affirms SF-1 KO mice as a model for gonad-dependent development of behavior. Second, we were particularly interested in whether SF-1 KO mice exhibit similar sex differences to gonadectomized WT mice. Such differences would likely be developmental-gonad independent, but were not found in the measures examined in this study. Finally, we hypothesized that if for any measure SF-1 KO mice of both sexes behaved differently than WT mice, then these differences may be due to alterations in the VMH. We found that aggressive behavior in the absence of hormone priming was significantly greater in SF-1 knockouts of both sexes than in WT mice. Further studies of SF-1 KO mice in the context of social, sexual, and aggressive behaviors, and brain mechanisms will help elucidate the relative contributions of genetic and gonadal factors for brain sexual differentiation.

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Figure 1.
Schematic representation of experimental protocols used with SF-1 KO and control mice.

Figure 2.
Schematic representation of behavioral testing paradigm.

Figure 3. Aggressive behaviors (means for each trial $\pm$ SEM: (a) number of bites, (b) attacks) displayed by WT control ($n = 4$ for each sex) and SF-1 KO ($n = 7$ for each sex) mice. Control mice were gonadectomized, none of the animals received hormonal treatment, and all were tested with a stimulus female for 45 min. A significant effect of genotype, but not of sex was noted. $*p < .05$. $**p < .01$.

Figure 4.

Attacks and bites (mean of four observations $\pm$ SEM) displayed by WT controls ($n = 4$ for each sex) and SF-1 KO ($n = 6$ for each sex) mice treated with TP and tested with a stimulus male for 15 min. (***) WT males were significantly different from all other groups, $p < .001$.

Figure 5.

Number of bites (mean of four observations $\pm$ SEM) by SF-1 KO mice against stimulus females during 8 trials. Trials 1 to 4 are tests without gonadal hormone replacement (hormonally naïve SF-1 KO mice) and trials 5 to 8 are tests with SF-1 KO mice treated with TP ($n = 6$ for both sexes).

Table 1
Number (M of Four Tests $\pm$ SEM) of Contacts and Anogenital Sniffs

Tested animal	Contacts	Anogenital sniffs	Contacts by stimulus female	Anogenital sniffs by stimulus female
WT f ($n = 4$)	321.6 [*] $\pm$ 45.7	24.3 $\pm$ 3.8	251.4 [*] $\pm$ 64.3	41.3 ^{**} $\pm$ 6.5
WT m ($n = 4$)	479.5 [*] $\pm$ 55.4	24.1 $\pm$ 6.6	116 [*] $\pm$ 12.4	31.9 ^{**} $\pm$ 7.1
KO f ($n = 7$)	240.9 $\pm$ 35.3	33.8 $\pm$ 7.2	39.7 $\pm$ 7.5	12.4 $\pm$ 3.0
KO m ($n = 7$)	156.7 $\pm$ 26.5	14.9 $\pm$ 4.1	25.3 $\pm$ 4.6	5.0 $\pm$ 2.3

Note. Conducted in 45-min tests with WT controls and SF-1 KO animals without sex steroid replacement tested with stimulus females. WT = wild type; f = female; m = male; KO = knockout.

* Significantly different between genotypes: $p < .01$.

** Significantly different between genotypes: $p < .001$.

Table 2
Number (M of Four Tests $\pm$ SEM) of Anogenital Sniffs

Tested animal	Anogenital sniffs toward stimulus female	Anogenital sniffs by stimulus female	Anogenital sniffs by stimulus male
WT f ($n = 4$)	8.6 ^{***} $\pm$ 1.7	4.1 $\pm$ 0.8	3.2 $\pm$ 1.2
WT m ($n = 4$)	5.7 ^{***} $\pm$ 1.7	0.5 ^{**} $\pm$ 0.1	0.1 [*] $\pm$ 0.01
KO f ($n = 7$)	13.4 $\pm$ 1.4	2.48 $\pm$ 0.6	3.03 $\pm$ 1.0
KO m ($n = 7$)	15.3 $\pm$ 1.4	3.4 $\pm$ 0.6	2.5 $\pm$ 1.0

Note. Conducted in tests adjusted to 15 min with TP treated WT controls and SF-1 KO animals. WT = wild type; f = female; m = male; KO = knockout.

* Significantly different between genotypes: $p < .05$.

** Significantly different between genotypes: $p < .01$.

*** Significantly different between genotypes: $p < .001$.