ORIGINAL ARTICLE

Developmental exposure to low levels of ethinylestradiol affects social play in juvenile male rats

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Abstract

Juvenile social play contributes to the development of adult social and emotional skills in humans and non-human animals and is therefore a useful endpoint to study the effects of endocrine disrupters on behavior in animal models. Ethinylestradiol (EE₂), a widely produced, powerful synthetic estrogen is widespread in the environment mainly because it is a component of the contraceptive pill. To understand whether clinical or environmental exposure to EE₂ during critical perinatal periods can affect male social play, we exposed 72 male Sprague–Dawley rats to EE₂ or vehicle either during gestation (from gestation day (GD) 5 through 20) or during lactation (from postnatal day (PND) 1 through 21). Two doses of EE₂ were used to treat the dams: a lower dose in the range of possible environmental exposure (4 ng/kg/day) and a higher dose similar to that received during contraceptive treatment (400 ng/kg/day). Social play was observed between PND 40 and 45. A principal component analysis (PCA) of frequencies of behavioral items observed during play sessions allowed to allocate behaviors to the two main components that we named aggressive-like play and defensive-like play. Aggressive-like play was increased by gestational and decreased by lactational exposure. Defensive-like play was decreased by treatment. For both types of play the lower dose (4 ng/kg/day) was as effective as the higher one. Total social activity was increased by gestational and decreased by lactational exposure. These findings provide further evidence that exposure to low and to very low doses of EE₂ during critical periods of development can affect essential aspects of social behavior, and that the timing of exposure is critical to understand its developmental action.

Keywords Endocrine disrupters · Ethinylestradiol · Xenoestrogens · Social play · Play fighting · Anogenital distance · Developmental windows · Cross-fostering · Rat

Abbreviations

AFP	α-Fetoprotein
AGD	Anogenital distance
AGI	Anogenital index
CNS	Central nervous system
EDCs	Endocrine disrupter chemicals
EE ₂	17α-Ethinylestradiol
ER	Estrogen receptor
GD	Gestation day
GLM	General linear model
PCA	Principal component analysis
PCs	Principal components
PND	Postnatal day
SHBG	Sex hormone-binding globulin
SD	Sprague–Dawley

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Introduction

Juvenile social play contributes to the development of adult social and emotional skills in humans and non human animals [1–6] and is ideal for studying the neurobiology of social development [5]. Rat juvenile social play is sensitive to chemical factors such as prenatal and neonatal hormones and is a useful behavioral marker of neurodevelopment as severe deficits in this behavior are associated with neurodevelopmental disorders [7]. It is well known that estrogen can exert an organizational effect on CNS and behavior in higher vertebrates during the early stages of development see [8–10]. Perinatal exposure to estrogen can modify behavioral developmental trajectories. In fact, a role for early estrogen in determining the sexual phenotype of the adult rodent brain was clearly established by classical studies that illustrated how exposure to aromatizable androgens is responsible for brain masculinization, whereas its lack leads to normal female brain development [9]. In fact, the mammalian brain is essentially feminine in the absence of early exposure to gonadal steroid [11] and is susceptible to the organizational action of sex hormones or of their mimics. Rat brain has been established as a useful model to study the effects of developmental exposure to estrogen and estrogenic endocrine disrupter chemicals (EDCs). There is strong evidence that the perinatal period is the most sensible time window for effects of EDCs on brain development, yet few studies have tried to tell apart the effects of gestational from those due to lactational exposure [12, 13]. This is crucial to better understand the time course of developmental EDCs action.

The development of play is influenced by perinatal testosterone through its 5α -reduced products [14]. However, recent research showed that estrogens or estrogenic EDCs are also involved in the development of play behavior in rats [15–19]. The synthetic estrogen ethinylestradiol (EE_2) is a powerful mimic of natural estrogen and is the active component of most contraceptive pills. Unintentional exposure of the developing human fetus can occur if oral contraception is continued during the early months of undetected pregnancy. Timms et al. [20] estimated that each year in the USA and Europe, almost 2 million women who use oral contraceptives become pregnant accidentally, primarily because of missed pills. Oral contraceptive pills often are taken for months until the unplanned pregnancy is discovered. Furthermore, EE_2 and other estrogenic compounds are used in hormone replacement therapy and osteoporosis treatment [21] and as growth enhancement products in veterinary medicine [22].

Due to its widespread pharmaceutical use and relatively long half-life, EE_2 has been detected in some river systems in the USA and Europe [23–25], and is a matter of concern for public and wildlife health [26].

Johnson and Williams [25] suggested that 40% of the ingested EE_2 is found free (deconjugated) in the environment. The disrupting effects of EE_2 at environmentally relevant levels on fish reproduction and behavior are well known [24, 27–30]. In mammals, pharmacological levels of EE_2 exert important effects on reproductive physiology and behavior [18, 31–34]. In humans, the assumption of EE_2 is mainly through fish food intake. In fact, in fishes, EE_2 is concentrated multiple times (480–720) relative to water [35]. Studies on terrestrial mammals at concentrations similar to the contraceptive dose of 400–800 ng/kg/day or lower are relatively scarce: administration of EE_2 during development can affect a variety of reproductive anatomical and physiological endpoints in mice and rats [20, 36–46] but see a lack of effects with 500 ng/kg/day in Mandrup et al. [34].

Behavior is a critical endpoint of estrogen action, and previous studies in Sprague–Dawley (SD) rats showed significant effects of a developmental administration (GD 5–PND 32) of very low (4 ng/kg/day) or low clinical EE_2 doses (400 ng/kg/day) on learning and memory [47], sexual behavior [48, 49], pain perception [50], anxiety [51] and social play [19].

In the present research, we studied the effects of EE_2 on play behavior of male SD rats exposed to this chemical in utero (GD5 to birth) or during lactation (PND 1–PND 21). The animals were exposed by treating their dams with two doses of EE_2 : a very low, environmentally relevant dose (4 ng/kg/day), or a clinical one (400 ng/kg/day). We studied play behavior in juvenile males maintained and observed in a social context with cagemates of the same age [52], a more naturalistic setting compared to dyadic encounters preceded by social isolation [2]. Isolation is a strong stressor per se [53] and, although it may enhance the emergence of effects on play [7], it represents an important confounding factor. Observations were carried out between 40 and 45 days of age: around this age males approach sexual maturity [54] yet their social play is not significantly different from that expressed at 35 days of age [16]. Pellis and Pellis [55] described in Long Evans hooded male and female rats a peak of play fighting around 41–45 days of age in same-sex pairs.

Materials and methods

Animals and treatment procedure

We used 72 juvenile SD male rats born and bred at the Human Physiology Institute, University of Siena (Italy), and exposed to EE_2 during gestation or lactation. To obtain the experimental subjects, we housed 100 female-male pairs of sexually mature Sprague–Dawley rats in 100 polysulfone cages (Tecniplast, Italy, 60 × 37 × 20 cm), provided with metal tops and a wire netting floors for the daily search of

the vaginal plug to detect the day of copulation (defined gestational day 0 or GD 0). On the same day, the male was removed, and the female was housed individually. We selected 72 dams that had been fertilized within the previous two days and transferred them in single cages. Half of the dams ($N=36$) were treated daily with either 4 ng/kg EE2 (Sigma-Aldrich; EE4, $N=12$), 400 ng/kg EE2 (EE400, $N=12$), or vehicle only (peanut oil, $N=12$) from GD 5 until weaning of the pups, whereas the other 36 dams were untreated. The treatment was administered orally with a pipette. This procedure appears to be much less stressful than gavage [56, 57]. On postnatal day (PND) 1, pups were removed from their mothers and gently placed in a cotton nest, weighed singularly with an analytical scale, and we measured their anogenital distance (AGD) with a caliper. AGD was defined as the distance between the anterior edge of the anus and the posterior edge of the genital tubercle when the area was naturally extended without stretch. Anogenital index (AGI) was calculated by dividing AGD by body weight. On the same day, the litters were culled to 4 females and 4 males and then cross-fostered. Pups born from treated dams were fostered to untreated dams so that their exposure to EE₂ or vehicle was confined to the gestational period, whereas pups born from untreated dams were fostered to treated dams so to be exposed to EE₂ or vehicle only during lactation (Table 1). At weaning (PND 21), all litters were separated from foster dams. At PND 32 one male for each litter was individually marked with cosmetic dye on the tail and randomly housed with 3 other males that had received the same treatment. No cage contained siblings. Thus, only one male per litter for a total of 72 males was used for the study, i.e. each experimental subject came from a different dam.

The animals were housed in polysulfone cages as previously described under a reversed light–dark cycle (dark 07.30–19.30) with a relative humidity of $60 \pm 10\%$. Food (Harlan Teklad soy-free AIN-76A diet) and water were supplied ad libitum throughout the experiment.

Table 1 Outline of the experimental groups

	Oil	EE4	EE400
Gestational (pups from treated dams fostered to untreated foster dams)	12	12	12
Lactational (pups from untreated dams fostered to treated foster dams)	12	12	12

The gestational animals received the treatment only in utero (GD 5–20) from their treated dams and at birth were fostered to untreated dams. The lactational group received no treatment in utero and were then exposed to the treatment only via the milk of their foster treated dams (PND 1–21). EE4 = 4 ng/kg/day; EE400 = 400 ng/kg/day

Behavioral testing

Observations were carried out during the dark phase, under dim red light combined with low indirect white light. All sessions were recorded with a video camera, and video recordings were analyzed with ‘The Observer Video Pro 4.0’ software (Noldus Information Technology, The Netherlands) by an observer blind to treatment.

Subjects were tested for social play between PND 40 and 45, an age at which social play is still vigorous and not significantly different from that expressed at 35 days of age [15, 16]. The four males from the same housing cage were tested together in a neutral arena ($60 \times 35 \times 35$ cm). At the beginning of the observation, just before the introduction of the rats into the arena, a small quantity of sawdust from the home cage was mixed to the clean one of the testing arena to facilitate habituation to the novel environment. After 1 min of familiarization, the behavior of the four animals was video-recorded for 15 min. Social behaviors of each individual were identified according to the ethogram described in Table 2, modified from Porrini et al. [16]. A behavior was attributed to the subject initiating the action. Testing of different experimental groups was balanced across time.

Animal welfare

The experiments described in this research were approved by the Ethical Committee of the Department of Physiology, University of Siena and followed European Community Council Directive 86/609/EEC and institutional guidelines.

Statistical analysis

Total social behavior (= all behavioral items involving any social interaction during the 15 min test) was analyzed by GLM to test the effects of treatment, timing of exposure, and home cage. To reduce the dimensions of the data set (and thus reduce the number of tests to be carried out) and identify correlated behavioral items (and thus eliminating auto-correlation), we carried out a principal component analysis (PCA) [58]. The Kaiser–Meyer–Olkin index (a measure of the proportion of variance in common between the different variables) was used to estimate the overall adequacy of the matrix [59], and communalities (a measure of the proportion of variance of each variance explained by the matrix) were calculated to for identifying variable contribution to the correlation matrix (the higher communality, the more the variable is associated to others), and exclude those variables whose contribution to the matrix was relatively less substantial, (i.e., communality < 0.6) or that had more than 50% of values equal to 0 [60].

Once extracted, the principal components (PCs) were rotated (with the Equamax procedure to capture the

Table 2 List of social behaviors considered

Social behaviors
Aggressive neck grooming (vigorous neck allogrooming)
Allo-grooming
Approach (moving toward another)
Bite
Boxing (both rats stand up facing each other and boxing with forepaws)
Charge
Chase
Crawl-over (moving over another)
Crawl-under (moving under another)
Flee
Genital sniffing
Lateral display (the animal orientates itself broadside to another animal)
On back (lying on the back with belly exposed to another)
Pinning (standing over the opponent with its forepaws on the ventral surface)
Pounce (bouncing over another)
Upright (with erect posture the rat exposes its belly to another)
Withdraw (all movements away from another)
Each behavior is performed by the focal subject

maximal amount of variance) to facilitate the interpretation of the different components, and Kaiser normalization was applied to reduce anomalies in the component loadings. Scores were saved using the Anderson-Rubin method to guarantee orthogonality between components [58]. The first three PCs were used as dependent variables into four general linear models (GLM) to study the effect of treatment, timing of exposure and home cage.

Analysis of variance using a GLM approach was also used to test differences between treatment groups for AGD, AGI and body weight, and between treatment groups and timing of exposure (and their interaction). For all analyses, we added ‘cage’ as a random factor to account for the effect of the social group. Post hoc comparisons were carried out using the Sidak test [61]. All tests were performed using IBM SPSS 25 (IBM®, Chicago, IL).

Results

Anatomical variables

Body weight at PND1 was not affected by treatment ($F_{2,146} = 1.45$; $p > 0.2$). AGD and AGI (AGD/body weight) at birth were significantly increased by gestational administration of EE₂ (AGD, $F_{2,146} = 6.68$, $p < 0.01$; AG index, $F_{2,146} = 11,654$, $p < 0.001$, Fig. 1a, b) in a dose dependent manner.

Total social activity

We computed total social activity by pooling all behavioral items involving any social interaction during the 15 min test (Table 2, Fig. 2). Timing of treatment was clearly significant ($F_{1,64} = 42.84$, $p < 0.001$); treatment per se was not significant ($F_{2,64} = 1.46$, $p = 0.24$) but a significant interaction of treatment $\times$ timing ($F_{2,64} = 11.44$, $p < 0.001$) was evident. Gestational EE400 treatment significantly increased social activity (EE400 vs oil, post hoc Sidak $p < 0.01$); while lactational one significantly decreased it (EE4 vs oil, post hoc Sidak $p < 0.05$; EE400 vs oil, post hoc Sidak $p < 0.05$). No cage effect ($F_{2,64} = 2.41$, $p = 0.09$) was detected.

PCA analysis

PCA applied to the frequencies of the behavioral items identified 3 components that explain 78.37% of the variance (Table 3). Principal component 1 (PC1) explained the largest percentage of variance (54.45%) and included most elements of aggressive play, and was therefore labeled as “aggressive-like play”. PC2 included most element of defensive play and was labeled as “defensive-like play” (variance explained = 12.66%). PC3 (11.25%) included mainly allogrooming. Based on the weight of each component and their internal coherence and their relevance to social behavior, we decided to consider only the first three components, explaining 78.37% of variance. Since

Fig. 1 Anogenital distance (mean $\pm$ SE) and Anogenital index (mean $\pm$ SE) at birth of male rats exposed to gestational treatment with 4 ng/kg/day (EE4) or 400 ng/kg/day (EE400) of EE2 or oil. **a** Anogenital distance was significantly increased by gestational admin-

istration of EE₂ ($F_{2,146}=6.68$, $p<0.01$) in a dose dependent manner; **b** Anogenital index was significantly increased by gestational administration of EE₂ ($F_{2,146}=11.654$, $p<0.001$) in a dose dependent manner

Fig. 2 Total social activity. Frequency ($n/15$ min) of total social activity of male rats exposed to gestational or lactational treatment with 4 ng/kg/day (EE4) or 400 ng/kg/day (EE400) of EE2. Box-whiskers show median, interquartiles, and range of individual values. Timing ($F_{1,64}=42.84$, $p<0.001$); treatment ($F_{2,64}=1.46$, $p=0.24$); treatment $\times$ timing ($F_{2,64}=11.44$, $p<0.001$)

the remaining PCs explained only a residual 21.63% of variance we excluded them from further analyses. GLM was applied to each component, using the individual component scores as variables, considering treatment (OIL, EE4, EE400), timing of administration (gestation or lactation), cage and interactions.

Table 3 Results of PCA applied to behaviors of male rats

	Components		
	1. Aggressive-like play	2. Defensive-like play	3. Allo-grooming
Aggressive grooming	0.865		
Pounce	0.857	0.457	
Chase	0.805		
Flee	0.710	0.531	
Bite	0.570	0.641	
On back		0.849	
Lateral display		0.756	
Withdraw	0.330	0.772	
Allo-grooming			0.981
Variance explained %	54.45	12.66	

Total variance explained: 78.37%. Only loadings $\geq \pm 0.3$ are shown. Component 3 is mainly represented by allo-grooming. Each behavior is performed by the focal subject. Those variables whose contribution to the matrix was relatively less substantial, (i.e., communality < 0.6) or that had more than 50% of values equal to 0 were excluded

PC1: aggressive-like play

Aggressive-like play (Fig. 3) was not significantly affected by treatment ($F_{2,64}=0.727$, $p=0.487$). However, the significance of the main factor timing ($F_{1,64}=8.154$, $p<0.01$), together with the interaction treatment $\times$ timing ($F_{2,64}=4.754$, $p<0.01$) indicate different effects of the treatment in the gestational and lactational periods: overall,

Fig. 3 Aggressive-like play. Principal component scores of aggressive-like play (PC1) performed by male rats exposed to gestational or lactational treatment with 4 ng/kg/day (EE4) or 400 ng/kg/day (EE400) of EE₂. Box-whiskers show median, interquartiles, and range of individual values. Timing ($F_{1,64}=8.154$, $p<0.01$); treatment ($F_{2,64}=0.727$, $p=0.48$); interaction treatment $\times$ timing ($F_{2,64}=4.754$, $p<0.01$)

gestational exposure increased the frequency of aggressive-like play, while lactational exposure decreased it. Cage had no significant effect ($F_{2,64}=2.688$, $p=0.07$).

PC2: defensive-like play

Overall defensive-like play (Fig. 4) was not affected by treatment ($F_{2,64}=2.08$, $p=0.133$). However, timing ($F_{1,64}=18.658$, $p<0.001$) and interaction between treatment and timing ($F_{2,64}=3.373$, $p<0.05$) were significant, showing a decrease due to lactational exposure regardless of dosage. Cage had no significant effect ($F_{2,64}=0.520$, $p=0.59$).

PC3: allogrooming

This component represented almost exclusively by allogrooming was not affected by treatment ($F_{2,64}=0.863$, $p=0.42$). Timing was significant ($F_{1,64}=5.577$, $p<0.05$) and interaction treatment $\times$ timing ($F_{2,64}=0.925$, $p=0.40$) was not significant. Cage had no significant effect ($F_{2,64}=0.271$, $p=0.76$).

Discussion

Low doses of EE₂ induce behavioral changes

Our findings showed that developmental exposure of male rats to low or very low doses of the synthetic estrogen EE₂

Fig. 4 Defensive-like play. Principal component scores of defensive-like play (PC2) performed by male rats exposed to gestational or lactational treatment with 4 ng/kg/day (EE4) or 400 ng/kg/day (EE400) of EE₂. Box-whiskers show median, interquartiles, and range of individual values. Timing ($F_{1,64}=18.658$, $p<0.001$); treatment ($F_{2,64}=2.08$, $p=0.13$); interaction treatment $\times$ timing ($F_{2,64}=3.373$, $p<0.05$)

significantly alters social play by modifying the frequencies of its aggressive and defensive components and that this effect may vary in relation of the timing of exposure. If play is essential for the refinement of social skills [2, 3], then a modification of play may have consequences on adult social behavior.

In our study, both doses of EE₂ produced significant effects on play behavior, an interesting finding given that the lowest dose (4 ng/kg/day) matches observed levels of environmental exposure [42]. The dose–effect relationship observed in our study is non-linear, confirming that at very low doses, hormonally active substances may have non-linear effects [62]. Aggressive-like play is increased after gestational, and decreased after lactational exposure. Defensive-like play was decreased by treatment. In general, lactational exposure was as effective or more effective than gestational exposure, which is in contrast with studies reviewed by Delclos et al. [36] reporting a limited transfer of EE₂ to newborns via milk in humans and rats.

How can low and very low doses of EE₂ affect behavior? EE₂ binds to estrogen receptors (ER), in particular ER α , with much higher affinity than endogenous estradiol [63]. In addition, EE₂ has a low affinity for α -fetoprotein (AFP) [64] and human sex hormone-binding globulin (SHBG) [65]. As a consequence, EE₂ is able to reach target areas in the brain and to affect physiology and behavior during critical time windows. However, one should be cautious in translating the present results to humans because of differences between man and rat in the biotransformation of EE₂ [66, 67], which

could result in more serious effects in rats. Effects of subtle variations in the concentration of hormones on development have been reported previously, as illustrated by the differences in adult behavior depending on the intrauterine position that affects the prenatal hormonal milieu [68, 69].

Effect of timing of exposure

Total social behavior, aggressive-like play, and defensive-like play were differently affected by gestational or lactational exposure to EE₂. To our knowledge it is the first time that this effect is shown, suggesting a difference between a prenatal and a postnatal critical window of sex steroid action. We are not aware of the mechanism underlying this difference. Maybe that different brain target areas are affected in different time windows, or that the same area is affected in different time windows.

Influence of treatment and timing is evident, looking at Total social activity: gestational exposure significantly increases it, while lactational one significantly decreases it. A similar trend was observed in aggressive components of social play. These components in our PCA analysis were grouped under PC1 (aggressive-like play) and accounted for 54.45% of the variance of PCA. PC1 differed significantly after gestational or lactational exposure. It increased after gestational exposure and decreased after lactational exposure. Defensive components of play accounted for 12.66% of the variance and were decreased by the treatment. This result is in agreement with the masculinizing effect on play caused by EE₂ during gestation. Again, there was a significant effect of timing, with lactational exposure decreasing this behavior.

It is clearly shown by a large number of studies that early effects of steroid hormones on mammalian brain and behavior take place in a perinatal time window, in rodents from late gestation to initial post-natal life [9]. In fact, by using a cross-fostering design we were able to tell apart the effects of pre- and post-natal exposure. The increase of total social activity and aggressive-like play observed after gestational exposure is in line with the widely described masculinizing effects of estrogens at the central level. However, our data show that this effect is reversed after lactational exposure, a difference that has not been reported by other researches.

A possible explanation of the difference between the effects of gestational and lactational exposure could be related to testosterone-induced activity of hypothalamic aromatase [70], which in male rats peaks at late gestation but rapidly declines after birth [71]: Tsuruo et al. [72] showed that fetal and neonatal aromatase-immunoreactive neurons coexpress ERs, and it is important to note that ERs have high affinity to EE₂ [63]. Since in rodent male fetuses the gonads are active in producing aromatizable androgens from about GD 18 [73], while in female fetuses

they are inactive in this period, availability of testosterone-derived estrogen at a central level in males could interfere with EE₂ action. According to this view, these differences should be absent in females: however, in a companion experiment conducted on females by our research group [19], we found some significant differences between gestational and lactational exposure to EE₂.

Developmental trajectories and the effect of maternal care

As far as lactational exposure is concerned, a possible effect of EE₂ on dam's maternal behavior, and consequently on pup's development and behavior, cannot be ruled out. In fact, alterations of maternal care can affect offspring [74, 75]. Our group showed that low doses of the weak estrogen bisphenol A administered through pregnancy to lactation can exert a mild but significant effect on maternal care of rat dams [76]. Thus, in the present research, the behavior of subjects exposed after birth to EE₂ through milk of EE₂ treated foster mothers could have been affected by changes of maternal behavior, an aspect that we did not measure. On the other hand, it must be noted that subjects exposed to EE₂ in utero were cared by non-treated foster mothers, and consequently the observed changes of their play behavior are a direct effect of EE₂.

Effect on anogenital distance

Anogenital distance (AGD) and anogenital index (AGI) were increased at birth by gestational exposure to EE₂ in a dose dependent mode: this increase is surprising since estrogens are known to act perinatally at the central level to masculinize brain, but a masculinizing action on male external genitalia at very low doses is not reported in literature. In a study by Corrieri et al. [47], SD male rats received through their mothers very low doses of EE₂ (4 ng/kg/day) or oil from GD 5 on, and a similar slight, though not significant, increase of AGD at birth was observed. Interestingly, a study by Do et al. [62] showed that in male mice gestational administration of Di(2-ethylhexyl) phthalate, an estrogenic chemical, increases fetal testosterone, AGD and AGI at very low doses but not at higher ones. These effects on AGD were observed only at low doses: a diet rich in the phytoestrogens genistein and diadzein during gestation had no effect on male rat AGD at birth [77]. In female rats, EE₂ was administered prenatally in low doses in a few studies where it had no effect on AGD at birth [19, 47]. To better understand the influence of developmental EE₂ exposure on male anatomy, we will

need further studies on other anatomical markers of sexual development and on fetal testosterone.

Concluding remarks

With an experimental protocol designed to mimic an environmental or clinical early exposure to EE₂, we observed robust effects of low and very low doses of EE₂ on social play, a perturbation that could influence the maturation of social behavior. We also highlighted that the effects on behavior of perinatal exposure to a sexual steroid can vary in relation to the timing (prenatal or postnatal) of exposure. An additional contribution of the present study is the demonstration that low and very low doses of a pure estrogen can be used as a tool to increase our understanding of the maturation of socio-sexual behavior. Previous work relying on castration/hormone replacement approaches often showed on–off effects of the hormones with consequent masking of those traits that typically respond in a dose-dependent manner. The high sensitivity of the behavioral endpoints examined in our study highlights the importance of implementing behavioral tests, to study the potential effects of low doses of endocrine disrupters.

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Compliance with ethical standards

Conflict of interests The authors declare that there is no conflict of interests associated with this paper.

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