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# Effects of pre-reproductive maternal enrichment on maternal care, offspring's play behavior and oxytocinergic neurons

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ABSTRACT

Potentiating social, cognitive, and sensorimotor stimulations the Environmental Enrichment (EE) increases levels of novelty and complexity experienced by individuals. Growing evidence demonstrates that parental EE experience, even occurring in the pre-reproductive phase, affects behavioral and neural developmental trajectories of the offspring.

To discover how the accumulation of early maternal complex experiences may inform and shape the social behavior of the following generation, we examined the effects of pre-reproductive enrichment of dams (post-natal days 21–72) on the play performances of their male and female adolescent offspring. Furthermore, we examined the effects of pre-reproductive enrichment on maternal behavior (during *post-partum* days 1–10) and male intruder aggression (on *post-partum* day 11). Since oxytocin modulates maternal care, social bonding, and agonistic behavior, the number of oxytocinergic neurons of the paraventricular (PVN) and supraoptic (SON) nuclei was examined in both dams and offspring.

Results revealed that enriched females exhibited higher levels of pup-oriented behaviors, especially Crouching, and initiated pup-retrieval more quickly than standard females after the maternal aggression test. Such behavioral peculiarities were accompanied by increased levels of oxytocinergic neurons in PVN and SON. Moreover, pre-reproductive maternal EE cross-generationally influenced the offspring according to sex. Indeed, male pups born to enriched females exhibited a reduced play fighting associated with a higher number of oxytocinergic neurons in SON in comparison to male pups born to standard-housed females.

In conclusion, pre-reproductive EE to the mothers affects their maternal care and has a cross-generational impact on the social behavior of their offspring that do not directly experiences EE.

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## 1. Introduction

A bulk of evidence indicates that environmental experiences can modify not only the neurobehavioral profiles of the directly exposed individual, but may even affect the next generations (Arai and Feig, 2011; Thayer and Kuzawa, 2011; Weaver et al., 2017). The

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inheritance of environmental experiences may occur through epigenetic modifications in the germ line or can be passed to the offspring directly from the mother via placenta or during lactation period (Bohacek and Mansuy, 2015; Caldji et al., 2011; Champagne and Curley, 2009; Champagne, 2010; Jirtle and Skinner, 2007; Ho and Burggren, 2010; Roth, 2012), and take different forms according to the negative or positive valence of the environmental stimuli and the period of exposure (e.g., during, pre-, and/or post-gestation) (Taouk and Schulkin, 2016). For instance, negative environmental stimulations, such as alcohol, drugs, and stress experienced by the mother during pregnancy negatively impact on physical and behavioral offspring's development (Charil et al., 2010; McMurray et al., 2008; Mueller and Bale, 2008; Van den Bergh et al., 2005; Weinstock, 2005). Parental age (Cannon, 2009; Malaspina et al., 2005; Perrin et al., 2007) as well as pre-reproductive stress (Shachar-Dadon et al., 2009) are risk factors for neuropsychiatric conditions in the offspring. *Post-partum* stress (Mashoodh et al., 2009) and maternal separation (Xiong et al., 2015; Franklin et al., 2010) influence anxiety and depressive-like behaviors, and induce changes in DNA methylation in the offspring.

Transgenerational effects have been also reported following the exposure to positive environmental stimulations, such as Environmental Enrichment (EE), which by increasing novelty and complexity levels experienced by individuals, potentiates behavioral performances and neural plasticity (Nithianantharajah and Hannan, 2006, 2009). Female offspring of dams enriched during pregnancy show reduced fetal hippocampal cell proliferation and adult anxiety behaviors (Maruoka et al., 2009). Moreover, maternal enrichment before and during gestation has the ability to influence offspring developmental trajectories improving or worsening specific motor, cognitive, and emotional performances of the progeny, and reduces global methylation levels in hippocampal and frontal areas of the offspring (Mychasiuk et al., 2012; Zuena et al., 2016). Maternal exposure to EE occurring during pregnancy and lactation enhances visual acuity and neurotrophin levels in the visual cortex of the offspring (Cancedda et al., 2004; Sale et al., 2004).

Interestingly, even the exposure to EE during the pre-reproductive phase either in females (Arai et al., 2009; Caporali et al., 2014; Cutuli et al., 2015, 2017; Dell and Rose, 1987; Leshem and Schulkin, 2012) and males (Mashoodh et al., 2012; Mychasiuk et al., 2012; Yeshurun et al., 2017) succeeds in significantly shaping the phenotype of the future offspring. Pre-reproductive maternal EE interventions also counteract the consequences of pre-reproductive stress on the offspring (Leshem and Schulkin, 2012). Finally, enrichment of the post-natal environment through “communal nesting” exerts beneficial transgenerational effects by reducing anxiety behaviors, increasing maternal behavior, and modifying oxytocin and vasopressin receptor densities of the offspring (Curley et al., 2009).

EE is also able to modify maternal behavior in females enriched before, during and/or after gestation (Cancedda et al., 2004; Caporali and Cutuli et al., 2015; Cutuli et al., 2015, 2017; Sale et al., 2004; Rosenfeld and Weller, 2012; Zuena et al., 2016) or even in standard females mated with enriched males (Mashoodh et al., 2012), by increasing mother's tendency to contact the pups in the very early phases of lactation (e.g., pup licking/grooming and crouching).

The present study follows our previous studies demonstrating that pre-reproductive maternal EE provides beneficial effects on motor (Caporali et al., 2014), cognitive (Cutuli et al., 2015), and affective (Cutuli et al., 2017) behaviors of the offspring. These multi-faceted modulations are supported by enhanced neuroplastic responses in neurotrophin (BDNF and NGF) levels in cerebellar, striatal, and hippocampal areas as well as in the number of glucocorticoid receptors in the amygdala. Starting from these observations, here we address the social play behavior of the male and female adolescent progeny of pre-reproductively enriched dams. Social play behavior is one of the earliest forms of non-mother-directed social behavior appearing in the ontogeny of mammalian species (Vanderschuren et al., 2012). This behavior is crucial for social and cognitive development, highly rewarding, and sex-dependent (Argue and McCarthy, 2015; Vanderschuren et al., 1997).

Since transgenerational changes may occur through changes in early mother-infant interactions (Meaney, 2010), we detailed maternal care of pre-reproductively enriched females by analysing maternal behavior during the first ten *post-partum* days (ppd), and then the maternal aggression and pup-retrieval following a male intrusion.

We also examined the effects of pre-reproductive maternal EE on oxytocinergic system, given that this system is known to regulate aspects of mother's and offspring's social behavior (Bosch, 2013; Crespi, 2016; Lee et al., 2009). In particular, we assessed the number of oxytocinergic neurons in the hypothalamic paraventricular (PVN) and the supraoptic (SON) nuclei (which mediate oxytocin synthesis and release) in dams and adolescent offspring.

## 2. Materials and methods

### 2.1. Pre-reproductive housing conditions of future mothers

Twenty female 21-day old Wistar rats were randomly assigned to enriched (N = 10) or standard (N = 10) housing conditions.

From post-natal day (pnd) 21 to pnd 72 Enriched Females (EF) were housed in a group of 10 in a large cage (100 × 70 × 90 cm) containing several toys and objects and a running wheel, as described in previous works (Caporali et al., 2014; Caporali and Cutuli et al., 2015; Cutuli et al., 2011, 2017). During the enrichment period the toys and objects were changed twice a week, while the feeding boxes and water bottles were moved to different cage areas once a week in order to encourage explorative behaviors.

Standard Females (SF) were pair-housed in standard cages (36 × 21 × 18 cm) containing wood sawdust, a red plastic tube and no toys. Food and water were delivered *ad libitum* through dispensers kept always in the same position. This procedure prevented an impoverished housing.

A 12/12 h dark/light cycle (light on between 07:00 a.m. and 07:00 p.m.) was applied to both EF and SF groups. On the pnd 72, EF were pair-housed in standard cages to be accustomed to the standard rearing condition. After a week, each EF and SF specimen in oestrus phase (Cutuli et al., 2017) was caged for 5 days with a conspecific standard-reared male rat (≈300 g) to allow mating. Afterwards, male rats were removed, and the females were maintained in standard home cages throughout pregnancy, delivery, and lactation, until offspring's weaning.

### 2.2. Pre-weaning experimental procedures of offspring and mothers

At birth (pnd 0), EF and SF dams' litters were quickly culled to reduce the pups to five males and five females. Litter size ranged from 10 to 14 pups with an average initial male sex ratio of 52.4% for EF and 50.4% for SF. Depending on the pre-reproductive maternal housing conditions, two groups of male and female pups were obtained. Notably, the difference in housing conditions affected the mothers in their pre-reproductive period and not the pups, which were reared in standard conditions.

As described below, after delivery the maternal behavior was observed in undisturbed conditions four times a day (3 during the

light cycle and 1 during the dark cycle) for ten days (from ppd 1 to ppd 10). On ppd 11 dams were tested for maternal aggression and pup-retrieval.

### 2.3. Post-weaning experimental procedures of offspring and mothers

At pups' weaning (pnd 21) dams were sacrificed, while two male and two female pups per dam were selected from each litter and pair-housed in standard conditions for two weeks. In this way, four groups of pups were obtained:

Male pups born to EF (N = 20; group name: ♂EF);
Male pups born to SF (N = 20; group name: ♂SF);
Female pups born to EF (N = 20; group name: ♀EF);
Female pups born to SF (N = 20; group name: ♀SF).

When adolescents (pnd 35), the animals were behaviorally evaluated in the Play behavior test and then sacrificed (pnd 38). For oxytocin quantification 4 dams per group and 3 pups per group (from 3 different mothers) were randomly selected.

The complete experimental design is reported in Fig. 1.

### 2.4. Behavioral testing

#### 2.4.1. Maternal care

*2.4.1.1. Maternal behavior observations.* Rats give birth to altricial newborns that - being helpless, hairless, blind, deaf, and poikilothermic - require an extensive committment of behavior, time, and resources (Kristal, 2009). Because of its elaborate, stereotyped, and comprehensive nature, rats' maternal behavior nicely represents the caretaking behavior of altricial mammalian species (Rosenblatt and Lehrman, 1963).

The behavior of EF and SF dams was observed daily from ppd 1 to ppd 10 (Caldji et al., 1998). Mother-pups interactions were recorded in undisturbed conditions for four 20-min observation periods (09:00 a.m., 12:30 a.m., 04:00 p.m., 07:30 p.m.). The observations collected during the dark phase of the light/dark cycle were performed under dim light illumination.

The following pup-oriented and non-pup-oriented components of maternal behavior were scored for duration and frequency (Cutuli et al., 2015; Ivy et al., 2008; Venerosi et al., 2008; Rees et al., 2004):

**- Pup-oriented Behaviors:**

- Retrieving: the dam picks up each pup in her mouth and carries it to the nest;
- Sniffing: the dam smells one or more pups;
- Licking: the dam licks or grooms any part of the pup's body, primarily the anogenital region;
- Nursing: the dam does not show obvious back-arching and/or leg extension, and only part of the litter is attached to her nipples (*low nursing*), or she is on her side with pups attached to her nipples (*side nursing*);
- Crouching: the dam lays over all pups with her extremities splayed and the back arched. This behavioral category encompasses both *low* and *high crouching* depending on the degree of the arch of the spinal column.

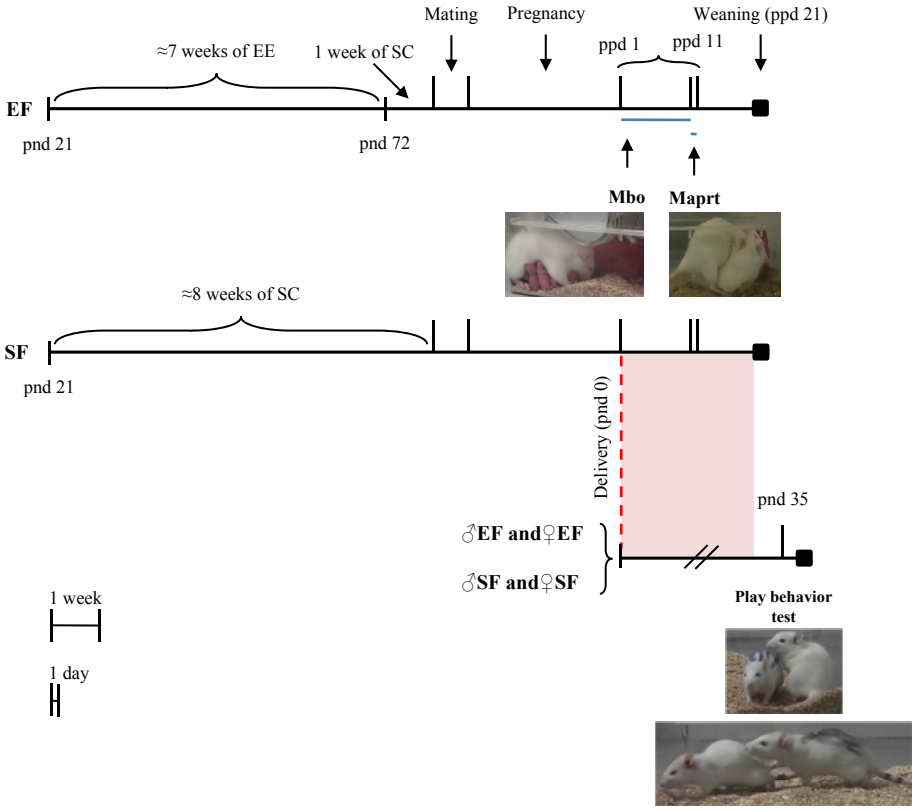


**Fig. 1. Timeline of the experimental procedures.** Experimental groups of female rats according to different pre-reproductive rearing conditions (EF, Enriched Females; SF, Standard Females; EE, Environmental Enrichment; SC, Standard Conditions). Experimental groups of pups (♂EF, male pups born to EF; ♀EF, female pups born to EF; ♂SF, male pups born to SF; ♀SF, female pups born to SF). The same group name abbreviations will be used in the following figure legends. Behavioral testing in dams (Mbo, Maternal behavior observations; Maprt, Maternal aggression and pup-retrieval test) and offspring (Play behavior test). Animals' perfusion and brain extraction for oxytocinergic neuron quantification (■).

- Nest building: the dam pushes and pulls the sawdust or the plastic tube toward the pups to form or adjust the nest.

**- Non-pup-oriented behaviors:**

- Digging: the dam nuzzles in the sawdust out of nest area, pushing and kicking it around using the snout and/or both fore- and hind-paws;
- Grooming: the dam wipes, licks, or scratches any part of its own body;
- Wall rearing: the dam is rearing on hindlimbs, while leaning (or not) with the forelimbs on the cage walls, often sniffing the air;
- Exploring: the dam moves around the cage and sniffs the substrate, but she does not carry pups or nesting material;
- Resting alone: the dam is lying down alone, out of the nest;
- Drink or eat: the dam feeds herself, out of the nest.

**Other behaviors:** behaviors different from the ones previously classified (such as immobility, transitional or mixed behaviors).

Manual scoring of the maternal behavior was performed by a researcher blind to pre-reproductive housing condition of the dams by using EthoVision XT (Noldus, The Netherlands).

*2.4.1.2. Maternal aggression and pup-retrieval test.* Maternal aggression is a protective behavior of the mother aimed at defending the offspring against a potentially dangerous intruder (Bosch, 2013). To test maternal aggression, on ppd 11 an unfamiliar, male intruder (belonging to the male rats used for mating, but not the father of the pups, and used only once) was marked by colored stripes on his back and tail, and placed for 10 min into the dam's home cage between 10:00 a.m. and 01:00 p.m. in bright light conditions. The pups were removed from the cage 2-3 min prior to the behavioral test and maintained together in a small box at 32° ± 1 °C, while the red plastic tube, generally used as nest, was left into the home cage (Venerosi et al., 2008; Wang and Storm, 2011). The removal of the pups from a dam just before an aggressive test does not diminish the expression of maternal aggression (Gammie and Stevenson, 2006). Each test session was video recorded and subsequently manually scored by a researcher blind to pre-reproductive housing condition of the dams by using EthoVision XT (Noldus, The Netherlands).

According to previously published evaluation protocols (Venerosi et al., 2008; Wang and Storm, 2011), duration, frequency, and latency of the following behavioral items were measured:

**- Fighting behaviors:**

- Attack: the dam attacks the intruder male by wrestling, kicking or pushing with forelimbs or biting him in the head, flank, and back regions. Chasing and clawing (without physical contact) were also recorded.
- Defense: the dam is forced into a prone position outside the nest, or she is inside the red plastic tube to prevent male's intrusion and/or to defend herself from his attack.

**- Social behaviors**

- Following: the dam follows the male intruder sniffing his ano-genital region;
- Mutual circling: the male intruder and the dam are sniffing each other's ano-genital regions;
- Social sniffing: the dam is sniffing the male intruder.

At the completion of the test, all pups were randomly scattered in the home cage and the latency to the first sniffing and retrieval of the pups was recorded with a cut-off of 5 min.

*2.4.2. Play behavior test*

In between weaning and puberty, rats display a characteristic form of social interaction known as social play behavior, play fighting or rough-and-tumble play (Vanderschuren et al., 2012). Such a play behavior of adolescent rats (35 days old) was assessed in a neutral standard cage (36 × 21 × 18 cm) with fresh bedding. The experiments took place in an experimental room to which the pups were habituated by having been exposed for 4 h the day before testing. Prior to testing, pups were given back and tail markings to distinguish individuals. The tested pairs encompassed same-sex partners belonging to the same kind of offspring (i.e., couples of female/male rats both born to enriched/standard dams). The partners were not litter- or cagemates.

Each encounter session lasted 15 min and was video recorded by using a frontally mounted video camera. After testing, pups were returned to their home cage.

An observer blind to the animal's experimental group manually scored the videos (EthoVision XT, Noldus, The Netherlands). The total amount of play behavior was scored for each couple of male and female rats. Namely, duration, frequency, and latency of the following behaviors were scored (Argue and McCarthy, 2015; Vanderschuren et al., 2012; van Kerkhof et al., 2013):

- Pouncing: nuzzling the nape of the partner's neck with the tip of the snout followed by a rubbing movement;
- Pinning: one animal is lying with its dorsal surface on the floor with the other animal standing over it;
- Boxing/wrestling: the two animals rapidly push, paw, bite, kick, and grab at each other;
- Chasing: pursuit of the partner which is running away.

Time spent in Social exploration (i.e., sniffing any part of the body of the partner, including the ano-genital area) was further analyzed.

*2.5. Tissue preparation for immunofluorescence*

The animals were deeply anesthetized and a transcardial perfusion with saline (0.9% NaCl) followed by 4% paraformaldehyde fixative in 0.1 M Phosphate-Buffered Saline (PBS; pH 7.4) was performed in order to preserve tissue. The brains were rapidly collected, equilibrated in a 30% sucrose phosphate buffer, frozen in dry ice, and stored at -80 °C.

The regions of interest, PVN and SON, were identified according to the rat stereotaxic atlas (Paxinos and Watson, 1998) and 40 μm coronal sections (from -0.80 to -1.88 mm to bregma) were cut with a freezing microtome and stored at -20° in an antifreeze solution (40% PBS, 30% ethylene glycol, 30% glycerol) until immunofluorescence staining.

Sections were stained for oxytocin labelling using fluorescent method. After a single wash with glycine for 10 min, sections were permeabilized with 0.3% Triton X-100 in PBS for 10 min. Thereafter, sections were incubated in a blocking solution containing 3% normal donkey serum (NDS) in 0.3% Triton X-100 in PBS for an hour to saturate the aspecific sites and then they were incubated in a blocking solution containing a mouse monoclonal anti-oxytocin antibody (1:1000, clone OT-NP, PS38, a generous gift from Dr. Hal Gainer, NIH, Bethesda MD USA) for 16-18 h at room temperature. To visualize the primary antibody, donkey secondary antibodies conjugated to Cy3 against mouse were used (Jackson Immunoresearch, West Grove, USA; 1:200 in PBS). Nuclei were observed incubating sections with Hoechst (1:500).

Cell numbers in PVN and SON were obtained with stereological analysis, by counting cells visualized with confocal microscopy throughout the whole rostrocaudal extent of these structures in free-floating coronal sections. Cell numbers obtained for each PVN and SON were divided for the corresponding area of the section, in order to obtain the average number of PVN or SON cells per square millimeter. Areas were obtained by tracing the outline of the whole PVN, or SON, identified by the presence of cell nuclei stained by Hoechst 33258 on a digital picture captured and measured using the I.A.S. software (Delta Sistemi, Rome, Italy).

### 2.6. Ethical issues

All efforts were made to minimize animal suffering and reduce the number of animals that were used, per the European Directive (2010/63/EU). All procedures were approved by the Italian Ministry of Health.

### 2.7. Statistical analysis

Statistical analyses were performed by using STATISTICA 7.0 (StatSoft). Since behavioral data did not conform to assumptions of normality, they were analyzed with non-parametric analyses of variance (Kruskal-Wallis H and Mann-Whitney U tests). Biochemical data were analyzed using Student's T-test.

To control for the alpha inflation the proportion of type I errors among all rejected null hypotheses, the False Discovery Rate (FDR) was set to 0.05. The FDR was estimated through the procedure described by Storey and Tibshirani (2003). The bootstrap procedure was used to estimate the $\pi$ 0 parameter (Storey, 2004). In our results, the 0.05 level of significance corresponded to an FDR $\leq$0.043.

## 3. Results

### 3.1. Maternal care

#### 3.1.1. Maternal behavior observations

EF emitted pup-oriented behaviors significantly longer than SF (Fig. 2). Conversely, SF displayed higher levels of non-pup-oriented behaviors. No differences between dam groups were found in frequency of pup-oriented and other behaviors, and in duration of other behaviors.

Detailed analyses on the single pup-oriented behaviors demonstrated that EF exhibited more Crouching and less Sniffing than SF (Fig. 3; Table 1A).

As for non-pup-oriented behaviors, EF dams emitted less Exploring, Wall Rearing, Resting alone, and Drink or eat than SF dams (Fig. 3; Table 1B). No differences between dam groups were evident in the remaining behaviors (Table 1C).

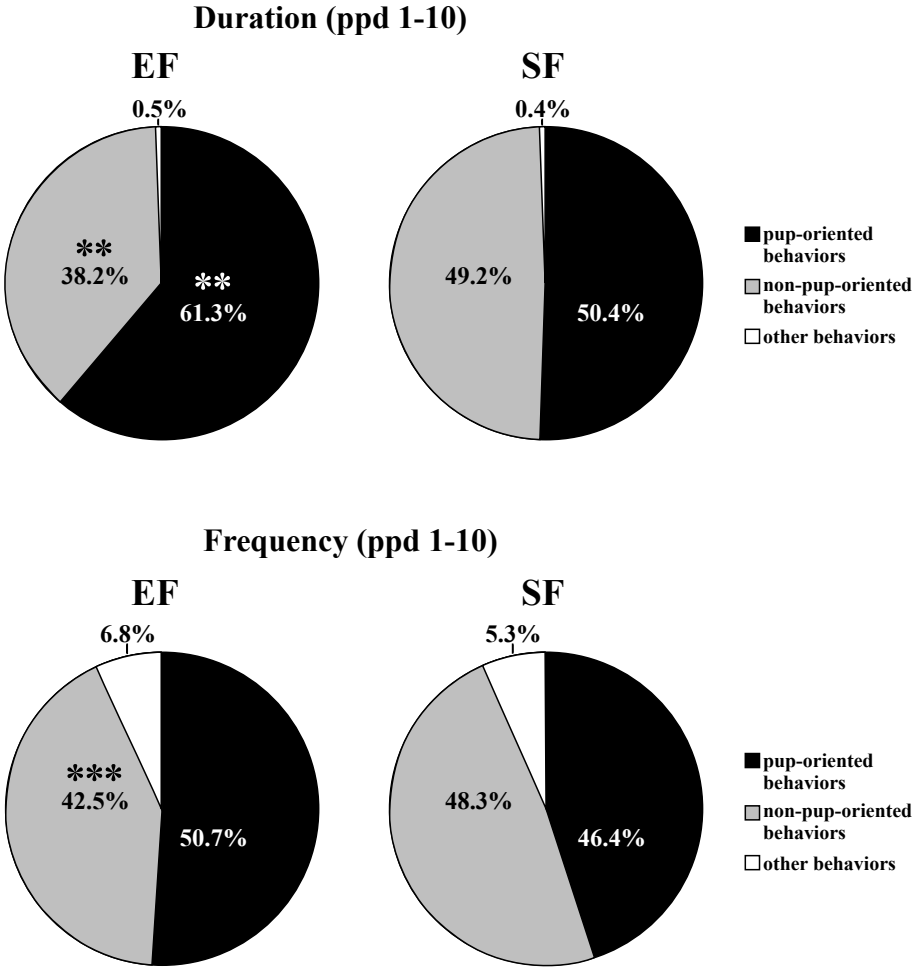


**Fig. 2. Maternal behavior observations.** Sum of duration and frequency of pup-oriented, non-pup-oriented, and other behaviors showed by EF and SF during the first ten *postpartum* days (ppd). Pie charts represent the percentage of each observed behavioral category. Results were obtained by performing Mann-Whitney U analyses. The asterisks represent the significance level of the differences between dam groups in each behavioral category. **p < 0.01, ***p = 0.001.

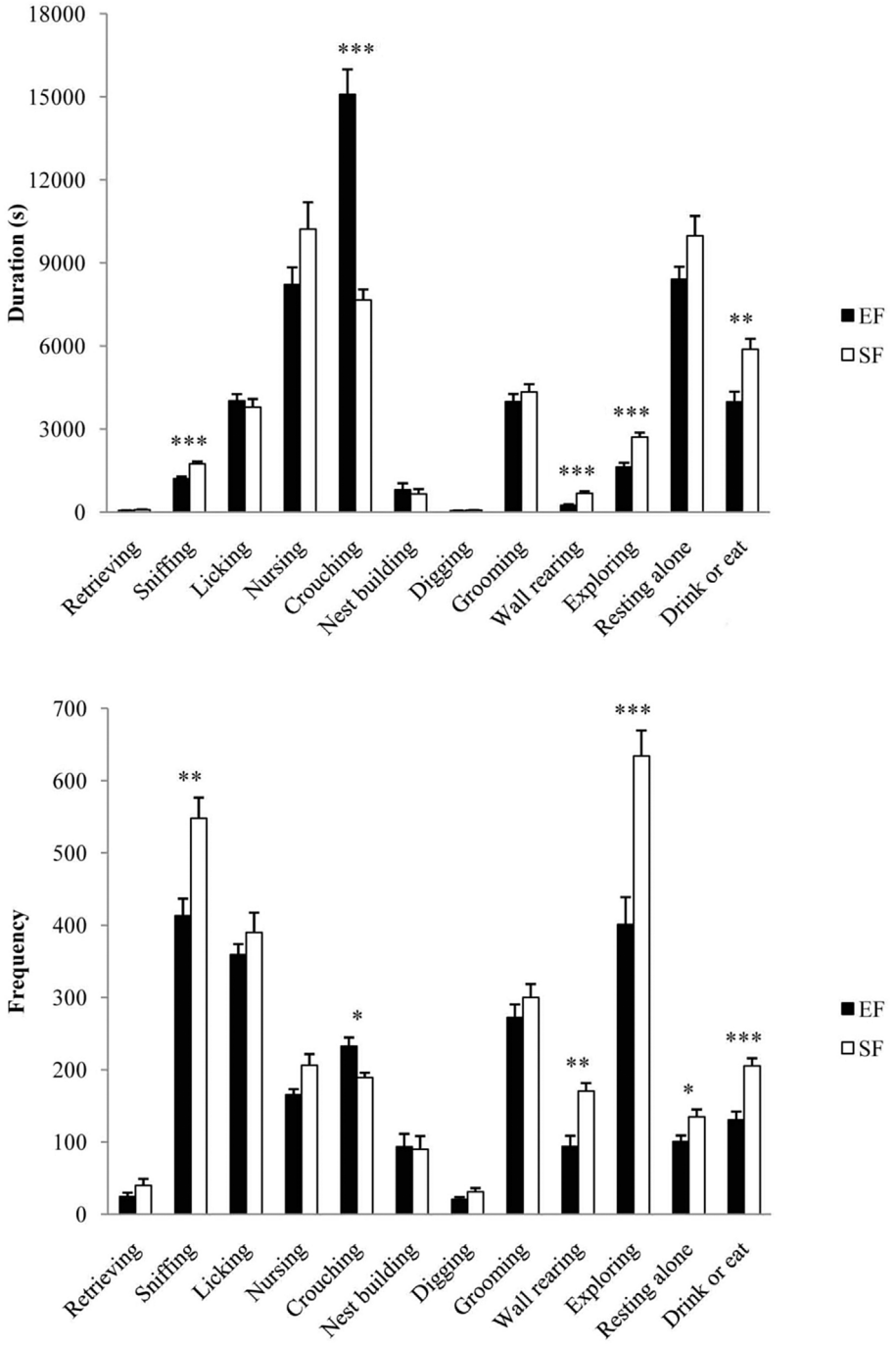


**Fig. 3. Pup-oriented and non-pup-oriented behaviors.** Total duration and frequency of pup-oriented and non-pup-oriented behaviors showed by EF and SF during the first ten *post-partum* days. Results were obtained by performing Mann-Whitney U analyses. *p < 0.05, **p < 0.01, ***p < 0.001.

### 3.1.2. Maternal aggression and pup-retrieval test

No differences were observed between EF and SF dams in Fighting and Social behaviors (Fig. 4A-E; Table 2). When the pups were returned to the mother after the encounter with the male intruder, all dams similarly sniffed the pups within the first 4 s (EF dams = 3.948 s ± 1.325 s; SF dams = 3.738 s ± 0.931 s; U = 46, Z = -0.302, p = 0.762). As for retrieving, 7 out of 10 EF dam and 4 out of 10 SF dams retrieved the pups ($\chi^2 = 1$, d. f. = 1, p = 0.317). Interestingly, EF dams retrieved the first pup significantly earlier than SF dams (U = 22, Z = -2.219, p = 0.026; Fig. 4F).

### 3.2. Play behavior test

Kruskal-Wallis tests performed on the total amount of rough-and-tumble play behavior revealed significant differences among the four groups of pups born to enriched and standard dams (♂EF, ♀EF, ♂SF, and ♀SF; Table 3). Namely, while in pups born to SF total duration and frequency of play behavior was higher in males than in females, in pups born to EF no sex difference was observed.

More detailed analyses performed on specific play behavior components demonstrated significant differences among adolescent pup groups, namely in Pouncing and Chasing. Conversely,

**Table 1**
**Maternal behavior.** Results of the Mann-Whitney U analyses performed on pup-oriented (A), non-pup-oriented (B), and other (C) behaviors emitted by EF and SF from ppd 1 to ppd 10.

| A. Pup-oriented behaviors | | |
|---|---|---|
| | Duration | Frequency |
| Sum (ppd1-10) | U = 10, Z = 3.024, **p=0.002** | U = 31, Z = -1.436, p = 0.151 |
| Retrieving (ppd 1-10) | U = 36, Z = -1.058, p = 0.290 | U = 37, Z = -0.983, p = 0.325 |
| Sniffing | U = 8, Z = -3.175, **p=0.0007** | U = 12, Z = -2.872, **p=0.004** |
| Licking | U = 41, Z = 0.680, p = 0.496 | U = 38.5, Z = -0.869, p = 0.385 |
| Nursing | U = 30, Z = -1.512, p = 0.131 | U = 26, Z = -1.814, p = 0.069 |
| Crouching | U = 0, Z = 3.779, **p=0.0002** | U = 19, Z = 2.343, **p=0.019** |
| Nest Building | U = 43, Z = 0.529, p = 0.597 | U = 49, Z = 0.076, p = 0.939 |
| **B. Non-pup-oriented behaviors** | | |
| | Duration | Frequency |
| Sum (ppd 1-10) | U = 10, Z = -3.024, **p=0.002** | U = 8, Z = -3.175, **p=0.001** |
| Digging | U = 38, Z = -0.907, p = 0.364 | U = 34, Z = -1.209, p = 0.226 |
| Grooming | U = 39, Z = -0.831, p = 0.406 | U = 37.5, Z = -0.945, p = 0.344 |
| Wall Rearing | U = 1, Z = -3.704, **p=0.0002** | U = 11, Z = -2.948, **p=0.003** |
| Exploring | U = 4, Z = -3.477, **p=0.0005** | U = 6, Z = -3.326, **p=0.0009** |
| Resting alone | U = 26, Z = -1.814, p = 0.069 | U = 22, Z = -2.117, **p=0.034** |
| Drink or eat | U = 13, Z = -2.797, **p=0.005** | U = 5, Z = -3.402, **p=0.0007** |
| **C. Other behaviors** | | |
| | Duration | Frequency |
| Sum (ppd 1-10) | U = 49, Z = 0.076, p = 0.939 | U = 46, Z = 0.303, p = 0.762 |

Pinning did not occur in females and occurred too infrequently or briefly for analyses in males (duration: ♂EF = 0.417 s ± 0.329 s; ♂SF = 0.876 s ± 0.529 s; frequency: ♂EF = 0.250 ± 0.197; ♂SF = 0.375 ± 0.207; latency: ♂EF = 842.467 s ± 45.382 s; ♂SF = 779.313 s ± 65.585 s).

As for male offspring (♂EF *vs.* ♂SF), ♂EF displayed less duration and frequency of the total social play behavior than ♂SF (Fig. 5A). In particular, Pouncing was the behavior emitted to a lower extent by ♂EF (Fig. 5B), with no differences in Boxing/wrestling and Chasing.

As for female offspring (♀EF *vs.* ♀SF), no differences were observed in total duration and frequency of play behavior (Fig. 5A). Anyway, a more in-depth analysis revealed some qualitative differences. In fact, ♀EF displayed more Pouncing and less Chasing than ♀SF (Fig. 5B). No differences were evident in Boxing/wrestling.

When male and female pups were compared depending on maternal housing (♂EF *vs.* ♀EF and ♂SF *vs.* ♀SF) significant differences were evident in Pouncing and Chasing behaviors. In particular, ♂EF showed shorter Pouncing duration and began Chasing earlier than ♀EF (Fig. 5B). On the contrary, ♂SF emitted more and earlier Pouncing in comparison to ♀SF (Fig. 5B).

No differences were found in Social exploration among the experimental groups (Suppl. Table).

### 3.3. Oxytocinergic neurons

EF showed a superior number of oxytocinergic neurons in both PVN (p = 0.036) and SON (p = 0.043) in comparison to SF (Fig. 6A-F). As for offspring, ♂EF displayed higher levels of oxytocinergic neurons in SON (p = 0.012), but not in PVN (p = 0.418) compared to ♂SF (Fig. 6G-L). No differences in oxytocinergic neurons were found in female offspring neither in PVN (p = 0.717) nor in SON (p = 0.175; Fig. 6M,N). No sex-dependent differences were found in the number of oxytocinergic neurons in PVN (♂EF *vs.* ♀EF: p = 0.09; ♂SF *vs.* ♀SF: p = 0.653) and SON (♂EF *vs.* ♀EF: p = 0.596; ♂SF *vs.* ♀SF: p = 0.385).

## 4. Discussion

In the present study, we evaluated the effects of pre-reproductive exposure to EE of female rats on their maternal care and on adolescent offspring's social play behavior. We analyzed the modifications induced by maternal enrichment on hypothalamic oxytocinergic neurons of both dams and pups.

In agreement with our previous studies (Caporali et al., 2014; Caporali and Cutuli et al., 2015; Cutuli et al., 2015, 2017) the pre-reproductive maternal EE modified behavioral and neural phenotype of both dams and pups. In fact, during the observation period enriched females exhibited higher levels of pup-oriented behaviors, especially Crouching. No differences in fighting and social behaviors were detected during maternal aggression test, but enriched dams retrieved the first pup earlier than standard females after the encounter with the male intruder. These behavioral features were associated with an increased number of oxytocinergic neurons either in PVN and SON. In addition, pre-reproductive exposure to EE of female rats cross-generationally influenced the social phenotype of their offspring in a sex-specific manner. In fact, male pups born to enriched females exhibited reduced play fighting behaviors and higher levels of oxytocinergic neurons in SON in comparison to male pups born to standard females. Conversely, no differences in the total amount of play behavior and number of oxytocinergic neurons was observed in female offspring born to enriched or standard females. Moreover, while the male offspring of standard dams showed higher levels of play behavior than females, the total amount of play behavior observed in male and female offspring of enriched dams was comparable. When the single play behavior components were analyzed, a different pattern emerged between male and female offspring of enriched dams, with males emitting less Pouncing, and females emitting more Pouncing and less Chasing.

### 4.1. Maternal care

In mammals the ultimate goal of reproductive behavior is the

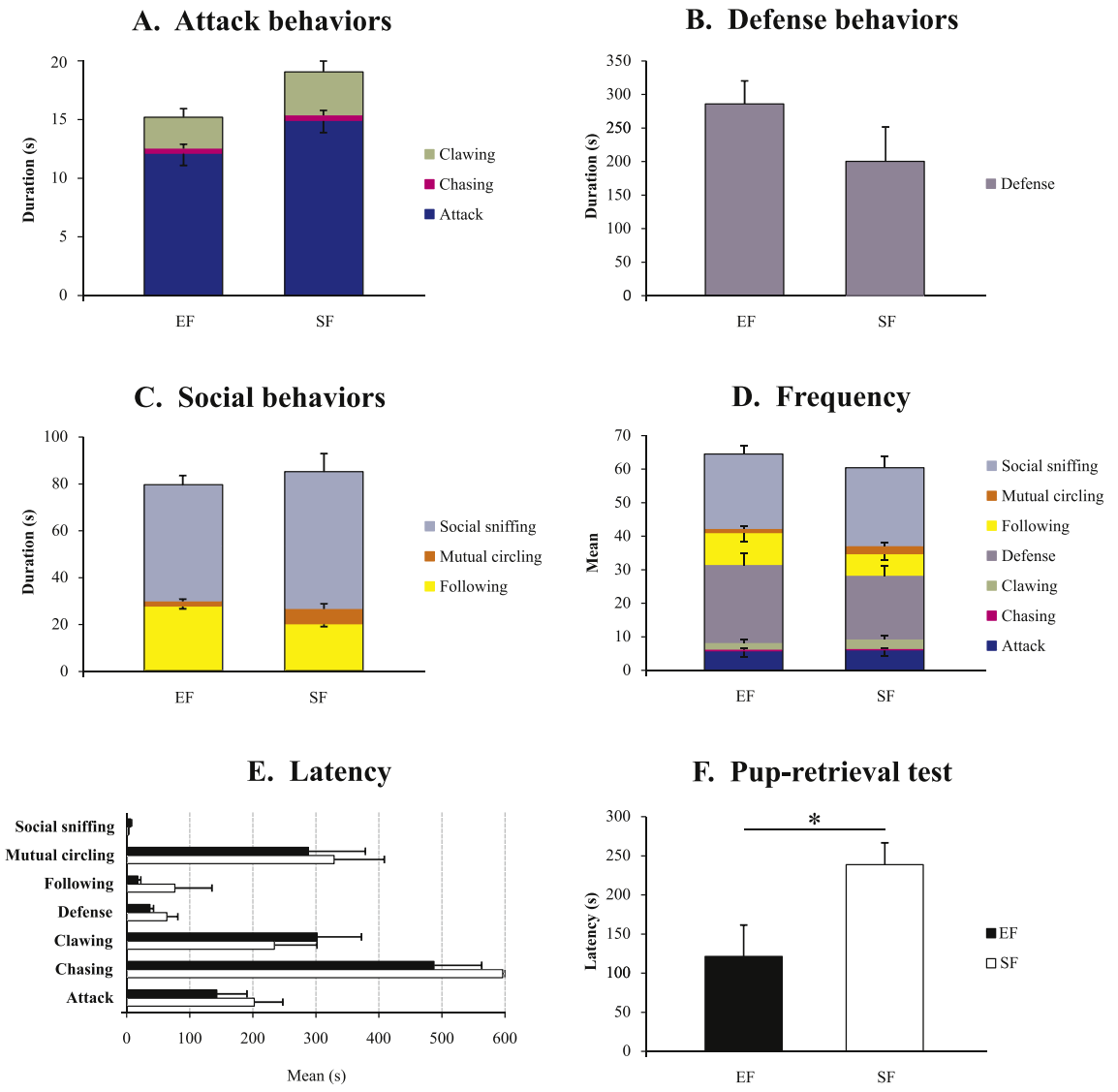


**Fig. 4. Maternal aggression and pup-retrieval test.** Duration, frequency, and latency of attack, defense, and social behaviors (A-E), and latency to retrieve the first pup (F) showed by EF and SF. Results were obtained by performing Mann-Whitney U analyses. *p < 0.05.

**Table 2**
**Maternal aggression.** Results of the Mann-Whitney U analyses performed on fighting and social behaviors emitted by EF and SF during maternal aggression test on ppd 11.

| Fighting behaviors | | | |
|---|---|---|---|
| | Duration | Frequency | Latency |
| Attack | U = 43, Z = -0.529, p = 0.596 | U = 47.5, Z = -0.191, p = 0.849 | U = 29, Z = -1.587, p = 0.112 |
| Chasing | U = 50, Z = 0, p = 1 | U = 48.5, Z = 0.162, p = 0.871 | U = 48, Z = -0.216, p = 0.829 |
| Clawing | U = 37, Z = -0.987, p = 0.324 | U = 34, Z = -1.225, p = 0.220 | U = 35.5, Z = 1.100, p = 0.271 |
| Defense | U = 35, Z = 1.134, p = 0.257 | U = 37.5, Z = 0.948, p = 0.343 | U = 43, Z = -0.529, p = 0.597 |
| Social behaviors | | | |
| | Duration | Frequency | Latency |
| Following | U = 37, Z = 0.983, p = 0.326 | U = 35.5, Z = 1.101, p = 0.271 | U = 47, Z = -0.227, p = 0.821 |
| Mutual circling | U = 37, Z = -1.015, p = 0.309 | U = 40, Z = -0.791, p = 0.429 | U = 41, Z = -0.703, p = 0.482 |
| Social sniffing | U = 37, Z = -0.983, p = 0.326 | U = 44.5, Z = -0.416, p = 0.677 | U = 26, Z = 1.814, p = 0.069 |

transmission of genetic information from one generation to the next. To succeed, adequate parental care and successful rearing of offspring until to reproductive age are indispensable. The maternal care in rats consist of a complex constellation of predictable and coordinated behaviors exhibited in preparation for the arrival of newborn as well as in the care and protection of the newborns (Kristal, 2009). In the present study we looked at maternal behavior since it is involved in epigenetic modifications underlying cross-generational inheritance.

Growing evidence indicates that EE can enhance maternal care

**Table 3**
**Play behavior test.** Results of the Kruskal-Wallis H and Mann-Whitney U tests performed on social play behaviors emitted by ♂EF, ♀EF, ♂SF, and ♀SF pups on pnd 35.

| A. Differences among the 4 groups | | | |
|---|---|---|---|
| | Duration | Frequency | Latency |
| Total play behavior | H (3, N = 40) = 11.305, p = **0.010** | H (3, N = 40) = 12.842, p = **0.005** | |
| Pouncing | H (3, N = 40) = 22.672, p < **0.00001** | H (3, N = 40) = 17.404, p = **0.0006** | H (3, N = 40) = 12.475, p = **0.006** |
| Boxing/wrestling | H (3, N = 40) = 2.803, p = 0.423 | H (3, N = 40) = 3.141, p = 0.370 | H (3, N = 40) = 3.320, p = 0.345 |
| Chasing | H (3, N = 40) = 5.659, p = 0.129 | H (3, N = 40) = 13.370, p = **0.004** | H (3, N = 40) = 14.726, p = **0.002** |
| B. Sex-dependent differences (♂EF*vs.*♀EF; ♂SF *vs.*♀SF) | | | |
| ♂EF*vs.*♀EF | | | |
| | Duration | Frequency | Latency |
| Total play behavior | U = 48, Z = 0.152, p = 0.879 | U = 31.5, Z = -1.405, p = 0.160 | |
| Pouncing | U = 14, Z = -2.733, p = **0.006** | U = 40, Z = -0.760, p = 0.447 | U = 38, Z = 0.911, p = 0.362 |
| Boxing/wrestling | U = 29, Z = 1.616, p = 0.106 | U = 29, Z = 1.658, p = 0.097 | U = 31, Z = -1.462, p = 0.144 |
| Chasing | U = 39, Z = 0.835, p = 0.404 | U = 26, Z = 1.825, p = 0.068 | U = 11, Z = -2.999, p = **0.003** |
| ♂SF *vs.*♀SF | | | |
| | Duration | Frequency | Latency |
| Total play behavior | U = 10, Z = -3.031, p = **0.002** | U = 12, Z = -2.889, p = **0.004** | |
| Pouncing | U = 8, Z = 3.182, p = **0.001** | U = 8, Z = 3.183, p = **0.001** | U = 4, Z = -3.485, p = **0.0005** |
| Boxing/wrestling | U = 38, Z = 0.970, p = 0.332 | U = 40, Z = 0.809, p = 0.418 | U = 40, Z = -0.809, p = 0.419 |
| Chasing | U = 46, Z = 0.303, p = 0.762 | U = 24, Z = 1.980, p = 0.048 | U = 24, Z = 1.970, p = 0.049 |
| C. Mother-dependent differences (♂EF*vs.* ♂SF; ♀EF*vs.*♀SF) | | | |
| ♂EF*vs.* ♂SF | | | |
| | Duration | Frequency | Latency |
| Total play behavior | U = 21, Z = -2.194, p = **0.028** | U = 21.5, Z = -2.158, p = **0.031** | |
| Pouncing | U = 12, Z = -2.875, p = **0.004** | U = 13.5, Z = -2.768, p = **0.006** | U = 32, Z = 1.362, p = 0.173 |
| Boxing/wrestling | U = 36, Z = -1.073, p = 0.283 | U = 48, Z = -0.154, p = 0.877 | U = 38, Z = -0.920, p = 0.358 |
| Chasing | U = 38, Z = -0.908, p = 0.364 | U = 29, Z = -1.590, p = 0.112 | U = 31, Z = -1.437, p = 0.151 |
| ♀EF*vs.*♀SF | | | |
| | Duration | Frequency | Latency |
| Total play behavior | U = 44, Z = 0.456, p = 0.648 | U = 40, Z = -0.765, p = 0.444 | |
| Pouncing | duration: U = 0, Z = 3.801, p = **0.0001** | U = 9, Z = 3.122, p = **0.002** | U = 8, Z = -3.193, p = **0.001** |
| Boxing/wrestling | U = 48, Z = 0.162, p = 0.871 | U = 50, Z = 0, p = 1 | U = 48, Z = -0.162, p = 0.871 |
| Chasing | U = 26, Z = -1,824, p = 0.068 | U = 15, Z = -2.673, p = **0.007** | U = 10, Z = 3.041, p = **0.002** |

by increasing nursing, licking, Licking/Grooming (LG), and Arched-Back Nursing (ABN) behaviors (Cancedda et al., 2004; Champagne and Meaney, 2007; Curley et al., 2009; Rosenfeld and Weller, 2012; Sale et al., 2004; Zuena et al., 2016), especially during the first week of lactation, and by modifying gene expression within the hypothalamus (Mashoodh et al., 2012). Recent works from our laboratory performed on ppd 1 - either by using a brief mother-pups separation as eliciting condition (Cutuli et al., 2015) or even in undisturbed conditions (Cutuli et al., 2017) - demonstrated that pre-reproductive maternal EE induces higher levels of pup-directed behaviors (e.g., licking, crouching, nest building activities), accompanied by increased BDNF levels in the frontal cortex (Cutuli et al., 2015). Here, we demonstrate that the early maternal care modifications induced by pre-reproductive maternal EE persist during the first 10 days of lactation. In fact, enriched dams emitted longer total amount of pup-oriented behaviors, in particular Crouching (two-fold higher than in SF), and exhibited non-pup-oriented behaviors (i.e., Exploring, Wall rearing, Resting alone, Drink or eat) more shortly and less frequently than standard dams.

Crouching is the most active and complex nursing posture that allows the mother to nourish and contact the entire litter. It is noteworthy to underlie the parallelism between the increase in the ABN component described by Meaney's group (Champagne and Meaney, 2007; Weaver et al., 2004) and the increase in Crouching behavior observed in EF. In both cases modifications in this maternal behavior are linked to differences in the offspring's behavioral performances. Our data are also in line with the increase in maternal behavior following the exposure to EE during and before gestation found by other researchers, especially in the earlier phases of lactation (Cancedda et al., 2004; Rosenfeld and Weller, 2012; Sale et al., 2004; Zuena et al., 2016). Nevertheless, for the sake of completeness, it has to be evidenced that some other studies investigating the effects of EE on maternal behavior reported no differences or even the reduction in specific maternal behaviors, such as the presence in the nest, nursing, and LG-ABN (Connors et al., 2015; Rosenfeld and Weller, 2012; Welberg et al., 2006; Zuena et al., 2016). However, since the EE protocols, exposure timing, strain and species used, and phase of lactation observed were different in the various researches, it is not surprising that divergent data were found.

In the present study pre-reproductively enriched females were also tested for the maternal aggression, a highly conserved behavior for protecting offspring. In line with a previous study (Friske and Gammie, 2005), we did not find any EE-induced difference in the measures of maternal aggression, while we observed that the enriched dams initiated pup-retrieval significantly more quickly than the standard dams. This finding confirms the enhanced proneness to contact the offspring we found during maternal behavior observations, and suggests a higher salience of pups for the enriched dams.

### 4.2. Play behavior in the adolescent offspring

Social play behavior starts around weaning and declines with

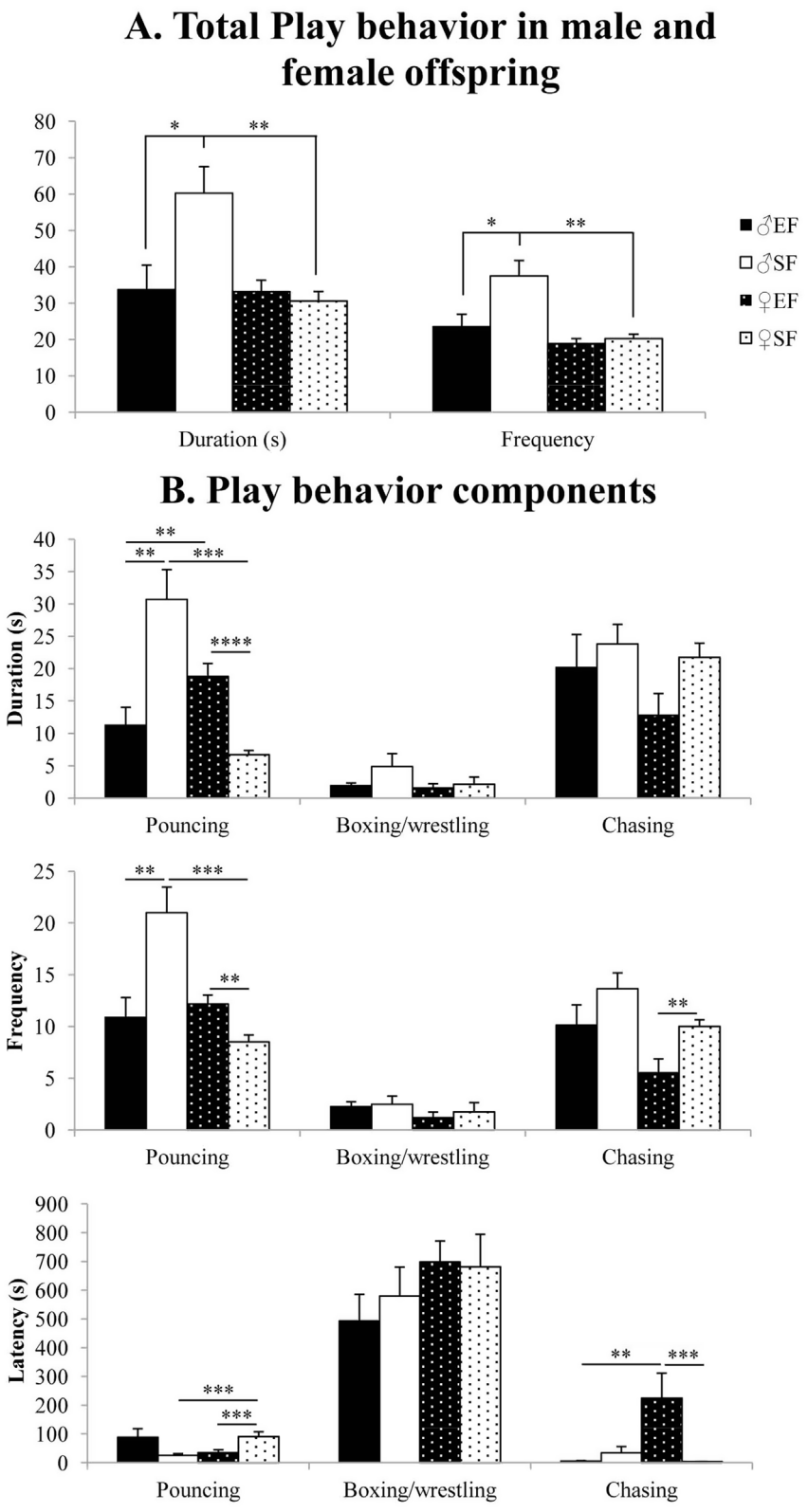


**Fig. 5. Play behavior test.** Duration, frequency, and latency of total Play behavior (A) and Play behavior components (B) emitted by ♂EF, ♀EF, ♂SF, and ♀SF pups on pnd 35. Results were obtained by performing Kruskal-Wallis H and Mann-Whitney U analyses. *$p < 0.05$, **$p < 0.01$, ***$p \leq 0.001$, ****$p < 0.0001$.

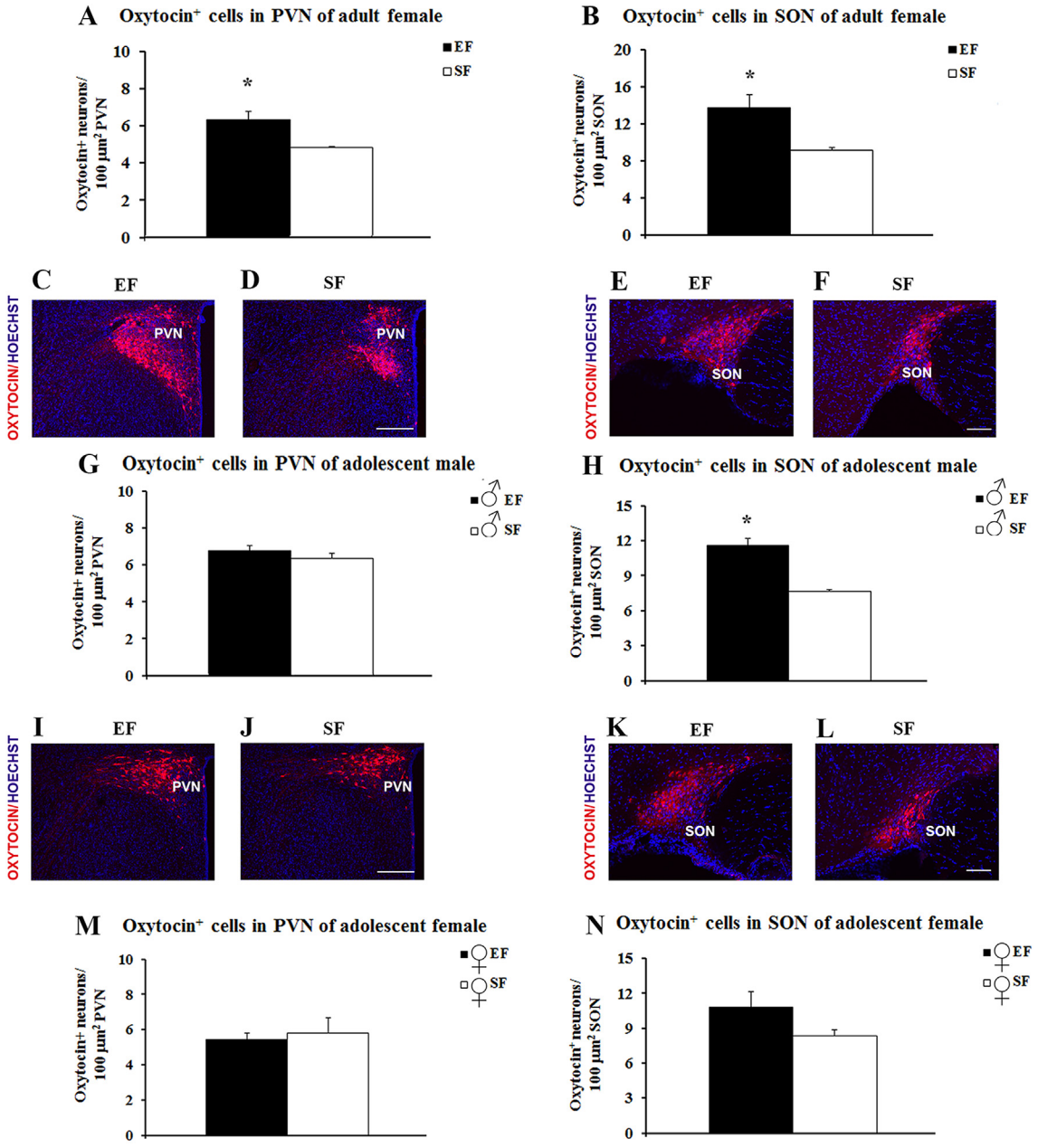


**Fig. 6. Oxytocinergic neurons.** Number of oxytocin-expressing neurons in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON). A-B. Graphics showing the increase in oxytocin-expressing neurons in PVN (A) and in SON (B) of EF. C-F. Representative images illustrating the rise of oxytocin-expressing neurons in PVN and in SON of EF. G, I, J. Diagram (G) and representative pictures (I,J) showing the number of oxytocin-expressing neurons in PVN of ♂EF and ♂SF. H, K, L. Diagram (H) and representative pictures (K,L) showing the enhanced number of oxytocin-expressing neurons in SON of ♂EF. M, N. Diagrams showing the number of oxytocin-expressing neurons in PVN (M) and SON (N) of ♀EF and ♀SF. Bars: 250 µm (panels C, D, I, J); 100 µm (panels E, F, K, L). Results were obtained by performing Student's T-tests. *$p < 0.05$.

the approach of sexual maturation (Vanderschuren et al., 1997). It is a complex behavior that requires both the ability to initiate social interactions and to respond appropriately to social signals from others (Argue and McCarthy, 2015). It is important for social and cognitive development, and it is highly rewarding being modulated by neural systems involved in reward and motivation, such as the opioid and dopaminergic systems (Pellis and Pellis, 2009; Vanderschuren et al., 1997). Rats are known to exhibit sex differences in rough-and-tumble play, with some studies reporting higher amounts of play in males (Argue and McCarthy, 2015; Lundberg et al., 2017; Olioff and Stewart, 1978; Zuena et al., 2016) and others in females, or no sex differences (Himmler et al., 2014; Panksepp, 1981; Panksepp and Beatty, 1980; Pellis et al., 1997; Thor and Holloway, 1984).

In the present study the adolescent offspring of female rats exposed to pre-reproductive EE exhibit remarkable peculiarities in social play behavior. The total amount of social play behavior was reduced in the male offspring of enriched dams, making it comparable to the female offspring's one. In spite of the similarity in its total amount, the pattern of play observed in the male and female offspring of enriched dams was different. In fact, males exhibited less Pouncing, while females displayed more Pouncing and less Chasing. These findings suggest that sex dimorphisms in brain and behavior development might be modified by pre-reproductive EE probably due to neuroendocrine factors (i.e., steroids, stress hormones, oxytocin), different maternal care during the neonatal

phase, or both. A different experimental design, like cross-fostering procedure, could help in disentangling the relative contribution of pre- and post-natal factors to such evident “feminization” of play behavior in male offspring.

In literature there is evidence for the influence of different EE protocols on offspring's social behavior in rats. Namely, as in the present study, pre-reproductive maternal EE reduces social interaction in male, but not in female progeny (Leshem and Schulkin, 2012). These findings indicate that social behavior is more vulnerable to transgenerational EE effects in male than female rats, consistently with the generally increased vulnerability of male rats to developmental disruption of social behavior (such as by gestational alcohol; Mooney and Varlinskaya, 2011).

Mixed results are reported for maternal EE exposure before and during gestation given in one study this enrichment protocol increased social play behavior in male, but not in female pups (Zuena et al., 2016), and in another one it specifically increased social contact time in the female offspring (Connors et al., 2015). Moreover, post-natal EE increased social interaction in both male and female rats (Leshem and Schulkin, 2012).

Interestingly, also variations in maternal care are associated with alterations in offspring's play fighting. For instance, reduced maternal LG stimulation selectively increase play fighting and aggressive social grooming in the male pups (Parent and Meaney, 2008), likely by increasing their sensitivity for the stimulating effects of play behavior in periadolescence. Besides social behavior alterations, low LG male offspring show also increased anxiety and sensitivity to stressful events, and reduced cognitive ability (Bredy et al., 2003; Caldji et al., 1998; Liu et al., 1997). These findings seem to be specular to the ones reported in our studies. In fact, in the present research increased maternal stimulation (i.e., more Crouching) selectively decreased play behavior in male pups born to enriched females. This behavioral pattern was not accompanied by alterations in Social exploration and in the motivation to approach a conspecific (Cutuli et al., 2015). Further, we previously demonstrated that male pups born to enriched females exhibited more active coping skills (Cutuli et al., 2017), and ameliorated motor (Caporali et al., 2014) and cognitive performances (Cutuli et al., 2015). Thus, it can be advanced that the increased maternal care received by enriched dams' offspring in the early phase of lactation may have affected male pups' behavioral phenotype by decreasing their sensitivity for the stimulating effects of play behavior.

The reduction in social play - and in particular in play solicitation (i.e., Pouncing) - exhibited by the enriched dams' male offspring appears thus as an intrinsic behavioral feature (rather than a mere behavioral deficit), possibly linked to a less rewarding value of play initiation (Vanderschuren et al., 1997) or to neurohormonal modifications, as discussed in the following section. Conversely, female offspring from EF were more prone to soliciting rough-and-tumble play by displaying more Pouncing than females from SF. Future studies aimed to clarify the underlying mechanisms of sex differences in play behavior induced by pre-reproductive maternal enrichment should take into account the role of differential maternal investment in male and female pups as well as the link among oxytocin, endocannabinoid system, and social reward (Argue et al., 2017; Trezza et al., 2012; Wei et al., 2015).

### 4.3. Oxytocinergic neurons

The neuropeptide oxytocin has been widely implicated in the regulation of social behavior in mammalian species, from rodents to humans. Oxytocin modulates maternal care, infant behavior, social bonding, sexual and agonistic behavior, social recognition, and group membership (Bosch, 2013; Crespi, 2016; Lim and Young, 2006). It is synthesized in magnocellular neurons of the hypothalamic PVN and SON, and it is transported along their axons to the posterior pituitary and released from there into the blood stream to act on target organs in the periphery (Veenema, 2012). Oxytocin synthesized in the PVN and SON is also released in forebrain and hindbrain regions (Veenema, 2012). Oxytocin exerts its effects on social behavior via activation of the oxytocin receptors expressed in many brain regions (including neocortical, limbic, hypothalamic, and brain stem areas) (Lee et al., 2009; Veenema, 2012). All oxytocinergic neurons in the SON are magnocellular neuroendocrine neurons, while the PVN also contains parvocellular oxytocinergic neurons that project within the brain (Sofroniew, 1980). It is unclear to what extent the influence of oxytocin on social behavior is attributable to the parvocellular or magnocellular oxytocinergic systems, because many of the action sites of oxytocin lack conspicuous innervation by oxytocin-containing fibers. Accordingly, it seems likely that oxytocin reaches these sites by volume transmission following release from possibly quite distant sites.

Neither sex differences in the oxytocin brain system nor sex-specific regulation of social behavior have been extensively clarified. The limited data in rodents and humans indicate that oxytocin synthesis in the hypothalamus is similar in both sexes, and there are few studies that have compared oxytocin receptors in the brain between males and females (Shansky, 2016). For example, Dumais et al. (2016) recently showed that, while baseline oxytocin release does not differ between sexes, male rats have a three-fold higher oxytocin receptor binding density than female rats in the posterior bed nucleus of the stria terminalis (pBNST), a sexually dimorphic area projecting to PVN neurons and implicated in the regulation of social behaviors. Oxytocin administration into this area prolongs the duration of social recognition in males only. Growing evidence in monkeys and humans indicates that oxytocin may produce opposite sex-specific effects (Gao et al., 2016). In particular, oxytocin tends to facilitate positive social judgments, social approach, kinship recognition, and altruism in women, while it can facilitate negative social judgments, social avoidance, competitor recognition, and selfishness in men (Gao et al., 2016). The neural basis of these sex-dependent behavioral effects of oxytocin has not yet been established.

Our findings demonstrate the influence of pre-reproductive maternal EE on the hypothalamic oxytocinergic neurons. In fact, enriched dams exhibited an increased number of oxytocinergic neurons in both PVN and SON. Further, their male offspring showed higher levels of oxytocinergic neurons in SON, but not in PVN, while the female offspring was unaltered. While it is well-known that oxytocin treatment increases the responsiveness of females to pups (Bales et al., 2007; Mogi et al., 2014) and the sociability during the peripuberal period (Bales et al., 2013, 2014; Bowen et al., 2011), less is known about the behavioral effects of environmental factors on the endogenous release of brain oxytocin. In fact, the literature upon the relations among enrichment, maternal care, and oxytocinergic modifications in the dams and offspring is sparse, and mainly takes into account oxytocin receptors. For example, the increased maternal behavior found in communally reared (Curley et al., 2009) and high LG-ABN (Francis et al., 2000) females is associated with an increase in oxytocin receptor expression in the lateral septum and central nucleus of the amygdala, respectively. In adult female, but not adult male offspring of high LG mothers oxytocin receptor binding is increased in the central nucleus of the amygdala and BNST (Francis et al., 2002). Post-weaning social enrichment enhances LG and oxytocin receptor binding of low LG offspring across generations (Champagne and Meaney, 2007).

Interestingly, a recent study demonstrated that oxytocin enables pup retrieval behavior by increasing the auditory cortical pup call responses in the dams (Marlin et al., 2015). Moreover, stable

individual differences in pup LG are abolished by oxytocin receptor blockade, and high LG mothers showed increased oxytocin expression in the medial preoptic area and PVN, and increased projections of oxytocin-positive cells from both these hypothalamic areas to the ventral tegmental area (Shahrokh et al., 2010). Thus, the increased maternal care of EF could be linked to their increased number of oxytocinergic neurons. Given the consistent oxytocin-dopamine interactions in the establishment and maintenance of social bonds (i.e., both motivation for maternal care and offspring's solicitation of care) involving midbrain dopaminergic reward systems (Crespi, 2016), the enriched females could be more attracted by their pups because of a more rewarding effect of nurturing behaviors. In turn their pups, especially the males one showing higher hypothalamic oxytocin levels, may be more motivated to the contact with their mother and thus more soliciting. Unfortunately, in the present study we cannot distinguish if the pre-reproductive maternal EE has potentiated oxytocinergic system of enriched females directly before gestation or indirectly after parturition, when they emit more pup-oriented behaviors. In the same manner, we are not able to define if mothers emit increased maternal care because their pups are more soliciting, and, if that were the case, we cannot define if male or female pups are more soliciting, and thus more contacted, since we have always analyzed the interactions between the mother and her entire litter.

Anyway, when adolescent, the male pups of enriched dams exhibited reduced play behavior and higher levels of oxytocinergic neurons in SON, the hypothalamic nucleus selectively activated after the display of offense (Kollack-Walker et al., 1997). Social play can be considered as a preparation for adult aggressive behavior (Aldis, 1975), so the reduced play here observed in enriched dams' male pups could be linked to neurohormonally-induced reduced aggressive tendencies (e.g, increased oxytocin levels). In fact, the anti-aggressive properties of the oxytocin during social challenges are well known (Crespi, 2016). For example, in naked mole-rats resident workers showing greater oxytocinergic neural activity are less aggressive than soldiers (Hathaway et al., 2016). On the contrary, in male rats the increase in aggressive behavior due to neonatal handling is accompanied by a reduced number of oxytocinergic neurons in the PVN (Todeschin et al., 2009).

Finally, the increased oxytocinergic modulation in pups can also be the result of epigenetic modifications linked to enriched dams' increased maternal care, as previously demonstrated (Champagne, 2008; Francis et al., 2002; Weaver et al., 2004).

The involvement of other hormones and neural systems cannot be excluded, given the lack of differences observed in female offspring of enriched dams either in oxytocin neurons and in the total amount of play behavior.

## 5. Conclusion

The present findings identify an influence of pre-reproductive enrichment of female rats on their maternal care and on the play fighting of their offspring, and suggest that early maternal care may modify social interactions during adolescence. As previously reported by other researchers (Leshem and Schulkin, 2012), social behavior is more vulnerable to transgenerational effects of pre-reproductive maternal enrichment in male than female rats.

It is interesting to speculate on the mechanisms of inheritance involved in the transmission of the environmental influence from mothers to the offspring. Several recent studies have provided evidence that maternal cross-generational inheritance is mediated by epigenetic modifications involving DNA methylation and subsequent changes in the expression of specific genes (Bohacek and Mansuy, 2013, 2015). Non-genetic marks fluctuate throughout lifetime and carry important information about previous experiences and faced environments, and their outcomes on the organism (Campos et al., 2014). Not all epigenetic transgenerational effects are transmitted through the germ line, but are passed on to the offspring directly from the mother during the first week of postnatal life (Meaney, 2010). In the present study we found neural modifications of the oxytocinergic system. Further investigations of enriched dams' and offspring's neuroendocrine function and hormonal status as well as of the epigenetic inheritance of neurohormonal signals will have major implications for our understanding of transgenerational impact of pre-reproductive maternal enrichment on offspring's behavioral traits.

## Authors' contributions

All authors participated in planning the research; DC, PC, PS-P, DL, and FF performed behavioral evaluations; SFV, EB, MP, PDB, and FG performed biochemical analyses; DC, SFV, and LP analyzed and interpreted data; all authors discussed and approved data; DC, SFV, and LP wrote the paper.

## Conflicts of interest

All authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2018.02.015.

## References

Aldis, O., 1975. Play Fighting. Academic press Inc., New York.

Arai, J.A., Li, S., Hartley, D.M., Feig, L.A., 2009. Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. J. Neurosci. 29, 1496–1502. https://doi.org/10.1523/JNEUROSCI.5057-08.2009.

Arai, J.A., Feig, L.A., 2011. Long-lasting and transgenerational effects of an environmental enrichment on memory formation. Brain Res. Bull. 85, 30–35. https://doi.org/10.1016/j.brainresbull.2010.11.003.

Argue, K.J., McCarthy, M.M., 2015. Characterization of juvenile play in rats: importance of sex of self and sex of partner. Biol. Sex Differ. 6, 16. https://doi.org/10.1186/s13293-015-0034-x.

Argue, K.J., VanRyzin, J.W., Falvo, D.J., Whitaker, A.R., Yu, S.J., McCarthy, M.M., 2017. Activation of both CB1 and CB2 endocannabinoid receptors is critical for masculinization of the developing medial amygdala and juvenile social play behavior. eNeuro 4. https://doi.org/10.1523/ENEURO.0344-16.2017 pii: ENEURO.0344–16.2017.

Bales, K.L., Perkeybile, A.M., Conley, O.G., Lee, M.H., Guoynes, C.D., Downing, G.M., Yun, C.R., Solomon, M., Jacob, S., Mendoza, S.P., 2013. Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. Biol. Psychiatry 74, 180–188. https://doi.org/10.1016/j.biopsych.2012.08.025.

Bales, K.L., Solomon, M., Jacob, S., Crawley, J.N., Silverman, J.L., Larke, R.H., Sahagun, E., Puhger, K.R., Pride, M.C., Mendoza, S.P., 2014. Long-term exposure to intranasal oxytocin in a mouse autism model. Transl. Psychiatry 4, e480. https://doi.org/10.1038/tp.2014.117.

Bales, K.L., van Westerhuyzen, J.A., Lewis-Reese, A.D., Grotte, N.D., Lanter, J.A., Carter, C.S., 2007. Oxytocin has dose-dependent developmental effects on pair-bonding and alloparental care in female prairie voles. Horm. Behav. 52, 274–279.

Bohacek, J., Mansuy, I.M., 2013. Epigenetic inheritance of disease and disease risk. Neuropsychopharmacology 38, 220–236.

Bohacek, J., Mansuy, I.M., 2015. Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. Nat. Rev. Genet. 16, 641–652. https://doi.org/10.1038/nrg3964.

Bosch, O.J., 2013. Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20130085. https://doi.org/10.1098/rstb.2013.0085.
Bowen, M.T., Carson, D.S., Spiro, A., Arnold, J.C., McGregor, I.S., 2011. Adolescent oxytocin exposure causes persistent reductions in anxiety and alcohol consumption and enhances sociability in rats. PLoS One 6, e27237. https://doi.org/10.1371/journal.pone.0027237.
Bredy, T.W., Humpartzoomian, R.A., Cain, D.P., Meaney, M.J., 2003. Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. Neuroscience 118, 571–576.
Caldji, C., Hellstrom, I.C., Zhang, T.Y., Diorio, J., Meaney, M.J., 2011. Environmental regulation of the neural epigenome. FEBS Lett. 585, 2049–2058. https://doi.org/10.1016/j.febslet.2011.03.032.
Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M., Meaney, M.J., 1998. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc. Natl. Acad. Sci. U. S. A 95, 5335–5340.
Campos, E.I., Stafford, J.M., Reinberg, D., 2014. Epigenetic inheritance: histone bookmarks across generations. Trends Cell Biol. 24, 664–674.
Cancedda, L., Putignano, E., Sale, A., Viegi, A., Berardi, N., Maffei, L., 2004. Acceleration of visual system development by environmental enrichment. J. Neurosci. 24, 4840–4848.
Cannon, M., 2009. Contrasting effects of maternal and paternal age on offspring intelligence: the clock ticks for men too. PLoS Med. 6, e42. https://doi.org/10.1371/journal.pmed.1000042.
Caporali, P., Cutuli, D., Gelfo, F., Laricchiuta, D., Foti, F., De Bartolo, P., Mancini, L., Angelucci, F., Petrosini, L., 2014. Pre-reproductive maternal enrichment influences offspring developmental trajectories: motor behavior and neurotrophin expression. Front. Behav. Neurosci. 8, 195. https://doi.org/10.3389/fnbeh.2014.00195.
Caporali, P., Cutuli, D., Gelfo, F., Laricchiuta, D., Foti, F., De Bartolo, P., Angelucci, F., Petrosini, L., 2015. Interaction does count: a cross-fostering study on transgenerational effects of pre-reproductive maternal enrichment. Front. Behav. Neurosci. 9, 320. https://doi.org/10.3389/fnbeh.2015.00320.
Champagne, F.A., 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. Front. Neuroendocrinol. 29, 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003.
Champagne, F.A., 2010. Epigenetic influence of social experiences across the lifespan. Dev. Psychobiol. 52, 299–311. https://doi.org/10.1002/dev.20436.
Champagne, F.A., Curley, J.P., 2009. Epigenetic mechanisms mediating the long-term effects of maternal care on development. Neurosci. Biobehav. Rev. 33, 593–600. https://doi.org/10.1016/j.neubiorev.2007.10.009.
Champagne, F.A., Meaney, M.J., 2007. Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. Behav. Neurosci. 121, 1353–1363.
Charil, A., Laplante, D.P., Vaillancourt, C., King, S., 2010. Prenatal stress and brain development. Brain Res. Rev. 65, 56–79. https://doi.org/10.1016/j.brainresrev.2010.06.002.
Connors, E.J., Migliore, M.M., Pillsbury, S.L., Shaik, A.N., Kentner, A.C., 2015. Environmental enrichment models a naturalistic form of maternal separation and shapes the anxiety response patterns of offspring. Psychoneuroendocrinology 52, 153–167. https://doi.org/10.1016/j.psyneuen.2014.10.021.
Crespi, B.J., 2016. Oxytocin, testosterone, and human social cognition. Biol. Rev. Camb. Philos. Soc. 91, 390–408. https://doi.org/10.1111/brv.12175.
Curley, J.P., Davidson, S., Bateson, P., Champagne, F.A., 2009. Social enrichment during postnatal development induces transgenerational effects on emotional and reproductive behavior in mice. Front. Behav. Neurosci. 3, 25. https://doi.org/10.3389/neuro.08.025.2009.
Cutuli, D., Berretta, E., Pasqualini, G., De Bartolo, P., Caporali, P., Laricchiuta, D., Sampedro-Piquero, P., Gelfo, F., Pesoli, M., Foti, F., Begega, A., Petrosini, L., 2017. Influence of pre-reproductive maternal enrichment on coping response to stress and expression of c-Fos and glucocorticoid receptors in adolescent offspring. Front. Behav. Neurosci. 11, 73. https://doi.org/10.3389/fnbeh.2017.00073.
Cutuli, D., Caporali, P., Gelfo, F., Angelucci, F., Laricchiuta, D., Foti, F., De Bartolo, P., Bisicchia, E., Molinari, M., Farioli Vecchioli, S., Petrosini, L., 2015. Pre-reproductive maternal enrichment influences rat maternal care and offspring developmental trajectories: behavioral performances and neuroplasticity correlates. Front. Behav. Neurosci. 9, 66. https://doi.org/10.3389/fnbeh.2015.00066.
Cutuli, D., Rossi, S., Burello, L., Laricchiuta, D., De Chiara, V., Foti, F., De Bartolo, P., Musella, A., Gelfo, F., Centonze, D., Petrosini, L., 2011. Before or after does it matter? Different protocols of environmental enrichment differently influence motor, synaptic and structural deficits of cerebellar origin. Neurobiol. Dis. 42, 9–20. https://doi.org/10.1016/j.nbd.2010.12.007.
Dell, P.A., Rose, F.D., 1987. Transfer of effects from environmentally enriched and impoverished female rats to future offspring. Physiol. Behav. 39, 187–190.
Dumais, K.M., Alonso, A.G., Immormino, M.A., Bredewold, R., Veenema, A.H., 2016. Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition. Psychoneuroendocrinology 64, 79–88. https://doi.org/10.1016/j.psyneuen.2015.11.007.
Francis, D.D., Champagne, F.C., Meaney, M.J., 2000. Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. J. Neuroendocrinol. 12, 1145–1148.
Francis, D.D., Young, L.J., Meaney, M.J., Insel, T.R., 2002. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. J. Neuroendocrinol. 14, 349–353.
Franklin, T.B., Russig, H., Weiss, I.C., Gräff, J., Linder, N., Michalon, A., Vizi, S., Mansuy, I.M., 2010. Epigenetic transmission of the impact of early stress across generations. Biol. Psychiatry 68, 408–415. https://doi.org/10.1016/j.biopsych.2010.05.036.
Friske, J.E., Gammie, S.C., 2005. Environmental enrichment alters plus maze, but not maternal defense performance in mice. Physiol. Behav. 85, 187–194.
Gammie, S.C., Stevenson, S.A., 2006. Effects of daily and acute restraint stress during lactation on maternal aggression and behavior in mice. Stress 9, 171–180. https://doi.org/10.1080/10253890600969106.
Gao, S., Becker, B., Luo, L., Geng, Y., Zhao, W., Yin, Y., Hu, J., Gao, Z., Gong, Q., Hurlemann, R., Yao, D., Kendrick, K.M., 2016. Oxytocin, the peptide that bonds the sexes also divides them. Proc. Natl. Acad. Sci. U. S. A 113, 7650–7654. https://doi.org/10.1073/pnas.1602620113.
Hathaway, G.A., Faykoo-Martinez, M., Peragine, D.E., Mooney, S.J., Holmes, M.M., 2016. Subcaste differences in neural activation suggest a prosocial role for oxytocin in eusocial naked mole-rats. Horm. Behav. 79, 1–7.
Himmler, S.M., Modlinska, K., Stryjek, R., Himmler, B.T., Pisula, W., Pellis, S.M., 2014. Domestication and diversification: a comparative analysis of the play fighting of the Brown Norway, Sprague-Dawley, and Wistar laboratory strains of (Rattus norvegicus). J. Comp. Psychol. 128, 318–327. https://doi.org/10.1037/a0036104.
Ho, D.H., Burggren, W.W., 2010. Epigenetics and transgenerational transfer: a physiological perspective. J. Exp. Biol. 213, 3–16. https://doi.org/10.1242/jeb.019752.
Ivy, A.S., Brunson, K.L., Sandman, C., Baram, T.Z., 2008. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. Neuroscience 154, 1132–1142. https://doi.org/10.1016/j.neuroscience.2008.04.019.
Jirtle, R.L., Skinner, M.K., 2007. Environmental epigenomics and disease susceptibility. Nat. Rev. Genet. 8, 253–262.
Kollack-Walker, S., Watson, S.J., Akil, H., 1997. Social stress in hamsters: defeat activates specific neurocircuits within the brain. J. Neurosci. 17, 8842–8855.
Kristal, M.B., 2009. The biopsychology of maternal behavior in nonhuman mammals. ILAR J. 50, 51–63. https://doi.org/10.1093/ilar.50.1.51.
Lee, H.J., Macbeth, A.H., Pagani, J.H., Young 3rd, W.S., 2009. Oxytocin: the great facilitator of life. Prog. Neurobiol. 88, 127–151. https://doi.org/10.1016/j.pneurobio.2009.04.001.
Leshem, M., Schulkin, J., 2012. Transgenerational effects of infantile adversity and enrichment in male and female rats. Dev. Psychobiol. 54, 169–186. https://doi.org/10.1002/dev.20592.
Lim, M.M., Young, L.J., 2006. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm. Behav. 50, 506–517.
Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., Meaney, M.J., 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277, 1659–1662.
Lundberg, S., Martinsson, M., Nylander, I., Roman, E., 2017. Altered corticosterone levels and social play behavior after prolonged maternal separation in adolescent male but not female Wistar rats. Horm. Behav. 87, 137–144. https://doi.org/10.1016/j.yhbeh.2016.11.016.
Malaspina, D., Reichenberg, A., Weiser, M., Fennig, S., Davidson, M., Harlap, S., Wolitzky, R., Rabinowitz, J., Susser, E., Knobler, H.Y., 2005. Paternal age and intelligence: implications for age-related genomic changes in male germ cells. Psychiatr. Genet. 15, 117–125.
Marlin, B.J., Mitre, M., D'amour, J.A., Chao, M.V., Froemke, R.C., 2015. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520, 499–504. https://doi.org/10.1038/nature14402.
Maruoka, T., Kodomari, I., Yamauchi, R., Wada, E., Wada, K., 2009. Maternal enrichment affects prenatal hippocampal proliferation and open-field behaviors in female offspring mice. Neurosci. Lett. 454, 28–32. https://doi.org/10.1016/j.neulet.2009.02.052.
Mashoodh, R., Franks, B., Curley, J.P., Champagne, F.A., 2012. Paternal social enrichment effects on maternal behavior and offspring growth. Proc. Natl. Acad. Sci. U. S. A 109, 17232–17238. https://doi.org/10.1073/pnas.1121083109.
Mashoodh, R., Sinal, C.J., Perrot-Sinal, T.S., 2009. Predation threat exerts specific effects on rat maternal behaviour and anxiety-related behaviour of male and female offspring. Physiol. Behav. 96, 693–702. https://doi.org/10.1016/j.physbeh.2009.01.001.
McMurray, M.S., Joyner, P.W., Middleton, C.W., Jarrett, T.M., Elliott, D.L., Black, M.A., Hofler, V.E., Walker, C.H., Johns, J.M., 2008. Intergenerational effects of cocaine on maternal aggressive behavior and brain oxytocin in rat dams. Stress 11, 398–410. https://doi.org/10.1080/10253890701850239.
Meaney, M.J., 2010. Epigenetics and the biological definition of gene x environment interactions. Child Dev. 81, 41–79. https://doi.org/10.1111/j.1467-8624.2009.01381.x.
Mogi, K., Ooyama, R., Nagasawa, M., Kikusui, T., 2014. Effects of neonatal oxytocin manipulation on development of social behaviors in mice. Physiol. Behav. 133, 68–75. https://doi.org/10.1016/j.physbeh.2014.05.010.
Mooney, S.M., Varlinskaya, E.I., 2011. Acute prenatal exposure to ethanol and social behavior: effects of age, sex, and timing of exposure. Behav. Brain Res. 216, 358–364. https://doi.org/10.1016/j.bbr.2010.08.014.
Mueller, B.R., Bale, T.L., 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J. Neurosci. 28, 9055–9065. https://doi.org/10.1523/JNEUROSCI.1424-08.2008.

Mychasiuk, R., Zahir, S., Schmold, N., Ilnytskyy, S., Kovalchuk, O., Gibb, R., 2012. Parental enrichment and offspring development: modifications to brain, behavior and the epigenome. Behav. Brain Res. 228, 294–298. https://doi.org/10.1016/j.bbr.2011.11.036.

Nithianantharajah, J., Hannan, A.J., 2006. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci. 7, 697–709.

Nithianantharajah, J., Hannan, A.J., 2009. The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders. Prog. Neurobiol. 89, 369–382. https://doi.org/10.1016/j.pneurobio.2009.10.001.

Olioff, M., Stewart, J., 1978. Sex differences in the play behavior of prepubescent rats. Physiol. Behav. 20, 113–115.

Panksepp, J., 1981. The ontogeny of play in rats. Dev. Psychobiol. 14, 327–332.

Panksepp, J., Beatty, W.W., 1980. Social deprivation and play in rats. Behav. Neurol. Biol. 30, 197–206.

Parent, C.I., Meaney, M.J., 2008. The influence of natural variations in maternal care on play fighting in the rat. Dev. Psychobiol. 50, 767–776. https://doi.org/10.1002/dev.20342.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, fourth ed. Academic, San Diego.

Pellis, S.M., Field, E.F., Smith, L.K., Pellis, V.C., 1997. Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. Neurosci. Biobehav. Rev. 21, 105–120.

Pellis, S.M., Pellis, V.C., 2009. The Playful Brain. OneWorld Publications, Oxford.

Perrin, M.C., Brown, A.S., Malaspina, D., 2007. Aberrant epigenetic regulation could explain the relationship of paternal age to schizophrenia. Schizophr. Bull. 33, 1270–1273.

Rees, S.L., Lovic, V., Fleming, A.S., 2004. Maternal behavior. In: Whishaw, I.Q., Kolb, B. (Eds.), The Behavior of the Laboratory Rat. Oxford University Press, Oxford, p. 289. https://doi.org/10.1093/acprof:oso/9780195162851.003.0027 (Chapter 27).

Rosenblatt, J.S., Lehrman, D.S., 1963. Maternal behavior in the laboratory rat. In: Reingold, H.L. (Ed.), Maternal Behavior in Mammals. Wiley, New York, pp. 8–57.

Rosenfeld, A., Weller, A., 2012. Behavioral effects of environmental enrichment during gestation in WKY and Wistar rats. Behav. Brain Res. 233, 245–255. https://doi.org/10.1016/j.bbr.2012.05.006.

Roth, T.L., 2012. Epigenetics of neurobiology and behavior during development and adulthood. Dev. Psychobiol. 54, 590–597. https://doi.org/10.1002/dev.20550.

Sale, A., Putignano, E., Cancedda, L., Landi, S., Cirulli, F., Berardi, N., Maffei, L., 2004. Enriched environment and acceleration of visual system development. Neuropharmacology 47, 649–660.

Shachar-Dadon, A., Schulkin, J., Leshem, M., 2009. Adversity before conception will affect adult progeny in rats. Dev. Psychol. 45, 9–16. https://doi.org/10.1037/a0014030.

Shahrokh, D.K., Zhang, T.Y., Diorio, J., Gratton, A., Meaney, M.J., 2010. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. Endocrinology 151, 2276–2286. https://doi.org/10.1210/en.2009-1271.

Shansky, R.M., 2016. Sex Differences in the Central Nervous System. Academic Press.

Sofroniew, M.V., 1980. Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. J. Histochem. Cytochem. 28, 475–478.

Storey, J.D., 2004. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. J. R. Stat. Soc. 66, 187–205. https://doi.org/10.1111/j.1467-9868.2004.00439.x.

Storey, J.D., Tibshirani, R., 2003. Statistical significance for genome wide studies. Proc. Natl. Acad. Sci. U. S. A 100, 9440–9445. https://doi.org/10.1073/pnas.1530509100.

Taouk, L., Schulkin, J., 2016. Transgenerational transmission of pregestational and prenatal experience: maternal adversity, enrichment, and underlying epigenetic and environmental mechanisms. J. Dev. Orig. Health Dis 7, 588–601.

Thayer, Z.M., Kuzawa, C.W., 2011. Biological memories of past environments: epigenetic pathways to health disparities. Epigenetics 6, 798–803.

Thor, D.H., Holloway Jr., W.R., 1984. Sex and social play in juvenile rats (Rattus norvegicus). J. Comp. Psychol. 96, 276–284.

Todeschin, A.S., Winkelmann-Duarte, E.C., Jacob, M.H., Aranda, B.C., Jacobs, S., Fernandes, M.C., Ribeiro, M.F., Sanvitto, G.L., Lucion, A.B., 2009. Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. Horm. Behav. 56, 93–100. https://doi.org/10.1016/j.yhbeh.2009.03.006.

Trezza, V., Damsteegt, R., Manduca, A., Petrosino, S., Van Kerkhof, L.W., Pasterkamp, R.J., Zhou, Y., Campolongo, P., Cuomo, V., Di Marzo, V., Vanderschuren, L.J., 2012. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J. Neurosci. 32, 14899–14908. https://doi.org/10.1523/JNEUROSCI.0114-12.2012.

Van den Bergh, B.R., Mulder, E.J., Mennes, M., Glover, V., 2005. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A Review. Neurosci. BioBehav. Rev. 29, 237–258.

van Kerkhof, L.W., Damsteegt, R., Trezza, V., Voorn, P., Vanderschuren, L.J., 2013. Social play behavior in adolescent rats is mediated by functional activity in medial prefrontal cortex and striatum. Neuropsychopharmacology 38, 1899–1909. https://doi.org/10.1038/npp.2013.83.

Vanderschuren, L.J., Niesink, R.J., Van Ree, J.M., 1997. The neurobiology of social play behavior in rats. Neurosci. BioBehav. Rev. 21, 309–326.

Vanderschuren, L.J.M.J., Achterberg, E.J.M., Baarendse, P.J.J., Damsteegt, R., Van Kerkhof, L.W.M., Trezza, V., 2012. Studying the neurobehavioral mechanisms of social behavior in adolescent rats. In: Spink, A.J., Grieco, F., Krips, O.E., Loijens, L.W.S., Noldus, L.P.J.J., Zimmerman, P.H. (Eds.), Proceedings of Measuring Behavior 2012, 8 Th International Conference on Methods and Techniques in Behavioral Research (Utrecht, The Netherlands, August 28-31, 2012. Noldus Information Technology, Wageningen, pp. 135–137.

Veenema, A.H., 2012. Toward understanding how early-life social experiences alter oxytocin- and vasopressin-regulated social behaviors. Horm. Behav. 61, 304–312. https://doi.org/10.1016/j.yhbeh.2011.12.002.

Venerosi, A., Cutuli, D., Colonnello, V., Cardona, D., Ricceri, L., Calamandrei, G., 2008. Neonatal exposure to chlorpyrifos affects maternal responses and maternal aggression of female mice in adulthood. Neurotoxicol. Teratol. 30, 468–474. https://doi.org/10.1016/j.ntt.2008.07.002.

Wang, Z., Storm, D.R., 2011. Maternal behavior is impaired in female mice lacking type 3 adenylyl cyclase. Neuropsychopharmacology 36, 772–781. https://doi.org/10.1038/npp.2010.211.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7, 847–854.

Weaver, I.C., Korgan, A.C., Lee, K., Wheeler, R.V., Hundert, A.S., Goguen, D., 2017. Stress and the emerging roles of chromatin remodeling in signal integration and stable transmission of reversible phenotypes. Front. Behav. Neurosci. 11, 41. https://doi.org/10.3389/fnbeh.2017.00041.

Wei, D., Lee, D., Cox, C.D., Karsten, C.A., Peñagarikano, O., Geschwind, D.H., Gall, C.M., Piomelli, D., 2015. Endocannabinoid signaling mediates oxytocin-driven social reward. Proc. Natl. Acad. Sci. U. S. A. 112, 14084–14089. https://doi.org/10.1073/pnas.1509795112.

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav. Immun. 19, 296–308.

Welberg, L., Thrivikraman, K.V., Plotsky, P.M., 2006. Combined pre- and postnatal environmental enrichment programs the HPA axis differentially in male and female rats. Psychoneuroendocrinology 31, 553–564.

Xiong, G.J., Yang, Y., Cao, J., Mao, R.R., Xu, L., 2015. Fluoxetine treatment reverses the intergenerational impact of maternal separation on fear and anxiety behaviors. Neuropharmacology 92, 1–7. https://doi.org/10.1016/j.neuropharm.2014.12.026.

Yeshurun, S., Short, A.K., Bredy, T.W., Pang, T.Y., Hannan, A.J., 2017. Paternal environmental enrichment transgenerationally alters affective behavioral and neuroendocrine phenotypes. Psychoneuroendocrinology 77, 225–235. https://doi.org/10.1016/j.psyneuen.2016.11.013.

Zuena, A.R., Zinni, M., Giuli, C., Cinque, C., Alemà, G.S., Giuliani, A., Catalani, A., Casolini, P., Cozzolino, R., 2016. Maternal exposure to environmental enrichment before and during gestation influences behaviour of rat offspring in a sex-specific manner. Physiol. Behav. 163, 274–287. https://doi.org/10.1016/j.physbeh.2016.05.010.