



Corticosterone administration immediately after peripuberty stress exposure does not prevent protracted stress-induced behavioral alterations

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ABSTRACT

Stress-related disorders are commonly associated with abnormalities in hypothalamic-pituitary-adrenal (HPA) axis activity. Preliminary studies with cortisol administration in the aftermath of trauma suggest that this HPA axis hormone can potentially prevent maladaptive behavioral and biological stress responses. However, the efficacy of glucocorticoid administration during the peripuberty period has not been tested yet, although this lifetime is a critical time window in brain development and is highly sensitive to the harmful effects of stress. To further examine the short and long-lasting impact of glucocorticoids treatment given during the post-peripubertal stress period, the present study utilized a rat model of peripubertal stress-induced psychopathology and animals were subjected to a battery of tests to assess anxiety-like behaviors, exploratory behavior and reactivity to novelty at late adolescence and sociability, anhedonia and stress coping behaviors at adulthood. All the experiments were performed in males and females to evaluate the potential behavioral sex differences. Overall, our results demonstrated that rats exposed to peripubertal stress show decreased sociability in adulthood without differences in anxiety and depression-like behaviors. Moreover, this study shows that the administration of corticosterone after stress exposure at peripuberty does not prevent stress-induced behavioral alterations. However, we observed that some stress-induced behavioural alterations and corticosterone responses are sex-specific. Thus, the data obtained highlight that delineating sex differences in stress-related studies may ultimately contribute to the development of effective therapeutic interventions for each sex.

1. Introduction

Stress-induced psychopathologies such as anxiety, depression, or post-traumatic stress disorder (PTSD) (McEwen et al., 2015) are devastating mental disorders with a large impact on public health (James et al., 2018; Trautmann et al., 2016). Current pharmacological treatments for these disorders show limited efficacy, resistance, and a long-delay onset of action (Cuijpers et al., 2020), therefore, the

development of more efficacious treatments remains a significant unmet medical need. The hypothalamic-pituitary-adrenocortical (HPA) axis is the main physiological stress system. Glucocorticoids (corticosterone in animals and cortisol in humans), the final products of the activated HPA axis, orchestrate a complex physiological and behavioral response to cope with stress and restore homeostasis (De Kloet et al., 2005).

Growing evidence supports a causal link between HPA dysregulation, aberrant cortisol levels, and the appearance of psychopathology

Abbreviations: CORT ratios, Corticosterone ratios; EP, elevated platform; HPA, hypothalamic-pituitary-adrenocortical; OF, open field; PN, postnatal day; PTSD, post-traumatic stress disorder; TMT, 2,4,5-Trimethylthiazole.

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(De Kloet et al., 2005). For instance, low cortisol levels in response to stress have been described in several psychopathological disorders, including depression (Wikgren et al., 2012), chronic fatigue syndrome (Roberts et al., 2004), anti-social personality disorder (Fairchild et al., 2018), or PTSD (Delahanty et al., 2000; Yehuda et al., 2006). In line with these observations, the administration of hydrocortisone to alter psychobiological trajectories following trauma exposure and reduce the incidence of psychopathology has been evaluated in the clinic. For instance, a single hydrocortisone administration after an acute traumatic event was shown to be efficient in attenuating anxiety and depression symptoms and subsequent PTSD (Schelling et al., 2001, 2004; Zohar et al., 2011a). While glucocorticoid-based pharmacotherapies appear to be a promising and efficient medication in preventing PTSD symptoms, not all clinical trials found beneficial effects (Carmi et al., 2022; Shaked et al., 2021) and further research is necessary (Florida et al., 2023). In preclinical studies, corticosterone administered after acute stress immobilization or acute exposure to predator odor significantly reduced anxiety-like behaviors and conditioned fear response (Cohen et al., 2008; Wingo et al., 2018). Thus, while single-dose approaches seem to be efficacious after acute stress in adults, treatment with corticosterone in early-time periods or after chronic adverse events has not been yet tested. It is widely known that the onset of stress-related psychopathologies occurs during late adolescence - a critical time of brain maturation- and chronic stress exposure during this period is a major risk factor in the development of psychopathologies (Heim and Binder, 2012; Solmi et al., 2022; Spear, 2000). Therefore, here we evaluate the effect of corticosterone treatment in reverting an animal model of chronic stress applied during peripuberty.

Interestingly, males and females display significant differences across the HPA axis caused by multiple factors, notably including sex chromosomes and developmental changes in gonadal hormone levels leading to sexually dimorphic physiological and behavioral responses to stress throughout the lifespan (Bale and Epperson, 2015), particularly during adolescence (Green and McCormick, 2016; Heck and Handa, 2019). For instance, rodent studies have shown that females secrete higher absolute concentrations of corticosterone than males (Goel et al., 2014) and in males, the rise in testosterone levels that occurs in puberty leads to an inhibition of the HPA axis (Evuarherhe et al., 2009). Unfortunately, the historical bias of not including enough female animals from preclinical studies has limited our understanding of the mechanisms underlying sex differences between males and females (Bangasser and Cuarenta, 2021; Lima et al., 2022). Indeed, we consider that taking into account the possible sex dimorphism in preclinical studies is crucial to understanding the gender gap that exists in the prevalence of psychopathologies (i.e., women show a two-fold greater risk as compared to men of developing depression) and the sex-specific efficacy and pharmacokinetics of some psychiatric drugs (Kuehner, 2017; LeGates et al., 2019). Considering this, our study aims to assess the effectiveness of corticosterone administration following stress exposure in mitigating the phenotype induced by a rat model of peripubertal stress in both male and female rats.

2. Material and methods

2.1. Animals

Experimental male and female Wistar Han rats were the offspring of breeding pairs purchased from a commercial breeder (Charles River Laboratories, France) and acclimated to our animal facility upon arrival. Juvenile male and female Wistar Han rats (3-weeks-old) used as unfamiliar juveniles for the social preference test were purchased from a commercial breeder (Charles River Laboratories, France) and acclimated in our animal facility upon arrival at least 1 week before the behavioral testing. Food and water were available *ad libitum*. Rats were maintained under standard housing conditions on a 12-hour light-dark cycle (lights on at 7:00 AM). Animals were paired-housed, except for

the period of the saccharine preference test, in which animals were single-housed. All experiments were performed with the approval of the Cantonal Veterinary Authorities (Vaud, Switzerland) and carried out in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU).

2.2. Peripubertal stress

The peripubertal stress paradigm (Fig. 1A) aims to model exposure to unpredictable stressful experiences of nonsocial nature during late childhood and puberty. On postnatal day (PN) 21 (PN21) rats were weaned and 2 rats (2 male or 2 female rats) from different litters were matched by weight and assigned to a new home cage. Rats from the same home cage were assigned to the same experimental group either Control (No Stress) or Stress. On 7 scattered days between PN28 and PN42, rats from the “Stress” group were exposed to fear-induction procedures. On PN28, rats were exposed to an open field (OF, a rectangular arena of 50 × 50 cm) for 5 min, then for 25 min to an elevated platform (EP, a 12 × 12 cm platform elevated 95 cm from the ground) and finally to tail punctures for blood collection. Tail blood samples were collected through a heparin-coated capillary tube (Microvette CB 300LH, Sarstedt, Cat #16.443) immediately after exposure to an EP (t_0) and 30 min later (t_{30}). During this interval, rats were placed in a novel cage and were prevented from direct physical contact with their cage mates. After the second blood sampling, animals were placed back in their home cage. On PN29, rats were placed in a novel cage (38 × 27.5 × 31 cm) and exposed to the synthetic fox odor 2,4,5-Trimethylthiazole (TMT) (Sigma-Aldrich, Cat #W332518) for 25 min followed by exposure to an EP for 25 min. On PN30, rats were exposed to an EP for 25 min followed by TMT exposure for 25 min and blood extraction (t_0 and t_{30}). On PN34, rats were exposed to TMT for 25 min. On PN36, rats were exposed to an EP for 25 min and an OF for 5 min. On PN40, rats were exposed to an EP for 25 min and to TMT for 25 min. On PN42, rats were exposed to an EP for 25 min followed by blood extraction (t_0 and t_{30}). Control animals were handled on the same days as their counterparts.

2.3. Corticosterone injections

Rats exposed to peripubertal stress and control littermates were intraperitoneally injected with corticosterone (Sigma-Aldrich, Cat #27840) at a dose of 25 mg/kg (Cohen et al., 2008) dissolved in 5 % DMSO 5 % PEG in 0.9 % NaCl (w/v) or vehicle [5 % DMSO 5 % PEG in 0.9 % NaCl (w/v)]. Stressed animals received the injection 1 hour after stress exposure or the first blood sampling. Control and stressed rats were injected with corticosterone or vehicle solution on three out of the seven days of the stress paradigm [PN28 (first day), PN34 and PN42 (last day)].

2.4. Bodyweight

Bodyweight was monitored during the period of peripubertal stress (PN28, PN34 and PN42) and at late adolescence- adulthood (PN58, PN62, PN69, PN76, PN90 and PN110).

2.5. Corticosterone measurement

Blood samples collected at t_0 and t_{30} during the stress paradigm were chilled until centrifugation at 9400 g at 4°C for 4 min. Corticosterone levels were not measured in control animals. Plasma was collected into new tubes and stored at -20°C until subsequent analysis. Corticosterone (dilution 1/40) was measured using an ELISA kit (Enzo Life Sciences, Cat #ADI-901-097). Corticosterone ratios (CORT ratios) were measured as follows. For each day (PN28, PN30 and PN42) CORT ratio t_{30-t_0} = (CORT t_{30} - CORT t_0) / (CORT t_{30} + CORT t_0).

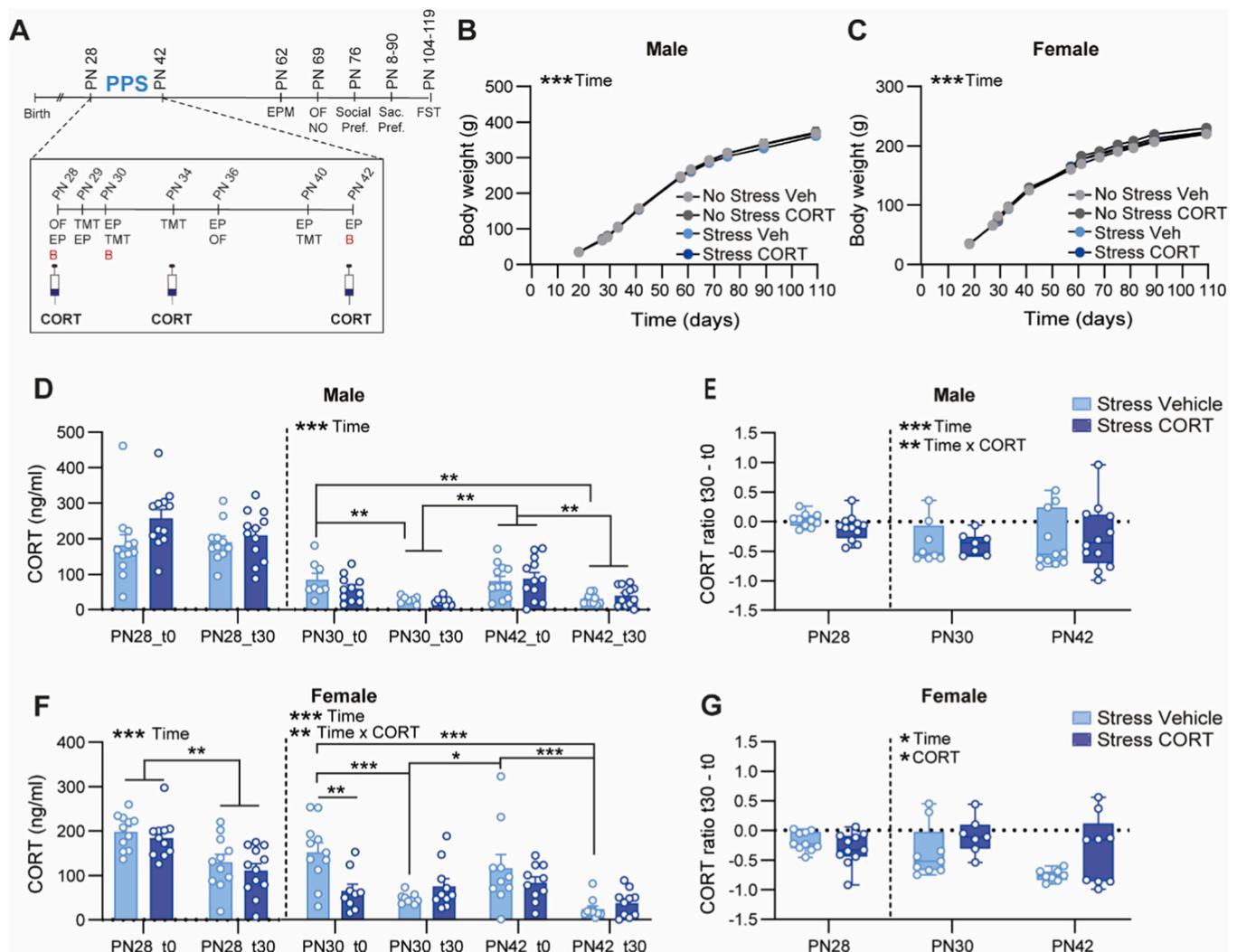


Fig. 1. Corticosterone administered after stress exposure during the peripubertal period does not impact body weight evolution but alters stress-induced corticosterone release in females: (A) Timeline of the experiment. The blood sampling was done immediately after the stress offset and then 30 min after the first blood extraction. Rats (male and female) were injected with vehicle (Veh) or corticosterone (CORT) at 25 mg/kg 1 hour after being exposed to stress or the first blood collection at PN28, PN34 and PN42. Body weight evolution in males (B) and females (C). Levels of plasma corticosterone in males (D) and females (E) measured after stress exposure. Corticosterone ratio t30 - t0 in males (F) and females (G). $N = 7-12/\text{group}$ (males) and $N = 7-12/\text{group}$ (females). Data are represented as mean \pm SEM (B, C, D and E) or as a boxplot showing the interquartile range with whiskers from minimum to maximum (F and G). Statistical analysis was done by t-student, two or three-way ANOVA with Holm-Sidak correction or mixed-effects model (REML) with Holm-Sidak correction. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. For detailed statistical information, see [Supplementary Table S1](#). PPS = Peripubertal stress; PN = postnatal; OF = open field; EP = elevated platform; B = blood extraction; TMT = 2,4,5- Trimethylthiazole; EPM = elevated plus maze; NO = novel object; Social Pref. = social preference; Sac. Pref. = saccharine preference; FST = forced-swim test; Veh = vehicle; CORT = corticosterone.

2.6. Determination of the estrous cycle phases

A non-invasive vaginal cytology was used to determine the phase of the estrous cycle in female rats before behavioral testing (for tests of only one day of duration: elevated plus maze, OF/novel object, social preference). Rats at the metestrus or diestrus phase of the cycle (high levels of progesterone) were selected for behavioral testing. Briefly, 50 μL of 0.9 % NaCl (w/v) was softly released on the aperture of the vagina with a sterile pipette tip. The liquid was drawn back into the tip and slowly load again in the vagina three consecutive times to collect enough cells. Next, the cell suspension was spread over a glass slide (Huberlab, Cat #10.0230.01), air-dried, and stained for 5 min with 0.1 % cresyl violet acetate (w/v) (Sigma-Aldrich, Cat #C5042) and washed for 1 minute in distilled water. The vaginal smear was examined immediately under a Brightfield microscope (200 x magnification). Estimation of the phase of the estrous cycle (proestrus, estrus, metestrus and diestrus) was based on the proportion of leucocytes, cornified

epithelial cells and nucleated epithelial cells (Ajayi and Akhigbe, 2020; Florido et al., 2021).

2.7. Animal behavior

2.7.1. Elevated plus maze test

Anxiety-like behavior was evaluated at PN62 using a five-minute exposure to the elevated plus maze. The maze consists of two opposing open arms (50 \times 10 cm) perpendicular to two closed arms (50 \times 10 \times 50 cm) that extend from a central platform (10 \times 10 cm) elevated 65 cm above the floor. Lighting was maintained at 15–16 lx on the open arms and 5–7 lx on the closed arms. The percentage of time spent on the open arms and the number of entries into the open arms were monitored using a video camera and analyzed with a computerized tracking system (Ethovision XT, Noldus Information Technology). The entire apparatus was cleaned with 5 % EtOH and dried properly between each trial.

2.7.2. Open field and novel object tests

On PN69, locomotor activity and exploratory behavior were evaluated using a rectangular OF (80 × 80 × 40 cm). The light was adjusted to a level of 8–10 lx in the center of the arena. Animals were introduced face to the wall of the arena and allowed to freely explore the arena for 10 min. Afterwards, a novel object was introduced in the center of the arena, and the animal was allowed to explore the object for another 5 min. Rat behavior was monitored using a video camera and analyzed with a computerized tracking system (Ethovision 11.0 XT, Noldus Information Technology). The entire apparatus was cleaned with 5 % EtOH and dried properly between each trial.

2.7.3. Social preference test

On PN76, social preference was evaluated using the three-chamber sociability test. The test was performed in a rectangular, three-chambered box, consisting of a center compartment (20 × 35 × 35 cm) and left and right compartments (30 × 35 × 35 cm). Dividing walls had retractable doorways allowing access to each chamber. Left and right compartments were equipped with a floor-fixed transparent Plexiglas cylinder (15 cm diameter), perforated with small holes, that contained either an unfamiliar juvenile rat (30 ± 2 days old, juvenile male for experimental rat male and juvenile female for experimental rat female) or an inanimate object (yellow plastic bottle). The cylinder permits visual, tactile, auditory and olfactory communication. The experimental rat was first placed in the middle chamber and allowed to habituate for 5 minutes. Thereafter, retractable doors were removed, and the rat was allowed to explore the left and right compartments for 10 min. Rat behavior was monitored using a video camera and the time spent sniffing either the juvenile or the novel object was manually scored offline (The Observer XT, Noldus IT) by an experimenter blind to animals' experimental conditions. The social preference ratio was calculated according to the formula: (time sniffing the juvenile - time sniffing the object) / total sniffing time. The entire apparatus was cleaned with 5 % EtOH and dried properly between each trial.

2.7.4. Saccharine preference test

Anhedonia was evaluated using the saccharine preference test between PN86–90. Rats were individually housed and acclimatized to a two-bottle choice (both bottles with drinking water) for 24 hours. For the next 3 days, rats were exposed to one bottle filled with drinking water and one bottle filled with a solution of 0.03 % saccharin (Sigma-Aldrich, Cat #S1002). Daily, the liquid consumption was calculated by weighting the bottles, and the position of the bottles was interchanged. Saccharin preference was calculated according to the formula: total saccharin consumption over the three days of test/ total consumption over the three days of test * 100.

2.7.5. Forced-swim test

Coping behavior was evaluated between PN104–119 with a forced-swim test. During the first day, rats were placed in a plastic beaker (25 cm diameter x 46 cm) containing 30 cm of water (25 °C) for 15 min. The next day, a second session of 5 min was performed. The time spent immobile and the total time spent by the animals exhibiting active behaviors (climbing or swimming) were manually scored using Clicker v1.13 by an experimenter blind to the experimental conditions. Immobility was defined as the time that the animal spent without making any movements beyond those required to keep its head above water. Climbing was characterized by forceful thrashing movements directed against the walls of the cylinder, while swimming was defined as moving all four paws in an active swimming motion. Additionally, animal behavior was monitored using a video camera and the total distance moved was analyzed with an automatic computerized tracking system (Ethovision 11.0 XT, Noldus Information Technology).

2.8. Statistical analysis

Data are presented as mean ± SEM or boxplot (interquartile range with whiskers showing the minimum to the maximum range). No power analysis was performed to determine the sample size. The sample size in each study was based on previous experience in our lab. *N* represents the number of independent animals. All statistical analyses were performed with Prism 8 (GraphPad Software, Inc.). Shapiro-Wilk normality test was used to test normality. The Student's *t*-test is used to compare the means between two groups. Two-way or three-way ANOVA was used to analyze the effect of stress, treatment corticosterone or time, and the interaction when applicable. A mixed-effects model (REML) was applied when there were missing values. *P* values were corrected for multiple comparisons using the Holm-Sidak method. All *P* < 0.05 were considered to be significant. **P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001. For data exclusion, Grubbs' test for outliers was performed in GraphPad with an alpha level of 0.05. Detailed statistical details and *N* are indicated in the figure legend or [supplementary tables](#).

3. Results

3.1. Corticosterone treatment after stress exposure during the peripubertal period does not affect body weight but modifies stress-induced corticosterone release in females

Corticosterone administration during the peripubertal period (Fig. 1A) did not affect body weight either in males (Fig. 1B) or females (Fig. 1C). Stress-induced release of corticosterone on the first day of stress exposure (PN28), measured after stress exposure (t0) and 30 minutes after (t30) was not affected in males (Fig. 1D), but corticosterone levels were reduced at t30 compared to t0 in females (Fig. 1F). Nevertheless, the release of corticosterone was altered over the time in both males and females in the following days (Fig. 1D, F). Otherwise, intraperitoneal corticosterone injections given 1 hour after stress sessions on PN28, PN30 and PN34 did not affect the corticosterone release in males (Fig. 1D). However, in females (Fig. 1F), corticosterone levels at PN30 t0 were higher in vehicle-treated animals compared to animals injected with corticosterone. These results show that in females, but not in males, corticosterone injection on the first day of stress exposure (PN28) affected the subsequent reactivity of the HPA axis and the consequent release of corticosterone in posterior stress exposures. Consequently, corticosterone levels at t30 compared to levels at t0 (CORT ratio t30 – t0) did not differ between vehicle and corticosterone-injected animals in males (Fig. 1E). However, there was a significant effect of both stress and corticosterone factors in CORT ratio t30-t0 in females (Fig. 1G), although the difference between vehicle- and CORT-administered animals CORT ratio did not reach statistical significance.

3.2. Corticosterone treatment after stress exposure during the peripubertal period has a mild impact on anxiety-like behaviors, exploration and reactivity to novelty

At late adolescence, three weeks after the termination of the stress protocol (PN62), animals were subjected to the elevated plus maze test. In male rats, we observed a significant interaction between stress and corticosterone (Fig. 2A, C), indicating that corticosterone treatment during peripuberty modulates anxiety-like behaviors differently in control and stressed animals. Even more, the analysis of anxiety-like behaviors (i.e., percent time spent in the open arms of the EPM) revealed that stressed rats show less anxiety-like behaviors than control littermates (Fig. 2A). Although the post-hoc test revealed non-significant results regarding the treatment effect, corticosterone appears to produce an anxiolytic effect in control animals, whereas it exhibits an opposite effect in stressed rats. Nevertheless, in female rats, performance in the elevated plus-maze was neither affected by stress nor by corticosterone injections (Fig. 2B, D).

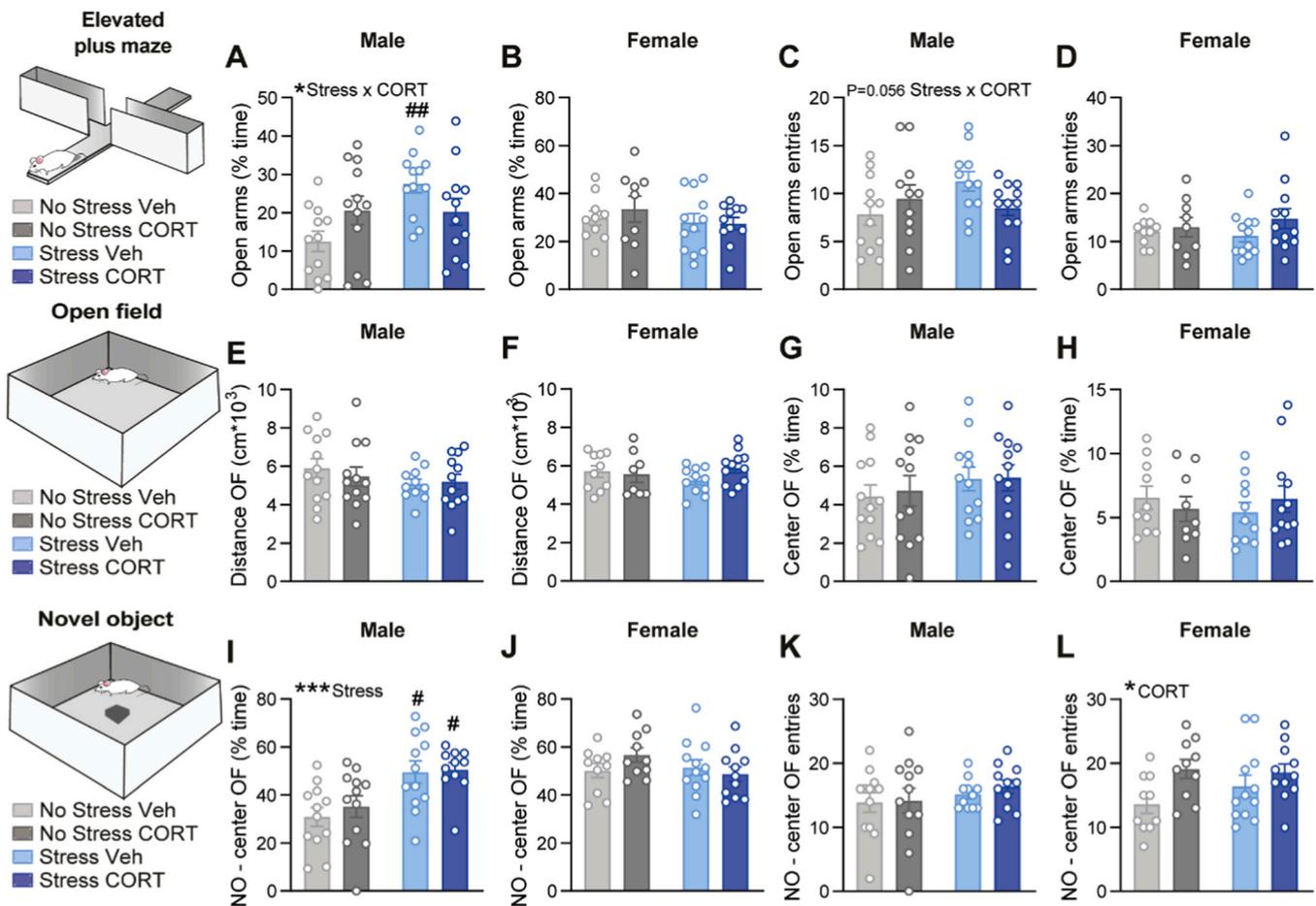


Fig. 2. Impact of corticosterone administered after stress exposure during the peripubertal period on anxiety-like behaviors, exploration and reactivity to novelty: Percentage of time in the open arms of the elevated plus maze (EPM) in males (A) and females (B). Number of entries in the open arms of the EPM in males (C) and females (D). Distance moved in the open field (OF) in males (E) and females (F). Percentage of time in the center of the OF in males (G) and females (H). Percentage of time in the center of the OF in the presence of a novel object (NO) in males (I) and females (J). Number of entries in the center of the OF in the presence of a NO in males (K) and females (L). N = 9–12/group (males) and N = 8–12/group (females). Data are represented as mean \pm SEM. Statistical analysis was done by two-way ANOVA (* $P < 0.05$, *** $P < 0.001$) with Holm-Sidak correction (# $P < 0.05$, ## $P < 0.01$ versus respective No Stress group). For detailed statistical information, see [Supplementary Table S2](#). Veh = vehicle; CORT = corticosterone.

Regarding locomotion, the total distance moved during the OF test was affected neither by stress nor by corticosterone treatment (Fig. 2E–F). In agreement, the percentage of time that the animals spent exploring the center OF was also not altered by stress or corticosterone, neither in males nor in females (Fig. 2G–H). However, when a novel object was introduced in the center of the arena, the behavior of the animals showed a clearly sexually dimorphic phenotype (Fig. 2I–L). In males, stressed animals -regardless of the treatment- showed an increase in the percentage of time in the center of the arena (Fig. 2I). In females, corticosterone-injected animals showed an increase in the number of entries in the center (Fig. 2L) without affecting the total percentage of time (Fig. 2K).

3.3. Corticosterone treatment after stress exposure during the peripubertal period does not prevent the protracted stress-induced alterations on sociability induced by peripuberty stress and has a mild impact on anhedonia and active-coping behavior observed in adulthood

Analysis of sociability with the three-chamber social preference test (Fig. 3A–F), revealed in both males and females that peripuberty stress produces a reduced social preference, expressed as a significant decrease in the interaction time with the juvenile rat (Fig. 3A–B) without differences in the interaction with the object (Fig. 3C–D). Moreover, we observed that both stress and corticosterone injections at peripuberty

lead to a reduction in social preference ratio only in females but not in males (Fig. 3E–F). The statistically significant decrease in the sociability ratio found only in stressed females injected with corticosterone might suggest that females are more vulnerable to the effects of early-life alterations.

Finally, we detected a statistically significant corticosterone x stress interaction in the percentage of saccharine preference in males, although the reduction in the preference induced by the peripuberty stress did not reach statistical significance (Fig. 4A). Conversely, saccharine preference was not altered by stress or corticosterone in females (Fig. 4B). Regarding the stress-coping strategy measured using the forced-swim test on Day 2, our results indicated that the duration of immobility, climbing, or swimming was not affected by either stress or corticosterone injections (Fig. 5A, B). Additionally, active-coping behavior was also assessed by measuring the mobility of the animals in the forced-swim test during a first exposure of 15 minutes (Day 1, habituation) (Fig. 5C, D) and a second exposure 24 hours after for 5 minutes (Day 2, test) (Fig. 5E–H). Neither stress nor corticosterone treatment had an impact on the total mobility in the forced swim test during Day 1 nor on Day 2 in males (Fig. 5C, E, G). However, we detected a statistically significant time x stress x corticosterone interaction in the total distance moved over the time on both days in female rats (Fig. 5D, F, H). Indeed, the post-hoc tests did not reach statistical significance, but stress appears to increase the distance moved similarly

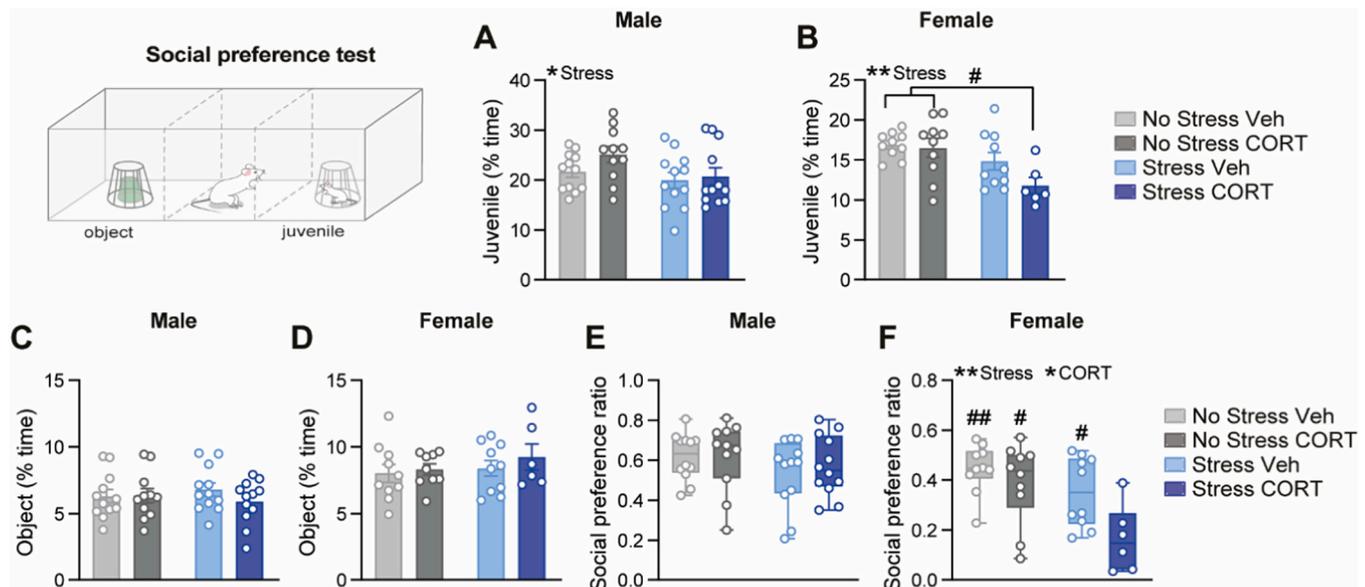


Fig. 3. Corticosterone administered after stress exposure during the peripubertal period reduces sociability in females: Percentage of time sniffing the juvenile in males (A) and females (B). Percentage of time sniffing the object in males (C) and females (D). Social preference ratio in males (E) and females (F). $N = 11\text{--}12/\text{group}$ (males) and $N = 6\text{--}10/\text{group}$ (females). Data are represented as mean \pm SEM (A–D) or as a boxplot showing the interquartile range with whiskers from minimum to maximum (E and F). Statistical analysis was done by two-way ANOVA ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$) with Holm-Sidak correction ($\# P \leq 0.05$, $\#\# P \leq 0.01$ versus Stress CORT group). For detailed statistical information, see [Supplementary Table S3](#). Veh = vehicle; CORT = corticosterone.

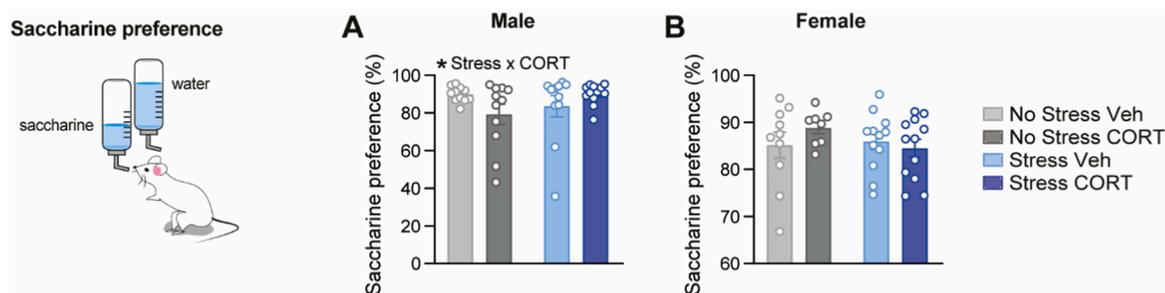


Fig. 4. Corticosterone administered after stress exposure during the peripubertal period does not affect anhedonia measured in adulthood: Saccharine preference in males (A) and females (B). $N = 11\text{--}12/\text{group}$ (males) and $N = 8\text{--}12/\text{group}$ (females). Data is represented as mean \pm SEM. Statistical analysis was done by two-way ANOVA with Holm-Sidak correction. $*P \leq 0.05$. For detailed statistical information, see [Supplementary Table S4](#). Veh = vehicle; CORT = corticosterone.

to the effect produced by corticosterone administration in control animals.

4. Discussion

To analyze the effect of corticosterone administration on the short and long-term effects of peripubertal stress, and whether these effects are sex-dependent, we used a subchronic peripubertal stress model in rats. The unpredictable chronic PPS paradigm used has been previously validated in rats. This peripubertal stress model was shown to elicit increased anxiety-like and depression-like behaviors and triggers long-lasting sociability deficits that parallel some neurobiological changes in brain regions (Márquez et al., 2013; Tzanoulinou et al., 2014a,b). Thus, male and female rats were subjected to an unpredictable stress protocol during the peripubertal period, which consists of seven days of exposure to fear-induction experiences of a non-social nature over fifteen days (Márquez et al., 2013). Control and stressed animals received an intraperitoneal injection of corticosterone or vehicle solution 1 hour after the stress exposure. Previous data suggest repeated intraperitoneal injections may be a mild stressor in these rats (Izumi et al., 1997; Stuart and Robinson, 2015). Therefore, to minimize the stress triggered by the repeated injections, mainly in control animals,

drugs were administered on three out of the seven stress days.

Evidence supports that aberrant stress-induced release of cortisol could be related to the appearance of psychopathology (De Kloet et al., 2005). Thus, to evaluate the effect of corticosterone administration in the aftermath of trauma on stress-induced corticosterone release, we analyzed the blood samples collected during the peripubertal stress protocol. It should be noted that corticosterone levels were not measured in control animals to avoid exposing them to possible stress induced by the blood collection. Not subjecting all animals to this procedure during adolescence could contribute to the behavioral differences found between control and stressed animals. Firstly, we observed a decrease in corticosterone release 30 minutes after the initial stress exposure in females, whereas corticosterone levels remained unchanged in males. Indeed, the developmental shifts that occur during adolescence might contribute to sex differences in HPA axis function (Green and McCormick, 2016). In line with this, corticosterone injections also affected the activity of the HPA axis and the consequent release of corticosterone in posterior stress exposures in females, but not in males. The low levels of corticosterone release in the corticosterone group compared to vehicle-injected animals found in females might be attributed to a lower glucocorticoid-mediated HPA axis negative feedback, as described for both female rats and women (Handa et al., 1994;

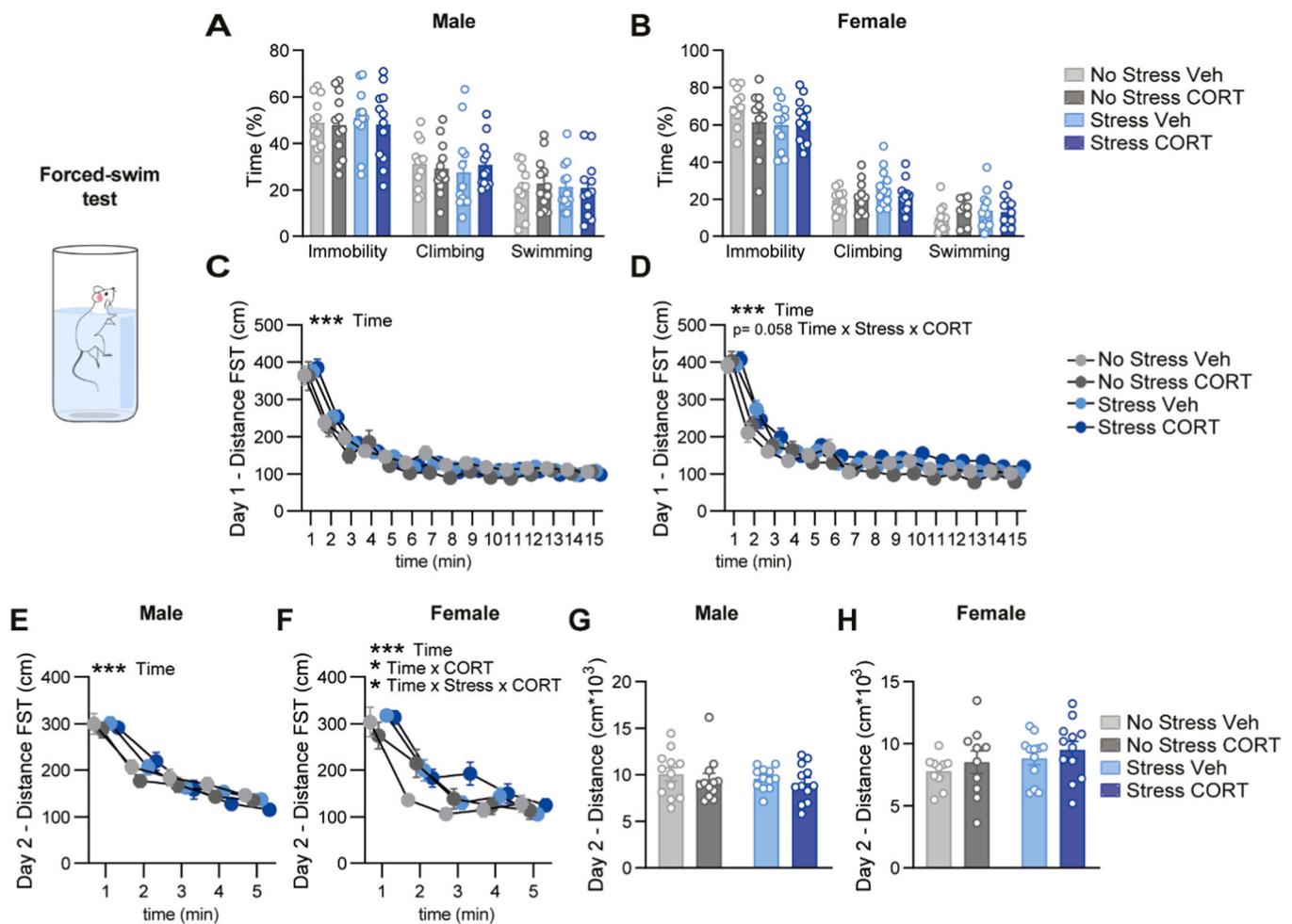


Fig. 5. Corticosterone administered after stress exposure during the peripubertal period does not affect active stress-coping behavior measured in the adulthood: Percentage of total time spent in immobility, climbing or swimming during the test session on Day 2 of the forced-swim test (FST in males (A) and females (B)). Distance moved in the forced-swim test (FST), represented by time bins of 1 min at Day 1 in males (C) and females (D). Distance moved in the FST, represented by time bins of 1 min at Day 2 in males (E) and females (F). Total distance moved in the FST at Day 2 in males (G) and females (H). $N = 11\text{--}12/\text{group}$ (males) and $N = 9\text{--}12/\text{group}$ (females). Data is represented as mean \pm SEM. Statistical analysis was done by two-way ANOVA with Holm-Sidak correction (E and F) or mixed-effects model (REML) (A-D). * $P \leq 0.05$, *** $P \leq 0.001$. For detailed statistical information, see [Supplementary Table S5](#). Veh = vehicle; CORT = corticosterone.

Kokras et al., 2019; Young et al., 1993, 2001). Moreover, sex dimorphism in HPA axis function has been widely described in rats, and numerous studies suggest that female rodents have higher glucocorticoid levels than males after HPA axis stimulation (Haleem et al., 1988; Yoshimura et al., 2003). This may be linked to the fact that low glucocorticoid responsiveness to stress has been implicated in fear extinction problems in preclinical models (Monari et al., 2024) and PTSD susceptibility in humans in a sex-dependent manner (Zimmerman et al., 2020).

When exploring the effect on anxiety-like behavior in late adolescence, we found that stress at peripuberty reduces anxiety-like behavior and increases novelty-seeking behavior in males. This finding is consistent with former observations showing that exposure to peripubertal stress exaggerates independence-building behaviors naturally happening during the period of transition from adolescence to adulthood (Laviola et al., 2003; Toledo-Rodriguez and Sandi, 2011). However, studies aimed at evaluating the long-term consequences of peripuberty stress on anxiety showed that peripubertal stress increases anxiety-like behaviors in male adult rats (Márquez et al., 2013; Walker et al., 2018). In previous studies, a reduction in anxiety-like behavior was observed in adult male rats either injected with corticosterone 1 hour after fear-conditioning (Wang et al., 2014) or exposed to predator scent (Cohen et al., 2008). Therefore, we cannot rule out that corticosterone injections at peripuberty affect the neural circuits regulating

anxiety-like behaviors as later manifested in adulthood. Indeed, the effects of corticosterone treatment on anxiety-like behavior have previously been related to glucocorticoids' capacity to modulate neural plasticity in the hippocampus (Albrecht et al., 2012; Zohar et al., 2011). The hippocampus serves as a key structure for glucocorticoid action in the brain, due to its high density of corticosteroid receptors and its function in regulating negative feedback to the HPA axis (de Kloet et al., 1998; Jacobson and Sapolsky, 1991).

Regarding sociability, a study showed that when Sprague-Dawley rats were exposed to maternal separation, only female pups displayed longer latency to contact a conspecific during a dyadic social interaction test (Farrell et al., 2016). The duration of social interactions was also found to be lower in adult female Wistar rats exposed to chronic social instability stress (Baranyi et al., 2005). The opposite phenotype, an increase in sociability, has been observed in female mice when they were exposed chronically to non-social stressors in adulthood using the chronic variable stress (Borrow et al., 2018; Dadomo et al., 2018) or the chronic swim stress protocol (Gasser et al., 2021). The inclusion of both males and females in the analysis was critical to demonstrate that adult male mice exposed to chronic variable stress do not show alterations in sociability, or in other words that this alteration in sociability was sex-specific (Borrow et al., 2018). Importantly, our results align with previous observations from our laboratory demonstrating that male

rodents exposed to peripubertal stress also show reduced time exploring a juvenile compared to control littermates (Morató et al., 2022; Tzanoulinou et al., 2014a). Overall, these pieces of evidence suggest that (i) females are more susceptible to the effects of stress on sociability than males and (ii) the directionality of the changes in sociability depends on the nature of the stressors and the period of stress exposure.

Moreover, corticosterone injections at peripuberty lead to a reduction in social preference ratio in females but not in males when measured in adulthood. In a previous study, performed exclusively in male rats (Veenit et al., 2013), reduced sociability was observed in adult animals (not exposed to stress) that were injected at peripuberty with low-dose corticosterone (5 mg/kg, as compared to 25 mg/kg used in the current study). Conversely, treatment with mifepristone, a glucocorticoid receptor antagonist, before stressor exposure at peripuberty, effectively prevented the increase in aggression observed in adulthood. It also prevented the habituation of plasma corticosterone responses observed throughout the peripubertal stress protocol (Papilloud et al., 2019). On the other hand, oral administration of a similar low-dose of corticosterone in drinking water for three weeks given to adult female mice induced a long-term reduction in sociability (Berger et al., 2019). Altogether, these published results suggest that both males and females show a similar sensitivity to low doses of corticosterone that mimics the glucocorticoid levels produced by substantial stress (Stein-Behrens et al., 1994). However, the present study suggests that male and female rats display different sensitivity to high doses of corticosterone. Importantly, human studies have shown that gender moderates the association between antisocial behavior and cortisol reactivity to stress in girls with more conduct problems showing higher cortisol response (Kobak et al., 2009). Based on previous findings, the effect of corticosterone during the peripubertal period could be related to its impact on the amygdala. Glucocorticoids are crucial in modulating social behaviors by affecting the amygdala, a key brain region involved in emotional processing and social behavior.

Indeed, stress during the peripubertal period induced increased amygdala activity in adulthood and abnormal amygdala functioning is often related to sociability deficits (Márquez et al., 2013; Tzanoulinou et al., 2014a). Besides the elevated glucocorticoid levels influence amygdala activity (Henckens et al., 2012), the glucocorticoid receptor activity in the central amygdala has been linked to the positive regulation of the HPA axis and behavioral changes induced by early life stress (Arnett et al., 2015; Ulrich-Lai and Herman, 2009). Nonetheless, further research would be necessary to corroborate the amygdala's involvement in this particular process.

5. Conclusions

This study shows that the administration of corticosterone after chronic stress exposure at peripuberty does not prevent protracted stress-induced behavioral alterations. Particularly, we observed that corticosterone treatment, accentuated rather than prevented, the sociability deficits exhibited by female stressed rats. Moreover, we observed that some stress-induced behavioral alterations and corticosterone responses are sex-specific. These results emphasize the importance of including sex as a biological variable in preclinical research aiming at finding new pharmacological opportunities for psychopathologies (Miller et al., 2017). Although further research with additional doses and time regimes is needed before drawing irrefutable conclusions, our results question the therapeutic value of high corticosterone doses in the prevention of stress-induced behavioral alterations when the nature of stress is chronic and occurs during peripuberty.

CRedit authorship contribution statement

Raul Andero: Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Carmen Sandi:** Writing – review &

editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Leire Romero:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Isabelle Guillot de Suduiraut:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jocelyn Grosse:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Laura Perez Caballero:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

None

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Author contributions

LPC, IGdS, RA, JG and CS conceived the project. IGdS and JG performed the experiments. IGdS, LPC, JG and LRR analyzed the data and interpreted the results. LPC and CS wrote the manuscript with input from all the authors, who together discussed the results and edited and approved the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2024.107164](https://doi.org/10.1016/j.psyneuen.2024.107164).

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