**Early life stress-induced vulnerability to postpartum mental disturbance: prolonged dysregulation of the HPA axis and behavior**

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**ABSTRACT**

**Objective:** Early life stress (ELS) increases the risk for postpartum depression (PPD). The patients with depression who experienced ELS tend to be treatment refractory. However, the mechanism by which ELS is involved in the pathophysiology of PPD remains unclear. We investigated the longitudinal effects of ELS on the hypothalamic-pituitary-adrenal (HPA) axis and behaviors in the postpartum period through mouse and human studies.

**Methods:** Behavioral tests (tail suspension, forced swim, and three-chamber social interaction tests) and measurement of plasma hormone levels were administered at postpartum in female mice with or without exposure to social isolation during late adolescence. Effects of a glucocorticoid receptor antagonist, a serotonin reuptake inhibitor, or a $GABA_A$ receptor modulator on their behaviors were examined at one week postpartum. Human plasma cortisol levels from women with ELS were also examined at second and third trimesters as well as two and six weeks postpartum.

**Results:** We observed that adolescent social isolation caused an aberrantly sustained elevation of glucocorticoids via dysregulation of the HPA axis, leading to long-lasting postpartum behavioral changes in female mice. The postpartum behavioral changes elicited by this ELS were not ameliorated by the medicines currently used for PPD treatment. However, a post-delivery treatment with a glucocorticoid receptor antagonist effectively ameliorated the behavioral changes in mice. We also demonstrated a significant impact of ELS on the HPA axis dysregulation and PPD in women.

**Conclusions:** We provide experimental evidence that suggest a mechanism-driven therapeutic strategy (repurposing a GR antagonist) for at least some cases of treatment refractory PPD.

## INTRODUCTION

Pregnancy and delivery are events accompanied by significant physical and psychological changes to the mother (1). Mood disturbances and cognitive impairments affecting mothers during the postpartum period are common and lead to serious mental health problems, which can consequently affect the child's development and behavior (2, 3). Adverse early life events or early life stress (ELS), such as poor family/social support or a history of psychiatric disorders, are known as predominant risk factors for postpartum depression (PPD) (1, 2, 4, 5). Women who experienced ELS are three times more likely to have PPD than women who did not experience ELS (6). Furthermore, patients with depression who experienced ELS tend to be treatment refractory (7, 8).

Mothers display endocrinological changes during pregnancy and the postpartum period. Such dynamic changes are observed in lactogenic hormones, including prolactin and oxytocin (9). The hypothalamic-pituitary-adrenal (HPA) axis is also activated during pregnancy and delivery, which results in an increase of glucocorticoid production (10-12). Furthermore, levels of estrogen, progesterone, and allopregnanolone are increased during pregnancy and followed by a precipitous drop-off after delivery (4, 13-16). Thus, the rapid decline in reproductive hormones immediately after delivery is believed to participate in the onset of PPD (4, 17). Consistent with this notion, postpartum injections of estrogen to women with PPD after delivery have shown some beneficial effects (18-21). Meanwhile, a sudden withdrawal from a two-month exposure of estradiol and progesterone at the supra-physiological doses can induce depressive symptoms in women who had a history of PPD (19). Analogously, a postpartum withdrawal from exogenous estrogen and progesterone injections in rodents lead to behavioral changes in the forced swim

test (FST), which has been frequently employed to discover and evaluate many antidepressants including those that are currently used for PPD (22-24). Thus, this rodent model has shed light on the biological and behavioral changes associated with the rapid decline in reproductive hormones after delivery. Nevertheless, these observations have not answered a crucial medical question of how ELS affects the pathophysiology of PPD at the mechanistic level. Development of relevant animal models is warranted to fill the knowledge gap.

The first-line pharmacological treatment for PPD is selective serotonin reuptake inhibitors (SSRIs), particularly sertraline due to its very low breastmilk transmission to infants (25). Although these are effective compared to placebo, only about 54% of patients respond according to a 2015 report (26), indicating a need for novel pharmacological treatments. Another limitation is that symptomatic relief only appears several weeks after the initial medication (27, 28). To overcome these limitations, an intravenous formulation of allopregnanolone (brexanolone), a positive allosteric modulator of $GABA_A$ receptors ($GABA_AR$), has been introduced to the repertoire of treatment for PPD (16). However, its drawbacks include cost and treatment accessibility (e.g., high price, intravenous administration, requirement of a Risk Evaluation and Mitigation Strategy, need for hospitalization) (16, 28-30). Another concern is a potential temporary separation from the infant during a critical period of mother-infant bonding (30). Thus, it is still important to investigate other treatment avenues for PPD. A mechanistic understanding of how ELS influences the disease pathophysiology may provide clues for novel treatment approaches. Therefore, we aimed to address this knowledge gap with a novel animal model that is useful for the pathophysiology of PPD in conjunction with ELS.

## RESULTS

### Long-lasting changes of postpartum behaviors in dams exposed to adolescent social isolation

In the present study, we applied an adolescent social isolation paradigm, which has been widely used as an ELS in mice (31-37), and examined dams' behaviors in the postpartum period. Experimental groups consisted of a) unstressed virgins, b) stressed virgins, c) unstressed dams and d) stressed dams (i.e. mice that were exposed to adolescent social isolation and gave the first-time birth to pups in adulthood) (**Figure S1**). We chose the tail suspension test (TST) and FST as major assays, because their frequent use in discovering and evaluating many antidepressants including those that are currently used for PPD: although it is very difficult to capture several features in human depression (face validity) by mouse behavioral assays, the utility of these tests that evaluate a switch from active to passive behavior upon an acute stress, underlying behavioral adaptation and survival, has been proven in pharmacological studies for depression (38). Indeed, in the drug discovery process for PPD, FST was used for the discovery of an allopregnanolone analog, $GABA_AR$ modulator (39). We did not observe any differences in the TST and FST among the four groups at postpartum days 0 and 1, respectively (**Figure 1**). Dams exposed to adolescent social isolation (stressed dams), compared with other three groups, showed increased immobility time during the TST and FST at postpartum days 7 and 8, respectively (**Figure 1**). The behavioral changes in stressed dams were prolonged for at least three weeks after delivery (**Figure 1**).

Social cognition in mothers may be regulated to monitor and interpret social signals from others, and safely navigate the new living environment (40). Thus, we examined the effect of adolescent

stress on dams' postpartum behavior in the three-chamber social interaction test (SIT) which is used to test the social communication ability in rodents (41). No behavioral differences among the groups were observed at postpartum day 0 in both sociability and social novelty recognition in the SIT (**Figure S2**). At postpartum days 7 and 21, stressed dams, compared with other three groups, showed a significant difference in social novelty recognition, but not in sociability (**Figure S2**). The postpartum behavioral changes in the TST, FST, and SIT in mice that experienced adolescent social isolation may represent clinically important aspects of PPD, such as delayed-onset and prolonged symptoms, and may be relevant to treatment refractory patients.

**Prolonged elevation of corticosterone levels and altered glucocorticoid signaling in dams exposed to adolescent social isolation: its pivotal role in long-lasting changes of postpartum behaviors**

Many studies have reported alterations in plasma hormone levels in patients with PPD and a correlation between hormonal changes and onset of PPD symptoms (9, 19-22, 42-44). Thus, we examined the plasma levels of estradiol, progesterone, prolactin, oxytocin, and corticosterone in our animal model. Although we observed physiological changes in the levels of estradiol, progesterone, prolactin, and oxytocin associated with pregnancy and delivery, no further differences in these changes were observed between unstressed and stressed dams at any time point (**Figure S3**). In contrast, the level of plasma corticosterone in both unstressed and stressed dams was increased after late pregnancy in comparison to virgin mice (**Figure 2A**). Corticosterone levels in unstressed dams began to decrease after delivery (**Figure 2A**). Notably, corticosterone levels in stressed dams were consistently higher than those in unstressed controls at 1 and 3 weeks postpartum (**Figure 2A**). These data suggest that a potential change in the

control of the HPA axis may occur in stressed dams, resulting in a prolonged elevation of corticosterone levels. Positive correlations between the levels of plasma corticosterone and behavioral changes in the TST and FST were observed at 1 week and 3 weeks postpartum (**Figure S4**).

The above results suggest that the alteration in the HPA axis may underlie the behavioral changes observed in stressed dams. To address the causal role directly, a selective GR antagonist, CORT113176, was orally administered from gestation day 14 to 24 h prior to behavioral testing at one-week postpartum when we first observed significant behavioral changes in stressed dams. The CORT113176 administration blocked the behavioral changes in the TST and FST in stressed dams at postpartum days 7 and 8, respectively (**Figure 2B**). This drug administration also blocked the behavioral changes in the social novelty recognition trial of the SIT in stressed dams at postpartum day 7 (**Figure S5**). These results support the hypothesis that prolonged activation of glucocorticoid signaling underlies long-lasting behavioral changes in dams exposed to adolescent social isolation.

**Molecular expression changes in the HPA axis in dams exposed to adolescent social isolation**

In the HPA axis, corticotropin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus triggers the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, leading to the production of glucocorticoids from the adrenal cortex, which in turn regulate GRs on the PVN and pituitary gland by a negative feedback mechanism (45, 46).

We hypothesized that mice exposed to adolescent social isolation might show molecular expression changes in the HPA axis after delivery, compared to mice without this adolescent social isolation. To address this question, we measured the mRNA levels of CRH and GR in the PVN, and those of CRH receptor1 (CRHR) and GR in the pituitary gland at 1 week postpartum when stressed dams start to show postpartum behavioral changes described above. We compared the expression data among stressed dams, unstressed dams, age-matched stressed virgins, and age-matched unstressed virgins. Delivery itself augmented the expression of GR in both the PVN and pituitary gland in unstressed mice (**Figure 2C**). In stressed dams, this delivery-associated augmentation of GR was not observed in the pituitary whereas it was still seen in the PVN (**Figure 2C**). In regard to the CRH-CRHR, we observed an upregulation of CRHR in the pituitary gland only in stressed dams (**Figure 2C**). The changes seen in the pituitary gland of stressed dams indicated that adolescent social isolation might affect at least the pituitary gland after delivery.

To validate this notion at the functional levels, we performed a low-dose dexamethasone (DEX) suppression test (47, 48) at 1 week postpartum. DEX, a synthetic glucocorticoid, does not effectively penetrate the blood-brain barrier (BBB) when used at low doses (e.g., 0.1 mg/kg) (47-49). The low dose of DEX binds to GR in the anterior pituitary gland, but not the PVN, because the anterior pituitary is located outside the BBB (49-51). Administration of a low dose (0.1 mg/kg) of DEX reduced the levels of ACTH and corticosterone in unstressed dams, whereas it failed to suppress these hormones in stressed dams (**Figure 2D**).

**Normalization of postpartum behavior changes in dams exposed to adolescent social isolation following a post-delivery treatment with a GR antagonist**

It is possible to hypothesize that the aforementioned mouse model may represent an important subset of PPD patients who experience ELS and resultant changes in the HPA axis. Because half of patients with PPD are refractory to SSRI treatment and these treatment refractory cases are likely to be associated with ELS (7, 8), we investigated the utility of our adolescent stress paradigm in modeling these clinically difficult cases. To this end, we examined the beneficial effects of post-delivery treatment with the SSRI, the $GABA_AR$ modulator, or the GR antagonist. Treatment with a SSRI (fluoxetine) for one-week post-delivery did not normalize the behavioral changes in TST and FST in stressed dams (**Figure 3**). Treatment with a $GABA_AR$ modulator (ganaxolone) was also ineffective (**Figure 3**). In contrast, a one-week post-delivery treatment with a GR antagonist (CORT113176) normalized the behavioral changes in both the TST and FST in stressed dams (**Figure 3**).

**A link between early life stress, a sustained increase in the glucocorticoid signaling, and PPD in humans**

To examine whether our preclinical model shows clinically-relevant biology and behavioral phenotypes, we assessed the possible influence of ELS on HPA axis function and postpartum mental conditions in humans. Specifically, we examined the relationship between ELS [a history of major mental illness, abnormal home environment, abnormal childhood behavior, and/or traumatic events], the HPA axis, and the development of PPD in 116 women. We observed the relationship between the development of PPD and ELS, in particular a history of mental illness (**Figures 4A** and **4B**). We then compared the level of plasma cortisol, as a surrogate of the HPA

axis activity, between non-PPD women who had a previous mental illness diagnosis and PPD patients who had a previous mental illness diagnosis. The former group showed an equivalent peak of glucocorticoids leading up to delivery followed by a gradual decline (**Figure 4C**). In contrast, cortisol levels in women with PPD who had a previous mental illness diagnosis exhibited both elevated and sustained levels of plasma cortisol until at least six weeks postpartum (**Figure 4C**).

## DISCUSSION

The main findings from the present study using a novel animal model are that adolescent social isolation elicits prolonged elevation of corticosterone levels and glucocorticoid signaling in mice, which in turn results in long-lasting postpartum behavioral changes in the TST, FST, and SIT. We pharmacologically proved that the alteration in the glucocorticoid signaling after delivery accounted for the behavioral changes. Of the most importance, we demonstrated that a short-term (one-week), post-delivery treatment, against this pathological mechanism is sufficient to block the behavioral changes in stressed dams, whereas other medicines currently used for PPD in the clinical setting are ineffective. Furthermore, we observed a sustained elevation of cortisol in patients with PPD with a history of mental illness. Together, we suggest that ELS may underlie long-lasting changes in the HPA axis and pathological symptoms at least in a subset of patients with PPD. The animal model with adolescent social isolation may be particularly useful for studying treatment refractory cases of PPD that are known to be associated with ELS. Given a GR antagonist is clinically available, this medicine may be utilized as an immediate and effective treatment for, at least a subset of, PPD. Our present data also suggest which clinical and biological stratifications will be effective in identifying the subset.

The mechanisms of how $GABA_AR$ modulators exert beneficial influences on PPD are still actively being studied (52-55). Intervention with $GABA_AR$-mediated neurotransmission in the PVN may be one of these mechanisms (52). Under our experimental conditions, neither a $GABA_AR$ modulator nor a SSRI ameliorated the behavioral changes. Although we selected the doses and administration routes for these compounds based on published protocols (56-59), further studies that test different doses and durations are warranted for further confirmation.

In the present study, we used an orally administered GR antagonist to directly translate the observation to humans. However, the systemic administration may possibly elicit confounding factors, in particular those associated with its impact on metabolic pathways (60, 61). Note that, at least in our experimental conditions, this GR antagonist (also the SSRI, and the $GABA_AR$ modulator that we used) did not affect body weight (**Figure S6**), suggesting that the influences of these medicines on body metabolism may be minimal.

The present data indicated molecular changes at least in the anterior pituitary gland in the HPA axis in our PPD model. Given that there were no differences in the levels of oxytocin and prolactin (released by the posterior lobe) between unstressed and stressed dams, it is unlikely that the posterior pituitary gland is affected in the present model. Further studies with region- or circuit-specific manipulations of the GR and related molecules will facilitate an understanding of the circuitry mechanism that is sensitive to ELS and its impact on HPA axis regulation and postpartum behaviors. There are specific brain regions that have been associated with ELS (45,

62). It will be worthwhile to study how the projections from the brain regions affected by ELS to the anterior pituitary are controlled in this PPD model.

Previous studies on PPD have frequently examined the roles of reproductive steroid hormones (4, 13-16). Although the goal of preclinical studies is usually to shed light on novel mechanisms, the success of following translational studies will be supported when multifaceted information from preclinical studies is well integrated. Therefore, in order to extrapolate this preclinical observation to human studies, more frequent sampling of blood and examination of glucocorticoid signaling, together with other reproductive steroid hormones, in greater detail will be beneficial.

Although other environmental stressors or complex genetic factors may also underlie PPD, the goal of the present study is to establish a straightforward model with a single environmental stressor under a homogenous genetic condition, which allowed us to dissect a causal role of an altered glucocorticoid signaling in long-lasting behavioral changes in the postpartum period. Future studies with this unique model may include to examine maternal behaviors, transgenerational transmission of risk to children reared by stressed mothers, involvement of the mineralocorticoid receptor, differences between depression during pregnancy and PPD, and involvement of genetics and epigenetics in the pathological mechanisms underlying PPD.

We underscored the potential of a GR antagonist for treatment of a subset of PPD. GR antagonists can be orally administered in treatment of Cushing syndrome (mifepristone) as well as in other ongoing clinical trials (63, 64). As summarized above, a post-delivery, short-term

treatment of a GR antagonist effectively normalizes postpartum behavioral changes in our mouse model, whereas medicines currently used for PPD treatment were ineffective within this condition. Accordingly, our data would support the notion that repurposing a GR antagonist may lead to an immediate improvement for at least some cases of treatment refractory PPD.

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## AUTHOR CONTRIBUTIONS

**M.N.** conceived and designed the project, and supervised **S.L.**, **D.J.W.**, and **K.K.** for practical experiments with the help of **S.K.** and the guidance of **A.S.**. Experiments were performed by **M.N.**, **S.L.**, **D.J.W.**, and **K.K.**. Data was analyzed by **M.N.**, **K.Y.**, and **K.K.**. The first manuscript draft was written by **M.N.** and **A.S.** with input from all the authors. **M.N.** and **A.S.** revised and edited the manuscript for the final version. **G.S.W.** provided expertise of endocrinology in a translationally relevant manner. **J.L.P.** provided human plasma samples with participants' information as well as expertise of postpartum mood disorders.

## DECLARATION INTERESTS

All authors declare that they have no competing interests.

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## FIGURE LEGENDS

**Figure 1. Long-lasting behavioral changes in the tail suspension and forced swim tests in dams exposed to adolescent social isolation.**

**A**, **B**, Immobility time (seconds) during the tail suspension (**A**) and forced swim tests (**B**) were assessed at postpartum days 0 and 1, postpartum days 7 and 8, and postpartum days 21 and 22, respectively. Behavioral changes emerged at 1 week postpartum and remained until at least 3 weeks postpartum. No changes across groups were observed immediately after delivery. N=10-12. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

**Figure 2. Prolonged elevation of corticosterone levels and altered glucocorticoid signaling in dams exposed to adolescent social isolation: its pivotal role in long-lasting changes of postpartum behaviors.**

**A**, Levels of corticosterone were measured at five time points: virgin, late pregnancy, 0 week postpartum, 1 week postpartum, and 3 weeks postpartum. Corticosterone levels in stressed dams were consistently higher than those in unstressed controls at 1 and 3 weeks postpartum. N=9-30. **B**, Causal effects of the GR on postpartum behavioral changes in the TST and FST. CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing) ameliorated the behavioral changes in the tail suspension and forced swim tests in stressed dams at postpartum days 7 and 8, respectively. The antagonist did not affect behavior in the TST and FST in unstressed dams. N=14-17. **C**, Differences in CRHR and GR mRNA levels in the pituitary, but not PVN, between unstressed and stressed dams. Differences in the mRNA levels of CRHR and GR in the pituitary were observed between unstressed and stressed dams at postpartum day 9. No difference in the mRNA levels of CRH and GR in the PVN was observed

between unstressed and stressed dams at postpartum day 9. There was no difference in the mRNA levels examined in this study between unstressed and stressed virgin mice. N=7. **D**, Changes in HPA axis negative feedback in stressed postpartum dams. In the dexamethasone (DEX) suppression test, DEX (0.1 mg/kg, *i.p.*) was administered at postpartum day 8. Treatment with low dose DEX reduced the levels of ACTH and corticosterone in unstressed dams, whereas it failed to suppress these hormones in stressed dams. N=11. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$; $^{\#\#}P<0.01$ versus unstressed virgins; $^{++}P<0.01$ versus stressed virgins.

**Figure 3. Normalization of postpartum behavior changes in dams exposed to adolescent social isolation following a post-delivery treatment with a GR antagonist.**

Mice were treated with a SSRI fluoxetine (18 mg/kg, *p.o.*), a $GABA_A$ receptor modulator ganaxolone (10 mg/kg, *i.p.*), or a GR antagonist CORT113176 (80 mg/kg, *p.o.*) once daily from postpartum day 0 to 24 h prior to behavioral testing. Post-delivery treatment with only the GR antagonist ameliorated the behavioral changes in the tail suspension and forced swim tests in stressed dams. N=12. Values are represented as mean ± SEM; $^{**}P<0.01$; $^{\#\#}P<0.01$ versus unstressed dams treated with the SSRI; $^{++}P<0.01$ versus unstressed dams treated with the $GABA_A$ receptor modulator; $^{\$\$}P<0.01$ versus unstressed dams treated with the GR antagonist.

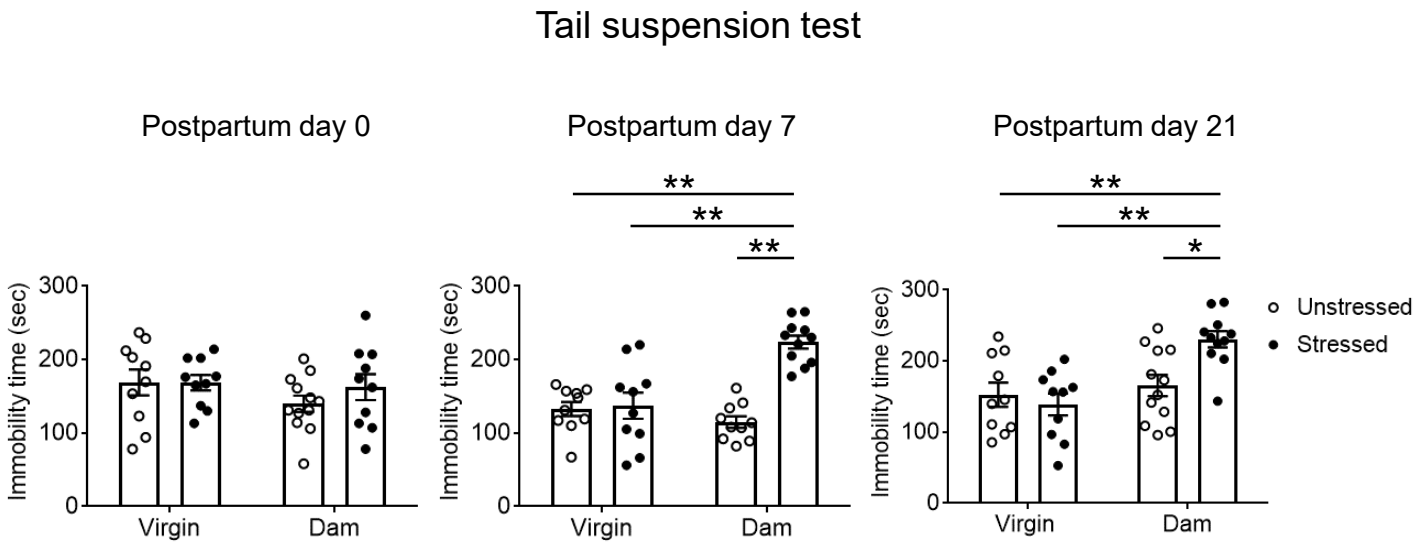
**Figure 4. A link between early life stress, a sustained increase in the glucocorticoid signaling, and PPD in humans.**

**A**, Significance of adverse early life events on PPD in human subjects. Participants with a history of mental illness were more likely to be diagnosed with PPD than those with other risk

factors. The white and black slices of the pie charts show the percentages of participants without and with PPD, respectively. N=116. **B**, Risk factors for PPD in human subjects. A history of mental illness was the most important risk factor positively correlated and linked to the development of PPD compared to childhood behavior, home environment, and traumatic events. N=116. **C**, A prolonged elevation in plasma cortisol levels in PPD patients with a history of mental illness. Participants with a history of mental illness and no diagnosis of PPD showed a significant decline in cortisol levels after delivery. Participants with a history of mental illness and PPD showed a sustained elevation of cortisol levels until at least 6 weeks postpartum. N=10-60. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

Figure 1

A


Tail suspension test
Postpartum day 0
Postpartum day 7
Postpartum day 21
Immobility time (sec)
300
200
100
0
Virgin
Dam
**
**
**
**
**
*
Unstressed
Stressed

B

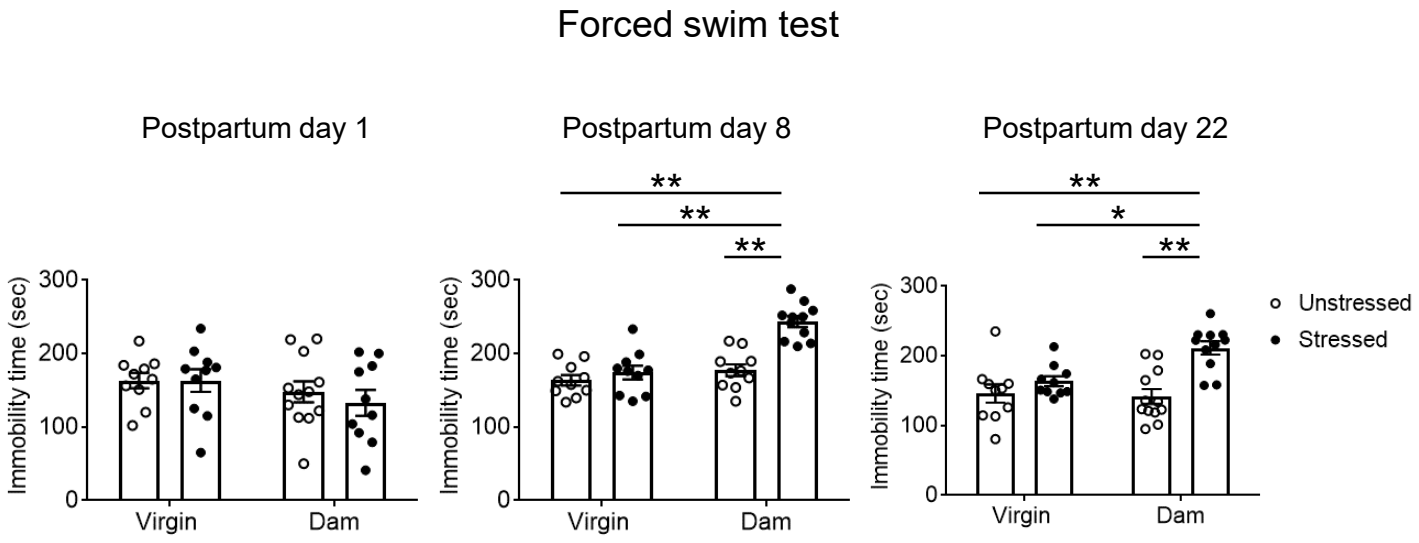
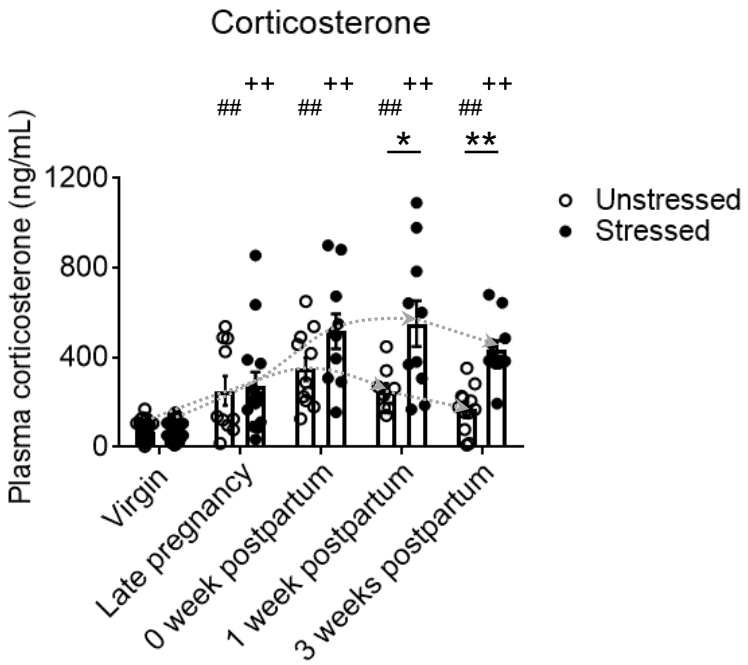
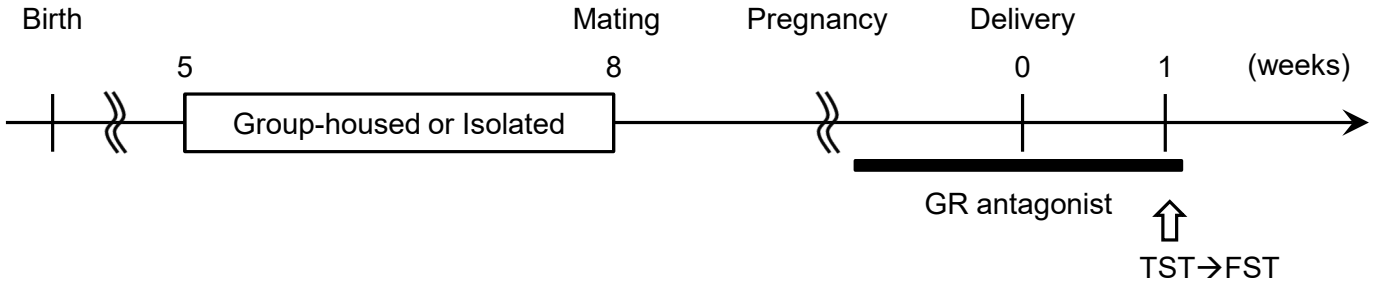

Forced swim test
Postpartum day 1
Postpartum day 8
Postpartum day 22
Immobility time (sec)
300
200
100
0
Virgin
Dam
**
**
**
**
*
**
Unstressed
Stressed

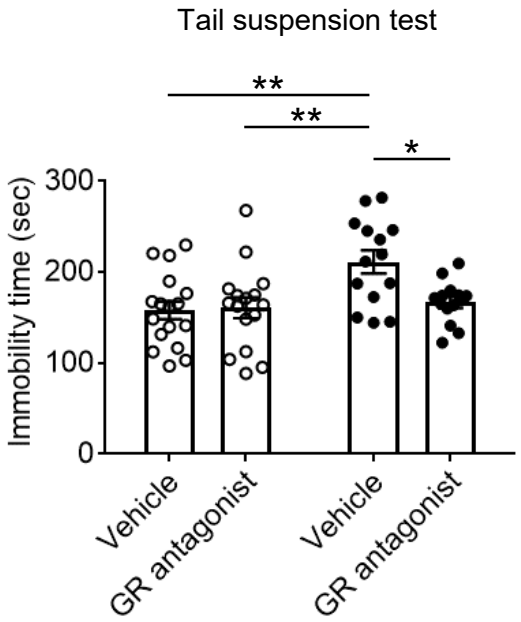
Figure 2

A


Corticosterone
Plasma corticosterone (ng/mL)
1200
800
400
0
++
##
*
**
Unstressed
Stressed
Virgin
Late pregnancy
0 week postpartum
1 week postpartum
3 weeks postpartum

B


Birth
Mating
Pregnancy
Delivery
5
8
0
1
(weeks)
Group-housed or Isolated
GR antagonist
TST→FST


Tail suspension test
Immobility time (sec)
300
200
100
0
**
**
*
Vehicle
GR antagonist
Vehicle
GR antagonist

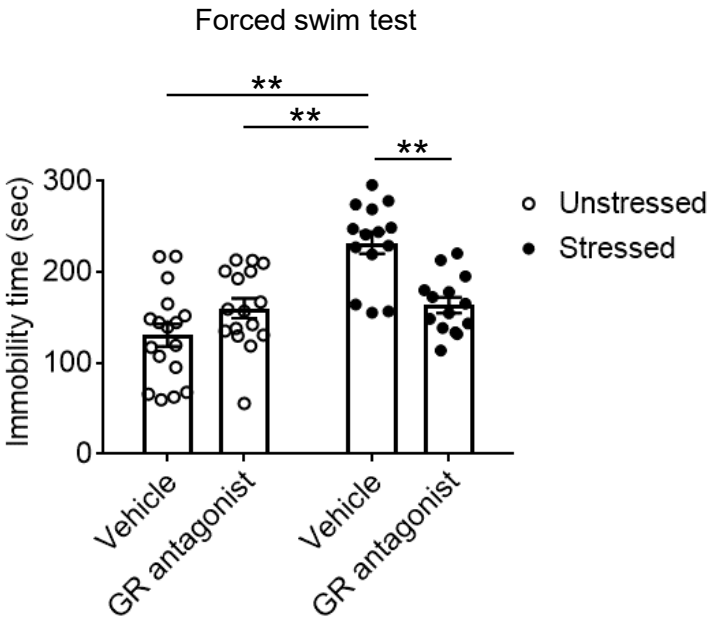

Forced swim test
Immobility time (sec)
300
200
100
0
**
**
**
Unstressed
Stressed
Vehicle
GR antagonist
Vehicle
GR antagonist

Figure 2 (cont'd)

C

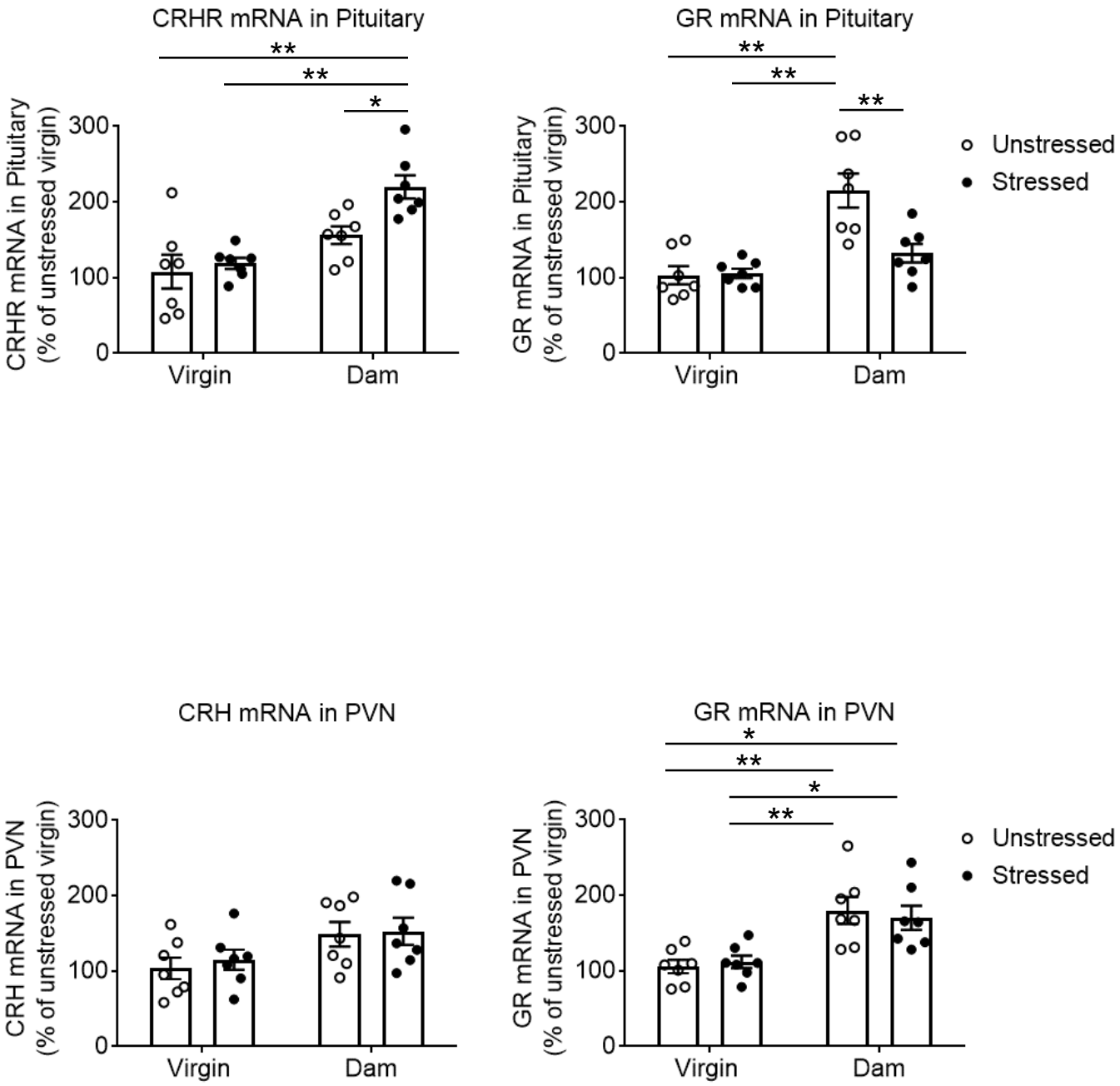


Figure 2 (cont'd)

D

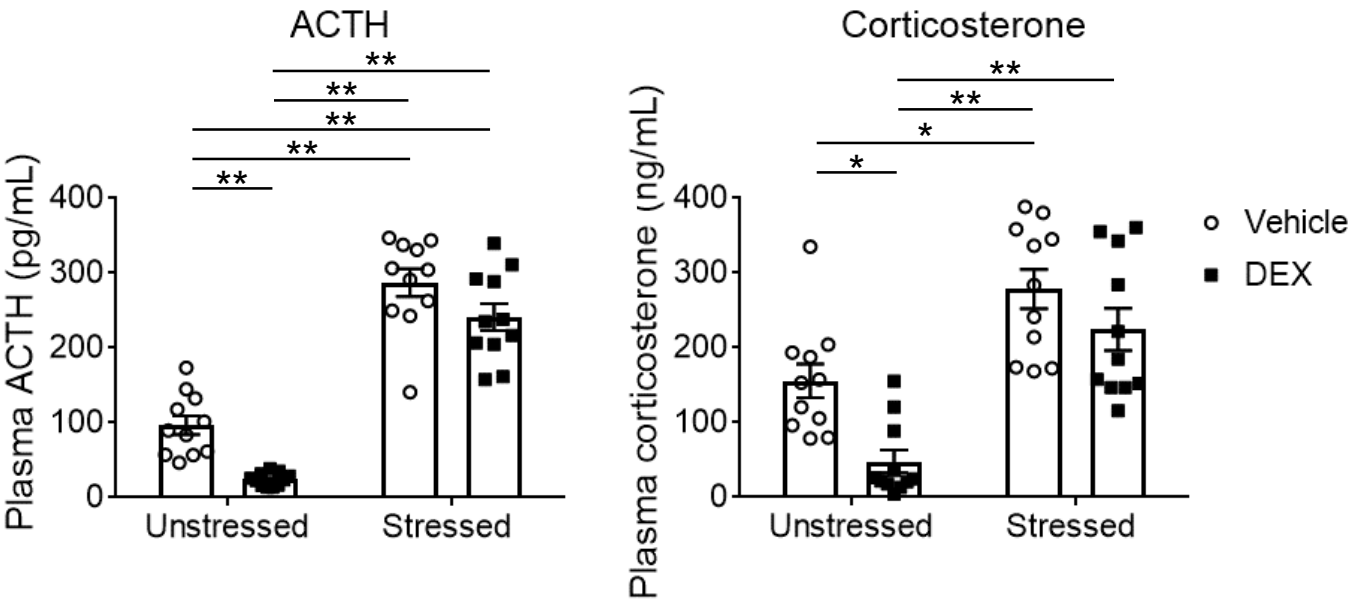


Figure 3

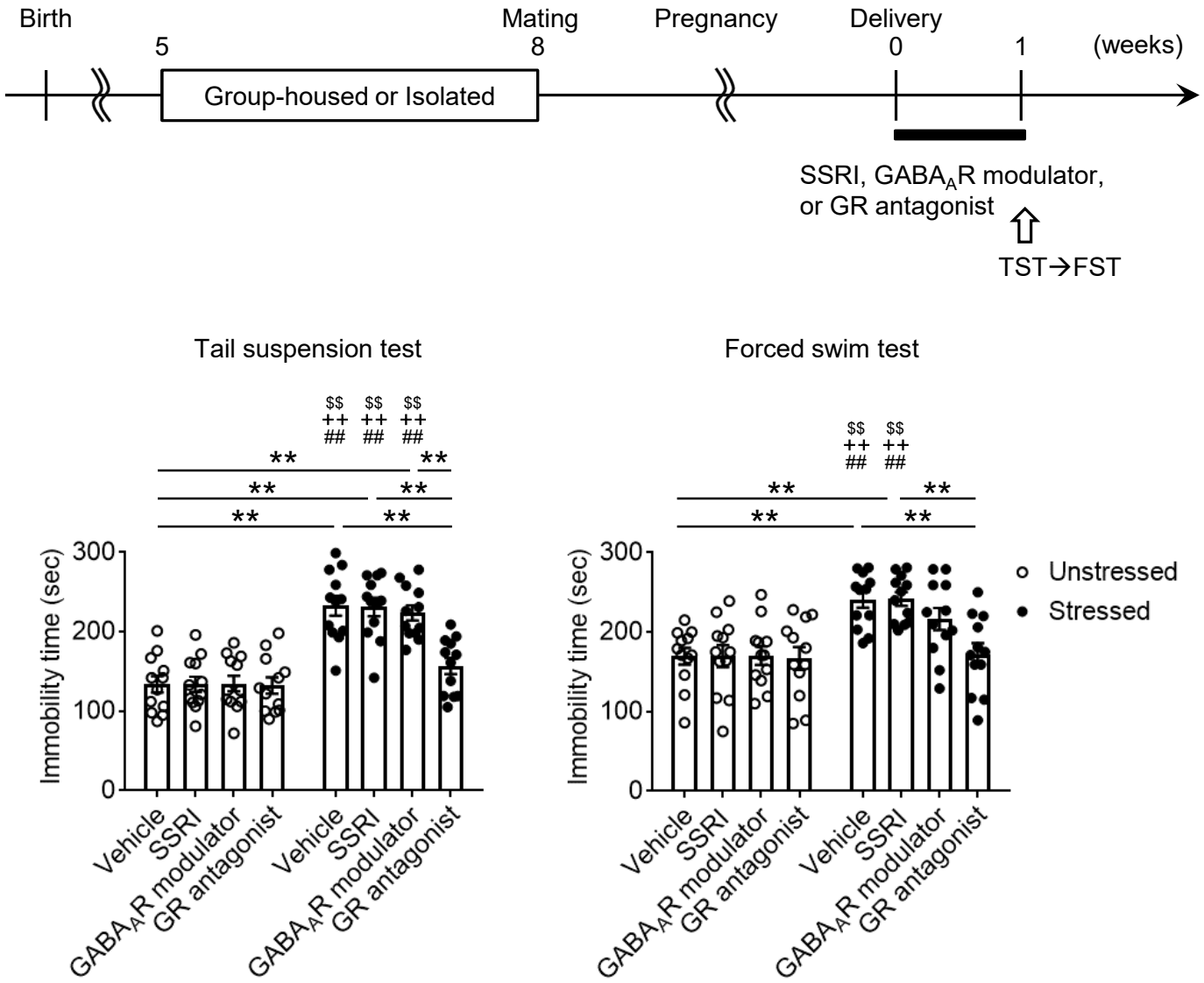
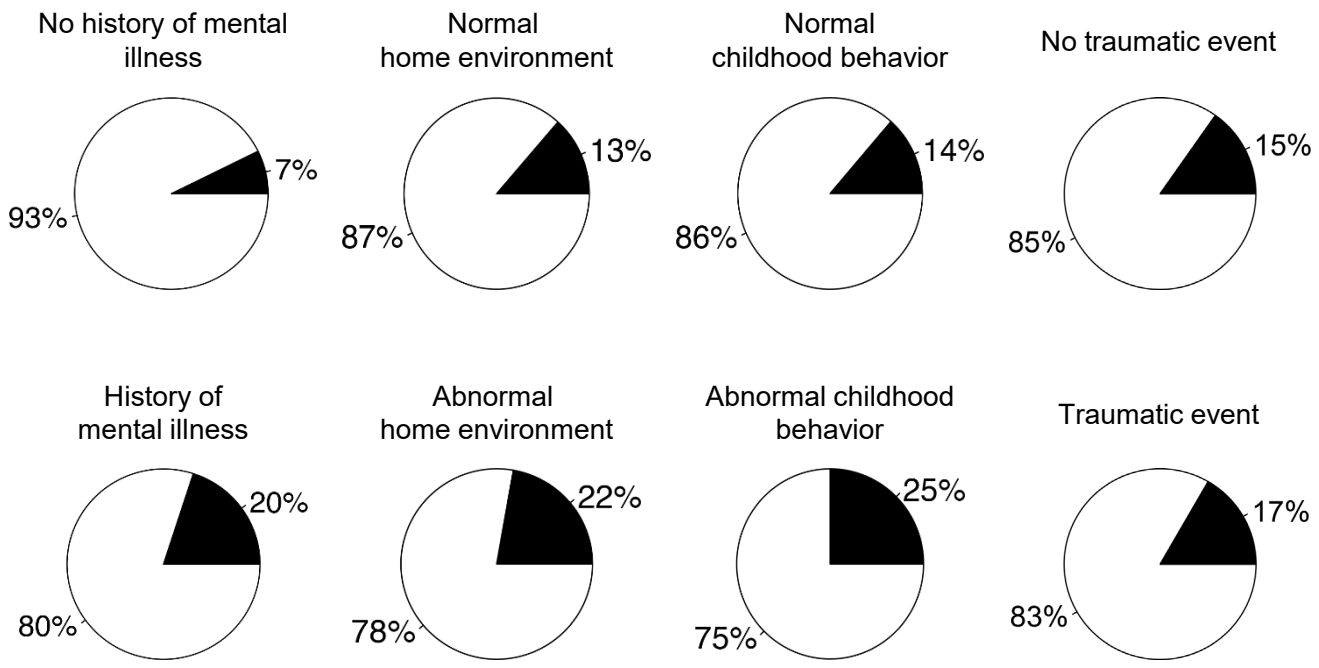

Birth
5
Mating
8
Pregnancy
Delivery
0
1
(weeks)
Group-housed or Isolated
SSRI, GABA$_A$R modulator,
or GR antagonist
TST→FST
Tail suspension test
Forced swim test
Immobility time (sec)
300
200
100
0
Vehicle
SSRI
GABA$_A$R modulator
GR antagonist
Unstressed
Stressed
**
$$
++
##

Figure 4

A


No history of mental illness
7%
93%
Normal home environment
13%
87%
Normal childhood behavior
14%
86%
No traumatic event
15%
85%
History of mental illness
20%
80%
Abnormal home environment
22%
78%
Abnormal childhood behavior
25%
75%
Traumatic event
17%
83%

B

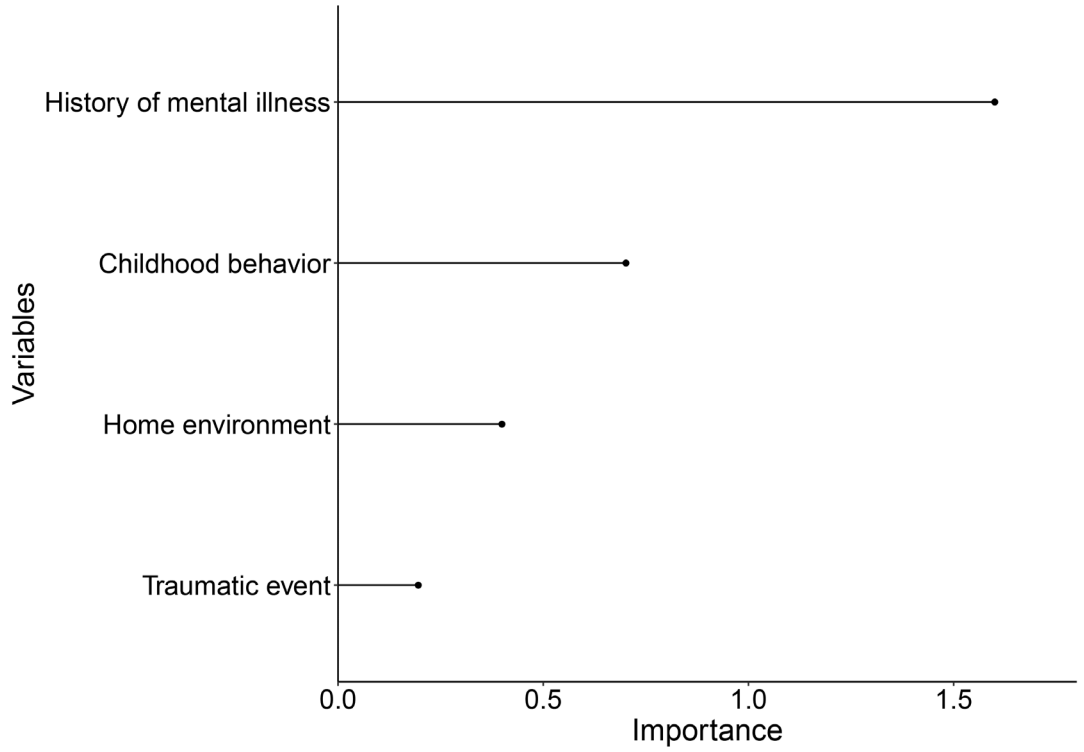
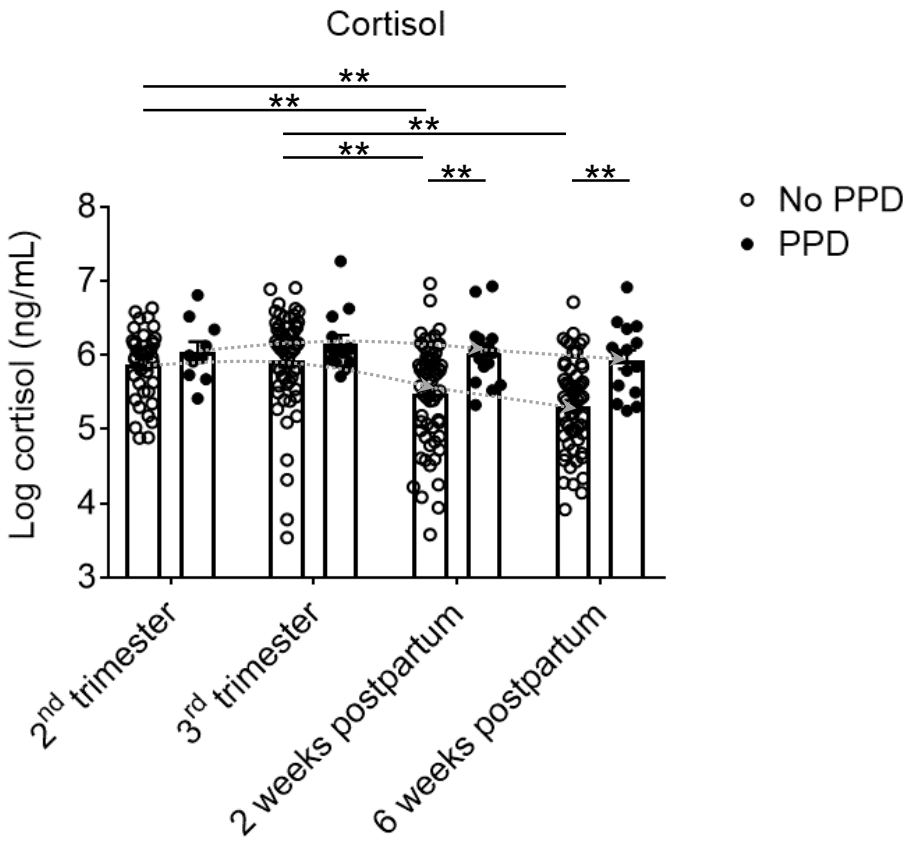

History of mental illness
Childhood behavior
Home environment
Traumatic event
Variables
0.0
0.5
1.0
1.5
Importance

Figure 4 (cont'd)

C



Data supplement for Niwa et al.,

**Early life stress-induced vulnerability to postpartum mental disturbance: prolonged dysregulation of the HPA axis and behavior**

The PDF file includes:

Supplemental Methods

Supplemental References

Figs. S1 to S6

Tables S1 and S2

## Supplemental Methods

**Animals.** To determine the effects of adolescent psychosocial stress on postpartum behaviors in adulthood, healthy virgin C57BL/6J female mice were exposed to mild isolation stress during late adolescence (from 5 to 8 weeks of age), which alone caused no endocrine or behavioral changes (1). Each mouse was then mated with a C57BL/6J male mouse at 8 weeks of age and gave birth to pups. This group was designated "stressed postpartum mice". Isolation consisted of no interaction with other mice and confinement to opaque wire-topped polypropylene cages, whereas group-housed mice were kept in clear wire-topped plastic cages (21×32×13 cm). Unstressed virgins, stressed virgins, unstressed dams, and stressed dams were age-matched in all the experiments. All mice were maintained under a controlled environment (23 ± 3˚C; 40 ± 5% humidity; light and dark cycles started at 7 am and 9 pm, respectively) with ad libitum access to food and water. All experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, under the animal protocols approved by the Institutional Animal Care and Use Committees at the Johns Hopkins University and the University of Alabama at Birmingham.

**Drug Treatments in mice.** To assess the influence of glucocorticoid signaling on behavioral changes related to mobility/despair and social cognition in stressed postpartum mice, the selective GR antagonist, CORT113176 (80 mg/kg, *p.o*, once a day, dissolved in dimethyl sulfoxide and further dissolved in saline containing 0.5 % hydroxypropyl methylcellulose + 0.1 % Tween 80), was orally administered from gestation day 14 to 24 h prior to behavioral testing. The three-chamber social interaction test (SIT) was performed at postpartum day 7 on a separate cohort. Unlike RU486 (mifepristone), which we have used previously (1, 2), CORT113176 does

not antagonize progesterone signaling (3-7). To examine the therapeutic effects of a GR antagonist (CORT113176), an antidepressant (fluoxetine), and an allopregnanolone analog (ganaxolone) on postpartum behaviors related to mobility/despair, mice were treated with CORT113176 (80 mg/kg, *p.o*, dissolved in dimethyl sulfoxide and further dissolved in saline containing 0.5 % hydroxypropyl methylcellulose + 0.1 % Tween 80), fluoxetine (18 mg/kg, *p.o.*, dissolved in deionized water), or a $GABA_A$ receptor modulator ganaxolone (10 mg/kg, *i.p.*, dissolved in saline containing 0.5 % Tween 80) once daily from postpartum day 0 to 24 h prior to behavioral testing. The tail suspension test (TST) and forced swim test (FST) were assessed at postpartum days 7 and 8, respectively. The doses and routes were selected based on published protocols (3, 8-10).

**Behavioral assays in mice.** Two cohorts (the first cohort was used for TST followed by FST, and the second cohort for SIT) for each postpartum time point were used. Different cohorts of mice were prepared to avoid the repeated exposure to stressful behavioral procedures (**Fig. S1**).
*Tail Suspension Test (TST)*: Female mice were suspended using a piece of tape attached to the tail. A trimmed 1000 µl pipette tip was used over the tail to prevent biting the tape. The duration of immobility was recorded for 6 minutes and calculated as follows: 360 (sec) - struggling time (sec) = immobility time (sec). Dams were tested at postpartum days 0, 7, and 21.
*Forced Swim Test (FST)*: At postpartum days 1, 8, and 22 (24 h after the TST), female mice were individually placed in a transparent glass cylinder (8 cm in diameter x 20 cm high) containing water at 23 ºC to a depth of 15 cm. The duration of immobility was measured for 6 minutes and calculated as follows: 360 (sec) - swimming time (sec) = immobility time (sec).

*Three-chamber Social Interaction Test (SIT)*: Female mice were individually placed in the center chamber with the two adjacent chamber doors open and allowed to habituate for 10 minutes to the three chambers for three consecutive days before testing. On postpartum days 0, 7, and 21, the same mouse was placed back in the center chamber for 5 minutes with the two chamber doors closed. In the "sociability" trial of the SIT, the subject mouse encountered a novel mouse (stranger 1) in a wire cage in one chamber and an empty wire cage in the other chamber for 10 minutes. Afterwards, the chamber doors were closed again, and the subject mouse was confined to the center chamber for five minutes. In the "social novelty recognition" trial, the subject mouse encountered stranger 1 (now familiar) and a novel mouse (stranger 2) in the previously empty wire cage. The time spent sniffing each wire cage was analyzed using ethovision computer software and manual counts.

**Quantitative real-time PCR.** The PVN and pituitary were rapidly dissected out 24 h following the last behavioral test. Total RNA was isolated by using the RNeasy Mini Kit and converted into cDNA with the SuperScript$^{TM}$ III First-Strand System for RT-PCR Kit. All reactions were carried out in duplicate using the 1 × Taqman master mix, 1 × Taqman probes for each gene (Crh, Chhr1, Gr, and β-actin) and 200 ng of cDNA template in a total volume of 20 µl. The β-actin Taqman probe was used as the internal control. Real-time reactions were performed on a Bio-Rad CFX96 Touch Real-Time PCR Detection System with standard PCR conditions (50°C for 2 min; 95°C for 10 min and 95°C for 15 s and 60°C for 1 min for 40 cycles). The expression levels were calculated as described previously (11).

**Measurement of plasma hormone levels in mice.** Blood was collected from the inferior vena cava under isoflurane anesthesia between 9 am and 11 am, 24 h following the FST. Levels of plasma corticosterone, estradiol, progesterone, oxytocin, and prolactin were assessed using commercially available enzyme immunoassay (EIA) kits (1) and measured at four time points [virgin, late pregnancy (gestation day 19), 0 week postpartum (postpartum day 2), and 1 week postpartum (postpartum day 9)]. Plasma corticosterone was also measured at 3 weeks postpartum (postpartum day 23) following the last behavioral testing. Plasma samples from different cohorts of mice at each time point were prepared to avoid the repeated exposure to stressful behavioral procedures. To measure the function of the HPA axis negative feedback loop, a dexamethasone (DEX) suppression test was performed at postpartum day 8. Dexamethasone is a corticosteroid that, at low doses, acts on the anterior pituitary gland to suppress adrenocorticotropic hormone (ACTH) release, which inhibits corticosterone release from the adrenal gland (12-14). DEX at low doses cannot pass the blood-brain barrier and can provide insight into the functionality of the HPA axis (15, 16). DEX (0.1 mg/kg, *i.p*) was administered to mice six hours prior to collecting blood samples at 5pm on postpartum day 8 (17, 18). Plasma ACTH and corticosterone levels after treatment with DEX were assessed using commercially available EIA kits as published (1).

**Human subjects.** Study participants were recruited through the Johns Hopkins Women's Mood Disorders Center and written informed consent was obtained by all patients. 116 pregnant women (18 years or older) with a history of mental illnesses underwent psychological interviews with the Structured Clinical Interview for DSM-IV Axis 1 Disorders (SCID) by study psychiatrists, and blood draws were conducted between 8:45 am and 4:45 pm at four time points across pregnancy and the postpartum period (2$^{nd}$ and 3$^{rd}$ trimester, 2 weeks postpartum, and 6

weeks postpartum). PPD was defined as developing major depression within six weeks of delivery and not during pregnancy (19). "History" refers to their psychiatric history up until their first visit (**Table S1**). Subjects were also assessed regarding childhood home environment, childhood behavior, and traumatic events (occurring before 17 years of age) (**Table S1**). Abnormal childhood home environment included physical abuse of kids or parents, sexual abuse, lots of fighting, food insecurity, or other stressful family environment. Abnormal childhood behavior included truancy, running away, setting fires, getting kicked out of school, harming of animals, stealing, or other antisocial types of behaviors. Traumatic events included death of a close friend/family member, major upheaval between parents, sexual assault, non-sexual assault, extreme illness/injury, or other major life events. Each traumatic event was scored from 1-7 based on the individual's subjective rating of how traumatic the event was. A score of zero indicated no event occurred for that participant.

**Measurement of plasma cortisol levels in humans.** Plasma cortisol levels were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Confounding effects of age, race, blood draw time, and medication (anti-psychotic, antidepressant SSRIs, and non-SSRI antidepressants) were adjusted for.

**Statistical Analysis.** Statistical analyses were performed using commercial software (GraphPad Prism 7, GraphPad Software, Inc., IBM SPSS statistics 26, IBM, and R version 3.5.1) (**Table S2**). Normality of data sets were tested using Shapiro-Wilk's test. For normally distributed data, statistical differences between two groups were calculated using the Student's t-test. Statistical differences among three groups or more were determined using a two-way ANOVA, followed by

a Bonferroni post hoc test. Corrections for multiple comparisons were made when appropriate. For non-normally distributed data, statistical differences between two groups were calculated using the Mann-Whitney test. Statistical differences among three groups or more were determined using a Kruskal-Wallis test followed by a Bonferroni post hoc test. Spearman's correlation or Pearson's correlation were used to examine correlations between plasma corticosterone levels and behavioral changes in TST and FST. For human data, the Fisher exact test was used to check if two categorical variables are related, for example, if PPD is related to traumatic events. Linear regression was employed to evaluate the contributions of risk factors to PPD. The t-values from the liner regression were used to estimate the impact of these factors on PPD. In addition, linear regression was conducted to evaluate the associations between cortisol values and PPD, with age, race, medication, and blood draw time as covariates. A value of $p < 0.05$ was considered statistically significant. All data are expressed as the mean ± SEM.

**Supplemental References**

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Figure S1

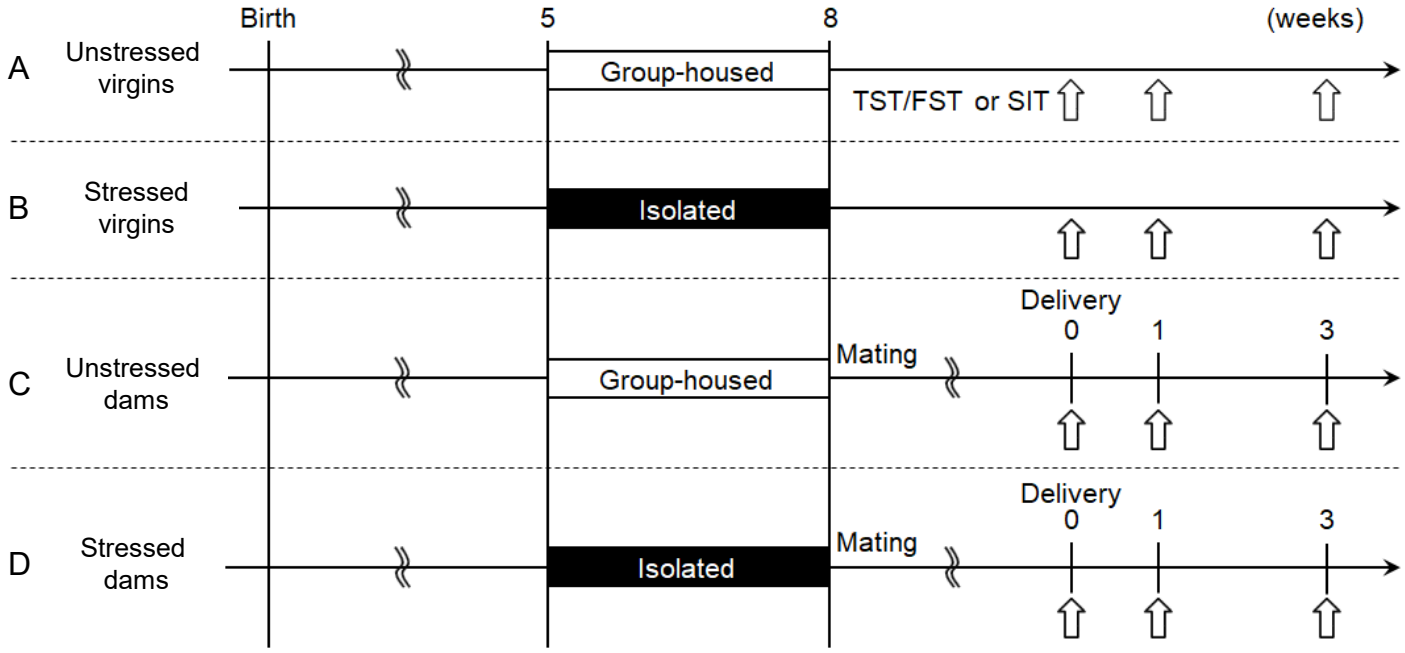



**Figure S1. Experimental schedule of the preclinical study. A**, Virgin female mice were group-housed. **B**, Virgin female mice were isolated from 5 to 8 weeks of age. **C**, Virgin female mice were group-housed, mated with a male mouse, and gave birth to pups. **D**, Virgin female mice were isolated from 5 to 8 weeks of age, mated with a male mouse, and gave birth to pups. TST, tail suspension test; FST, forced swim test; SIT, three-chamber social interaction test. Different cohorts of mice subjected to behavioral tests at 0 week, 1 week, and 3 weeks postpartum were studied to avoid the repeated exposure to stressful behavioral procedures.

Figure S2

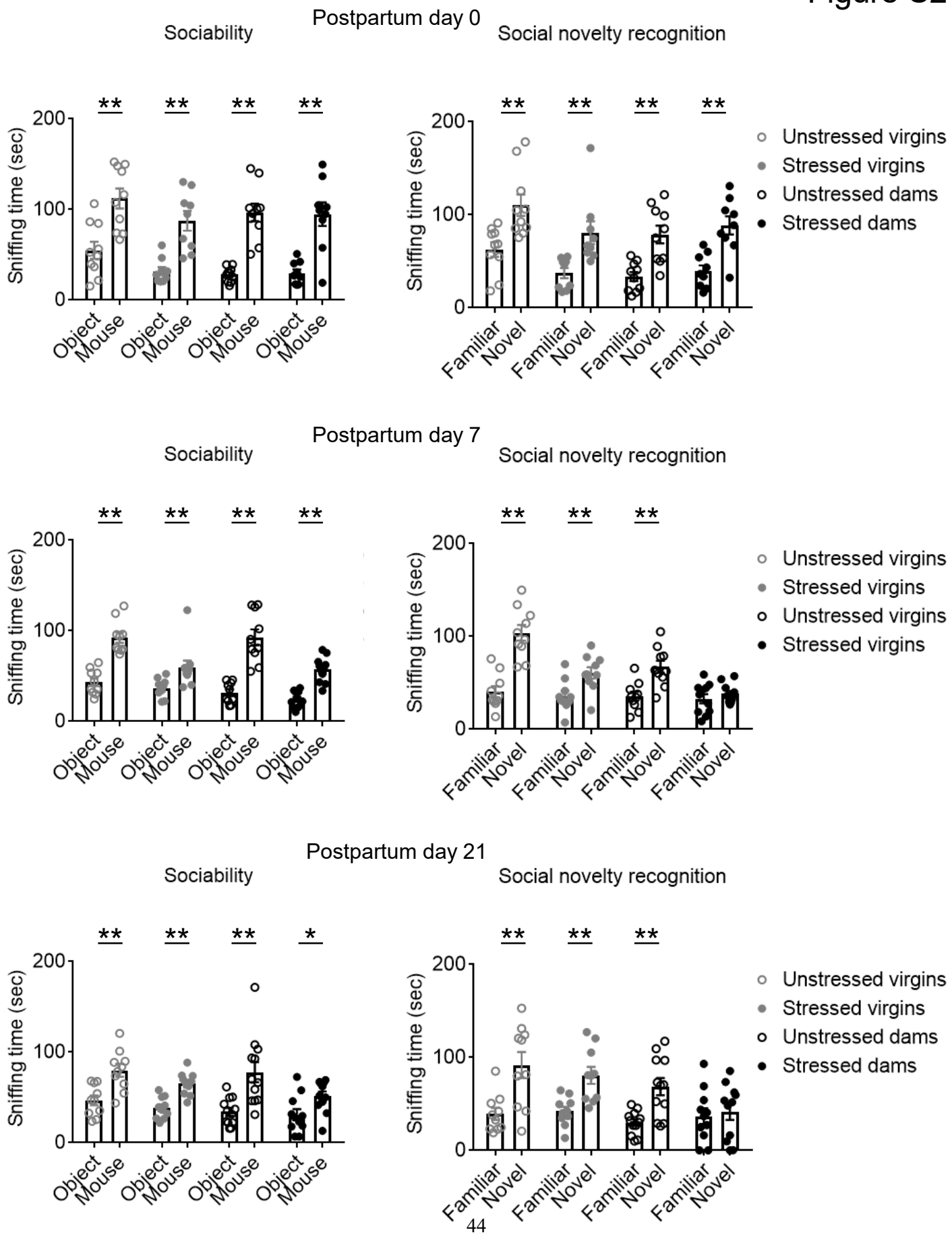

Postpartum day 0
Sociability
Social novelty recognition
Postpartum day 7
Sociability
Social novelty recognition
Postpartum day 21
Sociability
Social novelty recognition
Sniffing time (sec)
200
100
0
**
*
Object
Mouse
Familiar
Novel
Unstressed virgins
Stressed virgins
Unstressed dams
Stressed dams

**Figure S2. Long-lasting behavioral changes in the social interaction test in dams exposed to adolescent social isolation.** Sniffing time during the three-chamber social interaction test at postpartum days 0, 7, and 21. No changes in sociability or social novelty recognition among the four groups were observed at postpartum day 0. Behavioral changes in social novelty recognition, but not sociability, were observed in stressed dams at postpartum days 7 and 21. N=9-11. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

Figure S3

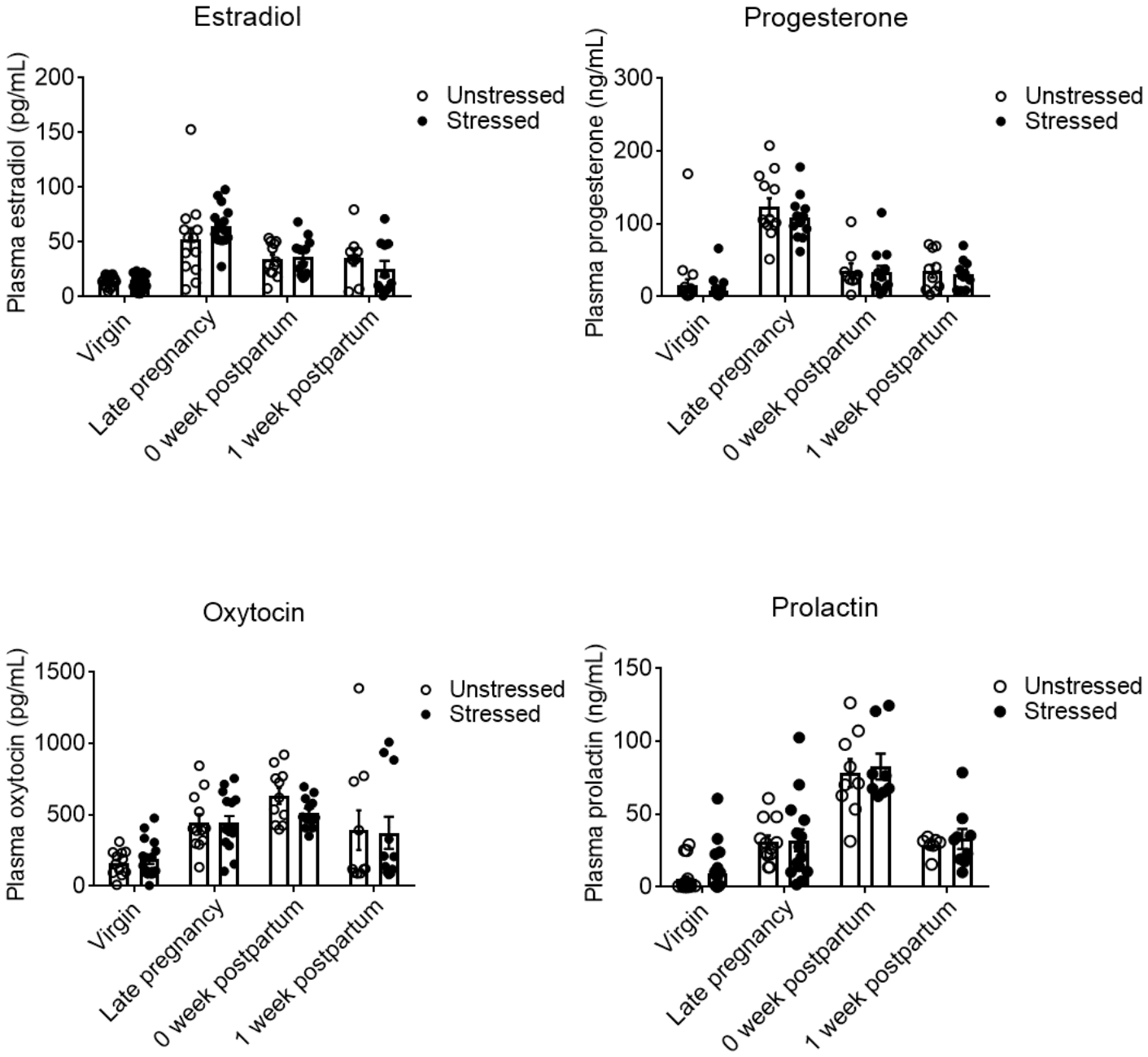



**Figure S3. No change in the levels of plasma estradiol, progesterone, oxytocin, and prolactin in mice exposed to adolescent social isolation.** Levels of estradiol, progesterone, oxytocin, and prolactin in plasma were measured at four-time points (virgin, late pregnancy, 0 week postpartum, and 1 week postpartum). No differences in the levels of estradiol, progesterone, oxytocin, and prolactin were observed between unstressed and stressed mice at any time point. N=9-20. Values are represented as mean ± SEM.

Figure S4

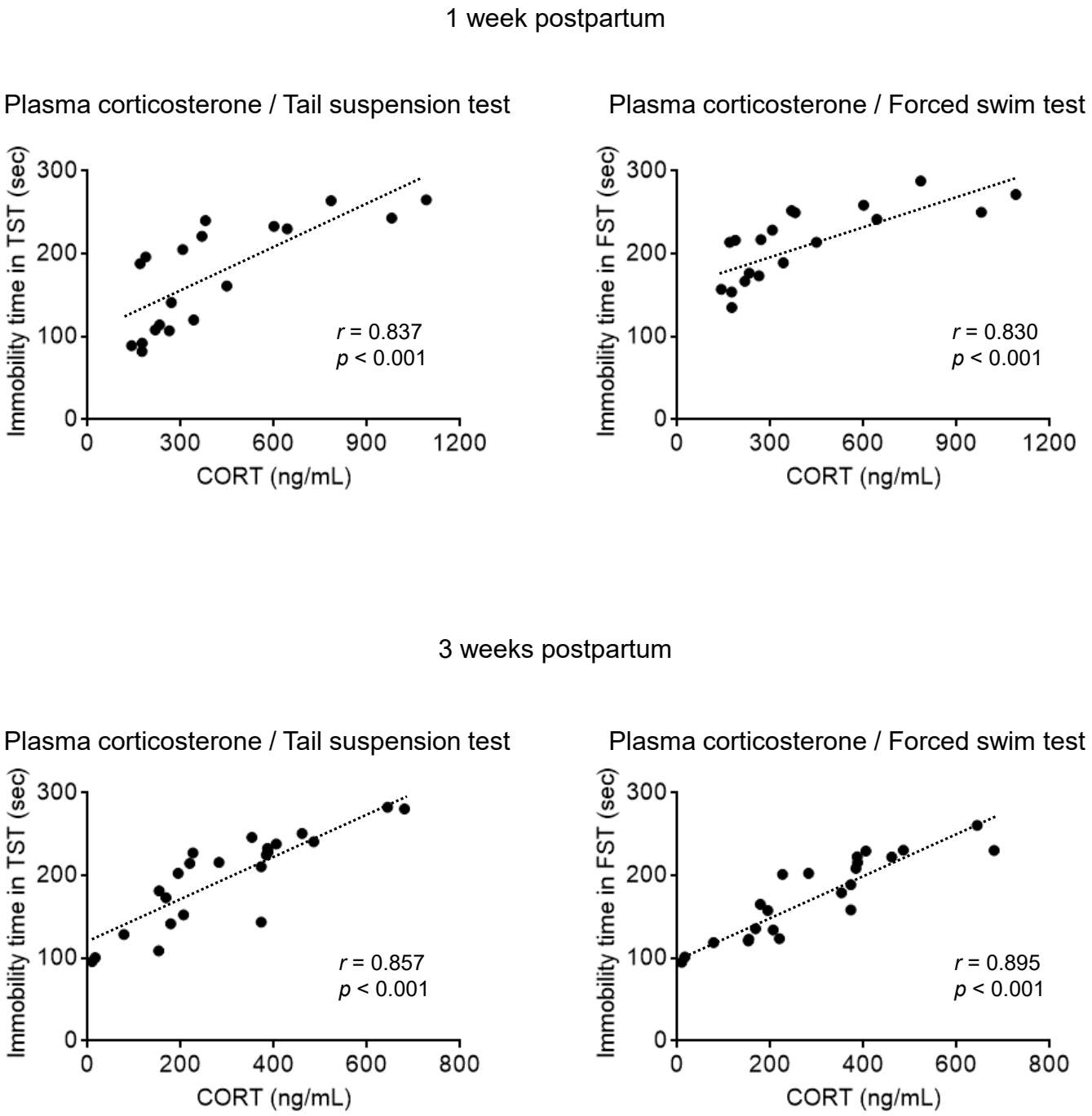



**Figure S4. Positive correlation between plasma corticosterone levels and immobility time in the tail suspension and forced swim tests at 1 week and 3 weeks after delivery.** The levels of plasma corticosterone (CORT) were positively correlated with immobility time in the tail suspension test (TST) and forced swim test (FST) at 1 week and 3 weeks after delivery. N=19-23. Spearman and Pearson rank correlation coefficients were examined for the data at 1 week and 3 week postpartum, respectively.

Figure S5

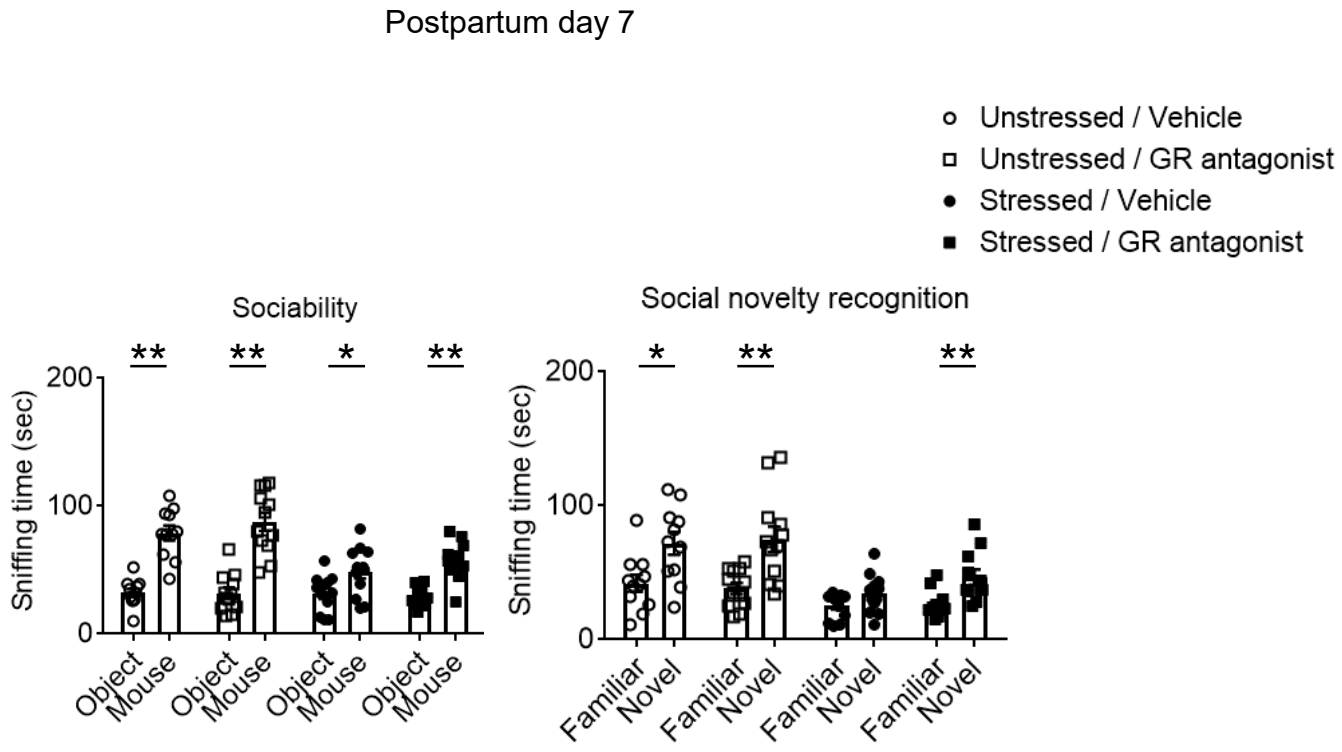
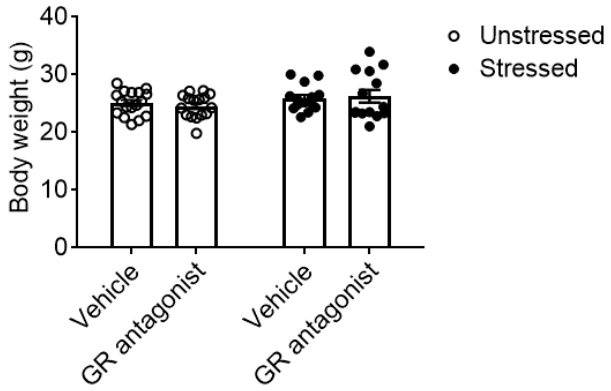


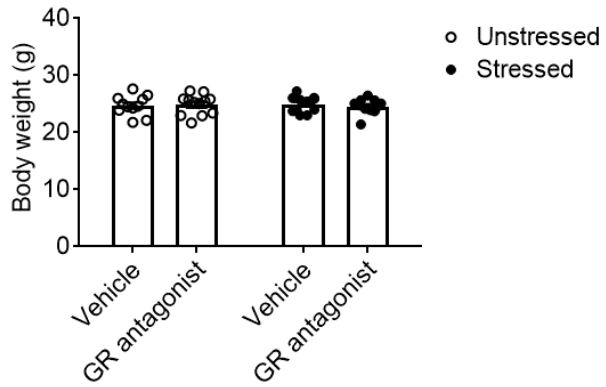

**Figure S5. Causal effect of the GR on postpartum behavioral changes in the social interaction test.** CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing), a selective GR antagonist, normalized the behavioral changes in social novelty recognition in the three-chamber social interaction test in stressed dams at postpartum day 7. N=11-12. Values are represented as mean ± SEM; ***P*<0.01 and **P*<0.05.

Figure S6

A




B




C

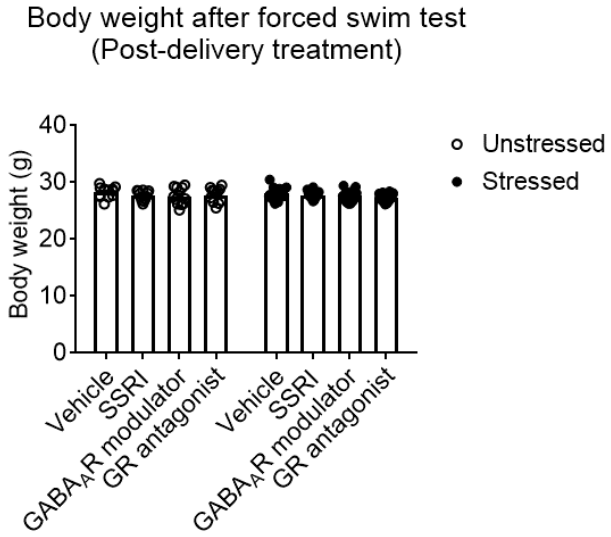


**Figure S6. No effect of a GR antagonist, a SSRI, and an allopregnanolone analog on body weight. A**, CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing), a selective GR antagonist, did not affect body weight after the forced swim test at postpartum day 8 in either group. **B**, CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing) had no effect on body weight after the three-chamber social interaction test at postpartum day 7 in any groups. N=11-12. **C**, Post-delivery treatment with a SSRI fluoxetine (18 mg/kg, *p.o.*), a $GABA_A$ receptor modulator ganaxolone (10 mg/kg, *i.p.*), or CORT113176 (80 mg/kg, *p.o.*) once daily from postpartum day 0 to 24 h prior to behavioral testing did not affect body weight after the forced swim test at postpartum day 8 in any of the groups. N=12. Values are represented as mean ± SEM.

**Table S1.** Human study participants' characteristics.

| Study participants (N=116) | | |
|---|---|---|
| **Characteristics** | **N** | **%** |
| *Age, years* | | |
| <24 | 1 | 0.9 |
| 25-29 | 23 | 19.8 |
| 30-34 | 59 | 50.9 |
| 35-39 | 26 | 22.4 |
| >40 | 7 | 6.0 |
| *Ethnicity* | | |
| Hispanic or Latino | 5 | 4.3 |
| Not Hispanic or Latino | 111 | 95.7 |
| *Race* | | |
| Black or African-American | 11 | 9.5 |
| White | 98 | 84.5 |
| Asian or Pacific Islander | 5 | 4.3 |
| **Personal history of psychiatric disorders** | **N** | **%** |
| Major Depression | 61 | 52.6 |
| General Anxiety | 29 | 25.0 |
| Substance Abuse | 9 | 7.8 |
| Other | 42 | 36.2 |
| No history | 34 | 29.3 |
| **Psychiatric medication use** | **N** | **%** |
| Anti-psychotic | 3 | 2.6 |
| SSRI | 21 | 18.1 |
| Non-SSRI antidepressant | 6 | 5.2 |
| **Home environment** | **N** | **%** |
| Normal | 83 | 71.6 |
| Other | 33 | 28.4 |
| **Childhood behavior** | **N** | **%** |
| Normal | 96 | 82.8 |
| Other | 20 | 17.2 |
| **Traumatic event(s) before 17 years old** | **# of occurrences** | **%** |
| Death of a close friend/family member | 44 | 37.9 |
| Major upheaval between parents | 30 | 25.9 |
| Sexual assault | 18 | 15.5 |
| Non-sexual assault | 13 | 11.2 |
| Extremely ill/injured | 9 | 7.8 |
| Major upheaval that shaped life/ personality | 34 | 29.3 |

**Table S2.** Statistical results.

| Figure | Experiment | Experimental day | Sample size | Data Structure | Statistical test | Values |
|---|---|---|---|---|---|---|
| 1A | Tail suspension test | Postpartum day 0 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 12; Stressed dams, 10 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,41) = 0.578, p = 0.452 |
| | | | | | Virgin, Dam | F(1,41) = 1.476, p = 0.232 |
| | | | | | Interaction | F(1,41) = 0.660, p = 0.422 |
| | | Postpartum day 7 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 10; Stressed dams, 11 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,40) = 23.651, p < 0.001 |
| | | | | | Virgin, Dam | F(1,40) = 8.657, p < 0.001 |
| | | | | | Interaction | F(1,40) = 20.123, p < 0.01 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 1 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Stressed virgins vs Unstressed dams | p = 1 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.001 |
| | | Postpartum day 21 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 12; Stressed dams, 11 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,42) = 12.371, p < 0.01 |
| | | | | | Virgin, Dam | F(1,42) = 2.971, p = 0.093 |
| | | | | | Interaction | F(1,42) = 7.002, p < 0.05 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 1 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.01 |
| | | | | | Stressed virgins vs Unstressed dams | p = 1 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.01 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.05 |
| 1B | Forced swim test | Postpartum day 1 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 12; Stressed dams, 10 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,41) = 0.257, p = 0.615 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | Virgin, Dam | F(1,41) = 2.393, p = 0.13 |
| | | | | | Interaction | F(1,41) = 0.257, p = 0.615 |
| | | Postpartum day 8 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 10; Stressed dams, 11 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,40) = 22.744, p < 0.001 |
| | | | | | Virgin, Dam | F(1,40) = 26.334, p < 0.001 |
| | | | | | Interaction | F(1,40) = 11.944, p < 0.001 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 1 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Stressed virgins vs Unstressed dams | p = 1 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.001 |
| | | Postpartum day 22 | Unstressed virgins, 10; Stressed virgins, 10; Unstressed dams, 12; Stressed dams, 11 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,42) = 17.662, p < 0.001 |
| | | | | | Virgin, Dam | F(1,42) = 4.432, p < 0.05 |
| | | | | | Interaction | F(1,42) = 6.314, p < 0.05 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 1 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Stressed virgins vs Unstressed dams | p = 1 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.05 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.001 |
| 2A | Measurement of plasma corticosterone levels | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum, 3 weeks postpartum | Unstressed / Virgin, 30; Stressed / Virgin, 29 | Normal Distribution | Student's *t*-test | p = 0.749 |
| | | | Unstressed / Late pregnancy, 10; Stressed / Late pregnancy, 14 | Non-normal Distribution | Mann-Whitney test | p = 0.752 |
| | | | Unstressed / 0 week postpartum, 11; Stressed / 0 week postpartum, 10 | Normal Distribution | Student's *t*-test | p = 0.082 |
| | | | Unstressed / 1 week postpartum, 9; | Normal Distribution | Student's *t*-test | p < 0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Stressed / 1 week postpartum, 10 | | | |
| | | | Unstressed / 3 weeks postpartum, 12; Stressed / 3 weeks postpartum, 11 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Unstressed / Virgin, 30; Unstressed / Late pregnancy, 10 | Non-normal Distribution | Mann-Whitney test | $p < 0.01$ |
| | | | Unstressed / Virgin, 30; Unstressed / 0 week postpartum, 11 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Unstressed / Virgin, 30; Unstressed / 1 week postpartum, 9 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Unstressed / Virgin, 30; Unstressed / 3 weeks postpartum, 12 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Stressed / Virgin, 29; Stressed / Late pregnancy, 14 | Non-normal Distribution | Mann-Whitney test | $p < 0.001$ |
| | | | Stressed / Virgin, 29; Stressed / 0 week postpartum, 10 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Stressed / Virgin, 29; Stressed / 1 week postpartum, 10 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Stressed / Virgin, 29; Stressed / 3 weeks postpartum, 11 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| 2B | Tail suspension test | Postpartum day 7 | Unstressed / Vehicle: 17, Unstressed / GR antagonist: 16, Stressed / Vehicle: 14, Stressed / GR antagonist: 14 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | $F(1,57) = 8.036$, $p < 0.01$ |
| | | | | | Vehicle, GR antagonist | $F(1,57) = 3.934$, $p = 0.052$ |
| | | | | | Interaction | $F(1,57) = 5.092$, $p < 0.05$ |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed / Vehicle vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | $p < 0.01$ |
| | | | | | Unstressed / Vehicle vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / GR antagonist vs Stressed / Vehicle | $p < 0.01$ |
| | | | | | Unstressed / GR antagonist vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / GR antagonist | $p < 0.05$ |
| | Forced swim test | Postpartum day 8 | Unstressed / Vehicle: 17, Unstressed / GR antagonist: 16, Stressed / Vehicle: 14, Stressed / GR antagonist: 14 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | $F(1,57) = 21.840$, $p < 0.001$ |
| | | | | | Vehicle, GR antagonist | $F(1,57) = 2.997$, $p = 0.089$ |
| | | | | | Interaction | $F(1,57) = 19.210$, $p < 0.001$ |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | Unstressed / Vehicle vs Unstressed / GR antagonist | p = 0.549 |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / Vehicle vs Stressed / GR antagonist | p = 0.427 |
| | | | | | Unstressed / GR antagonist vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / GR antagonist vs Stressed / GR antagonist | p = 1 |
| | | | | | Stressed / Vehicle vs Stressed / GR antagonist | p < 0.001 |
| 2C | Measurement of CRHR mRNA levels in pituitary | Postpartum day 9 | Unstressed virgins, 7; Stressed virgins; Unstressed dams, 7; Stressed dams, 7 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,24) = 5.99, p < 0.05 |
| | | | | | Virgin, Dam | F(1,24) = 24.05, p < 0.001 |
| | | | | | Interaction | F(1,24) = 2.998, p = 0.096 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 0.206 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Stressed virgins vs Unstressed dams | p = 0.571 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.001 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.05 |
| | Measurement of GR mRNA levels in pituitary | Postpartum day 9 | Unstressed virgins, 7; Stressed virgins, 7; Unstressed dams, 7; Stressed dams, 7 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,24) = 7.776, p < 0.05 |
| | | | | | Virgin, Dam | F(1,24) = 23.09, p < 0.001 |
| | | | | | Interaction | F(1,24) = 8.805, p < 0.01 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p < 0.001 |
| | | | | | Unstressed virgins vs Stressed dams | p = 1 |
| | | | | | Stressed virgins vs Unstressed dams | p < 0.001 |
| | | | | | Stressed virgins vs Stressed dams | p = 1 |
| | | | | | Unstressed dams vs Stressed dams | p < 0.01 |
| | Measurement of CRH mRNA levels in PVN | Postpartum day 9 | Unstressed virgins, 7; Stressed virgins; Unstressed dams, 7; Stressed dams, 7 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,24) = 0.237, p = 0.631 |
| | | | | | Virgin, Dam | F(1,24) = 7.032, p < 0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | Interaction | F(1,24) = 0.054, p = 0.818 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p = 0.315 |
| | | | | | Unstressed virgins vs Stressed dams | p = 0.217 |
| | | | | | Stressed virgins vs Unstressed dams | p = 0.833 |
| | | | | | Stressed virgins vs Stressed dams | p = 0.601 |
| | | | | | Unstressed dams vs Stressed dams | p = 1 |
| | Measurement of GR mRNA levels in PVN | Postpartum day 9 | Unstressed virgins, 7; Stressed virgins, 7; Unstressed dams, 7; Stressed dams, 7 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,24) = 0.017, p = 0.897 |
| | | | | | Virgin, Dam | F(1,24) = 24.4, p < 0.001 |
| | | | | | Interaction | F(1,24) = 0.341, p = 0.565 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed virgins vs Stressed virgins | p = 1 |
| | | | | | Unstressed virgins vs Unstressed dams | p < 0.01 |
| | | | | | Unstressed virgins vs Stressed dams | p < 0.05 |
| | | | | | Stressed virgins vs Unstressed dams | p < 0.01 |
| | | | | | Stressed virgins vs Stressed dams | p < 0.05 |
| | | | | | Unstressed dams vs Stressed dams | p = 1 |
| 2D | Measurement of plasma ACTH levels (Dexamethasone suppression test) | Postpartum day 8 | Unstressed / Vehicle: 11, Unstressed / DEX: 11, Stressed / Vehicle: 11, Stressed / DEX: 11 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,43) = 200.369, p < 0.001 |
| | | | | | Vehicle, DEX | F(1,43) = 17.033, p < 0.001 |
| | | | | | Interaction | F(1,43) = 0.845, p = 0.361 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed / Vehicle vs Unstressed / DEX | p < 0.01 |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / Vehicle vs Stressed / DEX | p < 0.001 |
| | | | | | Unstressed / DEX vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / DEX vs Stressed / DEX | p < 0.001 |
| | | | | | Stressed / Vehicle vs Stressed / DEX | p = 0.174 |
| | Measurement of plasma | Postpartum day 8 | Unstressed / Vehicle: 11, | | Kruskal-Wallis | |
| | | | | | H = 26.052 | p < 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | corticosterone levels (Dexamethasone suppression test) | | Unstressed / DEX: 11, Stressed / Vehicle: 11, Stressed / DEX: 11 | Non-normal Distribution | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed / Vehicle vs Unstressed / DEX | p < 0.05 |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | p < 0.05 |
| | | | | | Unstressed / Vehicle vs Stressed / DEX | p = 0.184 |
| | | | | | Unstressed / DEX vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / DEX vs Stressed / DEX | p < 0.001 |
| | | | | | Stressed / Vehicle vs Stressed / DEX | p = 0.229 |
| 2D | Measurement of CRHR mRNA levels in pituitary | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum, 3 weeks postpartum | Unstressed / Virgin, 7; Stressed / Virgin, 7 | Non-normal Distribution | Mann Whitney test | p = 0.535 |
| | | | Unstressed / Late pregnancy, 7; Stressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p = 0.470 |
| | | | Unstressed / 0 week postpartum, 7; Stressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Unstressed / 1 week postpartum, 7; Stressed / 1 week postpartum, 7 | Non-normal Distribution | Mann Whitney test | p = 0.456 |
| | | | Unstressed / 3 weeks postpartum, 7; Stressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.606 |
| | | | Unstressed / Virgin, 7; Unstressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p = 0.821 |
| | | | Unstressed / Virgin, 7; Unstressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p < 0.01 |
| | | | Unstressed / Virgin, 7; Unstressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.085 |
| | | | Unstressed / Virgin, 7; Unstressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.116 |
| | | | Stressed / Virgin, 7; Stressed / Late pregnancy, 7 | Non-normal Distribution | Mann Whitney test | p = 0.209 |
| | | | Stressed / Virgin, 7; Stressed / 0 week postpartum, 7 | Non-normal Distribution | Mann Whitney test | p < 0.05 |
| | | | Stressed / Virgin, 7; Stressed / 1 week postpartum, 7 | Non-normal Distribution | Mann Whitney test | p < 0.05 |
| | | | Stressed / Virgin, 7; Stressed / 3 weeks postpartum, 7 | Non-normal Distribution | Mann Whitney test | p =0.165 |
| | Measurement of GR mRNA levels in pituitary | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum, 3 weeks postpartum | Unstressed / Virgin, 7; Stressed / Virgin, 7 | Normal Distribution | Student's *t*-test | p = 0.477 |
| | | | Unstressed / Late pregnancy, 7; Stressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p < 0.05 |
| | | | Unstressed / 0 week postpartum, 7; Stressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.172 |
| | | | Unstressed / 1 week postpartum, 7; Stressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.275 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Unstressed / 3 weeks postpartum, 7; Stressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.368 |
| | | | Unstressed / Virgin, 7; Unstressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p < 0.05 |
| | | | Unstressed / Virgin, 7; Unstressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.100 |
| | | | Unstressed / Virgin, 7; Unstressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p < 0.05 |
| | | | Unstressed / Virgin, 7; Unstressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.133 |
| | | | Stressed / Virgin, 7; Stressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p = 0.853 |
| | | | Stressed / Virgin, 7; Stressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.782 |
| | | | Stressed / Virgin, 7; Stressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.488 |
| | | | Stressed / Virgin, 7; Stressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.912 |
| | Measurement of CRH mRNA levels in PVN | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum, 3 weeks postpartum | Unstressed / Virgin, 7; Stressed / Virgin, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.383 |
| | | | Unstressed / Late pregnancy, 7; Stressed / Late pregnancy, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.085 |
| | | | Unstressed / 0 week postpartum, 7; Stressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.923 |
| | | | Unstressed / 1 week postpartum, 7; Stressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.840 |
| | | | Unstressed / 3 weeks postpartum, 7; Stressed / 3 weeks postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.225 |
| | | | Unstressed / Virgin, 7; Unstressed / Late pregnancy, 7 | Non-normal Distribution | Mann-Whitney test | p < 0.01 |
| | | | Unstressed / Virgin, 7; Unstressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p < 0.01 |
| | | | Unstressed / Virgin, 7; Unstressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.209 |
| | | | Unstressed / Virgin, 7; Unstressed / 3 weeks postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p < 0.05 |
| | | | Stressed / Virgin, 7; Stressed / Late pregnancy, 7 | Non-normal Distribution | Mann-Whitney test | p < 0.05 |
| | | | Stressed / Virgin, 7; Stressed / 0 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p < 0.05 |
| | | | Stressed / Virgin, 7; Stressed / 1 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.848 |

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| | | | Stressed / Virgin, 7; Stressed / 3 weeks postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.338 |
| | Measurement of GR mRNA levels in PVN | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum, 3 weeks postpartum | Unstressed / Virgin, 7; Stressed / Virgin, 7 | Normal Distribution | Student's *t*-test | p = 0.789 |
| | | | Unstressed / Late pregnancy, 7; Stressed / Late pregnancy, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.949 |
| | | | Unstressed / 0 week postpartum, 7; Stressed / 0 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.565 |
| | | | Unstressed / 1 week postpartum, 7; Stressed / 1 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.949 |
| | | | Unstressed / 3 weeks postpartum, 7; Stressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.922 |
| | | | Unstressed / Virgin, 7; Unstressed / Late pregnancy, 7 | Normal Distribution | Student's *t*-test | p = 0.217 |
| | | | Unstressed / Virgin, 7; Unstressed / 0 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.385 |
| | | | Unstressed / Virgin, 7; Unstressed / 1 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.848 |
| | | | Unstressed / Virgin, 7; Unstressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.367 |
| | | | Stressed / Virgin, 7; Stressed / Late pregnancy, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.085 |
| | | | Stressed / Virgin, 7; Stressed / 0 week postpartum, 7 | Non-normal Distribution | Mann-Whitney test | p = 0.180 |
| | | | Stressed / Virgin, 7; Stressed / 1 week postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.445 |
| | | | Stressed / Virgin, 7; Stressed / 3 weeks postpartum, 7 | Normal Distribution | Student's *t*-test | p = 0.089 |
| 3 | Tail suspension test | Postpartum day 7 | Unstressed / Vehicle, 12; Unstressed / SSRI, 12; Unstressed / $GABA_AR$ modulator, 12; Unstressed / GR antagonist, 12; Stressed / Vehicle, 12; Stressed / SSRI, 12; Stressed / $GABA_AR$ modulator, 12; Stressed / GR antagonist, 12 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,88) = 108.30, p < 0.001 |
| | | | | | Drug treatment | F(3,88) = 6.266, p < 0.001 |
| | | | | | Interaction | F(3,88) = 5.712, p < 0.01 |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed / Vehicle vs Unstressed / SSRI | p = 1 |
| | | | | | Unstressed / Vehicle vs Unstressed / $GABA_AR$ modulator | p = 1 |
| | | | | | Unstressed / Vehicle vs Unstressed / GR antagonist | p = 1 |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | p < 0.001 |
| | | | | | Unstressed / Vehicle vs Stressed / SSRI | p < 0.001 |

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| | | | | | Unstressed / Vehicle vs Stressed / $GABA_AR$ modulator | $p < 0.001$ |
| | | | | | Unstressed / Vehicle vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / SSRI vs Unstressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Unstressed / SSRI vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / SSRI vs Stressed / Vehicle | $p < 0.001$ |
| | | | | | Unstressed / SSRI vs Stressed / SSRI | $p < 0.001$ |
| | | | | | Unstressed / SSRI vs Stressed / $GABA_AR$ modulator | $p < 0.001$ |
| | | | | | Unstressed / SSRI vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / Vehicle | $p < 0.001$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / SSRI | $p < 0.001$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / GABAAR modulator | $p < 0.001$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / GR antagonist vs Stressed / Vehicle | $p < 0.001$ |
| | | | | | Unstressed / GR antagonist vs Stressed / SSRI | $p < 0.001$ |
| | | | | | Unstressed / GR antagonist vs Stressed / $GABA_AR$ modulator | $p < 0.001$ |
| | | | | | Unstressed / GR antagonist vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / SSRI | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / GR antagonist | $p < 0.001$ |
| | | | | | Stressed / SSRI vs Stressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Stressed / SSRI vs Stressed / GR antagonist | $p < 0.001$ |
| | | | | | Stressed / $GABA_AR$ modulator vs Stressed / GR antagonist | $p < 0.01$ |
| | Forced swim test | Postpartum day 8 | Unstressed / Vehicle, 12; Unstressed / SSRI, 12; Unstressed / $GABA_AR$ modulator, 12; Unstressed / GR antagonist, 12; | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | $F(1,88) = 31.230$, $p < 0.001$ |
| | | | | | Drug treatment | $F(3,88) = 3.810$, $p < 0.05$ |

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| | | | Stressed / Vehicle, 12; Stressed / SSRI, 12; Stressed / $GABA_AR$ modulator, 12; Stressed / GR antagonist, 12 | | Interaction | $F(3,88) = 3.257$, $p < 0.05$ |
| | | | | | Post hoc multiple comparisons with a Bonferroni test | |
| | | | | | Unstressed / Vehicle vs Unstressed / SSRI | $p = 1$ |
| | | | | | Unstressed / Vehicle vs Unstressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Unstressed / Vehicle vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / Vehicle vs Stressed / Vehicle | $p < 0.001$ |
| | | | | | Unstressed / Vehicle vs Stressed / SSRI | $p < 0.001$ |
| | | | | | Unstressed / Vehicle vs Stressed / $GABA_AR$ modulator | $p = 0.168$ |
| | | | | | Unstressed / Vehicle vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / SSRI vs Unstressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Unstressed / SSRI vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / SSRI vs Stressed / Vehicle | $p < 0.01$ |
| | | | | | Unstressed / SSRI vs Stressed / SSRI | $p < 0.01$ |
| | | | | | Unstressed / SSRI vs Stressed / $GABA_AR$ modulator | $p = 0.439$ |
| | | | | | Unstressed / SSRI vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Unstressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / Vehicle | $p < 0.01$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / SSRI | $p < 0.01$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / GABAAR modulator | $p = 0.368$ |
| | | | | | Unstressed / $GABA_AR$ modulator vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Unstressed / GR antagonist vs Stressed / Vehicle | $p < 0.01$ |
| | | | | | Unstressed / GR antagonist vs Stressed / SSRI | $p < 0.01$ |
| | | | | | Unstressed / GR antagonist vs Stressed / $GABA_AR$ modulator | $p = 0.282$ |
| | | | | | Unstressed / GR antagonist vs Stressed / GR antagonist | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / SSRI | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / $GABA_AR$ modulator | $p = 1$ |
| | | | | | Stressed / Vehicle vs Stressed / GR antagonist | $p < 0.01$ |

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| | | | | | Stressed / SSRI vs Stressed / $GABA_AR$ modulator | p = 1 |
| | | | | | Stressed / SSRI vs Stressed / GR antagonist | p < 0.01 |
| | | | | | Stressed / $GABA_AR$ modulator vs Stressed / GR antagonist | p = 0.686 |
| 4A | Percentages of participants diagnosed without and with PPD | 2nd trimester, 3rd trimester, 2 weeks postpartum, 6 weeks postpartum | No history / No PPD, 38; No history / PPD, 3; history / No PPD, 60; history / PPD, 15 | Non-normal Distribution | Fisher exact test | p = 0.106 |
| | | | Normal home env / No PPD, 77; Normal home env / PPD, 12; Abnormal home env / No PPD, 21; Abnormal home env / PPD, 6 | Non-normal Distribution | Fisher exact test | p = 0.361 |
| | | | Normal child behav / No PPD, 83; Normal child behav / PPD, 13; Abnormal child behav / No PPD, 15; Abnormal child behav / PPD, 5 | Non-normal Distribution | Fisher exact test | p = 0.305 |
| | | | No traumatic event / No PPD, 78; No traumatic event / PPD, 14; Traumatic event / No PPD, 20; Traumatic event / PPD, 4 | Non-normal Distribution | Fisher exact test | p = 1 |
| 4B | Risk factors in postpartum mental deficits in humans | 2nd trimester, 3rd trimester, 2 weeks postpartum, 6 weeks postpartum | No history / No PPD, 38; No history / PPD, 3; history / No PPD, 60; history / PPD, 15 | Non-normal Distribution | Linear regression | p = 0.112 |
| | | | Normal home env / No PPD, 77; Normal home env / PPD, 12; Abnormal home env / No PPD, 21; Abnormal home env / PPD, 6 | Non-normal Distribution | Linear regression | p = 0.690 |
| | | | Normal child behav / No PPD, 83; Normal child behav / PPD, 13; Abnormal child behav / No PPD, 15; Abnormal child behav / PPD, 5 | Non-normal Distribution | Linear regression | p = 0.484 |
| | | | No traumatic event / No PPD, 78; No traumatic event / PPD, 14; Traumatic event / No PPD, 20; Traumatic event / PPD, 4 | Non-normal Distribution | Linear regression | p = 0.845 |
| 4C | Measurement of plasma cortisol levels in humans with a history of mental illness | 2nd trimester, 3rd trimester, 2 weeks postpartum, 6 weeks postpartum | No PPD 2nd trimester, 38; No PPD 3rd trimester, 56 | Normal Distribution | Linear regression | p = 0.647 |
| | | | No PPD 2nd trimester, 38; No PPD 2 weeks postpartum, 60 | Normal Distribution | Linear regression | p < 0.01 |
| | | | No PPD 2nd trimester, 38; No PPD 6 weeks postpartum, 60 | Normal Distribution | Linear regression | p < 0.001 |
| | | | No PPD 3rd trimester, 56; No PPD 2 weeks postpartum, 60 | Normal Distribution | Linear regression | p < 0.001 |
| | | | No PPD 3rd trimester, 56; No PPD 6 weeks postpartum, 60 | Normal Distribution | Linear regression | p < 0.001 |
| | | | No PPD 2 weeks postpartum, 60; No | Normal Distribution | Linear regression | p = 0.164 |

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| | | | PPD 6 weeks postpartum, 60 | | | |
| | | | PPD 2nd trimester, 10; PPD 3rd trimester, 14 | Normal Distribution | Linear regression | p = 0.514 |
| | | | PPD 2nd trimester, 10; PPD 2 weeks postpartum, 15 | Normal Distribution | Linear regression | p = 0.91 |
| | | | PPD 2nd trimester, 10; PPD 6 weeks postpartum, 15 | Normal Distribution | Linear regression | p = 0.545 |
| | | | PPD 3rd trimester, 14; PPD 2 weeks postpartum, 15 | Normal Distribution | Linear regression | p = 0.405 |
| | | | PPD 3rd trimester, 14; PPD 6 weeks postpartum, 15 | Normal Distribution | Linear regression | p = 0.184 |
| | | | PPD 2 weeks postpartum, 15; PPD 6 weeks postpartum, 15 | Normal Distribution | Linear regression | p = 0.593 |
| | | | No PPD 2nd trimester, 38; PPD 2nd trimester, 10 | Normal Distribution | Linear regression | p = 0.263 |
| | | | No PPD 3rd trimester, 56; PPD 3rd trimester, 14 | Normal Distribution | Linear regression | p = 0.105 |
| | | | No PPD 2 weeks postpartum, 60; PPD 2 weeks postpartum, 15 | Normal Distribution | Linear regression | p < 0.001 |
| | | | No PPD 6 weeks postpartum, 60; PPD 6 weeks postpartum, 15 | Normal Distribution | Linear regression | p < 0.001 |
| Supplementary Figures | | | | | | |
| S2 | Sociability in three-chamber social interaction test | Postpartum day 0 | Unstressed virgins, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed virgins, 9 | Non-normal Distribution | Mann Whitney test | p < 0.001 |
| | | | Unstressed dams, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed dams, 9 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | Social novelty recognition in three-chamber social interaction test | Postpartum day 0 | Unstressed virgins, 10 | Non-normal Distribution | Mann Whitney test | p < 0.01 |
| | | | Stressed virgins, 9 | Non-normal Distribution | Mann Whitney test | p < 0.001 |
| | | | Unstressed dams, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed dams, 9 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | Sociability in three-chamber social interaction test | Postpartum day 7 | Unstressed virgins, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed virgins, 10 | Non-normal Distribution | Mann Whitney test | p < 0.01 |
| | | | Unstressed dams, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed dams, 11 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | Social novelty recognition in three-chamber social interaction test | Postpartum day 7 | Unstressed virgins, 10 | Normal Distribution | Student's *t*-test | p < 0.001 |
| | | | Stressed virgins, 10 | Normal Distribution | Student's *t*-test | p < 0.01 |
| | | | Unstressed dams, 10 | Normal Distribution | Student's *t*-test | p < 0.01 |
| | | | Stressed dams, 11 | Normal Distribution | Student's *t*-test | p = 0.378 |

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| | Sociability in three-chamber social interaction test | Postpartum day 21 | Unstressed virgins, 10 | Normal Distribution | Student's *t*-test | $p < 0.01$ |
| | | | Stressed virgins, 10 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Unstressed dams, 12 | Normal Distribution | Student's *t*-test | $p < 0.01$ |
| | | | Stressed dams, 11 | Normal Distribution | Student's *t*-test | $p < 0.05$ |
| | Social novelty recognition in three-chamber social interaction test | Postpartum day 21 | Unstressed virgins, 10 | Normal Distribution | Student's *t*-test | $p < 0.01$ |
| | | | Stressed virgins, 10 | Normal Distribution | Student's *t*-test | $p < 0.01$ |
| | | | Unstressed dams, 12 | Normal Distribution | Student's *t*-test | $p < 0.001$ |
| | | | Stressed dams, 11 | Normal Distribution | Student's *t*-test | $p = 0.692$ |
| S3 | Measurement of plasma estradiol levels | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum | Unstressed / Virgin, 19; Stressed / Virgin, 20 | Normal Distribution | Student's *t*-test | $p = 0.363$ |
| | | | Unstressed / Late pregnancy, 13; Stressed / Late pregnancy, 16 | Non-normal Distribution | Mann Whitney test | $p = 0.121$ |
| | | | Unstressed / 0 week postpartum, 12; Stressed / 0 week postpartum, 12 | Normal Distribution | Student's t-test | $p = 0.713$ |
| | | | Unstressed / 1 week postpartum, 9; Stressed / 1 week postpartum, 11 | Non-normal Distribution | Mann Whitney test | $p = 0.656$ |
| | Measurement of plasma progesterone levels | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum | Unstressed / Virgin, 20; Stressed / Virgin, 20 | Non-normal Distribution | Mann Whitney test | $p = 0.496$ |
| | | | Unstressed / Late pregnancy, 13; Stressed / Late pregnancy, 16 | Normal Distribution | Student's t-test | $p = 0.267$ |
| | | | Unstressed / 0 week postpartum, 9; Stressed / 0 week postpartum, 12 | Non-normal Distribution | Mann Whitney test | $p = 0.702$ |
| | | | Unstressed / 1 week postpartum, 10; Stressed / 1 week postpartum, 11 | Normal Distribution | Student's t-test | $p = 0.702$ |
| | Measurement of plasma oxytocin levels | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum | Unstressed / Virgin, 12; Stressed / Virgin, 17 | Normal Distribution | Student's t-test | $p = 0.582$ |
| | | | Unstressed / Late pregnancy, 12, Stressed / Late pregnancy, 16 | Normal Distribution | Student's t-test | $p = 0.987$ |
| | | | Unstressed / 0 week postpartum, 11; Stressed / 0 week postpartum, 12 | Normal Distribution | Student's t-test | $p = 0.085$ |
| | | | Unstressed / 1 week postpartum, 10; Stressed / 1 week postpartum, 11 | Non-normal Distribution | Mann Whitney test | $p = 0.863$ |
| | Measurement of plasma prolactin levels | Virgin, Late pregnancy, 0 week postpartum, 1 week postpartum | Unstressed / Virgin, 19; Stressed / Virgin, 20 | Non-normal Distribution | Mann Whitney test | $p = 0.967$ |
| | | | Unstressed / Late pregnancy, 12; Stressed / Late pregnancy, 14 | Normal Distribution | Student's t-test | $p = 0.92$ |

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| | | | Unstressed / 0 week postpartum, 9; Stressed / 0 week postpartum, 8 | Non-normal Distribution | Mann Whitney test | p = 0.963 |
| | | | Unstressed / 1 week postpartum, 7; Stressed / 1 week postpartum, 9 | Non-normal Distribution | Mann Whitney test | p = 0.606 |
| S4 | Correlation between plasma corticosterone levels and immobility time in tail suspension test | 1 week postpartum | Unstressed dams, 9; Stressed dams, 10 | Non-normal Distribution | Spearman's correlation | r = 0.837, p < 0.001 |
| | Correlation between plasma corticosterone levels and immobility time in forced swim test | | | Non-normal Distribution | Spearman's correlation | r = 0.830, p < 0.001 |
| | Correlation between plasma corticosterone levels and immobility time in tail suspension test | 3 weeks postpartum | Unstressed dams, 12; Stressed dams, 11 | Normal Distribution | Pearson's correlation | r = 0.857, p < 0.001 |
| | Correlation between plasma corticosterone levels and immobility time in forced swim test | | | Normal Distribution | Pearson's correlation | r = 0.895, p < 0.001 |
| S5 | Sociability in three-chamber social interaction test | Postpartum day 7 | Unstressed / Vehicle, 11 | Normal Distribution | Student's t-test | p < 0.001 |
| | | | Unstressed / GR antagonist, 12 | Normal Distribution | Student's t-test | p < 0.001 |
| | | | Stressed / Vehicle, 12 | Normal Distribution | Student's t-test | p < 0.05 |
| | | | Stressed / GR antagonist, 11 | Normal Distribution | Student's t-test | p < 0.001 |
| | Social novelty recognition in three-chamber social interaction test | Postpartum day 7 | Unstressed / Vehicle, 11 | Normal Distribution | Student's t-test | p < 0.05 |
| | | | Unstressed / GR antagonist, 12 | Normal Distribution | Student's t-test | p < 0.01 |
| | | | Stressed / Vehicle, 12 | Non-normal Distribution | Mann Whitney test | p = 0.146 |
| | | | Stressed / GR antagonist, 11 | Non-normal Distribution | Mann Whitney test | p < 0.01 |
| S6A | Body weight after forced swim test | Postpartum day 8 | Unstressed / Vehicle: 17, Unstressed / GR antagonist: 16, Stressed / Vehicle: 14, Stressed / GR antagonist: 14 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,57) = 3.245, p = 0.077 |
| | | | | | Vehicle, GR antagonist | F(1,57) = 0.011, p = 0.917 |
| | | | | | Interaction | F(1,57) = 0.368, p = 0.546 |
| S6B | Body weight after social interaction test | Postpartum day 7 | Unstressed / Vehicle: 11, Unstressed / GR antagonist: 12, | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,42) = 0.016, p = 0.899 |

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| | | | Stressed / Vehicle: 12, Stressed / GR antagonist: 11 | | Vehicle, GR antagonist | F(1,42) = 0.086, p = 0.770 |
| | | | | | Interaction | F(1,42) = 0.365, p = 0.549 |
| S6C | Body weight after forced swim test | Postpartum day 8 | Unstressed / Vehicle, 12; Unstressed / SSRI, 12; Unstressed / $GABA_AR$ modulator, 12; Unstressed / GR antagonist, 12; Stressed / Vehicle, 12; Stressed / SSRI, 12; Stressed / $GABA_AR$ modulator, 12; Stressed / GR antagonist, 12 | Normal Distribution | Two-way ANOVA | |
| | | | | | Unstressed, Stressed | F(1,88) = 0.394, p = 0.532 |
| | | | | | Drug treatment | F(3,88) = 1.550, p = 0.207 |
| | | | | | Interaction | F(3,88) = 0.367, p = 0.777 |