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

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Early life stress (ELS) increases the risk for postpartum depression (PPD). Patients with depression who experienced ELS tend to be treatment refractory. Nevertheless, it remains elusive how ELS underlies the pathophysiology of PPD at the mechanistic level. Here we observed that adolescent social isolation in mice resulted in an aberrantly sustained elevation of glucocorticoids via hypothalamic-pituitary-adrenal (HPA) axis dysregulation and long-lasting behavioral deficits during the postpartum period. The postpartum behavioral changes elicited by ELS were not ameliorated by the medicines currently used for PPD in behavioral assays that are frequently used in drug discovery for human depression. However, post-delivery treatment with a glucocorticoid receptor antagonist effectively ameliorated the deficits. We also demonstrated a significant impact of ELS on the HPA axis dysregulation and PPD in humans. In summary, we show the validity and utility of a novel model and a novel option of treatment for a subset of treatment-refractory PPD.

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 as well as 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 and 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 showed some treatment effects[18-21]. Meanwhile, a sudden withdrawal from a 2-month exposure of estradiol and progesterone at 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 deficits in the forced swim test, a gold standard of mouse behavioral assay in drug discovery for human depression[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 beginning treatment[27,28]. To overcome these limitations, the intravenous formulation of allopregnanolone (brexanolone), a positive allosteric modulator of $GABA_A$ receptors ($GABA_AR$), has been introduced as a new effective medication to the repertoire of PPD treatments[16]. However, its drawbacks include high price and treatment inaccessibility (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. In order to obtain clues for novel treatment approaches, we aimed to elucidate how ELS affects the pathophysiology of PPD at the mechanistic level. We also aimed to build a novel animal model that allows us to probe the mechanistic relationship between ELS and PPD, since a lack of animal models reflecting the influence of ELS is one of the major knowledge gaps in our understanding of PPD.

In the present study, we used an adolescent social isolation paradigm, which has been widely used as an ELS in mice[31-35], 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 exposed to adolescent social isolation that gave birth to pups in adulthood) (**Extended Data Fig. 1**). We chose the tail suspension test (TST) and forced swim test (FST) as major assays: they are commonly used for examining the efficacy of antidepressants[36,37], and have indeed contributed to the drug discovery process for PPD, including the recent case of an allopregnanolone analog, $GABA_AR$ modulator[38]. We did not observe any behavioral deficits in the TST and FST among the four groups at postpartum days 0

and 1, respectively (**Fig. 1**). Dams exposed to adolescent social isolation showed increased immobility time during the TST and FST at postpartum days 7 and 8, respectively (**Fig. 1**). Behavioral deficits in stressed dams were prolonged for at least three weeks after delivery (**Fig. 1**).

Social cognition in mothers may be regulated to monitor and interpret social signals from others, and safely navigate the new living environment[39]. Thus, we examined the effect of adolescent stress on dams' postpartum social behavior in the three-chamber social interaction test (SIT) which is used to test the social communication ability in rodents[40]. No behavioral deficits among the groups were observed at postpartum day 0 in both sociability and social novelty recognition in the SIT (**Extended Data Fig. 2**). At postpartum days 7 and 21, stressed dams showed a significant behavioral deficit in social novelty recognition, but not in sociability (**Extended Data Fig. 2**). 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.

Many studies have reported alterations in plasma hormone levels in patients with PPD and a correlation between hormonal changes and onset of PPD symptoms[9,19-22,41-43]. 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 levels were observed between unstressed and stressed dams at any time point (**Extended Data Fig. 3**). In contrast, the level of plasma corticosterone in both unstressed and

stressed dams was increased at late pregnancy and 0 week postpartum in comparison to virgin mice (**Fig. 2a**). Corticosterone levels in unstressed dams began to decrease after delivery (**Fig. 2a**). Notably, corticosterone levels showed prolonged elevation in stressed dams at 1 week and 3 weeks postpartum (**Fig. 2a**). 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 (**Extended Data Fig. 4**). These results suggest the specificity of our mouse model for prolonged HPA axis disruption evident by the persistent elevation in levels of corticosterone after delivery. The sustained HPA axis dysregulation may underlie the behavioral deficits observed in stressed dams.

Given that the appearance of immobility in TST and FST, as well as social cognitive deficits matched with the sustained elevation of corticosterone in the time course in stressed dams, we hypothesized that prolonged activation of glucocorticoid receptor (GR) signaling underlay these behavioral deficits. To address this question, 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 deficits in stressed dams. GR antagonist administration ameliorated behavioral deficits in the TST and FST in stressed dams at postpartum days 7 and 8, respectively (**Fig. 2b**). CORT113176 administration also ameliorated the behavioral deficits in the social novelty recognition trial of the SIT in stressed dams at postpartum day 7 (**Extended Data Fig. 5**). These results support the hypothesis that prolonged activation of GR signaling underlies long-lasting behavioral deficits 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 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[44,45]. We hypothesized that this negative feedback mechanism may be dysregulated in our model and performed the dexamethasone (DEX) suppression test[46,47] at postpartum day 8 when we first observed significant behavioral deficits in stressed dams. Treatment with low dose (0.1 mg/kg) dexamethasone successfully reduced the levels of ACTH and corticosterone in unstressed dams, whereas it failed to suppress these hormones in stressed dams (**Fig. 2c**). These data suggest that HPA axis negative feedback mechanisms may be dysregulated in stressed dams.

We next measured the mRNA levels of CRH in the PVN, CRH receptor1 (CRHR) in the pituitary gland and GR in both regions at five distinct time points in the trajectory (from the virgin stage to 3 weeks postpartum). CRHR mRNA levels in the pituitary gland, but not CRH mRNA levels in the PVN, of stressed dams at 0 week postpartum (postpartum day 2) were increased in comparison to stressed virgin mice, while a decrease was observed in unstressed dams at 0 week postpartum compared to unstressed virgin mice (**Fig. 2d**). GR mRNA levels in the pituitary gland, but not the PVN, of stressed dams were blunted at all time points we examined, while levels in unstressed dams in late pregnancy were increased in comparison to unstressed virgin mice (**Fig. 2d**). Together, these results indicate that the prolonged increase in plasma glucocorticoids in stressed dams occurs in part through i) increased HPA axis signaling via increased CRHR mRNA expression in the pituitary gland and ii) disruption of negative feedback mechanisms via blunting of GR mRNA expression in the pituitary gland.

It is possible to hypothesize that the aforementioned mouse model may represent the cases of PPD who experience ELS and resultant changes in the HPA axis. 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 therefore investigated the utility of our adolescent stress paradigm in modeling these clinically difficult cases and 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 1 week post-delivery did not normalize the behavioral deficits in TST and FST in stressed dams (**Fig. 3**). Treatment with a $GABA_AR$ modulator (ganaxolone) was also ineffective (**Fig. 3**). In contrast, only a 1 week post-delivery treatment with a GR antagonist (CORT113176) was sufficiently effective and significantly ameliorated the behavioral deficits in both the TST and FST in stressed dams (**Fig. 3**).

To examine whether our preclinical model shows clinically-relevant biology and 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 traumatic events], the HPA axis, and the development of PPD in 116 women. Notably, a history of mental illness was the most important risk factor and was positively correlated and linked to the development of PPD when compared to other types of ELS (**Figs. 4a** and **4b**). We then questioned the biological impact of previous mental illness diagnosis and its relationship with PPD. To address this question, we compared the level of plasma cortisol, as a surrogate of the HPA axis activity, between non-PPD women who had previous mental illness diagnosis and PPD patients who had

previous mental illness diagnosis. The former group showed an equivalent peak of glucocorticoids leading up to delivery followed by a gradual decline (**Fig. 4c**), as seen in mouse unstressed dams (**Fig. 2a**). In contrast, cortisol levels in the latter group (PPD patients with a history of mental illness) exhibited both elevated and sustained levels of plasma cortisol until at least six weeks postpartum (**Fig. 4c**), mirroring our preclinical findings that links ELS with sustained elevation of plasma cortisol and abnormal behaviors (**Fig. 2a**).

The main findings from the present study using a novel animal model are that adolescent social isolation elicits aberrantly prolonged activation of GR signaling through HPA axis dysregulation, which in turn results in long-lasting postpartum behavioral deficits in the TST, FST, and SIT. Pharmacological inhibition of GR signaling in only the first week following delivery (short-term post-delivery treatment) is sufficient to ameliorate the behavioral deficits in stressed dams while other medicines currently used for PPD in the clinical setting are ineffective, supporting a causal role of prolonged activation of GR signaling in behavioral deficits observed in stressed dams. 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. Our novel animal model 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[48-51]. Intervention with $GABA_AR$-mediated neurotransmission in the PVN may be one of these mechanisms[48]. Under our experimental conditions, neither a $GABA_AR$ modulator nor a SSRI ameliorated the behavioral deficits. Although we selected the doses and administration routes for these compounds based on published protocols[52-55], further optimization of these conditions may be needed. Meanwhile, we underscored the pituitary gland, at least partly, as a fundamental center of the HPA axis dysregulation 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 likely that only the anterior pituitary gland may be dysregulated in the present model. Future studies should investigate other brain regions and circuits that may susceptible to ELS and pathologically affect the anterior pituitary gland.

We acknowledge the potential limitations of our study design, which also lead to opportunities for future research. We did not consider the contribution of many environmental stressors or complex genetic factors that may underlie PPD. Nevertheless, we have built a straightforward model with a single environmental stressor under a homogenous genetic condition, allowing us to dissect a causal role of prolonged GR signaling in long-lasting behavioral deficits in the postpartum period. Future studies may use our novel model to examine maternal behaviors, transgenerational transmission of risk to children reared by stressed mothers, involvement of the mineralocorticoid receptor, and involvement of genetics and epigenetics in the pathological mechanisms underlying PPD. Lastly, we underscored the potential of a GR antagonist for the treatment of a potential subset of PPD cases. GR antagonists, such as mifepristone, can be orally administered for the treatment of Cushing's syndrome as well as in other ongoing clinical

trials[56,57]. Although clinical trials are still needed, our data indicate that repurposing GR antagonists may lead to improvement in ELS-associated cases of treatment refractory PPD.

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Materials and 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 [32]. 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). 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 GR signaling on behavioral deficits 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 [32,33], CORT113176 does not antagonize

progesterone signaling [52,58-61]. 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[52-55].

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 (**Extended Data Fig. 1**).

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 ^{o}C 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™ 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[62].

Measurement of plasma hormone levels in mice. Blood was collected from the inferior vena cava under isoflurane anesthesia between 9am and 11am, 24 h following the FST. Levels of plasma corticosterone, estradiol, progesterone, oxytocin, and prolactin were assessed using commercially available enzyme immunoassay (EIA) kits[32] 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[63-65]. DEX at low doses cannot pass the blood-brain barrier and can provide insight into the functionality of the HPA axis [46,47]. DEX (0.1 mg/kg, *i.p*) was administered to mice six hours prior to collecting blood samples at 5pm on postpartum day 8. Plasma ACTH and corticosterone levels after treatment with DEX were assessed using commercially available EIA kits as published[32].

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 at four time points across pregnancy and the postpartum period (2nd and 3rd trimester, 2 weeks postpartum, and 6 weeks postpartum). PPD

was defined as developing major depression within six weeks of delivery and not during pregnancy[66]. "History" refers to their psychiatric history up until their first visit (**Extended Data Table 1**). Subjects were also assessed regarding childhood home environment, childhood behavior, and traumatic events (occurring before 17 years of age) (**Extended Data Table 1**). 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) (**Extended Data Table 2**). 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. Pearson's correlation was also performed to study the relationship between human cortisol (log transformed) and risk factors. The fisher exact test was used to check if two categorical variables are related, for example, if PPD is related to traumatic events. A value of $p < 0.05$ was considered statistically significant. All data are expressed as the mean ± SEM.

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Author contributions

M.N. conceived, designed, and supervised the project with the guidance of **A.S.** and **S.K.** 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 was written by **M.N.** and **A.S.** with input from all the authors. **M.N.**, **S.L**, **D.J.W.**, and **A.S.** revised and edited the paper. **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.

Competing interests

All authors declare that they have no competing interests.

Figure Legends

Fig. 1. **Long-lasting behavioral deficits related to postpartum mobility/despair in mice 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 deficits emerged at one week postpartum and remained until at least three weeks postpartum. No deficits across groups were observed immediately after delivery. N=10-12. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

Fig. 2. Prolonged activation of glucocorticoid signaling underlies postpartum behavioral deficits in mice exposed to adolescent social isolation. a, Levels of corticosterone were measured at five time points: virgin, late pregnancy, 0 week postpartum, 1 week postpartum, and 3 weeks postpartum. Adolescent isolation before pregnancy led to an increase in plasma corticosterone levels at one-week postpartum compared to unstressed controls. Notably, the prolonged levels in the stressed mice remained at three-week postpartum. N=9-30. **b**, Causal effects of the GR on postpartum behaviors related to mobility/despair. CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing) ameliorated the behavioral deficits in the tail suspension and forced swim tests in stressed postpartum mice at postpartum days 7 and 8, respectively. The antagonist did not affect behavior in the TST and FST in unstressed postpartum mice. N=14-17. **c**, Disruption of HPA axis negative feedback in stressed postpartum mice. In the dexamethasone (DEX) suppression test, DEX was administered at postpartum day 8 (0.1mg/kg, *i.p.*). A lack of suppression of ACTH and corticosterone was observed only in stressed postpartum mice. N=11. **d**, Dysregulation of CRHR and GR mRNA

levels in the pituitary, but not PVN. Homeostatic changes in the mRNA levels of CRHR and GR in the pituitary of stressed mice were disrupted. No difference in the mRNA levels of CRH and GR in the PVN was observed between unstressed and stressed mice at any time point. N=7. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$; $^{\#\#}P<0.01$ and $^{\#}P<0.05$ versus virgin unstressed mice; $^{++}P<0.01$ and $^{+}P<0.05$ versus virgin stressed mice.

Fig. 3. Therapeutic effect of a GR antagonist, compared with a SSRI and an allopregnanolone analog, on postpartum behavioral deficits related to mobility/despair in mice exposed to adolescent social isolation. Mice were treated with the 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 deficits 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.

Fig. 4. A link between adverse early life events, a sustained increase in 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 $^{*}P<0.05$.

Extended Data Fig. 1. 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.

Extended Data Fig. 2. **Long-lasting behavioral deficits related to postpartum social cognition in mice exposed to adolescent social isolation. a**-**c**, Sniffing time during the three-chamber social interaction test at postpartum days 0, 7, and 21. No deficits in sociability or social novelty recognition among the four groups were observed at postpartum day 0. Behavioral deficits in social novelty recognition, but not sociability, were observed in stressed postpartum mice at postpartum days 7 and 21. N=9-11. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

Extended Data Fig. 3. **No change in the** l**evels 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.

Extended Data Fig. 4. 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.

Extended Data Fig. 5. Causal effect of the GR on postpartum behavioral deficits related to social cognition in mice exposed to adolescent social isolation. CORT113176 (80 mg/kg, *p.o.*, once daily from gestation day 14 to 24 h prior to behavioral testing), a selective GR antagonist, ameliorated the behavioral deficits in social novelty recognition in the three-chamber social interaction test in stressed postpartum mice at postpartum day 7. N=11-12. Values are represented as mean ± SEM; $^{**}P<0.01$ and $^{*}P<0.05$.

Extended Data Table 1. 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

Extended Data Table 2. Statistical results.

Figure	Experiment	Experimental day	Sample size	Data Structure	Statistical test	Values
1a	Tail suspension test	Postpartum day 0	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10; Unstressed / Delivery (+), 12; Stressed / Delivery (+), 10	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,41) = 0.578, p = 0.452
					Delivery (-), Delivery (+)	F(1,41) = 1.476, p = 0.232
					Interaction	F(1,41) = 0.660, p = 0.422
		Postpartum day 7	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10; Unstressed / Delivery (+), 10; Stressed / Delivery (+), 11	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,40) = 23.651, p < 0.001
					Delivery (-), Delivery (+)	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 / Delivery (-) vs Stressed / Delivery (-)	p = 1
					Unstressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Unstressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.001
					Stressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Stressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.001
					Unstressed / Delivery (+) vs Stressed / Delivery (+)	p < 0.001
		Postpartum day 21	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10; Unstressed / Delivery (+), 12; Stressed / Delivery (+), 11	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,42) = 12.371, p < 0.01
					Delivery (-), Delivery (+)	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 / Delivery (-) vs Stressed / Delivery (-)	p = 1
					Unstressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Unstressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.01
					Stressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Stressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.01
					Unstressed / Delivery (+) vs Stressed / Delivery (+)	p < 0.05
1b	Forced swim test	Postpartum day 1	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10;	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,41) = 0.257, p = 0.615

			Unstressed / Delivery (+), 12; Stressed / Delivery (+), 10		Delivery (-), Delivery (+)	F(1,41) = 2.393, p = 0.13
					Interaction	F(1,41) = 0.257, p = 0.615
		Postpartum day 8	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10; Unstressed / Delivery (+), 10; Stressed / Delivery (+), 11	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,40) = 22.744, p < 0.001
					Delivery (-), Delivery (+)	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 / Delivery (-) vs Stressed / Delivery (-)	p = 1
					Unstressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Unstressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.001
					Stressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Stressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.001
					Unstressed / Delivery (+) vs Stressed / Delivery (+)	p < 0.001
		Postpartum day 22	Unstressed / Delivery (-), 10; Stressed / Delivery (-), 10; Unstressed / Delivery (+), 12; Stressed / Delivery (+), 11	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,42) = 17.662, p < 0.001
					Delivery (-), Delivery (+)	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 / Delivery (-) vs Stressed / Delivery (-)	p = 1
					Unstressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Unstressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.001
					Stressed / Delivery (-) vs Unstressed / Delivery (+)	p = 1
					Stressed / Delivery (-) vs Stressed / Delivery (+)	p < 0.05
					Unstressed / Delivery (+) vs Stressed / Delivery (+)	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; Stressed / 1 week postpartum, 10	Normal Distribution	Student's *t*-test	$p < 0.05$
			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	$F(1,60) = 8.036$, $p < 0.01$
					Vehicle, GR antagonist	$F(1,60) = 3.934$, $p = 0.052$
					Interaction	$F(1,60) = 5.092$, $p < 0.05$
					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	$F(1,63) = 26.002$, $p < 0.001$
					Vehicle, GR antagonist	$F(1,63) = 6.031$, $p < 0.05$
					Interaction	$F(1,63) = 23.406$, $p < 0.001$

					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 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 corticosterone levels (Dexamethasone suppression test)	Postpartum day 8	Unstressed / Vehicle: 11, Unstressed / DEX: 11, Stressed / Vehicle: 11, Stressed / DEX: 11	Non-normal Distribution	Kruskal-Wallis	
					H = 26.052	p < 0.001
					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	Non-	Mann Whitney test	p =

			postpartum, 7; Stressed / 1 week postpartum, 7	normal Distribution		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
			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;	Non-	Mann-Whitney test	p =

			Unstressed / 1 week postpartum, 7	normal Distribution		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(1,95) = 7.115, p < 0.01
					Drug treatment	F(3,95) = 0.834, p = 0.479
					Interaction	F(3,95) = 0.791, p = 0.502
					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 < 0.001
					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; Stressed / Vehicle, 12; Stressed / SSRI, 12; Stressed / $GABA_AR$ modulator, 12; Stressed / GR antagonist, 12	Normal Distribution	Two-way ANOVA	
					Unstressed, Stressed	F(1,95) = 31.876, p < 0.001
					Drug treatment	F(3,95) = 3.159, p < 0.05
					Interaction	F(3,95) = 3.547, 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
					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	2nd trimester, 3rd trimester, 2 weeks	No history / No PPD, 38; No history / PPD, 3; history / No PPD,	Non-normal Distribution	Linear regression	p = 0.112

	humans	postpartum, 6 weeks postpartum	60; history / PPD, 15			
			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 PPD 6 weeks postpartum, 60	Normal Distribution	Linear regression	p = 0.164
			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
Extended Data						
2	Sociability in	Postpartum	Unstressed / Delivery	Normal	Student's *t*-test	p <

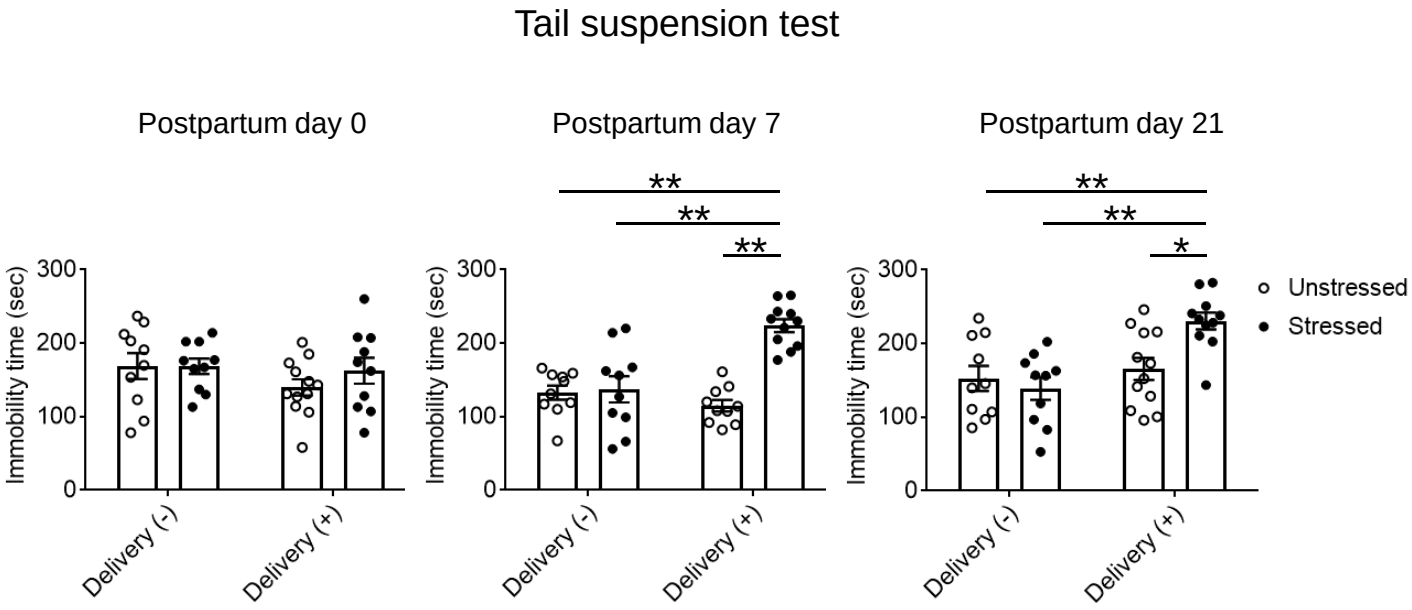
	three-chamber social interaction test	day 0	(-), 10	Distribution		0.001
			Stressed / Delivery (-), 9	Non-normal Distribution	Mann Whitney test	$p < 0.001$
			Unstressed / Delivery (+), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (+), 9	Normal Distribution	Student's *t*-test	$p < 0.001$
	Social novelty recognition in three-chamber social interaction test	Postpartum day 0	Unstressed / Delivery (-), 10	Non-normal Distribution	Mann Whitney test	$p < 0.01$
			Stressed / Delivery (-), 9	Non-normal Distribution	Mann Whitney test	$p < 0.001$
			Unstressed / Delivery (+), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (+), 9	Normal Distribution	Student's *t*-test	$p < 0.001$
	Sociability in three-chamber social interaction test	Postpartum day 7	Unstressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (-), 10	Non-normal Distribution	Mann Whitney test	$p < 0.01$
			Unstressed / Delivery (+), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (+), 11	Normal Distribution	Student's *t*-test	$p < 0.001$
	Social novelty recognition in three-chamber social interaction test	Postpartum day 7	Unstressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.01$
			Unstressed / Delivery (+), 10	Normal Distribution	Student's *t*-test	$p < 0.01$
			Stressed / Delivery (+), 11	Normal Distribution	Student's *t*-test	$p = 0.378$
	Sociability in three-chamber social interaction test	Postpartum day 21	Unstressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.01$
			Stressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.001$
			Unstressed / Delivery (+), 12	Normal Distribution	Student's *t*-test	$p < 0.01$
			Stressed / Delivery (+), 11	Normal Distribution	Student's *t*-test	$p < 0.05$
	Social novelty recognition in three-chamber social interaction test	Postpartum day 21	Unstressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.01$
			Stressed / Delivery (-), 10	Normal Distribution	Student's *t*-test	$p < 0.01$
			Unstressed / Delivery (+), 12	Normal Distribution	Student's *t*-test	$p < 0.001$
			Stressed / Delivery (+), 11	Normal Distribution	Student's *t*-test	$p = 0.692$
3	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	Virgin, Late pregnancy,	Unstressed / Virgin, 20; Stressed / Virgin, 20	Non-normal Distribution	Mann Whitney test	$p = 0.496$

	levels	0 week postpartum, 1 week postpartum	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
			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
4	Correlation between corticosterone levels and immobility in tail suspension test	1 week postpartum	Unstressed / Delivery (+), 9; Stressed / Delivery (+), 10	Non-normal Distribution	Spearman's correlation	r = 0.837, p < 0.001
	Correlation between corticosterone levels and immobility in forced swim test			Non-normal Distribution	Spearman's correlation	r = 0.830, p < 0.001
	Correlation between corticosterone levels and immobility in tail suspension test	3 weeks postpartum	Unstressed / Delivery (+), 12; Stressed / Delivery (+), 11	Normal Distribution	Pearson's correlation	r = 0.857, p < 0.001
	Correlation between corticosterone levels and immobility in forced swim test			Normal Distribution	Pearson's correlation	r = 0.895, p < 0.001
5	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	$p < 0.01$

Figure 1

a



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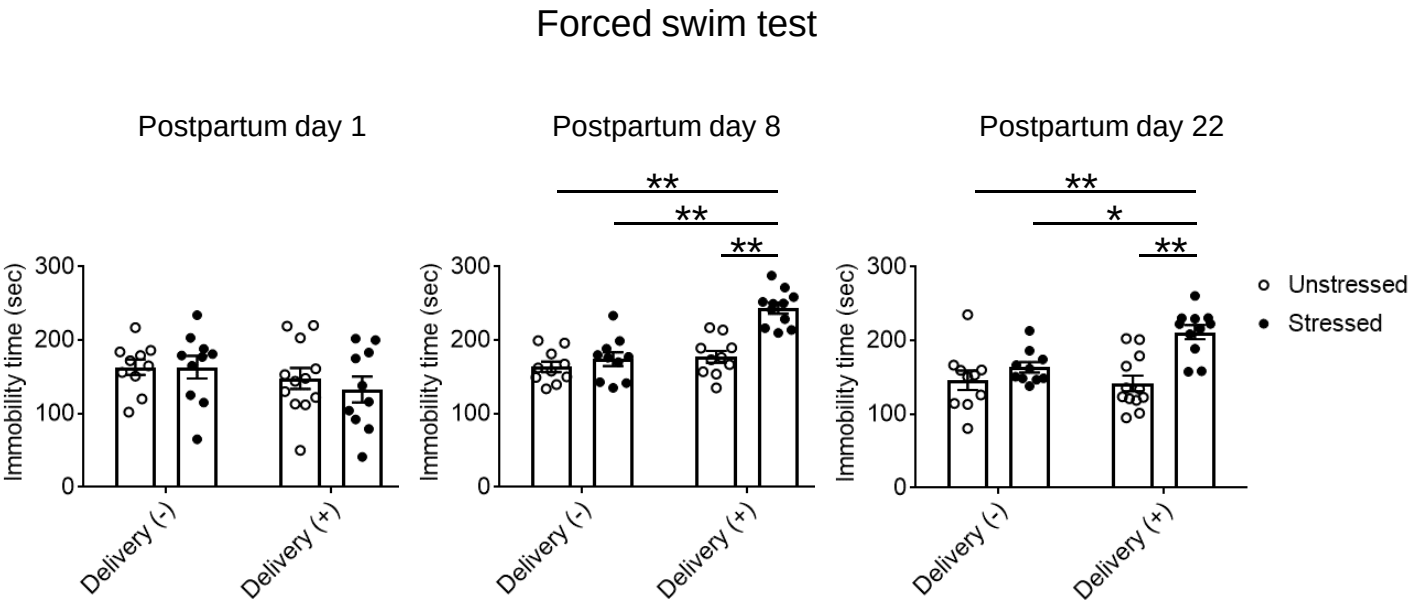
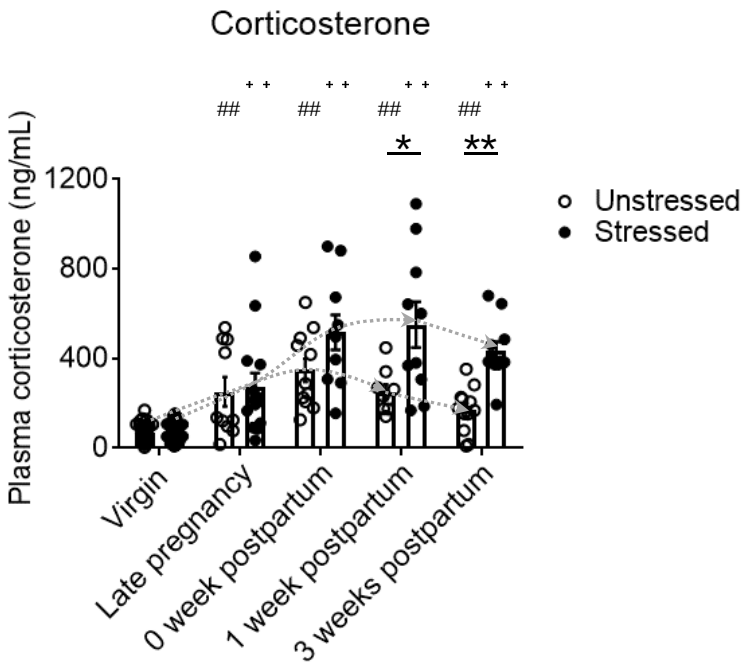


Figure 2

a



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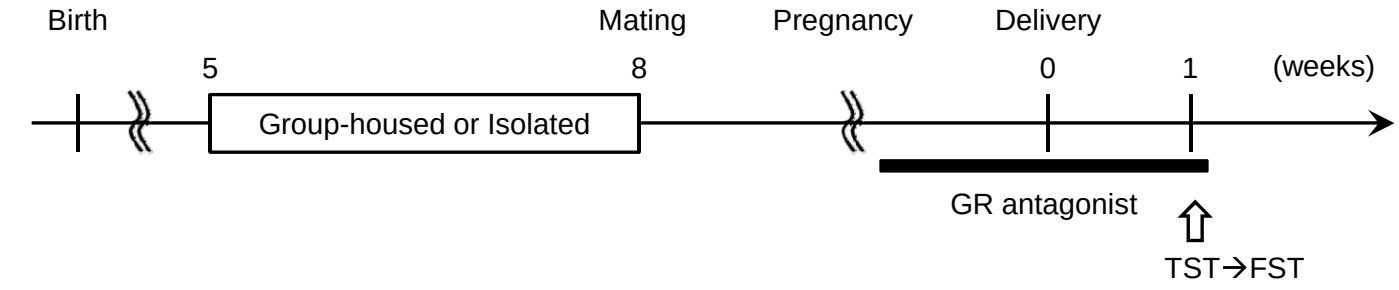

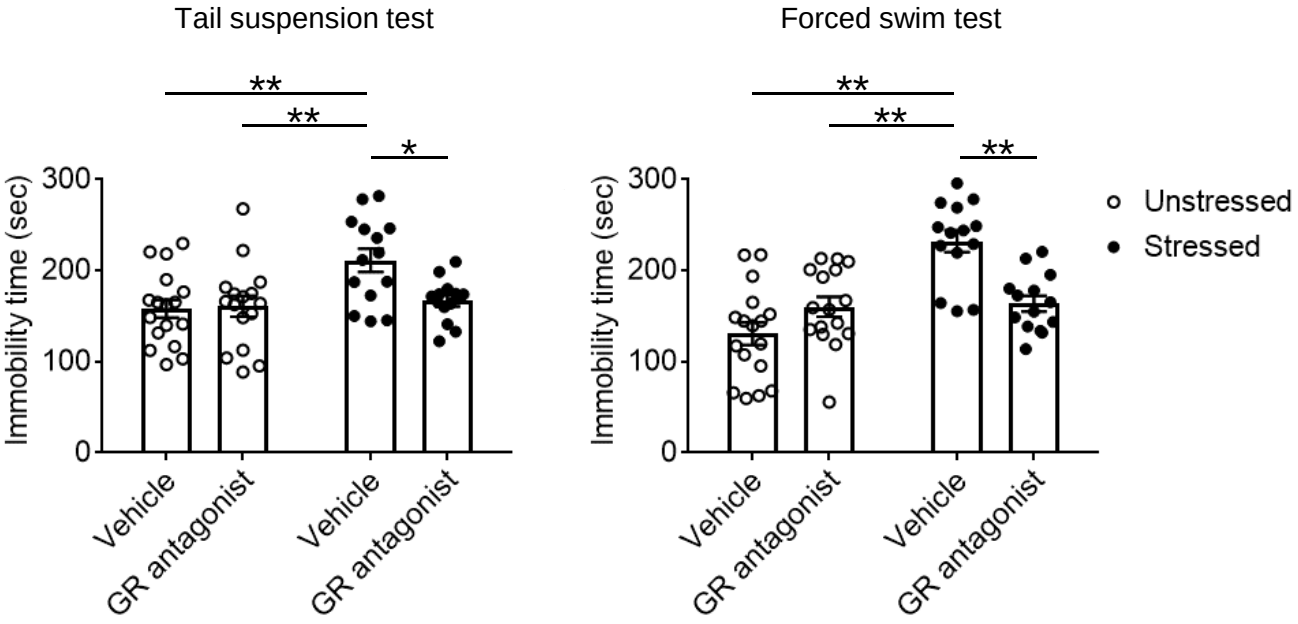

Figure 2 (1)

Figure 2 (cont'd)

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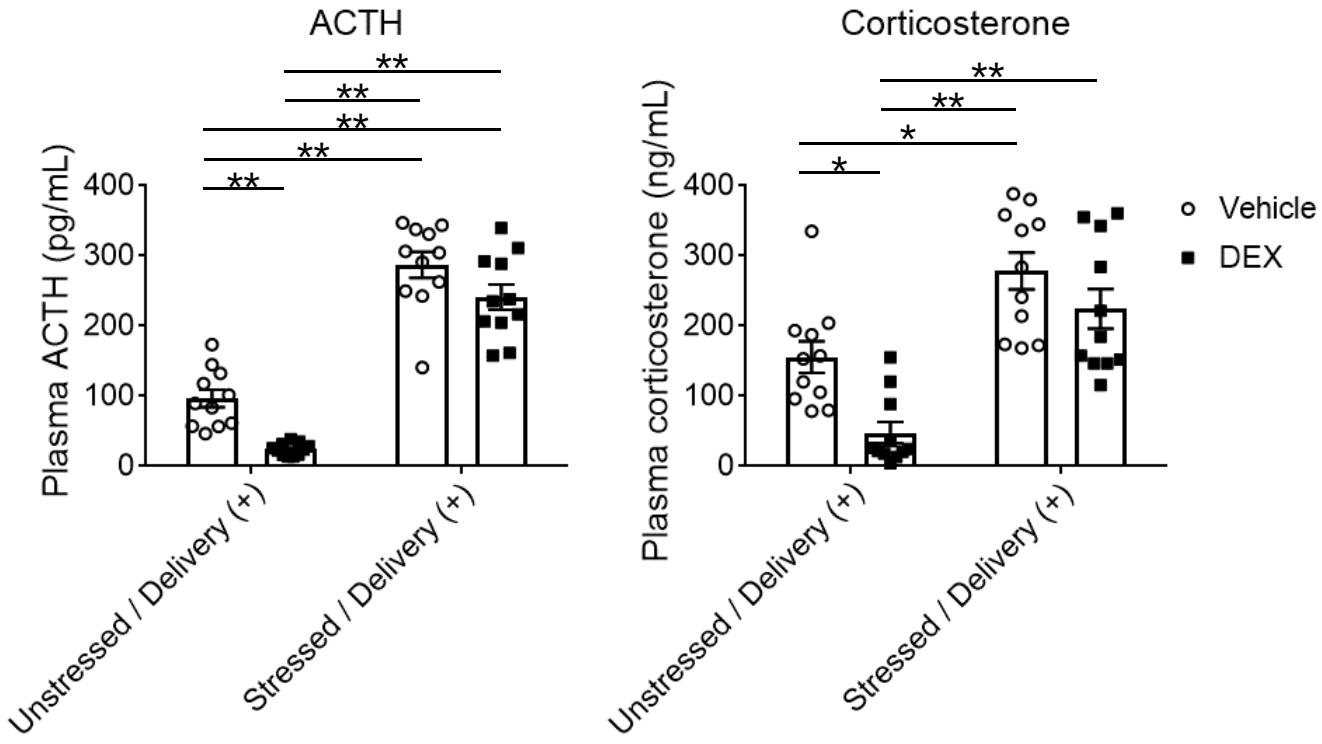

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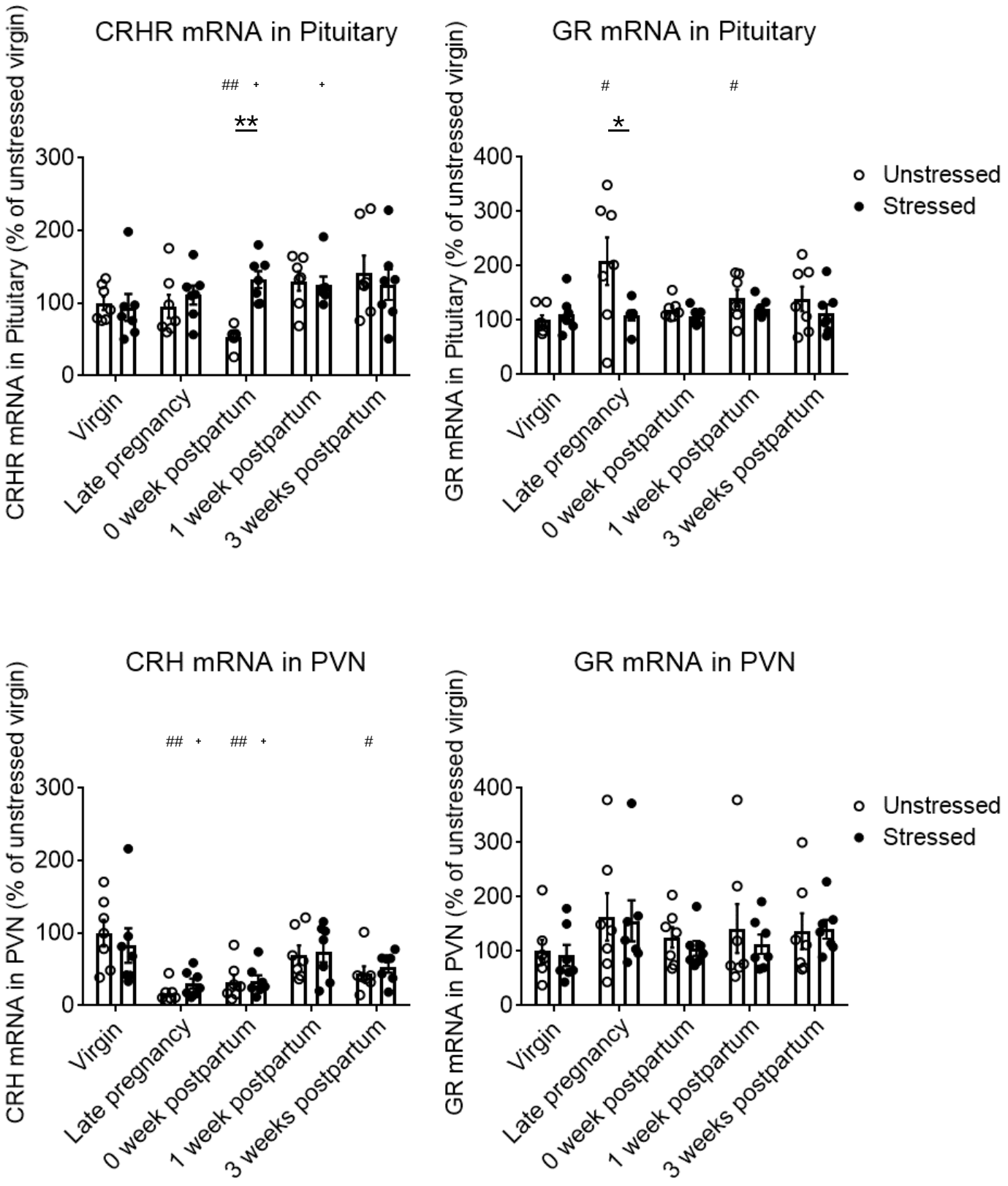


Figure 2 (3)

Figure 3

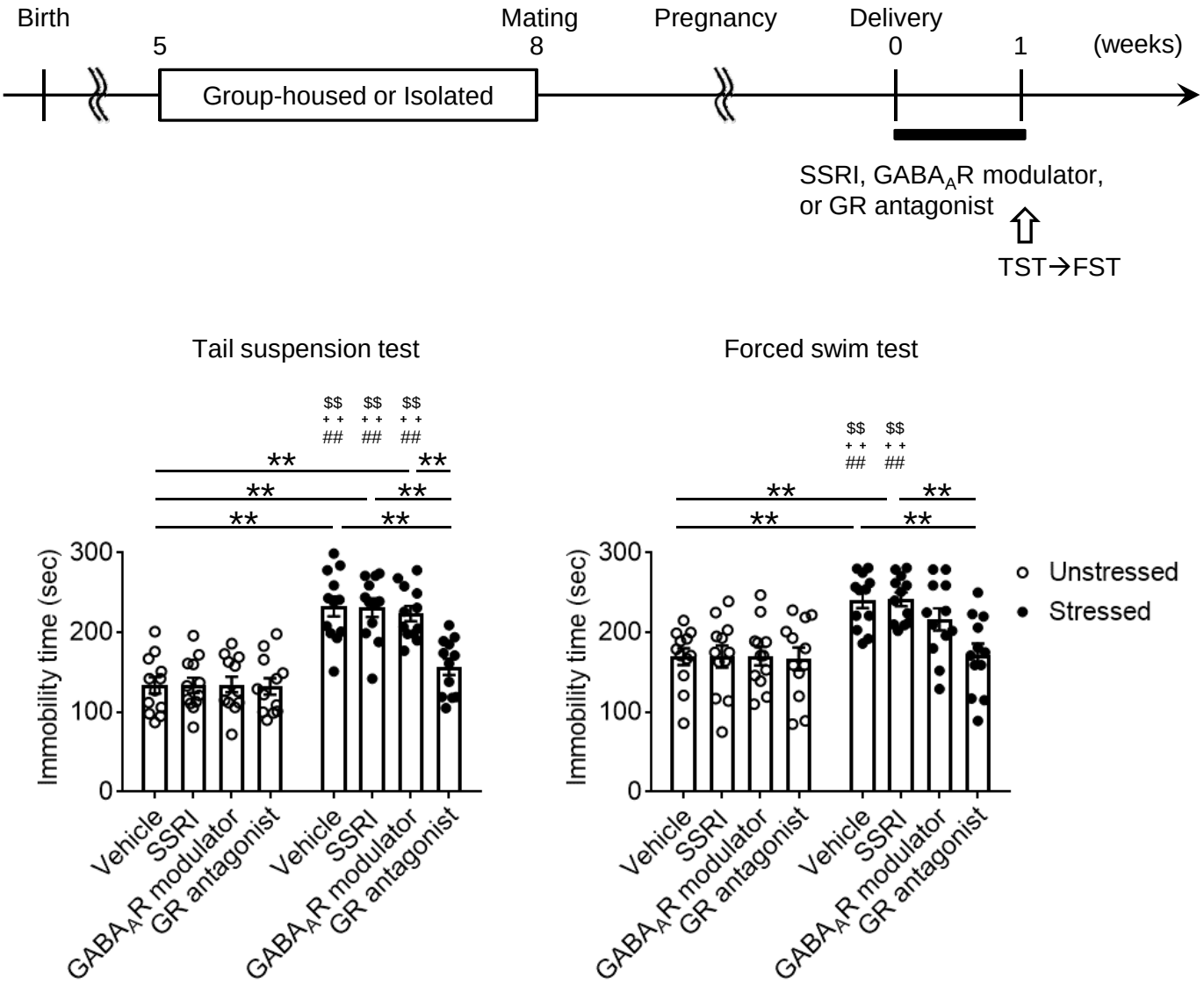
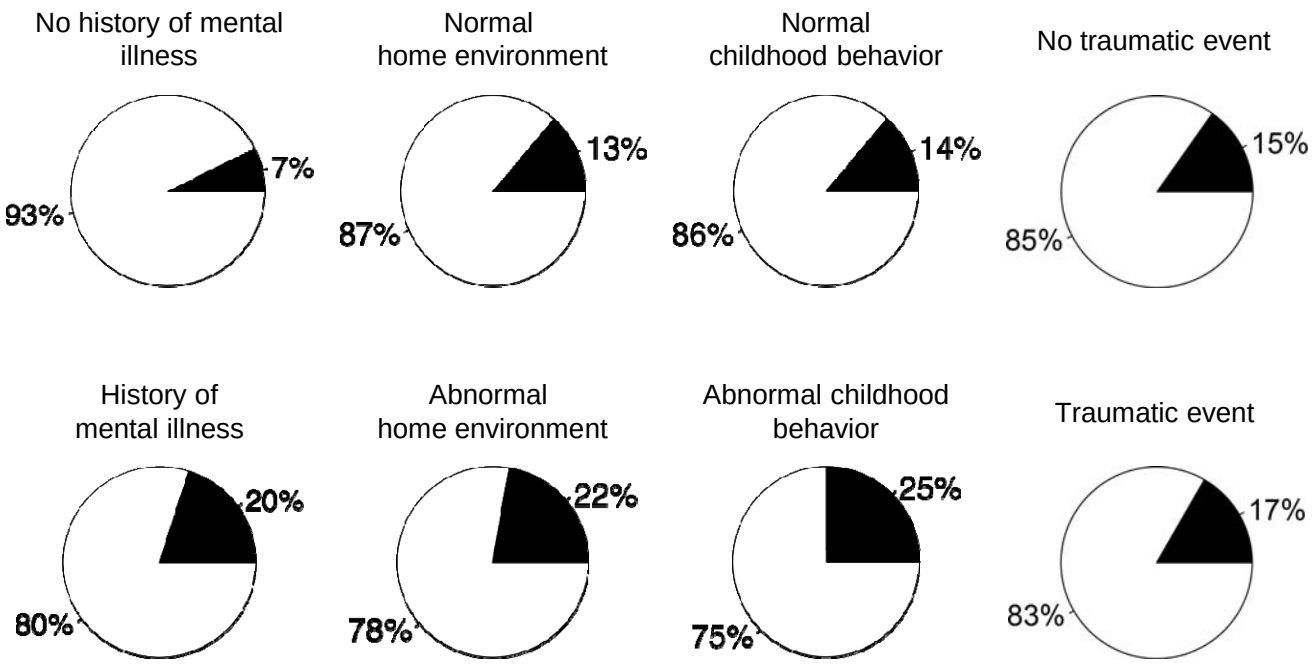


Figure 4

a



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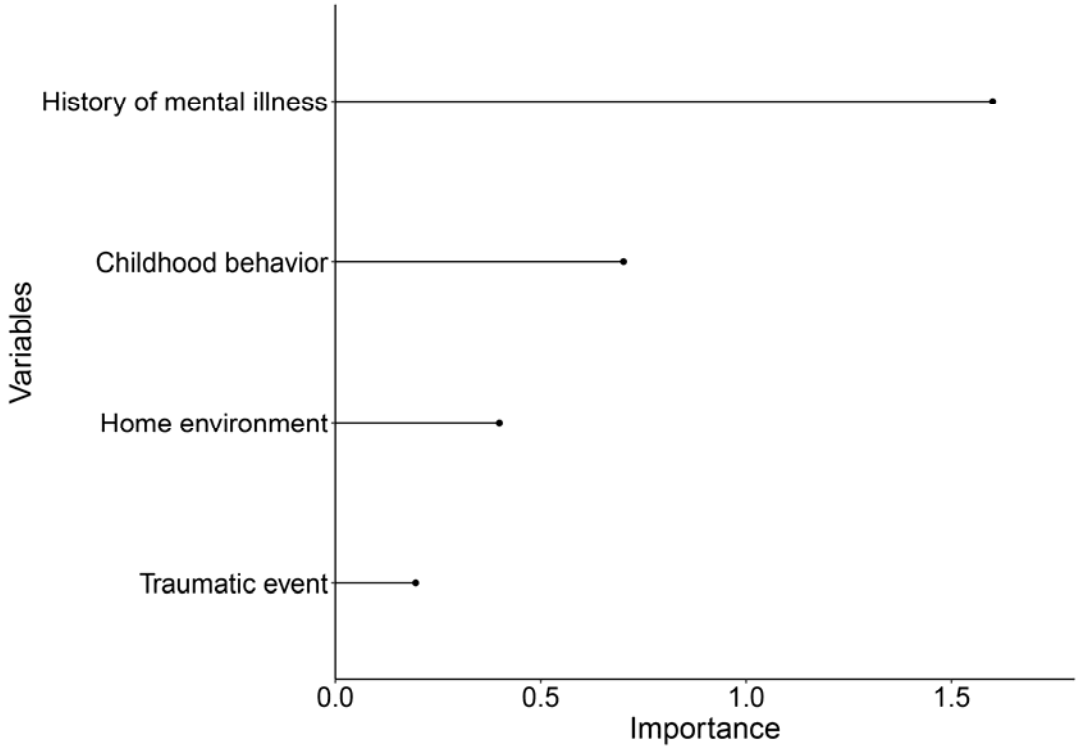

Figure 4 (cont'd)

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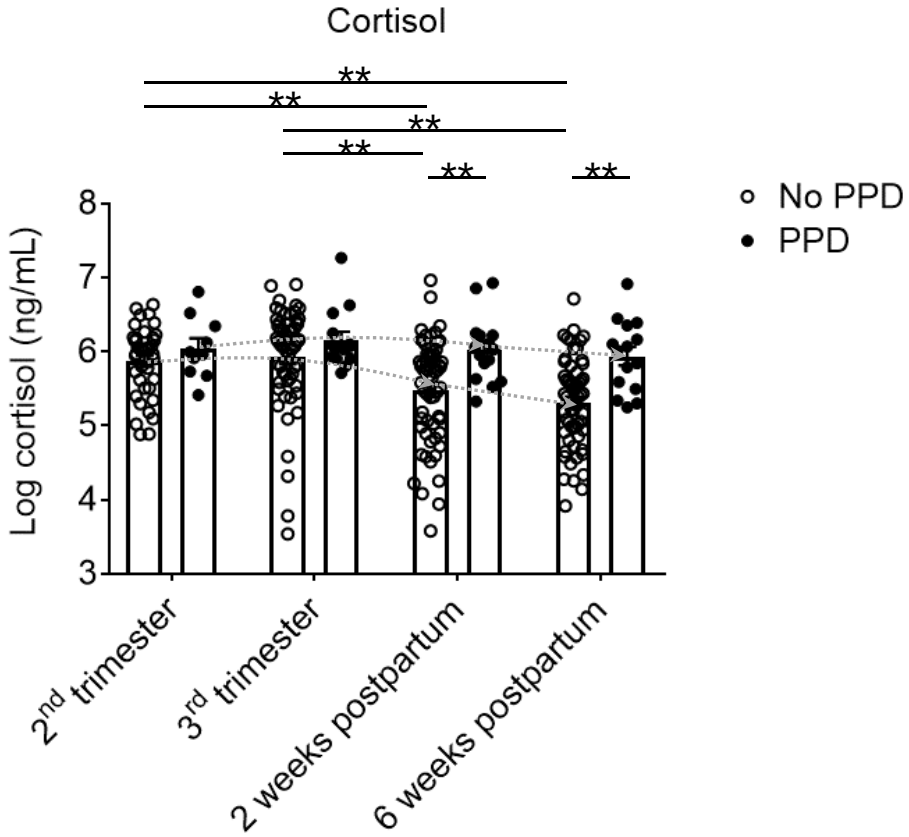
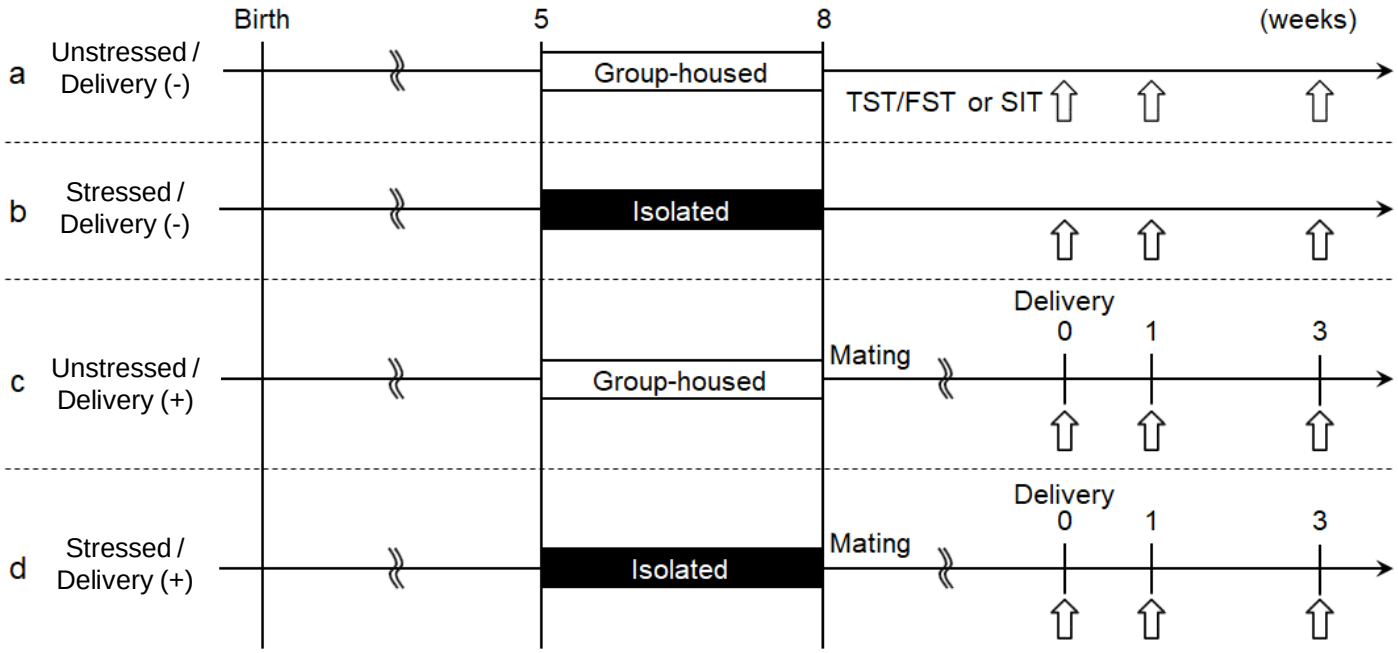
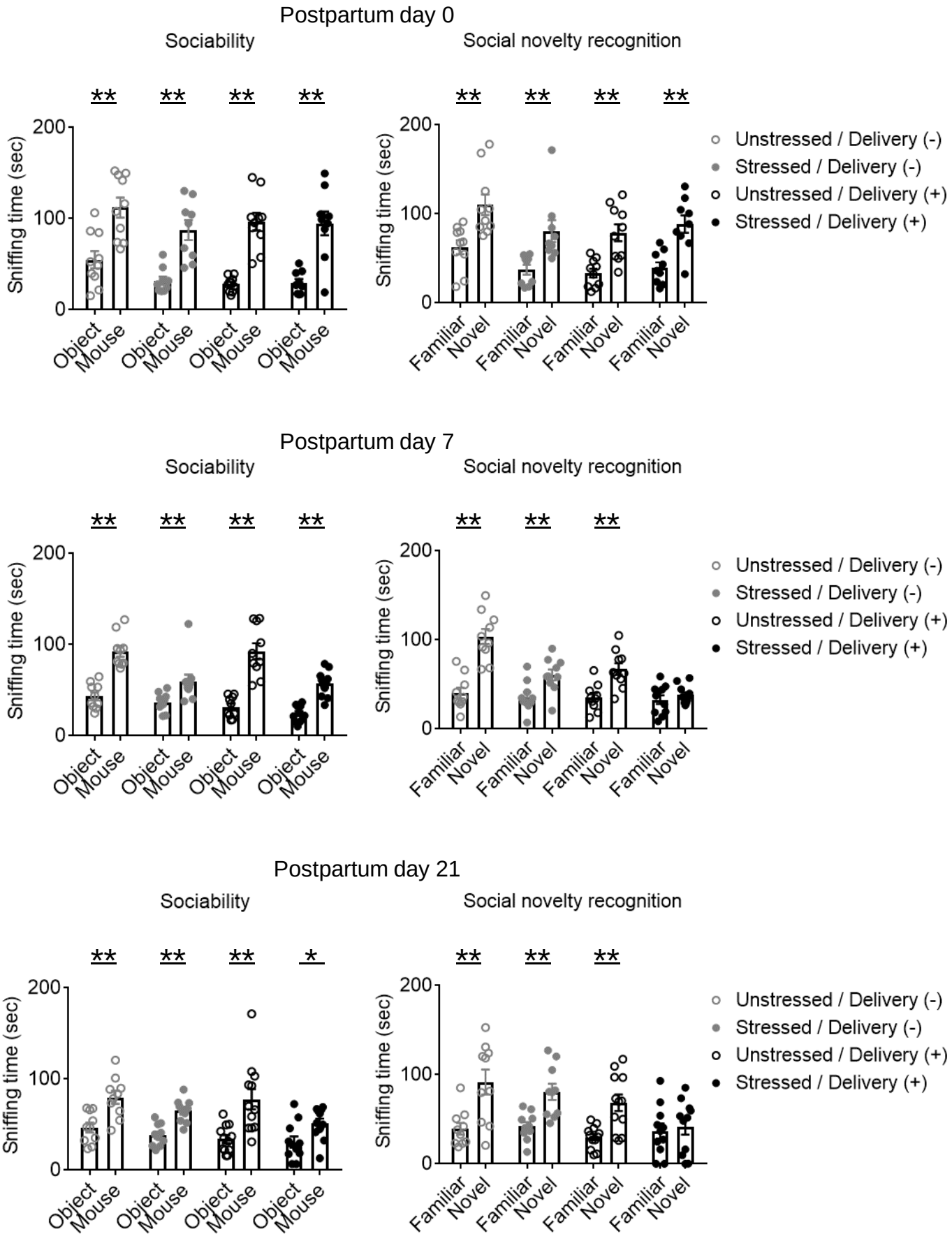


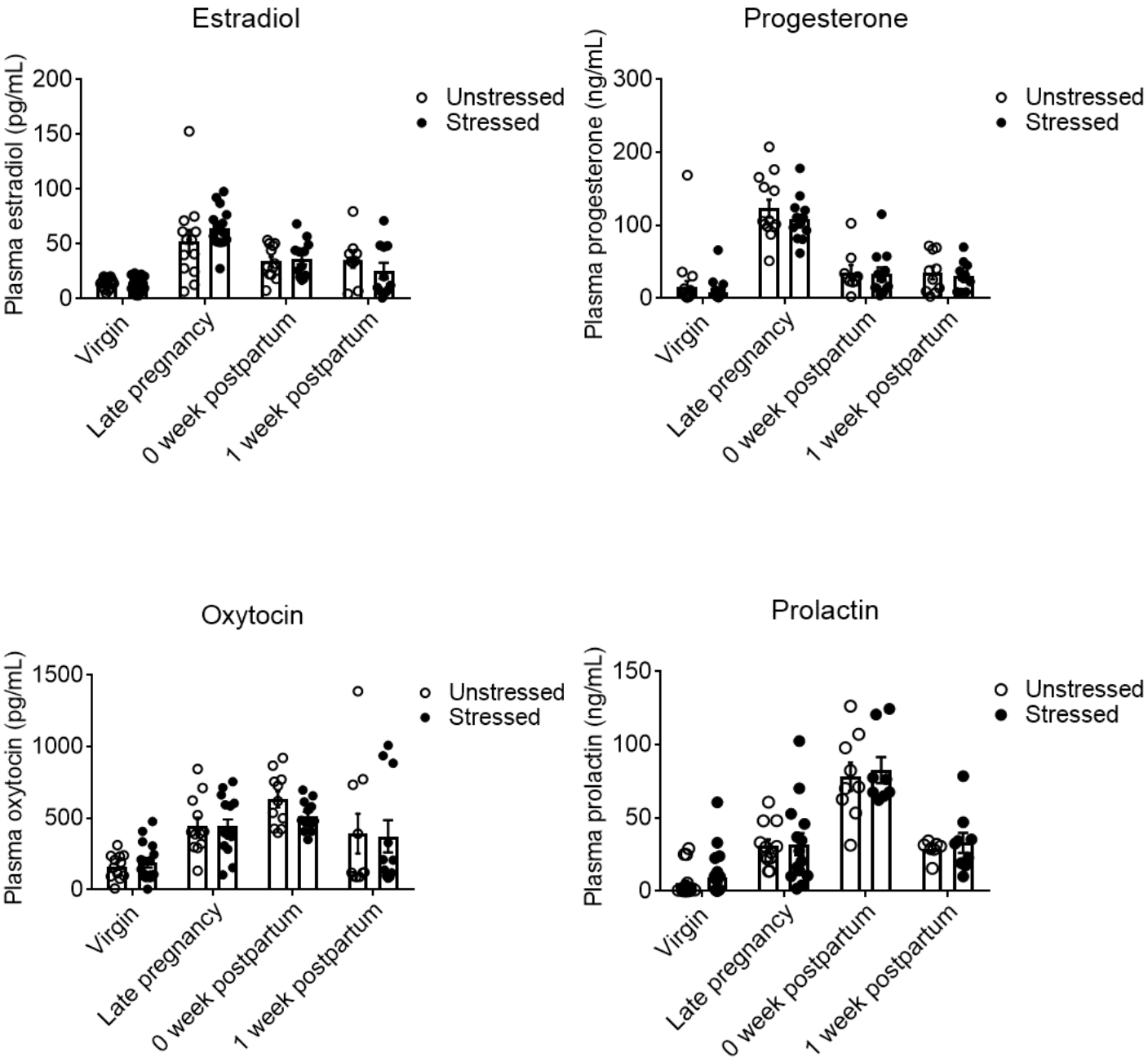
Extended Data Figure 1



Extended Data Figure 2



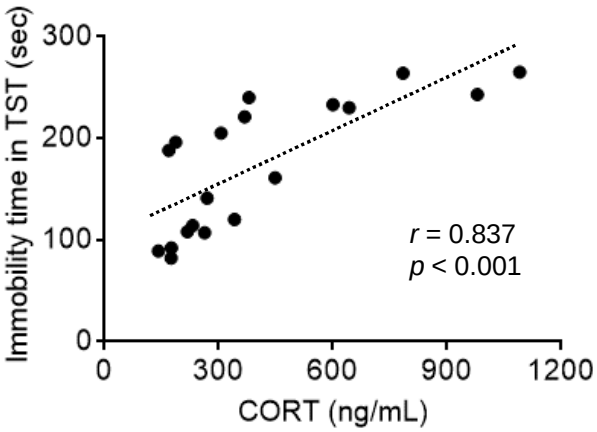
Extended Data Figure 3



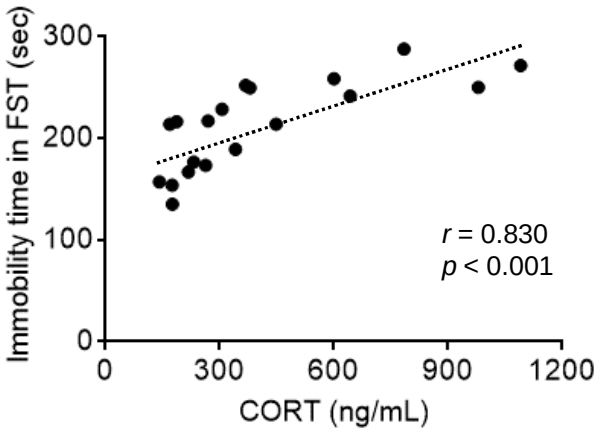
Extended Data Figure 4

1 week postpartum

Plasma corticosterone / Tail suspension test

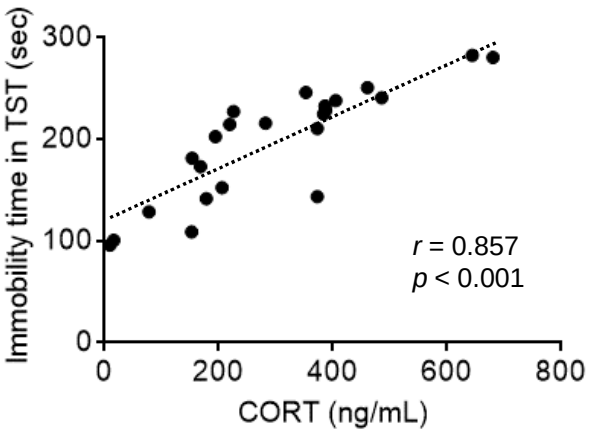


Plasma corticosterone / Forced swim test

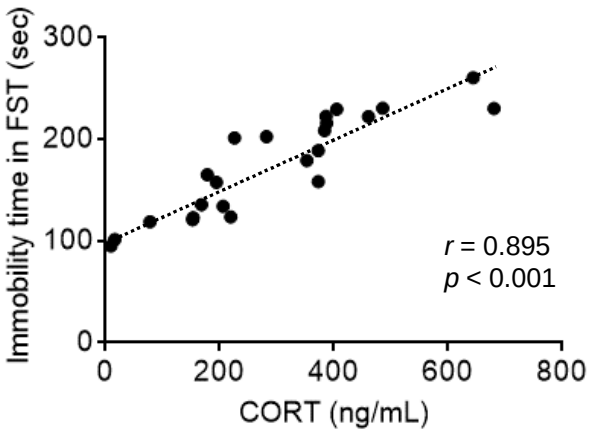


3 weeks postpartum

Plasma corticosterone / Tail suspension test



Plasma corticosterone / Forced swim test



Extended Data Figure 5