

RESEARCH ARTICLE

# Corticosterone response to gestational stress and postpartum memory function in mice

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## Abstract

Maternal stress is a common adversity during pregnancy. Gestational corticosterone alterations are thought to contribute to the etiology of postpartum behavioral disturbances. However, the impact of stress during pregnancy, in particular noise exposure, on gestational corticosterone fluctuations and spatial cognition in postpartum mice has not been fully understood yet. We hypothesized that noise exposure during pregnancy negatively affects gestational corticosterone levels and postpartum memory function in the dams similar to the physical stressors. Pregnant C57BL/6 mice were randomly assigned to either one of two stress conditions or a control condition. The noise stress (NS) was induced by presenting a loud intermittent 3000 Hz frequency on gestational days (GDs) 12, 14, and 16 for 24 hours, whereas the physical stress (PS) consisted of restraint and exposure to an elevated platform on GDs 12–16. Plasma corticosterone level was collected on GDs 11 and 17, and Morris water task (MWT) was carried out 30 days after parturition. Compared to the control group, the level of corticosterone in the stressed groups was significantly increased on GD17 relative to GD11. Significantly longer swim time and lower swim speed were observed in both stressed groups relative to the control group. Probe time was significantly shorter in the NS group than the other groups. The delta corticosterone level was significantly correlated with the swim time as well as the probe time in the three groups. Given the results, the adverse effects of gestational noise exposure on the hypothalamic pituitary-adrenal (HPA) axis activation and postpartum spatial learning and memory function were as large as/ or a bit stronger than the physical stresses. The findings suggest the significance of conservation against loud noise exposure in daily living, as well as need to further notice to the different aspects of gestational stress in mothers' behavior like offspring.

## OPEN ACCESS

**Citation:** Jafari Z, Mehla J, Afrashteh N, Kolb BE, Mohajerani MH (2017) Corticosterone response to gestational stress and postpartum memory function in mice. PLoS ONE 12(7): e0180306. <https://doi.org/10.1371/journal.pone.0180306>

**Editor:** Jodi Pawluski, Universite de Rennes 1, FRANCE

**Received:** December 18, 2016

**Accepted:** June 13, 2017

**Published:** July 10, 2017

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**Data Availability Statement:** All relevant data are within the paper.

**Funding:** This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant #40352 (MHM), Campus Alberta for Innovation Program Chair, Alberta Alzheimer Research Program (MHM), and a Canadian Institute for Advanced Research grant #43300 (BK). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Introduction

Stress, as an inherent component of modern life, has a major contribution in shaping the brain and behavior [1, 2]. The term “stress” has different definitions in the literature but in laboratory animal studies, it usually refers to the impact of an event on the organism and the

**Competing interests:** The authors have declared that no competing interests exist.

organism's response to it. Stress causes distress and produces emotional and cognitive changes in the pregnant animal and in her offspring subsequent to adaptive physiological responses and the release of hormones [3]. Exposure to environmental stresses during gestation, such as social, physical, or mental pressures, increases the risks of psychopathology in offspring; these adverse effects of environmental stress are well studied [4, 5]. The detrimental effect of gestational stress on parental care and maternal behavior are also very well investigated [6, 7]. However, a few studies examined the influence of stress during gestation on cognitive performance in mice postpartum [8, 9].

Reproductive experience has long-lasting effects in both rodent and human brains [10]. Rodent research has shown pregnancy, motherhood, and attendant offspring care cause changes in the brain and behavior; and, this has an impact on cognition, affect, and response to stress [11]. Motherhood has also been shown to augment hippocampal-dependent learning and memory [12, 13]. Thus, higher resilience to stress, decreased anxiety, and enhanced memory performance have been shown in primiparous (one pregnancy and bout of rearing) and multiparous (multiple pregnancies and bouts of rearing) rats compared with nulliparous rats without pregnancy experience [14]. The behavioural and cognitive changes last much longer after pregnancy, persisting even into old age [11, 14–16]. Interestingly, in a study of the acquisition of the Morris water task (MWT) by primiparous and nulliparous female rats exposed to gestational restraint stress, both groups of animals were impaired relative to controls; this suggests gestational stress can counteract the advantages of reproduction on postpartum learning and memory performance [14]. Stress-related hormones, in particular glucocorticoids, which adversely impair cognitive functions [5, 14, 17, 18], are presumed to play a significant role in the gestational stress effects. Whereas pregnancy itself highly increases secretion of the corticosterone [17], maternal stress markedly intensifies the corticosterone level leading to an impairment in hippocampal function in mothers and offspring [14, 17]. In spite of the importance of the gestational stress effects on hippocampal function in the mothers, to date there has been little research on it [17].

Noise, which can be defined as an unwanted unpleasant auditory stimulus, is a major environmental pollutant that can produce significant biochemical and neural changes in animals as well as in humans [17]. Noise can lead to negative health effects if the exposure time is prolonged and/or exceeds certain levels. Studies have frequently indicated that high levels of noise are markedly stressful and significantly enhance release of stress hormones, i.e., glucocorticoids, leading to impairments in learning and memory [17, 19, 20]. While evidence of an adverse impact of environmental noise exposure on public health (annoyance, disturbed sleep, increased the occurrence of hypertension and cardiovascular disease, and impaired cognitive performance) is growing [19, 21], noise exposure has been less investigated as a gestational stressor [9]. Thus, among the diverse types of gestational stressors, restraint is the most commonly used and noise is the least used stressor [4]. It is well known that the exposure to various environmental stresses, including noise [17, 22–24], causes morphological and non-morphological changes in the hippocampus and impacts hippocampal neurogenesis; a brain process which has been shown to continue until aging in a myriad of mammalian species [11, 25]. Little evidence, however, is available regarding the effects of gestational noise exposure on hippocampal plasticity and memory function in postpartum rodents.

The present study aimed to investigate the effects of two types of stressors during pregnancy, noise stress and physical stress, on gestational corticosterone levels and postpartum spatial learning and memory function in mice. Given the negative effects of loud noise levels on release of stress hormones and cognitive performance [17, 19], we hypothesized that gestational noise exposure strongly activates the hypothalamic–pituitary–adrenal (HPA) axis and adversely affects spatial learning and memory function in the postpartum dams as large as the

physical stressors [5, 26], i.e., restraint combined with placement on an exposed elevated platform in the present study.

## Material and methods

### Animals

All experiments were carried out in accordance with the Canadian Council of Animal Care and approved by the University of Lethbridge Animal Care Committee. All animals were given access to food and water ad libitum and were maintained on a 12:12-h light:dark cycle in a temperature-controlled breeding room (21°C) with less than 66±2 dB room noise level. Thirty female C57BL/6 mice between 8 to 11 weeks of age were individually mated with thirty male C57BL/6 mice in standard shoe-box cages at 4:00 pm. For recording of gestational length, the female mice were assessed three hours later at 7:00 pm and the next morning for breeding signs such as sperm plug and red/swollen vaginal opening [17]. If a plug or sperm was present, the female was considered possibly pregnant and removed from the male till the pregnancy confirmed, and then housed with 2–3 females by 1–2 days before parturition. Once a female was left with a male overnight, she was not paired with a male again until the lack of pregnancy was confirmed. The weight gain of the female mice was followed every day to confirmed pregnancy. On the gestational day (GD) 11, a weight gain of at least 3.5 g usually signifies conception has occurred. This method allows a determination of the length of gestation with a 0.5-day precision. When the pups were born, the dams were kept individually with the litters.

### Experimental design

Pregnant mice were randomly assigned to three groups consisting of two stress groups and one control group. The time range of the most reported gestational stress exposures is on GDs 14–21 [27–30]. We exposed animals to stress on GDs 12–16 because the corticogenesis process occurs from embryonic day 10 to 17 in mice, and the layer II/III, IV, and V mainly develop during GDs 12–16 [1, 31]. This timeframe also corresponds to the third trimester in human pregnancy when substantial neural development occurs [32].

**Physical stress (PS) group.** Two stressors, restraint and elevated platform (EP), were applied daily from GDs 12 through 16. For restraint, mice ( $n = 10$ ) were maintained in a transparent Plexiglas container (5 cm inner diameter), 20 minutes per day at 10:00 am. The container maintained the mice in a standing position without compression of the body [33, 34]. For the EP stressor, each mouse was placed on an elevated platform (1m height, 21×21 cm), 30 minutes twice a day at 9:00 am and 3:00 pm [35, 36].

**Noise stress (NS) group.** On gestational days (GDs) 12, 14, and 16, a female pregnant mouse was transferred into a standard cage and moved to a sound chamber. A speaker, which emitted an intermittent 3000 Hz frequency sound of 90 dB [37–39] for 1 sec duration and 15 sec inter-stimulus interval (ISI) [40], was placed in the cage. The sound pressure level was measured daily inside the cage without an animal (Tektronix RM3000, Digital Phosphor Oscilloscope). The mice ( $n = 10$ ) were exposed to the NS for 24 hrs starting at 8:00 am. Similar protocol was previously used in rats, including a low-frequency sound of 300 Hz for 1 sec in the intervals of 15 sec during 24 hrs. [40]. Here we used a 3000 Hz frequency tone, since (a) is audible by mice [41, 42] and (b) is relatively similar to environmental and traffic noises which are largely made up of low to mid frequency tones [43, 44]. The intensity of NS exposure used in previous studies was also between 95 to 130 dB [17, 37–39]. We applied an intermittent stimulus intensity (90 dB) to prevent noise-induced hearing loss. In addition, 24 hrs rest after every stress exposure will provide enough time for to recover from possible temporary threshold shifts [45].

**Control group.** There were two sets of control animals: one served as a control for NS dams and another was a control for PS dams. In NS control group, pregnant mice ( $n = 5$ ) on GDs 12, 14, and 16 were individually transferred into a standard cage and moved to a sound chamber. A silent speaker was placed in the cage. The mice were left undisturbed for 24 hrs starting at 8:00 am. In PS control group, pregnant mice ( $n = 5$ ) were removed daily from the home cage for 20 or 30 minutes (depending on the type of stressor) during GDs 12–16, transferred to the same testing room for the PS, left undisturbed, and then returned to their home cages. In the control group, no stress was given.

### Plasma corticosterone assay

The blood sampling procedure was performed at 7:30 to 8:30 am [46] on GDs 11 and 17, i.e., one day before starting stress regimen, and a day after finishing the stress exposure in the stressed groups. The blood samples were also collected from the control groups without stress using the same schedules. A commercially available enzyme-linked immunosorbent assay (ELISA) kit from Abcam (ab108821) was used to quantify the levels of corticosterone in the plasma. Approximately 0.1 ml of submandibular blood [47] was collected in heparin-coated tubes. These tubes were centrifuged at 6000 rpm at 4°C for 15 min [48] to collect the plasma [39]. Collected plasma samples then were stored at  $-80^{\circ}\text{C}$  until further analysis. The assay was carried out as per the manufacturer's instructions. All reagents were brought to room temperature before starting the assay. The assay was performed at room temperature ( $20\text{--}30^{\circ}\text{C}$ ). The plasma samples were diluted 1:100 into 1X diluent as suggested in the ELISA kit. Briefly, 25  $\mu\text{l}$  of standards or samples were added to the microplate wells. Twenty-five  $\mu\text{l}$  of biotinylated corticosterone was then added immediately to each well (on top of the sample or standard). The wells were tightly sealed with Parafilm and incubated for two hours at room temperature. After two hrs of incubation, the microplate was washed six times with 300  $\mu\text{l}$  of wash buffer using plate washer (ELX50 BioTek®). The plate then was inverted and tapped 4–5 times on absorbent paper towel to completely remove the liquid. Fifty  $\mu\text{l}$  of streptavidin-peroxidase conjugate then was added to each well and incubated for 30 minutes. After incubation, the microplate was again washed six times with 300  $\mu\text{l}$  of wash buffer using a plate washer. The plate then was tapped 4–5 times on absorbent paper towel to completely remove the liquid. After the liquid removal 50  $\mu\text{l}$  of chromogen substrate was added per well and incubated for 30 minutes. Finally, 50  $\mu\text{l}$  of stop solution was added to each well, leading to the color changing from blue to yellow. The optical density of corticosterone was read at 450 nm wavelength using a microplate reader (Synergy HT BioTek®) within 15 min after addition of stop solution. The concentration of corticosterone in samples was calculated using KC4 Bio-Tek® Microplate Data Collection and Analysis software. To reduce intra-plate variability, the coefficient of variation (CV) for all samples was determined using the same standards and controls across all plates, and only samples with a CV less than 10 percent were included in the analysis. The corticosterone concentration in plasma samples was expressed in ng/ml [17, 46, 49–52].

### Behavioral assessment

The MWT (acquisition and probe tests) was performed for the dams 30-days after parturition. Weaning occurred on postnatal days 21–23. The water task consisted of a pool (153 cm diameter) filled with water ( $23\text{--}25 \pm 1^{\circ}\text{C}$ ) up to a level of  $\sim 15$  cm from the top edge of the tank. The water was made opaque by non-toxic white tempura paint. The pool was located in a room rich with distal cues, which remained unobstructed throughout the duration of the experiment [53]. During all hidden platform trials, the platform was submerged  $\sim 1.0$  cm under the surface of the water. Each trial began with the mouse being placed in the pool in a pseudo-random

sequence at one of the four cardinal compass positions around the perimeter of the pool. The tank was divided into four quadrants, 1, 2, 3 and 4 using software, with starting points at north, west, east and south. The starting positions of the mice were located at the intersection of the quadrants, 4–5 cm away from the edge of the tank. Animals were trained with 4 trials per day for 8 consecutive days under regular room light (Water2100 Software vs.7, 2008). The acquisition trial was started by placing a mouse facing the wall of the tank at one of the 4 starting locations. Testing was stopped after the mouse reached the platform or, if the mouse did not find the platform, at the 60 second trial time limit. If a mouse found the platform within this 60 sec period, it was allowed to remain on the platform for five additional seconds. If it did not find the platform during the selected time, it was placed onto the platform for 15 sec by the experimenter before being returned to her home cage. Data were recorded using an automated tracking system (HVS Image Hampton, U.K.). Following each swim trial, the animal was dried with a soft fabric cloth and placed back into the home cage where it was allowed to rest for at least 5 min before the start of the next swim trial. The swim time (sec), swim speed (m/s), and swim distance (m) were calculated for analysis [54].

The probe trial was carried out on the ninth day, in which the platform was removed and each mouse was allowed to swim freely for 60 sec. In order to preclude the possible impact of working memory on retention [55] this trial was performed 24 hours after the last platform trial. The time spent in the quadrant where the platform had been located was measured.

## Statistical analysis

All statistical analyses were done using SPSS Statistics 24.0 using an alpha level of 0.05. We used the Kolmogorov–Smirnov test for normally distributed data. A Multivariate analysis of variance (MANOVA) was used to test for differences between the three studied groups: for different parameters of the MWT, the plasma corticosterone level on GD11 and GD17, and the delta corticosterone level (the difference in the corticosterone levels between GD11 and GD17). To make clear the possible effect of swim speed on the swim time we used a multivariate analysis of covariance (MANCOVA) to compare the three groups in swim time where swim speed was considered as a covariate. A repeated measures ANOVA was used to compare the eight days of training in the MWT, as well as the corticosterone levels on GDs 11 and 17 in each group. The Tukey post-hoc test was performed for multiple comparisons of group means in each measurement. The Pearson correlation coefficient test was used to determine the relationship between variables.

## Results

The ages of the female mice did not differ across the groups ( $F = 0.697$ ,  $p = 0.507$ ). Since no significant differences were observed between the two control groups in any of the measures used in this study ( $p > 0.05$ ), the results of the control groups were pooled together.

### Plasma corticosterone levels

**Corticosterone levels comparison between GD11 and GD17.** Blood collection was conducted on GDs 11 and 17 in the three groups. Table 1 compares the two corticosterone measures (ng/ml) across groups. No significant difference was found between the two plasma corticosterone measures in the control group. But in both stressed groups, particularly the NS group (Fig 1A), the corticosterone level was significantly higher on GD17 compared with GD11.

**Corticosterone level comparison among the groups.** As Table 2 shows, no significant difference was observed in the corticosterone levels (ng/ml) among the three groups on GD11.

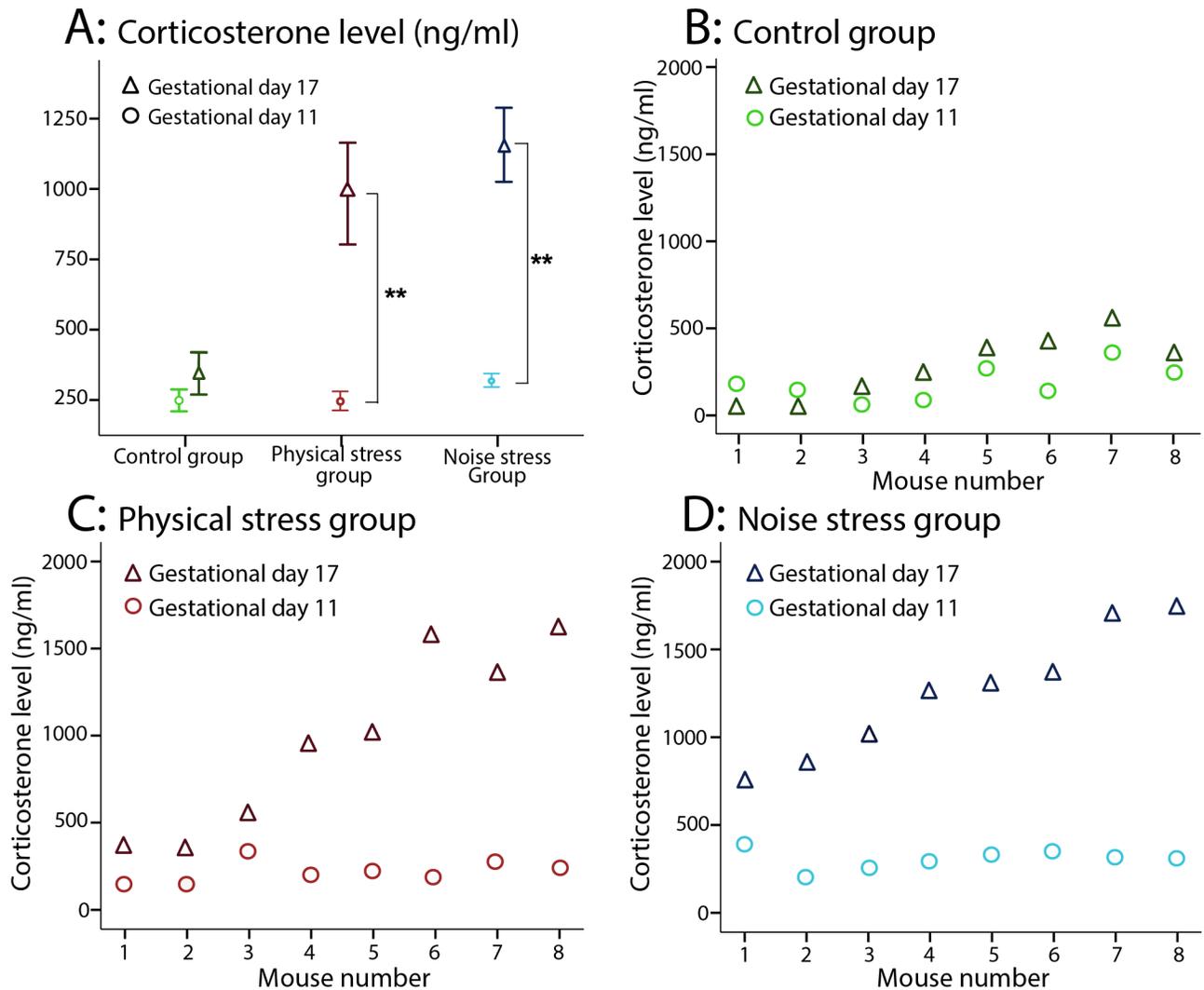
**Table 1. Comparison between corticosterone levels on gestational days 11 and 17 in every group.**

Corticosterone levels (ng/ml)	Mean difference (delta corticosterone)	F	p	$\eta^2$	Power
Control group	95.91	2.129	0.188	0.233	0.244
Physical stress group	736.64	17.933	<b>0.004</b>	0.719	0.950
Noise stress group	836.51	44.876	<b>&lt;0.001</b>	0.865	1.000

$\eta^2$  = estimates of effect size

<https://doi.org/10.1371/journal.pone.0180306.t001>

However, the corticosterone levels of the stressed groups were significantly higher compared with the control group on GD17. Similarly, the delta corticosterone levels were significantly higher in both stressed groups than the control group (Fig 1A, Table 2). Fig 1B, 1C and 1D



**Fig 1.** The corticosterone levels (ng/ml): A) A significant increase in corticosterone levels on gestational day (GD) 17 than GD11 in both stressed groups compared with the control group (○: GD11, △: GD17). Results reported as mean ± S.E.M. B) No significant difference in corticosterone levels between GD11 and GD17 in the control group. C) A significant difference in corticosterone levels between GD11 and GD17 in the PS group. D) A significant difference in corticosterone levels between GD11 and GD17 in the NS group. N = 8 in the three groups. Asterisks indicate \*p<0.05 or \*\*p<0.01.

<https://doi.org/10.1371/journal.pone.0180306.g001>

**Table 2. Comparison among the three groups in corticosterone levels on GDs 11 and 17.**

Corticosterone levels (ng/ml)	* Between groups' p-values			** Significant main effects			
	Control and PS	Control and NS	PS and NS	F	p	$\eta^2$	Power
GD11	0.967	0.414	0.385	1.628	0.220	0.134	0.305
GD17	<b>0.010</b>	<b>0.001</b>	0.378	9.875	<b>0.001</b>	0.485	0.967
Delta corticosterone	<b>0.006</b>	<b>0.002</b>	0.356	9.687	<b>0.001</b>	0.480	0.964

GD: gestational day,  $\eta^2$  = estimates of effect size, NS: noise stress, PS: physical stress.

\*The "between groups' p-values" show p-values for the between group comparisons.

\*\*The "significant main effects" indicate the statistical results of a significant main effect for every measure.

<https://doi.org/10.1371/journal.pone.0180306.t002>

demonstrate the corticosterone levels of each animal on GD11 and GD17 in control (n = 8), PS (n = 8), and NS groups (n = 8), respectively. The delta corticosterone level was noticeably high only in one animal in the control group (Fig 1B), while it was remarkably high in 5 out of 8 animals in the PS group (Fig 1C), and in all 8 animals in the NS group (Fig 1D).

### Morris water task (MWT)

**Swim time.** Table 3 compares the three groups in swim time (sec), swim speed (m/s), and the probe time (sec) in the MWT. Both stressed groups showed a significantly longer time to reach the platform relative to the control group. Fig 2 illustrates the learning process of the three groups during training days (Fig 2A) and in average (Fig 2B) in swim time. Also in the intra-group analysis, a significant difference was observed in swim time across the 8 days of training in every group (control group:  $F_{7,273} = 6.289$ ,  $p = 0.001$ , partial  $\eta^2 = 0.603$ , power = 0.997; PS group:  $F_{7,273} = 4.313$ ,  $p = 0.002$ , partial  $\eta^2 = 0.510$ , power = 0.967; NS group:  $F_{7,273} = 3.874$ ,  $p = 0.005$ , partial  $\eta^2 = 0.520$ , power = 0.937).

**Swim speed.** Both stressed groups showed a significantly lower swim speed (m/s) to reach the platform compared with the control group (Table 3). Fig 2 indicates the swim speed of the three groups during training days (Fig 2C) and in average (Fig 2D). However, no significant difference was found in swim speed across the 8 days of training in the control group ( $F_{7,273} = 1.163$ ,  $p = 0.324$ , partial  $\eta^2 = 0.028$ , power = 0.498). Nonetheless, in both stressed groups (PS group:  $F_{7,273} = 3.646$ ,  $p = 0.001$ , partial  $\eta^2 = 0.082$ , power = 0.974; and NS group:  $F_{7,273} = 4.014$ ,  $p < 0.001$ , partial  $\eta^2 = 0.092$ , power = 0.985), the difference in swim speed across training days was significant.

**Swim distance.** No significant differences were found among the three groups in swim distance (m) ( $F_{2,27} = 0.341$ ,  $p = 0.711$ , partial  $\eta^2 = 0.001$ , power = 0.105).

**Probe test.** The probe time (sec) was lower in the stressed groups relative to the control group (Fig 2E), and the difference between the NS group and the control group was significant

**Table 3. Comparison among the three groups in swim time, swim speed and probe time in the MWT.**

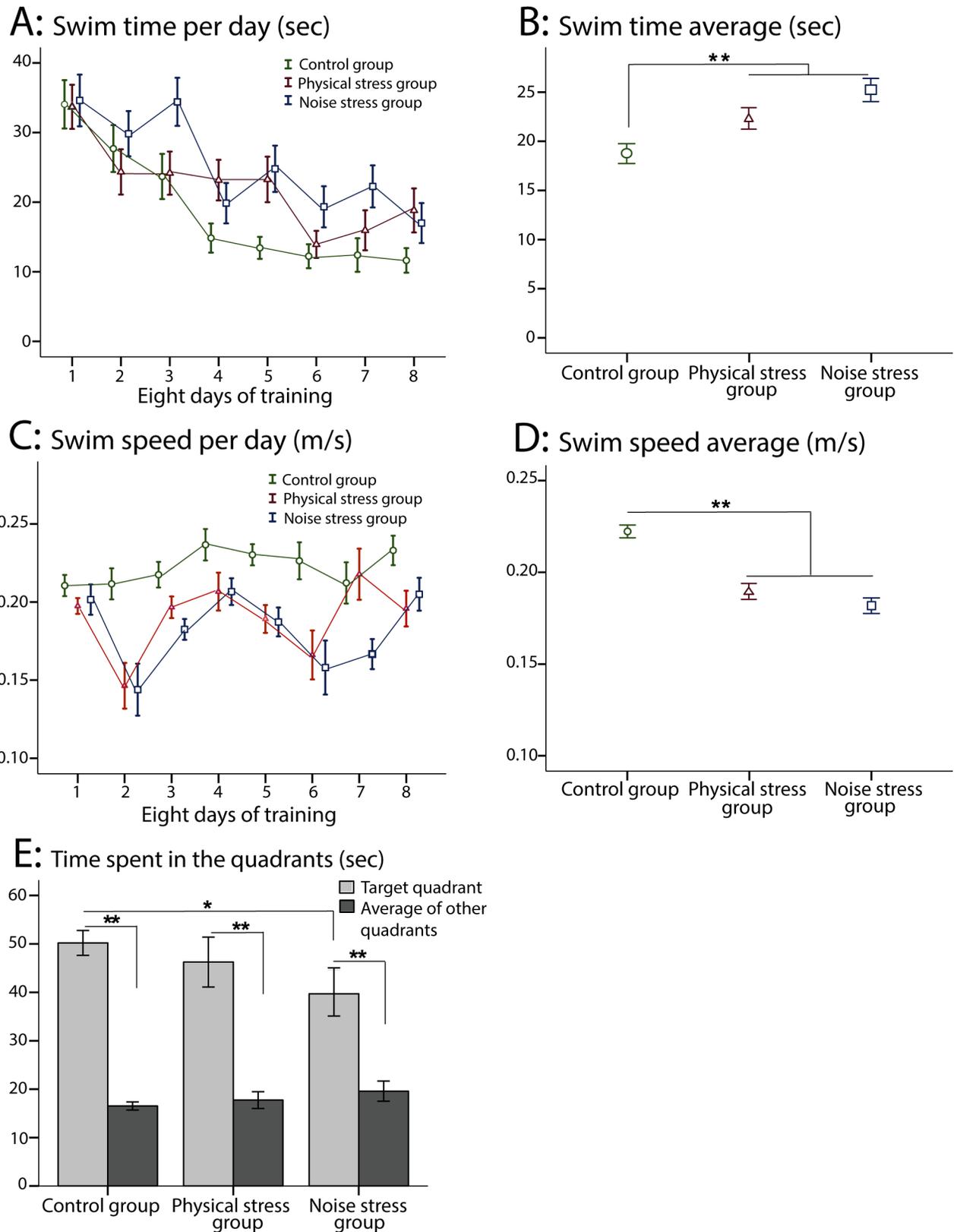
	* Between groups' p-values			** Significant main effects			
	Control and PS	Control and NS	PS and NS	F	p	$\eta^2$	Power
Swim time (sec)	<b>0.021</b>	<b>&lt;0.001</b>	0.071	8.421	<b>&lt;0.001</b>	0.019	0.965
Swim speed (m/s)	<b>0.021</b>	<b>&lt;0.001</b>	0.631	8.421	<b>&lt;0.001</b>	0.019	0.965
Probe time (sec)	0.463	<b>0.017</b>	0.095	3.541	<b>0.048</b>	0.260	0.588

MWT: Morris water task, NS: noise stress, PS: physical stress,  $\eta^2$  = estimates of effect size.

\*The "between groups' p-values" show p-values for the between group comparisons.

\*\*The "significant main effects" indicate the statistical results of a significant main effect for every measure.

<https://doi.org/10.1371/journal.pone.0180306.t003>



**Fig 2.** The Morris water task (MWT): A) The swim time (sec) across the 8 days of training in the three groups. B) The swim time average in the three groups (○: control group, Δ: physical stress (PS) group, □: noise stress (NS) group). A significantly higher swim time was observed in both stressed groups relative to the control group. C) The swim speed (m/s) during the 8 days of training in the three groups.

D) The swim speed average in the three groups. A significantly lower swim speed was shown in both stressed groups compared with the control group. F) A significant difference was observed between the NS stress group and the control group in probe time (sec). The difference between time spent in the target quadrant and mean of time spent in other quadrants was significant in the three groups.  $N = 10$  in the three groups. Results reported as mean  $\pm$  S.E.M. Asterisks indicate \* $p < 0.05$  or \*\* $p < 0.01$ .

<https://doi.org/10.1371/journal.pone.0180306.g002>

(Table 3). The difference between time spent in the target quadrant and average of time spent in other quadrants was significant in the three groups (control group:  $t = 12.484$ ,  $p = 0.001$ ; PS group:  $t = 5.946$ ,  $p = 0.001$ , NS group:  $t = 7.140$ ,  $p = 0.001$ ).

**Correlation between the corticosterone levels and MWT results.** A significant positive relationship was observed between the swim time and the delta corticosterone level in the three groups (control group:  $r = 0.762$ ,  $p = 0.028$ ; PS group:  $r = 0.881$ ,  $p = 0.004$ ; NS group:  $r = 0.905$ ,  $p = 0.002$ ). Furthermore, a significant negative correlation was found between the probe time and the delta corticosterone level in the three groups (control group:  $r = -0.760$ ,  $p = 0.026$ ; PS group:  $r = -0.714$ ,  $p = 0.041$ ; NS group:  $r = -0.738$ ,  $p = 0.037$ ).

## Discussion

The main findings of this study were: 1) gestational stress altered spatial learning in mouse dams that were examined 30 days postpartum; 2) the gestational corticosterone level was correlated with the spatial learning and memory function postpartum in all groups; and, 3) loud noise exposure during gestation negatively affected the HPA axis and impaired postpartum memory function as large as or a bit larger than the exposure to physical stressors.

To examine both acquisition and recall of the water task performance, we assessed learning across the 8 test days and recall on day 9 separately. Although a significant improvement in performance was observed in all three groups across training days of the MWT, the stressed groups, in particular the NS group, had a significantly longer latency to reach the platform relative to the control group. In the probe test, the NS group also spent significantly less time in the target quadrant than the other groups. The results demonstrated an adverse impact of both types of gestational stresses in spatial learning, as well as the negative effect of the noise stress in retention memory in the stressed dams compared with the controls. The findings are congruent with the past studies that reported impairment in spatial learning [56] and retention memory [57], which were correlated with increased gestational plasma corticosterone levels [17, 58], although no previous study explored the spatial learning and memory performance in the postpartum mice mothers who received stress during gestation.

The swim speed of the stressed groups was also significantly slower than the control group. Although the control animals showed a relatively constant speed during the eight days of training, the swim speed in both stressed groups was unstable over the days of training. Previous studies have indicated that regardless of confounding factors such as appetite or differences in body weight, rodents typically swim at similar speeds in the MWT, and changes in swim speed owing to a treatment are typically small. Therefore, a difference in swim speed might not account for a difference in learning the platform location [59]. In addition, in a factor analysis on MWT data in a large number of mice ( $n = 1500$ ), only latency to escape and probe time were highly related to learning, whereas the swim speed was not significantly related [59, 60]. In our study, however slower swim speed, with marked fluctuations over the days of training, might be related to the impact of the stress procedures on postpartum mental state, attentional skills, or mood as indicated in both human and animal studies [61–63]. This needs to be further explored in the future.

Pregnancy and the postpartum period have been shown as the times of maximal plasticity in both the mother's brain and behavior. Both short and long term time- dependent changes

in cognition and hippocampal neurogenesis, morphology, and electrophysiology have been correlated to pregnancy and mothering [10, 64, 65]. Rat studies have suggested that reproductive experience reduces anxiety throughout pregnancy and the postpartum period and generally enhance cognition, in particular spatial learning and memory [11]. Thus, it has been shown that there is better performance of both primiparous and multiparous females relative to nulliparous females on a dry-land maze at 6, 12, 18, and 24 months of age, long past their last reproductive experience [15], and similarly on the MWT at both 2 weeks and 16 months after weaning [14]. In the later study, whereas a single motherhood experience remarkably enhanced spatial memory and hippocampal synaptic plasticity (LTP) during the entire lifespan of female rats, gestational stress (restraint for 45 min three times each day during last week of pregnancy) totally abolished these positive effects. The authors argued stress-induced hormonal modifications, and in particular increased gestational corticosterone levels, which can impair hippocampal function [17, 18, 66] as one of the possible mediators [14]. The 11 $\beta$ -hydroxysteroid dehydrogenase-2 (11 $\beta$ -HSD-2) is an enzyme that is present in the placenta and central nervous system, and converts extra levels of corticosterone to relatively inactive products in normal pregnancies. In mid-gestation, the expression of this enzyme is greatly decreased in rodents [67–69], and it seems that gestational stress can decrease it further, and lead to long-lasting changes in both hippocampal neurogenesis and morphology.

In our study, the corticosterone level was significantly higher on GD17 than GD11 in both stressed groups compared with the control group. The interesting finding was the positive relationship between the delta corticosterone level and the swim time, as well as the negative relationship between the delta corticosterone level and the probe time in the three groups. Previous findings indicate the long-lasting dysfunction of the HPA axis under gestational stress [7, 70], as well as elevated corticosterone level in both dams and offspring [17, 70]. Increased corticosterone levels in our stressed mice are likely due to the effect of stress and also the gradual increase of exogenous corticosterone level during the course of pregnancy. As the relationships between delta corticosterone and swim speed/probe time were significant for both control and stressed groups, we argue that a greater increase in corticosterone levels across pregnancy predicts greater swim time and worse performance for the probe test, even when testing is performed weeks later.

There are some caveats of the study that should be considered. Although we used well-known protocols for exposing animals to NS and PS, these types of experimental stressors are different to some extent than those typical auditory and physical stress observed in natural environments. Thus, experimental conditions that provide more analogous models of stress to natural stressors are recommended for future studies. We also did not measure corticosterone at the time of spatial learning, which in hindsight would have provided some insight into the HPA activity at the time of the learning. In addition, including other female groups comprising a non-pregnant stressed group as well as a non-pregnant non-stress group might provide further information to shed light more on the effect of reproductive experience in the corticosterone alternations as well as the memory performance. Research into the neural mechanisms underlying cognitive changes owing to gestational stress in the rodent postpartum period is only beginning. It is clear that studies on hippocampal morphology and neurogenesis are needed to determine how pregnant mice exposed to gestational stress exhibit reactions to corticosterone fluctuations during pregnancy, early postpartum, lactation, weaning, and months later.

## Conclusion

This study was novel in using “noise stress” with pregnant mice to investigate the effect of stress during pregnancy on spatial learning and memory performance in the postpartum

dams. The results suggested an adverse effect of both gestational stresses, particularly noise exposure, on gestational corticosterone levels as well as postpartum learning and memory function relative to the controls. The strong relation between the MWT results and the delta corticosterone levels demonstrated the lasting effect of stress during gestation on postpartum memory performance. The relatively large effects of loud noise exposure during gestation demonstrate the importance of examining the role of environmental noise on pregnant mothers.

## Acknowledgments

We would like to acknowledge Gerlinde A.S. Metz for using her lab equipment for the corticosterone assay. We also thank Sean Lacoursiere (CCBN) for critical reading of the manuscript. This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant #40352 (MHM), Campus Alberta for Innovation Program Chair, Alberta Alzheimer Research Program (MHM), and a Canadian Institute for Advanced Research grant (BK). ZJ would like to thank the Iran University of Medical Sciences (IUMS) sabbatical leave committee for their approval of her study leave.

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## References

1. Kolb B, Mychasiuk R, Muhammad A, Gibb R. Brain plasticity in the developing brain. *Prog Brain Res*. 2013; 207:35–64. <https://doi.org/10.1016/B978-0-444-63327-9.00005-9> PMID: 24309250.
2. Jafari Z, Kolb BE, Mohajerani MH. Effect of acute stress on auditory processing: a systematic review of human studies. *Rev Neurosci*. 2016. <https://doi.org/10.1515/revneuro-2016-0043> PMID: 27718496.
3. Graignic-Philippe R, Dayan J, Chokron S, Jacquet AY, Tordjman S. Effects of prenatal stress on fetal and child development: a critical literature review. *Neurosci Biobehav Rev*. 2014; 43:137–62. <https://doi.org/10.1016/j.neubiorev.2014.03.022> PMID: 24747487.
4. Weinstock M. Prenatal stressors in rodents: Effects on behavior. *Neurobiol Stress*. 2016;29; 6:3–13. <https://doi.org/10.1016/j.ynstr.2016.08.004> PMID: 28229104.
5. Charil A, Laplante DP, Vaillancourt C, King S. Prenatal stress and brain development. *Brain Res Rev*. 2010; 65(1):56–79. <https://doi.org/10.1016/j.brainresrev.2010.06.002> PMID: 20550950.
6. Del Cerro MC, Perez-Laso C, Ortega E, Martin JL, Gomez F, Perez-Izquierdo MA, et al. Maternal care counteracts behavioral effects of prenatal environmental stress in female rats. *Behav Brain Res*. 2010; 208(2):593–602. <https://doi.org/10.1016/j.bbr.2010.01.003> PMID: 20079763.
7. Del Cerro MC, Ortega E, Gomez F, Segovia S, Perez-Laso C. Environmental prenatal stress eliminates brain and maternal behavioral sex differences and alters hormone levels in female rats. *Horm Behav*. 2015; 73:142–7. <https://doi.org/10.1016/j.yhbeh.2015.07.004> PMID: 26163152.

8. Salari AA, Fatehi-Gharehlar L, Motayagheni N, Homberg JR. Fluoxetine normalizes the effects of prenatal maternal stress on depression- and anxiety-like behaviors in mouse dams and male offspring. *Behav Brain Res.* 2016; 311:354–67. <https://doi.org/10.1016/j.bbr.2016.05.062> PMID: 27263073.
9. Jafari Z, Faraji J, Mirza Agha B, Metz GAS, Kolb BE, Mohajerani M.H. The Adverse Effects of Auditory Stress on Mouse Uterus Receptivity and Behaviour. *Sci Rep.* In Press.
10. Roes M, Galea LAM. Chapter 9—The Maternal Brain: Short- and Long-Term Effects of Reproductive Experience on Hippocampus Structure and Function in Adulthood A2—Shansky, Rebecca M. *Sex Differences in the Central Nervous System.* San Diego: Academic Press; 2016. p. 197–220.
11. Macbeth AH, Luine VN. Changes in anxiety and cognition due to reproductive experience: a review of data from rodent and human mothers. *Neurosci Biobehav Rev.* 2010; 34(3):452–67. <https://doi.org/10.1016/j.neubiorev.2009.08.011> PMID: 19761791.
12. Pawluski JL, Walker SK, Galea LA. Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Horm Behav.* 2006; 49(2):143–9. <https://doi.org/10.1016/j.yhbeh.2005.05.016> PMID: 15992800.
13. Pawluski JL, Vanderbyl BL, Ragan K, Galea LA. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. *Behav Brain Res.* 2006; 175(1):157–65. <https://doi.org/10.1016/j.bbr.2006.08.017> PMID: 17011053.
14. Lemaire V, Billard JM, Dutar P, George O, Piazza PV, Epelbaum J, et al. Motherhood-induced memory improvement persists across lifespan in rats but is abolished by a gestational stress. *Eur J Neurosci.* 2006; 23(12):3368–74. <https://doi.org/10.1111/j.1460-9568.2006.04870.x> PMID: 16820026.
15. Gatewood JD, Morgan MD, Eaton M, McNamara IM, Stevens LF, Macbeth AH, et al. Motherhood mitigates aging-related decrements in learning and memory and positively affects brain aging in the rat. *Brain Res Bull.* 2005; 66(2):91–8. <https://doi.org/10.1016/j.brainresbull.2005.03.016> PMID: 15982524.
16. Love G, Torrey N, McNamara I, Morgan M, Banks M, Hester NW, et al. Maternal experience produces long-lasting behavioral modifications in the rat. *Behav Neurosci.* 2005; 119(4):1084–96. <https://doi.org/10.1037/0735-7044.119.4.1084> PMID: 16187836.
17. Barzegar M, Sajjadi FS, Taleai SA, Hamidi G, Salami M. Prenatal exposure to noise stress: anxiety, impaired spatial memory, and deteriorated hippocampal plasticity in postnatal life. *Hippocampus.* 2015; 25(2):187–96. <https://doi.org/10.1002/hipo.22363> PMID: 25214446.
18. Lupien SJ, Lepage M. Stress, memory, and the hippocampus: can't live with it, can't live without it. *Behav Brain Res.* 2001; 127(1–2):137–58. PMID: 11718889.
19. Basner M, Babisch W, Davis A, Brink M, Clark C, Janssen S, et al. Auditory and non-auditory effects of noise on health. *Lancet.* 2014; 383(9925):1325–32. [https://doi.org/10.1016/S0140-6736\(13\)61613-X](https://doi.org/10.1016/S0140-6736(13)61613-X) PMID: 24183105;
20. Hu L, Yang J, Song T, Hou N, Liu Y, Zhao X, et al. A new stress model, a scream sound, alters learning and monoamine levels in rat brain. *Physiol Behav.* 2014; 123:105–13. <https://doi.org/10.1016/j.physbeh.2013.09.010> PMID: 24096192.
21. Stansfeld SA, Matheson MP. Noise pollution: non-auditory effects on health. *Br Med Bull.* 2003; 68:243–57. PMID: 14757721.
22. Liu L, Shen P, He T, Chang Y, Shi L, Tao S, et al. Noise induced hearing loss impairs spatial learning/memory and hippocampal neurogenesis in mice. *Sci Rep.* 2016; 6:20374. <https://doi.org/10.1038/srep20374> PMID: 26842803;
23. Kraus KS, Mitra S, Jimenez Z, Hinduja S, Ding D, Jiang H, et al. Noise trauma impairs neurogenesis in the rat hippocampus. *Neuroscience.* 2010; 167(4):1216–26. <https://doi.org/10.1016/j.neuroscience.2010.02.071> PMID: 20206235.
24. Gonzalez-Perez O, Chavez-Casillas O, Jauregui-Huerta F, Lopez-Virgen V, Guzman-Muniz J, Moy-Lopez N, et al. Stress by noise produces differential effects on the proliferation rate of radial astrocytes and survival of neuroblasts in the adult subgranular zone. *Neurosci Res.* 2011; 70(3):243–50. <https://doi.org/10.1016/j.neures.2011.03.013> PMID: 21514330.
25. Ortega-Martinez S. Influences of prenatal and postnatal stress on adult hippocampal neurogenesis: the double neurogenic niche hypothesis. *Behav Brain Res.* 2015; 281:309–17. <https://doi.org/10.1016/j.bbr.2014.12.036> PMID: 25546722.
26. Bustamante C, Bilbao P, Contreras W, Martinez M, Mendoza A, Reyes A, et al. Effects of prenatal stress and exercise on dentate granule cells maturation and spatial memory in adolescent mice. *Int J Dev Neurosci.* 2010; 28(7):605–9. <https://doi.org/10.1016/j.ijdevneu.2010.07.229> PMID: 20638465.
27. Belnoue L, Grosjean N, Ladeveze E, Abrous DN, Koehl M. Prenatal stress inhibits hippocampal neurogenesis but spares olfactory bulb neurogenesis. *PloS One.* 2013; 8(8):e72972. <https://doi.org/10.1371/journal.pone.0072972> PMID: 24009723.

28. Zhao D, Liu D, Chen X, Wang K, Zhang A, Kang J, et al. Prenatal stress disturbs hippocampal KIF17 and NR2B in spatial cognition in male offspring. *J Neurosci Res*. 2013; 91(4):535–44. <https://doi.org/10.1002/jnr.23172> PMID: 23239421.
29. Negron-Oyarzo I, Neira D, Espinosa N, Fuentealba P, Aboitiz F. Prenatal Stress Produces Persistence of Remote Memory and Disrupts Functional Connectivity in the Hippocampal-Prefrontal Cortex Axis. *Cereb Cortex*. 2015; 25(9):3132–43. <https://doi.org/10.1093/cercor/bhu108> PMID: 24860018.
30. Kolb B, Mychasiuk R, Muhammad A, Li Y, Frost DO, Gibb R. Experience and the developing prefrontal cortex. *Proc Natl Acad Sci U S A*. 2012; 109 Suppl 2:17186–93. <https://doi.org/10.1073/pnas.1121251109> PMID: 23045653.
31. Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. *Neurotoxicology*. 2007; 28(5):931–7. <https://doi.org/10.1016/j.neuro.2007.01.014> PMID: 17368774.
32. Ward ID, Zucchi FC, Robbins JC, Falkenberg EA, Olson DM, Benzies K, et al. Transgenerational programming of maternal behaviour by prenatal stress. *BMC Pregnancy Childbirth*. 2013; 13 Suppl 1:S9. <https://doi.org/10.1186/1471-2393-13-s1-s9> PMID: 23446000.
33. Erickson ZT, Falkenberg EA, Metz GA. Lifespan psychomotor behaviour profiles of multigenerational prenatal stress and artificial food dye effects in rats. *PloS one*. 2014; 9(6):e92132. <https://doi.org/10.1371/journal.pone.0092132> PMID: 24937660.
34. Muhammad A, Carroll C, Kolb B. Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex. *Neuroscience*. 2012; 216:103–9. <https://doi.org/10.1016/j.neuroscience.2012.04.041> PMID: 22542675.
35. Mychasiuk R, Gibb R, Kolb B. Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. *Synapse*. 2012; 66(4):308–14. <https://doi.org/10.1002/syn.21512> PMID: 22121047.
36. Arck PC, Merali FS, Manuel J, Chaouat G, Clark DA. Stress-triggered abortion: inhibition of protective suppression and promotion of tumor necrosis factor-alpha (TNF-alpha) release as a mechanism triggering resorptions in mice. *Am J Reprod Immunol*. 1995; 33(1):74–80. PMID: 7619237.
37. Haque SF, Izumi S, Aikawa H, Suzuki T, Matsubayashi H, Murano T, et al. Anesthesia and acoustic stress-induced intra-uterine growth retardation in mice. *J Reprod Dev*. 2004; 50(2):185–90. PMID: 15118245.
38. Kondoh E, Okamoto T, Higuchi T, Tatsumi K, Baba T, Murphy SK, et al. Stress affects uterine receptivity through an ovarian-independent pathway. *Hum Reprod*. 2009; 24(4):945–53. <https://doi.org/10.1093/humrep/den461> PMID: 19098291.
39. Mazurek B, Haupt H, Joachim R, Klapp BF, Stover T, Szczepek AJ. Stress induces transient auditory hypersensitivity in rats. *Hear Res*. 2010; 259(1–2):55–63. <https://doi.org/10.1016/j.heares.2009.10.006> PMID: 19840840.
40. Heffner HE, Heffner RS. Hearing ranges of laboratory animals. *J Am Assoc Lab Anim Sci*. 2007; 46(1):20–2. PMID: 17203911.
41. Heffner HE. Hearing in Glires: Domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. *J Acoust Soc Am*. 1980; 68(6):1584–99.
42. Chang TY, Beelen R, Li SF, Chen TI, Lin YJ, Bao BY, et al. Road traffic noise frequency and prevalent hypertension in Taichung, Taiwan: a cross-sectional study. *Environ Health*. 2014; 13(1):37. <https://doi.org/10.1186/1476-069X-13-37> PMID: 24886205.
43. Berglund B, Hassmen P, Job RF. Sources and effects of low-frequency noise. *J Acoust Soc Am*. 1996; 99(5):2985–3002. PMID: 8642114.
44. White DR, Boettcher FA, Miles LR, Gratton MA. Effectiveness of intermittent and continuous acoustic stimulation in preventing noise-induced hearing and hair cell loss. *J Acoust Soc Am*. 1998; 103(3):1566–72. PMID: 9514020.
45. Barriga C, Martin MI, Tabla R, Ortega E, Rodriguez AB. Circadian rhythm of melatonin, corticosterone and phagocytosis: effect of stress. *J Pineal Res*. 2001; 30(3):180–7. PMID: 11316329.
46. Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. *Lab Anim (NY)*. 2005; 34(9):39–43. <https://doi.org/10.1038/labani1005-39> PMID: 16195737.
47. Browne CA, Hanke J, Rose C, Walsh I, Foley T, Clarke G, et al. Effect of acute swim stress on plasma corticosterone and brain monoamine levels in bidirectionally selected DxH recombinant inbred mouse strains differing in fear recall and extinction. *Stress*. 2014; 17(6):471–83. <https://doi.org/10.3109/10253890.2014.954104> PMID: 25117886.

48. Malisch JL, Saltzman W, Gomes FR, Rezende EL, Jeske DR, Garland T Jr. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol Biochem Zool.* 2007; 80(1):146–56. <https://doi.org/10.1086/508828> PMID: 17160887.
49. Malisch JL, Breuner CW, Gomes FR, Chappell MA, Garland T Jr. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *Gen Comp Endocrinol.* 2008; 156(2):210–7. <https://doi.org/10.1016/j.ygcen.2008.01.020> PMID: 18329645.
50. Hare BD, Beierle JA, Toufexis DJ, Hammack SE, Falls WA. Exercise-associated changes in the corticosterone response to acute restraint stress: evidence for increased adrenal sensitivity and reduced corticosterone response duration. *Neuropsychopharmacology.* 2014; 39(5):1262–9. <https://doi.org/10.1038/npp.2013.329> PMID: 24280995.
51. Gong S, Miao YL, Jiao GZ, Sun MJ, Li H, Lin J, et al. Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. *PLoS One.* 2015; 10(2):e0117503. <https://doi.org/10.1371/journal.pone.0117503> PMID: 25699675.
52. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc.* 2006; 1(2):848–58. <https://doi.org/10.1038/nprot.2006.116> PMID: 17406317.
53. Akatsu S, Ishikawa C, Takemura K, Ohtani A, Shiga T. Effects of prenatal stress and neonatal handling on anxiety, spatial learning and serotonergic system of male offspring mice. *Neurosci Res.* 2015; 101:15–23. <https://doi.org/10.1016/j.neures.2015.07.002> PMID: 26163770.
54. Baldi E, Efofudebe M, Lorenzini CA, Bucherelli C. Spatial navigation in the Morris water maze: working and long lasting reference memories. *Neurosci Lett.* 2005; 378(3):176–80. <https://doi.org/10.1016/j.neulet.2004.12.029> PMID: 15781154.
55. Holscher C. Stress impairs performance in spatial water maze learning tasks. *Behavioural brain research.* 1999; 100(1–2):225–35. PMID: 10212070.
56. Benoit JD, Rakic P, Frick KM. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behav Brain Res.* 2015; 281:1–8. <https://doi.org/10.1016/j.bbr.2014.12.001> PMID: 25496779.
57. de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature.* 1998; 394(6695):787–90. <https://doi.org/10.1038/29542> PMID: 9723618.
58. Vorhees CV, Williams MT. Value of water mazes for assessing spatial and egocentric learning and memory in rodent basic research and regulatory studies. *Neurotoxicol Teratol.* 2014; 45:75–90. <https://doi.org/10.1016/j.ntt.2014.07.003> PMID: 25116937.
59. Lipp HP, Wolfer DP. Genetically modified mice and cognition. *Curr Opin Neurobiol.* 1998; 8(2):272–80. PMID: 9635213.
60. Reid KM, Taylor MG. Social support, stress, and maternal postpartum depression: A comparison of supportive relationships. *Soc Sci Res.* 2015; 54:246–62. <https://doi.org/10.1016/j.ssresearch.2015.08.009> PMID: 26463547.
61. Plamondon A, Akbari E, Atkinson L, Steiner M, Meaney MJ, Fleming AS. Spatial working memory and attention skills are predicted by maternal stress during pregnancy. *Early Hum Dev.* 2015; 91(1):23–9. <https://doi.org/10.1016/j.earlhumdev.2014.11.004> PMID: 25460253.
62. Pawluski JL, Csaszar E, Savage E, Martinez-Claros M, Steinbusch HW, van den Hove D. Effects of stress early in gestation on hippocampal neurogenesis and glucocorticoid receptor density in pregnant rats. *Neuroscience.* 2015; 290:379–88. <https://doi.org/10.1016/j.neuroscience.2015.01.048> PMID: 25655215.
63. Tomizawa K, Iga N, Lu YF, Moriwaki A, Matsushita M, Li ST, et al. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nat Neurosci.* 2003; 6(4):384–90. <https://doi.org/10.1038/nn1023> PMID: 12598900.
64. Kinsley CH, Lambert KG. The maternal brain. *Sci Am.* 2006; 294(1):72–9. PMID: 16468436.
65. Sapolsky RM. The physiological relevance of glucocorticoid endangerment of the hippocampus. *Ann N Y Acad Sci.* 1994; 746:294–304; discussion -7. PMID: 7825884.
66. Burton PJ, Smith RE, Krozowski ZS, Waddell BJ. Zonal distribution of 11 beta-hydroxysteroid dehydrogenase types 1 and 2 messenger ribonucleic acid expression in the rat placenta and decidua during late pregnancy. *Biol Reprod.* 1996; 55(5):1023–8. PMID: 8902213.
67. Diaz R, Brown RW, Seckl JR. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11beta-hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. *J Neurosci.* 1998; 18(7):2570–80. PMID: 9502816.
68. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci.* 2009; 3:19. <https://doi.org/10.3389/neuro.08.019.2009> PMID: 19826624,

69. Pedersen CA, Caldwell JD, McGuire M, Evans DL. Corticotropin-releasing hormone inhibits maternal behavior and induces pup-killing. *Life Sci.* 1991; 48(16):1537–46. PMID: [2016987](#).
70. Ohkawa T, Rohde W, Takeshita S, Dörner G, Arai K, Okinaga S. Effect of an acute maternal stress on the fetal hypothalamo-pituitary-adrenal system in late gestational life of the rat. *Exp Clin Endocrinol.* 1991; 98(2):123–9. <https://doi.org/10.1055/s-0029-1211108> PMID: [1663870](#).