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Does lateral habenula mediate effects of gestational stress on rat maternal behavior?

Bo Wang[a], **Ming Li**[b,*,1]

[a]College of Biological and Environmental Engineering/Key Laboratory of Natural Product Development and Anticancer Innovative Drug Research in Qinling, Xi'an University, Xi'an 710065, China

[b]Department of Psychology, Nanjing University, Nanjing 210023, China

Abstract

This exploratory study investigated the neural substrate underlying the effect of gestational stress on rat maternal behavior. We tested the hypothesis that the lateral habenula (LHb)-centered neural circuitry (e.g., raphe, ventral tegmental area, nucleus accumbens, etc.) mediates the maternal disruptive effect of gestational stress. Pregnant Sprague-Dawley rats were subjected to daily 30-min restraining stress from approximately gestation day (GD) 5 to 21, white noise from GD 5 to 12 and mild foot shock from GD 13 to 21. Maternal behavior in the home cage and pup retrieval on an elevated plus maze (EPM) were observed during the first postpartum week. The gestational stress reduced body weight gain of stressed females, and reduced time that they spent outside of the nest, a sign of increased maternal anxiety and hypervigilant parenting style. On the open arms of EPM, the stressed dams showed higher frequently sniffing pups than non-stressed ones. Testing with pups (pup exposure) on the EPM decreased c-Fos expression in the LHb in the non-stressed control dams, but it increased c-Fos expression in the dorsal and medial raphe regions of the control dams. Gestational stress reduced this pup effect in all three regions, implying that gestational stress attenuated the ability of pup exposure to activate the maternally relevant brain regions. Our findings indicate that gestational stress may act upon the LHb (as a putative center that mediates negative emotion) and its downstream projection sites (i.e., dorsal and median raphe) to compromise the quality of maternal care.

Keywords

Maternal behavior; Lateral habenula; C-fos immunohistology; Gestational stress; Elevated plus maze

*Correspondence to: Department of Psychology, School of Social and Behavioral Sciences, Nanjing University, Xianlin Avenue No. 163, Nanjing 210023, China. mingli@nju.edu.cn (M. Li).

[1]Previous affiliation: Department of Psychology, University of Nebraska-Lincoln, USA

CRediT authorship contribution statement

Ming Li: Writing – original draft, Writing – review & editing, Validation, Supervision, Software, Resources, Conceptualization. **Bo Wang:** Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

None.

1. Introduction

Stress during pregnancy is a major contributing factor to postpartum mental disorders such as anxiety, depression and psychosis. Peripartum stress can have long-term detrimental impacts on both mothers and the young. Depressed mothers are shown to exhibit more hostile, negative, and disengaged parenting (England and Sim, 2009; Paris et al., 2009), which in turn can adversely affect the physical, psychosocial, and neurobiological development of their children. Despite some recent efforts to identify the brain changes associated with peripartum stress (Leuner et al., 2014; Haim et al., 2016; Pawluski et al., 2016), little is known about the precise neural circuitry by which peripartum stress exerts its negative influence on maternal behavior. Such a neurocircuit is expected to interface the *stress* responsive network with the *maternal* behavior network. Based on extensive literature review, we hypothesize that the lateral habenula (LHb) might be one crucial hub in such a network that carries the stress signal to the maternal neurocircuitry (Li, 2022). The present study was designed to validate this hypothesis.

The LHb is an epithalamic nucleus connecting the forebrain (e.g., prefrontal cortex, nucleus accumbens) and monoaminergic systems in the midbrain and hindbrain [e.g., ventral tegmental area (VTA). sub-stantia nigra (SN), and raphe nuclei] (Aizawa et al., 2011; Zahm and Root, 2017). It plays a key role in encoding stress-induced effects, and aberrant hyperactivity in the LHb has been implicated in the pathophysiology of major depression and stress-related anxiety (Matsumoto and Hikosaka, 2007; 2009; Proulx et al., 2014). Interestingly, the LHb is also involved in the maternal neural circuit and plays a critical role in stimulating the nonhormonal onset of maternal behavior in rats (Yetnikoff et al., 2015). The LHb receives multiple projections from the brain regions critically involved in the mediation of maternal behavior, including the lateral preoptic area (LPOA), VTA, raphe, and peri-aqueductal gray (PAG) and indirect projections from the medial preoptic area (MPOA) and ventral bed nucleus of the stria terminalis (vBST) neurons via LPOA synapses (Numan and Insel, 2003; Zahm and Root, 2017). Also, excitotoxic lesions of the LHb produce deficits in all components of maternal behavior (pup retrieval, nest building, nursing behavior) (Corodimas et al., 1992, 1993; Matthews-Felton et al., 1995). In this study, we evaluated how gestational stress affects the LHb using the c-Fos immunohistochemistry technique. Specifically, we examined how gestational stress and pup exposure independently and jointly altered c-Fos expression (as a biomarker of neuronal activation) in the LHb-related neurocircuit in an attempt to determine whether the LHb is involved in the mediation both the stress effect and maternal response to pups.

2. Materials and methods

2.1. Animals

Virgin female Sprague-Dawley rats purchased from Charles River Inc. were used. They were housed individually in 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages under 12-h light/dark conditions and had access to standard laboratory rat chow and tap water *ad libitum*. At least one week after arrival, they were housed together with stud males for mating for one week. The first day of co-housing was designated gestation day 0 (GD 0). Adult female rats were housed individually one week later. The colony was maintained

with a controlled temperature (21 ± 1 °C) and a relative humidity of 45–60 %. All animal manipulations were reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee and were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental procedure

Pregnant females were randomly assigned to either the stressed group (n = 19) or non-stressed group (n = 19). We used a modified gestational stress paradigm in which stressed females were **restrained** twice daily for 30 min (each separated by 4 h) from approximately GD 5 to 21 in Plexiglas restrainers (19 cm wide × 9.8 cm deep × 14.6 high) that limited their movement. From GD 5 to 12, during the restraining period, stressed rats were additionally exposed to a randomly presented **white noise** (> 100 dB, 100 ms, with an averaged 146 s interval) for 12 times. From GD 13 to 21, they also received a **mild foot-shock** (0.6 mA, 0.5 s) for 7 times and the interval between each shock (averaged 2 min) was set randomly (80–160 s), in addition to the white noises during the stress period to increase the stress effect. Light and background noise (63 dB) was present during the entire testing session. Non-stressed females were handled 5 min daily during this period.

To assess the gestational stress effect on the mothers, mothers were weighed on GD 0, GD 7 and GD 21, as well as on postpartum day 1 (PPD 1) and PPD 7 after the pups were born. To assess the effect of gestational stress on the growth of litters, the entire litter was weighed, counted, and sexed on PPD 1 before culling to 4 males and 4 females. Litters were also weighed on PPDs 3, 5 and 7 until the time of sacrifice. We calculated the percentage weight gain of mothers as the absolute percentage difference of weight on any measured day minus weight on GD 0, divided by the weight on GD 0. Because each female became pregnant on different days (4–7) after co-housing, the actual stress period varied. On average, each rat received about 16.5 days of stress.

2.3. Maternal behavior test in the home cage

To assess the effect of gestational stress on maternal behavior after parturition, on PPD 2, 4 and 6, maternal behavior was tested in the home cage, as described in our previous studies (Zhao and Li, 2009). Maternal behavior test consisted of a 10-min of “undisturbed” maternal observation, followed by a 10-min pup retrieval test. In the “undisturbed” phase, we quantified the duration of *crouching/nursing behavior* (a rat positioning herself over the pups with legs splayed to accommodate the pups, including high and low crouching over postures), *pup licking/grooming* (a female opening her mouth, placing her tongue on the body of a pup and licking the body and anogenital area), *nest building* (a rat picking up nesting material in her mouth and transporting it back to the nest site or pushing the material with her forepaws toward the nest site), and *time spent off the nest*. In the 10-min pup retrieval test, 8 pups were first removed from the dam and the nest was destroyed. Ten seconds later, the pups were placed back in the cage at the corner diagonal to the original nest site or dam sleeping corner. Six hundred seconds (600 s) was assigned to non-responders who did not approach or retrieve the testing pups. The raters were blind to each dam’s treatment condition. Each test was recorded by video cameras and analyzed manually using a computer with an event-recording program (Boris, http://www.boris.unito.it).

2.4. Pup retrieval test on the elevated plus maze (EPM)

The gestational stress on maternal anxiety (as measured by the time spent on the open arms) and maternal motivation (as measured by pup retrieval latency and number of pups retrieved on the EPM) were assessed using the elevated plus maze (EPM) paradigm, as described in our previous study (Yang et al., 2015). On PPD 7, all postpartum rats were first brought to the experimental room and habituated to the test environment for at least 30 min. Then 12 subject rats from each group were tested for pup retrieval on the EPM with the remaining 7 rats tested on the EPM without pups, so that a potential stress and pup exposure interactive effect on the neuronal activation in various brain regions can be assessed. Thus, 4 groups were formed based on their stress history and whether they were exposed to pups or not on the EPM: stressed with pups (n = 12), stressed without pups (n = 7), non-stressed control with pups (n = 12), and non-stressed control without pups (n = 7). Mother rats tested without pups were placed in the center of the maze facing an open arm and allowed to freely explore the maze of 10 min. For those tested with pups, two pups (both sexes) were placed at the end of each open arm (a total of 4 pups used). Then subject mothers were placed in the central square facing a closed arm and allowed to freely explore the maze for 10 min. All experimental sessions were recorded by a digital video camera and analyzed using TopScan software (CleverSys, Inc., Reston, VA). Time in open arm and close arm was obtained using Viewer II (Biobserve), and percentage (% open arm time) in open arms was calculated as: 100 × time spent in the open arms/total time. An entry to an arm was defined as the all four feet entering the arm and % open arm entry was calculated as: 100 × number of entries to the open arms/-number of entries to all arms. The frequency of sniffing pups at the end of each open arm before retrieving them, number of pups picked up and carried back from an open arm to a closed arm, and pup retrieval latency were analyzed.

2.5. C-Fos immunohistochemistry

To examine how the EPM pup retrieval testing differentially influence the brain regions in stressed and control dams, rats were anesthetized with a lethal dose of sodium pentobarbital (100.0 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) immediately after EPM tests, then were overdosed and perfused as described in our previous work and their brains were extracted for c-Fos immunoreactivity staining (Zhao and Li, 2010, 2012). Free floating sections were repeatedly washed in cold wash buffer and preincubated with 0.3 % H_2O_2 (Sigma-Aldrich, USA) for 30 min at room temperature (RT). The sections were then rinsed 3 × 10 min in wash buffer and incubated with a rabbit polyclonal anti-c-Fos antibody (1:2000, Cat. No.sc-52, Santa Cruz, USA) for 48 h at 4 °C. After several rinsing in wash buffer, sections were then incubated with a biotinylated goat anti-rabbit secondary antibody (1:200 dilution, Vector Laboratories, Burlingame, CA, USA) in PBS containing 1 % normal goat serum for 2 h at RT. Next PBS rinsing was followed by incubation with avidin-biotin horseradish peroxidase complex (1:200 dilution, Vectastain Elite ABC Kit, Vector Laboratories) for 1 h on ice. After several washings in Tris buffer, the immunoreaction was visualized with peroxidase substrate (DAB Substrate Kit for Peroxidase, Vector Laboratories) for 1–5 min at RT. After staining, sections were mounted on gelatin-coated slides, air-dried, dehydrated and coverslipped. As a control, the primary antibody was substituted with normal goat serum. No corresponding nucleus or cytoplasm was immunostained in the control.

Microscopic images were captured with a digital camera (INFINITY lite, Canada) furnished with an Olympus CX41RF microscope (Japan) using × 10 objective lens. The number of positive cells characterized by clearly labeled nuclei was counted unilaterally in six serial sections with comparable anatomical levels across the treatment groups. We focused on the medial prefrontal cortex (mPFC), nucleus accumbens shell (NAs), medial preoptic area (MPOA), lateral habenula (LHb), medial nucleus of amygdala (MeA), ventral tegmental area (VTA), dorsal raphe (DR) and median raphe (MnR) because they are part of the maternal brain networks and have anatomical connections with the LHb (Li, 2022; Numan, 2020). With the help of ImageJ soft-ware, cell counts were made within a 500 × 500 μm^2 unit area of each region of interest by an experimenter blind to the treatment condition.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 20 software (SPSS Inc., Chicago, IL, USA). The percentage of weight gain of mothers and maternal behavior in the home cage were analyzed via repeated measures (using treatment and day as factors). The percentage of weight gain of pups was analyzed using repeated measures (using treatment and sex as factors). The parameters on EPM analyzed using the independentsample t test. The numbers of c-Fos were analyzed via two-way ANOVA, using a 2 × 2 design: (stressed versus non-stressed) × (pup: 4 pups versus no pups) followed by Post-hoc Bonferroni's correlation tests for multiple comparisons when necessary. Once the overall significant effects were determined, two-group comparisons were performed using Mann–Whitney U test. All data are presented as mean ± SEM. Differences were considered statistically significant if $P < 0.05$.

3. Results

As expected, stressed mothers showed a significantly reduced body weight gain from GD 14 to PPD 7 compared to females who were non-stressed ($F_{1,36} = 8.956$, $P = 0.005$). The weight gain of stressed mothers was significantly lower after one week's gestational stress (GD14: stressed mothers: $\overline{X} = 22.4$ % vs. non-stressed mothers: $\overline{X} = 26.74$ %, $P = 0.018$; GD 21: stressed mothers: $\overline{X} = 45.81$ % vs. non-stressed mothers: $\overline{X} = 54.20$ %, $P = 0.002$; PPD 1: stressed mothers: $\overline{X} = 4.87$ % vs. non-stressed mothers: $\overline{X} = 11.41$ %, $P = 0.002$; PPD 7: stressed mothers: $\overline{X} = 16.89$ % vs. non-stressed mothers: $\overline{X} = 21.38$ %, $P = 0.012$) (Fig. 1A). In addition, stressed mothers spent less time off the nest than non-stressed mothers ($F_{1,35} = 7.46$, $P = 0.01$), especially on PPD 2 (stressed mothers: $\overline{X} = 130.39$ vs. non-stressed mothers: $\overline{X} = 179.00$, $P = 0.045$) and PPD 6 (stressed mothers: $\overline{X} = 135.48$ vs. non-stressed mothers: $\overline{X} = 234.90$, $P = 0.017$) (Fig. 1B). The gestational stress did not affect the pup retrieval latency or other maternal responses (pup licking, crouching and nest building) during the 10-min of "undisturbed" maternal observation in the home cage (data not shown).

For the pup retrieval on the EPM, there was no significant difference between stressed mothers and non-stressed mothers on the number of pups retrieved, pup retrieval latencies and the percent time spent on the open arms, but stressed mothers were more likely to sniff pups at the end of each open arm than non-stressed mothers before retrieving them (stressed mothers: $\overline{X} = 6.27$ vs. non-stressed mothers: $\overline{X} = 3.75$, $t = 2.27$, $P < 0.05$) (Fig. 1C). Taken

together, behavioral results from this study show that the gestational stress was effective in reducing body weight gain and changed some aspects of maternal performance. It made dams less likely to leave the nest in the home cage and increased the frequency of sniffing pups on the EPM.

Both the gestational stress and testing with pups on the EPM influenced the number of Fos-like immunoreactivity (Fos-LIR) in various brain regions. Two-way ANOVA revealed a main effect of stress on Fos-LIR in the NAs ($F_{1,30}$ = 5.858, P = 0.022) and MPOA ($F_{1,30}$ = 15.532, P < 0.001), as stressed mothers had significantly fewer Fos-LIR in the NAs and MPOA compared to non-stressed mothers (stressed mothers: $\overline{X}$= 13.33 vs. non-stressed mothers: $\overline{X}$= 21.80) and MPOA (stressed mothers: $\overline{X}$= 54.22 vs. non-stressed mothers: $\overline{X}$= 69.27, Fig. 1D). There was also a main effect of testing with pups on the number of Fos-LIR in the NAs ($F_{1,30}$ = 7.496, P= 0.010), MPOA ($F_{1,30}$ = 18.743, P < 0.001), MeA ($F_{1,30}$ = 11.636, P = 0.002) and VTA ($F_{1,30}$ = 51.587, P < 0.001), as mother rats tested with pups had significantly more Fos-LIR in the MPOA (pups: $\overline{X}$= 61.75 vs. no pups: $\overline{X}$= 42.13), MeA (pups: $\overline{X}$= 32.04 vs. no pups: $\overline{X}$= 19.37) and VTA (pups: $\overline{X}$= 36.37 vs. no pups: $\overline{X}$= 7.95), but fewer Fos-LIR in the NAs (pups: $\overline{X}$= 17.61 vs. no pups: $\overline{X}$=25.39) compared to those tested without pups (Fig. 1E). Most importantly, there was a significant stress × pup testing interaction on the number of Fos-LIR in the LHb ($F_{1,30}$ = 7.358, P = 0.011), DR ($F_{1,30}$ = 4.954, P= 0.034) and MnR ($F_{1,30}$ = 7.828, P = 0.009) (Fig. 1F). In the LHb, non-stressed dams tested with pups had fewer Fos-LIR ($\overline{X}$= 17.38) than those tested without pups ($\overline{X}$= 34.80, $F_{1,30}$ = 11.921, P = 0.002), and this pup effect was abolished by the gestational stress, as there was no difference between stressed dams tested with pups and those tested without pups, suggesting that the LHb is sensitive to both stress and pup stimulation. In the DR and MnR, testing with pups increased Fos-LIR, but gestational stress significantly reduced this effect (DR: pups: $\overline{X}$= 36.47 vs. no pups: $\overline{X}$=15.49, $F_{1,30}$ = 29.79, P < 0.001; MnR: pups: $\overline{X}$= 24.84 vs. no pups: $\overline{X}$= 10.29, $F_{1,30}$ = 28.70, P < 0.001). Therefore, testing with pups altered neuronal activity in a region-specific and stress-specific way. It increased neuronal activity in the DR and MnR in the non-stressed dams, but decreased it in the LHb. Gestational stress reduced this effect of pups in all three regions. No significant stress and pup testing and their interaction was found regarding Fos-LIR in the mPFC. The representative Fos staining images were shown in Fig. 2.

4. Discussion

Our behavioral results showed that this gestational stress protocol had a strong effect on the body weight gain of the female rats from the late pregnant period to early postpartum period. This effect over lasted the entire stress period, and was still present even one week after the stress treatment (Fig. 1A). However, this stress only mildly disrupted maternal behavior. It did not impair pup retrieval, pup licking, nest building and pup crouching/nursing, four major components of maternal behavior in the home cage. It only decreased time outside of nest and pup sniffing frequency. The original protocol subjected pregnant females to restraining stress twice daily for 30 min (10:00 and 16:00) from gestation days (GD) 7–20. Stressed dams were reported to show a robust depressive-like behavior, impaired attention set shifting, and impaired maternal care, as they spend less time nursing, gathering and

grouping their litters under them (Smith et al., 2004; Leuner et al., 2014). They also showed a reduction in dendritic spine density in the nucleus accumbens and mPFC (Leuner et al., 2014; Haim et al., 2016). In this study, we added two more stressors (12 presentations of 100–120 dB, 40 ms white noise, and 7 presentations of 0.6 mA, 0.5 s footshock distributed periodically throughout the 30-min restraint period) with the intention to enhance the stress effect. Opposite to our expectation, the modified stress protocol did not produce more severe maternal impairment than the original one did. It was possible that more stressful events actually de-sensitized stress reactions of the pregnant females, so they may have developed an even better "buffer" system to deal with stress.

In this study, we observed that during the home-cage pup retrieval test, stressed mothers spent less time off the nest (more time inside the nest) than non-stressed mothers. At the beginning of the test, pups were removed from the dams, thus their continuous maternal care was disrupted by the experimenter. For the non-stressed dams, they quickly retrieved the pups back to their nest site. After exploring the cage few times, they re-settled down and engaged various maternal activities, mainly licking the pups, re-constructing the nest and crouching over the pups. The finding that stressed dams spent less time off the nest indicates that gestational stress may have disrupted the behavioral organization of maternal activities, or it may have increased the anxiety level of the dams, as both interpretations have been offered before (Gallo et al., 2019). Interestingly, the maternal behavioral pattern of stressed dams resembles what has been observed in dams subjected to a limited bedding and nesting stress (LBN) (Gallo et al., 2019). LBN dams also spend less time than controls off their nest, returned to their nest more frequently than control dams, but their overall maternal behavior was intact. The authors suggest that LBN "drives a form of stressed hypervigilant parenting associated with resources restriction", a type of parenting often associated with elevated anxiety of stressed dams. Our finding that stressed dams increased their pup sniffing frequency on the EPM might also reflect this stress-induced hypervigilant state, a speculation worth exploring in future studies.

As mentioned above, the primary goal of this study was to determine whether the LHb and related neurocircuit are involved in the mediation of stress-induced maternal disruption. This was achieved by examining how the gestational stress influenced the number of Fos-LIR in various brain regions when the dams were engaged in pup retrieval on the EPM. The idea was that if the LHb and related brain regions were involved in the mediation of stress-induced maternal disruption, Fos-LIR in the LHb should show a significant change and this change should be sensitive to the impact of both the gestational stress and testing with pups on the EPM. Our results regarding the change in the LHb was consistent with this idea. We found a significant stress × testing with pups interaction on the number of Fos-LIR in the LHb: testing with pups on the EPM decreased neuronal activity in the LHb, while gestational stress reduced this effect. We interprete this finding to indicate that pup stimulation, as a positive reward, may have an inhibitory effect on the aversively sensitive LHb neurons. Given the LHb's role in stress-induced anhedonia and amotivation (Nair et al., 2013; Yang et al., 2018), and its function in processing of negative emotional stimuli (Wirtshafter et al., 1994), it makeed sense for pup stimulation to inhibit the LHb neuronal activity (aversive signal) in order to activate maternal caregiving behaviors. Previous studies on the pup-induced Fos-LIR in the LHb are mixed. Some reported a pup presentation-

induced Fos-LIR increase in the LHb (Lonstein et al., 1998; Kalinichev et al., 2000), others have consistently reported negative results (Sheehan et al., 2000; Stack et al., 2000, 2002). This mixed result may be due to different levels of stress in the mother rats in different studies. It is possible that dams who exhibited an increase in Fos-LIR in the LHb had a relatively higher stress level than those who did not.

Interestingly, we also found that the neuronal activity in the DR and MnR were jointly influenced by gestational stress and pup presence on the EPM. In contrast to the effect on the LHb, testing with pups increased neuronal activity in the DR and MnR in the non-stressed control dams, and this pup effect was reduced by gestational stress. Both the DR and MnR have been implicated in rat maternal behavior, as lesions of the DR and/or MnR disrupt maternal behavior (Barofsky et al., 1983; Yurino et al., 2001; Holschbach et al., 2018). Because the LHb sends gluta-matergic input to the DR and MnR (Aizawa et al., 2011; Zahm and Root, 2017), and circuitry-level analysis suggests that the LHb mediates the aversive effects of stress by suppressing dopamine (DA) neurons in the VTA and 5-HT neurons in the raphe (Brown et al., 2016; Brown and Shepard, 2016; Metzger et al., 2017; Yang et al., 2018), it is possible that alterations of Fos-LIR in the DR and MnR are the consequence of pup- and stress-induced functional changes in the LHb.

For the main gestational stress effect, we found that stressed mothers had significantly fewer Fos-LIR in the NAs and MPOA compared to non-stressed mothers (Fig. 1C). The NAs and MPOA are important parts of the excitatory neural network that mediates maternal behavior (Stack et al., 2002; Li and Fleming, 2003; Numan et al., 2005), and reduction in the neuronal activity in these regions may be the reason why the stressed dams showed mildly impaired maternal care. For the main effect of testing with pups, we observed that mother rats tested with pups had significantly more Fos-LIR in the MPOA, MeA and VTA, but fewer Fos-LIR in the NAs compared to those tested without pups (Fig. 1D). The finding on the MPOA, MeA and VTA was consistent with many studies showing that pup cues increase Fos-LIR in these regions (Fleming and Walsh, 1994; Numan and Numan, 1995, 1997; Stack et al., 2000, 2002; Zhao and Li, 2012). The reduced Fos-LIR in the NAs is unexpected, as many of these studies find an opposite result (i.e., increased Fos-LIR) when the dams are tested with pups in the home cages. Different maternal testing conditions may be one of the reasons for the different findings. Overall, for the first time, this study demonstrated that the LHb and its projection sites (DR and MnR) is one important neural substrate that mediates the impact of gestational stress on maternal behavior. Future work could examine the functional interactions among the LHb, VTA, and raphe nuclei to see whether LHb mediates the stress effects on maternal care by dually modulating the VTA dopaminergic and the MR serotonergic system. It is also important to further determine the behavioral mechanisms by which the LHb disrupts maternal behavior. Identifying the behavioral and neural processes involved in gestational stress-altered maternal behavior may contribute to the foundational knowledge needed to develop novel strategies for postpartum depression and anxiety treatment.

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References

Aizawa H, Amo R, Okamoto H, 2011. Phylogeny and ontogeny of the habenular structure. Front. Neurosci 5, 138. [PubMed: 22203792]

Barofsky AL, Taylor J, Tizabi Y, Kumar R, Jones-Quartey K, 1983. Specific neurotoxin lesions of median raphe serotonergic neurons disrupt maternal behavior in the lactating rat. Endocrinology 113, 1884–1893. [PubMed: 6685026]

Brown PL, Shepard PD, 2016. Functional evidence for a direct excitatory projection from the lateral habenula to the ventral tegmental area in the rat. J. Neurophysiol 116, 1161–1174. [PubMed: 27358317]

Brown PL, Palacorolla H, Brady D, Riegger K, Elmer GI, Shepard PD, 2016. Habenula-induced inhibition of midbrain dopamine neurons is diminished by lesions of the rostromedial tegmental nucleus. J. Neurosci 37, 217–225.

Corodimas KP, Rosenblatt JS, Morrell JI, 1992. The habenular complex mediates hormonal stimulation of maternal behavior in rats. Behav. Neurosci 106, 853–865. [PubMed: 1445661]

Corodimas KP, Rosenblatt JS, Canfield ME, Morrell JI, 1993. Neurons in the lateral subdivision of the habenular complex mediate the hormonal onset of maternal-behavior in rats. Behav. Neurosci 107, 827–843. [PubMed: 8280392]

England M, Sim LJ, 2009. Depression in Parents, Parenting, and Children. National Academies Press, Washington (DC).

Fleming AS, Walsh C, 1994. Neuropsychology of maternal behavior in the rat: c-fos expression during mother-litter interactions. Psychoneuroendocrinology 19, 429–443. [PubMed: 7938344]

Gallo M, Shleifer DG, Godoy LD, Ofray D, Olaniyan A, Campbell T, Bath KG, 2019. Limited bedding and nesting induces maternal behavior resembling both hypervigilance and abuse. Front. Behav. Neurosci 13, 167. [PubMed: 31402857]

Haim A, Albin-Brooks C, Sherer M, Mills E, Leuner B, 2016. The effects of gestational stress and selective serotonin reuptake inhibitor antidepressant treatment on structural plasticity in the postpartum brain–a translational model for postpartum depression. Horm. Behav 77, 124–131. [PubMed: 25997412]

Holschbach MA, Vitale EM, Lonstein JS, 2018. Serotonin-specific lesions of the dorsal raphe disrupt maternal aggression and caregiving in postpartum rats. Behav. Brain Res 348, 53–64. [PubMed: 29653128]

Kalinichev M, Rosenblatt JS, Nakabeppu Y, Morrell JI, 2000. Induction of c-fos-like and fosB-like immunoreactivity reveals forebrain neuronal populations involved differentially in pup-mediated maternal behavior in juvenile and adult rats. J. Comp. Neurol 416, 45–78. [PubMed: 10578102]

Leuner B, Fredericks PJ, Nealer C, Albin-Brooks C, 2014. Chronic gestational stress leads to depressive-like behavior and compromises medial prefrontal cortex structure and function during the postpartum period. PLoS One 9, e89912. [PubMed: 24594708]

Li M., 2022. Lateral habenula neurocircuits mediate the maternal disruptive effect of maternal stress: a hypothesis. Zool. Res 43, 166–175. [PubMed: 35008132]

Li M, Fleming AS, 2003. The nucleus accumbens shell is critical for normal expression of pup-retrieval in postpartum female rats. Behav. Brain Res 145, 99–111. [PubMed: 14529809]

Lonstein JS, Simmons DA, Swann JM, Stern JM, 1998. Forebrain expression of c-fos due to active maternal behaviour in lactating rats. Neuroscience 82, 267–281. [PubMed: 9483519]

Matsumoto M, Hikosaka O, 2007. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447, 1111–1115. [PubMed: 17522629]

Matsumoto M, Hikosaka O, 2009. Representation of negative motivational value in the primate lateral habenula. Nat. Neurosci 12, 77–84. [PubMed: 19043410]

Matthews-Felton T, Corodimas KP, Rosenblatt JS, Morrell JI, 1995. Lateral habenula neurons are necessary for the hormonal onset of maternal behavior and for the display of postpartum estrus in naturally parturient female rats. Behav. Neurosci 109, 1172–1188. [PubMed: 8748966]

Metzger M, Bueno D, Lima LB, 2017. The lateral habenula and the serotonergic system. Pharmacol. Biochem. Behav 162, 22–28. [PubMed: 28528079]

Nair SG, Strand NS, Neumaier JF, 2013. DREADDing the lateral habenula: a review of methodological approaches for studying lateral habenula function. Brain Res. 1511, 93–101. [PubMed: 23085473]

Numan M., 2020. The Parental Brain: Mechanisms, Development, and Evolution. Oxford University Press, New York.

Numan M, Insel TR, 2003. The Neurobiology of Parental Behavior.

Numan M, Numan MJ, 1995. Importance of pup-related sensory inputs and maternal performance for the expression of Fos-like immunoreactivity in the preoptic area and ventral bed nucleus of the stria terminalis of postpartum rats. Behav. Neurosci 109, 135–149. [PubMed: 7734069]

Numan M, Numan MJ, 1997. Projection sites of medial preoptic area and ventral bed nucleus of the stria terminalis neurons that express fos during maternal behavior in female rats. J. Neuroendocrinol 9, 369–384. [PubMed: 9181491]

Numan M, Numan MJ, Schwarz JM, Neuner CM, Flood TF, Smith CD, 2005. Medial preoptic area interactions with the nucleus accumbens-ventral pallidum circuit and maternal behavior in rats. Behav. Brain Res 158, 53–68. [PubMed: 15680194]

Paris R, Bolton RE, Weinberg MK, 2009. Postpartum depression, suicidality, and mother-infant interactions. Arch. Women's Ment. Health 12, 309–321. [PubMed: 19728036]

Pawluski JL, Lambert KG, Kinsley CH, 2016. Neuroplasticity in the maternal hippocampus: relation to cognition and effects of repeated stress. Horm. Behav 77, 86–97. [PubMed: 26122302]

Proulx CD, Hikosaka O, Malinow R, 2014. Reward processing by the lateral habenula in normal and depressive behaviors. Nat. Neurosci 17, 1146–1152. [PubMed: 25157511]

Sheehan TP, Cirrito J, Numan MJ, Numan M, 2000. Using c-fos immunocytochemistry to identify forebrain regions that may inhibit maternal behavior in rats. Behav. Neurosci 114, 337–352. [PubMed: 10832795]

Smith JW, Seckl JR, Evans AT, Costall B, Smythe JW, 2004. Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. Psychoneuroendocrinology 29, 227–244. [PubMed: 14604603]

Stack EC, Edward C, Numan M, 2000. The temporal course of expression of c-fos and fos B within the medial preoptic area and other brain regions of postpartum female rats during prolonged mother-young interactions. Behav. Neurosci 114 (609–609. [PubMed: 10883811]

Stack EC, Balakrishnan R, Numan MJ, Numan M, 2002. A functional neuroanatomical investigation of the role of the medial preoptic area in neural circuits regulating maternal behavior. Behav. Brain Res 131, 17–36. [PubMed: 11844569]

Wirtshafter D, Asin KE, Pitzer MR, 1994. Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. Brain Res. 633, 21–26. [PubMed: 8137158]

Yang Y, Qin J, Chen W, Sui N, Chen H, Li M, 2015. Behavioral and pharmacological investigation of anxiety and maternal responsiveness of postpartum female rats in a pup elevated plus maze. Behav. Brain Res 292, 414–427. [PubMed: 26159828]

Yang Y, Wang H, Hu J, Hu H, 2018. Lateral habenula in the pathophysiology of depression. Curr. Opin. Neurobiol 48, 90–96. [PubMed: 29175713]

Yetnikoff L, Cheng AY, Lavezzi HN, Parsley KP, Zahm DS, 2015. Sources of input to the rostromedial tegmental nucleus, ventral tegmental area, and lateral habenula compared: a study in rat. J. Comp. Neurol 523, 2426–2456. [PubMed: 25940654]

Yurino H, Tsukahara S, Koranyi L, Yamanouchi K, 2001. Inhibitory effect of postpartum lesions or cuts in median raphe nucleus on maternal behavior in female rats. Zool. Sci 18, 1225–1230.

Zahm DS, Root DH, 2017. Review of the cytology and connections of the lateral habenula, an avatar of adaptive behaving. Pharmacol. Biochem. Behav 162, 3–21. [PubMed: 28647565]

Zhao C, Li M, 2009. Sedation and disruption of maternal motivation underlie the disruptive effects of antipsychotic treatment on rat maternal behavior. Pharmacol. Biochem. Behav 92, 147–156. [PubMed: 19041338]

Zhao C, Li M, 2010. c-Fos identification of neuroanatomical sites associated with haloperidol and clozapine disruption of maternal behavior in the rat. Neuroscience 166, 1043–1055. [PubMed: 20096751]

Zhao C, Li M, 2012. Neuroanatomical substrates of the disruptive effect of olanzapine on rat maternal behavior as revealed by c-Fos immunoreactivity. Pharmacol. Biochem. Behav 103, 174–180. [PubMed: 22960130]

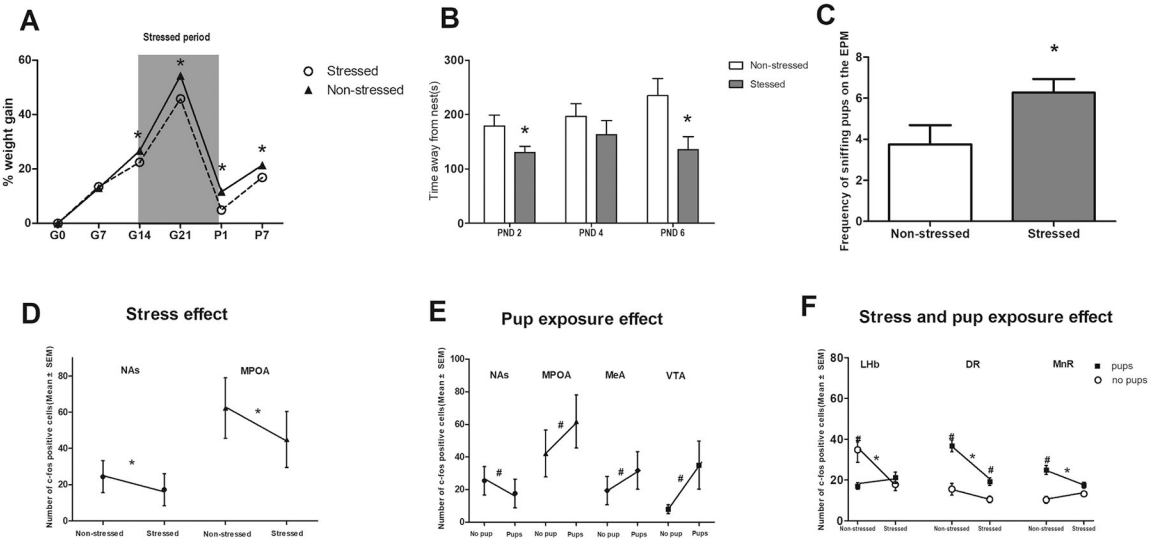

Fig. 1.

(A) Effect of gestational stress on the percentage weight gain in mothers (stressed, non-stressed mothers, n = 19/group, sc) from mating to postpartum day 7. G represents gestation, P represents postpartum, grey shadow represents stressed period for stressed mothers. (B) Duration time of outside nest in home cage. (C) Frequency of sniffing pups on the EPM. (D) The main effect of gestational stress on Fos expression (stressed, non-stressed mothers, n = 19/group, sc). (E) The main effect of pup exposure on the EPM on Fos expression (with pups = 24, without pups = 14). (F) Interactive effect of gestational stress and pup exposure on c-Fos expression (stressed + pups = 12, stressed + no pup = 7, non-stressed + pups = 12, non-stressed + no pup = 7). NAs, nucleus accumbens shell; MPOA, medial preoptic area; LHb, lateral habenula; MeA, medial nucleus of amygdale; VTA, ventral tegmental area; DR, dorsal raphe; MnR, median raphe. Data are expressed as mean ± SEM, respectively.

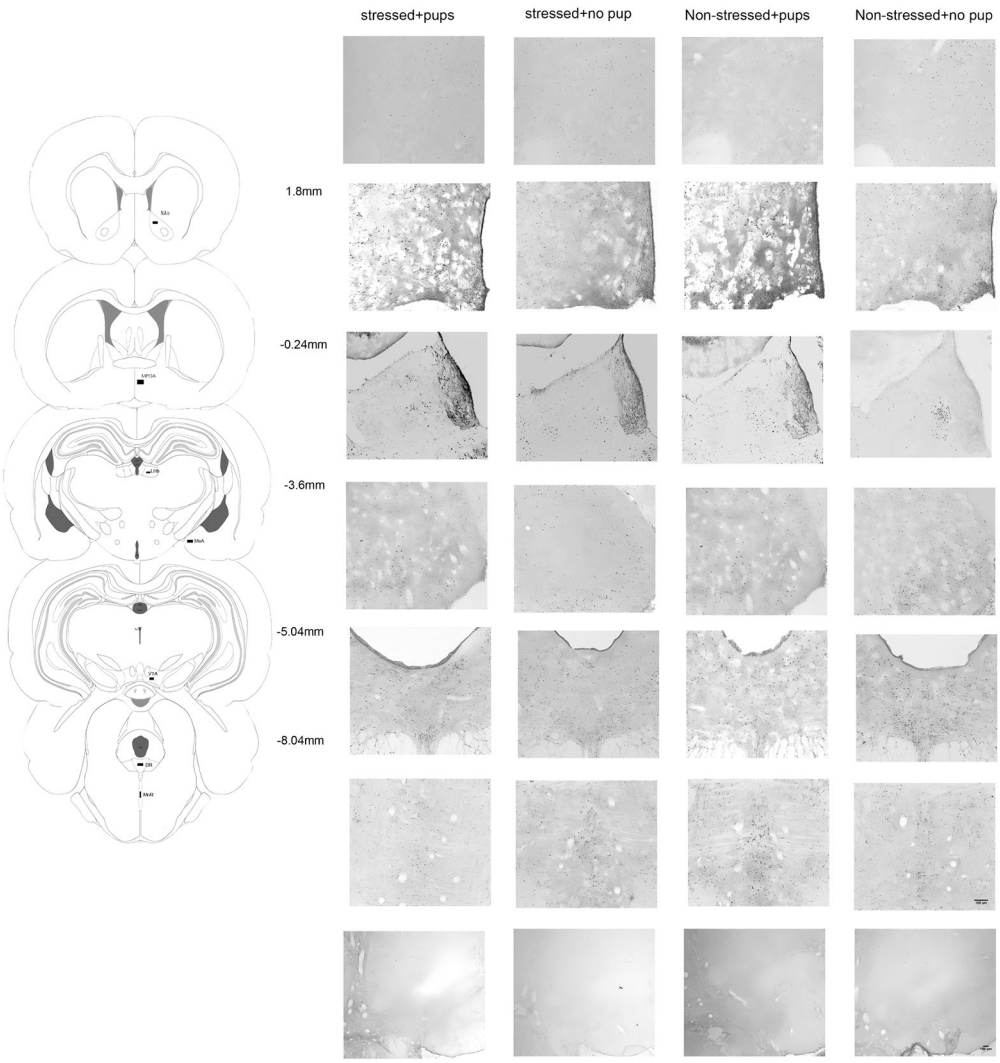

Fig. 2.

Brain atlas diagrams depict the areas viewed in the representative images of various brain regions (A), and representative Fos staining photomicrographs in these regions (B). NAs, nucleus accumbens shell; MPOA, medial preoptic area; LHb, lateral habenula; MeA, medial nucleus of amygdale; VTA, ventral tegmental area; DR, dorsal raphe; MnR, median raphe. Scale bar = 100 μm (NAs, MPOA, LHb, MeA, VTA and DR used the same scale bar).