




Astrocytic Hydrogen Sulfide Regulates Supraoptic Cellular Activity in the Adaptive Response of Lactating Rats to Chronic Social Stress

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Abstract

Maternal social stress among breastfeeding women can be adapted in chronic process. However, neuroendocrine mechanisms underlying such adaptation remain to be identified. Here, we report the effects of 2 hr/day unfamiliar male rat invasion (UMI) stress on maternal behaviors in lactating rats during postpartum day 8 (UMI8) to postpartum day 12 (UMI12). Rat dams at UMI8 presented signs of maternal anxiety, depression, and attacks toward male intruder. These changes partially reversed at UMI12 except the sign of anxiety. In the supraoptic nucleus (SON), UMI12 but not UMI8 significantly increased the expression of c-Fos and phosphorylated extracellular signal-regulated protein kinase 1/2. At UMI8 but not UMI12, length of glial fibrillary acidic protein (GFAP, astrocytic cytoskeletal element) filaments around oxytocin (OT) neurons was significantly longer than that of their controls; the amount of GFAP fragments at UMI12 was significantly less than that at UMI8. Expression of cystathionine β -synthase (CBS, enzyme for H₂S synthesis) at UMI12 was significantly higher than that at UMI8. CBS expression did not change significantly in the somatic zone of the SON but decreased significantly at the ventral glia lamina at UMI8. In brain slices of the SON, aminooxyacetate (a CBS blocker) significantly increased the expression of GFAP proteins that were molecularly associated with CBS. Aminooxyacetate also reduced the firing rate of OT neurons whereas Na₂S, a donor of H₂S, increased it. The adaptation during chronic social stress is possibly attributable to the increased production of H₂S by astrocytes and the subsequent retraction of astrocytic processes around OT neurons.

Keywords

astrocyte, glial fibrillary acidic protein, hydrogen sulfide, lactation, social stress

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Introduction

Maternal stress is a unique form of social stress and can disrupt maternal behaviors and lactation performance (Yaribeygi et al., 2017; Murgatroyd et al., 2015). Notably, these behavioral changes can be adapted in chronic process of social stress (Murgatroyd et al., 2015). It is known that maternal behaviors are regulated by both behavior-modulating brain regions and hypothalamic neuropeptide. Among many behavior-regulating brain regions, the supraoptic nucleus (SON), paraventricular nuclei (PVN), the medial amygdala, and the medial prefrontal cortex (mPFC) have been implicated in neural regulation of maternal behaviors (Murgatroyd et al., 2015; Kim et al., 2016; Gao et al., 2018;

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Jurek & Neumann, 2018). Thus, identification whether and how these brain regions are involved in bodily response to acute social stress is essential for understanding the adaptive response of the dams to chronic social stress.

Changes in oxytocin (OT) neuronal activity are known to play an essential role in the disorder of maternal behaviors (Olza et al., 2020; Witteveen et al., 2020). OT neurons are mainly located in the SON and PVN (Dyball & Koizumi, 1969; Gainer et al., 1977; Russell et al., 1981) and their activity is widely studied in lactation rats. During suckling, OT neurons exhibit periodic burst-like firing activity that depends on the maturation of hypothalamic machinery for the milk-ejection reflex around parturition (Salm et al., 1988; Wakerley et al., 1994; Hatton & Wang, 2008). This machinery involves coordinative activities of many neurochemical events, particularly OT secretion in the hypothalamus (Neumann et al., 1993) that is under intensive regulation of astrocytic plasticity (Tweedle & Hatton, 1977; Tweedle & Hatton, 1982). Under resting condition, astrocytic processes provide a physical barrier among adjacent neurons, and influence the uptake of various neuroactive substances and interneuronal interactions; their retraction from the surrounding of OT neurons during lactation increases the excitability of OT neurons and the extent of their coordination (Salm et al., 1985, 1988; Hatton et al., 1992; Bonfanti et al., 1993). The retraction of astrocytic processes under the influence of suckling and/or OT further increases OT neuronal activity in the form of synchronized burst-like firing, which is associated with the increased expression of phosphorylated extracellular signal regulated protein kinase1/2 (pERK1/2) in a membrane subcortical localization (Wang & Hatton, 2007). By contrast, astrocytic process expansion following the burst firing inhibits OT neuronal activity, particularly the synchronization. Notably, glial fibrillary acidic protein (GFAP, astrocytic cytoskeletal element) filament is highly representative for astrocytic morphological plasticity (Wang & Hatton, 2009b) and thus can be used as an indicator of astrocytic morphological plasticity. Clarification of the expression of these molecules is pivotal for understanding the role of OT neurons in this adaptive response.

While astrocytic morphological plasticity plays an essential role in astrocytic regulation of OT neuronal activity by influencing interneuronal interactions, astrocytes can also release gliotransmitters (Parpura & Zorec, 2010; Tasker et al., 2012) to alter OT neuronal activity, such as ATP and adenosine (Ponzio et al., 2006). In terms of synchronized burst firing, the diffusible gaseous gliotransmitter in the hypothalamus (Ruginsk et al., 2015) is particularly meaningful for OT neuronal activity as evidenced in the postinhibition excitation effect of nitric oxide on the burst firing of OT neurons (Okere et al., 1996). In the modulation of OT/vasopressin neuronal activity, a downstream effector of nitric oxide is hydrogen sulfide (H_2S) that is likely from astrocytes in the ventral glial lamina (VGL) (Coletti et al., 2019). H_2S can not only activate protein kinase A (Shao et al., 2011) that increases

burst firing (Li et al., 2021a), but also increase intracellular calcium in astrocytes (Nagai et al., 2004; Nii et al., 2021), which is known to promote retraction of astrocytic processes (Li et al., 2020b), thereby potentially facilitating OT neuronal activity. In social stress evoked by separation of newborns from the mothers, abnormal astrocytic plasticity and malfunction of OT neurons have been identified in rats (Wang & Hatton, 2009a; Liu et al., 2019; Li et al., 2020a). However, neurochemical modulation of OT neuronal activity for the adaptive response of OT neurons to social stress in breastfeeding women, particularly involving H_2S from astrocytes, remains largely unexplored.

We hypothesized that the adaptive responses of breastfeeding mothers to chronic social stress result from increased H_2S production and retraction of astrocytic processes that can be evoked by H_2S . Both factors facilitate OT neuronal activity and then influence maternal behaviors. This hypothesis was tested in a rat model of chronic social stress set by 2 hr/day unfamiliar male rat invasion (UMI) in the cage of lactating rats during postpartum day (PPD) 8 to 12.

Materials and Methods

All procedures in this study were conducted in accordance with NIH Guidelines for the Care and Use of Animals and approved by the Animal Care and Use Committees of Harbin Medical University, Harbin, China (No.31471113).

Preparation of UMI rat Model

Adult Sprague-Dawley virgin female rats (7–8 weeks old/200–230 g) and male rats (30–40 weeks old/300–400 g for breeding) were used. All rats were housed with free access to food and water in a room of 21–23 °C and 12/12 hr light/dark cycle. After adaption for 5 days, one breeder male and one virgin female rat were housed in the same cage until parturition (Figure 1). Lactating rats were randomly divided into control day 8 (CTR8), UMI day 8 (UMI8), CTR12, and UMI12 at PPD 1 and the number of pups was adjusted to 10 per litter. The UMI dams were set by cohabiting with a nonfather male rat/intruder with the lactating rat for 2 hr per day during PPD8 to PPD12, a method modified from a previous report (Murgatroyd et al., 2015).

Open Field Test

Open field test (OFT) was conducted to evaluate the level of anxiety-like behavior in rats at PPD6 before formal experiment. A video camera was fixed above the center of the open field box and connected to a computer for recording behaviors of rat dams. Before the observation, rat dams with the litters were placed in the test room for 12 hr to adapt for the test environment. The OFT was performed during 8–12 am. Rats were allowed to move freely for 5 min while video recording was performed and saved. The parameters to be

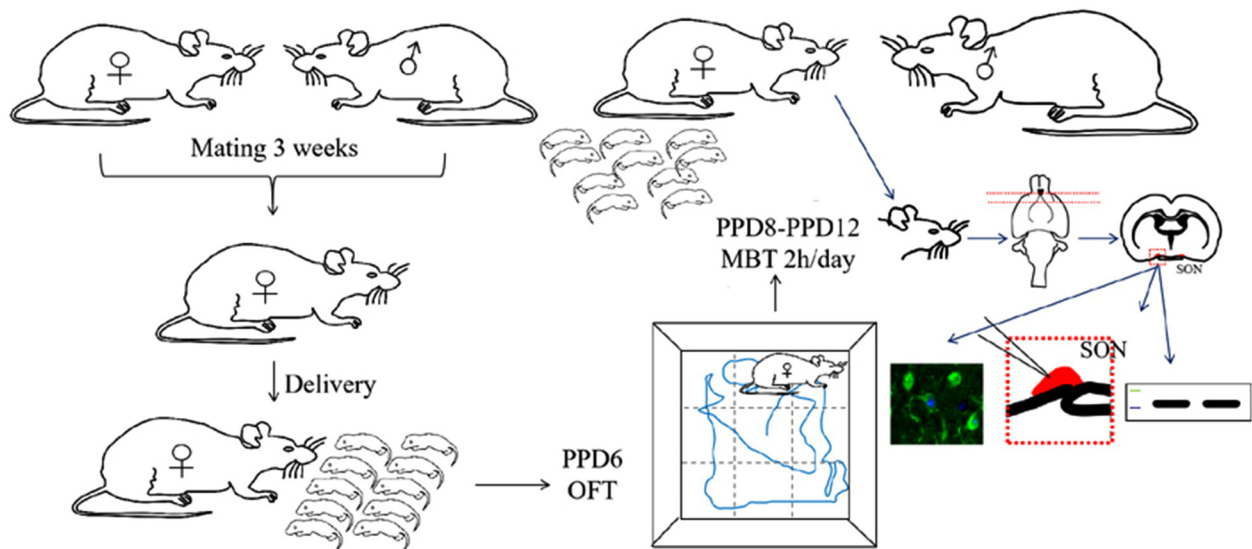


Figure 1. Schematic drawing of the experimental procedures and basal anxiety evaluation. Rats were housed in the laboratory to adapt the environment at least 5 days before the experiment. After mating with a male rat, getting gestation and then parturition in ~3 weeks, the biological father rats were separated from the dams and each dam was housed with a litter of 10 pups. Open field test (OFT) was applied to test the basal anxiety/exploration abilities of the dams at PPD6 and maternal behavior tests were performed at PPD8 and PPD12, respectively, to evaluate the behavioral changes. Finally, the brain and blood were sampled after decapitation for further examination with a variety of methods.

CTR = control; MBT = maternal behavior test; PPD = postpartum day; UMI = unfamiliar male invasion.

analyzed included the amount of time spending in still/immobility and self-grooming and the number of entering the central square.

Maternal Behavior Observation and Tissue Preparation

Maternal behaviors are a set of behaviors of the mother towards the offspring and are different from common social behaviors. Maternal behaviors in rodents include nest building, retrieval, contact, nursing, anogenital licking and maternal attack, and so on. A reduction in maternal interest toward the pups is the core sign of maternal depression, particularly anogenital licking (Pedersen & Boccia, 2002; Wang et al., 2007). Observation of maternal behavior and lactation performance was basically the same as previously described (Li et al., 2020c, 2021a). The dams were adapted in the test box (52.5 cm × 52.5 cm × 45 cm) for 12 hr before observation on PPD8 and PPD12. Then, we separated the pups from their dams 1 hr before observation. The pups were then returned to their dams after weighing litter's body weight for calculating litter's body weight gains (LBWGs) with/without UMI 2 hr later. Subsequently, a male rat was introduced into the test box in a different square of four horizontal four squares of the test box. During 2 hr observation, dam's behavior was video-taped and used for blindly analyzing maternal behavior later. The behaviors included high-leaning, shivering

behaviors, the latency of pup retrieval, duration of anogenital licking, the number of attacks, the latency and duration of suckling, and LBWGs in addition to the general behavior described above.

Following the observation, the brains of dams were sampled at 30 min later, immediately following decapitation and the pups were euthanized by carbon dioxide inhalation for 4–5 min. The brains were removed and soaked in ice-cold artificial cerebrospinal fluid (aCSF) that contained (in mM) 126 NaCl, 3 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 1.3 NaH₂PO₄, 26 NaHCO₃, 10 Glucose, 305 mOsm/kg water, pH 7.4, and gassed with a mixture of 95% O₂ and 5% CO₂. The hypothalamus in one side of the brain was cut into coronal sections of 2 mm thickness and the SON was punched out for extracting proteins for Western blots. The other side of the brain was fixed in 4% paraformaldehyde for 3 days for immunohistochemistry.

Western Blots

The procedures were the same as previously described (Li et al., 2021a). Briefly, the lysates were prepared in a lysis buffer (20115ES60, Yesen, Shanghai) using a tissue lyser. Protein concentration was measured at 562 nm wavelength using bicinchoninic acid assay agent (20201ES76, Yesen, Shanghai). After denaturation, proteins of 30 µg in 20 µL were separated on 10% sodium dodecyl sulfate-

polyacrylamide gel electrophoresis gel and then transferred onto polyvinylidenedifluoride membrane. The nonspecific binding sites in the membrane were blocked with 5% bovine serum albumin in Tris-buffered solution containing 0.1% Tween 20 for 2 hr at room temperature, and then incubated overnight at 4 °C with antibody against extracellular signal regulated protein kinase1/2 (ERK1/2, sc-376852, anti-mouse antibody, 1:500, Santa Cruz Biotechnology), pERK1/2 (sc-136521, anti-mouse antibody, 1:500, Santa Cruz Biotechnology, RRID: AB_10856869), c-Fos (ab190289, anti-rabbit antibody, 1:500, Abcam, RRID: AB_2737414), cystathionine β -synthase (CBS, an enzyme for H₂S synthesis, ab135626, 1:500, anti-rabbit antibody, Abcam, RRID: AB_2814659), GFAP (OM254428, 1:500, anti-rabbit antibody, Omnimabs), or β -tubulin (2128 s, 1:500, anti-rabbit antibody, CST, RRID: AB_823664) overnight. After rinsing, the protein membranes were further incubated with HRP-conjugated secondary antibodies (A0216, HRP-labeled Goat Anti-Mouse IgG, RRID: AB_2860575; A0208, HRP-labeled Goat Anti-Rabbit IgG, RRID: AB_2757022; Beyotime Biotechnology, Shanghai). Protein bands were visualized with an automated chemiluminescence imaging analysis system (Tanon 5200, Shanghai). The protein bands were quantified by multiplying average intensity and pixel numbers using Image J or Photoshop software, which was further corrected with the intensity of their corresponding loading protein, β -tubulin or ERK1/2.

Notably, detection of c-Fos and pERK1/2 in Western blots was for evaluation of cellular activation, in which c-Fos reflects general increase in cellular metabolic activity and pERK1/2 is more closely related to the signaling process. In their analysis, β -tubulin and ERK1/2 were used as loading controls of c-Fos and pERK1/2, respectively.

Coimmunoprecipitation

Assaying molecular association between GFAP and CBS in lactation rats used the same protocol as previously described (Li et al., 2021a). Briefly, total tissue lysates were pre-cleared with protein A/G agarose beads (sc-2003, RRID: AB_10201400) to reduce nonspecific binding. Immunoprecipitating antibody against GFAP (1.0 μ g) was added to 500 μ l protein lysis buffer containing 600 μ g protein lysates and the reaction was incubated overnight at 4 °C on a rotator to form immunocomplex. The immunocomplex was captured by 50 μ l slurry of protein A/G agarose beads with gently rocking for 2 hr at 4 °C. The beads were then collected by a pulse centrifugation. After removing the supernatant and washing, the beads were resuspended in 2X sample buffer (25 μ l, P0015B, Beyotime, Shanghai), heated for 10 min at 100 °C to dissociate target proteins from the beads in the immunocomplex and denature them, and then spun down to collect supernatant for running Western blots.

GFAP and CBS expressions were detected as described above.

Immunohistochemistry

The method was basically the same as previously described (Wang & Hatton, 2009b). In brief, the brain with intact optic chiasm was cut into 80 μ m-thick coronal sections. In immunostaining, the plasma membrane was permeabilized with 0.3% Triton X-100 for 1 hr, and nonspecific binding sites to antibodies were blocked with 5% bovine serum albumin for 2 hr. The sections were then incubated with primary antibodies against c-Fos, pERK1/2, CBS, GFAP (ab4674, 1:500, anti-chicken antibody, Abcam, RRID: AB_304558), or OT neurophysin (OT-NP, MABN844, anti-mouse antibody, 1:3000, Merck Millipore) at 4 °C overnight. Species-matched secondary antibodies (ab150171, Alexa Fluor 647-labeled Donkey Anti-Chicken IgG; ab150074, Alexa Fluor 555-labeled Donkey Anti-Rabbit IgG, RRID: AB_2636997; ab150109, AlexaFluor 488-labeled Donkey Anti-Mouse IgG, RRID: AB_2571721; Abcam, Cambridge, UK) were applied for 2 hr at room temperature. Lastly, Hoechst (bisbenzimidazole, B2261, Sigma, 0.5 μ g/ml, 30 min, RRID: AB_1589326) was used to label nuclei. Sections were examined with a confocal microscope (Eclipse Ti, Nikon). To reduce the variability of results from different loci, sections of equivalent location in the SON from different groups were used for comparisons. Specificity of the antibodies was verified by applying no-primary and no-secondary antibody control staining.

Preparation of Fresh Brain Slices and In-vitro Treatments

The method was the same as previously described (Li et al., 2020c). In brief, rats were decapitated with a guillotine; 300 μ m brain slices were quickly removed and placed in ice-cold oxygenated slice solution containing (in mmol/L): 213 sucrose, 2.5 KCl, 5 MgCl₂, 3 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose, 305 mOsm/kg, pH 7.4. The slices were then transferred into aCSF and incubated at 37 °C for 30 min before maintenance at room temperature. The slices were divided into the following groups: CTR, aminooxyacetate (AOA, 5 mmol/L, CBS inhibitor, HY-107994, MCE, Shanghai), OT (10 pmol/L, O6379, Sigma-Aldrich, Shanghai), AOA+OT. In AOA+OT group, OT was applied for 5 min and AOA was applied 5 min before OT application. After treatments, the SON was punched out in ice-cold aCSF and then used for protein analyses.

Whole-Cell Patch-Clamp Recordings

After preparation, the slices were incubated at 37 °C in the aCSF for 30 min and then at room temperature until

recordings. During recordings, 35 °C aCSF was superfused via a gravity-fed perfusion system at a rate of 1.5–2 ml/min. Patch pipette-filling solution contained (in mmol/L): 145 K-gluconate, 10 KCl, 1 MgCl₂, 10 HEPES, 1 EGTA, 0.01 CaCl₂, 2 Mg-ATP, and 0.5 Na₂-GTP, pH 7.3, adjusted with KOH. In the recording, a patch electrode was guided onto the soma of a SON neuron under visual observation with a microscope. Electrical signals were filtered and sampled at 5 kHz and then collected with Clampex 10 software and a 700B through an analog-to-digital converter (1550, Molecular Devices). Data were analyzed using Clampfit 10 software amplifier (Molecular Devices).

Ten minutes after obtaining stable recording in whole-cell configuration, Na₂S (S818266, Sigma-Aldrich, Shanghai) in 1 mM was applied for 10 min through the perfusion system. Estimates of the amount that actually exists as free H₂S in solution should be lower (3%–10% of the initial concentration) in the open recording chamber. We prepared our Na₂S solution immediately before use and the final working solution of Na₂S was approximately at 30–100 μM according to the evaluation on NaHS usage of Kuksis and Ferguson in 2015 (Kuksis & Ferguson, 2015).

Data Analysis

Data analyses were carried out using the same methods as previously described (Li et al., 2021a). In immunohistochemistry, we selected OT neurons in the SON with somatic diameter ≥20 μm as the target to count the number of positive cells of c-Fos, pERK1/2 and CBS staining. To measure the length of GFAP filaments, we averaged the length of five longest GFAP filaments with a minimal length of 50 μm and a diameter wider than 2 μm to get one value in the visual field of the somatic zone of the SON. Each value was from one SON section of one rat. In patch-clamp recordings, the firing rate was measured and expressed as relative value to the basal firing rate (1.0).

Data were expressed as mean ± SEM. All analyses were performed using SigmaStat program (SPSS 19, Chicago, IL) software. Two- or one-way analysis of variance (ANOVA) was used for comparison among four groups and followed by Bonferroni post-hoc test to compare two treatments. Student's *t*-test was used for comparison between two groups. *p* < .05 was considered statistically significant.

Results

Evaluation of Anxiety Levels of Dams Before Experiment

Through evaluation of anxiety levels of dams before UMI experiments, the rats with high anxiety levels were ruled out from further experiments. Remaining rats in the two groups showed similar frequency in their presence in center entries (3.72 ± 0.29, *n* = 19 in CTR; 3.58 ± 0.51, *n* = 14 in UMI;

p = .79 by Student's *t*-test), the duration of immobility (57.98 ± 3.6 s, *n* = 19 in CTR; 58.08 ± 3.51 s, *n* = 14 in UMI; *p* = .99 by Student's *t*-test), and the duration of self-grooming (35.74 ± 5.60 s, *n* = 19 in CTR; 38.19 ± 8.46 s, *n* = 14 in UMI, *p* = 0.80 by Student's *t*-test). This evaluation allows the study to be performed with high comparability.

Maternal Behavior

Male intruder can cause maternal anxiety, depression, and hypogalactia in rat dams (Murgatroyd et al., 2015). To verify this finding, we further examined it with a different protocol in the present study. In general, there was no significant difference in high-leaning and rearing behaviors between the CTR8 and UMI8 in the first 10 min (16.08 ± 2.31, *n* = 12 at UMI8 vs. 13.42 ± 1.73, *n* = 12 at CTR8, *p* = 1.000 by two-way ANOVA). However, UMI12 significantly reduced this behavior (12.67 ± 1.78, *n* = 12 at UMI12 vs. 20.75 ± 1.61, *n* = 12 at CTR12, *p* = .047 by two-way ANOVA; Figure 2A). Moreover, the number of shivering at UMI8 increased significantly compared to CTR8 (4.57 ± 0.98, *n* = 14 at UMI8 vs. 0.44 ± 0.29, *n* = 18 at CTR8, *p* = .000 by two-way ANOVA). However, there was no significant difference in the number of shivering in PPD12 (2.25 ± 0.90, *n* = 8 at UMI12 vs. 0.75 ± 0.25, *n* = 12 at CTR12, *p* = .955 by two-way ANOVA; *p* = 0.0006 between UMI12 and UMI8 by two-way ANOVA; Figure 2B). The duration of self-grooming was significantly longer at UMI8 (123.90 ± 11.68 s, *n* = 14 at UMI8 vs. 30.00 ± 4.46 s, *n* = 18 at CTR8, *p* = .000 by two-way ANOVA) and at UMI12 (100.60 ± 18.16 s, *n* = 9 at UMI12 vs. 32.25 ± 6.81 s, *n* = 12 at CTR12, *p* = .0004 by two-way ANOVA). Relative to UMI8, the duration reduced significantly at UMI12 (*p* = .000 between UMI8 and UMI12 by two-way ANOVA; Figure 2C).

Next, we observed maternal interests toward pups in the first 10 min at UMI8 and UMI12, respectively. The latency of pup retrieval was significantly increased at UMI8 (4.57 ± 1.91 min, *n* = 11 at UMI8 vs. 0.66 ± 0.16 min, *n* = 17 at CTR8; *p* = .021 by two-way ANOVA); however, it was not significantly longer at UMI12 (2.42 ± 0.88 min, *n* = 8 at UMI12 vs. 1.01 ± 0.29 min, *n* = 11 at CTR12; *p* = 1.000 by two-way ANOVA; Figure 2D). The duration of anogenital licking was significantly reduced at UMI8 (0.44 ± 0.18 min, *n* = 12 at UMI8 vs. 2.74 ± 0.24 min, *n* = 12 at CTR8; *p* = .000 by two-way ANOVA), which became insignificant at UMI12 (2.43 ± 0.33 min, *n* = 12 at UMI12 vs. 2.86 ± 0.37 min, *n* = 12 in CTR 12; *p* = 1.000 by two-way ANOVA). Relative to UMI8, the duration at UMI12 became longer (*p* = .000 by two-way ANOVA; Figure 2E). Lastly, the number of maternal attacks to the male rat at UMI12 reduced significantly relative to that at UMI8 (2.00 ± 0.53, *n* = 7 at UMI12 vs. 9.92 ± 1.42, *n* = 13 at UMI8, *p* < .001 by independent Student's *t*-test, Figure 2F).

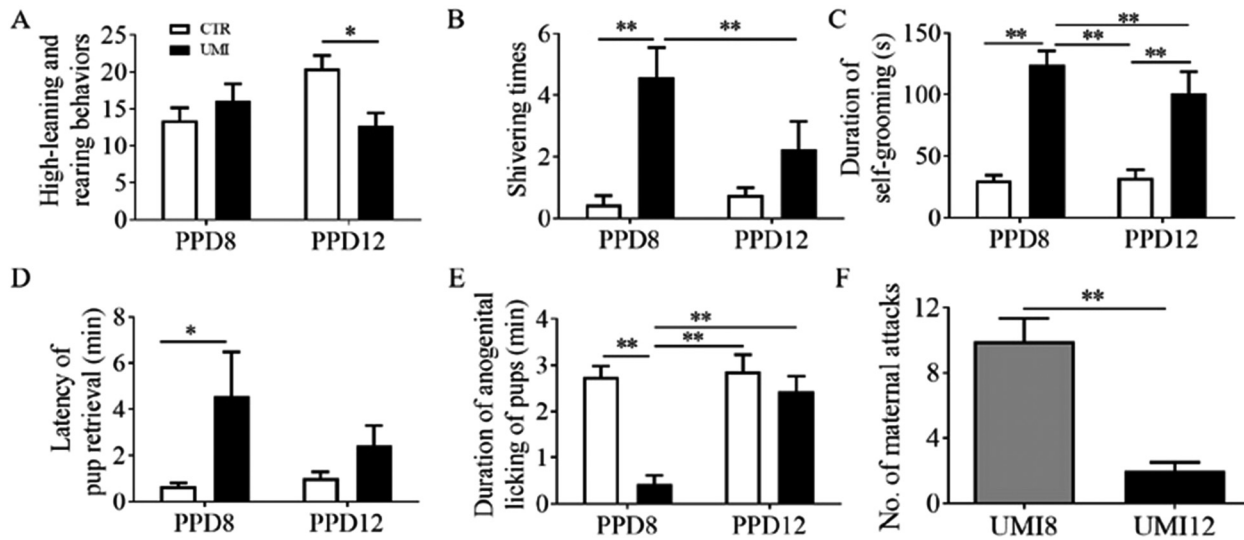


Figure 2. Effects of UMI on maternal behavior. (A–F) Bar graphs summarizing the incidence of high-leaning and rearing behaviors (A), shivering times (B), duration of self-grooming (C), the latency of pup retrieval (D), duration of anogenital licking (E), and the number of maternal attacks (F).

* $p < .05$, ** $p < .001$ by two-way ANOVA.

CTR = control; UMI = unfamiliar male invasion; ANOVA = analysis of variance.

Lactation Performance

Lactation performance is an essential component of maternal behavior. In this study, we analyzed the lactation behavior in 2 hr. Latency of suckling was significantly increased at UMI8 (32.42 ± 4.77 min, $n = 12$ at UMI8 vs. 7.27 ± 0.52 min, $n = 18$ at CTR8, $p = .000$ by two-way). The latency of suckling at UMI12 did not differ significantly from that at CTR12 but was significantly shorter than that at UMI8 (14.30 ± 4.77 min, $n = 9$ at UMI12 vs. 7.34 ± 0.73 min, $n = 12$ at CTR12, $p = .747$ by two-way ANOVA and $p = .001$ between UMI12 and UMI8 by two-way

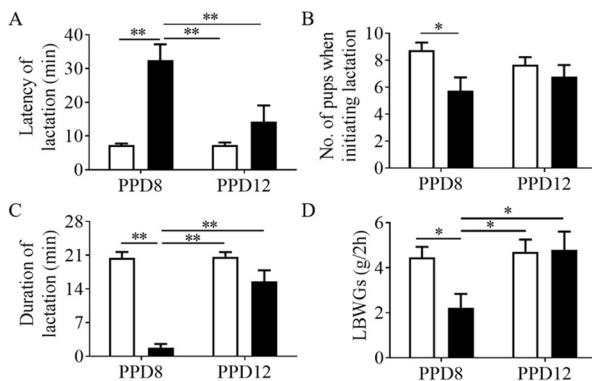


Figure 3. Effects of UMI on lactation performance of rat dams. (A–D) Bar graphs showing the latency of lactation (A), the number of pups when dams started suckling (B), the duration of lactation (C) and LBWGs (litter's body weight gains (g)/2 hr (D)).

* $p < .05$, ** $p < .01$ by two-way ANOVA. CTR = control; UMI = unfamiliar male invasion; ANOVA = analysis of variance.

ANOVA; Figure 3A). The number of pups at the beginning of suckling was significantly smaller at UMI8 (5.75 ± 0.98 , $n = 12$ at UMI8 vs. 8.77 ± 0.56 , $n = 17$ at CTR8, $p = .028$ by two-way ANOVA) but not at UMI12 (7.67 ± 0.57 , $n = 12$ at UMI12 vs. 6.78 ± 0.88 , $n = 9$ at CTR12, $p = 1.000$ by two-way ANOVA; Figure 3B). Correspondingly, duration of suckling in the first 30 min was significantly shorter at UMI8 than at CTR8 (1.76 ± 0.81 min, $n = 13$ at UMI8 vs. 20.43 ± 1.26 min, $n = 16$ at CTR8, $p = .000$ by two-way ANOVA). However, the difference was insignificant in PPD12 (15.57 ± 2.29 min, $n = 9$ at UMI12 vs. 20.71 ± 0.96 min, $n = 12$ at CTR12, $p = .091$ by two-way ANOVA) while the latency at UMI12 was significantly longer than that at UMI8 ($p = .000$ by two-way ANOVA; Figure 3C). At the end of 2 hr observation, the LBWGs of 10 pups were significantly smaller at UMI8 (2.23 ± 0.62 g, $n = 12$ at UMI8 vs. 4.46 ± 0.47 g, $n = 18$ at CTR8, $p = .036$ by two-way ANOVA) but not at UMI12 (4.79 ± 0.82 g, $n = 9$ at UMI12 vs. 4.72 ± 0.54 g, $n = 12$ at CTR12, $p = 1.000$ by two-way ANOVA) compared to their controls. The weight gain at UMI12 was significantly higher than that at UMI8 ($p = .046$ between UMI8 and UMI12 by two-way ANOVA; Figure 3D).

Activity of Neurons in the PVN, Amygdala, and mPFC

To identify whether the adaptive behavior in UMI dams was associated with activities of maternal behavior-associated brain regions, we analyzed the expressions of c-Fos and pERK1/2 in the PVN, amygdala and mPFC with Western

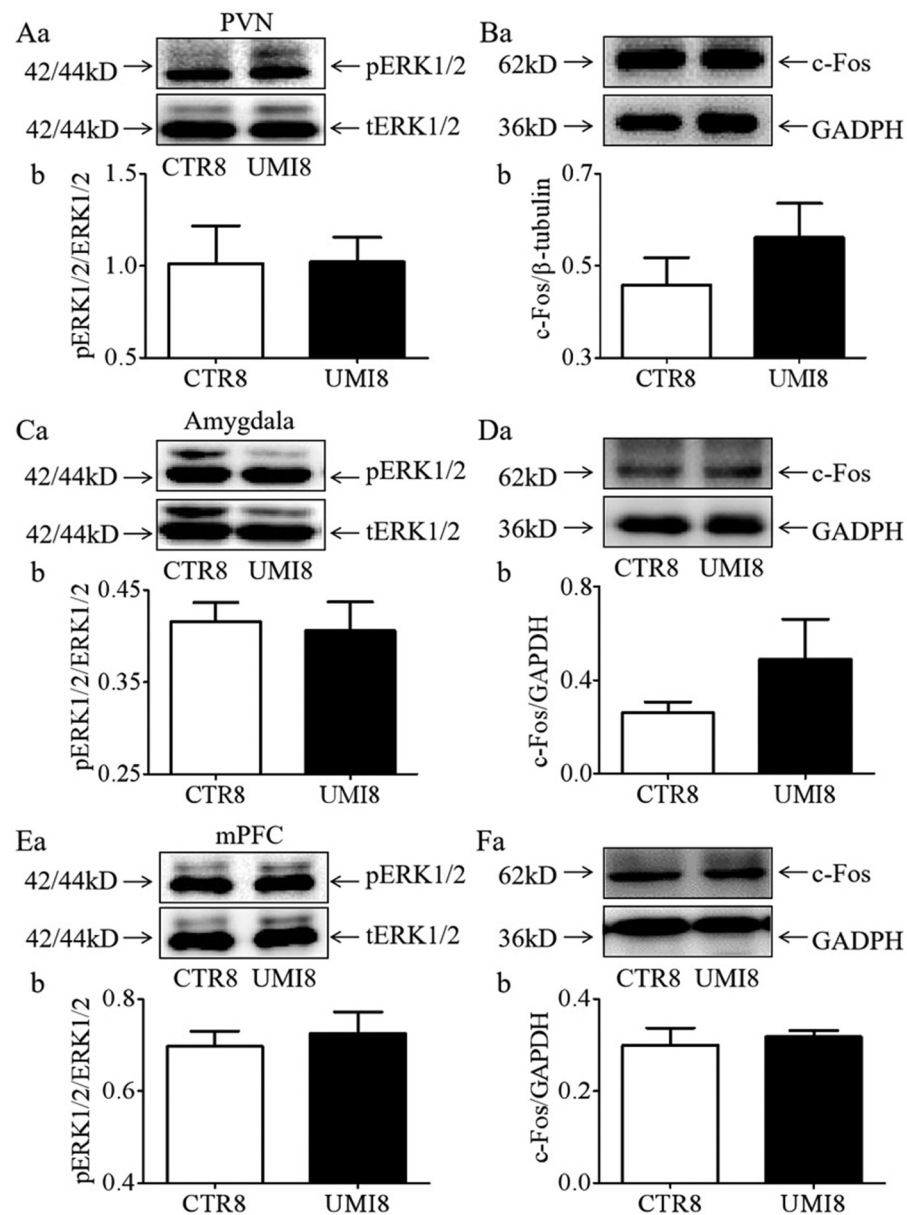


Figure 4. Effects of UMI on the expression of pERK1/2 and c-Fos in the PVN, amygdala, and mPFC. (A–B) Representative Western blot bands (a) and the summary graphs (b) showing the expression of pERK1/2 (A, $n = 6$) and c-Fos (B, $n = 6$) under different experimental conditions in the PVN. (C–D) Representative Western blot bands (a) and the summary graphs (b) showing the expression of pERK1/2 (C, $n = 6$) and c-Fos (D, $n = 6$) under different experimental conditions in amygdala. (E–F) Representative Western blot bands (a) and the summary graphs (b) showing the expression of pERK1/2 (E, $n = 7$) and c-Fos (F, $n = 4$) under different experimental conditions in mPFC. OT-NP = oxytocin neurophysin; CTR = control; UMI = unfamiliar male invasion; pERK1/2 = phosphorylated extracellular signal-regulated protein kinase1/2; mPFC = medial prefrontal cortex; PVN = paraventricular nucleus.

blots in PPD8. In the PVN, there was no significant difference in the expressions of pERK1/2 (1.02 ± 0.13 , $n = 6$ at UMI8 vs. 1.01 ± 0.2 , $n = 6$ at CTR8, $p = .97$ by Student's *t*-test; Figure 4A) and c-Fos (0.56 ± 0.07 , $n = 6$ at UMI8 vs. 0.46 ± 0.06 , $n = 6$ at CTR8, $p = .3$ by *t*-test; Figure 4B). In the amygdala, the differences were statistically insignificant between CTR8 and UMI8 in the expression of pERK1/2 (0.41 ± 0.03 , $n = 6$ at UMI8 vs. $0.42 \pm$

0.02 , $n = 6$ at CTR8, $p = .793$ by *t*-test; Figure 4C) and c-Fos (0.49 ± 0.07 , $n = 6$ at UMI8 vs. 0.26 ± 0.05 , $n = 6$ at CTR8, $p = .225$ by *t*-test; Figure 4D). Similarly, in the mPFC, the expressions of pERK1/2 (0.72 ± 0.05 , $n = 7$ at UMI8 vs. 0.72 ± 0.03 , $n = 7$ at CTR8, $p = .999$ by *t*-test; Figure 4E) and c-Fos (0.32 ± 0.01 , $n = 4$ at UMI8 vs. 0.30 ± 0.04 , $n = 4$ at CTR8, $p = .663$ by *t*-test; Figure 4F) were not different significantly between the two groups.

The Activity of SON Neurons in UMI Dams

The milk-ejection reflex and maternal behaviors are both regulated by OT and the SON is a representative nucleus releasing OT. Thus, we further observed the activity of SON cells to identify whether the adaptive behavior in UMI dams is associated with changes in OT neuronal activity. In immunohistochemistry, there was no significant difference in the colocalization of c-Fos puncta with OT-NP staining in OT neurons in the SON between different groups (0.96 ± 0.02 , $n=6$ at UMI8 vs. 0.98 ± 0.02 , $n=7$ at CTR8, $p=1.000$ by two-way ANOVA; 0.95 ± 0.03 , $n=5$ at UMI12 vs. 0.98 ± 0.02 , $n=7$ at CTR12, $p=1.000$ by two-way ANOVA; Figure 5Ab). In Western blots, expressions of c-Fos did not change significantly at UMI8 (0.79 ± 0.09 , $n=10$ at UMI8 vs. 0.72 ± 0.07 , $n=10$ at CTR8, $p=1.000$ by two-way ANOVA); however, c-Fos expression at UMI12 was significantly higher than that at CTR12 (0.94 ± 0.07 , $n=10$ at UMI12 vs. 0.63 ± 0.06 , $n=10$ at CTR12, $p=0.031$ by two-way ANOVA; Figure 5B).

Consistently, there was no significant difference in the colocalization of pERK1/2 and OT-NP in the SON among the four groups in immunohistochemistry (0.24 ± 0.09 , $n=7$ at UMI8 vs. 0.37 ± 0.09 , $n=7$ at CTR8, $p=1.000$ by two-way ANOVA; 0.43 ± 0.10 , $n=7$ at CTR12; 0.45 ± 0.10 , $n=7$ at UMI12; $p=1.000$ by two-way ANOVA; Figure 6Ab). In Western blots, UMI8 did not significantly influence pERK1/2 expression (0.62 ± 0.05 , $n=10$ at UMI8 vs. 0.64 ± 0.04 , $n=10$ at CTR8, $p=1.000$ by two-way ANOVA); however, UMI12 significantly increased pERK1/2 expression (0.78 ± 0.03 , $n=10$ at UMI12 vs. 0.59 ± 0.03 , $n=10$ at CTR12, $p=0.016$ by two-way ANOVA; Figure 6B) in the SON.

Effects of UMI on Astrocytic Plasticity in the SON

OT neuronal activity is under intense modulation of astrocytic plasticity that is largely reflected by GFAP plasticity (Li et al., 2020b). Thus, we further analyzed the effects of UMI on GFAP expression in the SON. In immunohistochemistry (Figure 7A), the length of GFAP filaments was significantly longer at UMI8 than at CTR8 ($113.56 \pm 4.52 \mu\text{m}$, $n=5$ at UMI8 vs. $85.96 \pm 5.40 \mu\text{m}$, $n=5$ at CTR8, $p=0.006$ by two-way ANOVA). At PPD12, the length of GFAP filaments at UMI12 did not differ significantly from that at CTR12 ($75.40 \pm 3.36 \mu\text{m}$, $n=5$ at UMI12 vs. $91.44 \pm 5.78 \mu\text{m}$, $n=5$ at CTR12; $p=0.197$ by two-way ANOVA) but was significantly shorter than that at UMI8 ($p=0.0003$ by two-way ANOVA).

GFAP has highly morphological plasticity because its assembling and polymerizing states can be changed quickly in response to environmental challenges. GFAP monomer of 50 kD is composed of head, body and tail domains and its decomposition forms GFAP fragments (Li et al., 2020b). Transition of GFAP monomer to filamentous form is

associated with increased fragmentation of GFAP monomer (Wang et al., 2017). To clarify polymerizing states of GFAP under different experimental conditions, we further examined the expression of GFAP monomer and fragments in Western blots. The results showed that expressions of GFAP at 55 kD did not differ significantly among different groups (0.31 ± 0.05 , $n=5$ at UMI8 vs. 0.34 ± 0.07 , $n=5$ at CTR8, $p=1.00$ by two-way ANOVA; 0.22 ± 0.02 , $n=5$ at UMI12 vs. 0.25 ± 0.03 , $n=5$ at CTR12, $p=1.00$ by two-way ANOVA). Moreover, expression levels of GFAP fragments did not differ significantly between UMI8 and CTR8 (0.59 ± 0.06 , $n=5$ at UMI8 vs. 0.36 ± 0.07 , $n=5$ at CTR8, $p=.0804$ by two-way ANOVA) and between UMI12 and CTR12 (0.22 ± 0.05 , $n=5$ at UMI12 vs. 0.407 ± 0.05 , $n=5$ at CTR12, $p=.059$ by two-way ANOVA). Relative to UMI8, GFAP fragment levels at UMI12 reduced significantly ($p=.002$ between UMI8 and UMI12 by two-way ANOVA; Figure 7Bb).

CBS Expression in UMI and Effect of H₂S on OT Neuronal Activity

H₂S can be a downstream effector of OT receptor (OTR) and nitric oxide (Wang et al., 2019b), and the changes in its expression possibly account for the relative increase in OT neuronal activity at UMI12. CBS is a key enzyme for H₂S production and assaying CBS expression can reflect H₂S production (Cong et al., 2020). Thus, we assayed the expression of CBS in the SON. In immunohistochemistry (Figure 7Aa), there was no significant difference in the expression levels of CBS in the SON as a whole among the four groups. However, the fluorescence intensity of CBS-positive cells in the VGL was significantly decreased at UMI8 (367.77 ± 44.88 , $n=6$ at UMI8 vs. 541.69 ± 24.63 , $n=8$ at CTR8, $p=.033$ by two-way ANOVA), which became insignificant between UMI12 and CTR12 (424.64 ± 47.04 , $n=4$ at UMI12 vs. 447.87 ± 24.63 , $n=11$ at CTR12, $p=1.00$ by two-way ANOVA).

In Western blots, there was no significant difference in CBS protein levels in the SON between PPD8 and UMI8 or PPD12 and UMI12 (0.21 ± 0.02 , $n=7$ at UMI8 vs. 0.28 ± 0.01 , $n=7$ at CTR8, $p=.549$ by two-way ANOVA; 0.36 ± 0.03 , $n=7$ at UMI12 vs. 0.30 ± 0.04 , $n=7$ at CTR12, $p=.662$ to UMI12 by two-way ANOVA). However, CBS protein levels at UMI12 increased significantly relative to UMI8 ($p=.004$ by two-way ANOVA; Figure 7C). In coimmunoprecipitation of the SON slices ($n=12$) from three rats, molecular association between GFAP and CBS was identified (Figure 7Da), in which AOA and OT did not change the molecular association significantly. In addition, the extensively identified excitatory effect of OT on OT neurons (Yamashita et al., 1987) did not occur following AOA pretreatment (Figure 7D).

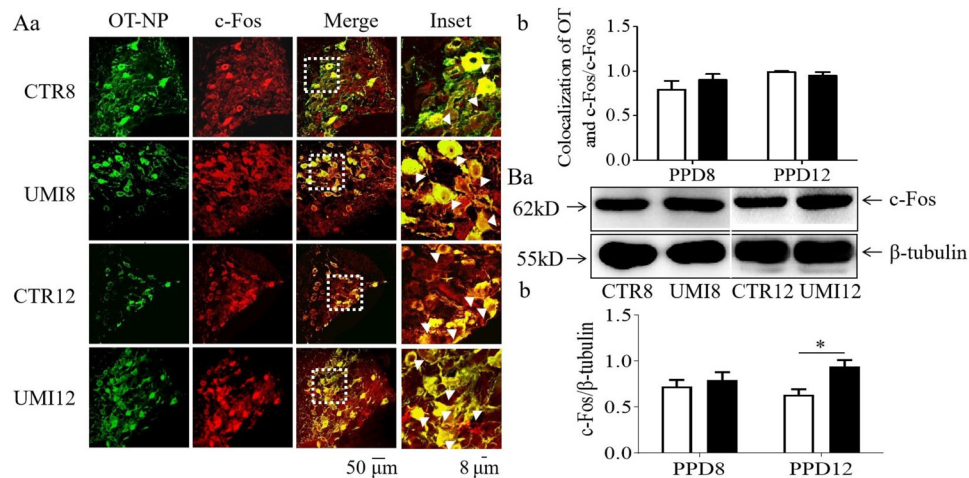


Figure 5. Effects of UMI on the expressions of c-Fos in the SON. (A) Confocal microscopic images (a) showing (from left to the right): OT-NP (in green), c-Fos (in red), their merges and inset (from dashed squares) and the bar graph (b) summarizing the analysis of ratio of c-Fos-positive OT neurons. The arrowheads point to c-Fos in OT neuron staining ($n = 7$ in CTR, $n = 6$ at UMI8 and $n = 5$ at UMI12). (B) Representative Western blot bands (a) and the summary graph (b) showing the expression of c-Fos ($n = 10$) under different experimental conditions.

Note: Ba is a recombination of the original gel after removing irrelevant protein bands.

* $p < .05$ by two-way ANOVA.

OT-NP = oxytocin neurophysin; CTR = control; UMI = unfamiliar male invasion; ANOVA = analysis of variance; SON = supraoptic nucleus.

The dual change in CBS expressions suggests that the adaptive response in chronic social stress is related to the relative increase of CBS in astrocytes. To test this possibility, we observed effects of blocking CBS on the expression of GFAP with and without OT in Western blots in the SON from brain slices of male rats. As shown in Figure 8A, AOA treatment for 10 min significantly increased the expression of GFAP at 55 kD protein in the SON (0.44 ± 0.1 , $n = 6$ in AOA vs. 0.24 ± 0.01 , $n = 6$ in CTR, $p = .0303$ by Student's *t*-test). OT had no significant effect on this AOA effect (0.33 ± 0.1 , $n = 6$ in OT; 0.40 ± 0.08 , $n = 6$ in AOA + OT, $p = .575$ by one-way ANOVA test).

In the experiments performed above, the effect of AOA on protein expressions may be influenced by the permeating time of drugs into the deep layer of slices. To compensate this insufficiency, we tested effect of AOA on OT neuronal activity by use of whole-cell patch-clamp recording from OT neurons on the surface of the slices. The results revealed that AOA decreased the firing rate of OT neurons (0.19 ± 0.07 of basal firing, $n = 10$ in AOA, $p < .001$ by paired *t*-test; Figure 8B). By contrast, Na_2S , a donor of H_2S significantly increased the firing rate of OT neurons (0.24 ± 0.07 of basal firing, $n = 4$ in Na_2S , $p = .02$ by paired *t*-test; Figure 8C).

Discussion

The present study reveals that acute social stress (i.e., UMI8) can increase anxiety-like behavior and evoke maternal depression and hypogalactia, which, except anxiety-like behavior, is improved/adapted during chronic social stress (i.e., UMI12).

The adaptive changes are likely due to the activation of OT neurons by increasing astrocytic H_2S production and the subsequent retraction of astrocytic processes around OT neurons, both of which can increase OT neuronal activity even if there is no activation of OTR-associated signaling events. The remaining sign of anxiety likely results from the activation of vasopressin neurons.

Effects of UMI on Maternal Behavior

Exposing rat dams to male intruder stress impairs maternal care and lactation while increasing maternal anxiety and aggression, which can be adapted in chronic processes (Murgatroyd et al., 2015). This finding is supported by the present result that acute social stress causes maternal anxiety, depression and hypogalactia, which are weakened at chronic social stress as demonstrated at UMI12.

As stated in the introduction, many brain regions are involved in bodily response to acute social stress and thus we examined the activity of several brain regions. However, analyzing expressions of pERK1/2 and c-Fos in the PVN, mPFC, and amygdala at UMI8 in Western blots showed no significant difference from that at CTR8 (Figure 4). Therefore, we further considered the involvement of behavior-regulating hormones.

Maternal behavior-regulating hormones (Demura, 1994) are mainly produced in the hypothalamus or act on the hypothalamus (Matsushita et al., 2019), which include OT, glucocorticosteroid, estrogen and others (Liu & Herbison, 2016; Swaab et al., 2005). Estrogen mainly functions shortly

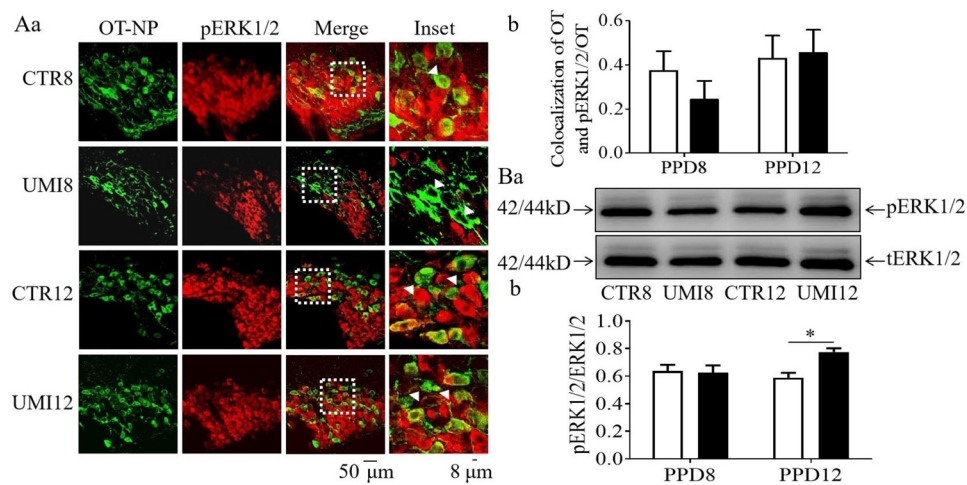


Figure 6. Effects of UMI on the expression of pERK1/2 in the SON. A. Confocal microscopic images (a) showing (from left to the right): OT-NP (in green), pERK1/2 (in red), their merges and inset ($n = 7$ from the dashed squares). The arrowheads point to pERK1/2 in non-OT neuron stains and the bar graph (b) summarizing the analysis of ratio of c-Fos-positive OT neurons (OT). (B) Representative Western blot bands (a) and the summary graphs (b) showing the expression of pERK1/2 (B, $n = 10$) under different experimental conditions.

* $p < .05$; ** $p < .01$ by two-way ANOVA.

OT-NP = oxytocin neurophysin; CTR = control; UMI = unfamiliar male invasion; pERK1/2 = phosphorylated extracellular signal-regulated protein kinase1/2; ANOVA = analysis of variance; SON = supraoptic nucleus.

before parturition and influences the initiation of maternal behavior (Russell et al., 2001). Activities of corticotropin-releasing hormone (CRH) neurons and hypothalamic-pituitary adrenal (HPA) axis exert an inhibitory role in maternal behavior (Klampfl & Bosch, 2019). However, the responses of HPA axis to various stresses are blunted during lactation (Smotherman et al., 1976), likely due to buffering effects of the release surge of OT during suckling (Yang et al., 2019). In our preliminary study, male rat intruders reduced but did not increase CRH expression in the PVN of rat dams (Liu et al., unpublished data). Thus, the signs of maternal anxiety and depression are not likely due to the activation of HPA axis. By contrast, OT is not only essential for the milk-ejection reflex (Hou et al., 2016) but also a key hormone regulating maternal behavior (Pedersen & Boccia, 2002; Meyer-Lindenberg et al., 2011). Thus, we focused on OT neuronal activity and its regulation.

OT Neuronal Activity and Maternal Behavior

It has been observed extensively that maternal behaviors depend on OT actions (Boutet et al., 2006; Bosch, 2011; Okabe et al., 2017; Riem et al., 2020). In the present study, acute social stress as shown at UMI8 significantly reduced lactation efficiency, which is a clear sign of the failure in the milk-ejection reflex that is normally driven by OT neuronal activity and OT secretion (Wakerley et al., 1994; Hatton & Wang, 2008). Thus, the disorders in maternal behavior of UMI dams can be attributable to disorders in OT neuronal

activity. By contrast, the adaptive behaviors and lactation performance are in agreement with the reports that the maternal sensitivity to adverse stimuli is reduced during lactation (Heinrichs et al., 2002; Murgatroyd et al., 2015).

In the present study, the relatively increased LBWGs of UMI12 versus UMI8 suggests that OT neuronal activity relatively increased at UMI12. Since increased OT neuronal activity suppresses social fear (Menon et al., 2018), the increased SON activity at UMI12 as shown in c-Fos and pERK1/2 expressions also suggests that the adaptive reactions of rat dams during chronic social stress are associated with increased OT neuronal activity or the efficiency of OT release. An increased OT secretion may occur during the milk-ejection reflex but not at nonsuckling period, judging from the improvement of LBWGs at UMI12, which needs to be clarified in further observation.

Another important finding is that the increased pERK1/2 and c-Fos proteins in the SON were not accompanied with increases in their presence in the somata of OT neurons at UMI12 (Figure 5 and 6). Since the SON contains OT neurons, vasopressin neurons, and astrocytes (Hou et al., 2016), the increased c-Fos and pERK1/2 expressions detected in Western blots should be attributable to vasopressin neurons and/or astrocytes although it remains to be excluded a robust increase in OTergic processes. The proposal is supported by the relative increase in the number of pERK1/2-positive non-OT neurons in UMI dams and the increased CBS expression at the VGL at UMI12. An increase in vasopressin neuronal activity in UMI dams can explain the remained anxiety-like reaction since vasopressin can promote anxiety (Csikota et al., 2016). Lastly, activation of astrocytes can

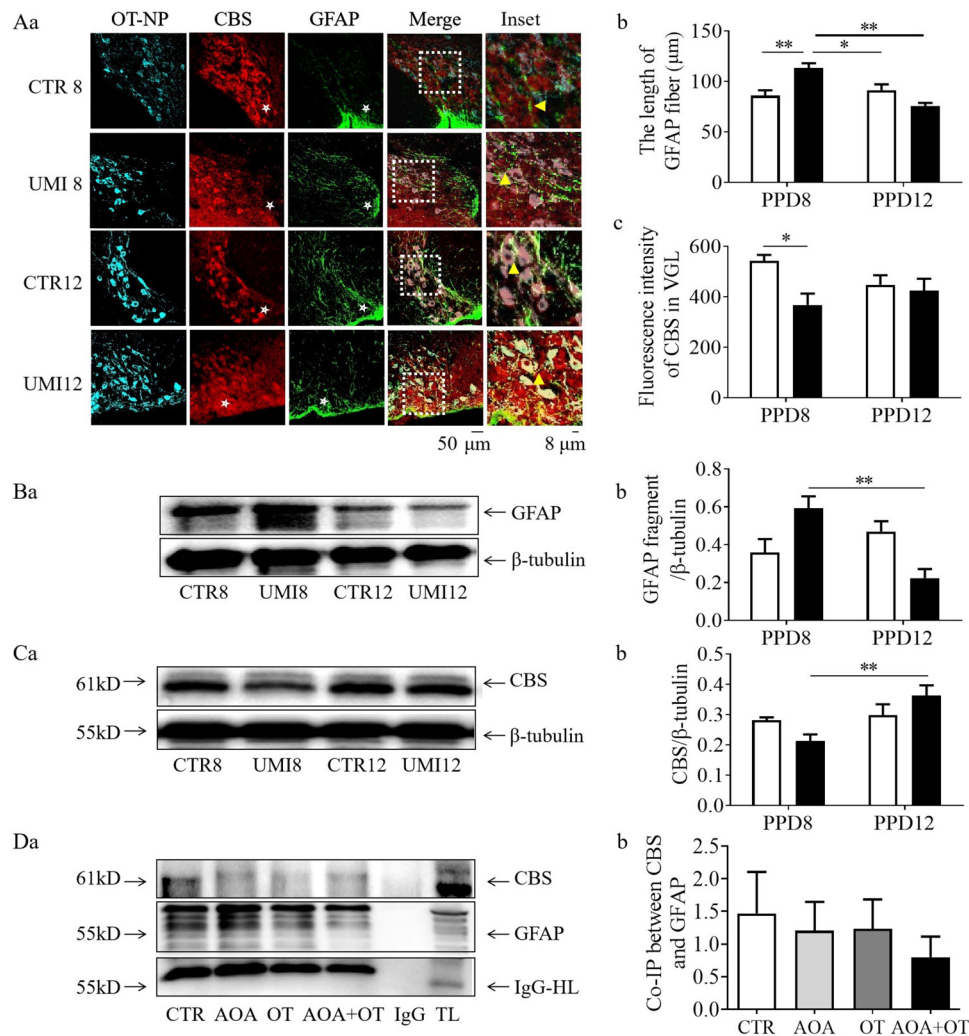


Figure 7. Effects of UMI on GFAP and CBS expressions in the SON. A. Confocal microscopy of immunofluorescence showing (from left to the right): OT-NP (in cyan), CBS (in red), GFAP (in green), their merges and the enlarged insets ($n = 5$ from the dashed squares). The stars indicate the VGL and arrowheads point to GFAP filaments near CBS-positive neurons. The summary graphs (b) showing GFAP filament/fiber length and fluorescence intensity of CBS-positive cells in ventral glial lamina (VGL), c). (B–C) Representative Western blot bands (a) and the summary graphs (b) showing GFAP fragments (B, $n = 5$) and CBS (C, $n = 7$), respectively.

* $p < .05$; ** $p < .01$ by two-way ANOVA. (D) Effects of AOA on OT-evoked changes in the molecular associations between GFAP and CBS. CBS = cystathionine β -synthase; CTR = control; GFAP = glial fibrillary acidic protein; OT-NP = oxytocin neurophysin; TL = total lysis; IgG-HL = immunoglobulin G-heavy chain; UMI = unfamiliar male invasion; ANOVA = analysis of variance; SON = supraoptic nucleus.

contribute to the restoration of OT neuronal activity during chronic social stress as further discussed below.

Astrocytic Regulation of OT Neuronal Activity During Social Stress

During lactation, suckling can elicit astrocytic morphological plasticity (Wang & Hatton, 2009b) and other neurochemical events including intrahypothalamic OT release (Neumann et al., 1993; Li et al., 2021b), patterned synaptic input (Wang & Hatton, 2004; Popescu et al., 2019), intercellular gap junctional coupling (Wang et al., 2019a), OTR-associated signaling events (Hatton &

Wang, 2008), and activation of some ion channels (Li et al., 2020c, 2021a). Among them, astrocytic plasticity plays an essential role as evidenced in previous (Wang & Hatton, 2009b; Wang et al., 2017) and the present observations.

In the present study, chronic social stress caused significant changes in astrocytic plasticity. As shown in Figure 7, consistent with the initial increase and then decrease in the expression levels of GFAP fragments, the length of GFAP filaments increased first at UMI8 and decreased at UMI12 (relative to UMI8). It is known that increased GFAP fragments are correlated with increased extension of GFAP filaments (Wang et al., 2017) and the extension of GFAP filaments and the associated

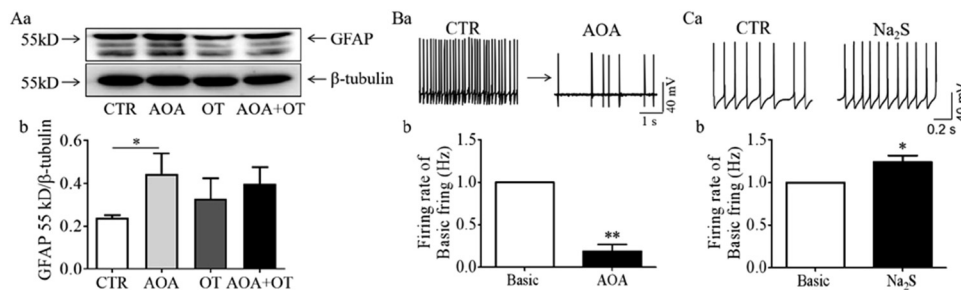


Figure 8. Effects H₂S on GFAP expression and OT neuronal activity in the SON. (A) Western blot bands (a) and the summary graph (b) showing the expression of GFAP in the SON of brain slices ($n = 6$) with CTR, AOA, OT, and AOA plus OT, respectively. * $p < .05$ compared with CTR by Student's t -test. (B) Representative episodes (a) and summary graph (b) showing effects of CBS inhibitor, AOA, on the firing activity of OT neurons ($n = 10$). (C) Representative episodes (a) and summary graph (b) showing effects of Na₂S on the firing activity of OT neurons ($n = 4$). ** $p < .01$ by Student's t -test.

AOA = aminoxyacetate; CTR = control; GFAP = glial fibrillary acidic protein; CBS = cystathionine β -synthase; SON = supraoptic nucleus.

expansion of astrocytic processes indicate increased astrocytic inhibition of OT neuronal activity (Wang & Hatton, 2009b). Thus, extension of GFAP filaments around OT neurons during acute social stress can inhibit OT neuronal activity. By contrast, the relatively reduced GFAP fragments and reduced GFAP filaments mirror the facilitation of astrocytic process retraction on OT neuronal activity (Li et al., 2020b). Therefore, the dual morphological plasticity of astrocytes plays an important role in the changes in OT neuronal activity in at different stages of social stress as previously reviewed (Wang et al., 2020).

The contribution of this dual astrocytic plasticity to the maternal behaviors is likely associated with its influence on synchronized activation of individual OT neurons and the resultant pulsatile release of OT. Pulsatility of OT secretion is determined by a synchronized burst-like firing activity among OT neurons in the SON and PVN (Belin et al., 1984; Wang et al., 1996). The synchronized burst firing activity triggers pulsatile OT release from neural terminals, which causes milk letdown in the mammary glands (Hou et al., 2016). Obviously, expansion of astrocytic processes enforces their barrier effect on adjacent OT neurons and blocks their mutual interactions, thereby weakening the coordination of their electrical activity and burst synchrony and causing hypogalactia. Moreover, OT neurons also have axon collaterals that innervate maternal behavior-regulating brain regions (Mason et al., 1984). Thus, synchronized burst firing of OT neurons and the resultant pulsatile OT release can influence maternal behaviors (Nissen et al., 1998) and milk letdown (Higuchi et al., 1985) simultaneously. By contrast, disruption of such synchrony results in disorders in maternal behaviors.

Notably, at nonsuckling period, OT neurons remain at relatively low activity state (Wang et al., 1996; Wang & Hatton, 2007) and thus, even no significant increase in pERK1/2 and c-Fos expressions was present in OT

neurons in our samples at UMI12, synchronized burst can still be evoked during suckling due to the shortening of GFAP filaments, thereby helping the synchronization and restoration of maternal behaviors.

Interaction Between H₂S and Astrocytic Plasticity in the Adaptation

At molecular levels, GFAP plasticity and OT neuronal activity are associated with the expression of CBS. CBS is the main source of H₂S in human brain, and has obvious colocalization with astrocytes (Enokido et al., 2005). The present study confirms the expression of CBS in astrocytes in the SON by showing the heavy labeling of CBS at the VGL and by presenting direct molecular association between GFAP and CBS (Figure 7).

Further analysis also highlights a feedback effect of H₂S on astrocytic plasticity as shown in Figure 7A that the increased length of GFAP filaments at UMI8 is accompanied with a trend of decrease in CBS expression. By contrast, the expression of CBS in the SON significantly increased at UMI12 relative to UMI8, which is accompanied with decrease in the length of GFAP filaments. Thus, the reduction of CBS or decrease in H₂S production increases GFAP expression and expansion of astrocytic processes in acute social stress while increased CBS expression results in retraction of GFAP filaments and astrocytic processes. Correspondingly, the excitability of OT neuronal activity, particularly the synchronized burst firing, decreases in acute social stress while at chronic social stress, the excitability of OT neurons recovers. The changes in OT neuronal activity can subsequently change the milk yield (i.e., LBWGs) and maternal behaviors. This proposal is supported by the direct inhibitory effect of AOA and excitatory effect of Na₂S on the firing rate of OT neurons.

The excitatory effect of H₂S can also explain the remaining anxiety effect of chronic social stress. As a diffuse

neurotransmitter/gliotransmitters (Yang et al., 2021), increased CBS expression/H₂S production can also activate vasopressin neurons that can cause anxiety (Plasencia et al., 2019) at chronic social stress. Notably, AOA-increased expression of 50 kD GFAP proteins (Figure 8A) seems against the finding that increased GFAP monomers are associated with retraction of GFAP filaments (Wang et al., 2017) and thus it is necessary to identify the positioning of GFAP filaments in astrocytic somata versus processes. However, the blockade of AOA on OT-evoked change in GFAP expression (Wang & Hatton, 2009b) supports a proposal that H₂S could be one signaling event in OT actions.

Conclusion

As a whole, increased interactions between astrocytes and OT neurons by the mediation of H₂S are likely responsible for the adaptation of rat dams to chronic social stress. This study provides unique references for understanding the mental health issues of breastfeeding women who suffer from social stress as well as therapeutic targets for clinical managements.

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Authors' Contribution

DYL and HTL wrote the first draft; HTL and DYL collected data; HTL, DYL, and HYW analyzed data; SJ, XW, SL, and GC participated in discussion and revision significantly; XYL supervised the study; YFW conceived the study and edited the draft.



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