Neurobiology of Stress 27 (2023) 100570

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr

The mineralocorticoid receptor and extra-synaptic NMDA receptor in the lateral habenula involve in the vulnerability to early life stress in the maternal separation model

Miseon Kang [a,c], Jun-mo Chung [a], Jihyun Noh [b,**], Jeongyeon Kim [c,*]

[a] Department of Brain and Cognitive Sciences, Brain Disease Research Institute, Ewha Woman's University, Seoul, South Korea
[b] Department of Science Education, College of Education, Dankook University, Yongin, South Korea
[c] Emotion, Cognition & Behavior Research Group, Korea Brain Research Institute, Daegu, South Korea

ARTICLE INFO

Handling Editor: Dr. Tallie Z Baram

Keywords:
Stress vulnerability
Lateral habenula
Extra-synaptic LTD
Mineralocorticoid receptor

ABSTRACT

The lateral habenula (LHb) plays a pivotal role in regulating emotional responses during stress reactions, and its hyperactivity has been associated with depression. Recently it has been demonstrated that chronic early-life stress results in individual differences in stress vulnerability among rodents. However, how synaptic function in the LHb varies between susceptibility and resilience to early life stress remains elusive. In this study, we used a maternal separation model to assign animals with different stress vulnerabilities into groups and investigated the synaptic responses in the LHb. Our findings indicate that synaptic long-term depression (LTD) was impaired and extra-synaptic LTD was enhanced in the LHb of the susceptible group. To mimic the synaptic alteration in stress situations, when administered corticosterone, a stress hormone, the intervention appeared to impair synaptic LTD in the LHb of the control group, through the activation of mineralocorticoid receptors (MR). Indeed, there was an up-regulation of MR mRNA observed in the susceptible group. Following there was an up-regulation of both NR2A and NR2B subunits in the LHb. These results indicated that MR and extra-synaptic NMDA receptors in LHb are critically engaged in the susceptibilities to stress. Furthermore, our findings propose potential therapeutic targets for alleviating stress-related symptoms.

1. Introduction

Lateral habenula (LHb) modulates and regulates emotional changes, and potentiates stress-induced depression-like behavior in animal models (Li et al., 2011; Shabel et al., 2012; Tchenio et al., 2017; Valentinova and Mameli, 2016). In stressed animals, increased neuronal activity in the LHb has been observed (Li et al., 2011, 2013; Valentinova and Mameli, 2016; Yang et al., 2018). This heightened activity is closely associated with depressive behavior. Conversely, the inhibition of the enhanced neuronal activity within the LHb is linked with significant relief in stress response (Li et al., 2011, 2013; Kang et al., 2020). Consequently, stress triggers alterations in excitatory neurotransmission in the LHb. It is thereby crucial to modulate neuronal activation within the LHb to effectively manage emotional changes.

The hyperactivity in the LHb is associated with depression-like behavior in animal models. Inactivation of the aberrant LHb activity induced relief in stress responses (Cui et al., 2019, Li et al., 2011; Li et al., 2013; Yang et al., 2018). In stressed animals, an increase in neuronal activity has been observed in both presynaptic and postsynaptic neurons of the LHb. According to previous studies, impairment of the eCB-dependent signaling promotes depressive-like behavior (Valentinova and Mameli, 2016; Park et al., 2017). And then, up-regulated βCaMKII mediates postsynaptic AMPAR trafficking associated with synaptic potentiation (Li et al., 2013). Recent studies suggest that dysfunction in astroglial potassium channels may play a role in the pathophysiology of depression. The up regulation of Kir4.1 channels in the LHb of depressed animal models may contribute to the development of depressive symptoms (Yang et al., 2018). Altogether, neuronal activity of the LHb region plays key role in emotional changes.

We previously reported that an increase in glutamate release due to stress can induce extra-synaptic LTD by inhibiting glutamate transporters. In addition, the regulation of extra-synaptic LTD depends on the

* Corresponding author.
** Corresponding author.
E-mail addresses: jihyun2@dankook.ac.kr (J. Noh), jykim@kbri.re.kr (J. Kim).

https://doi.org/10.1016/j.ynstr.2023.100570
Received 31 May 2023; Received in revised form 5 September 2023; Accepted 10 September 2023
Available online 18 September 2023

activation of the NMDA receptor and it is unaffected by acute stress (Kang et al., 2020). However, the mechanisms of extra-synaptic LTD regulation in relation to delayed exposure from acute or chronic stress based on stress vulnerability require further investigations. According to Tchenio et al., early-life chronic stress such as maternal separation causes LHb hyperactivity by reducing $GABA_BR$-G-protein coupled inwardly rectifying potassium channel (GIRK) function. The $GABA_B$--GIRK signaling alteration will worsen depression-like behavioral symptoms (Tchenio et al., 2017). However, the effect of stress vulnerability, which is caused by exposure to early-life chronic stress, on the long-term regulatory mechanism of LHb synapse efficiency has never been investigated. In addition, it is still necessary to examine whether stress vulnerability has an impact on the modulation mechanism of the extra-synaptic LTD.

Repeated exposure to stress activates the hypothalamic-pituitary-adrenal (HPA)-axis in our bodies to release cortisol (corticosterone), a stress hormone that maintains homeostasis. These stress hormones act on the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) to control the homeostasis mechanism for stress (Reul and Kloet, 1985; Prager and Johnson, 2009). However, long-term chronic stress exposure causes an imbalance in homeostatic mechanisms, leading to various behavioral and physiological changes (Karatsoreos et al., 2010; Schoech et al., 2011; McEwen 2004). Recently, research on the control of neuronal activity in several brain regions such as hippocampus, amygdala and anterior cingulate cortex that are modulated by stress hormones has been conducted (Kim et al., 2019; Harrewijn et al., 2020; Souza-Talarico et al., 2011). However, there is still a lack of research on the regulatory mechanisms of synaptic plasticity through corticosterone and specific corticosterone receptors. This study established an early life stress model to investigate whether stress vulnerability results in changes in synaptic plasticity and intrinsic properties. Additionally, the study investigated the modulation mechanism of synaptic plasticity, which is mediated by stress hormones.

2. Materials and methods

2.1. Animal

Outbred Sprague Dawley pregnant rats (TP 10) were purchased from Orient Bio Inc. (Seongnam, South Korea). Prior to giving birth, the pregnant rats were well-rested following conditions in the animal facility. The animals were maintained on a 12-h light-dark cycle and had free access to food and water in a specific-pathogen-free facility with controlled temperature (22 ± 2 °C), humidity (50 ± 10%), and lighting (150–300 lux). All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Korea Brain Research Institute.

2.2. Maternal separation model

We established the maternal separation model by modifying a part of the method in a previous study (Kalinichev et al., 2002). Briefly, we separated half of the pups (P3), regardless of sex, randomly from their dam rat for 15 days (4 h/day, from 10:00 a.m. to 2:00 p.m.) and placed in separate cages located in a distinct room to avoid communication between the pups and their dam rat using ultrasonic vocalization. All pups were labeled using a marker pen (SAKURA #130) and re-marked every week to keep track of each of them. During separation, the isolated pups rested on a wireless heating pad (at 37 °C) to stabilize their body temperature. Then, the pups were returned to their home cage and sufficiently given maternal care with their siblings. We provided handling steps for both non-separated and separated pups to minimize the impact of other stressors. After the maternal separation procedure was completed, we assigned siblings with maternal care to the control group and the separation group without maternal care, which were subcategorized to the resilient and susceptible groups using the principal component analysis (PCA) with immobility times of the FST and TST in all experimental groups. We used only adolescent male rats for behavioral, electrophysiological, and molecular experiments.

2.3. Depressive-like behavior test

2.3.1. Forced swim test (FST)

Behavioral experiments were performed as previously described by Porsolt et al. (1977). The transparent acrylic cylinder used was 40 cm and 20 cm in height and diameter, respectively, and it was filled with tap water set at room temperature (25 °C) to 26 cm from the bottom of the cylinder. On the first day, all rats swam in the water cylinder for 10 min in the habituation session. On the next day, the rats were placed in an acrylic cylinder containing water for 6 min (including an acclimation time of 1 min) for the FST test session. We conducted all sessions in a counterbalanced manner. Which we chose a rat one by one in each experimental group and a total of two rats chosen in each group were used at the same time for the FST test. During the FST, we recorded depressive-like behavior utilizing a video tracking system (Camcorder, Sony). After each session, the rats were dried with a paper towel gently and returned to their home cages. The immobility time during the test was measured for the analysis.

2.3.2. Tail suspension test (TST)

The TST was based on the method described by Steru et al. (1985). A TST apparatus (60 × 40 × 22 cm, Scitech Korea Inc., South Korea) was used for the test. All experimental groups of rats performed the TST in an apparatus consisting of four separated partitions at 10 cm intervals, ensuring that their movements would not affect each other. This rat's tail was suspended with a suspension bar (above 25 cm from the apparatus bottom). We conducted all sessions in a counterbalanced manner, total of four rats chosen in each experimental group were performed at the same time. In the habituation session, rats were conducted and adapted for 6 min. In the test session, climbing and immobility times were determined using a video tracking system (Camcorder, Sony) for 6 min. After TST experiments, the rats were returned to their home cages, The immobility times were manually counted for all data sets.

2.4. Electrophysiology experiment

2.4.1. Brain slice preparation

All male rats were anesthetized using isoflurane (Hana Pham.) via aspiration. Brain slices, sagittal sections containing LHb (400 μm thick), were prepared from 17 to 24-day-old rats using vibratome (VT1200, Leica) with an ice-cold sucrose solution (in mM: sucrose, 201; $NaHCO_3$, 26; glucose, 10; KCl, 3; NaH_2PO_4, 1.25; $MgCl_2$, 3; and $CaCl_2$, 1 (saturated with 95% O_2/5% CO_2). And then, slices were transferred to an incubation chamber filled with artificial cerebrospinal fluid (aCSF) containing the following (in mM): NaCl, 126; $NaHCO_3$, 26; glucose, 10; $MgSO_4$, 1.3; NaH_2PO_4, 1.25; KCl, 3; and $CaCl_2$,) at 21–23 °C during 1 h for the recovery. For recording, the slices were placed in a recording chamber and continuously superfused with oxygenated aCSF at a flow rate of 2–3 mL/min and temperature of 30 ± 2 °C using an in-line heater (Warner Instrument, Hamden, CT).

2.4.2. Extracellular field recording

To measure the field excitatory postsynaptic potentials (fEPSPs), recording glass capillary filled with aCSF solution with a tip impedance of 4–8 MΩ (World Precision Instruments, Inc., Sarasota, FL, USA, #1B150F-4) was placed on the LHb and stimulating electrode (FHC, Bowdoin, Me, USA; 125 μm/Rnd/25 μm Pt-lr, concentric bipolar electrodes, #30215) was placed into the stria medullaris (SM) fibers. Signals were obtained using WinLTP 2.31 software (winltp.com, and The University Bristol, UK) and National Instruments Data Acquisition (NI DAQ, BNC-2110, USA). To investigate intrinsic property changes in the LHb

due to early life stress, we measured the input-output response (I/O curve, stimulus intensities from 0 to 200 μA) and paired-pulse ratio (PPR, inter-stimulus intervals; 25, 50, 100, and 200 ms). To exclude the GABAergic transmission, *f*EPSP sweeps were recorded in the presence of picrotoxin (100 μM) diluted with aCSF solution. Before the induction of synaptic plasticity, we recorded a stable baseline for 15 minutes with consistent stimulus intensity. The initial *f*EPSP was recorded with half of the maximum intensity (from 80 to 100 μA). After obtaining stable baseline for 30 min, LTD was induced by applying 900 pulses at 1 Hz (LFS) through a concentric bipolar electrode with the same stimulation intensity used during baseline experiments. And then, LFS-induced LTD was maintained for over 60 min. The *f*EPSP slope was used to assess the changes in synaptic strength. The time course diagram shows averaged normalized *f*EPSP slope values over all recording times. Merged *f*EPSP traces presented an average 40 *f*EPSP sweeps before and after LFS. All electrophysiological data sets were obtained one by one from a rat in each experimental group. The sample size is equivalent to the number of rats used in the experiment.

2.5. Corticosterone assay

A corticosterone HS (high sensitivity) EIA kit (Immunodiagnostic Systems Ltd, IDS Ltd; Boldon, United Kingdom, #AC-15F1) was used to determine corticosterone concentration. Blood was drawn from the control and experimental groups' rats and centrifuged at 3000 rpm for 15 min (Eppendorf centrifuge 5810 R, Brinkmann Instruments Co., Westbury, NY) to obtain blood serum only. The experimental procedure was conducted in accordance with the corticosterone HS EIA kit manufacturer's guidelines. All sample sizes were calculated in blood serum per mouse.

2.6. Drugs

$_{DL}$-TBOA ($_{DL}$-threo-β-benzyloxyaspartate, *Cat.* #1223), Mifepristone ((11β,17β)-11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one, *Cat.* #1479), (+)-MK-801 maleate ((+)-5-methyl-10,11-dihydroxy-5H-dibenzo (a,d)cyclohepten-5,10-imine, *Cat.* #0924), Spironolactone ((7α,17α)-7-(acetylthio)-17-hydroxy-3-oxo pregn-4-ene-21-carboxylic acid γ-lactone, *Cat.* #2968), Corticosterone (Tetrasodium hydrate, 11β,21-Dihydroxy-4-pregnene-3,20-dione, *Cat.* #27840) an d SR-95531 (2-(3-Carboxypropyl)-3-amino-6-(4 methoxyphenyl)pyridazinium bromide, *Cat.* #S106) were obtained from the Tocris Cookson (Bristol, UK). Ketamine ((R,S)-ketamine hydrochloride, Yuhan Corporation, Seoul, Korea) was diluted with aCSF solution (50 mg/mL, 100 μM). For drug application, the aCSF solution was replaced with a drug solution for at least 15 min before LFS.

2.7. Gene expression analysis

2.7.1. RNA extraction and cDNA synthesis

To evaluate relative gene expression changes, we first performed RNA extraction for the control and separation groups. For tissue preparation, we obtained coronal brain slices including LHb region with a thickness of 400 μm using a Leica vibratome (Leica, #VT1200) and micro-dissected the LHb (AP −2.2 mm to −4.2 mm from bregma) in an ice-cold aCSF solution under a stereomicroscope (Olympus, #SZ61). We prepared an LHb tissue sample pooled from four rats. The tissue lysis solution consists of β-mercaptoethanol in RLT solution (10 μL/ml, sample pooling; 350 μl RLT solution/tube addition). Carrier RNA (20 μg) was added prior to tissue lysis (5 μL of a 4 ng/μL solution). RNA extraction was performed using an RNeasy Micro Kit (*Cat.*# 74,004). The cD\NA was synthesized with the use of the Reverse Transcription 5× Premix (random hexamer, ELPIS-Biotech, Deajon, Korea, EBT-1514) for 60 min at 40 °C, followed by enzyme inactivation for 5 min at 92 °C.

2.7.2. Quantitative real time-PCR

Quantitative real-time PCR was performed using a Real-Time PCR 7500 Fast system (Applied Biosystems, Foster City, CA, USA) and qPCR 2× Master Mix with SYBR Green and ROX dye (ELPIS-Biotech, Deajon, Korea, EBT-1802). The PCR cycling was performed for 3 min at 94 °C, followed by 40 cycles for 10 s at 94 °C, 10 s at 55 °C, and 30 s at 72 °C. β-actin was used for normalization, and relative gene expression was calculated according to a previously reported method (Schoech et al., 2011). The following oligonucleotides pairs were used: NR2A forward primer sequence (5′-3′); GAC GGT CTT GGG ATC TTA AC, NR2A reverse primer sequence (3′-5′); TGA CCA TGA ATT GGT GCA GG, NR2B forward primer sequence (5′-3′); CAA GAA CAT GGC CAA CCT, NR2B reverse primer sequence (3′-5′); GGT ACA CAT TGC TGT CCT GC, mineralocorticoid receptor (MR) forward primer sequence (5′-3′); CCA AAG GCT ACC ACA GTC TC, MR reverse primer sequence (3′-5′); TCC CAG ACC GAC TAT TGT CT, glucocorticoid receptor (GR) forward primer sequence (5′-3′); AGG CAG TGT GAA ATT GTA TCC CAC, GR reverse primer sequence (3′-5′); GAG GCT TAC AAT CCT CAT TCG GTG T, GLAST forward primer sequence (5′-3′); GGG TTT TCA TTG GAG GGT TGC, GLAST reverse primer sequence (3′-5′); CCA CGG GTT TCT CTG GTT CAT, GLT-1 forward primer sequence (5′-3′); GGG TCA TCC TGG ATG GAG GT, GLT-1 reverse primer sequence (3′-5′); CGT GTC ATA AAC GGA CTG, *β*-actin forward primer sequence (5′-3′); GGA GAT TAC TGC CCT GGC TCC TA, and *β*-actin reverse primer sequence (3′-5′); GAC TCA TCG TAC TCC TGC TG. All oligonucleotides were synthesized using BIONICS (Korea).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to examine significant differences among groups with post-hoc test, *Tukey's multiple comparisons* between groups. In electrophysiological experiments, repeated measure two-way ANOVA was performed to examine the interaction effect of the variations among groups with *Tukey's multiple comparisons*. The significant difference between groups was determined using *unpaired Student's t-test* for comparisons or *paired Student's t-test*. To avoid arbitrarily splitting the continuous data from FST and TST into susceptible and resilient groups, we performed principal component analysis (PCA). Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed by using GraphPad Prism software 10.0.02 version (GraphPad Software, La Jolla California United States). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Vulnerability to early life stress in maternal separation rat model

To test vulnerability to chronic early life stress, we utilized the maternal separation stress paradigm regarding to the previous literatures (Kalinichev et al., 2002; Vetulani, 2013) where postnatal 3-day-old male rats were isolated postnatal 3-day-old male rats from their dam for 4 h/day for 15 days (Fig. 1A). In FST and TST test for measuring stress related behavioral changes such as depressive-like behavior, separation group showed increased the immobility time compared with the control group (Suppl. Fig. 1A and B, FST; control group; 25.81 ± 4.17 s, n = 23, separation group; 61.18 ± 8.05 s, n = 39, TST; control group; 64.96 ± 93.54 s, n = 25, separation group; 93.54 ± 7.78 s, n = 41). Despite experiencing maternal separation, some rats exhibited similar level of immobility time compared to the control group. To further classify stress vulnerability with unbiased manner, we conducted a multiple principal component analysis (PCA) involving immobility times from FST and TST between the control and separation groups (Fig. 1B–C). As depicted in Fig. 1B, some of the separated rats, which overlap with the control group, are classified as stress-resilient groups. In addition, the remaining separated rats, from R1 to R3, are categorized into susceptible groups (yellow shades outlined by dashed lines). Based on the cluster identified

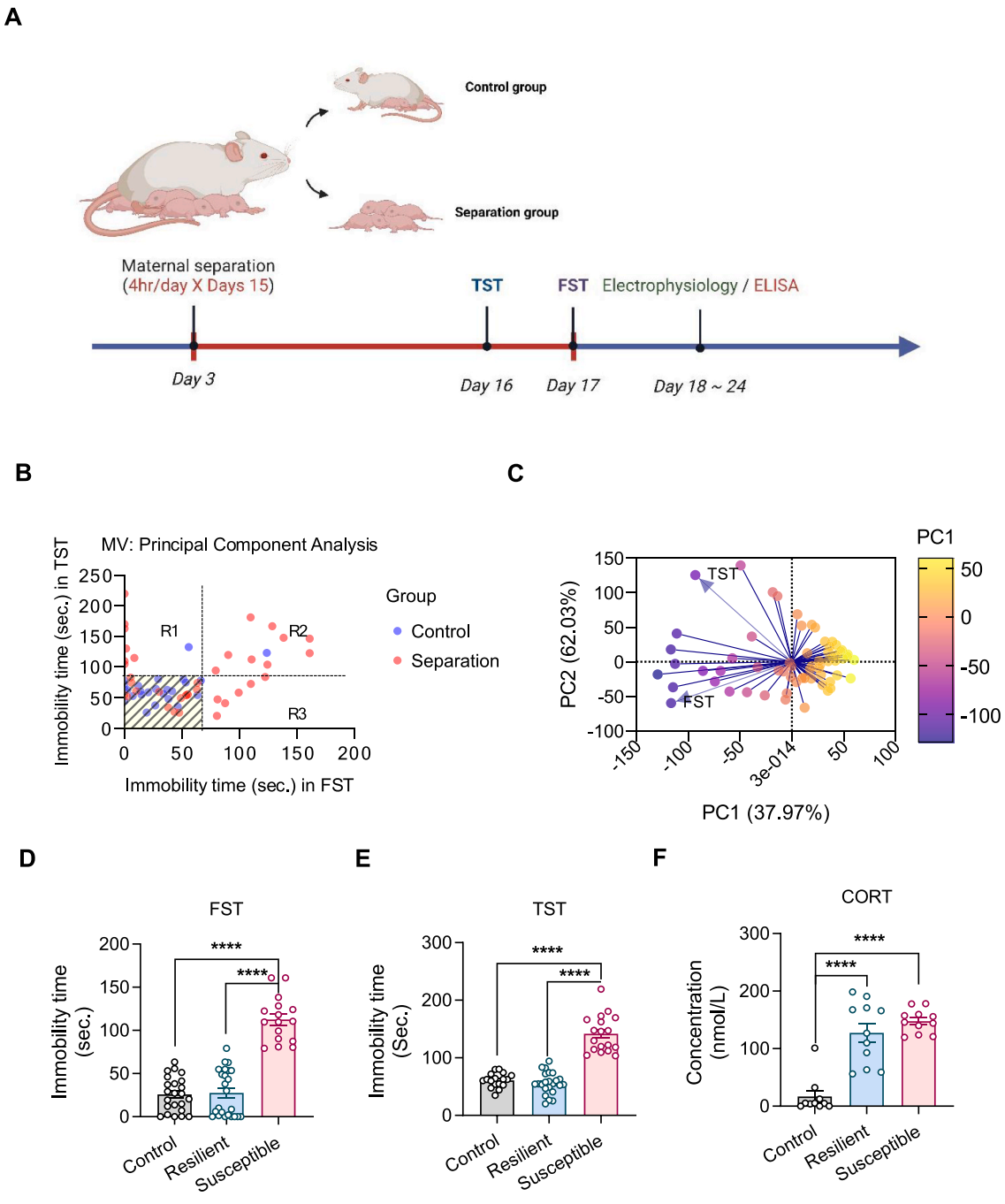


Fig. 1. Early life stress induces physiological and behavioral changes. (A) Graphical illustration of the experimental flowchart. Maternal separation stress paradigm in P3–P17 rats (Red line). Some male rats that were given maternal care were isolated from the dam rat for 4 h per day for 15 days. (B) Multiple valuables chart of immobility time between FST and TST. A deviant crease-lined cluster indicates stress-resilient rats (yellow box). R1, R2, and R3 indicate stress-susceptible rats. (C) Principal component analysis (PCA) biplot with immobility times between FST and TST in early life stress model (PC1; 37.97%, PC2; 62.03%). (D and E) Classification of the stress vulnerability group for maternal separation model using the FST and TST analysis. Statistical significance was analyzed using one-way ANOVA with *Tukey's multiple comparison test*. Data were presented with the mean ± S.E.M. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

by PCA analysis, we reorganized and presented the bar graphs displaying the changes of FST and TST immobility times in the control, resilient, and susceptible groups (Fig. 1D, FST; control group, 25.81 ± 4.17 s, n = 23, resilient group, 27.67 ± 5.52 s, n = 24, one-way ANOVA with *Tukey's multiple comparisons test,* control vs resilient; ns, $P = 0.9632$, control vs susceptible; ****$P < 0.0001$, resilient vs susceptible; ****$P < 0.0001$) (Fig. 1E, TST; control group, 60.95 ± 2.97. Sec., n = 17, resilient group, 55.97 ± 3.95 s, n = 23, one-way ANOVA with *Tukey's multiple comparisons test*, control vs resilient; ns, $P = 0.7688$, control vs susceptible; ****$P < 0.0001$, resilient vs susceptible; ****$P < 0.0001$). Taken together, these findings suggest that chronic early-life stress induces individual differences in stress vulnerability in adolescent rats, as the resilient group, is distinguished from the susceptible group rats.

3.2. The alteration of intrinsic properties and synaptic plasticity in the early life stress model

As shown in Fig. 1, we were able to classify separation groups into susceptible group and resilient group, which have different stress vulnerabilities caused by maternal separation (Fig. 2A). Previous studies have reported that acute stress causes changes in the intrinsic properties and synaptic plasticity of the LHb (Kang et al., 2020; Tchenio et al., 2017; Valentinova and Mameli, 2016). To determine whether chronic exposure to the stress caused by maternal separation leads to the hyperactivity of the LHb neurons, we investigated intrinsic properties, such as the input-out response (I/O curve) and paired-pulse ratio (PPR) (Fig. 2C and D).

First, to determine whether stress vulnerability causes changes in synaptic efficacy, the stria medullaris fiber was gradually increased from 0 to 200 μA to measure synaptic responses (Fig. 2B). In the resilient group, I/O curves did not show significant changes compared to the control group. In contrast, we observed an increased *f*EPSP amplitude that was significantly enhanced from that of the other groups in the susceptible group (Fig. 2C, two-way ANOVA test *with Turkey's multiple comparisons*, $****P < 0.0001$, F (16, 64) = 12.25). Second, we recorded the PPR to determine synaptic release probability. The PPR recorded from the *f*EPSP amplitudes across varying inter-stimulus intervals (25, 50, 100, and 200 ms intervals) was not different between the control and resilient groups. However, in the susceptible group, the PPR decreased at an inter-stimulus interval of 25 ms (Fig. 2D, two-way ANOVA test *with Tukey's multiple comparisons test*, $*P = 0.0121$, F (6, 27) = 3.421). In summary, despite exposure to early life stress, pups exhibit changes in intrinsic properties depending on their stress vulnerability. In addition, when they are vulnerable to stress, there is an enhancement in synaptic efficiency due to an increase in the release probability of the glutamate transmitter.

When LFS-induced LTD was observed in the control group rats that did not experience separation from their dam after early life stress, control rats showed a stable LFS-induced LTD (Fig. 2E and F, control group, 71.4 ± 3.3%, n = 5). Even following maternal separation, LFS resulted in a decrease in *f*EPSP slopes and steadily induced LTD in the resilient group, which did not exhibit helplessness behavior (Fig. 2E and F, resilient group, 72.3 ± 6.4%, n = 5). There was no significant difference in the LTD between the resilient group and the control group. However, LFS failed to induce LTD in stress-susceptible rats after maternal separation, and *f*EPSP slope reverted to the baseline level (Fig. 2E and F, susceptible group, 100.0 ± 4.0%, n = 4, one-way ANOVA with *Tukey's multiple comparisons test*, control *vs* resilient; ns, $P = 0.9904$, control vs susceptible; $**P = 0.0051$, resilient vs susceptible; $**P = 0.0063$). Collectively, these results suggest that as the vulnerability to stress increased, LHb displayed more excitable synaptic responses, such as enhanced synaptic efficacy and impairment of LTD.

3.3. The effect of stress hormone on synaptic plasticity

In rats exposed to stress, the activation of the HPA axis leads to an increase in corticosterone levels (Johnson et al., 2006; Raubenheimer et al., 2006), which plays a crucial role in modifying synaptic plasticity across various brain regions (Bodnoff et al., 1995; Martin et al., 2009; Majcher-Maślanka et al., 2018). To assess the changes in stress hormone levels, we collected blood serum from rats following the established maternal separation model (Fig. 1F). Stress hormone concentrations in the blood serum were analyzed using an enzyme-linked immunosorbent assay (ELISA). The corticosterone concentration in the blood serum from the control group rats was found to be 16.53 ± 9.86 ng/mL. However, corticosterone levels in rats exposed to early stress showed a ten-fold increase. When the separation groups were subdivided and analyzed based on their levels of stress susceptibility, both the resilient and susceptible groups exhibited elevated corticosterone levels (Fig. 1F, control group, 16.53 ± 9.86 ng/mL, n = 10; resilient group, 127.5 ± 16.36 ng/mL, n = 11; susceptible group, 147.6 ± 6.22 ng/mL, n = 11, one-way ANOVA with *multiple Tukey's comparisons test*, control vs resilient; $****P < 0.0001$, control vs susceptible; $****P < 0.0001$, resilient vs susceptible; ns, $P = 0.4439$).

We conducted recordings of *f*EPSPs of a specific magnitude in the LHb of control group. Following the stabilization of *f*EPSPs for about 15 min, corticosterone was treated in the bath. Throughout the corticosterone administration (CORT, 10 μM), LFS failed to induce LTD (Fig. 3A and B, 102.3 ± 1.7%, n = 4). Therefore, LTD inhibition in rats exposed to stress resulted from corticosterone administration. It is noteworthy that corticosterone largely binds to and activates either the mineralocorticoid receptor (MR) or glucocorticoid receptor (GR) (McKay and Cidlowski, 2003; Taves et al., 2011). To determine whether the activation of specific corticosterone receptors, such as MR and GR, is involved in LTD impairment in the LHb, spironolactone (MR antagonist) and mifepristone (GR antagonist) were used to induce LTD. Pretreatment with both mifepristone and corticosterone failed to reverse the impairment of LTD (Fig. 3A and B, CORT + mifepristone; 102.6 ± 2.4%, n = 4), indicating that mifepristone did not affect corticosterone-induced LTD impairment. In contrast, spironolactone recovered corticosterone-induced LTD impairment. It suggests that spironolactone and corticosterone blocked MR, leading to LFS-induced LTD induction (Fig. 3C and D, CORT + Spironolactone; 80.6 ± 1.4%, n = 5, one-way ANOVA *with Tukey's multiple comparisons test*, CORT vs CORT + Mifepristone; ns, $P = 0.9936$, CORT vs CORT + Spironolactone; $****P < 0.0001$, CORT + Mifepristone vs CORT + Spironolactone; $****P < 0.0001$). Therefore, corticosterone-induced LTD impairment is caused by MR activation. To confirm the continuity of these stress hormones, we tried LTD induction again after a 60 min washout. Even after washing corticosterone with CSF solution for 1-h, LTD was still impaired (Fig. 3C and D, CORT; 102.3 ± 1.66%, n = 4, aCSF; 101.5 ± 1.77%, n = 4). Taken together, chronic exposure to early life stress was found to cause synaptic modification in the LHb, and the direction of synaptic plasticity was determined by stress sensitivity. Considering these stress responses, corticosterone is likely to regulate synaptic plasticity by activating the MR.

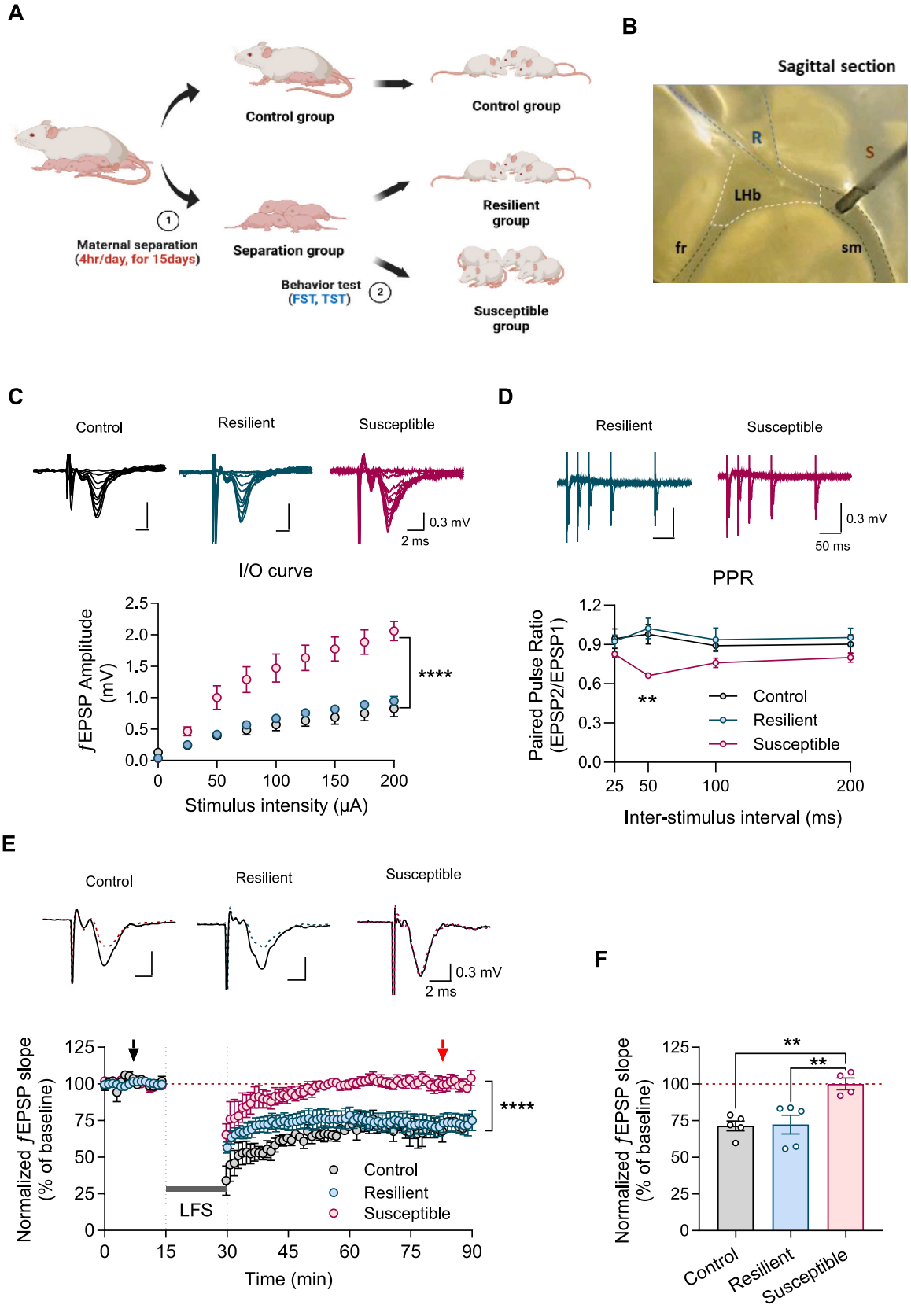
3.4. Modification of the extra-synaptic LTD associated with stress vulnerability

In Figs. 2 and 3, we confirmed that stress susceptibility resulted in increased glutamate release probability and synaptic efficacy. Because electrophysiological evidence for increased glutamate release probability and synaptic efficiency suggests that the possibility of extra-synaptic glutamate spillover cannot be ruled out, we also investigated the effects of stress vulnerability on extra-synaptic LTD.

Initially, we checked whether the exposure of the LHb to stress hormones could interfere with LTD induction at extra-synaptic sites in the control group. We confirmed the stable induction of the extra-synaptic LTD in the presence of DL-TBOA (selective glutamate transporter blocker, 10 μM) as well as CORT-induced LTD impairment in the LHb of control group (Fig. 4A and B, DL-TBOA; 84.35 ± 2.06%, n = 5, one-way ANOVA with *Tukey's multiple comparisons test*, CORT vs aCSF; ns, $P = 0.9571$, CORT vs DL-TBOA; $***P = 0.0001$, aCSF vs DL-TBOA; $***P = 0.0002$). This suggests that extra-synaptic LTD is not affected, even if the LHb is exposed to stress hormones.

Secondly, we examined the effects of extra-synaptic LTD on stress vulnerability. As illustrated in Fig. 4A, a stable LTD induction was observed in the resilient group. Therefore, treatment with MK-801, a selective synaptic NMDA receptor blocker, inhibited synaptic LTD. After pretreatment with DL-TBOA for 30 min, extra-synaptic LTD was induced (Fig. 4C and D, MK-801; 99.0 ± 1.5%, n = 6, DL-TBOA; 80.5 ± 3.2%, n = 6, *Paired t-test*, $**P = 0.0082$). In other words, it was confirmed that synaptic and extra-synaptic LTD mechanisms were not impaired in the LHb of the resilient group rats.

Third, we confirmed extra-synaptic LTD induction in the susceptible



(caption on next page)

Fig. 2. Potentiation of the synaptic efficacy in the LHb region associated with stress susceptibility. (A) Graphical illustration for maternal separation model separated into stress vulnerability. (B) Photograph of the sagittal section of LHb tissue. Sites of the recording electrode (R) and stimulus electrode (S) in brain slices containing the LHb. Field potentials (*f*EPSPs) were obtained in the LHb by stimulating the stria medullaris (SM). R, recording electrode; S; Stimulus electrode; LHb; Lateral habenula; fr, fasciculus retroflexus fiber; sm, stria medullaris fiber. Changes in intrinsic properties in the LHb region for the maternal separation model (C) Input-Output response (I–O curves) for control, resilient, and susceptible groups (stimulus intensities, from 0 to 200 μA) *Top panel:* *f*EPSP merge traces show the changes in input-output response for stimulus intensities (black traces: control group, blue traces: resilient group, red traces: susceptible group). (D) The paired-pulse ratio (PPR) is different for control, resilient, and susceptible groups depending on inter-stimulus intervals (25, 50, 100, and 200 ms) *Top panel:* *f*EPSP merge traces show changes in paired pulse stimulation (blue traces: resilient, red traces: susceptible) (E and F) Stress susceptibility mediates synaptic modifications in the LHb. Summary histograms show the LFS-induced LTD in the early life stress model. Statistical significance was analyzed using one-way ANOVA with *post-hoc test, Tukey's multiple comparison test*. Data were presented with the mean ± S.E.M. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

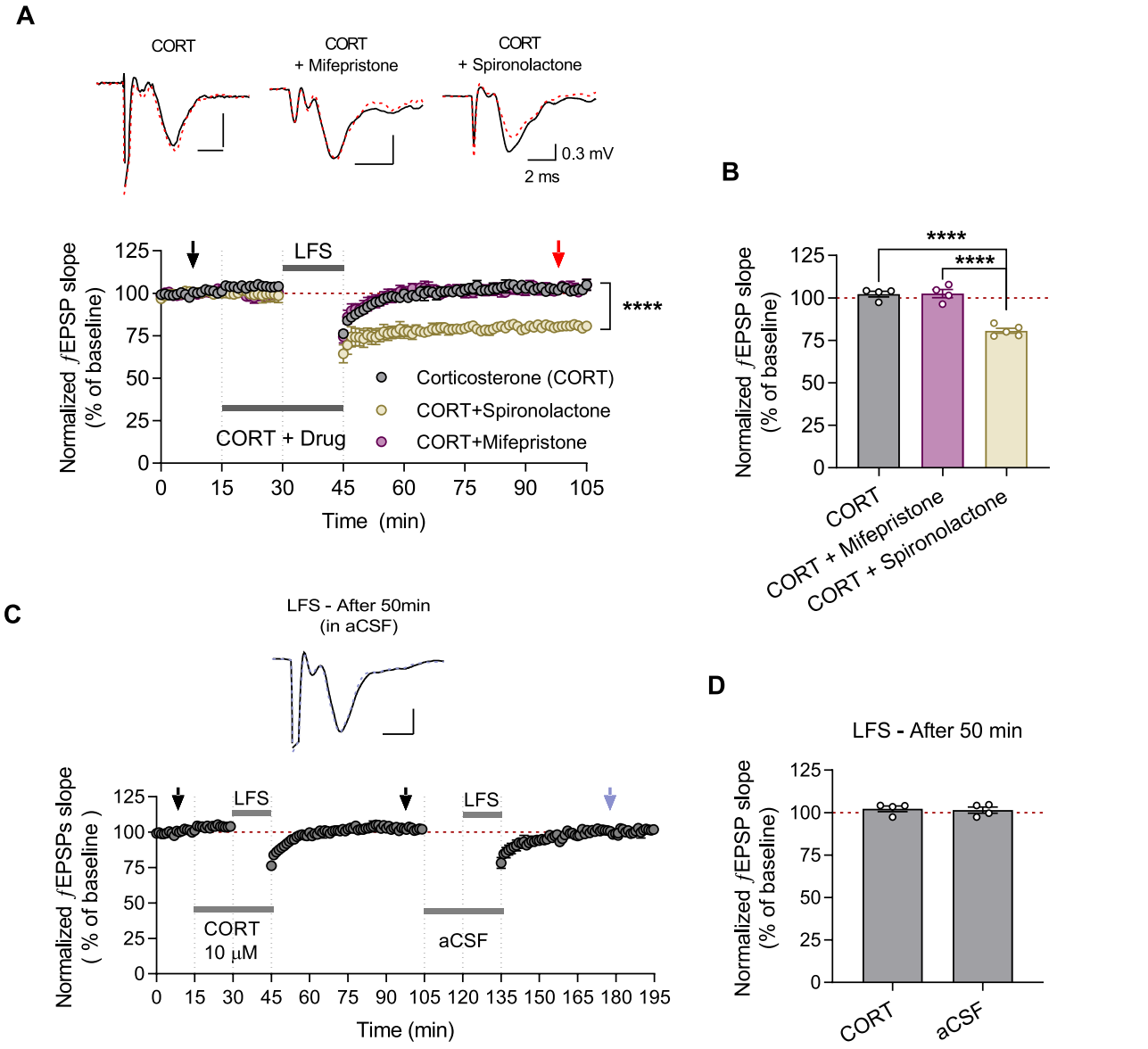


Fig. 3. Stress hormone impaired synaptic depression in the LHb region by stimulating mineralocorticoid receptors. (A and B) In control rats, corticosterone (CORT, 10 μM) induced impairment of the LTD in LHb. Spironolactone (10 μM), a mineralocorticoid receptor inhibitor, completely blocked corticosterone-induced LTD impairment, but mifepristone, a glucocorticoid receptor inhibitor, had no effect. Stress hormones modulate synaptic depression via mineralocorticoid receptors. (C and D) The effect of CORT persists even after washing out CSF for a 1 h. Data were presented with the mean ± S.E.M.

group. Along with the results illustrated in Fig. 2A, the susceptible group showed impairment of the LFS-induced LTD in the absence of MK-801 (Fig. 4E and F). After synaptic LTD impairment was identified, extra-synaptic LTD was induced by LFS during DL-TBOA application (Fig. 4E and F, LFS, 99.0 ± 3.0%, n = 4; DL-TBOA, 66.9 ± 2.9%; n = 4, *Paired t-test*, ***$P = 0.0003$*). Extra-synaptic LTD was much more enhanced in the susceptible group than in the resilient group. Summarily, the extra-synaptic LTD was preserved or increased, even though stress vulnerability caused synaptic LTD impairment in the LHb (Fig. 4G, Resilient; 82.37 ± 2.0%, n =68; Susceptible, 66.86 ± 2.93%, n = 4, *Unpaired t-test*, **$P = 0.0018$*).

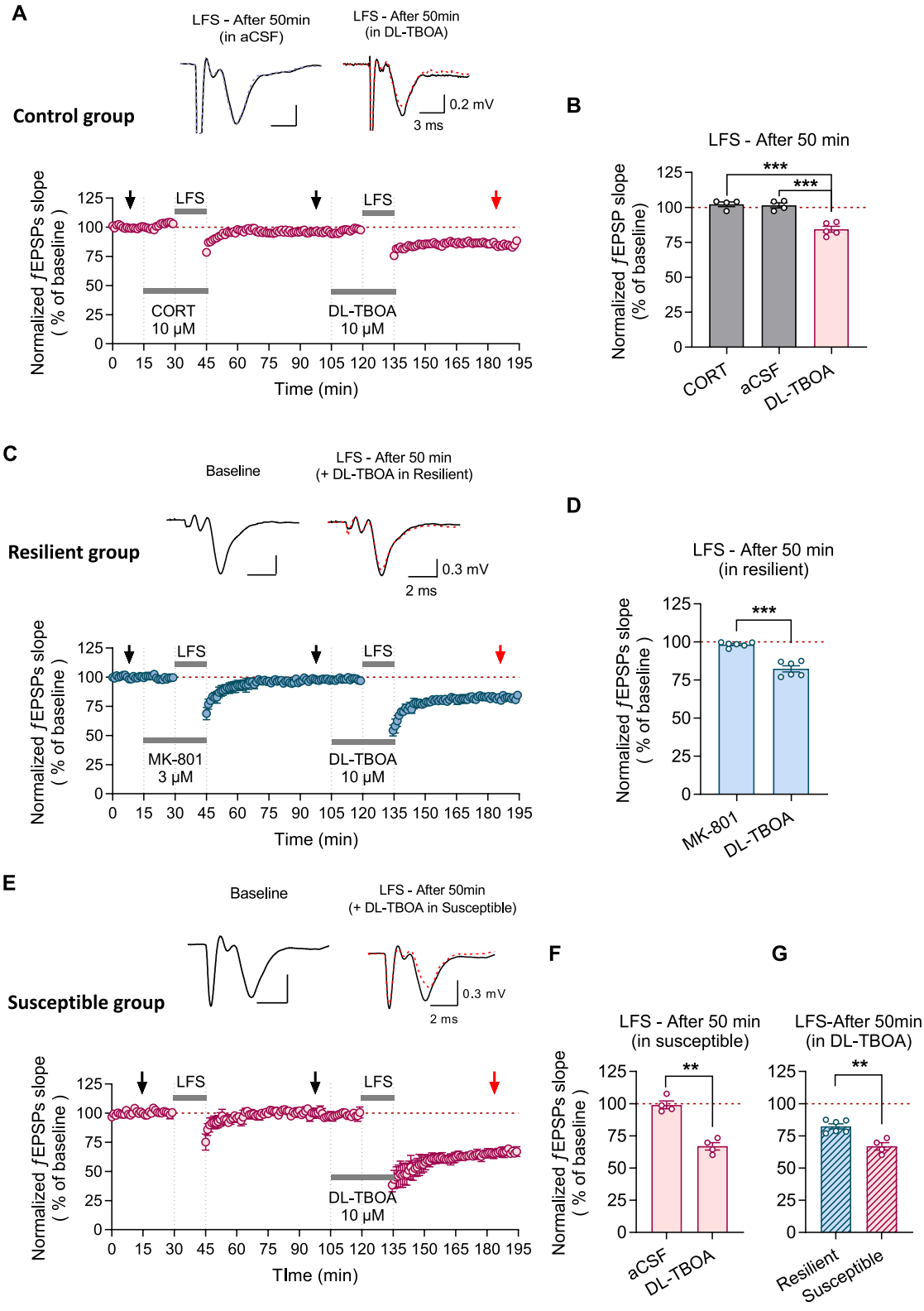


Fig. 4. Sensitivity of the extra-synaptic LTD in the stress vulnerability. (A and B) In control rats, the application of corticosterone failed to induce LTD and completely recovered to baseline levels. Even after washing corticosterone with aCSF for 1 h, LTD impairment continued. Summary histograms comparing the effects of LFS applied 50 min after LTD induction. The LTD was calculated 50 min after the end of LFS in the control group. (C and D) Preservation of extra-synaptic LTD in the resilient group. Summary histograms for changes of the *f*EPSP slope after 50 min of the applied LFS. (E and F) Extra-synaptic LTD facilitated in the LHb region of the susceptible group compared with the resilient group. Summary histograms show the induction of the LTD after 50 min of the applied LFS. All *f*EPSP traces show the changes in the baseline (black trace) and 50 min after LTD induction (red dotted trace). Data were presented with the mean ± S.E.M. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Validation of the gene profiling response to stress vulnerability

-Accordingly, previous studies have suggested that early-life stress induce modification of glutamate signaling-related molecules such as GLAST, GLT-1, NR2A and NR2B by responding to stressor (Own et al., 2013; Roceri et al., 2002; Zeng et al., 2020). NMDA receptors and glutamate transporters critically mediate the intrinsic properties and extra-synaptic LTD.

Therefore we performed qRT-PCR analysis to validate the gene expression of the LHb region for the maternal separation model

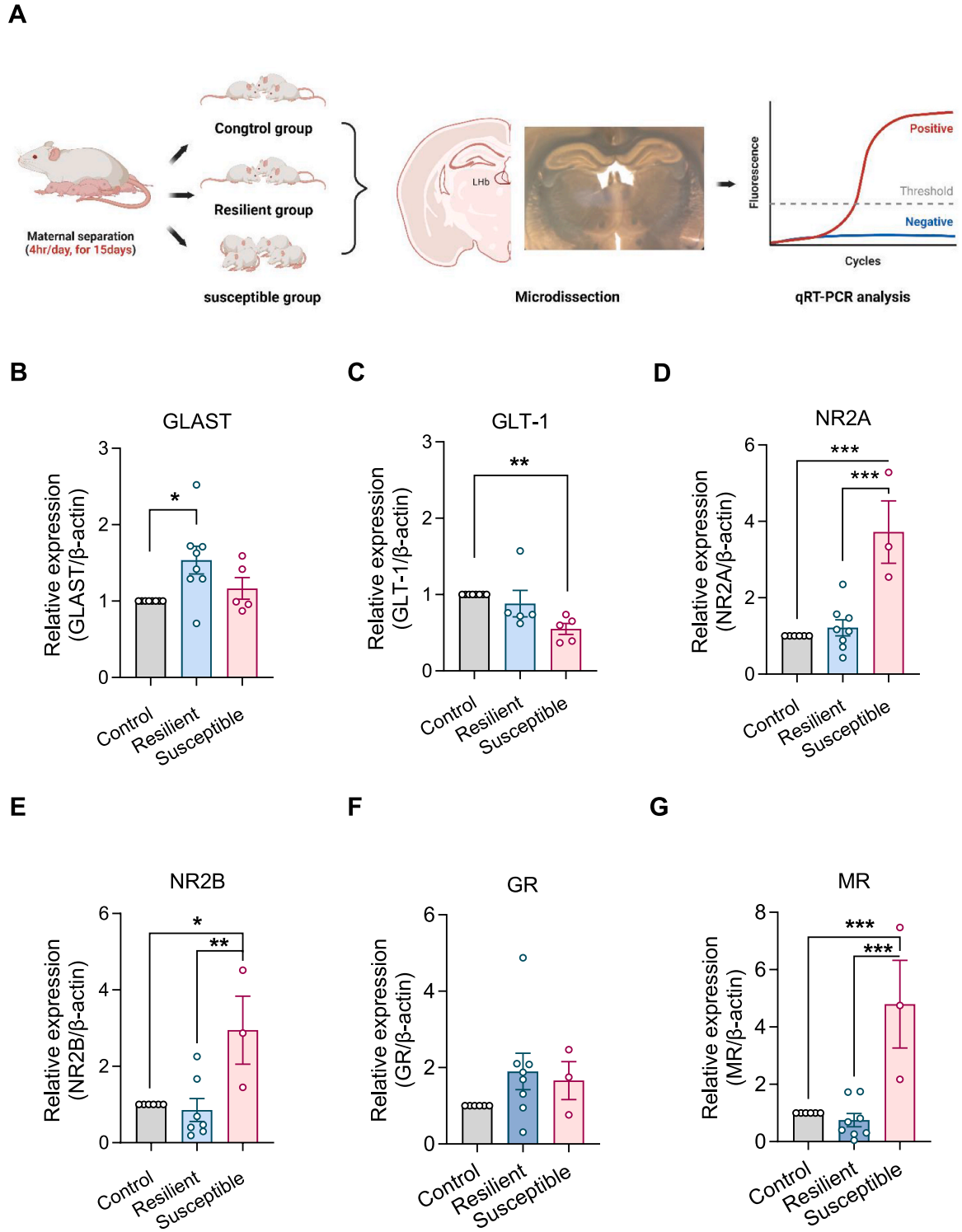


Fig. 5. Validation of the gene expression for the maternal separation model (A) Experimental paradigm for quantitative real time-PCR analysis. (B–G) Validation of the mRNA gene expression of the corticosterone receptors (MR, GR), glutamate transporter (GLAST and GLT-1) and NMDA receptor subunits (NR2A, NR2B) in the lateral habenula for the early life stress model. Statistical significance was analyzed using one-way ANOVA test with *post-hoc tests*, *Tukey's multiple comparison test* (***$P = 0.0003$). CORT; corticosterone, MR; mineralocorticoid receptor, GR; glucocorticoid receptor. Statistical significance was analyzed using one-way ANOVA test with *post-hoc test*. Data were presented with the mean ± S.E.M.

(Fig. 5A). In the early life stress model, the relative GLAST (EAAT1) gene expression in the LHb of the resilient group was slightly higher than that in the control group (Fig. 5B). In addition, in the susceptible group, there was no difference in the relative gene expression of GLAST compared to that in the control group (Fig. 5B, one-way ANOVA with *Tukey's multiple comparisons test*, control vs resilient; $*P = 0.0138$, control vs susceptible; ns, $P = 0.6383$, resilient vs susceptible; ns, $P = 0.1878$). GLT-1 (EAAT2), a glutamate transporter, was significantly reduced in the susceptible group (Fig. 5C, one-way ANOVA with *Tukey's multiple comparisons test*, control vs resilient; ns, $P = 0.6174$, control vs susceptible; $**P = 0.0066$, resilient vs susceptible; ns, $P = 0.0705$). Gene expression validation of the NMDAR subtypes containing NR2A and NR2B subunit was analyzed in rats chronically exposed to maternal separation (Fig. 5D and E). The NR2A and NR2B subtypes were significantly upregulated in the susceptible group compared with the control group. In contrast, gene expression in the resilient and control groups was similar level (Fig. 5D, one-way ANOVA with *Turkey's multiple comparisons test*, control vs resilient; ns, $P = 0.8309$, control vs susceptible; $***P = 0.0002$, resilient vs susceptible; $***P = 0.0002$) (Fig. 5E, one-way ANOVA with *Turkey's multiple comparisons test*, control vs resilient; ns, P = 0.9440, control vs susceptible; $*P = 0.0121$, resilient vs susceptible; $**P = 0.0064$). In conclusion, it appears that GLT-1 modification in the susceptible group causes synaptic potentiation. Thus, it was predicted that the increased expression of NR2A and NR2B affects extra-synaptic LTD induction. We used quantitative RT-PCR analysis to track changes in the MR and GR to genetically validate corticosterone receptor changes according to stress vulnerability (Fig. 5E and F). Using quantitative RT-PCR analysis, we detected no relative genetic change in the GRs in the control, resilient, and susceptible groups in the early life stress models (Fig. 5F, one-way ANOVA *with Tukey's multiple comparisons test*, control vs resilient; ns, $P = 0.2577$, control vs susceptible; ns, $P = 0.6339$, resilient vs susceptible; ns, $P = 0.9345$). However, the MR gene was upregulated in the susceptible group compared to that in the control and resilient groups (Fig. 5G, one-way ANOVA *with Tukey's multiple comparisons test*, control vs resilient; ns, $P = 0.9083$, control vs susceptible; $***P = 0.0007$, resilient vs susceptible; $***P = 0.0003$).

4. Discussion

This study provides that synaptic modification in the LHb region is associated with stress vulnerability in an early-life stress model. Interestingly, some of the pups who experienced maternal separation stress showed depression-like behavior while the others apparently showed stress-resilient behavioral patterns when they grew to adolescence. Therefore, we examined electrophysiological properties and molecular analyses by subdividing these behavioral changes into stress-resilient and stress-susceptible groups. In the resilient group, both synaptic and extra-synaptic LTD seemed unaffected by early life stress. However, in the susceptible group, extra-synaptic LTD was enhanced, even when synaptic LTD was impaired. This alteration in synaptic plasticity, instigated by stress, appears to be initiated by corticosterone and modulated through MR activation. Taken together, our findings suggest that extra-synaptic NMDA receptor-dependent LTD seems to be strengthened when it is vulnerable to stress. In addition, the potentiation of glutamatergic transmission following stress exposure is driven by CORT-activated MR.

Our findings reveal that susceptibility to stress in rats is associated with an inhibition of the synaptic LTD and an enhancement of extra-synaptic LTD in the LHb region. We previously reported that glutamate spillover leads to the stable induction of extra-synaptic LTD in acute stress models by inhibiting glutamate transporter (Kang et al., 2020). We propose that the decline in glutamate transporters (GLT-1) following chronic early-life stress may affect glutamate spillover, leading to the induction of extra-synaptic LTD (Figs. 4E and 5C). According to previous studies, maternal separation with early weaning impaired glutamate transporter such as GLT-1 (EAAT2) associated with neuronal hyperactivity through impairment of the neuron-glia integrity (Acosta, 2018; Zeng et al., 2020). Furthermore, it has been observed that extra-synaptic LTD was increased in the hippocampal CA1 region of stressed rats, which might be caused by the increased expression of extra-synaptic NR2B-containing NMDAR (Wong et al., 2007; Yang et al., 2005). Our findings suggest that the increase in extra-synaptic LTD may be linked to increased expression of NMDAR. This is because there was a significant change in the protein expression level of NR2A and NR2B, among other NMDAR subtypes, in the LHb of susceptible group rats that had experienced maternal separation, compared to control group rats (Fig. 5D and E). Recently, Yang et al. suggested that NMDAR aroused burst firing with low voltage-sensitive T-type calcium channels and caused an increase in neural activity. Considering that the NMDAR inhibition caused by ketamine may have an antidepressant effect, using NMDAR as a therapeutic target requires more attention (Cui et al., 2019; Stone et al., 2012; Shepard et al., 2018; Yang et al., 2018). According to our results, ketamine suppressed extra-synaptic LTD in LHb region (Supple Fig. 2). While being damaged by ketamine, extra-synaptic NMDA R-dependent LTD, a defensive mechanism against stress, is highly probable to cause hyperactivity of LHb and intensify depressive symptoms.

The hyperactivation of neuronal activity in the LHb is closely associated with depression-like behaviors, such as learned helplessness and anhedonia. In addition, electrophysiological research on long-lasting depression is important because the increased inhibition of glutamatergic transmission in the LHb improved helplessness and had an antidepressant effect (Li et al., 2011; Li et al., 2013, Cui et al., 2019). Stress damages the eCB_1R signaling pathway in pre-synaptic neurons, resulting in the increased release of glutamate and the diminished expression of GLT-1 (Cui et al., 2014; Kang et al., 2018, Lyu et al., 2021; Arjmand et al., 2022). As a result, the induction threshold of extra-synaptic LTD is further lowered. Our findings indicate that extra-synaptic LTD is strengthened in the LHb region of the resilient group to stress. If GLT-1 is inhibited, NMDAR-dependent LTD in the extra-synaptic region can effectively control the neuronal activity of the LHb, which can be excessively increased by stress.

In addition, LTD suppression was completely reversed by spironolactone, an MR antagonist (Fig. 3). In other words, LTD inhibition in stressed rats arose from both corticosterone, which was increased by the HPA axis and MR activation. As shown in the results above, the inhibition of LTD was recovered by the activation of MR. Generally, MR increases neuronal excitability through dual pathways (Olijslagers et al., 2008). In the CA1 region of the hippocampus, extracellular signal-regulated kinases 1/2 (ERK1/2), resulting from MR activity, cause an increased probability of glutamate release in presynaptic neurons (Olijslagers et al., 2008). In addition, the G-protein coupled receptor (MR) caused a decrease in K^+ current in the postsynaptic neuron and enhanced neuronal excitability (Olijslagers et al., 2008). As mentioned above, MR-mediated neuronal excitability may lead to LTD suppression.

Previous studies reported that early life stress causes dysfunction of the kappa opioid receptor signaling pathway. This abnormal mechanism ultimately leads to hyperactivation of the intrinsic excitability in the LHb associated with early-life stress (Simmons et al., 2020). In particular, the corticotropin-releasing factor (CRF) signaling, known as key factor in behavior and emotional response to stress, has been reported to induce the inhibition of GABA transmission through retrograde eCB signaling leading to LHb hyperactivation in male rats (Authement et al., 2018). We confirmed the mechanism of synaptic alteration associated with stress vulnerability in male adolescent rats. Further study is required to understand the synaptic mechanisms that mediate stress vulnerability in female adolescents.

According to the findings of this study, extra-synaptic LTD was either induced or increased by stress. This means that an increase in glutamate release due to extra-synaptic LTD may prevent an increase in neuronal excitability. Extra-synaptic LTD may play an inhibitory role in regulating neuronal excitability, which may be excessively enhanced by

stress and serves as a system to prevent serotonin and dopamine deficiency. Extra-synaptic LTD will be effective in attenuating LHb hyperactivity if glutamate spillover activates its mechanism by blocking glutamate reuptake when rats are vulnerable to stress. Our data clearly support the claim that extra-synaptic LTD and MR functionally regulate vulnerability to early-life stress. Based on these results, the targeted control of extra-synaptic LTD and MR, which can regulate neuronal excitability often magnified by stress, may serve as an innovative treatment strategy for disorders related to serotonin and dopamine deficiency. This could potentially reduce hyperactivity in the LHb and provide a diagnosis for the status of stress vulnerability.

Ethics

All experiments were carried out in accordance with procedures approved in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Ewha Womans University (EWU, IACUC 18-010) and carried out in accordance with IACUC of Korea Brain Research Institute (KBRI, M1-IACUC-21-00034).

CRediT authorship contribution statement

Miseon Kang: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Formal analysis, Funding acquisition. **Jun-mo Chung:** Conceptualization, Resources. **Jihyun Noh:** Conceptualization, Funding acquisition, Supervision. **Jeongyeon Kim:** Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Jihyun Noh reports financial support was provided by Korea Ministry of Science and ICT. Miseon Kang reports financial support was provided by Korea Ministry of Science and ICT.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by KBRI basic research program through the Korea Brain Research Institute funded by the Ministry of Science and ICT (grant numbers 23-BR-02-02 and 23-BR-03-04 to J.K.), Basic Science Research through the National Research Foundation (NRF) of Korea funded by the Ministry of Science and ICT (NRF-2022R1F1A1063437 to M.K. & NRF–2023R1A2C2005346 to J.N.). Experimental diagrams were created with BioRender.com (Fig. 1A, license#*WA257MR7Z3;* Fig. 5A, *license #FW258NQZ7G;* Graphical abstract, *license#XR257OL3H7*).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2023.100570.

References

Acosta, G.B., 2018. Early life experience, maternal separation, and Involvement of GABA and glutamate transporters. GABA And Glutamate: New Developments In Neurotransmission Research 5 (12), 9.

Arjmand, S., Landau, A.M., Varastehmoradi, B., Andreatini, R., Joca, S., Wegener, G., 2022. The intersection of astrocytes and the endocannabinoid system in the lateral habenula: on the fast-track to novel rapid-acting antidepressants. Mol. Psychiatr. 1–12. PMID: 35585261.

Authement, M.E., Langlois, L.D., Shepard, R.D., Browne, C.A., Lucki, I., Kassis, H., Nugent, F.S., 2018. A role for corticotropin-releasing factor signaling in the lateral habenula and its modulation by early-life stress. Sci. Signal. 11 (520), eaan6480. PMID: **29**511121, PMCID: PMC5861378.

Bodnoff, S.R., Humphreys, A.G., Lehman, J.C., Diamond, D.M., Rose, G.M., Meaney, M. J., 1995. Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J. Neurosci. 15 (1), 61–69. PMID: **7823152**, PMCID: PMC6578287.

Cui, W., Mizukami, H., Yanagisawa, M., Aida, T., Nomura, M., Isomura, Y., et al., 2014. Glial dysfunction in the mouse habenula causes depressive-like behaviors and sleep disturbance. J. Neurosci. 34 (49), 16273–16285. PMID: **25**471567, PMCID: PMC6608483.

Cui, Y., Hu, S., Hu, H., 2019. Lateral habenular burst firing as a target of the rapid antidepressant effects of ketamine. Trends Neurosci. 42 (3), 179–191. PMID: 30823984.

Harrewijn, A., Vidal-Ribas, P., Clore-Gronenborn, K., Jackson, S.M., Pisano, S., Pine, D.S., Stringaris, A., 2020. Associations between brain activity and endogenous and exogenous cortisol–A systematic review. Psychoneuroendocrinology 120, 104775. PMID: **32592873,** PMCID: PMC7502528.

Johnson, S.A., Fournier, N.M., Kalynchuk, L.E., 2006. Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. Behav. Brain Res. 168 (2), 280–288. PMID: **16386319**.

Kalinichev, M., Easterling, K.W., Plotsky, P.M., Holtzman, S.G., 2002. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long–Evans rats. Pharmacol. Biochem. Behav. 73 (1), 131–140. PMID: **12076732**.

Kang, S., Li, J., Bekker, A., Ye, J.H., 2018. Rescue of glutamate transport in the lateral habenula alleviates depression-and anxiety-like behaviors in ethanol-withdrawn rats. Neuropharmacology 129, 47–56. PMID: 29128307 PMCID: PMC5714683.

Kang, M., Noh, J., Chung, J.M., 2020. NMDA receptor-dependent long-term depression in the lateral habenula: implications in physiology and depression. Sci. Rep. 10, 17921. PMID: **33087**756, PMCID: PMC7578045.

Karatsoreos, I.N., Bhagat, S.M., Bowles, N.P., Weil, Z.M., Pfaff, D.W., McEwen, B.S., 2010. Endocrine and physiological changes in response to chronic corticosterone: a potential model of the metabolic syndrome in mouse. Endocrinology 151 (5), 2117–2127.

Kim, J.S., Han, S.Y., Iremonger, K.J., 2019. Stress experience and hormone feedback tune distinct components of hypothalamic CRH neuron activity. Nat. Commun. 10 (1), 1–15. PMID: **31836701**, PMCID: PMC6911111.

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C.D., Schulz, D., Henn, F., Malinow, R., 2011. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature 470 (7335), 535–539. PMID: **21350486,** PMCID: PMC3285101.

Li, K., Zhou, T., Liao, L., Yang, Z., Wong, C., Henn, F., Malinow, R., Hu, H., 2013. βCaMKII in lateral habenula mediates core symptoms of depression. Science 341 (6149), 1016–1020. PMID: 23990563, PMCID: PMC3932364.

Lyu, S., Guo, Y., Zhang, L., Tang, G., Li, R., Yang, J., et al., 2021. Downregulation of astroglial glutamate transporter GLT-1 in the lateral habenula is associated with depressive-like behaviors in a rat model of Parkinson's disease. Neuropharmacology 196, 108691. PMID: 34197892.

Majcher-Maślanka, I., Solarz, A., Wędzony, K., Chocyk, A., 2018. Previous early-life stress modifies acute corticosterone-induced synaptic plasticity in the medial prefrontal cortex of adolescent rats. Neuroscience 379, 316–333. PMID: **29608944**.

Martin, S., Henley, J.M., Holman, D., Zhou, M., Wiegert, O., van Spronsen, M., et al., 2009. Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. PLoS One 4 (3), e4714. PMID: **19305644**, PMCID: PMC2659165.

McEwen, B.S., 2004. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann. N. Y. Acad. Sci. 1032 (1), 1–7. PMID: **15677391**.

McKay, L.I., Cidlowski, J.A., 2003. Physiologic and Pharmacologic Effects of Corticosteroids, vol. 6. Holland-Frei cancer medicine.

Olijslagers, J.E., De Kloet, E.R., Elgersma, Y., Van Woerden, G.M., Joëls, M., Karst, H., 2008. Rapid changes in hippocampal CA1 pyramidal cell function via pre-as well as postsynaptic membrane mineralocorticoid receptors. Eur. J. Neurosci. 27 (10), 2542–2550. PMID: **18547242**.

Own, L.S., Iqbal, R., Patel, P.D., 2013. Maternal separation alters serotonergic and HPA axis gene expression independent of separation duration in c57bl/6 mice. Brain Res. 1515, 29–38. PMID: 23548594.

Park, H., Rhee, J., Lee, S., Chung, C., 2017. Selectively impaired endocannabinoid-dependent long-term depression in the lateral habenula in an animal model of depression. Cell Rep 20, 289–296. PMID: 28700932.

Porsolt, R.D., Le Pichon, M., Jalfre, M.L., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266 (5604), 730–732. PMID: 559941.

Prager, E.M., Johnson, L.R., 2009. Stress at the synapse: signal transduction mechanisms of adrenal steroids at neuronal membranes. Sci. Signal. 2 (86) re5-re5. PMID: 19724063.

Raubenheimer, P.J., Young, E.A., Andrew, R., Seckl, J.R., 2006. The role of corticosterone in human hypothalamic–pituitary–adrenal axis feedback. Clin. Endocrinol. 65 (1), 22–26. PMID: **16817814**.

Reul, J.M.H.M., Kloet, E.D., 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117 (6), 2505–2511. PMID: 2998738.

Roceri, M., Hendriks, W.J.A.J., Racagni, G., Ellenbroek, B.A., Riva, M.A., 2002. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol. Psychiatr. 7 (6), 609–616. PMID: 12140784.

Schoech, S.J., Rensel, M.A., Heiss, R.S., 2011. Short-and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: a review. Current Zoology 57 (4), 514–530.

Shabel, S.J., Proulx, C.D., Trias, A., Murphy, R.T., Malinow, R., 2012. Input to the lateral habenula from the basal ganglia is excitatory, aversive, and suppressed by serotonin. Neuron 74, 475–481. PMID: **22578499,** PMCID: PMC3471532.

Shepard, R.D., Langlois, L.D., Browne, C.A., Berenji, A., Lucki, I., Nugent, F.S., 2018. Ketamine reverses lateral habenula neuronal dysfunction and behavioral immobility in the forced swim test following maternal deprivation in late adolescent rats. Front. Synaptic Neurosci. 10, 39. PMID: **30425634**, PMCID: PMC6218426.

Simmons, S.C., Shepard, R.D., Gouty, S., Langlois, L.D., Flerlage, W.J., Cox, B.M., Nugent, F.S., 2020. Early life stress dysregulates kappa opioid receptor signaling within the lateral habenula. Neurobiol. Stress 13, 100267.

Souza-Talarico, J.N.D., Marin, M.F., Sindi, S., Lupien, S.J., 2011. Effects of stress hormones on the brain and cognition: evidence from normal to pathological aging. Dementia & Neuropsychologia 5, 8–16. PMID: **29213714**, PMCID: PMC5619133.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85 (3), 367–370. PMID:3923523.

Stone, J.M., Dietrich, C., Edden, R., Mehta, M.A., De Simoni, S., Reed, L.J., et al., 2012. Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. Mol. Psychiatr. 17 (7), 664–665. PMID: 22212598,PMCID:PMC3883303.

Taves, M.D., Gomez-Sanchez, C.E., Soma, K.K., 2011. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. Am. J. Physiol. Endocrinol. Metabol. 301 (1), E11–E24. PMID: 21540450, PMCID: PMC3275156.

Tchenio, A., Lecca, S., Valentinova, K., Mameli, M., 2017. Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. Nat. Commun. 8 (1), 1135. PMID: 29074844, PMCID: PMC5658350.

Valentinova, K., Mameli, M., 2016. mGluR-LTD at excitatory and inhibitory synapses in the lateral habenula tunes neuronal output. Cell Rep. 16 (9), 2298–2307. PMID: 27545888, PMCID:PMC5009114.

Vetulani, J., 2013. Early maternal separation: a rodent model of depression and a prevailing human condition. Pharmacol. Rep. 65 (6), 1451–1461. PMID: 24552992.

Wong, T.P., Howland, J.G., Robillard, J.M., Ge, Y., Yu, W., Titterness, A.K., et al., 2007. Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. Proc. Natl. Acad. Sci. U. S. A. 104 (27), 11471–11476. PMID: **17592137,** PMCID: PMC2040922.

Yang, C.H., Huang, C.C., Hsu, K.S., 2005. Behavioral stress enhances hippocampal CA1 long-term depression through the blockade of the glutamate uptake. J. Neurosci. 11–25 (17), 4288–4293. PMID: **15**858055, PMCID: PMC6725105.

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

Zeng, H., Zhang, X., Wang, W., Shen, Z., Dai, Z., Yu, Z., et al., 2020. Maternal separation with early weaning impairs neuron-glia integrity: non-invasive evaluation and substructure demonstration. Sci. Rep. 10 (1), 1–14. PMID: 33173142, PMCID: PMC7656452.