Corticotropin releasing factor neurons in the visual cortex mediate long-term changes in visual function induced by early adversity

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ABSTRACT

Early adversity can cause malfunction of the visual system in adulthood. Adult female but not male mice undergoing early chronic mild stress (ECMS) maintain ocular dominance (OD) plasticity after the critical period. How early stressful experiences have a long-term impact on it is largely unknown. Here, we observed a wide distribution of corticotropin-releasing factor (CRF)-positive neurons, which mainly colocalized with a subpopulation of GABAergic interneurons in the mouse primary visual cortex (V1). Optogenetic activation of CRF-positive neurons transfected with AAV-ChR2 evoked inhibitory currents in nearby pyramidal cells. ECMS induced a reduction in the expression of CRF mRNA in adult mouse V1. Chemogenetic activation of V1 CRF neurons impaired OD plasticity in adult ECMS females. We further showed that local administration of the corticotropin releasing factor receptor 1 (CRFR1) antagonist via an osmotic minipump into the visual cortex mimicked OD plasticity in adult ECMS females. Whole-cell recording in layer 2/3 pyramidal neurons revealed that the CRFR1 antagonist reduced the short-term depression (STD) of evoked inhibitory postsynaptic current (IPSC) in females but not in males. Likewise, CRF agonists have the opposite effect. In summary, our findings indicate that the local CRF-CRFR1 system within V1 may mediate the long-term and sex-dependent effect of early stress experiences on visual plasticity and provide a target for the prevention of visual deficits in adults with a history of early-life adversity.

1. Introduction

Stressful experiences can cause structural and functional damage in various brain regions, including the prefrontal cortex and amygdala (Aristen, 2009; McEwen et al., 2016). Similarly, stress has both short- and long-term effects on the visual system. Acute stress amplifies early visual and inferior temporal responses to picture presentation (Henckens et al., 2009). Stress potentiates early and attenuates late stages of visual processing (Schackman et al., 2011). Participants in the high-stress condition respond faster, but less accurately, than participants in the low-stress condition in the visual search task (Rued et al., 2019). Meanwhile, stressful experiences have a long-term impact on the development of the visual system. Maltreated children with reactive attachment disorder show a reduced gray matter volume of the visual cortex (Fujisawa et al., 2018). Neglect, which is related to stress, reduces visual attention and visual memory in children (MD et al., 2009). Internationally adopted children are more likely to have vision and hearing deficits (Eckerle et al., 2014). In an early adversity model in which mice were subjected to unpredictable chronic mild stress from postnatal day 2 (P2) to P8, we observed delayed maturation of the visual cortex in a sex-dependent manner (Liu et al., 2020). However, how these stressors affect the visual cortex in the long term is still unknown.

After receiving an unpleasant, disgusting or threatening stress signal, individuals exhibit a series of stress responses, including autonomic

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nervous response and neuroendocrine response (Myers et al., 2017). CRF (also referred to as corticotropin-releasing hormone, CRH), a 41 amino acid peptide, functions as a major regulator of the HPA axis in response to stress (Vale et al., 1981). Acute stress or chronic stress can raise CRF within a few seconds, while early-life stress may cause long-term abnormal expression of CRF (Chen et al., 2012; Fenoglio et al., 2005; Rice et al., 2008). Abnormal levels of CRF are involved in various physiological and behavioral processes, including learning and memory and anxiety/depression-like behaviors (Hooper et al., 2018; Raadsheer et al., 1995). Outside the HPA axis, CRF acts as a regulator of CNS synaptic transmission and participates in plasticity within specific CNS neuronal circuits, which may provide a coping mechanism to deal with daily and life-long stressors. It has been reported that both CRF mRNA and CRF neurons exist in the primary visual cortex (Dedic et al., 2019; Peng et al., 2017), while their functions are unknown.

The shift in ocular dominance (OD) distribution induced by monocular deprivation reflects the plasticity of the primary visual cortex. Due to the limitation of the critical period, 4 days of monocular suture in normal adult animals cannot induce the deviation of ocular dominance (Sato and Stryker, 2008). The level of excitation/inhibition is the key to regulating adult OD plasticity. In adult animals, increasing the NR2B/NR2A ratio or downregulating the level of GABAergic inhibition reinstates OD plasticity (Harauzov et al., 2010; Li et al., 2018; Liu et al., 2015). In cultured hippocampal neurons, CRF reduces n-methyl-D-aspartate (NMDA) current through the activation of CRFR1 (Sheng et al., 2008). CRF also modulates the release of glutamate and GABAergic neurotransmission in various brain regions (Blank et al., 2002; Nie et al., 2004; Tan et al., 2004). Thus, we hypothesized that CRF neurons in V1 mediate the long-term effect of early stress experiences on visual plasticity.

In this study, we found that V1 CRF neurons in the primary visual cortex were coexpressed with VIP- and SST-positive interneurons, which form GABAergic synapses with pyramidal neurons. Adult ECMS mice showed reduced expression of V1 CRF mRNA, and chemogenetic activation of V1 CRF neurons prevented OD plasticity in adult ECMS females. CRFR1 has high affinity binding with CRF and plays a distinct role in different brain regions. The CRFR1 antagonist restored OD plasticity only in adult females. We further used whole-cell patch clamp recording to examine the underlying mechanism. The frequency and amplitude of the miniature inhibitory postsynaptic current (mIPSC) of neurons in V1 were delivered into V1 of the left hemisphere or both hemispheres (co-injected point, the scalp was sutured with a sterile suture needle. Subsequent experiments were conducted after approximately 4 weeks of viral expression.

2. Materials and methods

2.1. Animals

Male and female C57BL/6J mice were purchased from Vital River Laboratories (Beijing, China). Crh-IRES-Cre transgenic female and male mice were provided by Zhou Jiangning’s research group (Hefei, China), and tdTomato Cre reporter Ai9 mice were obtained from Zhang Xiaohui’s research group (Beijing, China). Crh-IRES-Cre; Ai9 mice were derived from crosses of the Crh-IRES-Cre and Ai9 genotypes. One male and two females were reared in a standard cage and placed in a 12-h reversed light–dark cycle with food and water provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Science and Technology of China.

2.2. Animal model

Early Chronic Mild Stress. Dam and litters as a whole were subjected to unpredictable chronic mild stress (Liu et al., 2020). One stressor was selected randomly each day. After receiving stress, the dam and litters were returned to their original cages. Males and females were fed separately after P21.

2.3. Eyelid suture

Monocular deprivation (MD) was performed by suturing the eyelids of mice under 0.5–3% isoflurane anesthesia. After surgery, we checked the mice and applied erythromycin ophthalmic ointment to the sutured eye daily to prevent eye opacity due to inflammation. For MD animals, eyelids were kept closed for 4 days. The mice with eyes coming open during lid suture before recording were excluded from subsequent experiments.

2.4. Osmotic minipump implantation

One day before MD, the mice were anesthetized with 1–2% isoflurane and fixed on a stereotaxic instrument. Erythromycin ophthalmic ointment was applied to protect the eyes. Body temperature was kept constant at 37 °C. In the monocular area of the primary visual cortex, a 0.5 mm hole was drilled 2.0 mm lateral to the midline and 1.0 mm anterior to the lambda. Then, the infusion cannula was inserted 1 mm below the surface of the skull. We placed the connected minipump (0.5 μl/h, ALZET 1007D) under the skin on the back of the neck. The exposed skull was covered with cyanoacrylate and dental cement. CRF, receptor antagonist NBI 35965 (0.44 ng/d, diluted with PBS, APE) or 0.01 M PBS were directly infused into the visual cortex through an osmotic minipump for 5 days.

2.5. Virus injection

Before surgery, mice (approximately 6 weeks) were anesthetized with a mixture (i.p.) of ketamine (0.1 mg/g) and xylazine (0.01 mg/g) and fixed on a stereotaxic instrument. For manipulation of CRF neurons in V1, 300 nl of AAV virus of pAAV-hSyn-DIO-mCherry (2.34 × 10^13, genome copies/ml, Ohio Technology, Shanghai), pAAV-hSyn-DIO-hM4Di(Gi)-mCherry (5.86 × 10^12, genome copies/ml, Ohio Technology, Shanghai), pAAV-hSyn-DIO-hM3Dq(Gq)-mCherry (4.57 × 10^12, genome copies/ml, Ohio Technology, Shanghai) and AAV-DIO-hCRHR2-GFP (4.84 × 10^12, genome copies/ml, Ohio Technology, Shanghai) was delivered into V1 of the left hemisphere or both hemispheres (coordinates: x: anteroposterior, 1.0 mm; medial side, 2.75 mm; dorsoventral, 0.55–0.65 mm) of Crh-IRES-Cre mice in 15 min through a 1 μl microsampler. After finishing each injection, the needle was left for 5 min to spread the virus completely. After removing the needle from the injected point, the scalp was sutured with a sterile suture needle. Subsequent experiments were conducted after approximately 4 weeks of viral expression.

2.6. Drug administration

Clozapine N-oxide (CNO) administration: CNO was diluted to 0.5 mg/ml with physiological saline. The mice were given a dose of 5 mg/kg at a concentration of 0.5 mg/ml one hour before the experiment.

2.7. In vivo electrophysiological recording

Mice were anesthetized and maintained with urethane (2 g/kg, i.p.) and chlorprothixene (5 mg/kg, i.m.) and placed in a stereotaxic frame. Body temperature was continuously monitored and maintained at 37 °C. A craniotomy was performed over the binocular primary visual cortex (V1b) for recording. For single-unit recording, a computer-generated moving bar was presented on a monitor that was positioned 23 cm from the mouse’s eyes. Cortical responses to each eye were recorded within the entire thickness of the primary visual cortex. The mean firing rates of spontaneous and visually evoked activities were computed from
peristimulus time histograms. In each mouse, 18–25 cells were recorded in 4–5 vertical penetrations that were evenly spaced (at least a 200 μm interval) across the mediolateral extent of V1b to avoid sampling bias. Only the cells with a receptive field within 20° from the vertical meridian were included in our sample. Cells were assigned to OD categories according to the seven category scheme of Hubel and Wiesel (Hubel and Wiesel, 1970). The CBI value of each mouse was calculated as follows: \( \frac{\sum n_I \times (C - I)}{\sum n_I + \sum n_C} \), where \( C \) and \( I \) were the evoked contralateral and ipsilateral responses, respectively.

2.8. Real-time PCR

The mouse brain was quickly obtained under deep anesthesia with ether. The primary visual cortex was dissected and placed in a sterilized EP tube with 400 μl of TRIzol reagent (Invitrogen, USA). The tissue was thoroughly ground, after which RNA was extracted and dissolved in approximately 20 μl of DEPC water (slightly adjusted according to the size of the material on the bottom of the specific sample tube). After measuring the RNA content of each sample and uniformity cDNA concentration, cDNA (Takara) was synthesized using reverse transcriptase. The synthesized cDNA was diluted 20-fold with dddH2O, after which the diluted DNA was amplified using a SYBR Green PCR Kit (Takara). The specific primer design was as follows: primers 50-CTGCCACAGCT-TACCCCTAC-30 and 50-CCATTTCCAGGCGAACATC-30 amplified mouse GR; primers 50-AGGGGGCCATCTGAGAAGAT-30 and 50-CATGTTAGGGGCGCTCTC-30 amplified mouse CRF; primer mouse β-actin was 50-ATCCTTATGTTGGTACAGC-30 and 50-CATCTTTT-CAGGTGGCCCTTAG-30. The target gene was calculated for quantitative analysis using the 2-ΔΔCt method.

2.9. Immunohistochemistry

After anesthetizing adult Crh-IRES-Cre; Ai9 mice with a mixture (i.p.) of ketamine (0.1 mg/g) and xylazine (0.01 mg/g), the mice were transcardially perfused with ice-cold PBS, followed by 4% paraformaldehyde in PBS. Brains were extracted and postfixed in 4% paraformaldehyde overnight at 4 °C and then cryoprotected with 30% sucrose in 0.1 M PBS for 2 days at 4 °C. Forty millimeter thick coronal sections were cut with a cryostat microtome (Leica CM1950). Free-floating sections were permeabilized and blocked with 0.5% Triton X-100 and 5% BSA for 1.5 h at room temperature. After permeabilization and blocking, the sections were immunostained by incubation with anti-rabbit GABA (1:500, Sigma, G6642), anti-guinea pig parvalbumin (1:500, SY5Y, 195 004), anti-rabbit glutamate (1:500, Sigma, A2052), anti-rabbit VIP (1:2000, ImmunoStar, 20077) and anti-goat somatostatin (1:50, sc-7819, Santa) antibodies at 4 °C for 24–48 h. After washing, sections were conjugated with a suitable Alexa 488 secondary antibody, GABA/glutamate/VIP (goat anti-rabbit, 1:500, Jackson Immuno), PV (donkey anti-guinea pig-Cy3, 1:500, Jackson Immuno), SST (donkey anti-goat, 1:500, Life, 11055) for 2 h at room temperature. To identify nuclei, sections were counterstained for 10 min with Hoechst 33342 (2 mg/ml, Sigma, B2261) or DAPI (SC-24941). Subsequently, the brain slices were transferred to a glass slide and mounted with an antifading agent (Vector CA 94010). Photography was performed under constant parameters of the LSM 710 confocal laser scanning microscope (Zeiss). Finally, statistical analysis was performed on the number of neurons using ImageJ.

2.10. Whole-cell patch clamp recording

Coronal slices from the visual cortex (300 μm thick) were prepared from postnatal 3-month-old male and female mice using a tissue slicer (Vibratome 3000; Vibratome) in ice-cold dissection buffer containing the following (in mM): 212.7 sucrose, 3 KCl, 1.25 NaH2PO4, 3 MgCl2, 1 CaCl2, 26 NaHCO3, and 10 dextrose, bubbled with 95% O2/5% CO2. The slices were immediately transferred to ACSF at 35 °C for 30 min before recordings. The recipe of ACSF was similar to the dissection buffer, except that sucrose was replaced with 124 mM NaCl, and the concentrations of MgCl2 and CaCl2 were changed to 1 mM and 2 mM, respectively. All recordings were performed at 30 °C. Pyramidal cells in layer 2/3 of visual cortex areas were identified visually under infrared differential interference contrast optics on the basis of their pyramidal somata and prominent apical dendrites.

To isolate GABA receptor-mediated mIPSCs from pyramidal cells in the visual cortex, 1 μM TTX, 20 μM CNQX, and 100 μM D,L-APV were added to the ACSF. mIPSCs were recorded at a holding potential (Vh) of −60 mV as inward current with the Cs-based internal solution consisting of the following (in mM): 120 CsCl, 8 NaCl, 2 EGTA, 10 HEPES, 5 QX-314, 4 ATP, 10 Na-phosphocreatine and 0.5 GTP, pH 7.4, at 270–290 mOsm. The recordings were obtained using an Integrated Patch-Clamp Amplifier (Sutter Instrument, Novato, CA, USA) controlled by Igor 7 software (WaveMetrics, Portland, OR, USA) filtered at 3 kHz and digitized at 10 kHz. Acquired mIPSCs were analyzed using the Mini Analysis ProgramTM (Synaptosoft, Decatur, GA). The threshold for detecting mIPSCs was set at 3 times the Root Mean Square (RMS) noise. There was no significant difference in the RMS noise in the mIPSCs between individual mice. For the computation of kinetic parameters, 400–500 fully isolated events of mIPSCs were averaged.

Evoked IPSCs were recorded by whole-cell voltage-clamp mode in the presence of 20 μM CNQX and 100 μM D,L-APV. A concentric bipolar stimulating electrode with a tip diameter of 125 μm (FHC) was placed in layer 4 of the visual cortex. The distance between the stimulating and recording electrodes was kept at 50–100 μm. Patch pipettes (2–4 mΩ) were filled with the internal solution consisting of the following (in mM): 120 CsCl, 8 NaCl, 2 EGTA, 10 HEPES, 5 QX-314, 4 ATP, 10 Na-phosphocreatine and 0.5 GTP, pH 7.4, at 270–290 mOsm. Only cells with series resistance <20 mΩ and input resistance >100 mΩ were studied. Cells were excluded if input resistance changed >15% or series resistance changed >10% over the experiment. Data were filtered at 3 kHz and digitized at 10 kHz using Igor Pro (WaveMetrics).

For the optogenetic experiment, the visual cortex of Crh-IRES-Cre mice was infected with AAV-DIO-ChR2-GFP, and V1 brain slices were prepared 3 weeks later. Single photostimulation (470 nm, 1 ms width, 10 mW; POLYGRON 400, Migettec) was used to induce photoevoked IPSCs, and repetitive photostimulation (30 s of light pulse, 470 nm, 1 ms width, 20 Hz, 10 mW) was used to activate CRF neurons.

2.11. Statistical analysis

All data are presented as the mean ± SEM. Statistical significance between two groups was analyzed by Student’s t-test or one-way ANOVA with post hoc Tukey’s test. The paired Wilcoxon signed rank test was used to compare the two sets of matching data. To determine differences among multiple groups, two-way ANOVA with post hoc Tukey’s test was performed. The Kolmogorov–Smirnov test (K–S test) was used to compare the cumulative distributions of the two groups. Differences were considered to be significant with a p value less than 0.05.

3. Results

3.1. CRF neurons colocalize with subtypes of inhibitory neurons in the V1 and form GABAergic synapses with pyramidal neurons

To assess the existence of CRF neurons in V1, we generated Crh-IRES-Cre; Ai9 mice that labeled CRF-positive neurons with tdTomato and stained the nuclei with Hoechst 33342 (Fig. S1). We found a laminated distribution of CRF neurons in V1, which is similar to a previous study in other neocortices (Gallopin et al., 2006). Most CRF neurons were
3.3. Inhibition of V1 CRF-CRFR1 signaling reinstates OD plasticity in adult mice

To directly examine the potential contribution of V1 CRF neurons to the restoration of adult OD plasticity, we used a pharmacogenetic DREADD approach to selectively express the engineered inhibitor-coupled receptor hM4Di in V1 CRF neurons of adult mice (Fig. 3A). We injected hM4Di and mCherry viruses into the binocular regions of adult Crh-IRES-Cre mice. One month later, CNO (5 mg/kg, i.p.) was administered daily beginning one day before 4 days of monocular deprivation, and then the OD distribution was assessed with single unit recording in V1. In adult CRH<sup>mCherry</sup> mice, 4-day MD with CNO application did not induce the OD shift (Fig. 3B), suggesting that neither virus injection nor CNO itself had an effect on OD plasticity. In adult <sup>hM4Di</sup> mice, 4-day MD with CNO application induced a significant shift in the OD distribution. The CBI of the <sup>hM4Di</sup> group was significantly lower than that of the CRH<sup>mCherry</sup> group after MD (Fig. 3C, <i>F</i>(1,10) = 28.14, <i>p</i> < 0.001 for mCherry/hM4Di; <i>F</i>(1,10) = 0.05, <i>p</i> = 0.945 for sex; <i>F</i>(1,10) = 0.04, <i>p</i> = 0.844 for interaction; mCherry Male vs hM4Di Male, <i>p</i> = 0.013; mCherry Male vs mCherry Female, <i>p</i> < 0.009; mCherry Female vs hM4Di Female, <i>p</i> = 0.021; hM4Di Male vs hM4Di Female, <i>p</i> = 0.997), which was also supported by the cumulative ocular dominance index (ODI) distribution curve (Fig. 3D, <i>p</i> < 0.001, KS-test). As shown in Fig. 3E, the evoked firing rates in response to eye deprivation in the <sup>hM4Di</sup> mice were significantly reduced following MD (contralateral eye: CRH<sup>mCherry</sup> mice: 13.6 ± 0.3, CRH<sup>hM4Di</sup> mice: 10.9 ± 0.3, <i>p</i> < 0.001; ipsilateral eye: CRH<sup>mCherry</sup> mice: 7.0 ± 0.3; CRH<sup>hM4Di</sup> mice: 9.3 ± 0.3, <i>p</i> < 0.05, two-way ANOVA). These results suggest that reducing the activity of CRF neurons in the visual cortex can trigger OD plasticity in adult mice. It should be noted that adult females and males showed similar restoration of OD plasticity after the downregulation of CRF neuron activity (<i>p</i> = 0.85). The alteration of excitation/inhibition is closely correlated with the recovery of adult OD plasticity. To reveal the underlying molecular mechanism of the restoration of OD plasticity by inhibition of V1 CRF neurons, we next performed western blotting to measure the expression levels of several well-known proteins that contribute to the inhibition/excitation balance. We found that the expression levels of two main GABA synthetic enzymes (GAD65 and GAD67) were significantly altered in both male and female adults after DREADD inhibition of CRF neurons for 5 days. The expression level of NR2B was slightly upregulated (Fig. 3F top, <i>p</i> = 0.06, <i>t</i>-test) in males and significantly increased (<i>p</i> < 0.010, <i>t</i>-test) in females (Fig. 3F bottom). The ratio of NR2B/NR2A expression was markedly upregulated in both adult males and females, confirming the correlation between the increase in the NR2B/NR2A expression ratio and the restoration of adult plasticity.

CRFR1 is a vital downstream target of CRF, and its mRNA is widely distributed in the visual cortex, while CRFR2 mRNA is confined to subcortical structures (Henckens et al., 2010). Thus, we administered the CRFR1 antagonist NBI 35965, through an osmotic minipump into the visual cortex one day prior to and through 4 days of MD. Surprisingly, female mice, but not males, showed a significant OD shift after 4 days of MD with injection of NBI 35965 (Fig. 3H). With NBI 35965 treatment, the CBI value after 4 days of MD of the female mice was significantly lower than that of the male mice (Fig. 3L, <i>p</i> < 0.01, <i>t</i>-test). The cumulative curve of ODI distribution was significantly different between the two groups (Fig. 3L, <i>p</i> < 0.001, KS-test). These results indicate that CRFR1 signaling modulates adult OD plasticity in mice in a female-specific manner.

3.4. CRFR1 increases the short-term depression of IPSC in visual cortical layer 2/3 in females

To examine the mechanism underlying CRF-CRFR1-mediated sex differences in the adult visual cortex, we performed whole-cell
Fig. 1. CRF neurons are coexpressed with inhibitory neurons in the L2/3 of V1. (A) The coexpression of CRF neurons and glutamatergic neurons, GABAergic neurons, SST neurons and VIP neurons in adult mice. Scale bar = 20 μm; red indicates CRF neurons, and green indicates the other types of neurons. (B) The distribution of CRF neurons in excitatory neurons (glutamate, n = 4 mice, 7 slices/mice) and inhibitory neurons (GABA, n = 3 mice, 7 slices/mice). (C) The left panel shows the percentage of PV neurons expressed in CRF neurons, and the right panel shows the percentage of CRF neurons expressed in PV neurons (n = 3 mice, 8 slices/mouse). (D) The left panel shows the percentage of SST neurons expressed in CRF neurons, and the right panel shows the percentage of CRF neurons expressed in SST neurons (n = 4 mice, 8 slices/mouse). (E) The left panel shows the percentage of VIP neurons expressed in CRF neurons, and the right panel shows the percentage of CRF neurons expressed in VIP neurons (n = 4 mice, 7 slices/mouse). (C and D) Data represent the mean ± SEM. (F) The left panel shows the schematic of V1 injection of AAVs in Crh-IRES-Cre mice (n = 4 mice) and recording configuration in acute slices (n = 16 cells). The middle panel shows the example traces of photo-evoked IPSCs before and after PTX addition. The right panel is the quantification of IPSC amplitude without PTX and with PTX. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
recordings in layer 2/3 pyramidal neurons in both sexes of adult mice. The amplitude and frequency of miniature inhibitory postsynaptic currents (mIPSCs) with and without CRFR1 antagonist were essentially identical in both adult males and females (Fig. 4A–F, p > 0.5, paired t-test). Next, we examined the effects of CRF-CRFR1 on the STD of evoked IPSCs. We probed the STD with 20 stimulation pulses at 30 Hz because the effects of age on the STD are prominent at this frequency (Jiang et al., 2005; Liu et al., 2020). There was no sex difference in the steady-state level of STD before administration (p = 0.69, unpaired t-test). STD was quantified as the amplitude of the IPSC during the steady state (average of the last 5 responses) normalized to the first response. Strong decreases in the amplitude of evoked IPSCs were observed in both sexes of adult mice, with an STD of approximately 0.3 ± 0.001 for males and 0.3 ± 0.002 for females (Fig. 5A top, p < 0.001, paired t-test). Interestingly, the steady-state level of STD was reduced after the administration of the CRFR1 antagonist in female mice but not males (Fig. 5A bottom). Furthermore, the amplitude of STD was enhanced after the administration of the CRFR1 agonist in female mice but not in males (Fig. 5B, top, female, p < 0.001; bottom, male, p > 0.5, paired t-test). Last, we optogenetically activated V1 CRF neurons with 20 Hz blue light stimulation for 30 s and recorded the STD in the visual cortex slices from female mice. The steady-state level of STD was enhanced after the activation of V1 CRF neurons, which was similar to the result after the activation of downstream CRFR1 with agonist (Fig. 5C, p < 0.001, paired t-test). These results suggest that CRH-CRHR1 signaling enhanced the GABA release probability from GABAergic neurons in a female-specific manner.
Fig. 3. Inhibition of V1 CRF-CRFR1 signaling sex-specifically modulates OD plasticity in adult mice. (A) Upper panel, schematic of the experimental procedure. Lower left panel, stereotaxic injection of AAVs into V1. Lower right panel, illustration of V1 infections with mCherry and hM4Di in Crh-IRES-Cre. Scale bar = 500 μm. Enlarge view shown in inset, Scale bar = 50 μm. (B) OD histograms for CRH<sup>mCherry</sup> (3 males and 3 females) and CRH<sup>hM4Di</sup> (4 males and 4 females) mice with 4-d MD. (C) Summary of CBI of each group shown in B. The horizontal line represents the average of the data. Error bars, data represent the mean ± SEM; each symbol represents an animal in C. ***p < 0.001, **p < 0.01 two-way ANOVA with post hoc Tukey’s test. (D) Cumulative distribution of ODI shown in B. (E) The evoked firing rates in response to the deprived eye and deprived eye in CRH<sup>mCherry</sup> and CRH<sup>hM4Di</sup> mice after 4-d MD. mCherry: n = 122 cells; hM4di: n = 164 cells. ***p < 0.001, Student’s t-test. (F) Left panel, sample western blots of β-actin, NR2B and NR2A in males (n = 6 mice/group, left upper panel) and females (n = 7 mice/group, left lower panel). Right panel, quantified expression levels of NR2B/NR2A ratio, NR2B and NR2A in males (right upper panel) and females (right lower panel) normalized to β-actin. ***p < 0.001, *p < 0.05, Student’s t-test. (G) Schematic of minipump administration of NBI 35965 and electrophysiological recording. H. OD histograms in males (n = 4 mice) and females (n = 4 mice) administered NBI 35965 after 4-d MD. I. Cumulative distribution of ODI shown in H. Inserted is the CBIs.
4. Discussion

Compared with the cognitive deficits caused by stress, the role of CRF signals in the sensory system has received less attention. A few studies have reported the acute role of CRF neurons in the retina and dorsal lateral geniculate nucleus (dLGN) in the visual pathway. CRF-expressing amacrine cells in the mouse retina form GABAergic synapses with ON alpha ganglion cells and inhibit their firing during positive contrast (Park et al., 2018). CRF administration increases sensory-evoked discharges of neurons in the dLGN of the thalamus in anesthetized rats (Zitnik et al., 2014). However, the role of the V1 CRF signal as a local neuromodulator in functional plasticity has not been investigated extensively. Here, our study in CRF transgenic mice identifies that CRF interneurons in the primary visual cortex mediate the regulation of visual plasticity by early stressful experiences. We further indicate the role of CRF/CRFR1 signaling in sex differences in visual plasticity.

4.1. V1 CRF neurons mediate the acute and long-term effects of stress on visual function

CRF shows widespread expression across the central neural system and plays distinct roles in different brain regions (Chen et al., 2020; Hooper et al., 2018; Paretkar and Dimitrov, 2018). CRF neurons in the prefrontal cortex are directly engaged in the tail suspension challenge (Chen et al., 2020). These CRF neurons colocalize with fewer excitatory neurons (Ketchesin et al., 2017) but a higher proportion of GABAergic interneurons. In this study, we characterized V1 CRF neurons as unique subtypes of GABAergic interneurons. Specifically, V1 CRF neurons coexpressed VIP and SST with a proportion exceeding 40%, while none with PV (Fig. 1C) in the superficial layers (Fig. 1). However, it is worth noting that the number of fluorescent-labeled CRF neurons in our reporter mouse line (Ai9) may be underestimated due to its sensitivity to Cre and strength of fluorescent reporter. The low colocalization of CRF and PV neurons in visual cortex might be ignored. Previous study has shown that the distribution of Cre positive population is various in different reporter mouse lines (Wang et al., 2021). Meanwhile, optogenetic activation of V1 CRF neurons evoked GABAA receptor-mediated IPSC in pyramidal neurons (Fig. 1). This is consistent with previous studies showing that CRF neurons in the neocortex are coexpressed with VIP and SST chemical markers (Gallopin et al., 2006; Kubota et al., 2011). Our results indicated that CRF neurons in the visual cortex might serve as novel targets for regulating OD plasticity in adulthood after early adversity. Both SST neurons and VIP neurons participate in the regulation of OD plasticity in adult mice. However, short-term silencing of SST neurons or activation of VIP neurons enhances visual cortical plasticity in adult mice (Fu et al., 2015). SST interneurons inhibit PV
interneurons twice as much as local pyramidal excitatory neurons (Cottam et al., 2013). Activation of VIP neurons inhibits SST neurons and disinhibits excitatory pyramidal neurons (Fu et al., 2014). It should be noted that not all VIP and SST neurons are CRF-positive neurons. Thus, whether there is a potential difference between VIP- and SST-CRF-expressing neurons in response to ECMS warrants further investigation.

Numerous studies have shown that CRF is inextricably linked to the long-term effects on brain structure and function induced by early stress (Fenoglio et al., 2006; Ivy et al., 2010). Our study revealed that ECMS led to a long-lasting downregulation of CRF mRNA levels in the visual cortex (Fig. 2). Chemogenetic activation of V1 CRF neurons blocked the abnormal OD plasticity in ECMS mice (Fig. 2). Our previous study showed that the increase in GABAergic inhibition by the GABAA receptor agonist diazepam has no effect on the OD plasticity of adult ECMS females. Therefore, the effect of activating V1 CRF neurons is mainly mediated by increasing the CRF signal, not by enhancing the inhibition. This hypothesis is further confirmed by the results that chemogenetic inhibition of the activity of CRF neurons in the V1 did not directly result in a decrease in the levels of GAD67/GAD65 in the visual cortex (Fig. S2). We also showed that acute stress increased the expression of V1 CRF mRNA, which is consistent with previous studies in other brain regions (Hsu et al., 1998; Locci et al., 2021; Meng et al., 2011). Taken together, CRF neurons in the visual cortex mediate both the short-term

![Figure 5](image-url)

**CRFR1 increases the STD of IPSC in visual cortical layer 2/3 of females.** (A) Example traces of frequency-dependent depression of IPSCs recorded from females and relative amplitudes of IPSCs after application of NBI 35965 in females (n = 6 mice/20 cells, top). Example traces of frequency-dependent depression of IPSCs recorded from females and relative amplitudes of IPSCs after application of NBI 35965 in males (n = 3 mice/10 cells, bottom). (B) Example traces of frequency-dependent depression of IPSCs recorded from females and relative amplitudes of IPSCs after application of stress in females (n = 5 mice/13 cells, top). Example traces of frequency-dependent depression of IPSCs recorded from females and relative amplitudes of IPSCs after application of stress in males (n = 4 mice/10 cells, bottom). (C) The left upper panel shows the schematics of AAV injection and acute slice recording. Left lower panels show example traces of frequency-dependent depression of IPSCs before and after blue light stimulation recorded from Cnrh-IRES-Cre female mice. Right panel, relative amplitudes of IPSCs before and after blue light stimulation (n = 2 mice/6 cells). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
and long-term impact of stress experience on visual function. How early adversity affects V1 CRF neurons is still unknown. In fact, many studies have been devoted to investigating how early stress interferes with CRF activity. In adult maternal deprived rats, the CRF promoter showed a significant decrease in the methylation percentage of the cyclic AMP response element in the two CpGs (CpG1 and CpG2), suggesting that HPA axis hypersensitivity caused by neonatal stress leads to long-term enhancement of CRF transcriptional activity in PVN (Chen et al., 2012). Early life stress leads to an increase in CRF, which promotes the overgrowth of mossy fibers in the hippocampus. The synapses formed by abnormal moss fibers on CA3 pyramidal cells are excitatory (glutamatergic), which may promote progressive excitotoxic damage to these cells (Brunson et al., 2001, 2005). These studies can shed light upon future studies on the underlying mechanisms. Stress does not increase the number of PVN CRF-positive neurons but only increases their activity (Walker et al., 2019; Wamsteeker Cusulin et al., 2013). Thus, we speculate that ECMS did not change CRF-positive cell numbers in the visual cortex during development.

4.2. The CRF-CRFR1 system in the visual cortex mediates sex differences in visual plasticity after stress

Sex differences in the CRF signaling pathways in response to stress have been reported in studies of rats and mice. However, most studies are mainly limited to the protein and mRNA expression levels of CRF/CRFR1 after acute stress or early adversity, which vary with both brain regions and species (Locci et al., 2021; Rosinger et al., 2017; Sterrenburg et al., 2012). CRFR1 signaling has been shown to mediate sex differences in various brain regions, including the medial prefrontal cortex (Viola et al., 2019), the dorsal raphe nucleus (Howerton et al., 2014), the anteroventral periventricular nucleus and the paraventricular hypothalamus (Rosinger et al., 2020). The physiological role of CRF signaling in sex differences remains elusive. We showed that inhibition of V1 CRF neurons restored OD plasticity in both adult males and females. However, blockade of the CRF downstream receptor with a CRFR1 antagonist restored OD plasticity only in adult females but not males. Consistently, the CRFR1 antagonist and agonist bidirectionally regulated the STD of IPSCs of V1 layer 2/3 pyramidal neurons in adult females but not in males (Fig. 5A–B). These results suggest that CRFR1 plays a role at the presynaptic site of GABAergic synapses in a sex-specific manner. Moreover, our results showed that the CRFR1 antagonist had no effect on the frequency and amplitude of mIPSCs of V1 pyramidal neurons in either sex (Fig. 4). Meanwhile, CRFR1 antagonist reduces mIPSC frequency in the medial central amygdala of adult male but not female Sprague Dawley rats (Rouzer and Diaz, 2021). This may be because the release of basal CRF in the visual cortex requires the activation of presynaptic CRF neurons. When we used the optogenetic method to directly activate V1 CRF neurons, the STD of IPSCs in adult female mice was enhanced (Fig. 5C). These findings indicate that CRFR1 potentiates the probability of GABA release to pyramidal neurons in females but not in males. Our data add more evidence to the contribution of CRF/CRFR1 signaling to sex differences.

In conclusion, this study highlights the physiological role of local CRF/CRF1 signaling within V1 and how it mediates the long-term effect of early adversity on visual plasticity. Our results also enhance the understanding of how CRF/CRFR1 signaling affects brain function in a sex-specific manner.

Declaration of competing interest

There is no conflict of interest between the authors.

CRediT authorship contribution statement

Yueqin Liu: designed the experiments, built the animal model, performed and analyzed the in vivo electrophysiological experiments, biochemical experiments and Q-PCR, wrote the original draft, and modified the paper. Sitong Li: performed and analyzed the in vitro electrophysiological experiments, wrote the original draft, and modified the paper. Xinxin Zhang: built the animal model and performed and analyzed the biochemical experiments. Laijian Wang: performed and analyzed the in vitro electrophysiological experiments. Ziming Li: performed and analyzed the in vitro electrophysiological experiments. Wei Wu: conducted the monocular deprivation and double-blind experimental design. Xinya Qin: performed and analyzed the Q-PCR. Jiangning Zhou: modified the paper. Chenchen Ma: performed and analyzed the in vitro electrophysiological experiments. Wei Meng: built the animal model and performed and analyzed the biochemical experiments. Xi Kuang: built the animal model and performed and analyzed the biochemical experiments. Fei Yin: built the animal model and performed and analyzed the biochemical experiments. Qianhui Xia: built the animal model and performed and analyzed the biochemical experiments. Bin Jiang: modified the paper. Yupeng Yang: designed the experiments, wrote the original draft, and modified the paper.

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Appendix A. Supplementary data

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