American College of Neuropsychopharmacology

www.nature.com/npp

ARTICLE

Check for updates

Adolescent social isolation induces distinct changes in the medial and lateral OFC-BLA synapse and social and emotional alterations in adult mice

Hiroshi Kuniishi[1,2,4]✉, Yuko Nakatake[1], Masayuki Sekiguchi[1,3] and Mitsuhiko Yamada[1]

Early-life social isolation is associated with social and emotional problems in adulthood. However, neural mechanisms underlying how social deprivation impairs social and emotional development are poorly understood. Recently, the orbitofrontal cortex (OFC) and basolateral amygdala (BLA) have been highlighted as key nodes for social and emotional functions. Hence, we hypothesize that early social deprivation disrupts the information processing in the OFC-BLA pathway and leads to social and emotional dysfunction. Here, we examined the effects of adolescent social isolation on the OFC-BLA synaptic transmission by optogenetic and whole-cell patch-clamp methods in adult mice. Adolescent social isolation decreased social preference and increased passive stress-coping behaviour in adulthood. Then, we examined excitatory synaptic transmissions to BLA from medial or lateral subregions of the OFC (mOFC or lOFC). Notably, adolescent social isolation decreased the AMPA/NMDA ratio in the mOFC-BLA synapse in adulthood, while the ratio was increased in the lOFC-BLA synapse. Furthermore, we optogenetically manipulated the mOFC-BLA or lOFC-BLA transmission in behaving mice and examined the effects on social and stress-coping behaviours. Optogenetic manipulation of the mOFC-BLA transmission altered social behaviour without affecting passive stress-coping behaviour, while optogenetic manipulation of the lOFC-BLA transmission altered passive stress-coping behaviour without affecting social behaviour. Our results suggest that adolescent social isolation induces distinct postsynaptic changes in the mOFC-BLA and lOFC-BLA synapses, and these changes may separately contribute to abnormalities in social and emotional development.

Neuropsychopharmacology (2022) 47:1597–1607; https://doi.org/10.1038/s41386-022-01358-6

INTRODUCTION

Because the early-life period is a critical window for brain development, the early-life environment is thought to influence the development of cognitive and emotional functions [1–4]. In particular, early-life social experiences could influence individuals' social and emotional functions in adulthood [5–7]. Human studies have reported that early-life social adversity, such as neglect and social deprivation, is associated with psychiatric disorders, which include social and emotional problems such as depression [8–10]. In addition, several animal studies have reported that deprivation of social experience in early life induces alterations of social and emotional behaviours [11–17]. It is important to understand the influence of early social adversity on the brain circuit for the understanding of pathologies and the development of novel treatments in psychiatric diseases.

The prefrontal cortex (PFC) is thought to play a crucial role in the top-down regulation of subcortical structures involving emotion and social cognition [18–22], and early-life social adversity may induce social and emotional problems through the disruption of information processing in PFC-subcortical pathways. Recent rodent studies have mainly focused on the medial subregion of PFC (mPFC)-subcortical circuits and reported that early social deprivation might cause dysregulations of mPFC-subcortical pathways and contributes to social and aggressive abnormalities in rodents [23–27]. However, the effects of early social isolation on subcortical pathways from other prefrontal subregions are still unclear. The orbitofrontal cortex (OFC), a ventral subregion of the PFC (Brodmann area 10, 11, 12, and 47 [28]), has been highlighted as a key structure for social and emotional regulations [29–32]. Several human studies have reported that OFC lesions alter the emotional response and sociality [28, 33–35], and abnormal activity is observed in several psychiatric disorders, such as depression and personality disorders, which associates with a history of early life adversity [36–39]. The OFC receives multi-sensory inputs, has reciprocal projections to several subcortical structures and is involved in emotional processing, decision making, and behavioural flexibility [31, 32]. In particular, the OFC sends a dense projection to the amygdala, which is a key structure for the generation of emotion and social cognition [19, 31, 40, 41]. Several imaging studies have reported that abnormal connectivity between the OFC and amygdala was observed in patients with a history of early social

[1]Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan. [2]United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, Osaka University, Osaka, Japan. [3]Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan. [4]Present address: Division of Development of Mental Functions, Research Center for Child Mental Development, University of Fukui, Fukui, Japan. ✉email: kuniishi@u-fukui.ac.jp

Received: 18 February 2022 Revised: 17 May 2022 Accepted: 31 May 2022
Published online: 13 June 2022

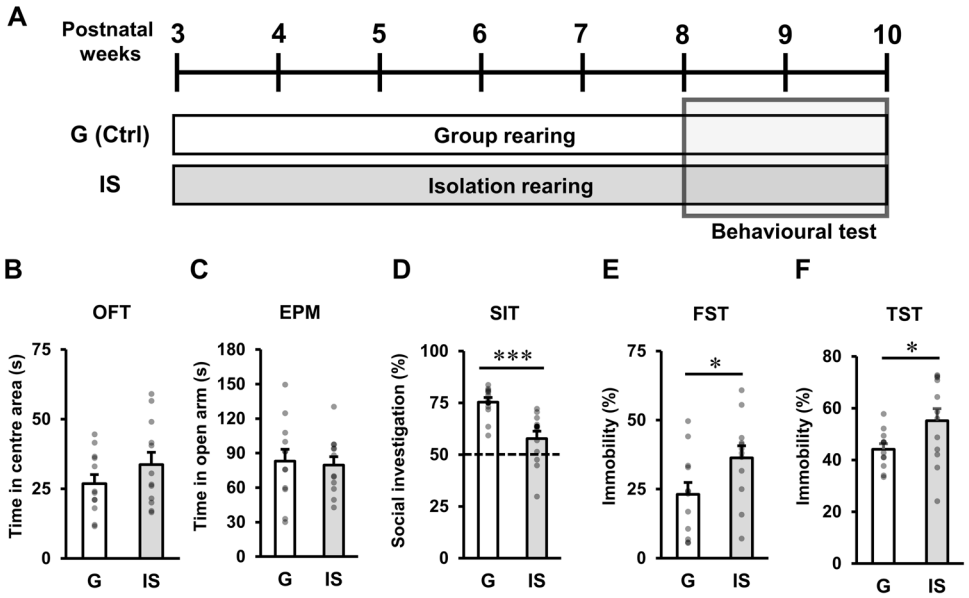


Fig. 1 Adolescent social isolation decreased social behaviour and increased passive stress-coping behaviour in mice. A Time schedule of the experiments. Subjected mice (IS group) were singly housed in plastic cages during adolescence (3–8 weeks of age) and subjected to behavioural tests in adulthood (8 weeks of age~). Control mice (G group) were housed in groups of 3–4 in the plastic cages during the same period. **B** Time spent in the centre area in the OFT. $t(22) = -1.258$, $p = 0.222$, unpaired t test. **C** Time spent in the open arm in the EPM. $t(22) = 0.2737$, $p = 0.787$, unpaired t test. **D** Percentage of social investigation in the SIT. The social investigation ratio was calculated as follows: (time spent investigating the mice cage / [time spent investigating the mice cage + time spent investigating the empty cage]) x 100. $t(22) = 4.209$, $p < 0.001$, unpaired t test. **E** Time of immobility in the FST. $t(22) = -2.167$, $p = 0.041$, Student's t test. **F** Time of immobility in the TST. $t(15.307) = -2.166$, $p = 0.046$, unpaired t test. Open bars represent group-reared mice (G, $n = 12$). Filled bars represent socially isolated mice (IS, $n = 12$). *: $p < 0.01$, ***: $p < 0.001$.

adversity [42, 43]. Furthermore, recent optogenetic and chemogenetic studies have reported that manipulation of OFC-BLA transmission impairs social and emotional behaviour in rodents [44, 45]. Taken together, these reports suggest that social adversity may cause OFC-amygdala disruption and contributes to social and emotional abnormalities. However, detailed effects of the social adversity on the OFC-BLA circuits and the causative mechanism underlying social and emotional abnormalities remain unknown.

The OFC is divided into further subregions, and recent studies have suggested that these subregions have functional heterogeneity [46–48]. Especially, human functional imaging studies have reported that the medial part of the OFC (mOFC) involves positive valence, while the lateral part of the OFC (lOFC) involves negative valence [49–51]. Based on these reports, it can be suggested that early social deprivation may cause distinct effects on mOFC and lOFC functions in a subregion-dependent manner, and these changes may induce distinct emotional abnormalities. Here, to address this hypothesis, we examined the effects of adolescent social deprivation on the synaptic transmission in the mOFC-amygdala and lOFC-amygdala pathways using optogenetic and whole-cell patch-clamp methods. Moreover, we examined the effects of optogenetic manipulation of the mOFC-BLA or lOFC-BLA transmission on social and emotional behaviours to clarify the relationship between OFC-amygdala transmission and behavioural abnormalities induced by early social deprivation in mice.

MATERIALS AND METHODS

Detailed procedures are described in Supplementary Information.

Animals

Male C57BL/6 J mice (purchased at 3 weeks of age, 4 weeks of age at viral injection surgery, CLEA, Tokyo, Japan) were used. Animals were housed under controlled temperature (23 ± 1 °C) and light (12 h light/dark cycle, light onset at 08:00 AM) conditions with food and water ad libitum. Our experimental procedures complied with the guidelines of the National Institute of Neuroscience and the National Centre of Neurology and Psychiatry and were approved by the Institutional Animal Investigation Committee (approval number: 2021037). For social isolation, the socially isolated mice (IS) were housed singly in clear plastic cages (17 cm × 24 cm × 12 cm, CL-0103-2 Mouse TPX, CLEA) from adolescence to adulthood (3–9 weeks of age, Fig. 1A). Control mice (G) were housed in groups of 3–4 in cages of the same size as IS mice during the same period. All individual cages were placed in the same rack in the same room and were not covered by any kind of opaque shroud. Thus, visual, auditory, and olfactory information from other cages was not fully obstructed. During 8–9 weeks of age, the mice performed behavioural and electrophysiological tests. Different animals were used for the behavioural and electrophysiological tests.

Behavioural tests

All behavioural tests were conducted during the light phase of the illumination cycle (between 10:00 a.m. and 16:00 p.m.). On the day of the test, the animals were transported to the testing room and left in their home cages for at least 1 h before the test. The animals in the experimental and control groups were tested alternately to avoid diurnal variation bias.

Stereotaxic surgery

Mice were anaesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/kg) and head-fixed using a stereotaxic instrument (Narishige, Tokyo, Japan). Virus solutions (AAV5-CaMKIIa-EYFP, AAV5-CaMKIIa-ChR2-EYFP, AAV5-CaMKIIa-eNpHR3.0-EYFP obtained from University of North Carolina Vector Core, and AAV5-CaMKIIa-hM3D(q)-mcherry, obtained from Addgene) were pressure-injected into the mOFC or lOFC using a 10 µL Hamilton syringe driven by an infusion pump (UMP-3, World Precision Instruments, FL, USA). Four to five weeks after the viral injection, the mice were subjected to behavioural and electrophysiological experiments. For optogenetic manipulation in behaving mice, a dual-LED optic cannula (TeleLCD-B-5-500-6.2 or TeleLCD-Y-5-500-6.2, BRC Nihon Bioresearch, Hashima, Japan) was implanted into the BLA 3 weeks after the AAV injection. After the surgery, the mice were housed individually and given 7 days to recover prior to behavioural testing.

Electrophysiology

Whole-cell patch-clamp recordings were performed as previously described [44, 52]. The animals were anesthetised with sevoflurane between 10:00 a.m. and 15:00 p.m. and the acute slices were prepared immediately. Brain coronal slices (300 μm thick) containing the amygdala were prepared in artificial cerebrospinal fluid (aCSF; containing 125 mM NaCl, 4.4 mM KCl, 1.5 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 2.5 mM $CaCl_2$); pH 7.4, 290–300 mOsm/L using a linear slicer (Dosaka EM Co., Ltd., Kyoto, Japan). Before being transferred to a recording chamber, the slices were maintained for at least 30 min at room temperature in aCSF. During recording, brain slices were perfused (3.0 ml/min, gravity flow) with aCSF maintained at 28–32 °C. Patch-clamp recordings were taken from pyramidal-shaped principal neurons, which were surrounded by ChR2-EYFP-positive fibres in the BLA. To activate ChR2, blue light (465 nm) was delivered to the recorded cell through a 63x objective lens (duration = 0.1 −3 ms, 1.8–70.7 × 10-7 J under the objective lens [52]), using an LED lamp (LEX-2B, BrainVision, Tokyo, Japan). Patch electrodes (resistance 4-7 MΩ) were filled with a solution containing 105 mM CsOH, 30 mM CsCl, 10 mM HEPES, 0.5 mM EGTA, 1 mM $MgCl_2$, 12 mM Na-phosphocreatine, 3 mM Mg-ATP, 0.5 mM Na-GTP, 0.1 mM spermine, 1 mM QX-314, pH 7.3 with gluconic acid, 295 mOsm/L for the measurement of synaptic response and 105 mM K-gluconate, 30 mM KCl, 10 mM HEPES, 0.5 mM EGTA, 1 mM MgCl2, 12 mM Na-phosphocreatine, 4 mM Mg-ATP, 0.5 mM Na-GTP, pH 7.3, 295 mOsm/L for the measurement of intrinsic

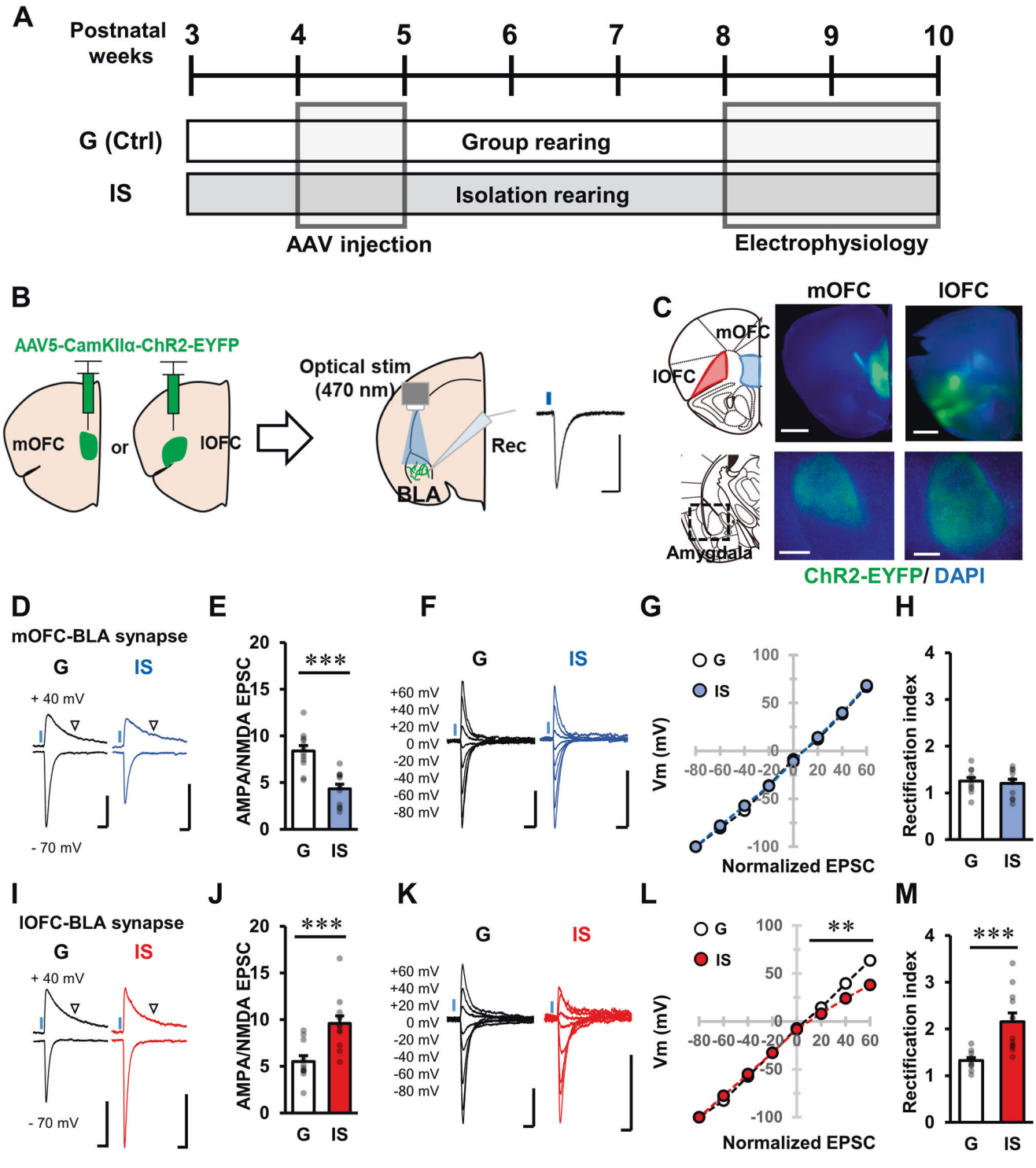


Fig. 2 Adolescent social isolation induced distinct effects on postsynaptic properties in the medial and lateral OFC-BLA pathways. A Time schedule of the experiments. **B** Schematic representations of the optogenetic isolation of mOFC-BLA or lOFC-BLA synaptic transmissions in the acute brain slice. **C** Representative photographs of ChR2-EYFP expression in the viral injection site in the mOFC (left top) or lOFC (right top, scale bar: 1 mm) and projection axon terminals in the BLA (left and right bottom, scale bar 250 μm). **D** Representative traces of AMPAR (at −70 mV) and ANPA/NMDA-mixed EPSCs (at +40 mV) in the mOFC-BLA synapse obtained from G (left) and IS mice (right). NMDAR-EPSC is measured at 60 ms after blue light stimulation. **E** The AMPA/NMDA current ratio in the mOFC-BLA synapse. $t(22) = 6.042$, $p < 0.001$, unpaired *t* test. G $n = 12$ cells, IS: $n = 12$ cells from 3 mice. **F** Representative traces of AMPAR-EPSC in the mOFC-BLA synapse at various holding potentials. **G** The current-voltage relationship in the mOFC-BLA synapse (at all holding potentials, $p > 0.05$, unpaired *t* test or Mann–Whitney U test). **H** The rectification index (EPSC amplitude at −60 mV / EPSC amplitude at +60 mV) in the mOFC-BLA synapse. $t(20) = 0.419$, $p = 0.680$, unpaired *t* test. G: $n = 11$ cells from 4 mice, IS: $n = 12$ cells from 3 mice. **I** Representative traces of AMPAR- and ANPA/NMDA-mixed EPSCs in the lOFC-BLA synapse. **J** The AMPA/NMDA current ratio in the lOFC-BLA synapse. $t(21) = -3.906$, $p < 0.001$, unpaired *t* test. G: $n = 11$ cells, IS: $n = 12$ cells from 3 mice. **K** Representative traces of AMPAR-EPSC in the lOFC-BLA synapse at various holding potentials. **L** The current-voltage relationship in the lOFC-BLA synapse (at holding potentials + 20, +40, and +60 mV, $p < 0.01$, unpaired *t* test). **M** The rectification index in the lOFC-BLA synapse. $t(13.538) = -4{,}238$, $p < 0.001$, unpaired *t* test. G: $n = 10$ cells, IS: $n = 12$ cells from 3 mice. Open bars represent G mice. Filled bars represent IS mice. Scale bar: 20 ms, 100 pA. **: $p < 0.01$, ***: $p < 0.001$.

excitability. The electrophysiological signal was amplified and filtered at 5 kHz using a MultiClamp 700B patch-clamp amplifier (Axon Instruments, Union City, CA, USA). Data were digitised at 50 kHz and acquired using ClampX software (version 10.6, Axon Instruments).

Optogenetic stimulation in behaving mice

To optogenetically manipulate OFC-BLA transmissions in behaving mice, we virally expressed ChR2 for activation or NpHR for inhibition, into the mOFC or lOFC. Control mice were expressed EYFP in the mOFC or lOFC. Then, optogenetic stimulation was performed using a wireless optogenetic stimulation system (Teleopto, BRC Nihon Bioresearch) through LED optic cannula implanted into BLA. Blue light (470 nm, 10-16 mW, 10 ms, 10 Hz for ChR2 [44]) or yellow light (590 nm, 7–14 mW, for NpHR [53]) was delivered under the control of an LED remote controller (TeleRemocon, BRC Nihon Bioresearch) and an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan). In the SIT, the mice were subjected to optical stimulation during a 5 min test session on the first day and without optical stimulation for 5 min on the next day. In the TST, mice were performed the test without optical stimulation in the first half (3 min) of the test session and with stimulation in the second half (3 min). In the OFT, the mice were subjected to optical stimulation in the first half (3 min) of the test session and did not receive stimulation in the second half (3 min).

Statistical analyses

Bar graphs represent the mean ± standard error of the mean. Each grey dot represents an individual value. All statistical analyses were performed using the EZR statistical software [54]. All data were analysed using the Shapiro-Wilk test to examine the sample distribution and using the F-test to examine homoscedasticity. Statistical comparisons between two groups were performed using two-sided unpaired *t* tests for Gaussian distribution or the Mann–Whitney U test for non-Gaussian distribution. For the unpaired *t* tests, homoscedastic and heteroscedastic data were analysed using the Student's *t* test and Welch's *t* test, respectively. For multiple comparisons, homoscedastic and heteroscedastic data were analysed by one-way ANOVA followed by Tukey's honestly significant difference post hoc test and Kruskal-Wallis test followed by Steel-Dwass test, respectively. The effect of drugs on the amplitude of the EPSC was analysed using a repeated-measures ANOVA followed by an unpaired *t* test. Statistical significance was set at $p < 0.05$.

RESULTS

Adolescent social isolation altered social and stress-coping behaviours in adulthood

To deprive early-life social experience, we applied the adolescent social isolation paradigm to male mice and performed behavioural and electrophysiological analyses in socially isolated mice (IS) and control mice (G) after maturation (Fig. 1A). Social isolation did not induce a significant effect on the body weight at 8 weeks of age (Fig. S1A). To evaluate the effect of social isolation on anxiety-like behaviours, we performed the open-field test (OFT) and elevated plus-maze (EPM) test. Social isolation did not induce significant effects on the time in the centre area (Fig. 1B) and spontaneous locomotor activity (Fig. S1B) in the OFT. Correspondingly, social isolation did not significantly alter the time in the open arm (Fig. 1C) and spontaneous locomotor activity (Fig. S1C) in the EPM. These data suggest that adolescent social isolation does not alter anxiety-like behaviours and spontaneous activity. Then, we performed the social interaction test (SIT) to evaluate the effect of social isolation on social behaviour. As previously reported [11, 15, 17], social isolation decreased social preference, which was shown by the ratio of exploring the cage containing an unfamiliar mouse to exploring the empty cage during the SIT (Fig. 1D). Next, we performed the forced swim test (FST) and tail suspension test (TST) to evaluate the effect of social isolation on passive stress-coping behaviour. As previously reported [13, 14], social isolation increased the immobility in the FST and TST (Fig. 1E, F). These data suggest that adolescent social isolation decreases sociality and increases passive stress-coping behaviours in mice.

Adolescent social isolation induced distinct postsynaptic changes and intrinsic excitability in the mOFC-BLA and lOFC-BLA pathways in adulthood

Recently, optogenetic and chemogenetic studies have demonstrated that mOFC-BLA and lOFC-BLA pathways involve the regulation of social and passive stress-coping behaviours [44, 45]. Hence, the adolescent social isolation paradigm might disrupt synaptic transmission in the mOFC-BLA and lOFC-BLA pathways and alter social and stress-coping behaviours in mice. To confirm the projection pattern from the mOFC and lOFC to the BLA in mice, we injected AAV vectors expressing EYFP or mCherry into the mOFC and lOFC in the same hemisphere (Fig. S2A, B), respectively. Four weeks after injection, we observed the projection sites in the BLA. We found that mOFC sends strong projections to medial and dorsal parts of the BLA, while lOFC projects to the entire BLA (Fig. S2C). Then, we isolated mOFC-BLA and lOFC-BLA excitatory synaptic transmissions using optogenetics and patch-clamp methods. To isolate the OFC-BLA transmission, we injected an AAV vector expressing a ChR2 into the mOFC or lOFC in mice (Fig. 2A, C, S3A). Four to five weeks after the injection, ChR2-expressing axon terminals were observed in the BLA (Fig. 2C). We conducted whole-cell recordings in BLA pyramidal neurons in acute brain slices obtained from these mice. ChR2-expressing axons were activated by local blue light irradiation, and light-evoked synaptic transmissions were recorded (Fig. 2B). We then examined the effects of adolescent social isolation on the mOFC-BLA and lOFC-BLA excitatory transmissions using this method in adulthood. First, we measured the relative ratio of AMPA receptor (AMPAR)-mediated currents to N-methyl-D-aspartate receptor (NMDAR)-mediated currents in excitatory transmissions (AMPA/NMDA ratio) to provide a history of past plasticity in excitatory synapses [55] (Fig. S3B). Notably, social isolation decreased the AMPA/NMDA ratio in the mOFC-BLA synapse (Fig. 2D, E) while increased in the lOFC-BLA synapse (Fig. 2I, J). In the amygdala, synaptic strengthening is associated

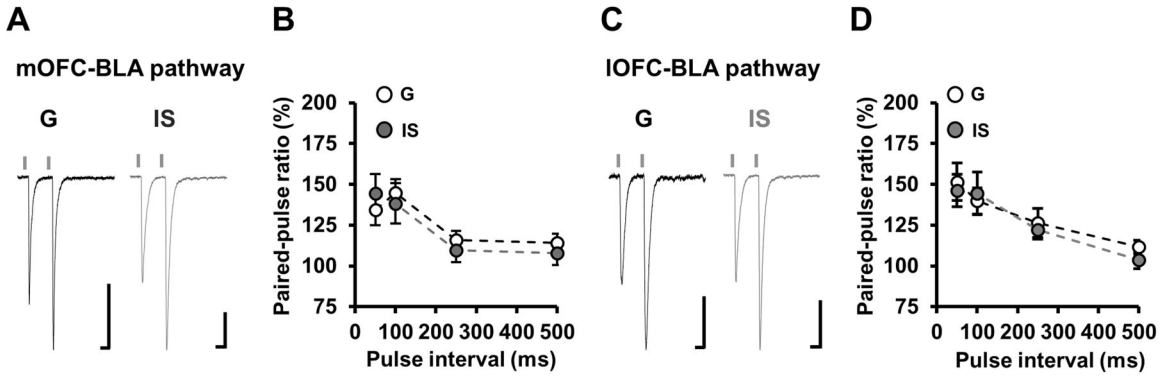


Fig. 3 Adolescent social isolation did not induce significant effects on the paired-pulse ratio in both mOFC-BLA and lOFC-BLA synapses. **A** Representative traces of EPSCs in the mOFC-BLA synapse evoked by paired optical stimulation (50 ms interval) at −70 mV. **B** The paired-pulse ratio in the mOFC-BLA synapse at 50, 100, 250, 500 ms pulse-interval (at all pulse intervals, $p > 0.05$, unpaired *t* test or Mann–Whitney U test). G: $n = 14$ cells from 4 mice. IS: $n = 14$ cells from 6 mice. **C** Representative traces of EPSCs in the lOFC-BLA synapse evoked by paired optical stimulation (50 ms interval) at −70 mV. **D** The paired-pulse ratio in the lOFC-BLA synapse (at all pulse intervals, $p > 0.05$, unpaired *t* test). G: $n = 14$ cells from 3 mice, IS: $n = 14$ cells from 5 mice.

with the synaptic insertion of inwardly rectifying calcium-permeable AMPARs (CP-AMPARs) [56]. To examine whether CP-AMPARs were presented into the OFC-BLA synapse, we measured the current-voltage relationships of AMPARs and examined inward-rectification, an indicator of the presence of CP-AMPARs, in the mOFC-BLA and lOFC-BLA synapses. Social isolation reduced the amplitude of the outward current in the AMPAR excitatory postsynaptic current (EPSC) (Fig. 2K, L) and increased the rectification index in the lOFC-BLA synapse (Fig. 2M) but not in the mOFC-BLA synapse (Fig. 2F–H). Similarly, social isolation increased the sensitivity to the CP-AMPARs blocker, NASPM in the lOFC-BLA synapse, but not in the mOFC-BLA synapse (Fig. S4A, B). These results indicate that adolescent social isolation promoted synaptic insertion of CP-AMPARs in the lOFC-BLA but not in the mOFC-BLA pathways. Taken together, these data suggest that adolescent social isolation induces distinct changes in postsynaptic properties in the mOFC-BLA and lOFC-BLA pathways in mice. In addition, we examined the effect of social isolation on the paired-pulse ratio, an indicator of presynaptic short-term plasticity, and found that social isolation did not induce significant effects in mOFC-BLA and lOFC-BLA synapses (Fig. 3A–D).

Moreover, we examined the effects of intrinsic excitability in neurons receiving input from the mOFC and lOFC (Fig. S3C, S5A). Interestingly, adolescent social isolation decreased action potential frequency (Fig. S5B, C) and increased the magnitude of after depolarization in mOFC-target neurons (Fig. S5H). Meanwhile, lOFC-target neurons showed increased action potential frequency and input resistance (Fig. S5I, J, M) and decreased magnitude of after depolarization (Fig. S5O).

Notably, the lOFC projects to mOFC projecting (medial and dorsal parts) and non-projecting areas (lateral and ventral parts) in the BLA (Fig. S2B). We separately analysed the AMPA/NMDA ratio and intrinsic excitability in the lOFC-BLA pathway focusing on putative mOFC projecting and non-projecting areas in the BLA. Social isolation increased the AMPA/NMDA ratio in both these areas in the BLA (Fig. S6A). In addition, lOFC-target neurons in mOFC non-projecting area in the BLA increased intrinsic excitability by the social isolation. While, lOFC-target neurons in mOFC projecting area did not significantly alter intrinsic excitability by the social isolation (Fig. S6B).

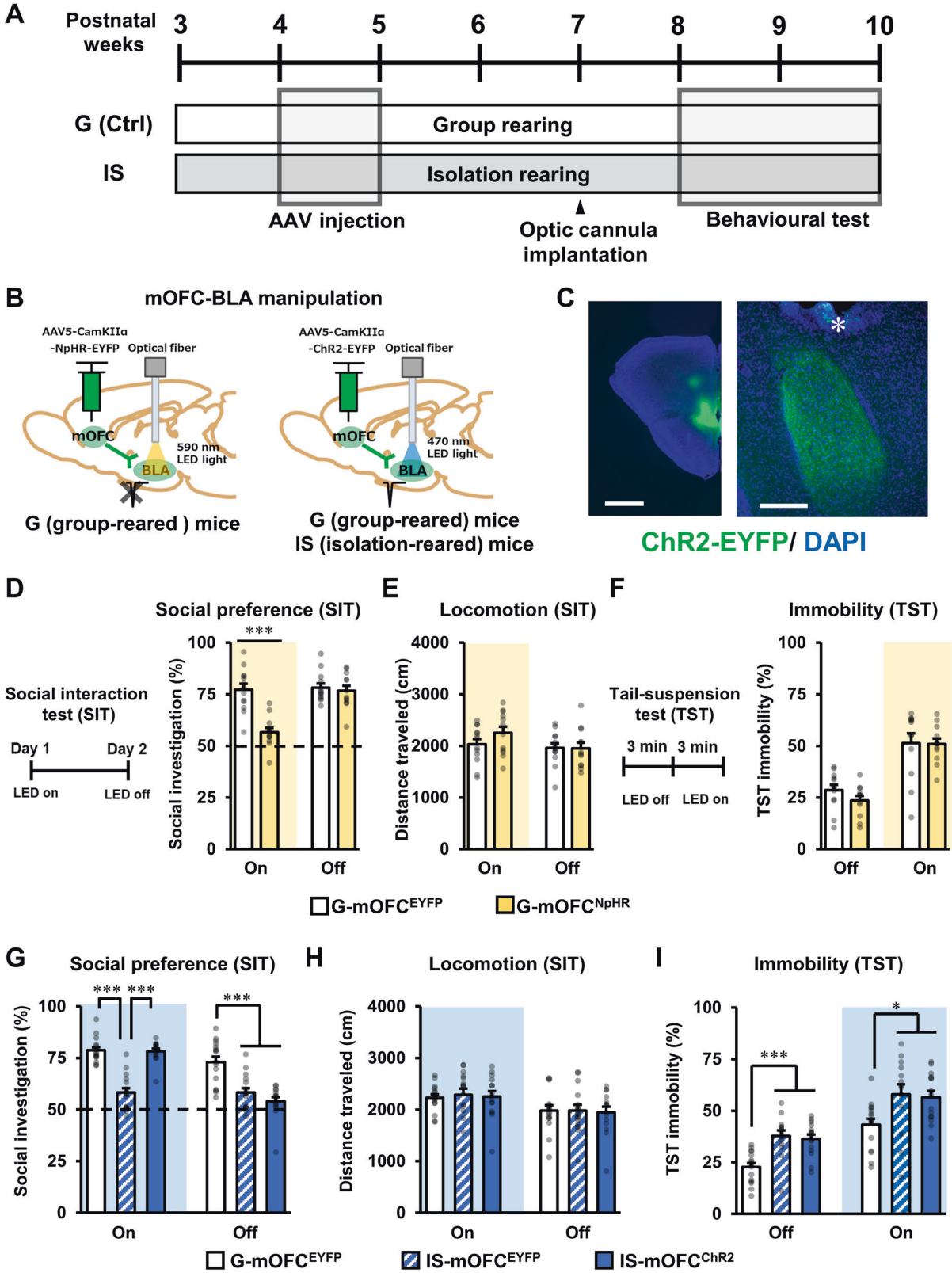
mOFC-BLA transmission modulated social behaviour but not passive stress-coping behaviour

In electrophysiological tests, we found that adolescent social isolation induced synaptic changes in the mOFC-BLA and lOFC-BLA pathways, and these changes might contribute to alterations in social and passive stress-coping behaviours. To address the possible causal relationship between the OFC-BLA transmission and social and stress-coping behaviours, we optogenetically manipulated the mOFC-BLA or lOFC-BLA transmission during the SIT and TST (Figs. 4A–C, S7A, B, 5A, B, S8A, B).

First, we examined whether mOFC-BLA inactivation induces abnormalities in social and stress-coping behaviours in group-reared mice. On the first day of the SIT, LED light was applied to the subjected mice during the test session, revealing that the NpHR-expressing mice (G-mOFC^{NpHR}) showed decreased social preference compared with the EYFP-expressing mice (G-mOFC^{EYFP}) (Fig. 4D). On the next day, the mice were subjected to the SIT without LED application, and there were no significant differences in social preference and locomotor activity between G-mOFC^{EYFP} and G-mOFC^{NpHR} mice (Fig. 4D, E). In the TST, the mice were subjected to the test without the LED application during the first half of the session and with the LED application during the last half of the session. There was no significant difference in immobility between the G-mOFC^{EYFP} and G-mOFC^{NpHR} mice in both light-off and light-on periods (Fig. 4F). Next, we examined whether the mOFC-BLA activation rescues behavioural abnormalities in IS mice. We expressed ChR2 in the mOFC and applied blue LED light into the BLA of mice during the SIT and TST. In the light-on period, EYFP-expressing IS mice (IS-mOFC^{EYFP}) showed decreased social preference compared with EYFP-expressing G mice (G-mOFC^{EYFP}), and ChR2-expressing IS mice (IS-mOFC^{ChR2}) showed increased social preference compared with IS-mOFC^{EYFP} mice (Fig. 4G). In the light-off period, both IS-mOFC^{EYFP} and IS-mOFC^{ChR2} showed decreased social preference compared with G-mOFC^{EYFP} mice without significant changes in locomotor activity (Fig. 4G, H). In the TST, IS-mOFC^{EYFP} and IS-mOFC^{ChR2} mice showed greater immobility than G-mOFC^{EYFP} mice, and there was no significant difference in immobility between IS-mOFC^{EYFP} and IS-mOFC^{ChR2} mice in both light-off and light-on periods (Fig. 4I). These results suggest that a decrease in the mOFC-BLA transmission could induce social deficits, and mOFC-BLA activation would alleviate the social deficits in IS mice without an effect on the stress-coping behaviour.

lOFC-BLA transmission modulated passive stress-coping behaviour but not social behaviour

Then, we examined whether lOFC-BLA activation induces abnormalities in social and stress-coping behaviours in group-reared mice. ChR2-expressing (G-lOFC^{ChR2}) mice did not show significant changes in social preference and locomotor activity compared with EYFP-expressing (G-lOFC^{EYFP}) mice in the SIT in



both light-on and light-off periods (Fig. 5C, D). On the other hand, consistent with our previous study [44], G-lOFC^{ChR2} mice showed increased immobility in the TST during the light-on period but not off period compared with G-lOFC^{EYFP} mice (Fig. 4E). Finally, we examined whether lOFC-BLA inactivation rescues behavioural abnormalities in IS mice using NpHR and yellow LED light. In the SIT, both EYFP-expressing (IS-lOFC^{EYFP}) and NpHR-expressing IS (IS-lOFC^{NpHR}) mice showed decreased social preference compared with G-lOFC^{EYFP} mice, but there was no significant difference in social preference between IS-lOFC^{EYFP} and IS-lOFC^{NpHR} mice in

Fig. 4 mOFC-BLA pathway modulates social but not passive stress-coping behaviour. A Time schedule of the experiments. **B** Schematic representations of the viral injection and LED optic fibre placement for optogenetic manipulation of the mOFC-BLA transmission. **C** Representative photograph of ChR2-EYFP expression in the injection site (left, scale bar: 1 mm) and ChR2-EYFP-expressing axon terminal from the mOFC and optic fibre tips in the BLA (right, scale bar: 250 μm). Asterisk represents the fibre tip. **D** The experimental procedure for optogenetic manipulation of the mOFC-BLA pathway during the SIT (left). The mice were subjected to the test with optical stimulation on the first day (On) and without the stimulation on the second day (Off). The percentage of social investigation in the SIT with mOFC-BLA inactivation (right). **E** The total distance travelled in the SIT with mOFC-BLA inactivation. Open bars represent group-reared EYFP-expressing mice. (G-mOFC^{EYFP}, $n = 13$). Filled bars represent group-reared NpHR-expressing mice (G-mOFC^{NpHR}, $n = 12$). **F** The experimental procedure for optogenetic manipulation during the TST (left). The mice were subjected to the test without optical stimulation in the first half (3 min) and with optical stimulation in the second half (3 min) of the test session. The time of immobility in the TST with mOFC-BLA inactivation (right). G-mOFC^{EYFP}: $n = 12$, G-mOFC^{NpHR}: $n = 12$. **G** The percentage of social investigation in the SIT with mOFC-BLA activation. **H** The total distance travelled in the SIT with mOFC-BLA activation. Open bars represent EYFP-expressing group-reared mice (G-mOFC^{EYFP}: $n = 16$). The shaded bar represents EYFP-expressing isolated mice (IS-mOFC^{EYFP}, $n = 16$). Filled bars represent ChR2-expressing isolated mice (IS-mOFC^{ChR2}, $n = 15$). **I** The time of immobility in the TST with mOFC-BLA activation. G-mOFC^{EYFP}: $n = 16$, IS-mOFC^{EYFP}: $n = 14$, IS-mOFC^{ChR2}: $n = 15$. *: $p < 0.05$, ***: $p < 0.001$. All statistical values are described in Supplementary Table S1.

both light-on and light-off periods (Fig. 5F). There was also no significant difference in locomotor activity in both light-on and light-off periods (Fig. 5G). In the TST, IS-lOFC^{NpHR} mice showed decreased immobility compared with IS-lOFC^{EYFP} mice during the light-on period but not during the light-off period (Fig. 5H). These results suggest that hyperactivation of lOFC-BLA transmission could enhance passive stress-coping behaviour, and lOFC-BLA inhibition could alleviate the increased passive stress-coping behaviour in IS mice without an effect on social preference. Notably, mOFC-BLA and lOFC-BLA manipulation did not change spontaneous activity and the time in the centre area in the OFT (Fig. S9).

DISCUSSION

In the present study, we focused on projecting pathways from two distinct subregions of the OFC (mOFC and lOFC) to the BLA and examined the effect of adolescent social isolation on synaptic transmission in these two pathways after maturation. We found that adolescent social isolation induced distinct postsynaptic changes in the mOFC-BLA and lOFC-BLA pathways, as shown by the increment or decrement of the AMPA/NMDA ratio. Notably, optogenetic manipulations of these two pathways demonstrated that the mOFC-BLA and lOFC-BLA projections modulated social and stress-coping behaviours, respectively. These results suggest that mOFC-BLA and lOFC-BLA transmissions could separately modulate social and emotional behaviours, and adolescent social isolation might alter these behaviours through postsynaptic modifications in these pathways. This is the first study to compare the effects of social isolation on mOFC-BLA and lOFC-BLA synaptic transmissions. Our findings provide novel insights into the orbitofrontal functional heterogeneity and identify neural circuit mechanisms underlying the early social deprivation-induced behavioural changes.

Adolescent social environment has an impact on the development of medial and lateral OFC-BLA circuits

Our results demonstrated that adolescent social isolation results in the mOFC-BLA and lOFC-BLA synaptic abnormalities in adulthood. It is generally accepted that adolescence is a critical window period for experience-dependent development or reorganization of neuronal circuits [57, 58]. The synaptic development of the PFC-amygdala circuit is also achieved during this period [59, 60]. In addition, rodent social play behaviour emerges and reaches its peak during adolescence [61], and several studies have demonstrated that adolescent social play or interaction with conspecific alters neural activity in PFC regions, including the OFC in rodents [62, 63]. It is possible that deprivation of adolescent social interaction by isolation disrupts the social experience- and activity-dependent synaptic development or reorganization of neuronal circuits in the mOFC-BLA and lOFC-BLA pathways.

In this study, we investigated adolescent social isolation but did not examine the effect of social isolation in adulthood. It is possible that social isolation in adulthood also induces changes in OFC-BLA circuits. However, previous studies demonstrated that social isolation in early adolescence but not in late-adolescent or adulthood induced social deficits [17, 64]. In addition, another study reported that social isolation in adolescence, but not adulthood, disrupted instrumental response updating, which involves OFC function [65]. Together with the present findings, social isolation in adolescence, which corresponds with the developmental window of the PFC-amygdala circuit, might have a greater impact on OFC-BLA function than adult isolation. Moreover, previous studies have reported that the length of social isolation is important for increments of passive stress-coping [13, 66]. To further understanding the effects of social isolation on OFC-BLA function and development, detailed studies focusing on the timing and duration of social isolation are needed.

Adolescent social isolation induces distinct changes in postsynaptic properties and intrinsic excitability in the mOFC-BLA and lOFC-BLA pathways

Human imaging and lesion studies have suggested that the mOFC is involved in positive emotion and social processing [33, 51], and a previous animal study demonstrated that BLA projecting neurons in the mOFC regulate social behaviour in mice [45]. In this study, we found that adolescent social isolation-induced postsynaptic changes, as shown by the decrease in the AMPA/NMDA ratio in the mOFC-BLA synapse. AMPARs are the primary mediators of fast excitatory transmission, and AMPA/NMDA ratio is a proxy for glutamatergic synaptic strength in the amygdala [67]. Our data suggest that adolescent social isolation decreases the efficiency of postsynaptic excitatory transmission in the mOFC-BLA synapse and induces social deficits. This possibility was supported by our results demonstrating that mOFC-BLA synaptic inhibition by optogenetics induced social deficits in control mice similar to socially isolated mice. A previous study reported that peri-adolescent stress induced abnormal functional connectivity mOFC and medial and central amygdala and abnormalities in social and aggressive behaviours in rats [68]. Similarly, our finding emphasised the impact of adolescent environment on mOFC-amygdala function and social behaviour.

On the other hand, it has been suggested that the lOFC is involved in negative emotion processing [51]. In addition, our previous study demonstrated that the lOFC-BLA pathway regulates passive stress-coping [44]. In this study, we found that adolescent social isolation induced postsynaptic changes, as shown by the increase in the AMPA/NMDA ratio in the lOFC-BLA synapse, contrary to the mOFC-BLA synapse. This result suggests that adolescent social isolation increases the efficiency of postsynaptic excitatory transmission in the lOFC-BLA synapse and leads to an increment of passive stress-coping. This possibility

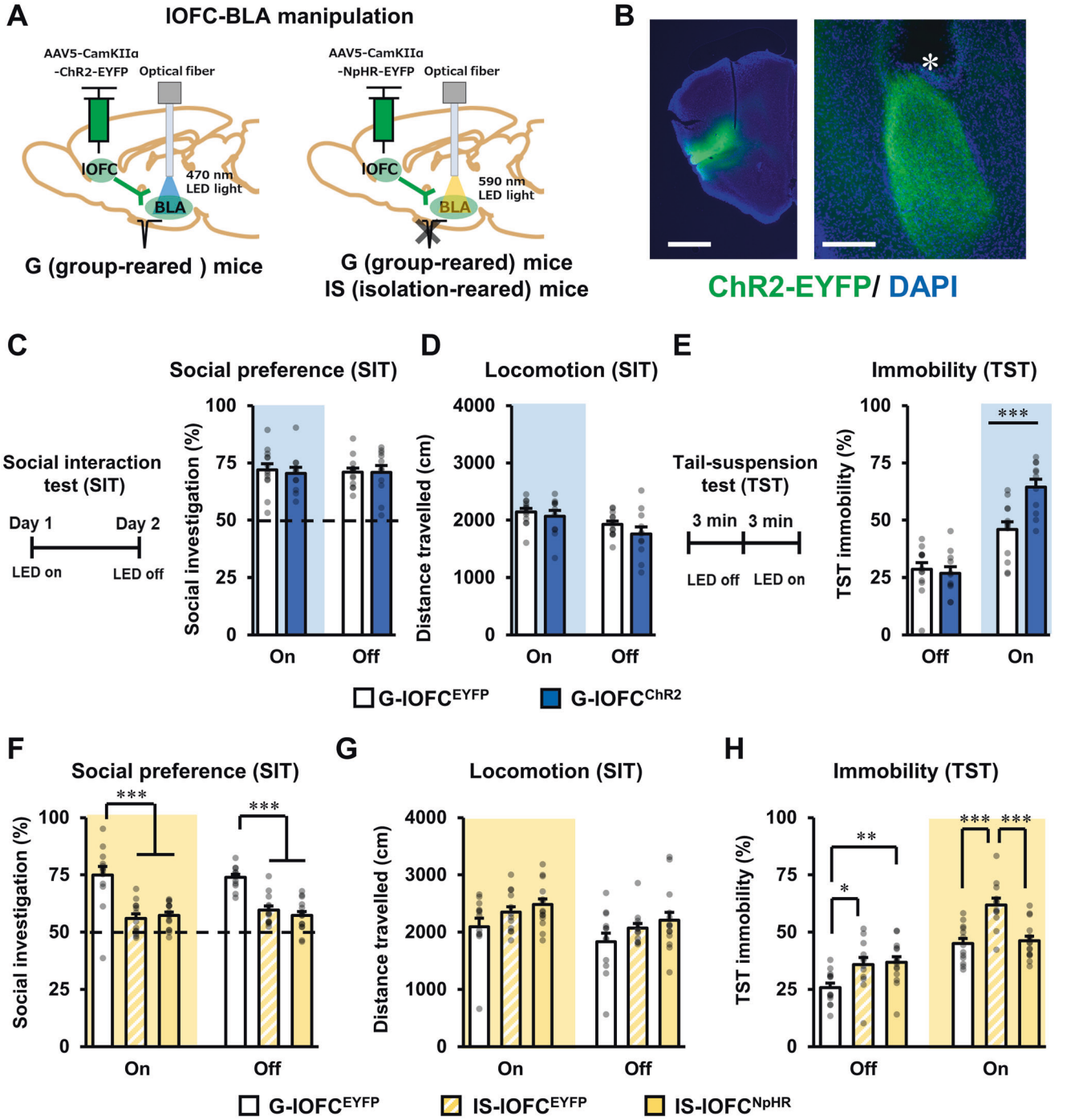


Fig. 5 lOFC-BLA pathway modulates passive stress-coping but not social behaviour. A Schematic representation of optogenetic manipulation of the lOFC-BLA transmission. **B** Representative photograph of ChR2-EYFP expression in the injection site (left, scale bar: 1 mm) and ChR2-EYFP-expressing axon terminal from the lOFC and optic fibre tips in the BLA (right, scale bar: 250 μm). The asterisk represents the fibre tip. **C** Experimental procedure for optogenetic manipulation of the lOFC-BLA pathway during the SIT (left). Percentage of social investigation in the SIT with lOFC-BLA activation (right). **D** Total distance travelled in the SIT with lOFC-BLA activation. Open bars represent group-reared EYFP-expressing mice. (G-lOFC^{EYFP}, $n = 13$). Filled bars represent group-reared ChR2-expressing mice (G-lOFC^{ChR2}, $n = 11$). **E** The experimental procedure for optogenetic manipulation during the TST (left). The time of immobility in the TST with lOFC-BLA activation (right). G-lOFC^{EYFP}: $n = 13$, G-lOFC^{ChR2}, $n = 11$. **F** Percentage of social investigation in the SIT with lOFC-BLA inactivation. **G** Total distance travelled in the SIT with lOFC-BLA inactivation. Open bars represent EYFP-expressing group-reared mice (G-lOFC^{EYFP}, $n = 13$). The shaded bar represents EYFP-expressing isolated mice (IS-lOFC^{EYFP}, $n = 13$). Filled bars represent ChR2-expressing isolated mice (IS-lOFC^{NpHR}, $n = 15$). **H** Time of immobility in the TST with lOFC-BLA inactivation. G-lOFC^{EYFP}: $n = 13$, IS-lOFC^{EYFP}: $n = 12$, IS-lOFC^{NpHR}: $n = 15$. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. All statistical values are described in Supplementary Table S2.

was supported by our results demonstrating that the lOFC-BLA synaptic activation by optogenetics increased passive stress-coping in control mice similar to socially isolated mice. In addition, we found adolescent social isolation increased inward-rectification of AMPARs current, an indicator of synaptic recruitment of CP-AMPARs, in the lOFC-BLA synapse. CP-AMPARs were reported to have Ca^{2+} permeability and implied to contribute to the plasticity of synaptic transmission and structure [69, 70]. Our previous study demonstrated that stress-induced recruitment of CP-AMPARs in the lOFC-BLA pathway contributed to increased passive stress-coping in mice [44]. Similarly, CP-AMPARs-induced changes in synaptic transmission or plasticity might increase passive stress-coping behaviour in socially isolated mice.

In addition to synaptic changes, we found that adolescent social isolation decreased intrinsic excitability in neurons receiving mOFC inputs, but increased it in neurons receiving lOFC inputs in the BLA. It is possible that these changes in intrinsic excitability also contribute to abnormalities in social and stress-coping behaviours.

Notably, adolescent social isolation induced distinct synaptic and changes in the mOFC-BLA and lOFC-BLA pathways. The difference in the prefrontal subregion-dependent responsivity to the early social environment may contribute to the distinct synaptic changes. In fact, it was reported that lOFC neurons had an increased dendritic spine density [65], while the density of dendritic spines was decreased by social isolation in mPFC neurons [71]. Further, in rats, adolescent stress decreased glutamic acid decarboxylase 65/67 expression in the mOFC, but not in the lOFC [72]. It is also possible that the mOFC and lOFC have different cellular responsiveness to social isolation, and it might lead to distinct synaptic changes in the downstream pathways in the BLA.

Distinct modulation of the mOFC-BLA and lOFC-BLA pathways on social and passive stress-coping behaviours

In this study, the mOFC-BLA optogenetic manipulation modulated social behaviour without affecting passive stress-coping behaviour, while the lOFC-BLA manipulation altered passive stress-coping behaviour without affecting social behaviour. These results suggest that the mOFC and lOFC would separately modulate social and stress-coping behaviours. This pathway-selective modulation effect of the mOFC and lOFC on social and passive stress-coping behaviours might be due to the heterogeneity of downstream BLA neurons. Several studies have indicated that dissociable neuronal subsets within the BLA have different functions, and these subsets induce distinct synaptic plasticity by reward or aversive conditioning [67, 73]. Notably, a single-cell imaging study reported that some subsets of BLA neurons were activated during social interaction [74]. mOFC neurons might preferentially project to these social subsets of neurons, while the lOFC might project to others within the BLA. Moreover, these separated neuronal subsets may separately modulated social and passive stress-coping behaviours.

We observed a distinct spatial arrangement of mOFC and lOFC projections in the BLA. These data indicated that neurons in medial and dorsal parts of the BLA received inputs from both the mOFC and lOFC. Meanwhile, neurons in ventral and lateral parts of the BLA preferentially receive inputs from the lOFC but not mOFC. These differences might contribute to separate mechanisms of regulation of social and stress-coping behaviours, where social isolation induces distinct changes in synaptic transmission and intrinsic excitability in the mOFC-BLA and lOFC-BLA pathways. Interestingly, several studies have demonstrated that neurons in the medial and dorsal BLA preferentially project to the nucleus accumbens, involved in reward and social processing, while neurons in the lateral and ventral BLA project to the central amygdala, involved in aversive behaviours [67, 73, 75]. Future functional imaging or electrophysiological recordings focusing on input-output specificity or the spatial arrangement in the BLA by anterograde and retrograde transsynaptic methods may clarify this issue.

Clinical implications of early social deprivation-induced alterations in the OFC-BLA pathway

In human functional imaging studies, abnormal activities in the mOFC, lOFC, and amygdala were observed in patients with psychiatric disorders that are associated with early-life social adversity [42, 43]. In the present study, we showed that early social deprivation could change excitatory transmissions in the OFC-amygdala synapse. These synaptic changes might underlie abnormal functional connectivity. This speculation raises the possibility that manipulation of the neuroplasticity in the mOFC-BLA and lOFC-BLA circuits could provide an effective therapeutic approach for early social adversity-related psychiatric symptoms. Interestingly, high frequency transcranial direct current stimulation or repetitive transcranial magnetic stimulation could target the mOFC or lOFC neural activity, and it has been reported that these manipulations have some positive effects on patients with obsessive-compulsive and depressive disorders [76–78]. These non-invasive neuromodulation therapies also might be effective for emotional and social problems in social adversity-related psychiatric disorders.

Limitations and future implications

In this study, we found that social isolation caused changes in synaptic transmission and excitability in OFC-BLA pathways. However, how the mOFC-BLA and lOFC-BLA circuits are activated during social behaviour and immobility in vivo and the effects of social isolation are still unclear. Although a previous study using fiberphotometry showed activation of BLA projecting neurons in the mOFC during social approach [45], further research on OFC-BLA activity during behaviour with fiberphotometry or unit recordings is needed to improve our understanding of OFC-BLA functions in social and stress-coping behaviours, and the effect of social isolation.

Another limitation of the present study is that we used only male mice. However, several studies have reported sex differences in the effects of social isolation in rodents [25, 79, 80]. Therefore, to understand whether social isolation induces sex-related changes in OFC-BLA circuits, further research in female mice is warranted.

In addition, the behavioural and electrophysiological tests were performed in different animals in this study. However, behavioural testing followed by electrophysiological analysis in the same animals allows to better examine correlations or perform clustered analysis. In the future, these experiments may allow a further understanding of the effect of social isolation on OFC-BLA function.

REFERENCES

1. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci. 2001;24:1161–92.
2. Bick J, Nelson CA. Early adverse experiences and the developing brain. Neuropsychopharmacology. 2016;41:177–96.
3. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev. 2003;27:3–18.
4. Blakemore SJ, Choudhury S. Development of the adolescent brain: implications for executive function and social cognition. J Child Psychol Psychiatry. 2006;47:296–312.
5. Baarendse PJ, Counotte DS, O'Donnell P, Vanderschuren LJ. Early social experience is critical for the development of cognitive control and dopamine modulation of prefrontal cortex function. Neuropsychopharmacology 2013;38:1485–94.
6. Cacioppo JT, Fowler JH, Christakis NA. Alone in the crowd: the structure and spread of loneliness in a large social network. J Pers Soc Psychol. 2009;97:977–91.
7. Orben A, Tomova L, Blakemore SJ. The effects of social deprivation on adolescent development and mental health. Lancet Child Adolesc Health. 2020;4:634–40.
8. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. Dev Psychobiol. 2010;52:671–90.
9. Naughton AM, Maguire SA, Mann MK, Lumb RC, Tempest V, Gracias S, et al. Emotional, behavioral, and developmental features indicative of neglect or

emotional abuse in preschool children: a systematic review. JAMA Pediatr. 2013;167:769–75.
10. Nemeroff CB. Paradise lost: the neurobiological and clinical consequences of child abuse and neglect. Neuron 2016;89:892–909.
11. Liu J, Dupree JL, Gacias M, Frawley R, Sikder T, Naik P, et al. Clemastine enhances myelination in the prefrontal cortex and rescues behavioral changes in socially isolated mice. J Neurosci. 2016;36:957–62.
12. Lukkes JL, Watt MJ, Lowry CA, Forster GL. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. Front Behav Neurosci. 2009;3:18.
13. Matsumoto H, Omata N, Kiyono Y, Mizuno T, Mita K, Kosaka H. Paradoxical changes in mood-related behaviors on continuous social isolation after weaning. Exp Brain Res. 2021;239:2537–50.
14. Sargin D, Oliver DK, Lambe EK. Chronic social isolation reduces 5-HT neuronal activity via upregulated SK3 calcium-activated potassium channels. eLife. 2016;5:e21416.
15. Swire M, Kotelevtsev Y, Webb DJ, Lyons DA, Ffrench-Constant C. Endothelin signalling mediates experience-dependent myelination in the CNS. eLife. 2019;8: e49493.
16. Tulogdi A, Toth M, Barsvari B, Biro L, Mikics E, Haller J. Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats. Dev Psychobiol. 2014;56:49–57.
17. Makinodan M, Rosen KM, Ito S, Corfas G. A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 2012;337:1357–60.
18. Dixon ML, Thiruchselvam R, Todd R, Christoff K. Emotion and the prefrontal cortex: an integrative review. Psychol Bull. 2017;143:1033–81.
19. Gangopadhyay P, Chawla M, Dal Monte O, Chang SWC. Prefrontal-amygdala circuits in social decision-making. Nat Neurosci. 2021;24:5–18.
20. Kober H, Barrett LF, Joseph J, Bliss-Moreau E, Lindquist K, Wager TD. Functional grouping and cortical-subcortical interactions in emotion: a meta-analysis of neuroimaging studies. NeuroImage. 2008;42:998–1031.
21. Wager TD, Davidson ML, Hughes BL, Lindquist MA, Ochsner KN. Prefrontal-subcortical pathways mediating successful emotion regulation. Neuron 2008;59:1037–50.
22. Murray EA, Fellows LK. Prefrontal cortex interactions with the amygdala in primates. Neuropsychopharmacology. 2022;47:163–79.
23. Yamamuro K, Bicks LK, Leventhal MB, Kato D, Im S, Flanigan ME, et al. A prefrontal–paraventricular thalamus circuit requires juvenile social experience to regulate adult sociability in mice. Nat Neurosci. 2020;23:1240–1252.
24. Park G, Ryu C, Kim S, Jeong SJ, Koo JW, Lee YS, et al. Social isolation impairs the prefrontal-nucleus accumbens circuit subserving social recognition in mice. Cell Rep. 2021;35:109104.
25. Tan T, Wang W, Liu T, Zhong P, Conrow-Graham M, Tian X, et al. Neural circuits and activity dynamics underlying sex-specific effects of chronic social isolation stress. Cell Rep. 2021;34:108874.
26. Toth M, Tulogdi A, Biro L, Soros P, Mikics E, Haller J. The neural background of hyper-emotional aggression induced by post-weaning social isolation. Behav Brain Res. 2012;233:120–9.
27. Biro L, Toth M, Sipos E, Bruzsik B, Tulogdi A, Bendahan S, et al. Structural and functional alterations in the prefrontal cortex after post-weaning social isolation: relationship with species-typical and deviant aggression. Brain Struct Funct. 2017;222:1861–75.
28. Szczepanski SM, Knight RT. Insights into human behavior from lesions to the prefrontal cortex. Neuron. 2014;83:1002–18.
29. Downar J. Orbitofrontal cortex: a 'non-rewarding' new treatment target in depression? Curr Biol. 2019;29:R59–R62.
30. Kuniishi H, Ichisaka S, Matsuda S, Futora E, Harada R, Hata Y. Chronic inactivation of the orbitofrontal cortex increases anxiety-like behavior and impulsive aggression, but decreases depression-like behavior in rats. Front Behav Neurosci. 2016;10:250.
31. Rempel-Clower NL. Role of orbitofrontal cortex connections in emotion. Ann NY Acad Sci. 2007;1121:72–86.
32. Rolls ET. The functions of the orbitofrontal cortex. Brain Cogn. 2004;55:11–29.
33. Beer JS, John OP, Scabini D, Knight RT. Orbitofrontal cortex and social behavior: integrating self-monitoring and emotion-cognition interactions. J Cogn Neurosci. 2006;18:871–9.
34. Hornak J, Bramham J, Rolls ET, Morris RG, O'Doherty J, Bullock PR, et al. Changes in emotion after circumscribed surgical lesions of the orbitofrontal and cingulate cortices. Brain. 2003;126:1691–712.
35. Kuusinen V, Cesnaite E, Perakyla J, Ogawa KH, Hartikainen KM. Orbitofrontal lesion alters brain dynamics of emotion-attention and emotion-cognitive control interaction in humans. Front Hum Neurosci. 2018;12:437.
36. Drevets WC. Orbitofrontal cortex function and structure in depression. Ann NY Acad Sci. 2007;1121:499–527.
37. Wolf RC, Thomann PA, Sambataro F, Vasic N, Schmid M, Wolf ND. Orbitofrontal cortex and impulsivity in borderline personality disorder: an MRI study of baseline brain perfusion. Eur Arch Psychiatry Clin Neurosci. 2012;262:677–85.
38. Johnson JG, Cohen P, Brown J, Smailes EM, Bernstein DP. Childhood maltreatment increases risk for personality disorders during early adulthood. Arch Gen Psychiatry. 1999;56:600–6.
39. Martins-Monteverde CMS, Baes CVW, Reisdorfer E, Padovan T, Tofoli SMC, Juruena MF. Relationship between depression and subtypes of early life stress in adult psychiatric patients. Front Psychiatry. 2019;10:19.
40. Janak PH, Tye KM. From circuits to behaviour in the amygdala. Nature. 2015;517:284–92.
41. Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron. 2005;48:175–87.
42. Goetschius LG, Hein TC, Mitchell C, Lopez-Duran NL, McLoyd VC, Brooks-Gunn J, et al. Childhood violence exposure and social deprivation predict adolescent amygdala-orbitofrontal cortex white matter connectivity. Dev Cogn Neurosci. 2020;45:100849.
43. Jedd K, Hunt RH, Cicchetti D, Hunt E, Cowell RA, Rogosch FA, et al. Long-term consequences of childhood maltreatment: Altered amygdala functional connectivity. Dev Psychopathol. 2015;27:1577–89.
44. Kuniishi H, Yamada D, Wada K, Yamada M, Sekiguchi M. Stress induces insertion of calcium-permeable AMPA receptors in the OFC-BLA synapse and modulates emotional behaviours in mice. Transl Psychiatry. 2020;10:154.
45. Li A, Jing D, Dellarco DV, Hall BS, Yang R, Heilberg RT, et al. Role of BDNF in the development of an OFC-amygdala circuit regulating sociability in mouse and human. Mol Psychiatry. 2021;26:955–73.
46. Burks JD, Conner AK, Bonney PA, Glenn CA, Baker CM, Boettcher LB, et al. Anatomy and white matter connections of the orbitofrontal gyrus. J Neurosurg. 2018;128:1865–72.
47. Izquierdo A. Functional heterogeneity within rat orbitofrontal cortex in reward learning and decision making. J Neurosci. 2017;37:10529–40.
48. Mar AC, Walker AL, Theobald DE, Eagle DM, Robbins TW. Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat. J Neurosci. 2011;31:6398–404.
49. Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol. 2004;72:341–372.
50. O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C. Abstract reward and punishment representations in the human orbitofrontal cortex. Nat Neurosci. 2001;4:95–102.
51. Cheng W, Rolls ET, Qiu J, Liu W, Tang YQ, Huang CC, et al. Medial reward and lateral non-reward orbitofrontal cortex circuits change in opposite directions in depression. Brain. 2016;139:3296–309.
52. Yamada D, Wada K, Sekiguchi M. Modulation of long-term potentiation of cortico-amygdala synaptic responses and auditory fear memory by dietary polyunsaturated fatty acid. Front Behav Neurosci. 2016;10:164.
53. Cui Q, Li Q, Geng H, Chen L, Ip NY, Ke Y, et al. Dopamine receptors mediate strategy abandoning via modulation of a specific prelimbic cortex-nucleus accumbens pathway in mice. Proc Natl Acad Sci USA. 2018;115:E4890–E99.
54. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transpl. 2013;48:452–58.
55. Kauer JA, Malenka RC. Synaptic plasticity and addiction. Nat Rev Neurosci. 2007;8:844–58.
56. Arruda-Carvalho M, Clem RL. Pathway-selective adjustment of prefrontal-amygdala transmission during fear encoding. J Neurosci. 2014;34:15601–9.
57. Hensch TK. Critical period plasticity in local cortical circuits. Nat Rev Neurosci. 2005;6:877–88.
58. Reh RK, Dias BG, Nelson CA 3rd, Kaufer D, Werker JF, Kolb B, et al. Critical period regulation across multiple timescales. Proc Natl Acad Sci USA. 2020;117:23242–51.
59. Arruda-Carvalho M, Wu WC, Cummings KA, Clem RL. Optogenetic examination of prefrontal-amygdala synaptic development. J Neurosci. 2017;37:2976–85.
60. Tottenham N, Gabard-Durnam LJ. The developing amygdala: a student of the world and a teacher of the cortex. Curr Opin Psychol. 2017;17:55–60.
61. Thor DH, Holloway WR Jr. Social play in juvenile rats: a decade of methodological and experimental research. Neurosci Biobehav Rev. 1984;8:455–64.
62. Avale ME, Chabout J, Pons S, Serreau P, De Chaumont F, Olivo-Marin JC, et al. Prefrontal nicotinic receptors control novel social interaction between mice. Faseb J. 2011;25:2145–55.
63. Kim Y, Venkataraju KU, Pradhan K, Mende C, Taranda J, Turaga SC, et al. Mapping Social Behavior-Induced Brain Activation at Cellular Resolution in the Mouse. Cell Rep. 2015;10:292–305.
64. Rivera-Irizarry JK, Skelly MJ, Pleil KE. Social Isolation Stress in Adolescence, but not Adulthood, Produces Hypersocial Behavior in Adult Male and Female C57BL/6J Mice. Front Behav Neurosci. 2020;14:129.
65. Hinton EA, Li DC, Allen AG, Gourley SL Social isolation in adolescence disrupts cortical development and goal-dependent decision-making in adulthood, despite social reintegration. eNeuro. 2019;6:ENEURO.0318-19.

66. Han RT, Kim YB, Park EH, Kim JY, Ryu C, Kim HY, et al. Long-term isolation elicits depression and anxiety-related behaviors by reducing oxytocin-induced gabaergic transmission in central amygdala. Front Mol Neurosci. 2018;11:246.
67. Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, et al. A circuit mechanism for differentiating positive and negative associations. Nature 2015;520:675–8.
68. Márquez C, Poirier GL, Cordero MI, Larsen MH, Groner A, Marquis J, Magistretti PJ, et al. Transl Psychiatry. 2013;3:e216.
69. Fortin DA, Davare MA, Srivastava T, Brady JD, Nygaard S, Derkach VA, et al. Long-term potentiation-dependent spine enlargement requires synaptic Ca2+-permeable AMPA receptors recruited by CaM-kinase I. J Neurosci. 2010;30:11565–75.
70. Henley JM, Wilkinson KA. Synaptic AMPA receptor composition in development, plasticity and disease. Nat Rev Neurosci. 2016;17:337–50.
71. Silva-Gomez AB, Rojas D, Juarez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Res. 2003;983:128–36.
72. Tzanoulinou S, García-Mompó C, Riccio C, Grosse J, Zanoletti O, Dedousis P, et al. Neuroligin-2 expression in the prefrontal cortex is involved in attention deficits induced by peripubertal stress. Neuropsychopharmacology 2016;41:751–61.
73. Beyeler A, Chang CJ, Silvestre M, Leveque C, Namburi P, Wildes CP, et al. Organization of valence-encoding and projection-defined neurons in the basolateral amygdala. Cell Rep. 2018;22:905–18.
74. Fustinana MS, Eichlisberger T, Bouwmeester T, Bitterman Y, Luthi A. State-dependent encoding of exploratory behaviour in the amygdala. Nature. 2021;592:267–71.
75. O'Leary TP, Sullivan KE, Wang L, Clements J, Lemire AL, Cembrowski MS. Extensive and spatially variable within-cell-type heterogeneity across the basolateral amygdala. Elife. 2020;9:e59003.
76. Grover S, Nguyen JA, Viswanathan V, Reinhart RMG. High-frequency neuromodulation improves obsessive-compulsive behavior. Nat Med. 2021;27:232–38.
77. Feffer K, Fettes P, Giacobbe P, Daskalakis ZJ, Blumberger DM, Downar J. 1Hz rTMS of the right orbitofrontal cortex for major depression: Safety, tolerability and clinical outcomes. Eur Neuropsychopharmacol. 2018;28:109–17.
78. Schutter DJ, van Honk J. Increased positive emotional memory after repetitive transcranial magnetic stimulation over the orbitofrontal cortex. J Psychiatry Neurosci. 2006;31:101–4.
79. Pisu MG, Garau A, Boero G, Biggio F, Pibiri V, Dore R, et al. Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. Neuroscience. 2016;320:172–82.
80. Senst L, Baimoukhametova D, Sterley TL, Bains JS. Sexually dimorphic neuronal responses to social isolation. Elife. 2016;5:e18726.

ACKNOWLEDGEMENTS

We would like to thank Ms. Yasuko Nakamura, Ms. Hiromi Fujita, Ms. Masako Shikama, Ms. Yoshiko Hara, Mr. Shuhei Kayashima, and Dr. Misa Yamada for their assistance with the experiments and animal care. We would like to thank Editage (www.editage.com) for English language editing.

AUTHOR CONTRIBUTIONS

HK, MY, and MS conceived and designed the experiments. HK and YN performed the experiments and analysed the data. HK, MY, and MS contributed to writing the manuscript. All authors discussed the results and implications and critiqued the manuscript.

FUNDING

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. This research was supported by KAKENHI (grant number: 18K15533, 21J01636), an Intramural Research Grant (grant number: 30-1 and 3-1) for Neurological and Psychiatric Disorders funded by the National Centre of Neurology and Psychiatry (NCNP) and research grant from Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics, Japan.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-022-01358-6.

Correspondence and requests for materials should be addressed to Hiroshi Kuniishi.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.