Neurosci. Bull. October, 2019, 35(5):841–852
https://doi.org/10.1007/s12264-019-00373-w

www.neurosci.cn
www.springer.com/12264

ORIGINAL ARTICLE

Enhancement of Aggression Induced by Isolation Rearing is Associated with a Lack of Central Serotonin

Yiqiong Liu[1,8] · Yunong Sun[7] · Xiaoyan Zhao[2] · Ji-Young Kim[3] · Lu Luo[4] · Qian Wang[4] · Xiaolu Meng[6] · Yonghui Li[6] · Nan Sui[6] · Zhou-Feng Chen[3] · Chuxiong Pan[2] · Liang Li[4,5] · Yan Zhang[1,8]

Received: 10 September 2018 / Accepted: 13 January 2019 / Published online: 11 April 2019

Abstract Isolation rearing (IR) enhances aggressive behavior, and the central serotonin (5-hydroxytryptamine, 5-HT) system has been linked to IR-induced aggression. However, whether the alteration of central serotonin is the cause or consequence of enhanced aggression is still unknown. In the present study, using mice deficient in central serotonin *Tph2*$^{-/-}$ and *Lmx1b*$^{-/-}$, we examined the association between central serotonin and aggression with or without social isolation. We demonstrated that central serotonergic neurons are critical for the enhanced aggression after IR. 5-HT depletion in wild-type mice increased aggression. On the other hand, application of 5-HT in *Lmx1b*$^{-/-}$ mice inhibited the enhancement of aggression under social isolation conditions. Dopamine was downregulated in *Lmx1b*$^{-/-}$ mice. Similar to 5-HT, L-DOPA decreased aggression in *Lmx1b*$^{-/-}$ mice. Our results link the serotoninergic system directly to aggression and this may have clinical implications for aggression-related human conditions.

Keywords 5-HT · Aggression · Social isolation · Dopamine · Lmx1b

Introduction

The central serotonin (5-hydroxytryptamine, 5-HT) system plays a role in regulating aggressive behaviors [1–4]. In humans, aggressive personality disorders have been associated with alterations of central 5-HT activity [5–9], 5-HT receptor 2A [10, 11], and the 5-HT transporter [12, 13]. Also, reactive aggression in patients with attention deficit/hyperactivity disorder is associated with 5-HT levels in the central nervous system [14, 15]. In patients with schizophrenia, the level of serotonin transporter in blood lymphocytes and platelets as well as its genetic variants is associated with an aggressive phenotype [16–18]. Since enhanced aggressive behaviors are associated with low

Yiqiong Liu, Yunong Sun, Xiaoyan Zhao have contributed equally to work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12264-019-00373-w) contains supplementary material, which is available to authorized users.

✉ Liang Li
liangli@pku.edu.cn

✉ Yan Zhang
yanzhang@pku.edu.cn

[1] State Key Laboratory of Membrane Biology, College of Life Sciences, Peking University, Beijing 100871, China

[2] Department of Anesthesiology, Beijing Tongren Hospital, Capital Medical University, Beijing 100005, China

[3] Department of Anesthesiology, Department of Psychiatry, Department of Developmental Biology, Center for the Study of Itch, Washington University School of Medicine, Saint Louis, MO 63110, USA

[4] School of Psychological and Cognitive Sciences, Beijing Key Laboratory of Behavior and Mental Health, Peking University, Beijing 100871, China

[5] Beijing Institute for Brain Disorders, Beijing 100069, China

[6] Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China

[7] Hendrix College, Conway, AR 72032, USA

[8] PKU-IDG/McGovern Institute for Brain Research, Beijing 100871, China

5-HT function [19–22], 5-HT may suppress aggression. However, there has been controversy regarding whether isolation rearing (IR) increases or decreases the 5-HT level in the central nervous system. Some studies have shown that early maternal deprivation significantly reduces the level of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brain of infant monkeys throughout their early development from 14 to 150 days of age [23, 24]. Low central 5-HIAA concentrations are strongly related to excessive aggression [25, 26]. Also, 5-HT immune-reactivity decreases in the anterior hypothalamus of maternally-separated rats and this 5-HT loss is correlated with the display of aggression [27, 28]. In addition, in IR rats the terminals of serotonergic neurons disappear from the CA region of the hippocampus [29] and the basal turnover of serotonin decreases significantly in the nucleus accumbens [30, 31]. Although the basal level of 5-HT in the prefrontal cortex is unaffected by IR, 5-HT release in this cortical structure under the condition of amphetamine-challenge becomes attenuated in isolates [32]. Nevertheless, other studies have shown that IR increases the 5-HT level in all brain regions except for the raphe nuclei [33, 34], particularly in the prefrontal cortex and nucleus accumbens [35].

IR from weaning in laboratory animals produces a range of persistent behavioral changes in the young adult, including hyperactivity in response to novelty and amphetamine, and altered responses to conditioning; it is an effective stressful manipulation in early life that affects brain development [36, 37], causing enhanced aggression [21, 38–42] and other behavioral and cognitive deficits. This isolation rearing time is a pretty effective early-life stage for the brain development. [36, 37, 43–51]. Since IR also affects brain structures and neurotransmission [29, 30, 32, 46, 52–62], some of these changes may underlie the IR-related enhancement of aggression.

To investigate whether 5-HT is responsible for enhanced aggression in IR mice, we investigated the effects of IR on aggressive behavior in both wild type (WT) and 5-HT-deficient mice either lacking the essential enzyme for synthesizing 5-HT (tryptophan hydroxylase 2, Tph2) [63] or lacking LIM-homeobox-transcription factor 1 β (Lmx1b) [64]. We also investigated the effects of 5-HT depletion in WT mice and the supply of 5-HT to $Lmx1b^{-/-}$ mice.

Materials and Methods

Animals, Genotyping, and Social Isolation

The animal maintenance and handling followed the regulations of Peking University Animal Care and Use Committee. The experimental procedures were approved by the Peking University Animal Care and Use Committee and followed the NIH Guidelines regarding the care and use of animals for experimental procedures. *ePet1*-Cre/$Lmx1b^{flox}$/$Lmx1b^{flox}$ ($Lmx1b^{-/-}$), *ePet1*-Cre/$Lmx1b^{flox/+}$ ($Lmx1b^{+/-}$) and $Tph2^{-/-}$ mice were kind gifts from Dr. Zhou-Feng Chen (Washington University, St. Louis, MO) [63, 64]. $Lmx1b^{+/+}$ refers to mice that were not crossed with the Cre line. All WT C57BL/6J and knockout mice were maintained under a 12 h/12 h light/dark cycle and housed with their mothers for 27 days after birth and before weaning (usually on day 21 after birth). Food and water were supplied *ad libitum*. On day 28, male mice were selected for experiments and genotyped. Genomic DNA was extracted from the tail. The primers used to examine the *Lmx1b* and *Tph2* lines were as previously described [63]. The social-isolation (SI) groups were then singly housed for 4 weeks. The controls were divided into groups of 5 mice per cage (24×17×12 cm) and socially-reared for 4 weeks. Moreover, all the mice were kept in the same room so that they had the same visual, auditory, and olfactory contact with other animals. The handling was kept to the minimum necessary. Mice from the same litter were assigned equally to isolation and social housing conditions.

Resident-Intruder Test

To test the aggression of the group-housed and SI mice, a male intruder mouse (8 weeks of age, socially reared) was put into the resident cages at 17:00–18:00 and the behavior was videotaped (Quickcam, Logitech, Lausanne) overnight for 12 h. The aggression was measured as the frequency of fighting, latency to the first attack, and the average duration of fighting within the first 3 h of recording. The experimenters were blind to the genotype and housing conditions of the mice.

Open Field

Open field tests began after a 30-min acclimation period in the test room in a transparent 43×43×30.5 cm open field box (ENV-520, Med Associates, Fairfax). Each mouse was placed in the center of the box and the travel distance and rearing behavior were recorded for 1 h. The box was cleaned thoroughly after each test.

Elevated Plus Maze

The elevated plus maze apparatus consisted of four 32-cm long, 5-cm wide arms in the shape of a cross, 50 cm above the ground. Two arms were open and the others were closed by 15-cm walls. Illumination of the test room was kept constant with two floor lamps throughout the test (10

lumens). Mice were located in the center of the cross, of which head leaned against the walls at the beginning of the test. The total time the mice stayed in the open and closed arms during 5 min was measured and the numbers of times mice entered the open and closed arms were counted.

Beam Walking

The beam-walking test was conducted using a wooden beam 8 mm in diameter and 1 m long. The beam was placed 30 cm above a table and was horizontally connected to a box at one end for the mouse to escape. The mouse was placed at one end of the beam and allowed to walk to the box in 5 trials/day for 3 training days. After training, the mouse was placed on the beam and the average time to cross the beam and the numbers of hind-paw slips from 5 trials were recorded.

Rotarod

The rotarod test (IITC Life Science, Woodland Hills, CA) was conducted by placing a mouse on the apparatus rotating at 30 rpm for 5 training trials on 5 days. After training, the latency to falling from the rotarod was recorded for 5 test trials.

Acoustic Startle Response

The acoustic startle reflex was measured with an apparatus consisting of a non-restrictive Plexiglas cylinder (4 cm inner diameter, 13 cm long) mounted on a Plexiglas platform. The apparatus was placed in a ventilated, sound-attenuated chamber and cylinder movement was measured by a piezoelectric element mounted under each cylinder. Startle stimuli were presented through a high-frequency loudspeaker located 33 cm above the startle cylinders. The background noise was 70 dB. Startle magnitudes were sampled every millisecond during a 200-ms period beginning at the onset of the startle stimulus. The mice were allowed 5 min at the beginning of the test to become familiar with the environment and then sound stimuli were delivered at random intervals. The stimulus intensities were 90, 100, and 110 dB. All intensities were presented in a pseudo-random order, 10 times each. The maximum peak response in every 200 ms was recorded (startle amplitude) as an index of vigilance or arousal.

Forced Swimming

The forced swimming test was carried out using a Plexiglas cylinder 18 cm in diameter containing 25 cm of water at 21°C–22°C. Each mouse was placed in the water, left for 15 min, and then removed. The same mouse was placed back into the cylinder 24 h after the first test and the total immobility period during 10 min was recorded.

Tail Suspension

The tail-suspension test was performed by suspending a mouse at $\sim$2 cm from the tip of the tail, 30 cm above a table for 10 min. The total immobile time was recorded.

Passive Avoidance Test

In the passive avoidance experiment, the apparatus consisted of two chambers of equal size connected by an arched channel. One chamber was enclosed by white walls and the other by black walls. The floor of black chamber was made up of several electrodes for shocks. Four infrared light beams detect the location of the animal. The mice were placed in the white chamber at the beginning of training, with the middle channel open to allow free movement from one side to the other. When all four paws of a mouse were in the black chamber, the channel was closed and a shock was delivered (0.6 mA for 1 s), then the mouse remained in the black chamber for the time remaining. The whole trial time was 300 s. After 24 h, each mouse was tested in the same manner as training, but without receiving a shock. The latency to the first entry into the black chamber was recorded as a test of inhibitory avoidance memory.

Morris Water Maze

The Morris water maze apparatus consisted of a circular pool 120 cm in diameter and 50 cm in height. The maze was placed in a room in which cues were available on the walls. The pool contained 30 cm of water at 21°C–22°C. The hidden platform was a 10-cm circular plate 2 cm below the water surface. First, all of the animals were placed in the room to become familiar with the environment. On the next day, the mice were trained to escape onto the hidden platform. The pool was conceptually divided into four quadrants of equal area. The hidden platform was placed in the center of one quadrant throughout the experiments. Each of the four cardinal points in the perimeter of the pool was randomly used as a starting point. A trial started when a mouse was placed in the pool facing the cues and ended when the it escaped onto the platform. In each trial, the escape latency, swimming speed, and total swimming distance to the hidden platform were recorded. If a mouse failed to escape within 60 s, the trial was terminated with an escape latency defined as 60 s. All the mice were tested 4 times per day for 5 days. On day 6, a probe trial was carried out, in which the time each mouse spent in each quadrant without the platform was recorded.

Drug Treatments

An indwelling cannula was implanted in the brain. All mice performed behavioral tasks on day 6 after surgery[65]. 5-HT (500 nmol/L, 10 μL; Sigma, St. Louis, MO) was injected intracerebroventricularly. The behavioral tests and measurement of 5-HT/5-HIAA and bioamine levels were performed 30 min after injection. WT mice were injected intracerebroventricularly with para-chlorophenylalanine (pCPA) (200 μg, 10 μL; Sigma) or 1% Tween saline control for 3 days before behavioral tests and the 5-HT/5-HIAA levels were measured. L-DOPA (Sigma) was dissolved in 0.1 mol/L HCl, neutralized by 0.1 mol/L NaOH, and then injected intracerebroventricularly (100 μg, 10 μL). The aggression test was performed 30 min after drug injection.

Measurement of 5-HT/5-HIAA and Bioamine Levels

The levels of 5-HT, 5-HIAA, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the indicated regions were measured by high-performance liquid chromatography (HPLC). After cervical dislocation, the brain was removed on ice. The indicated regions were dissected out and weighted. For measurement of 5-HT and 5-HIAA, the tissues were homogenized on ice in buffer containing 0.2 mol/L perchloric acid, 100 μmol/L EDTA, and 100 ng/mL isoproterenol. For measurement of DA, DOPAC and HVA, the tissues were put into HeGA buffer (catecholamine/monoamine preservative solution containing 0.1 mol/L glacial acetic acid, 0.1 mmol/L EDTA, 0.12% oxidized L-glutathione, pH 3.7), homogenized, and centrifuged at 15000 g for 30 min at 4°C. After filtering through 0.45 μm membrane, samples were injected into HPLC with electrochemical detection (HPLC-ECD) with an auto-sampler (L-2200, Hitachi, Tokyo, Japan). The samples were separated at 25°C with a reversed-phase analytical column (Eicom, Japan) and eluted at 0.5 mL/min at 30°C with 0.1 mol/L sodium acetate, 17% methanol, 190 mg/L sodium L-octanesulfonate, and 5 mg/L EDTA. The levels were measured by an electrochemical detector (2465, Waters, Massachusetts, USA).

Sexual Preference Determination

All mice were sexually naïve. After SI or social rearing for 4 weeks, an 8-week-old female C57BL/6J mouse and an 8-week-old male C57BL/6J mouse were placed into a cage with each experimental male mouse. The mating and mounting behaviors were observed for 10 min. The first mounting choice over male or female was used to determine the sexual preference of the home-caged male mouse.

Statistical Evaluation

Statistical significance was assessed by one-way, two-way, or three-way analysis of variance (ANOVA). Sheffé's test was applied *post hoc* for significant differences shown by ANOVAs. A P value < 0.05 or < 0.01 was taken to indicate statistical significance.

Results

Central 5-HT-Deficient Mice Showed Enhanced Aggression After SI

Tph2 is the essential synthetic enzyme converting tryptophan into 5-hydroxytryptophan (5-HTP) which can then be converted into 5-HT. $Tph2^{-/-}$ mice showed a remarkable decrease in the brain 5-HT level [63]. All behavioral experiments were carried out independently on 8-week-old mice. Under social rearing conditions, after the intruder was introduced (Fig. 1A), $Tph2^{-/-}$ mice showed more attacks, a shorter latency to the first attack, and a longer fighting time than WT mice (Fig. 2A, C, E, $P < 0.01$). In the SI groups, $Tph2^{-/-}$ mice showed a significant increase in fighting frequency and a decrease in first-fight latency (Fig. 2A, C, $P < 0.01$). However, the increase of average attack duration was not significant (Fig. 2E, $P = 0.896$). In contrast to $Tph2^{-/-}$ mice, $Tph2^{+/-}$ mice did not show significant differences in fighting frequency, first-fight latency and average attack duration compared with the WT in both the socially reared and isolated groups (Fig. 2A, C, E). There was an interaction between central 5-HT loss and social isolation in the regulation of frequency and duration, but there is no interaction on the regulation of latency (Fig. 2A, C, E, two-way ANOVA, F_{fre} (2, 42) = 10.45, F_{lat} (2, 42) = 0.2267, F_{dur} (2, 42) = 4.353). There are two types of tryptophan hydroxylase 1 (Tph), Tph1 and Tph2. Although many reports have shown that Tph2 is expressed in the central and Tph1 in the peripheral nervous system, there is also evidence that Tph1 is present in the central nervous system [1]. Therefore, $Tph2^{-/-}$ mice could have remaining 5-HT in their central nervous system. To further investigate the role of 5-HT in the development of aggression, we used conditional Lmx1b-knockout mice in which almost all the serotonergic neurons in the central nervous system are deleted from embryogenesis to adulthood [63, 64]. $Lmx1b^{-/-}$ mice showed a dramatic increase of aggressive behavior in the SI groups (Fig. 2B, D, F, $P < 0.01$ for frequency, first-fight latency, and average duration). There was interaction between central 5-HT loss and social isolation on the regulation of frequency and latency, but is no interaction on the regulation of duration (Fig. 2B, D, F, two-way

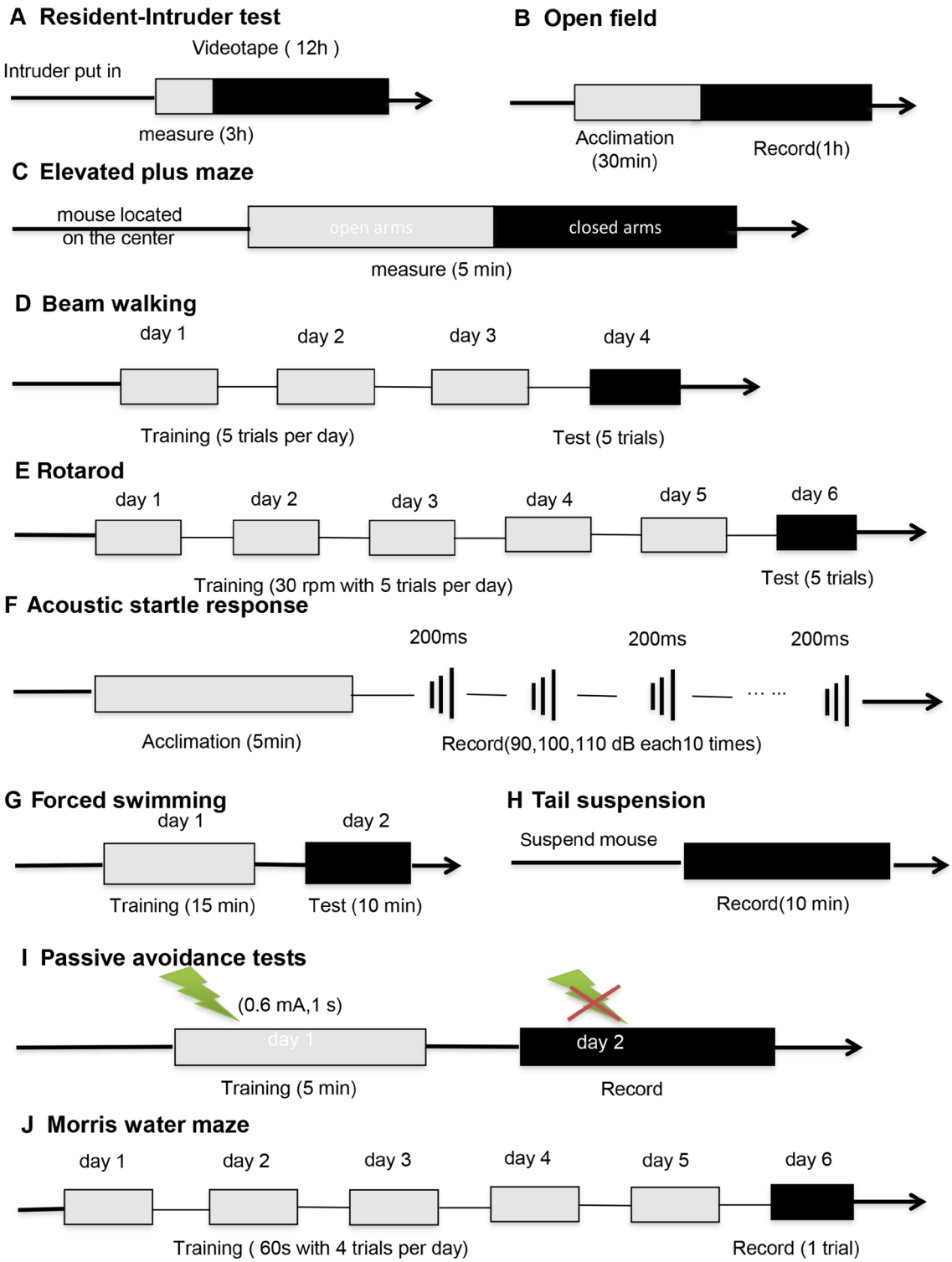

Fig. 1 Schematics of experimental methods. **A** Resident-intruder test. **B** Open field. **C** Elevated plus maze. **D** Beam walking. **E** Rotarod test. **F** Acoustic startle response. **G** Forced swimming. **H** Tail suspension. **I** Passive avoidance test. **J** Morris water maze.

ANOVA, F_{fre} (3, 56) = 22.28, F_{lat} (3, 56) = 3.924, F_{dur} (3, 56) = 2.58).

There is evidence that 5-HT transporter blockade impairs motor activity in mice [66] and mice with 5-HT_{1B} receptor-knockout are hyperactive [67]. We then examined the motor functions of 5-HT-deficient mice. The results of the open field, elevated plus maze, beam walking, and rotarod tests (Fig. 1B–E) demonstrated no significant

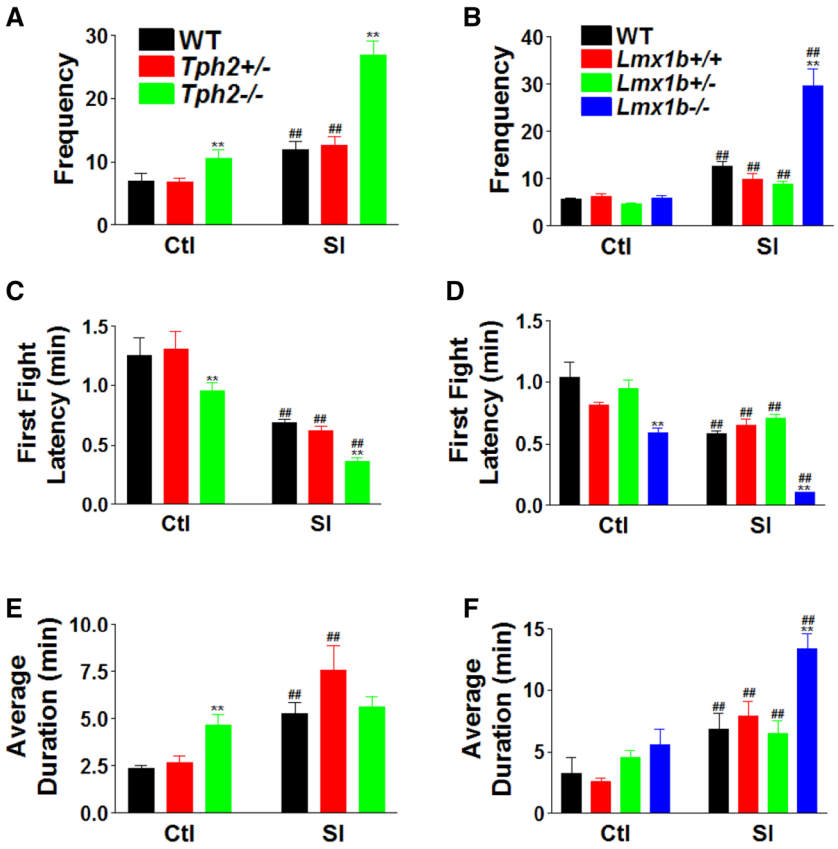

Fig. 2 Central 5-HT-deficient mice showed enhanced aggression. *Tph2*$^{-/-}$ (**A, C, E**) and *Lmx1b*$^{-/-}$ mice (**B, D, F**) showed greater aggression as measured by the frequency of attack, latency to first attack, and average duration of fighting than WT mice in both the socially-reared (Ctl) and isolated (SI) groups (n = 8/group). In the *Tph2* group, there was interaction between central 5-HT loss and social isolation on the regulation of frequency and duration, but no interaction on the regulation of latency (F_{fre} (2, 42) = 10.45, F_{lat} (2, 42) = 0.2267, F_{dur} (2, 42) = 4.353). In the *Lmx1b* group, there was interaction between central 5-HT loss and social isolation on the regulation of frequency and latency, but no interaction on the regulation of duration (F_{fre} (3, 56) = 22.28, F_{lat} (3, 56) = 3.924, F_{dur} (3, 56) = 2.58). Data are represented as mean ± SEM. $^{**}P < 0.01$ *vs* WT mice, $^{\#\#}P < 0.01$ *vs* socially-reared mice.

differences in *Tph2*$^{+/-}$, *Tph2*$^{-/-}$, *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$, and *Lmx1b*$^{-/-}$ mice compared to WT mice in both socially grouped and isolated animals (Fig. S1A–N), indicating that *Tph2*$^{-/-}$ and *Lmx1b*$^{-/-}$ mice had normal locomotor activity. The acoustic startle reflex (Fig. 1F) is used as a simple and objective indicator of emotionality and attention in rodents and humans [68]. Our data on the startle responses of WT and 5-HT-deficient mice to 90, 100, and 110 dB sounds showed no significant differences (Fig. S1 O, P). 5-HT has also been suggested to play roles in emotions such as anxiety and depression [69, 70]. Surprisingly, our data on the forced-swimming, tail-suspension, and passive-avoidance tests (Fig. 1G–I) showed no noticeable differences between *Tph2*$^{-/-}$, *Lmx1b*$^{-/-}$, and WT mice in both the socially-reared and SI groups (Fig. S2A–F). Cognitive functions are also influenced by the serotonergic system [69] and SI [71]. Our results from the Morris water maze (Fig. 1J) indicated that spatial learning and memory was not altered in the socially and isolated *Lmx1b*$^{-/-}$ (Fig. S2G–J), *Tph2*$^{-/-}$ (Fig. S2K–N), and WT mice. Taken together, our data suggested that mice with central 5-HT deficiency showed more aggression in SI group than WT mice, which was not due to differences in their locomotor function, emotions, and spatial learning/memory.

Central 5-HT was Responsible for the Aggressive Phenotype

Since 5-HT plays important roles in both the central and peripheral systems, especially the digestive system [1], we decided to minimize the central 5-HT action in WT mice by intracerebroventricular injection of pCPA (200 μg, 10 μL). This treatment led to a dramatic reduction in the levels of 5-HT and its metabolite 5-HIAA in various brain areas (Fig. 3A, C, $P < 0.01$). pCPA-treated mice showed intense enhancement of aggression after SI, similar to that found in 5-HT-deficient mice (Fig. 3E, F, $P < 0.01$). In the other hand, when *Lmx1b*$^{-/-}$ mice were injected intracerebroventricularly with 5-HT (500 nmol/L, 10 μL), the levels of

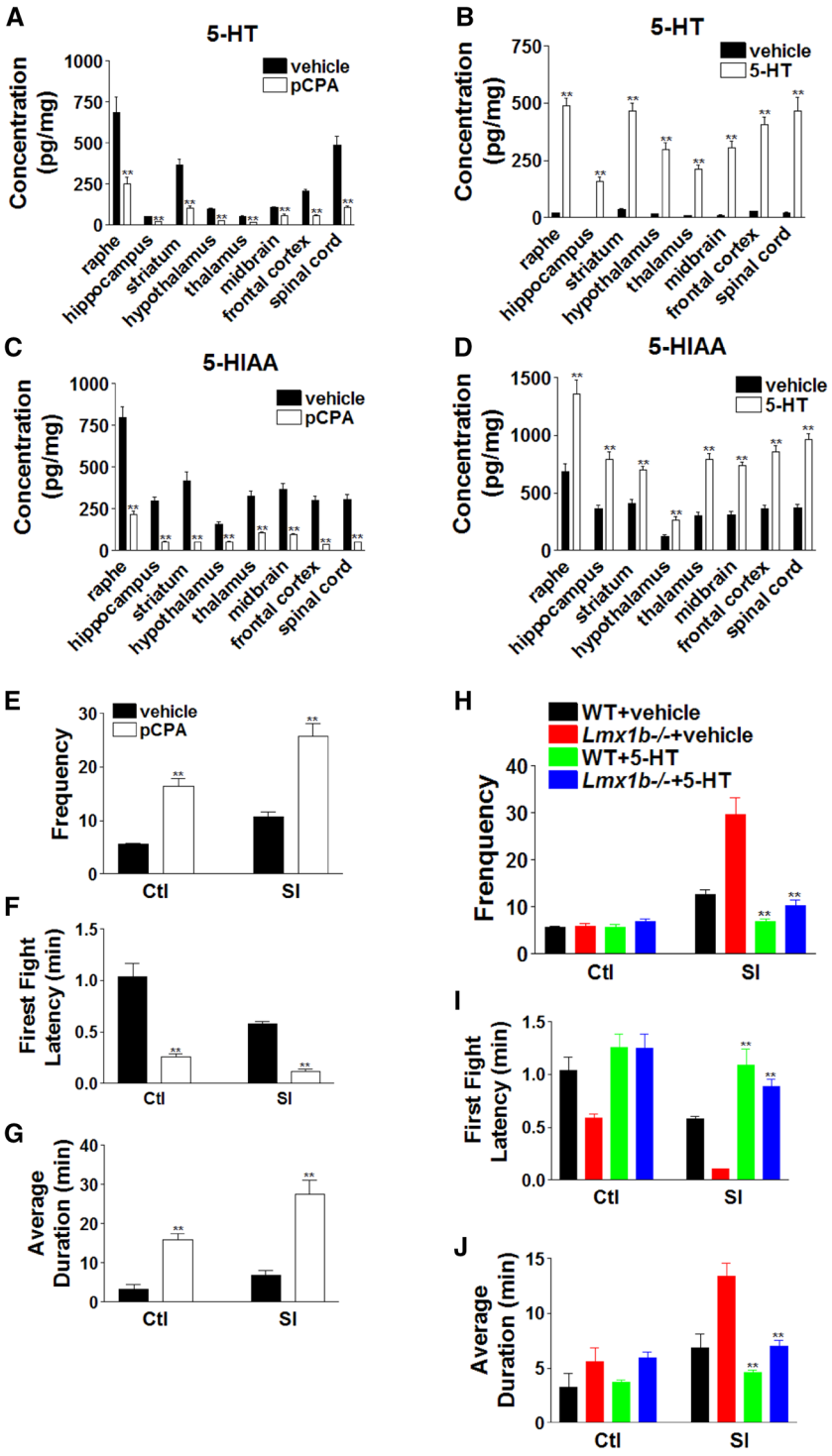

Fig. 3 Depletion of 5-HT with pCPA increased aggression in WT mice and 5-HT reversed the enhanced aggression in *Lmx1b*$^{-/-}$ mice. **A, C** Levels of 5-HT and 5-HIAA in various brain regions decreased with central pCPA treatment (n = 3/group). **E, F, G** Aggression increased in WT mice treated with pCAP centrally (n = 8/group). **B, D** Levels of 5-HT and 5-HIAA in various brain regions increased with central 5-HT treatment (n = 3/group). **H, I, J** Aggression decreased in *Lmx1b*$^{-/-}$ mice treated with 5-HT centrally (n = 8/group). Data represent mean ± SEM. $^{**}P < 0.01$ *vs* vehicle-treated group.

5-HT and 5-HIAA increased (Fig. 3B, D, $P < 0.01$). 5-HT rescued the aggressive phenotype in *Lmx1b*$^{-/-}$ mice (Fig. 3H, I, J, $P < 0.01$). The results of both depletion and rescue experiments confirmed that 5-HT is responsible for the enhanced aggression in *Lmx1b*$^{-/-}$ mice.

5-HT-deficient mice have been reported to exhibit mis-regulated sexual preference [63]; *Lmx1b*$^{-/-}$ mice show loss of sexual preference in male-female and male-male mounting [63]. Since sexual competition is one of the potential drives for aggressive behavior, we investigated whether the sexual preference of *Lmx1b*$^{-/-}$ mice affects their aggression. By introducing one sexually-naïve male mouse and one female mouse together to the home cage of the tested male mouse and observing the first mounting

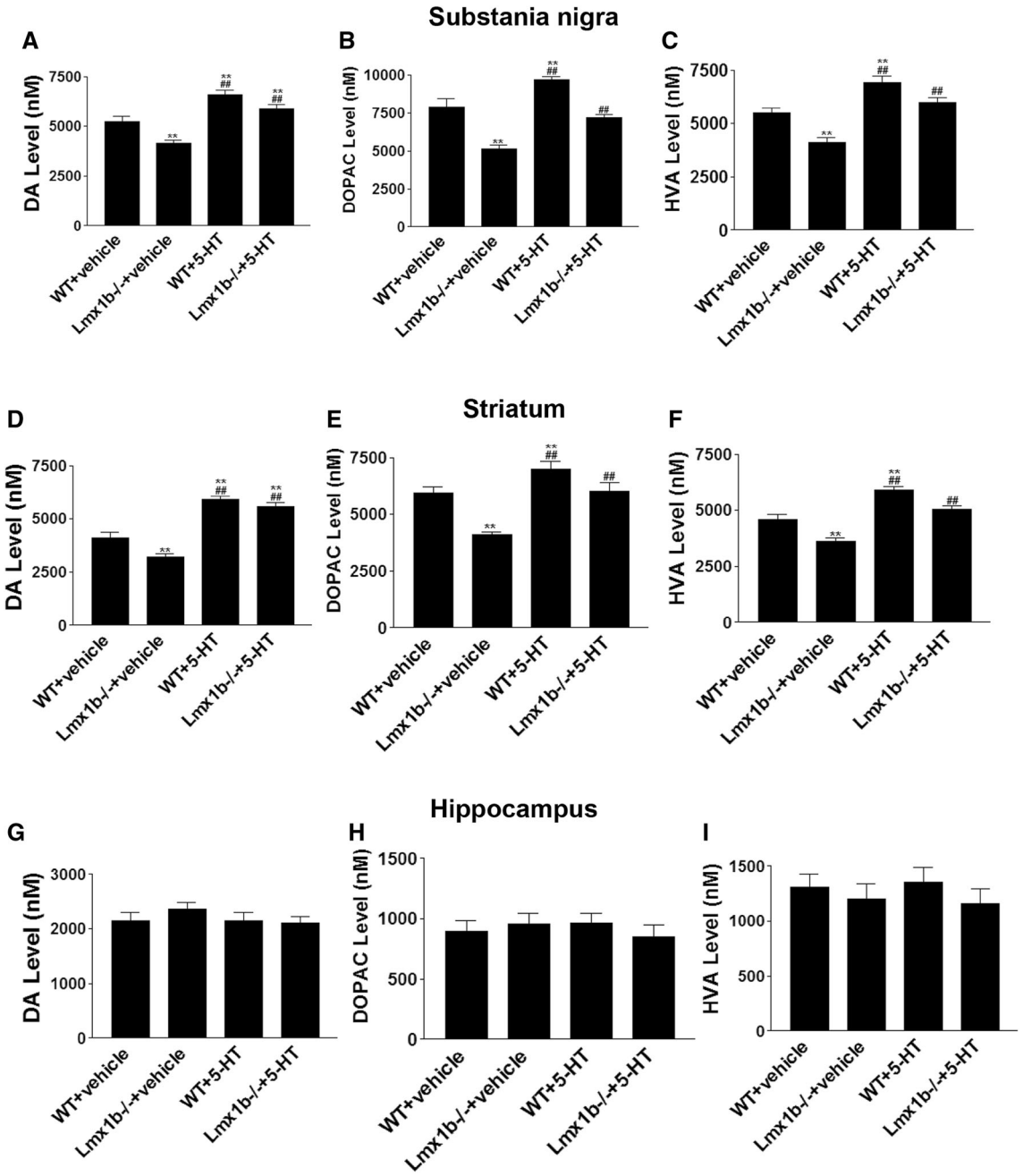

Fig. 4 Levels of DA and its metabolites were downregulated in *Lmx1b*$^{-/-}$ mice. The levels of DA, DOPAC, and HVA were measured in the substantia nigra (**A**–**C**), striatum (**D**–**F**), and hippocampus (**G**–**I**). DA and its metabolites decreased in the substantia nigra and striatum but not in the hippocampus (n = 3/group). Data represent mean ± SEM. $^{**}P < 0.01$ *vs* WT mice, $^{\#\#}P < 0.01$ *vs* vehicle treatment group.

choice of the tested male mouse, we divided the WT and *Lmx1b*$^{-/-}$ mice into two groups according to their sexual preference: a group favoring male and a group favoring female. The groups neither showed a significant difference in baseline aggression in the socially-reared condition nor in the enhancement of aggression in the SI condition (Fig. S3 A–C), suggesting that sexual preference did not affect aggression in *Lmx1b*$^{-/-}$ mice.

DA Signaling Was Involved in Aggression of *Lmx1b*$^{-/-}$ Mice

The DA system interacts with the serotonin system and mediates various functions. Selective serotonin reuptake inhibitors (SSRIs) and 5-HT transporter blockers reduce DA signaling [66, 72–75], and the 5-HT1A receptor is involved in DA release in the frontal cortex of mice

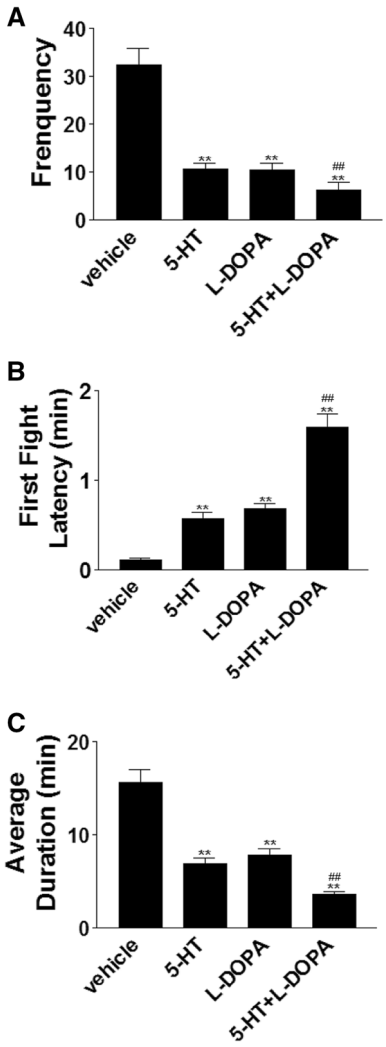

Fig. 5 L-DOPA reversed the enhanced aggression, while central delivery of L-DOPA reduced the aggression in socially-isolated *Lmx1b*$^{-/-}$ mice (n = 8/group). Data represent mean + SEM. $^{**}P < 0.01$ *vs* vehicle treatment group, $^{\#\#}P < 0.01$ *vs* 5-HT and L-DOPA alone groups.

[76–78]. We further investigated whether DA signaling is involved in the enhanced aggression in *Lmx1b*$^{-/-}$ mice. In the substantia nigra and striatum, the levels of DA as well as its metabolites DOPAC and HVA, significantly decreased in *Lmx1b*$^{-/-}$ mice, and this was reversed by 5-HT treatment (Fig. 4A, B, C, $P < 0.01$; Fig. 4C, D, E $P < 0.01$). However, the levels of DA and its metabolites did not change in the hippocampus in WT and *Lmx1b*$^{-/-}$ mice with or without 5-HT application (Fig. 4G, H, I), suggesting that 5-HT regulates DA levels, and this might alter the aggressive behavior in *Lmx1b*$^{-/-}$ mice. To confirm that DA is involved in aggression and that regulation of DA levels modulates aggression, L-DOPA was injected intracerebroventricularly in socially-isolated *Lmx1b*$^{-/-}$ mice. The aggression was largely reduced with L-DOPA treatment compared to vehicle treatment (Fig. 5, $P < 0.01$). Interestingly, applying 5-HT and L-DOPA together further decreased the aggression compared to application of 5-HT or L-DOPA alone (Fig. 5, $P < 0.01$), suggesting an additive effect of the 5-HT and DA systems.

Discussion

The SI animal model has been used to study the mechanisms underlying schizophrenia for many years [70]. Aggression is significantly enhanced after SI [70]. It is widely accepted that the serotonin system is related to the development of schizophrenia and aggression [13, 79, 80]. However, definite confirmation of its causative role in aggression and enhanced aggression under SI condition has not been achieved. By using two mouse lines with deficits in central 5-HT, we demonstrated that depletion of central 5-HT greatly increased aggression in the socially-reared environment and largely amplified the enhancement of aggression in the socially -isolated condition. Pharmacological depletion of central 5-HT in WT mice mimicked the effects in 5-HT-deficient mouse lines. Furthermore, 5-HT successfully rescued the phenotype of increased aggression in *Lmx1b*$^{-/-}$ mice. We also excluded the possibility that locomotion, emotional state, and learning/memory may influence aggression, since *Lmx1b*$^{-/-}$ did not differ from WT mice in the above measurements, which is consistent with the findings of the group that originally produced this mouse line [64]. 5-HT has been suggested to play roles in emotions such as anxiety and depression. Moreover, depression-like behaviors can also change after social isolation [69, 70]. Surprisingly, our data from the forced swimming, tail suspension, and passive avoidance tests showed no notable differences between *Tph2*$^{-/-}$, *Lmx1b*$^{-/-}$, and WT mice in both the socially-reared and SI groups. Thus, although several mouse lines were used to address the relationship between central 5-HT deficiency and anxiety- and depression-like behaviors, we cannot exclude the possibility that the effects on these behaviors are caused indirectly by compensations or other unknown changes associated with the loss of 5-HT transmitter or serotonergic neurons.

In fact, the anxiety- and depression-like behaviors in Tph2-deficient mice are still a controversial problem [81, 82]. The deletion of 5-HT1A receptors increases anxiety, but reduces immobility in the forced swimming test [83]. However, effects on anxiety- and depression-like behaviors of manipulations of the 5-HT system were not evident in our 5-HT-deficient mice, neither in the socially-reared nor the SI groups. A previous study demonstrated that 5-HT deficiency in the brain may not be sufficient to

lead to depression- and anxiety-like behaviors in mice [84]. Nevertheless, our results link central 5-HT directly to aggressive behavior.

We also found that DA signaling is involved and modulates aggression. A recent study reported that the prefrontal DA and 5-HT systems in male isolation-reared mice are activated by stimulation, and then abnormal behaviors are induced in these mice, such as aggression and hyperactivity [85]. When the serotonin production is decreased in Tph2-deficient mice, the degradation of serotonin has been reported to decrease so as to maintain the brain serotonin levels. These results suggested that the abnormal behavior found in Tph2-deficient mice is not caused by the decreased TPH2 activity [86]. Since the primary change in *Lmx1b*$^{-/-}$ mice is the deletion of central serotoninergic neurons and loss of most of the central 5-HT activity from embryonic day 16.5 [64] without affecting DA signaling, we conclude that the changes in the levels of DA and its metabolites in various brain areas are secondary to 5-HT modulation. It is possible that the 5-HT system interacts closely with the DA system and the absence of 5-HT neurons and its activity induces the downregulation of DA levels or dopaminergic neurons either during development or adulthood. Like 5-HT, L-DOPA reverses the aggression in *Lmx1b*$^{-/-}$ mice, indicating that L-DOPA, besides 5-HT, could be a potential treatment to relieve extreme aggressive behavior in human conditions such as schizophrenia. Furthermore, applying 5-HT and L-DOPA together induce an additive response, suggesting that the serotoninergic and dopaminergic systems might be parallel pathways in regulating aggression, so interference with either one or both pathways might improve aggressive conditions.

Further research focused on understanding the differential regulation, modulation, and function of serotoninergic and dopaminergic systems may shed light on the development of effective disease-treatment strategies. Importantly, the development of more specific drugs targeted at this may have considerable application prospects in aggression-related human conditions and in the treatment of clinical and social problems.

Acknowledgements We thank Dr. Jian-Xu Zhang (Institute of Zoology, Chinese Academy of Sciences) and Dr. Yan Liu (Peking University) for reading the manuscript and helpful suggestions. This work was supported by the National Natural Science Foundation of China (81425009, 31630028, 91632305, 30950030, 31170988, and 81671044), and the National Basic Research Development Program (973 Program) of China (2009CB522002).

Conflicts of interest The authors declare no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work.

References

1. Waider J, Araragi N, Gutknecht L, Lesch KP. Tryptophan hydroxylase-2 (TPH2) in disorders of cognitive control and emotion regulation: a perspective. Psychoneuroendocrinology 2011, 36: 393–405.
2. Olivier B. Serotonin: A never-ending story. Eur J Pharmacol 2015, 753: 2–18.
3. Bell R, Hobson H. 5-HT1A receptor influences on rodent social and agonistic behavior: a review and empirical study. Neurosci Biobehav Rev 1994, 18: 325–338.
4. Dolan M, Deakin WJ, Roberts N, Anderson I. Serotonergic and cognitive impairment in impulsive aggressive personality disordered offenders: are there implications for treatment? Psychol Med 2002, 32: 105–117.
5. Coccaro EF, Lee R, Kavoussi RJ. Aggression, suicidality, and intermittent explosive disorder: serotonergic correlates in personality disorder and healthy control subjects. Neuropsychopharmacology 2010, 35: 435–444.
6. Russo S, Kema IP, Bosker F, Haavik J, Korf J. Tryptophan as an evolutionarily conserved signal to brain serotonin: molecular evidence and psychiatric implications. World J Biol Psychiatry 2009, 10: 258–268.
7. McCloskey MS, Ben-Zeev D, Lee R, Berman ME, Coccaro EF. Acute tryptophan depletion and self-injurious behavior in aggressive patients and healthy volunteers. Psychopharmacology (Berl) 2009, 203: 53–61.
8. Lanctot KL, Herrmann N, Eryavec G, van Reekum R, Reed K, Naranjo CA. Central serotonergic activity is related to the aggressive behaviors of Alzheimer's disease. Neuropsychopharmacology 2002, 27: 646–654.
9. Suri D, Teixeira CM, Cagliostro MKC, Mahadevia D, Ansorge MS. Monoamine–sensitive developmental periods impacting adult emotional and cognitive behaviors. Neuropsychopharmacology 2015, 40: 88–112.
10. Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS, *et al.* Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. Biol Psychiatry 2010, 67: 1154–1162.
11. Soloff PH, Chiappetta L, Mason NS, Becker C, Price JC. Effects of serotonin-2A receptor binding and gender on personality traits and suicidal behavior in borderline personality disorder. Psychiatry Res Neuroimaging 2014, 222: 140–148.
12. Silva H, Iturra P, Solari A, Villarroel J, Jerez S, Jimenez M, *et al.* Fluoxetine response in impulsive-aggressive behavior and serotonin transporter polymorphism in personality disorder. Psychiatr Genet 2010, 20: 25–30.
13. Bortolato M, Pivac N, Muck Seler D, Nikolac Perkovic M, Pessia M, Di Giovanni G. The role of the serotonergic system at the interface of aggression and suicide. Neuroscience 2013, 236: 160–185.
14. Stadler C, Zepf FD, Demisch L, Schmitt M, Landgraf M, Poustka F. Influence of rapid tryptophan depletion on laboratory-provoked aggression in children with ADHD. Neuropsychobiology 2007, 56: 104–110.
15. Kotting WF, Bubenzer S, Helmbold K, Eisert A, Gaber TJ, Zepf FD. Effects of tryptophan depletion on reactive aggression and aggressive decision-making in young people with ADHD. Acta Psychiatr Scand 2013, 128: 114–123.
16. Barkan T, Peled A, Modai I, Barak P, Weizman A, Rehavi M. Serotonin transporter characteristics in lymphocytes and platelets of male aggressive schizophrenia patients compared to non-aggressive schizophrenia patients. Eur Neuropsychopharmacol 2006, 16: 572–579.

17. Frisch A, Finkel B, Michaelovsky E, Sigal M, Laor N, Weizman R. A rare short allele of the serotonin transporter promoter region (5-HTTLPR) found in an aggressive schizophrenic patient of Jewish Libyan origin. Psychiatr Genet 2000, 10: 179–183.
18. Steinert T HK. External validity of studies on aggressive behavior in patients with schizophrenia: systematic review. Clin Pract Epidemiol Ment Health 2012, 8: 74–80.
19. Linnoila VM, Virkkunen M. Aggression, suicidality, and serotonin. J Clin Psychiatry 1992, 53 Suppl: 46–51.
20. Miczek KA, Fish EW, De Bold JF, De Almeida RM. Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma-aminobutyric acid systems. Psychopharmacology (Berl) 2002, 163: 434–458.
21. Veenema AH. Early life stress, the development of aggression and neuroendocrine and neurobiological correlates: what can we learn from animal models? Front Neuroendocrinol 2009, 30: 497–518.
22. Westergaard GC, Suomi SJ, Higley JD, Mehlman PT. CSF 5-HIAA and aggression in female macaque monkeys: species and interindividual differences. Psychopharmacology (Berl) 1999, 146: 440–446.
23. Shannon C, Schwandt ML, Champoux M, Shoaf SE, Suomi SJ, Linnoila M*, et al.* Maternal absence and stability of individual differences in CSF 5-HIAA concentrations in rhesus monkey infants. Am J Psychiatry 2005, 162: 1658–1664.
24. Shrestha SS, Nelson EE, Liow JS, Gladding R, Lyoo CH, Noble PL*, et al.* Fluoxetine administered to Juvenile monkeys: effects on the serotonin transporter and behavior. Am J Psychiatry 2014, 171: 323–331.
25. Higley JD, King ST Jr., Hasert MF, Champoux M, Suomi SJ, Linnoila M. Stability of interindividual differences in serotonin function and its relationship to severe aggression and competent social behavior in rhesus macaque females. Neuropsychopharmacology 1996, 14: 67–76.
26. Higley JD, Mehlman PT, Higley SB, Fernald B, Vickers J, Lindell SG*, et al.* Excessive mortality in young free-ranging male nonhuman primates with low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations. Arch Gen Psychiatry 1996, 53: 537–543.
27. Veenema AH, Blume A, Niederle D, Buwalda B, Neumann ID. Effects of early life stress on adult male aggression and hypothalamic vasopressin and serotonin. Eur J Neurosci 2006, 24: 1711–1720.
28. Diamantopoulou A, Raftogianni A, Stamatakis A, Alikaridis F, Oitzl MS, Stylianopoulou F. Denial of reward in the neonate shapes sociability and serotonergic activity in the adult rat. PLoS One 2012, 7: e33793.
29. Whitaker-Azmitia P, Zhou F, Hobin J, Borella A. Isolation-rearing of rats produces deficits as adults in the serotonergic innervation of hippocampus. Peptides 2000, 21: 1755–1759.
30. Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G*, et al.* Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 2000, 100: 749–768.
31. Soga T, Teo CH, Cham KL, Idris MM, Parhar IS. Early-life social isolation impairs the gonadotropin-inhibitory hormone neuronal activity and serotonergic system in male rats. Front Endocrinol (Lausanne) 2015, 6: 172.
32. Dalley JW, Theobald DE, Pereira EA, Li PM, Robbins TW. Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. Psychopharmacology (Berl) 2002, 164: 329–340.
33. Miura H, Qiao H, Ohta T. Attenuating effects of the isolated rearing condition on increased brain serotonin and dopamine turnover elicited by novelty stress. Brain Res 2002, 926: 10–17.
34. Yorgason JT, Calipari ES, Ferris MJ, Karkhanis AN, Fordahl SC, Weiner JL*, et al.* Social isolation rearing increases dopamine uptake and psychostimulant potency in the striatum. Neuropharmacology 2016, 101: 471–479.
35. Han X, Wang W, Shao F, Li N. Isolation rearing alters social behaviors and monoamine neurotransmission in the medial prefrontal cortex and nucleus accumbens of adult rats. Brain Res 2011, 1385: 175–181.
36. Weiss IC, Domeney AM, Moreau JL, Russig H, Feldon J. Dissociation between the effects of pre-weaning and/or post-weaning social isolation on prepulse inhibition and latent inhibition in adult Sprague–Dawley rats. Behav Brain Res 2001, 121: 207–218.
37. Kosten TA, Kim JJ, Lee HJ. Early life manipulations alter learning and memory in rats. Neurosci Biobehav Rev 2012, 36: 1985–2006.
38. Matsumoto K, Uzunova V, Pinna G, Taki K, Uzunov DP, Watanabe H*, et al.* Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. Neuropharmacology 1999, 38: 955–963.
39. Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders. Brain Res Brain Res Rev 2001, 37: 110–115.
40. Koike H, Ibi D, Mizoguchi H, Nagai T, Nitta A, Takuma K*, et al.* Behavioral abnormality and pharmacologic response in social isolation-reared mice. Behav Brain Res 2009, 202: 114–121.
41. Toth M, Halasz J, Mikics E, Barsy B, Haller J. Early social deprivation induces disturbed social communication and violent aggression in adulthood. Behav Neurosci 2008, 122: 849–854.
42. Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996, 75: 27–32.
43. Arakawa H. Interaction between isolation rearing and social development on exploratory behavior in male rats. Behav Processes 2005, 70: 223–234.
44. Paulus MP, Bakshi VP, Geyer MA. Isolation rearing affects sequential organization of motor behavior in post-pubertal but not pre-pubertal Lister and Sprague-Dawley rats. Behav Brain Res 1998, 94: 271–280.
45. Geyer MA, Wilkinson LS, Humby T, Robbins TW. Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biol Psychiatry 1993, 34: 361–372.
46. Jones GH, Marsden CA, Robbins TW. Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: neurochemical correlates. Behav Brain Res 1991, 43: 35–50.
47. Li N, Ping J, Wu R, Wang C, Wu X, Li L. Auditory fear conditioning modulates prepulse inhibition in socially reared rats and isolation-reared rats. Behav Neurosci 2008, 122: 107–118.
48. Li N, Wu X, Li L. Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats. Behav Pharmacol 2007, 18: 135–145.
49. Reboucas RC, Schmidek WR. Handling and isolation in three strains of rats affect open field, exploration, hoarding and predation. Physiol Behav 1997, 62: 1159–1164.
50. Varty GB, Paulus MP, Braff DL, Geyer MA. Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biol Psychiatry 2000, 47: 864–873.
51. Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology 1994, 10: 61–72.

52. Day-Wilson KM, Jones DN, Southam E, Cilia J, Totterdell S. Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle. Neuroscience 2006, 141: 1113–1121.
53. Harte MK, Powell SB, Reynolds LM, Swerdlow NR, Geyer MA, Reynolds GP. Reduced N-acetylaspartate in the temporal cortex of rats reared in isolation. Biol Psychiatry 2004, 56: 296–299.
54. Heidbreder CA, Foxton R, Cilia J, Hughes ZA, Shah AJ, Atkins A, *et al.* Increased responsiveness of dopamine to atypical, but not typical antipsychotics in the medial prefrontal cortex of rats reared in isolation. Psychopharmacology (Berl) 2001, 156: 338–351.
55. Jones GH. Social isolation and individual differences: behavioural and dopaminergic responses to psychomotor stimulants. Clin Neuropharmacol 1992, 15 Suppl 1 Pt A: 253A–254A.
56. Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav 1992, 43: 17–35.
57. Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci Behav Physiol 2003, 33: 13–29.
58. Muchimapura S, Mason R, Marsden CA. Effect of isolation rearing on pre- and post-synaptic serotonergic function in the rat dorsal hippocampus. Synapse 2003, 47: 209–217.
59. Preece MA, Dalley JW, Theobald DE, Robbins TW, Reynolds GP. Region specific changes in forebrain 5-hydroxytryptamine1A and 5-hydroxytryptamine2A receptors in isolation-reared rats: an in vitro autoradiography study. Neuroscience 2004, 123: 725–732.
60. Gilabert-Juan J, Moltó MD, Nacher J. Post-weaning social isolation rearing influences the expression of molecules related to inhibitory neurotransmission and structural plasticity in the amygdala of adult rats. Brain Res 2012, 1448: 129–136.
61. Quan MN, Tian YT, Xu KH, Zhang T, Yang Z. Post weaning social isolation influences spatial cognition, prefrontal cortical synaptic plasticity and hippocampal potassium ion channels in Wistar rats. Neuroscience 2010, 169: 214–222.
62. Han X, Wang W, Shao F, Li N. Isolation rearing alters social behaviors and monoamine neurotransmission in the medial prefrontal cortex and nucleus accumbens of adult rats. Brain Res 2011, 1385: 175–181.
63. Liu Y, Jiang Y, Si Y, Kim JY, Chen ZF, Rao Y. Molecular regulation of sexual preference revealed by genetic studies of 5-HT in the brains of male mice. Nature 2011, 472: 95–99.
64. Zhao ZQ, Scott M, Chiechio S, Wang JS, Renner KJ, Gereau RWt, *et al.* Lmx1b is required for maintenance of central serotonergic neurons and mice lacking central serotonergic system exhibit normal locomotor activity. J Neurosci 2006, 26: 12781–12788.
65. Seyer B, Pham V, Albiston AL, Chai SY. Cannula implantation into the lateral ventricle does not adversely affect recognition or spatial working memory. Neurosci Lett 2016, 628: 171–178.
66. Morelli E, Moore H, Rebello TJ, Gray N, Steele K, Esposito E, *et al.* Chronic 5-HT transporter blockade reduces DA signaling to elicit basal ganglia dysfunction. J Neurosci 2011, 31: 15742–15750.
67. Brunner D, Buhot MC, Hen R, Hofer M. Anxiety, motor activation, and maternal-infant interactions in 5HT1B knockout mice. Behav Neurosci 1999, 113: 587–601.
68. Li L, Du Y, Li N, Wu X, Wu Y. Top-down modulation of prepulse inhibition of the startle reflex in humans and rats. Neurosci Biobehav Rev 2009, 33: 1157–1167.
69. Marsden CA, King MV, Fone KC. Influence of social isolation in the rat on serotonergic function and memory–relevance to models of schizophrenia and the role of 5-HT receptors. Neuropharmacology 2011, 61: 400–407.
70. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. Nature 2010, 468: 203–212.
71. Ibi D, Takuma K, Koike H, Mizoguchi H, Tsuritani K, Kuwahara Y, *et al.* Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. J Neurochem 2008, 105: 921–932.
72. Settle EC, Jr. Antidepressant drugs: disturbing and potentially dangerous adverse effects. J Clin Psychiatry 1998, 59 Suppl 16: 25–30; discussion 40–22.
73. Cassano P, Fava M. Tolerability issues during long-term treatment with antidepressants. Ann Clin Psychiatry 2004, 16: 15–25.
74. Damsa C, Bumb A, Bianchi-Demicheli F, Vidailhet P, Sterck R, Andreoli A, *et al.* "Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. J Clin Psychiatry 2004, 65: 1064–1068.
75. Preskorn SH. Reboxetine: a norepinephrine selective reuptake pump inhibitor. J Psychiatr Pract 2004, 10: 57–63.
76. Ago Y, Sakaue M, Baba A, Matsuda T. Selective reduction by isolation rearing of 5-HT1A receptor-mediated dopamine release in vivo in the frontal cortex of mice. J Neurochem 2002, 83: 353–359.
77. Song N, Xie J. Iron, Dopamine, and alpha-synuclein interactions in at-Risk dopaminergic neurons in Parkinson's disease. Neurosci Bull 2018, 34: 382–384.
78. Qian A, Wang X, Liu H, Tao J, Zhou J, Ye Q, *et al.* Dopamine D4 receptor gene associated with the frontal-striatal-cerebellar loop in children with ADHD: a resting-state fMRI study. Neurosci Bull 2018, 34: 497–506.
79. Shin JK, Malone DT, Crosby IT, Capuano B. Schizophrenia: a systematic review of the disease state, current therapeutics and their molecular mechanisms of action. Curr Med Chem 2011, 18: 1380–1404.
80. Hayes DJ, Greenshaw AJ. 5-HT receptors and reward-related behaviour: a review. Neurosci Biobehav Rev 2011, 35: 1419–1449.
81. Savelieva KV, Zhao S, Pogorelov VM, Rajan I, Yang Q, Cullinan E, *et al.* Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. PLoS One 2008, 3: e3301.
82. Belmaker RH, Agam G, Bersudsky Y. Role of GSK3beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A 2008, 105: E23; author reply E24.
83. Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, *et al.* Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A 1998, 95: 14476–14481.
84. Jia YF, Song NN, Mao RR, Li JN, Zhang Q, Huang Y, *et al.* Abnormal anxiety- and depression-like behaviors in mice lacking both central serotonergic neurons and pancreatic islet cells. Front Behav Neurosci 2014, 8: 325.
85. Ago Y, Araki R, Tanaka T, Sasaga A, Nishiyama S, Takuma K, *et al.* Role of social encounter-induced activation of prefrontal serotonergic systems in the abnormal behaviors of isolation-reared mice. Neuropsychopharmacology 2013, 38: 1535–1547.
86. Mosienko V, Matthes S, Hirth N, Beis D, Flinders M, Bader M, *et al.* Adaptive changes in serotonin metabolism preserve normal behavior in mice with reduced TPH2 activity. Neuropharmacology 2014, 85: 73–80.