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Neurological mechanism of Xiaochaihutang's antidepressant-like effects to socially isolated adult rats

Jie Ma[a], Chun Fu Wu[a], Fang Wang[a], Jing Yu Yang[a], Ying Xu Dong[a], Guang Yue Su[b], Kuo Zhang[a], Zhi Qian Wang[a], Long Wen Xu[a], Xing Pan[a], Ting Shuo Zhou[a], Ping Ma[a] and Shao Jiang Song[c]

[a]Department of Pharmacology, [b]Department of Functional Food and Wine and [c]Department of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

Keywords
5-HT_{1A} receptor; chronic social isolation stress; neurogenesis; neurotransmitter

Correspondence
Chun Fu Wu, Department of Pharmacology, Shenyang Pharmaceutical University, Box 31, 103 Wenhua Road, 110016 Shenyang, China.
E-mail: wucf@syphu.edu.cn

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Abstract

Objectives Xiaochaihutang (XCHT) has antidepressant effects in multiple animal models of depression in our previous studies. But the antidepressant effects and exact mechanisms of XCHT in a rat model of chronic social isolation stress (CSIS) have never been studied. We therefore aimed to investigate the effects of XCHT on depressive/anxiety-related behaviours of CSIS-exposed rats and understand the neurological mechanism involving neurogenesis.

Methods We established the CSIS model and then investigated the effects of XCHT on behavioural change. HPLC-MS/MS was adopted to quantify neurotransmitter levels in the cerebrospinal fluid (CSF). Immunofluorescence technology was used to study the effects of XCHT on neurogenesis; while expressions of 5-HT_{1A} receptor signalling pathway in the hippocampus were measured using Western blotting.

Key Findings Xiaochaihutang significantly alleviated depressive/anxiety-like behaviours of CSIS-exposed rats. XCHT significantly regulated levels of monoamine neurotransmitters in the CSF without affecting Glu, GABA and ACh. XCHT also significantly increased neurogenesis in CSIS-exposed rats. Additionally, XCHT reversed CSIS-induced decrease of 5-HT_{1A} receptor expression and promoted the expression of BDNF in the hippocampus.

Conclusion Our results suggest that XCHT could significantly regulate the depressive/anxiety-like behaviours induced by CSIS, which are likely attributed to the promotion of hippocampal neurogenesis and neurotrophin expressions through the activation of serotonergic system.

Introduction

Depression is a prevalent and life-threatening mental illness that affects around 20% of the population worldwide.[1] Xiaochaihutang (XCHT) was recorded as a classic prescription in traditional Chinese medicine in 'ShangHan Lun' written by Zhang Zhongjing in the Chinese Eastern Han Dynasty.[2] In this prescription, XCHT was used to treat 'Shaoyang syndrome', in which the core symptom such as upset and anepithymia was similar to the clinical feature of depression. Moreover, XCHT has been reported to be clinically used for the treatment of depressive disorders in China.[3,4] Our previous studies have demonstrated that XCHT could significantly alleviate depressive-like behaviours in several depressive animal models,[2,5,6] which suggest that XCHT has the potential to become a novel antidepressant for its extensive therapeutic effect for depression.

The mechanisms of depression and antidepressant are multifactorial. The widely accepted hypothesis suggests that the antidepressant-induced elevation of monoamine transmitter levels results in an enhanced expression of neurotrophins leading to increased hippocampal neurogenesis.[7,8] In our previous study, we found that XCHT could reverse the behavioural alternations and regulate monoamine neurotransmitters levels in the hippocampus of rats exposed to chronic unpredictable mild stress.[2] Nevertheless, the molecular and cellular mechanisms underlying the antidepressant effects of XCHT remain unknown.

Social isolation is especially stressful for social individuals.[9] Under laboratory conditions, chronic social isolation stress (CSIS) mimics the psychosocial stress that may effectively model a depressive-like state. In previous studies, we have used several depressive animal models including model of chronic unpredictable mild stress (CUMS) to evaluate the antidepressant effect of XCHT. CUMS, which contains various mild unpredictable stressors lasting for weeks prominently models chronic physical stressful events exposed to human during the development of depression.[10] As chronic social isolation stress represents a kind of psychosocial stress, this model is considered particularly important since it closely mimics the pathogenesis of depression in adult individuals who lack social interaction rather than physical stress.[11,12] Many previous studies have shown that social isolation affects behaviours such as increased aggression and behavioural despair, HPA function and neurogenesis.[11,13] Moreover, previous studies demonstrated that CSIS induced alternation of 5-HT level and 5-HT_{1A} receptor in the hippocampus,[14,15] which have been shown to play important roles in neurogenesis responsible for the activities of serotonergic antidepressants.[16] However, the antidepressant effects of XCHT on the CSIS model have not yet been investigated. This is the first study to investigate the effect of XCHT on depressive/anxiety-related behaviours alternation of CSIS-exposed rats and the possible underlying molecular and cellular mechanism involving neurogenesis and neurotrophin expressions.

Materials and Methods

Drugs

Xiaochaihutang consists of a mixture of seven Chinese herbs: 12 g of Radix Bupleuri (*Bupleurum chinense* DC.), 9 g of Ginseng (*Panax ginseng* C.A.Mey.), 9 g of Radix Scutellariae (*Scutellaria baicalensis* Georgi), 9 g of Rhizoma Pinelliae [*Pinellia ternata* (Thunb.) Makino], 6 g of Rhizoma Zingiberis recens (*Zingiber officinale* Roscoe), 6 g of Radix Glycyrrhizae (*Glycyrrhiza uralensis* Fisch.) and 9 g of Fructus Jujubae (*Ziziphus jujuba* Mill.).[2] The herbs were purchased from the Tongrentang Chinese Pharmaceutical Co. Ltd. (Shenyang, China, 2012) and authenticated by Professor Jincai Lu (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China) according to the Chinese Pharmacopoeia (The Pharmacopoeia Commission of PRC, 2010).

The extraction method of XCHT is the same as that reported in our laboratory, and forty-four components in the extracted powders were identified using the UPLC-MS/MS method described in our previous study where the phytochemical profile was presented.[5] In this study, the extracted powders of XCHT were dissolved in distilled water to get three dosages (0.6, 1.7 and 5.0 g/kg) equivalent to the clinical doses.

Fluoxetine (FLU), as a positive control, was obtained from Lilly S. A. (Alcobendas, Spain).

Isolation stress and drug administration

Adult male Sprague Dawley rats (weighing 250–270 g) were obtained from the Experimental Animal Centre of Shenyang Pharmaceutical University. Rats were reared in a constant environment (23 ± 1 °C, 12-h light/dark cycle) with free access to food and water except during the sucrose preference test. They were allowed to acclimatize for 3 weeks with training for sucrose preference before the experiment. All animal experiments were approved by the Animal Ethics Committee of Shenyang Pharmaceutical University (Permit Number: SYPU-IACUC-2014-W-0312-207), and all experimental procedures were according to the guidelines for animal experimentation of Shenyang Pharmaceutical University. Every effort was made to minimize animal suffering.

Adult male rats were divided into control (four animals per cage) or CSIS groups. For the social isolation procedure, the rats were individually housed in plastic cages measuring 37 × 26 × 17 cm and subjected to social isolation stress for 6 weeks in a separate quiet room.[11,13] During the isolation process, rats had normal auditory and olfactory experience, but were deprived of any visual or tactile contact with other rats. Animals were left undisturbed except for daily XCHT administration and cage changing.

Rats in the CSIS group were divided into 5 groups: CSIS vehicle, CSIS-FLU (20 mg/kg), CSIS-XCHT (0.6 g/kg), CSIS-XCHT (1.7 g/kg) and CSIS-XCHT (5.0 g/kg) groups. The doses mentioned above refer to the crude drug dosage.[2] Drugs were given orally once per day from the beginning of the isolation stress lasting for 6 weeks till the end of the behavioural test (for the experimental timeline, see Figure 1).

Behavioural experiments

Sucrose preference test

The sucrose preference (SP) test was performed weekly according to our previous study.[2] Rats (n = 15/group) were food and water deprived for a period of 24 h before the sucrose preference test. Then, two bottles containing premeasured tap water and 1% sucrose solution were placed on the cage, and fluid intake was monitored for 1 h. The sucrose preference was expressed as follows:

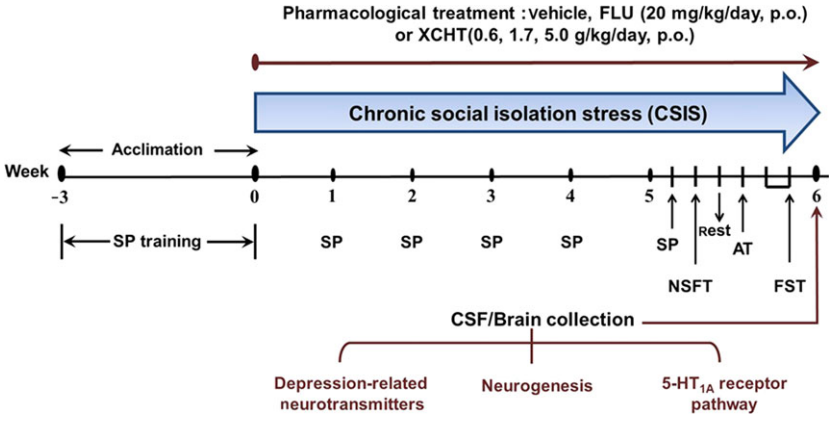

Figure 1 Experimental timeline: chronic social isolation stress (CSIS) and Xiaochaihutang (XCHT) treatments lasted for 6 weeks. Sucrose preference (SP) was measured once a week for 6 weeks. The novelty-suppressed feeding test (NSFT), isolation-induced aggression test (AT) and forced swimming test (FST) were performed at the end of the experiment (Week 6). After the end of the FST, the cerebrospinal fluid (CSF) and brains were harvested and used for neurochemical analysis.

$$\text{sucrose preference} = \frac{\text{sucrose consumption}}{\text{sucrose consumption} + \text{water consumption}} \times 100\%$$

Novelty-suppressed feeding test

The novelty-suppressed feeding (NSF) test was conducted as previously described with slight modifications.[17] Rats (n = 15/group) were introduced into a novel open field (100 × 100 × 40 cm), from the corner after 48 h of food deprivation. A single food pellet was placed on a white paper in the centre of the box. The latency to chew the pellet was recorded. Immediately afterwards, rats were returned to their home cage, and the amount of food consumed within 10 min was measured.

Forced swimming test

The forced swimming test (FST) is widely used for the evaluation of depressive behaviour and prediction of the antidepressant drugs efficacy in rodent animals. It is based on the observation that animals in an enclosed environment will become immobile after an initial period of vigorous activity, which can be reduced by effective antidepressant drugs.[18,19] In brief, rats (n = 15/group) were subjected to a pretest session for 15 min followed by a 5-min test session 24 h later. All rats were individually placed in a cylinder (50 cm in height, 20 cm in diameter) filled with water at 25 °C to a depth of 30 cm. The immobility duration during the 5 min was measured. Immobility was defined as the absence of all movement except minor movement required for the rats to keep its head above the surface.

Isolation-induced aggression test

The test was carried out as previously described.[20] An intruder rat was introduced into the cage of the resident rat for 15 min. The investigated behaviours performed by the resident rat against the intruder are as follows: the latency to first attack, the frequency of attacking/biting and duration of aggression (n = 15/group).

Neurotransmitters analysis

Twenty-four hours after FST, rats (n = 8–9/group) were anaesthetized with chloral hydrate (350 mg/kg, i.p.) and mounted in a stereotaxic frame. CSF samples were then collected by punctuation of the cisterna magna. A Thermo TSQ Quantum Access MAX mass spectrometer coupled to a Thermo ACCELA HPLC system was used for neurotransmitters analysis. The CSF preparation procedure and HPLC/MS-MS conditions are shown in the Appendix S1.

Immunofluorescence for Ki-67 and doublecortin

After the end of the behavioural tests, rats (n = 6 per group) after CSF collection were anesthetized with chloral hydrate (350 mg/kg), and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brains were then removed and transferred to the 30% sucrose solutions at 4 °C until they sank. The tissue was cut into 25-μm coronal sections through the hippocampus for immunofluorescence analysis.

The immunofluorescence method was performed as previously described.[6] The sections were rehydrated and blocked with goat serum (8% in 0.3% Triton X-100) for

1 h at 37 °C followed by incubation with polyclonal rabbit Ki-67 primary antibody (1 : 1000; Abcam, Cambridge, UK) and doublecortin (DCX) primary antibody (1 : 500; Abcam) overnight at 4 °C. Sections were then incubated for 1 h at 37 °C with secondary antibodies including Cy3-conjugated goat anti-rabbit IgG (H + L) (1 : 100; Proteintech, Chicago, USA) and Alexa Fluor 488-conjugated goat anti-rabbit IgG (H + L) (1 : 200; Proteintech, Chicago, USA). For Ki-67 staining, DAPI (1 : 300; Sigma-Aldrich, St. Louis, MO, USA) was added for incubation and covered slips with antifade mounting medium (Pro-long Gold; Invitrogen, California, USA). A Nikon C2 Plus confocal microscope was used for image acquisition.

Western blotting analysis

We used Western blot to analyse the expressions of the 5-HT_{1A} receptor, p-AKT/AKT, p-ERK/ERK, p-CREB/CREB and BDNF. Animals ($n = 4$ per group) after CSF collection were decapitated and their brains were rapidly removed and dissected. The hippocampal tissue samples were homogenized in lysis buffer. Protein extracts were then separated by SDS-PAGE electrophoresis. The following primary antibodies including p-CREB (#9198, 1:1000), CREB (#9197, 1:1000), p-AKT (#9614, 1:1000), AKT (#4685, 1:1000), p-ERK (#4370, 1:1000), ERK (#4695, 1:1000) were from Cell Signaling Technology (Cell Signaling, Beverly, MA, USA). 5-HT1A receptor (ab79230, 1:1000) and BDNF (ab108383, 1:1000) antibodies were from Abcam Technology (Abcam, Cambridge, UK).

Data analysis

Data were analysed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA), and results are expressed as mean ± SEM. The statistical significance was determined by one-way ANOVA followed by Tukey's HSD test. $P < 0.05$ was considered as indicative of statistical significance. All analyses were performed using GraphPad (La Jolla, CA, USA) Prism 5.

Results

Effects of Xiaochaihutang on the behaviours of chronic social isolation stress-exposed rats

Chronic social isolation stress induced a decrease of sucrose preference to 53 ± 6.9% compared with control group after 5 weeks ($P < 0.05$) while co-administration with XCHT (1.7 and 5.0 g/kg) suppressed the decrease of sucrose preference (each $P < 0.05$) (Figure 2a). CSIS significantly prolonged the immobility duration in the FST, compared with control group ($P < 0.001$). However, a decrease in immobility duration was observed with co-administration of XCHT (1.7 and 5.0 g/kg) ($P < 0.05$ and $P < 0.01$, respectively) (Figure 2b). Meanwhile, we found XCHT had no significant effect on locomotor activity (Figure S1). This suggested that XCHT exerted an antidepressant effect in FST rather than a psychostimulant effect. In the NSF test, we found that CSIS led to a significant increase in latency to feed (Figure 2c), which was reversed by co-administration of XCHT (1.7 and 5.0 g/kg; all $P < 0.001$). Similarly, in Figure 2d, CSIS reduced the home food consumption in 10 min compared with the control group, and this reduction could be reversed by XCHT (0.6, 1.7 and 5.0 g/kg). In the isolation-induced aggression test, we revealed that CSIS significantly decreased the latency to attack and increased the bouts of aggression and the aggression duration in 15 min. However, the increased aggressive behaviour could be alleviated after XCHT (1.7 g/kg) and FLU (20 mg/kg) treatment as illustrated in Figure 2(e–g) and Table S1.

Effects of Xiaochaihutang on neurotransmitters in cerebrospinal fluid samples of chronic social isolation stress-exposed rats

Effects of XCHT on neurotransmitters in CSF of rats are shown in Tables 1 and S1. We found that CSIS significantly reduced 5-HT and DA levels of CSF compared with control group ($P < 0.05$). XCHT (0.6, 1.7, 5.0 g/kg) treatment increased the level of 5-HT in the CSF of isolated rats ($P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively), and only XCHT (0.6 g/kg) elevated DA level ($P < 0.05$). Moreover, XCHT (0.6, 1.7, 5.0 g/kg) significantly reversed CSIS-induced decrease of DOPAC ($P < 0.01$, $P < 0.001$ and $P < 0.01$, respectively), the primary metabolite of DA, without affecting 5-HIAA level. Our data showed a significant decrease of HVA, the secondary metabolite of DA in isolated rats, and XCHT (0.6, 1.7, 5.0 g/kg) significantly reversed this decrease (all $P < 0.01$). Furthermore, XCHT had no significant effect on GABA, Glu and ACh levels of isolated rats in CSF.

Effects of Xiaochaihutang on neurogenesis in the hippocampus of chronic social isolation stress-exposed rats

Chronic social isolation stress significantly reduced cell proliferation, as indicated by Ki-67 immunoreactivity (Figure 3 and Table S1). Six weeks of chronic XCHT (1.7, 5.0 g/kg) and FLU (20 mg/kg) treatment induced a significant increase in the number of Ki-67-positive cells ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively). CSIS caused in a reduction in the number of DCX-positive cells, compared with control (Figure 4 and Table S1). The decreased

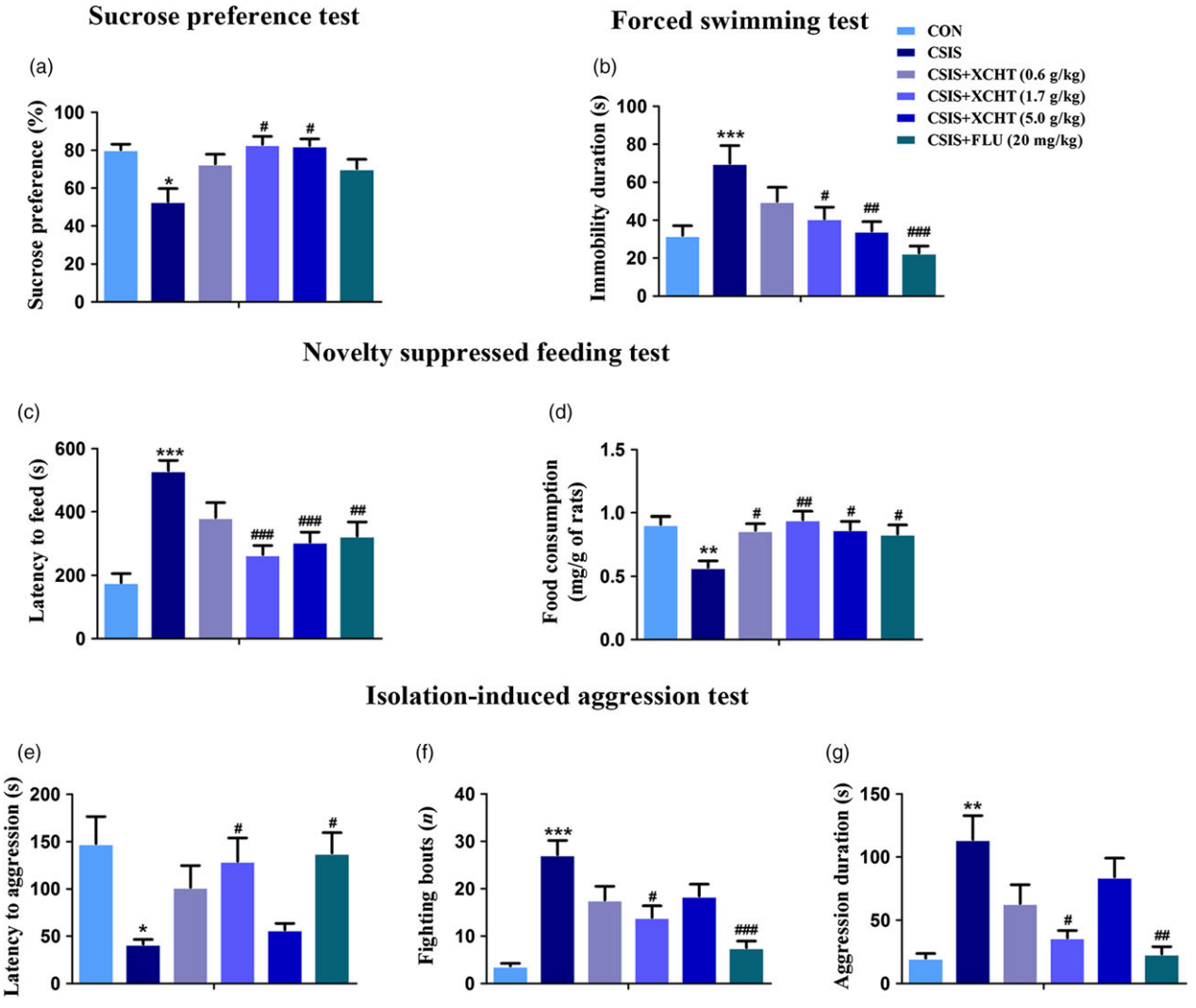

Figure 2 Effects of Xiaochaihutang (XCHT) on depressive-like and anxiety-like behaviours of chronic social isolation stress (CSIS)-exposed rats. The data from the following tests are as follows: sucrose preference (SP) (a), forced swimming test (FST) (b), novelty-suppressed feeding test (NSFT) (c and d) and aggression test (AT) (e–g). Data represent mean ± SEM; n = 13–14 per group. For statistical significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with CSIS group.

number of DCX-positive cells in the CSIS group was reversed by XCHT treatment at the doses of 1.7 and 5.0 g/kg (each $P < 0.05$).

Effects of Xiaochaihutang on the protein expressions of the 5-HT$_{1A}$ receptor signalling pathway

There was a significant difference in 5-HT$_{1A}$ receptor expression between the CSIS and control groups ($P < 0.01$). Chronic XCHT (1.7, 5.0 g/kg) treatment reversed these alterations and significantly increased hippocampal 5-HT$_{1A}$ receptor expression ($P < 0.01$). A similar result was observed in FLU treatment. We found that p-AKT level was decreased after CSIS, compared with control group ($P < 0.05$), and this decrease was reversed by chronic treatment of XCHT (1.7 g/kg) and FLU (20 mg/kg) ($P < 0.05$ and $P < 0.001$, respectively). Expression of p-ERK was also significantly decreased in the CSIS group ($P < 0.001$), while both XCHT (0.6, 1.7, 5.0 g/kg) and FLU (20 mg/kg) administration increased p-ERK expression. We further found that XCHT (5.0 g/kg) or FLU (20 mg/kg) treatment significantly increased the p-CREB expression in rats exposed to CSIS (each $P < 0.05$). Importantly, the decrease in BDNF level in the hippocampus induced by CSIS was reversed with XCHT (1.7, 5.0 g/kg) or FLU (20 mg/kg) treatment ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively) (Figure 5 and Table S1).

Table 1 Effects of chronic Xiaochaihutang (XCHT) administration on neurotransmitters and the metabolites in cerebrospinal fluid (CSF) of rats following chronic social isolation stress (CSIS)

Group	Dose	5-HT	5-HIAA	DA	DOPAC	HVA	Glu	GABA	ACh
CON	–	0.55 ± 0.08	282.3 ± 20.9	0.20 ± 0.02	7.39 ± 0.36	11.5 ± 1.2	174.1 ± 14.7	1.95 ± 0.19	1.70 ± 0.37
CSIS	–	0.22 ± 0.02*	254.9 ± 11.4	0.14 ± 0.01*	4.80 ± 0.46**	6.3 ± 1.1*	190.7 ± 27.8	1.29 ± 0.25	1.22 ± 0.22
CSIS + XCHT	0.6 g/kg	0.43 ± 0.03$^{\#\#}$	306.1 ± 17.1	0.19 ± 0.01$^{\#}$	7.52 ± 0.55$^{\#\#}$	12.5 ± 1.3$^{\#\#}$	148.1 ± 7.6	2.08 ± 0.27	1.50 ± 0.27
	1.7 g/kg	0.41 ± 0.04$^{\#}$	288.0 ± 9.8	0.17 ± 0.01	7.94 ± 0.52$^{\#\#\#}$	13.0 ± 0.8$^{\#\#}$	163.7 ± 20.3	2.60 ± 0.60	1.48 ± 0.16
	5.0 g/kg	0.41 ± 0.03$^{\#\#}$	274.8 ± 33.1	0.17 ± 0.01	7.58 ± 0.35$^{\#\#}$	12.3 ± 0.6$^{\#\#}$	166.5 ± 28.9	1.44 ± 0.08	1.91 ± 0.40
CSIS + FLU	20 mg/kg	1.20 ± 0.13$^{\#\#}$	183.5 ± 10.9	0.21 ± 0.01$^{\#\#}$	8.05 ± 0.74$^{\#\#\#}$	13.7 ± 1.6$^{\#\#\#}$	167.2 ± 20.3	1.86 ± 0.20	1.92 ± 0.48

Data are shown as mean ± SEM with units of ng/ml (n = 8–9 per group). For statistical significance, *$P < 0.05$, **$P < 0.01$, compared with control group; $^{\#}P < 0.01$, $^{\#\#}P < 0.01$, $^{\#\#}P < 0.001$, compared with CSIS group.

Discussion

Chronic social isolation stress can induce many behavioural and neurochemical alternations in social animals.[21] In the present study, it induced depressive-like behaviours in the form of anhedonia and behavioural despair and anxiety-like behaviours in the form of altered novelty-suppressed feeding response and elevated isolation-induced aggression. Our findings suggested that XCHT (1.7, 5.0 g/kg), the effective doses in our previous study,[2] could significantly alleviate the depressive/anxiety-like behaviours of rats induced by CSIS.

Monoaminergic deficiency has long been thought to underlie depressive disorders.[22] Our previous studies have demonstrated that XCHT enhanced the monoaminergic activity in several brain regions of animal models of depression.[2,5,6] Many studies reported that depressed individuals were associated with reduced monoamine neurotransmitter levels in the CSF, which provided a direct reflection of the biochemical changes in the brain in response to pathological processes.[23] However, few studies reported the levels of neurotransmitters in the CSF of depressive animals, especially in rats exposed to CSIS. To our knowledge, this is the first study on panels of neurotransmitters in the CSF from CSIS-exposed rats and to confirm the effect of XCHT on neurotransmitters in the CSF by HPLC-MS/MS.

Our studies showed that CSIS significantly reduced 5-HT level of rats in the CSF. On the other hand, XCHT could significantly reverse the decreased 5-HT level induced by CSIS, and it is accompanied by the alleviation of aggressive behaviour, one reported behavioural alteration induced by CSIS, which is modulated by brain 5-HT.[24] We also found that isolation stress decreased DA and DOPAC levels in the CSF, which was reversed by XCHT treatment. This result was in accordance with our previous studies in a rat model of chronic unpredictable mild stress and two mouse models of behavioural despair.[2,6] In our study, CSIS did not affect Glu, GABA and ACh levels in the CSF, and these also showed no significant changes after XCHT treatment. Therefore, our results indicated that the effect of XCHT on depressive behaviours might be related to the alteration of monoamine neurotransmitter levels.

Adult neurogenesis and neurotrophins are two critical regulators responsible for depression.[7] A milestone of depression and antidepressant activity is altered hippocampal neurogenesis.[25] Previous studies have shown that social isolation affects hippocampal neurogenesis in many rodent species[26,27] and Ki-67 and DCX can serve as markers of adult hippocampal neurogenesis in rodents. Moreover, CSIS often induces the alteration of the 5-HT_{1A} receptor that is thought to play a central role in modulating depressive/anxiety-related behaviours.[28] It has also been demonstrated that the 5-HT_{1A} receptor was required for

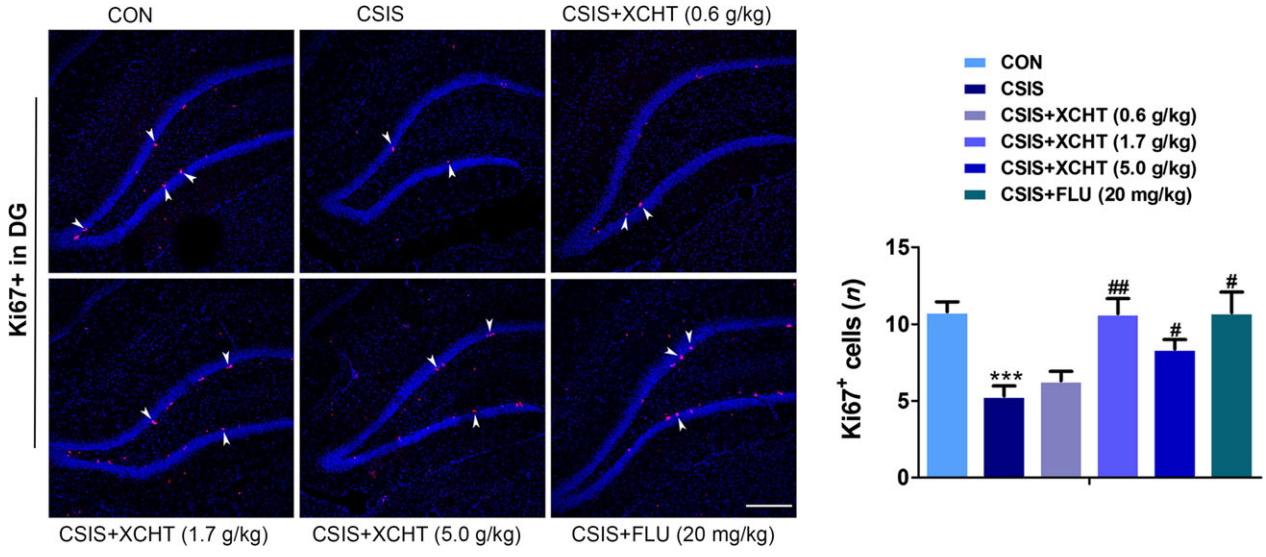

Figure 3 Effects of Xiaochaihutang (XCHT) on Ki-67 expression in the DG of the hippocampus of chronic social isolation stress (CSIS) rats. Bar = 100 μm. Data are shown as mean ± SEM; n = 3–4 per group. ***$P < 0.001$ compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with CSIS group.

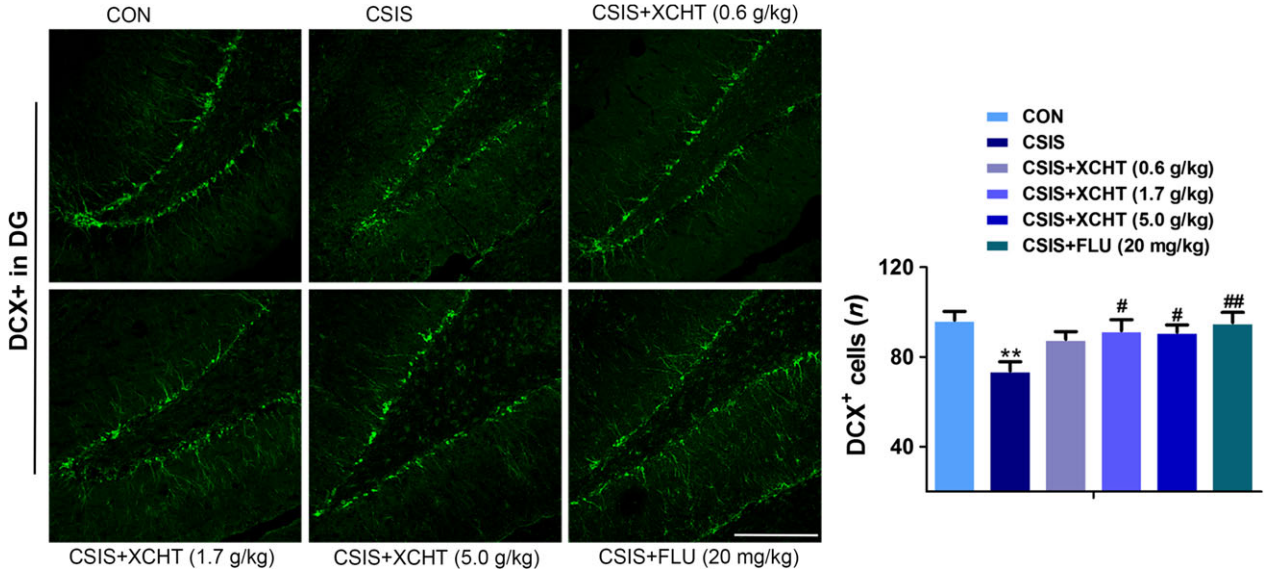

Figure 4 Effects of Xiaochaihutang (XCHT) on DCX expression in the DG of the hippocampus of chronic social isolation stress (CSIS) rats. Bar = 100 μm. Data are expressed as mean ± SEM; n = 3–4 per group. For statistical significance, **$P < 0.01$, compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with CSIS group.

serotonergic antidepressants' effects on hippocampal neurogenesis.[16,29] In our previous study, we found that HRG, defined as the core in compatibility of XCHT, significantly increased hippocampal neurogenesis in non-stressed mice.[6] It was assumed that hippocampal neurogenesis might be implicated in the antidepressant effect of XCHT on CSIS-exposed rats.

We obtained the results that XCHT treatment could promote Ki-67, DCX and the 5-HT_{1A} receptor expressions. XCHT induced a significant increase in the number of Ki-67 in the dentate gyrus, which is expressed in dividing cells throughout the entire mitotic process and is used to identify proliferating cells.[30] XCHT also increased the expression of DCX, a microtubule-associated protein, used

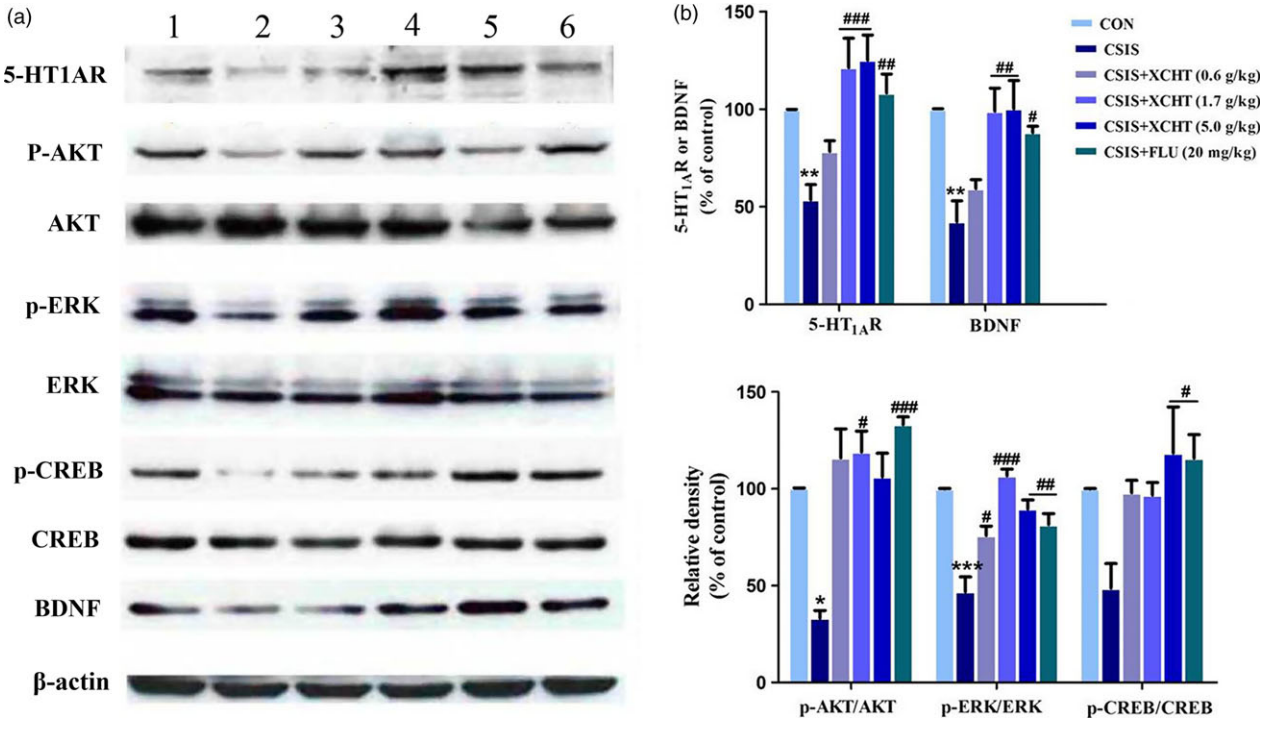

Figure 5 Effects of Xiaochaihutang (XCHT) on the protein expressions of the 5-HT_{1A} receptor signalling pathway in the hippocampus of chronic social isolation stress (CSIS)-exposed rats. The representative images (a) are as follows: Band 1: control; Band 2: CSIS; Band 3: CSIS + XCHT (0.6 g/kg); Band 4: CSIS + XCHT (1.7 g/kg); Band 5: CSIS + XCHT (5.0 g/kg); Band 6: CSIS + FLU (20 mg/kg). Each column (b) represents mean ± SEM; $n = 3$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with CSIS group.

as an indicator of neurogenesis in the dentate gyrus.[31] Moreover, we found that 5-HT_{1A} receptor expression in the hippocampus was downregulated in the CSIS group, but this was reversed by XCHT at doses of 1.7 and 5.0 g/kg. This indicates that activation of the 5-HT_{1A} receptor might be involved in adult hippocampal neurogenesis responsible for the antidepressant effects of XCHT.

It has been demonstrated that the 5-HT_{1A} receptor mediates 5-HT-induced neurogenesis in the treatment of depression-related behaviours.[32] This suggested that the change in 5-HT level could cause the stimulation of receptors with subsequent effects on downstream signalling cascades leading to increased expression of neurotrophins responsible for neurogenesis. The 5-HT_{1A} receptor directly modulates hippocampal ERK and AKT activities, both of which affect the neurogenesis of newborn cells in hippocampus.[33] CREB is an upstream transcriptional activator of BDNF, and they both play an important role in neuronal proliferation, differentiation, survival and synaptic plasticity.[34]

In this study, our data revealed that both the protein expressions of p-ERK and p-AKT were upregulated by XCHT in CSIS-exposed rats. Moreover, we observed an increase in the p-CREB and BDNF protein levels in the hippocampus of CSIS-exposed rats after 6 weeks of XCHT treatment, which corresponds with our previous study in CUMS.[2] This indicates that the antidepressant effect of XCHT on chronic isolated rats might operate via increasing 5-HT levels, causing the activation of the 5-HT_{1A} receptor that then promotes downstream CREB and BDNF expressions in the hippocampus leading to increased neurogenesis.

As a traditional Chinese herbal formula containing multiple components, XCHT may exert the antidepressant effects in more ways than one. Correspondingly, XCHT has been shown to produce the U-shaped or inverted U-shaped curves in most behavioural tests and biochemistry experiments, which is similar to our previous study in TST and FST of mice.[6] This phenomenon is common after traditional Chinese medicine treatment.[35–37] Therefore, it is important to identify and elucidate active constituents to help clarify the therapeutic basis of XCHT. Forty-four components, including saikosaponins, ginsenoside, baicalin, liquiritin, wogonoside, baicalein, wogonin, oroxylin A and saikosaponin A, have been identified and quantified using ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) by our group.[5,38] Multiple constituents including baicalin, ginsenoside, liquiritin and

so on have been detected in the serum[6] of mice after administration of XCHT, which have been reported to exert significant antidepressant effect in models of depression,[39–41] suggesting that these compounds might be active ingredients and need to be further studied. Furthermore, considering the importance of baicalin derived from Radix Scutellariae in XCHT for depression therapy, we are exploring the antidepressant effect of baicalin on depressive animals. However, as the components are complex, the identification of active components is in progress and deserves further research.

Conclusion

Our results demonstrate that XCHT has a therapeutic effect on CSIS-induced depression. Moreover, this is the first study on panels of neurotransmitters in the CSF and to confirm the effect of XCHT on neurotransmitters of CSIS-exposed rats by HPLC-MS/MS. Above all, the current results suggest that the antidepressant effects of XCHT on CSIS-exposed rats might be related to promoting hippocampal neurogenesis and neurotrophin expressions through the activation of the serotonergic system. This study provides new evidence for the therapeutic effect of XCHT against different kinds of depression and suggests that XCHT needs to be further investigated as a potential antidepressant therapeutic.

Declarations

Conflict of interest

The Authors declared that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental procedures.

Figure S1. Effects of XCHT on locomotor activity of CSIS-exposed rats after 5-week XCHT treatment. Data represent mean ± SEM; $n = 13$ rats per group.

Table S1. *F* values listed in statistical analysis.