Dynamic changes of serotonin levels induces depression-like behaviour in mice with high-fat diet

Yan Chen

College of Pharmacology Sciences, Zhejiang University of Technology

Lifang Chen

College of Pharmacology Sciences, Zhejiang University of Technology

Tingting Gong

Laboratory Animal Center, Zhejiang University of Technology

Haotian Liu

College of Pharmacology Sciences, Zhejiang University of Technology

Shengfeng Wang

Laboratory Animal Center, Zhejiang University of Technology

Yunxia Fang

College of Pharmacology Sciences, Zhejiang University of Technology

Yongyong Zhang

College of Life Sciences, China Jiliang University

Weiguang shan (✉ crh8888@126.com)

College of Pharmacology Sciences, Zhejiang University of Technology

Article

Keywords:

Posted Date: December 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1178329/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Introduction of SSRI as the first-line pharmacotherapy options for depression, which purpose is to increase the concentrations of 5-HT in brain and help reduce depressive symptoms. However, there are still many limitations to the effectiveness of these treatment strategies, 30-40% of patients do not respond to treatment at all, and even suicide occurs. In the present study, we revealed that increasing brain 5-HT concentration is not the best treatment options. When we injected sufficient amounts of 5-HT into the lateral ventricular region of mice, it actually caused depression-like behavior in the animals. The second study on high-fat fed induces depressive symptoms in mice demonstrated that 5-HT concentration in brain showed a tendency to increase first and then decrease. Both experiments indicated that if SSRI are administrated to the patient in the acute phases of depression, it may lead to exacerbation of mental disorders or suicidal tendencies.

Introduction

Major depression disorder (MDD) is a highly prevalent and debilitating mental illness that has a significant impact on more than 16% of the global population and is one of the major causes of the severe global health and socio-economic burden¹. In middle-income countries (including China)-accounts for an estimated three-quarters of the global burden these conditions. MDD patients have several clinical symptoms: persistent dark emotions and other problems, such as suicidal thoughts, sleep disorders, altered appetite, and cognitive impairment^{2,3}. The complexity of the 5-HT system and the role of other neurochemical systems suggest that depression may be caused by a variety of pathological mechanisms, and the common denominator is undoubtedly a conduction disorder in the central 5-HT synapse⁴. Fourteen different serotonin receptor have been identified, and these receptors are subdivided into different families, labeled 1, 2, 3, 4, 5, 6, and 7. The subtypes in each family are marked with letters (for example, a, b, c). Some receptors are thought to be involved in the pathogenesis of various central nervous system disease⁵. Among these functional receptors, the serotonin receptor 5-HT_{1A} (5-HT_{1A}R) seems to play a key role in neuropathology depression^{6,7}. 5-HT_{1A}R is involved in the regulation of depression and anxiety⁸. 5-HT_{1A}R deficient mice have been shown to exhibit increased anxiety-related responses, fear of classical conditioning, and increased freezing behavior⁹. Reduced 5-HT_{1A}R levels have been shown to cause anxiety and stress disorders in mice and primates^{10,11}. In addition, studies have shown that lower 5-HTT(5-hydroxytryptamine transporter) expression in humans is associated with higher levels of generalized anxiety and depression^{12,13}. Rodent studies have shown that 5-HTT knockout (Ko) rodents exhibit more anxiety-and depression-like behavior¹⁴. Based on this theory, selective 5-HT reuptake inhibitors (SSRIs) are widely prescribed as antidepressants, and becoming a first-choice treatment for depression. Patients with depression usually require long-term treatment with antidepressant drugs and those with major depression often require polytherapy. However, most antidepressant drugs are far from ideal and they often possess undesirable side effects while lacking the ability to manage these conditions in the long term. For example, Selective 5-HT reuptake inhibitors

(SSRIs), including paroxetine or sertraline are some well-known limitations of antidepressant drugs, and there has been concern that used antidepressant drugs might be associated with an increased risk of suicidal ideation in pediatric patients¹⁵. Up to now, the cause of depression is not very clear, but it can be sure that some factors such as biology, psychology and social environment, participate in the process of depression. However, little is known about the role of dietary pattern in the development of depressive disorders. Several cross-sectional studies found a significantly lower prevalence of depression symptoms for persons with a low consumption of fast-food (hamburgers, sausages, pizza)¹⁶. Similarly, depressive symptoms were associated positively with consumption of fast-food, snacks and high-energy sweets¹⁷. Moreover, a recent analysis showed that a food pattern rich in processed or fried foods, refined grains, sugary products and high-fat dairy products was significantly related to perceived stress¹⁸. In spite of many studies show a potentially detrimental association between the consumption of high-fat diet and the risk of depression. But, people would rather choose fast-food than 'healthy' dietary pattern, such as the Mediterranean diet, because they think consumption of fast-food is a lifestyle, especially there is significant proportion among teenagers who hold this view. While the determining factors of depression are complex, the mechanism by which a high-fat diet causes depression is not yet understood. Due to the fact that multiple studies have previously shown that high daily intake of saturated fats and refined carbohydrates often leads to depression and cognitive impairment, and it has been linked to impair the serotonin system, which includes decreased tryptophan concentration, impaired 5-HT synthesis or release, and postsynaptic 5-HT receptor dysfunction^{19, 20, 21}. Collectively, these findings emphasize the existence of common molecular pathway of serotonin system between both high-fat diet and major depression. However, there is little research that explicitly demonstrates whether the decrease of 5-HT level in the brain is not the only cause of depression-like behavior induced by high-fat diet. In this study, the authors first intervened in mice with diet to induce the occurrence of depression-like behavior, determine the changes in 5-HT levels in the brain, and then set the brain stereotactic injection group to confirm that the excessive 5-HT content in the brain may also be another cause of depression-like behavior in mice. In addition to the behavioral assessment, the expression and other biochemical indicators of 5-HT-associated proteins and genes were also examined in each group. Research on epigenetics has made great strides over the past few decades²². Epigenetics is defined as the stably heritable changes to the regulation of gene expression through modifications in chromatin structure and DNA methylation without altering DNA sequences²³. Epigenetic modifications, including DNA methylation, histone modification, positioning of histone variants and gene regulation of miRNA, are potentially reversible. DNA methylation, one of the most important epigenetic modifications, has been proofed proven to be associated with depression in many studies^{24, 25, 26}. For this purpose, we evaluated the methylation levels of 5-HTT and 5-HT_{1A} in promoter region CPG islands in diet intervention and brain stereotaxic injection groups were evaluated in the study.

Results

Increases in bodyweight by HFD Exposure. To study the effects of metabolic disorder on depressive-like behaviours, mice were fed HFD for 18 weeks. Behavioral tests were performed on mice at weeks 3, 5, 11 and 17 (Fig.1a) and biochemical indicators were determined after testing. The timeline is shown in Fig.1a. As expected, the HFD diet progressively increased body weight over the weeks (Fig.1b).

Promotes of depression behaviors by HFD exposure. HFD exposure promotes depression behaviors as demonstrated by behavioral tests. HFD-fed mice were tested for depressive-like symptoms using behavioural tests. There was a significant difference in behavior between the STD group and the high-fat group at the beginning of 5 weeks. In the OFT, 5 and 11 weeks of an HFD significantly decreased the time spent in this compartment. In FST and TST (Fig.2c and 2d), as compared to STD groups, the immobility time of HFD group increased at 5 and 11 weeks, respectively. At the 17th week, the sugar and water preference of the high fat group was significantly lower than that of the STD group (Fig.2a). In the open field test (Fig. 2b) the percentage of central residence decreased, at the same time, the time of immobility in the tail suspension test (Fig.2c) and forced swimming test (Fig.2d) was significantly increased. At the behavioral level, mice showed signs of depression.

Dynamic changes of 5-HT level in the brain of HFD exposed group. The monoamine neurotransmitter 5-HT is believed to be directly or indirectly involved in regulating one's mood, and its dysfunction is related to the pathophysiology of depression. Studies have shown that the occurrence of depression-like behavior is caused by a reduction in 5-HT content in the brain. Interestingly, the dynamic changes were not observed in 5-HT levels in the HFD groups (Fig.3). The 5-HT levels of hippocampus from each group were detected. 5-HT levels of the HFD group were a dynamic change process as compared to the STD group. Levels of 5-HT significantly increased at 5 and 11 weeks and then decreased at 17th week in the HFD mice.

Brain injection of 5-HT leading to the occurrence of depression-like behavior. To further understand the relationship between 5-HT and the development of depressive-like behaviours, another model of stereotactic infusions was used. In behavioral test experiments, depression-like behavior was found in the model group as compared to the control group. In the OFT (Fig.4a), there was decrease in time spent in the centre as compared to the control group and depression-like behavior. The TST (Fig.4b) and FST (Fig.4c) were used to further determine the depression level in mice; the immobility time significantly increased in both tests in the model mice. The mice in the model group showed depressive-like symptoms.

Brain hippocampal neurons damage in high-fat diet and 5-HT mice. When compared with the normal group, the mice in the HFD group showed significant difference in some behavioral indexes in the 5th week. Therefore, the degree of hippocampal neuron injury was observed after 5, 11 and 17 weeks of ethology in the diet intervention group and brain-targeted injection group. Under the light microscope, the DG neurons in the STD group were arranged regularly, the cell layer was rich, the cell membrane and cytoplasm were intact, and the nucleus was round and large. In the HFD group, the number of hippocampal neurons decreased, the structure was disordered, the neurons shrank, the cell morphology

was irregular, the intercellular space was enlarged, the nucleolus was not obvious, and some neurons were vacuolated, especially at 17 weeks (Fig.5.a). In the stereotactic injection experiment, when compared with the Control Group, the number of hippocampal neurons in the Model group was significantly decreased, the neurons were disordered, the neurons were atrophied, the shapes were irregular, the intercellular spaces were enlarged and some neurons were vacuolated (Fig.5.b).

High-fat diet leading to depression-like behavior because 5-HT pathway is destroyed in the brain.In order to determine whether the depression-like behavior induced by HFD in mice is due to the influence of 5-HT pathway-related genes and proteins, 5-HTT and 5-HT_{1A} were measured and it was found that the expression of 5-HTT and 5-HT_{1A} was dynamic. At the level of gene and protein, the expression level of mRNA (Fig. 6a) and protein (Fig. 6b) in the HFD group increased significantly in the 5th week, and then decreased in the 11th week until the 17th week, there was a significant difference.

An overdose of 5-HT in the brain leading to damage of the 5-HT nerves.In order to further investigate whether the single decrease of 5-HT in the brain is not the main cause of depression-like behavior in mice, the present study first measured the level of 5-HT in the brain of the stereotactic injection group (Fig6.2a) and found that the level of 5-HT in the brain of the model group was higher than that of the control group. At the same time, the expression of 5-HTT and 5-HT_{1A} in the stereotactic brain injection group was determined at the gene and protein levels. The results showed (Fig6.2b and Fig6.2c) that when compared with the control group, the mRNA and protein expressions of 5-HTT and 5-HT_{1A} in the model group decreased with statistical significance, indicating that stereotaxic injection could damage 5-HT neural energy.

Higher methylation levels in the 5-HT-related gene promoter region resulting in abnormal protein expression.In order to further study the relationship between 5-HT related protein expression and epigenetics, DNA methylation was detected on the CPG islands of the promoter region of the 5-HT related gene. In the diet intervention experiment, as compared to the STD group(Fig7.a), the mean methylation level of 5-HT_{1A} and 5-HTT promoter regions in HFD mice at 11 weeks was higher than that of the STD mice and the same result was obtained at 17 weeks. It was hypothesized that the elevated mean methylation level in the promoter regions of both genes might be due to elevated 5-HT levels in the brain. Thus, the level of methylation in these two gene promoter regions in the stereotaxic mice was also measured, and it was found that the promoter region of 5-HTT gene in model group was hypermethylated, as compared to the control mice(Fig7.b).

Discussion

Effective management of obesity and its associated emotional complications is a challenging task unless underlying neurobiological mechanisms are fully understood^{32,33}. In this study, mice were fed either a high-fat diet or a normal diet for 17 weeks, and since the serotonin system is a major factor in mood regulation³⁴, therefore, at 3, 5, 11, 17 weeks, ethology and 5-HT neuroenergy analysis were carried out to determine the relationship between the high-fat diet and the serotonin system. Recent studies have

shown that some 5-HT receptor subtypes, of which 5-HT_{1A}, play an important role in anxiety and depression relief³⁴. Glucose preference test, open field test, forced swimming, tail suspension test and pathological observation were used to determine depression-like features and hippocampal injury, and 5-HT content in brain was assessed by ELISA Kit. The results suggest that a high-fat diet leads to depressive-like behavior and damage to hippocampal neurons, which may be due to more than a decrease in 5-HT in the brain. It is a dynamic process of rising and then falling. To further investigate whether depression-like presentation in mice on a high-fat diet may be due to elevated levels of 5-HT in the presenile brain, a brain stereotactic injection group was set up, and the data demonstrated that the depression-like appearance in the model group showed that the hippocampal neurons were damaged, when compared with control mice. Interestingly, these results are different from the 5-HT theory³⁵ proposed by earlier scholars, and it allow us to speculate that 5-HT levels in patients is not reduced but substantially increased in the early stages of depression. Elevated serotonin levels makes postsynaptic 5-HT receptor 5-HT_{1A} insensitive. At the same time, the continuous increase of serotonin concentration in synaptic cleft will enhance of 5-HTT transport rate and elicits a dose-dependent increase of serotonin uptake or recycles in presynaptic membrane, which eventually triggers a negative feedback mechanism that leads to the decrease of serotonin secretion. This explains the reason why the patient with depression take the 5-HT inhibitors in the early stage of depression can increase the amount of 5-HT in the synaptic cleft and further damage the 5-HTT, leading to the suicidal tendency of patients.

It is now recognized that there is a physiological and pharmacological relationship between the 5-HT_{1A} receptor and 5-HTT in the regulation of 5-HT neurotransmission⁽³¹⁾. Membrane serotonin transporter (5-HTT) is the main regulator of 5-HT neurotransmission³⁶ in the brain and controls the reuptake of 5-HT in the synaptic cleft. Studies have shown that 5-HTT gene knockout leads to desensitization³⁷ and a decrease in the density and gene expression of 5-HT_{1A} receptors in the Dorsal raphe nucleus⁽³¹⁾. To further determine the relationship between the high-fat diet and the serotonin system, this study also measured the expression of 5-HT_{1A}R and 5-HTT genes and proteins in the brains of mice from each group. In the diet intervention group, when compared with the normal group, the expression of 5-HT_{1A}R and 5-HTT in the cerebral cortex of the high-fat group increased first and then decreased in the whole feeding period. In combination with the previous high-fat group, the 5-HT level in the brain of mice showed a tendency to increase at first and then decrease. In order to maintain the homeostasis of 5-HT, it was transported back to the vesicles or decomposed, and 5-HTT expression was increased. However, the 5-HT_{1A} receptor in the postsynaptic membrane was stimulated by 5-HT, and the 5-HT_{1A} receptor expression was increased. At 11 weeks, 5-HT levels were still high in the brains of mice that were still on a high-fat diet. It is the long-term excessive 5-HT stimulation that makes 5-HTT and 5-HT_{1A} to be activated all the time, leading to desensitization, resulting in the decrease of 5-HTT and 5-HT_{1A} expression and the destruction of the 5-HT nervous energy system in the brain. There is abundant evidence that the 5-HT_{1A} receptor plays an important role in the regulation of 5-HT release^{36, 38, 39}. Since the 5-HT nervous system was destroyed at 11 weeks, the 5-HT release decreased and the 5-HT level in the brain decreased in the 17 week high-fat group, and the expression of 5-HTT and 5-HT_{1A} receptor decreased. The data from the

stereotactic experiment also showed that the expression of 5-HTT and 5-HT_{1A}R decreased in the gene and protein levels in the model group when compared with the control group.

A recent review of 67 studies concluded that there is evidence that DNA methylation variants are associated with depression risk⁴⁰, when exploring the specific causes of the decline in the expression of 5-HTT and 5-HT_{1A}R genes. In order to explore the relationship between the decrease of the front protein expression and the DNA methylation, the DNA methylation of 5-HTT and 5-HT_{1A}R in the cerebral cortex of each group of mice was determined. It was found that in the diet intervention group, the hypermethylation level of 5-HTT and 5-HT_{1A} in the brain of the hyperlipidemic group at the 11th and 17th week was in line with the trend of protein expression as compared with the normal group, it is suspected that the previous decline in the expression of 5-HTT and 5-HT_{1A} was due to the hypermethylation of their corresponding genes. In the brain stereotactic injection group, it is noteworthy that the level of DNA methylation of the gene corresponding to the down-regulation of 5-HTT expression was increased in the model group when compared with the control group, but the level of DNA methylation of the gene corresponding to the down-regulation of 5-HT_{1A} expression was not increased. It is hypothesized in the present study that this may be due to the fact that the high-fat diet is a chronic stimulus, but the brain-targeted injection is an acute stimulus, and the mice respond to acute and chronic stress differently. Of course, this result is open to further discussion.

In conclusion, the findings of the current study suggest that the mechanism of depression-like behavior induced by high-fat diet in mice is not only due to the decrease of 5-HT levels in the brain, indeed, it may be due to a dynamic process in which the level of 5-HT in the brain increases first and then decreases. At the same time, it is thought that long-term high level of 5-HT stimulation will lead to DNA methylation of 5-HT neuroenergy related genes, and then damage 5-HT neuroenergy. This study can explain the low efficacy of monoamine drugs to some extent, and provide a theoretical basis for the development of antidepressant drugs.

Materials And Methods

Animals. The experiment was divided into two parts. The first was divided into dietary intervention experiment. In order to prove that the depression-like characterization in mice in the dietary intervention experiment is not only due to the 5-HT level reduction in the brain, the brain stereotactic experiment was established. Specific-pathogen-free C57BL/6J male mice (n = 70), approximately 3–4 weeks old. This study was approved by the Ethics Committee of Zhejiang University of Technology Animal Care. Male mice were housed under a 12-h light/dark cycle at 23 ± 2°C. After one week of adaptation, the C57BL/6J were randomly divided into Standard Diet Group (STD, n = 24) and high fat diet group (HFD, n = 24). The duration of dietary intervention was 18 weeks. In the brain stereotactic experiment, the C57BL/6J were randomly divided into Control group (saline injection, n = 10) and Model Group (5-HT injection, n = 10) for 14 days. In the dietary intervention experiment, body weight was monitored weekly and the degree of depression was assessed using behavioral tests and other biochemical indicators at 3, 5, 11, and 17

weeks. In the brain stereotactic experiment, the degree of depression was assessed using behavioral tests and other biochemical indicators.

Methods

Stereotactic Infusions. In this study, 5-hydroxytryptamine was injected into the lateral ventricular region of C57BL/6J mice. In brief, each mouse was immobilized in a stereotactic frame (RWD) after being anesthetized with 0.5% pentobarbital. The following coordinates were adopted: anteroposterior = -0.58mm, mediolateral = +1mm, dorsoventral = -2.2mm. The custom Cannula (RWD) was implanted into the lateral ventricle area of each mouse according to the coordinates, and the incision was sutured. After 7 days of recovery, 5-HT and 0.9% sodium chloride (0.9% NaCl) were injected into each mouse at a rate of 1 ml/min for 14 days, respectively.

Behavioral assays

Behavioral tests were conducted to assess the depression phenotype in the following sequence: sucrose preference test, open field, tail suspension and forced swim test. During behavioral test, researchers kept quiet, removed each animal's excrement on time, and tested each group alternately.

Open field test [OFT]. Since OFT was used for assessment of anxiety-like behavior by recording the time across the central square, OFT was carried out to assess anxiety-like behavior in this study. Each mouse was placed in the center of the open field arena of white box (45 cm × 45 cm) and locomotor activity was recorded for 5 min 30s by an overhead camera. Mice showed higher levels of depression and less central residence time. After OFT of each mouse, alcohol was sprayed to remove the peculiar smell of the previous mouse and avoid affecting the next experiment results²⁷.

Sucrose preference test [SPT]. Sucrose preference was used to measure depressive-like behavior, where decreased sucrose preference was considered as anhedonia^{28, 29}. In this test, the mice were fed in a single cage. Two bottles of 1% sucrose solution per cage were given to the test rodents for 24 h, next, two bottles, one bottle with 1% sucrose solution, and the other bottle with water fed for 24 h, and then deprived of water for 24 h. After 24 h, 1% sucrose solution and purified water were respectively placed in each cage, fed for 24 h, and the positions of the two bottles were changed every 12 h to prevent the potential influence of position preference. During the experiment, the weight of the water bottle was weighed, and the formula for calculating sucrose preference (%) = sucrose intake/ (sucrose intake + water intake) × 100% .

Tail suspension test and forced swimming test (TST and FST). Depressive-like behavior was assessed by tail suspension and forced swimming tests^{30, 31}. Mice with increased depression showed increased immobility time. In the first test, mice were suspended by the tail on a horizontal bar (at around 50 cm from the floor) with adhesive tape, and immobility time (in seconds) was recorded during the last 4 min of the 6 min test. Mice were considered motionless when they did not exhibit escape-oriented behavior and passively hung without any body movements. The forced swimming test, was performed using glass

drums (20 cm diameter, 45 cm height). Before the test, the cylinders were filled with 15 cm of water (25± 1°C), and the test mice were individually placed in the cylinders during the last 4 min of the 6 min test. The immobility period was considered when animal floated in the water without behavior oriented to find an exit or made only the necessary movements to keep his head out of the water. The time of immobility was recorded for 6 min. In this condition, mouse developed escape-orientated behaviors interspersed with temporally enhancement bouts of immobility.

Sample Collection and Preparation.After all the behavior tests, brains from each mouse were collected, the samples were transferred to EP tubes and stored at -80°C for later analysis.

CORT ELISA.The content of 5-HT in mice brain tissue was determined using an ELISA kit according to the instruction provided by Shanghai Meilian Biotechnology Co, Ltd (China).

Morphological observations.Mice were transcardially perfused with saline, then followed by a 4% neutral paraformaldehyde solution. The brain was post-fixed for 24 h at 4°C and after that, they were embedded in paraffin and cut into 4 μm thick slices. Sections of cortex and hippocampus were stained with hematoxylin and eosin (HE) for histopathological evaluation.

Total RNA isolation and quantitative RT-PCR.All the kits used in this work were from Shanghai Biyuntian Biotechnology Co., Ltd. Total RNA was extracted and purified using an RNAeasy™ kit (Beyotime, Shanghai), following the manufacturer's protocol. The cDNA was amplified using the BeyoFast™ SYBR Greeline by Primer 3 Plus. The comparative threshold cycle (Ct) method was employed for quantification of transcripts according to the manufacture's protocol (Applied Biosystems). Measurement of ΔCt was performed at least three times per sample. Amplification of the single product was confirmed by monitoring the dissociation curve. A control gene (glyceraldehyde-3-phosphate dehydrogenase) (GAPDH) was used for normalization to control for possible fluctuations of the target transcripts. The target genes were 5-HTT and 5-HT_{1A} (Table 1).

Table 1Primer sequences for real-time PCR

Name of gene	Primer sequence
Mouse 5-HTT	F: TATCCAATGGGTACTCCGCAG
	R: CCGTTCCCCTTGGTGAATCT
Mouse 5-HT _{1A}	F: GACAGGCGGCAACGATACT
	R: CCAAGGAGCCGATGAGATAGTT
Mouse GAPDH	F:GAAGGTCGGTGTGAACGGATTTG
	R: CATGTAGACCATGTAGTTGAGGTCA

Western blotting analysis. The total protein was prepared with brain tissue, 40 µg of protein was loaded into SDS-PAGE gels and transferred onto PVDF membranes. After blocked with 5% skim milk in TBST buffer for 1 h at RT, the membranes were subsequently incubated with primary antibodies at 4°C overnight. Primary antibodies used are listed as follows in the following: β-actin and 5-HTT purchased from protein (1:500); 5-HT_{1A}R obtained from abcam (1:1000). Then, it was rinsed with TBST buffer 3 times, incubated with Er Kang at room temperature for 1 h then rinsed with TBST buffer 3 times and exposed to Ecl developer. Membranes were exposed by chemiluminescence developing agents. The intensity of the targeting bands was quantified using ImageJ 1.51 software. Protein levels were normalized to β-actin levels in respective blots.

DNA methylation. According to the manufacturer's instructions, Genomic DNA was extracted from about 100 mg of cerebral cortex using the Bioteke corporation (Beijing, China). DNA purity was assessed using the nanodrop 2000 (Thermo Fisher Scientific). The methylation of 5-HT_{1A} and 5-HTT was commissioned by Quantitative analysis in Beijing, China.

Statistical analysis. All figures were performed using Graph Pad Prism 8.0. Data were expressed as the mean ± Standard Error of Mean (SEM). Two-tailed Student's t-test was used for statistical analysis of data. Statistical significance was defined as $p < 0.05$.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Declarations

Data availability

Source data are provided as a Source Data.xlsx. Source data are provided with this paper.

Author contributions Weiguang Shan and Yan Chen contributed to the conception of the study; Lifang Chen performed the experiment; Tingting Gong contributed significantly to analysis and manuscript preparation; Yan Chen and Yongyong Zhang performed the data analyses and wrote the manuscript; Yunxia Fang and Shengfeng Wang helped perform the analysis with constructive discussions.

Competing interests

The authors declare no competing interests

Acknowledgements

This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant No. LY20C140006.

References

1. Kessler RC, *et al.* The Epidemiology of Major Depressive Disorder Results From the National Comorbidity Survey Replication (NCS-R). *JAMA* **289**, 3095–3105 (2003).
2. Kalmoe MC, Janski AM, Zorumski CF, Nagele P, Palanca BJ, Conway CR. Ketamine and nitrous oxide: The evolution of NMDA receptor antagonists as antidepressant agents. *Journal of the Neurological Sciences* **412**, 116778 (2020).
3. Sheehan DV, Nakagome K, Asami Y, Pappadopulos EA, Boucher M. Restoring function in major depressive disorder: A systematic review. *Journal of affective disorders* **215**, 299–313 (2017).
4. Ślifirski G, Król M, Turło J. 5-HT Receptors and the Development of New Antidepressants. *International journal of molecular sciences* **22**, (2021).
5. Woolley DW, Shaw E. A BIOCHEMICAL AND PHARMACOLOGICAL SUGGESTION ABOUT CERTAIN MENTAL DISORDERS. *Proceedings of the National Academy of Sciences of the United States of America* **40**, 228–231 (1954).
6. Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C. Serotonin type-1A receptor imaging in depression. *Nuclear medicine and biology* **27**, 499–507 (2000).
7. Hirvonen J, *et al.* Decreased brain serotonin 5-HT_{1A} receptor availability in medication-naïve patients with major depressive disorder: an in-vivo imaging study using PET and [carbonyl-¹¹C]WAY-100635. *The international journal of neuropsychopharmacology* **11**, 465–476 (2008).
8. Wu S, Comings DE. A common C-1018G polymorphism in the human 5-HT_{1A} receptor gene. *Psychiatric genetics* **9**, 105–106 (1999).
9. Klemenhagen KC, Gordon JA, David DJ, Hen R, Gross CT. Increased fear response to contextual cues in mice lacking the 5-HT_{1A} receptor. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* **31**, 101–111 (2006).
10. Barros M, *et al.* Anxiolytic-like effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 in non-human primates. *European journal of pharmacology* **482**, 197–203 (2003).
11. Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin_{1A} receptor. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 10734–10739 (1998).
12. Bleys D, Luyten P, Soenens B, Claes S. Gene-environment interactions between stress and 5-HTTLPR in depression: A meta-analytic update. *Journal of affective disorders* **226**, 339–345 (2018).
13. You JS, Hu SY, Chen B, Zhang HG. Serotonin transporter and tryptophan hydroxylase gene polymorphisms in Chinese patients with generalized anxiety disorder. *Psychiatric genetics* **15**, 7–11 (2005).
14. Schipper P, Nonkes LJ, Karel P, Kiliaan AJ, Homberg JR. Serotonin transporter genotype x construction stress interaction in rats. *Behavioural brain research* **223**, 169–175 (2011).
15. Hammad TA, Laughren T, Racoosin J. Suicidality in pediatric patients treated with antidepressant drugs. *Archives of general psychiatry* **63**, 332–339 (2006).

16. Liu C, *et al.* Perceived stress, depression and food consumption frequency in the college students of China Seven Cities. *Physiology & behavior* **92**, 748–754 (2007).
17. Jeffery RW, *et al.* Reported food choices in older women in relation to body mass index and depressive symptoms. *Appetite* **52**, 238–240 (2009).
18. Sánchez-Villegas A, Toledo E, de Irala J, Ruiz-Canela M, Pla-Vidal J, Martínez-González MA. Fast-food and commercial baked goods consumption and the risk of depression. *Public health nutrition* **15**, 424–432 (2012).
19. Kim M, Bae S, Lim KM. Impact of High Fat Diet-induced Obesity on the Plasma Levels of Monoamine Neurotransmitters in C57BL/6 Mice. *Biomolecules & therapeutics* **21**, 476–480 (2013).
20. Zemdegs J, *et al.* Metformin Promotes Anxiolytic and Antidepressant-Like Responses in Insulin-Resistant Mice by Decreasing Circulating Branched-Chain Amino Acids. *The Journal of neuroscience: the official journal of the Society for Neuroscience* **39**, 5935–5948 (2019).
21. Zemdegs J, Quesseveur G, Jarriault D, Pénicaud L, Fioramonti X, Guiard BP. High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. *British journal of pharmacology* **173**, 2095–2110 (2016).
22. Felsenfeld G. A brief history of epigenetics. *Cold Spring Harbor perspectives in biology* **6**, (2014).
23. López-Jaramillo P, Camacho PA, Forero-Naranjo L. The role of environment and epigenetics in hypertension. *Expert review of cardiovascular therapy* **11**, 1455–1457 (2013).
24. Bayles R, *et al.* Methylation of the SLC6a2 gene promoter in major depression and panic disorder. *PloS one* **8**, e83223 (2013).
25. Januar V, Ancelin ML, Ritchie K, Saffery R, Ryan J. BDNF promoter methylation and genetic variation in late-life depression. *Translational psychiatry* **5**, e619 (2015).
26. Okada S, *et al.* The potential of SLC6A4 gene methylation analysis for the diagnosis and treatment of major depression. *Journal of psychiatric research* **53**, 47–53 (2014).
27. Seibenhener ML, Wooten MC. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of visualized experiments: JoVE*, e52434 (2015).
28. Yin C-Y, *et al.* A novel method for automatic pharmacological evaluation of sucrose preference change in depression mice. *Pharmacological Research* **168**, 105601 (2021).
29. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nature reviews Neuroscience* **14**, 609–625 (2013).
30. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. *Journal of visualized experiments: JoVE*, (2015).
31. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The tail suspension test. *Journal of visualized experiments: JoVE*, e3769 (2012).
32. Anderson EJ, *et al.* 60 YEARS OF POMC: Regulation of feeding and energy homeostasis by α -MSH. *Journal of molecular endocrinology* **56**, T157-174 (2016).

33. Waterson MJ, Horvath TL. Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding. *Cell metabolism* **22**, 962–970 (2015).
34. Wang L, Zhang Y, Du X, Ding T, Gong W, Liu F. Review of antidepressants in clinic and active ingredients of traditional Chinese medicine targeting 5-HT_{1A} receptors. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **120**, 109408 (2019).
35. Haase J, Brown E. Integrating the monoamine, neurotrophin and cytokine hypotheses of depression—a central role for the serotonin transporter? *Pharmacology & therapeutics* **147**, 1–11 (2015).
36. Mannoury la Cour C, Boni C, Hanoun N, Lesch KP, Hamon M, Lanfumey L. Functional consequences of 5-HT transporter gene disruption on 5-HT(1a) receptor-mediated regulation of dorsal raphe and hippocampal cell activity. *The Journal of neuroscience: the official journal of the Society for Neuroscience* **21**, 2178–2185 (2001).
37. Li Q, Wichems C, Heils A, Lesch KP, Murphy DL. Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT_{1A}) in 5-HT transporter knock-out mice: gender and brain region differences. *The Journal of neuroscience: the official journal of the Society for Neuroscience* **20**, 7888–7895 (2000).
38. McDevitt RA, Neumaier JF. Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J Chem Neuroanat* **41**, 234–246 (2011).
39. Blier P, de Montigny C. Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain. *Synapse (New York, NY)* **1**, 470–480 (1987).
40. Li M, D'Arcy C, Li X, Zhang T, Joobor R, Meng X. What do DNA methylation studies tell us about depression? A systematic review. *Translational psychiatry* **9**, 68 (2019).

Figures

Figure 1

Increase in bodyweight by Long-term high-fat diet. Error bars represent SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ significantly different from STD; two-tailed Student's t-test. Source data are provided as a Source Data file.

Figure 2

Long-term high-fat diet induction of depressive-like phenotype. Sugar preference (%) in the SPT **(a)**, time spent in the centre in the OFT **(b)**, immobility time in the FST **(c)**, immobility time in the TST **(d)** in mice fed an STD or HFD ($n=12$) for 3, 5, 11 or 17 weeks. SPT (5week), $p=0.0011$; SPT (11week), $p<0.0001$; SPT (17week), $p<0.0001$. OFT (5week), $p<0.0001$; OFT (11week), $p=0.0401$; OFT (17week), $p=0.0149$. FST (5week), $p<0.0001$; FST (11week), $p=0.1610$; FST (17week), $p=0.0124$. TST (5week), $p=0.4831$; TST (11week), $p<0.0001$; TST (17week), $p=0.0016$. two-tailed Student's t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$ significantly different from STD. Error bars represent SEM. Source data are provided as a Source Data file.

Figure 3

Long-term high-fat diet induction of 5-HT level changes in mice fed on STD or HFD. 5-HT (5week), $p = 0.0239$; 5-HT (11week), $p = 0.0002$; 5-HT (17week), $p = 0.0102$. two-tailed Student's t-test. * $p < 0.05$ and *** $p < 0.001$ significantly different from STD; Error bars represent SEM, Source data are provided as a Source Data file.

Figure 4

Depression-like phenotype induced by excessive injection of 5-HT. Time spent in the centre in the OFT (a), immobility time in the TST (b) and immobility time in the FST (c) in the control mice (injected with vehicle) or model mice (injected with 5-HT) ($n = 5$). OFT, $p = 0.0032$; TST, $p = 0.0244$; FST, $p = 0.0095$. two-

tailed Student's t-test. [#]*p* < 0.05 and ^{##}*p* < 0.01 significantly different from control; Error bars represent SEM. Source data are provided as a Source Data file.

Figure 5

Long-term high-fat diet leads to 5-HT nerve injury. HE staining of hippocampal DG area in long-term high-fat inducing 5-HT level changes in mice fed STD or HFD (a) and in control or model group(b).

Figure 6

6.1 The expression of 5-HT_{1A} and 5-HTT in HFD is in a dynamic process. **a** The mRNA expression of 5-HT_{1A} and 5-HTT in various mouse tissues (n=3). 5-HT_{1A} (5week), $p=0.0045$; 5-HT_{1A} (11week), $p=0.0049$; 5-HT_{1A} (17week) $p=0.0134$. 5-HTT (5week), $p=0.0306$; 5-HTT(11week), $p=0.0109$; 5-HTT(17week) $p=0.0101$. **b** The protein expression of 5-HT_{1A} and 5-HTT in various mouse tissues (n=3). 5-HT_{1A} (5week), $p=0.0098$; 5-HT_{1A} (11week), $p=0.0003$; 5-HT_{1A} (17week) $p=0.0043$. 5-HTT (5week), $p=0.0108$; 5-HTT (11week), $p=0.0052$; 5-HTT (17week) $p=0.0045$. two-tailed Student's t-test. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ significantly different from STD. Error bars represent SEM. Source data are provided as a Source Data file. **6.2 Excessive 5-HT leading to damage of the 5-HT nerves.** **a** 5-HT levels in the brain (n=4). $p=0.0094$. **b** the mRNA expression of 5-HT_{1A} and 5-HTT in control and model mouse tissues (n=3). 5-HT_{1A}, $p=0.0014$; 5-HTT, $p=0.0019$; **c** the protein expression of 5-HT_{1A} and 5-HTT in control and model mouse tissues (n=3). 5-HT_{1A}, $p<0.0001$; 5-HTT, $p=0.0035$; two-tailed Student's t-test. # $p<0.05$, ## $p<0.01$, ### $p<0.001$ and #### $p<0.0001$, significantly different from control. Error bars represent SEM. Source data are provided as a Source Data file.

Figure 7

Methylation of the promoter region of 5-HT related gene leading to the decrease of the corresponding protein expression. **a** Methylation levels of 5-HT_{1A} and 5-HTT genes in STD or HFD groups (n=3). 5-HT_{1A}(11week), $p=0.0173$; 5-HT_{1A} (17week), $p=0.0087$. 5-HTT (11week), $p=0.045$; 5-HTT(17week), $p=0.0181$. **b** Methylation levels of 5-HTTgene in the control or model groups (n=3). $p=0.0028$. Error bars represent SEM, * $p<0.05$ and ** $p<0.01$ significantly different from STD, ## $p<0.01$ significantly different from Control group; two-tailed Student's t-test. Source data are provided as a Source Data file.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Sourcedata.xlsx](#)