Absence of gut microbiota affects lipid metabolism in the prefrontal cortex of mice

Jian-Jun Chen (✉ chenjianjun@cqmu.edu.cn)

Chongqing Medical University

Jing Xie

Chongqing University Central Hospital

Ben-hua Zeng

Huazhong Agricultural University

Wen-wen Li

Chongqing Medical University

Shun-jie Bai

the First Affiliated Hospital of Chongqing Medical University

Chanjun Zhou

Chongqing Medical University

Wei Chen

Shanghai Applied Protein Technology Co. Ltd

Hong Wei

Huazhong Agricultural University

Peng Xie (✉ xiepeng@cqmu.edu.cn)

Chongqing Medical University

Article

Keywords: gut microbiota, lipid metabolism, metabolites, prefrontal cortex, host behaviors

Posted Date: February 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-96615/v2>

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

[Read Full License](#)

Version of Record: A version of this preprint was published at Neurological Research on October 7th, 2019. See the published version at <https://doi.org/10.1080/01616412.2019.1675021>.

Abstract

Objectives: Lipid metabolism is closely associated with many important biological functions. Here, we conducted this study to explore the effects of gut microbiota on the lipid metabolism in the prefrontal cortex of mice.

Methods: Germ-free (GF) mice, specific pathogen-free (SPF) and colonized GF (CGF) mice were used in this study. The open field test (OFT), forced swimming test (FST) and novelty suppressed feeding test (NSFT) were conducted to assess the changes in general behavioral activity. The liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was used to obtain the lipid metabolites. Both one-way analysis of variance (one-way ANOVA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were used to obtain the key differential lipid metabolites.

Results: The behavioral tests showed that compared to SPF mice, GF mice had more center distance, more center time, less immobility time and less latency to familiar food. Meanwhile, 142 key differential lipid metabolites between SPF mice and GF mice were identified. These lipid metabolites mainly belonged to glycerophospholipids, glycerolipids, sphingolipids, and saccharolipids. The gut microbiota colonization did not reverse these changed behavioral phenotypes, but could restore 25 key differential lipid metabolites.

Discussion: These results showed that the absence of gut microbiota could influence host behaviors and lipid metabolism. Our findings could provide original and valuable data for future studies to further investigate the microbiota-gut-brain axis.

Introduction

Gut microbiota is an assortment of microorganisms inhabiting in the mammalian gastrointestinal tract. It estimates that the ratio of the number of micro-organisms in gut to the number of human cells is much closer to 1:1 [1]. Recently, due to the important role of gut microbiota on the pathogenesis of diseases and host health, it obtains more and more attention [2-5]. Clemente JC et al. reported that it played a major role in health and disease in humans [6]. Previous studies reported that gut microbiota could influence the brain function through the microbiota-gut-brain axis [7, 8]. Our previous study found that the gut microbiota could be a potential causative factor in depression through affecting the carbohydrate and amino acid metabolism [9].

Lipid is an important constituent of cell membranes and a major form of energy storage, which plays a critical role in a variety of important biological functions. Gut microbiota, as a key regulator of lipid metabolism [10], could significantly affect the central and peripheral lipid metabolism: i) the disorderly of gut microbiota could disturb the lipid peroxidation, liver fatty acid production and triglyceride storage [11]; ii) the differentially expressed genes in the striatum and hippocampus of germ-free (GF) mice were

mainly related to the steroid metabolism [12]; iii) our previous work found that the differentially expressed genes in the hippocampus of GF mice were mainly involved in lipid metabolism [13]. These findings indicated that lipid metabolism might be a key pathway for gut microbiota to influence the host brain function and behaviors.

Recently, lipid metabolism was found to be related to the pathogenesis of neuropsychiatric diseases [14-18]. However, very few studies have been conducted to examine the effects of gut microbiota on the host lipid metabolism. The prefrontal cortex had a close relationship with emotion, complex cognitive behavior, social behavior, personality expression. Its basic activity was considered to be orchestration of thoughts and actions according to internal goals. Therefore, in this study, we used GF mice, specific pathogen-free (SPF) mice and colonized GF (CGF) mice to study the effects of gut microbiota on the lipid metabolism in the prefrontal cortex of mice. Our results would provide original and valuable data for future studies to further investigate the microbiota-gut-brain axis.

Materials And Methods

Animal model

GF and SPF male Balb/c mice were obtained from the Department of Laboratory Animal Science of the 3rd Military Medical University (Chongqing, China). To prevent the normalization of gut microbiota, GF mice were housed in flexible film gnotobiotic isolators before conducting experiments. To verify the germ-free status of mice, the Chinese Laboratory Animal-Microbiological Standards and Monitoring (GB 14922.2-2011) was used to test the feces and skin of mice. At the same time, SPF mice were housed in the standard animal facility. CGF mice were obtained by placing GF mice (5-6 weeks) into the cages with bedding and fecal matter from SPF mice. This method was previously proved to be effective at restoring a normal gut microbiota [19]. All mice were kept under the standard conditions (standard rodent diet; water *ad libitum*; humidity 55±5%; temperature of 21-22°C; and 12 hours light-dark cycle with lights on at 7:30 am). The Ethics Committee of Chongqing Medical University reviewed and approved this study, and we performed the experiments strictly according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

Behavioral tests

Before each test, all male mice were transferred into the behavioral testing room for acclimation at least half-hour. All behavioral tests were conducted between 8:00 am and 5:00 pm by the observers who were blinded to the animal genotypes. The video-computerized tracking system (SMART, Panlab, Barcelona, Spain) was used to videotape and quantify all behavioral tests. To reduce stress, all behavioral tests were conducted in a soundproof and isolated room.

Open field test (OFT) (8-9 weeks old; GF, n=8; CGF, n=8; SPF, n=8): Each mouse was allowed to freely explore the open field box (width, 45cm; height, 45cm; length 45cm) for six minutes. The video tracking system only recorded the spontaneous activities in the last five minutes. The total distance was used as

an index of locomotor activity, and center time and center distance were used as the indexes of anxiety-like behavior.

Novelty suppressed feeding test (NSFT) (8-9 weeks old; GF, n=8; CGF, n=8; SPF, n=8): Each mouse was allowed to freely explore the open field box for up to ten minutes. A single food pellet on a circular piece of white filter paper (diameter, 12cm) was put in the center of the box. The test would be ended when the mouse chewed a part of food pellet. Food consumption was quantified for five minutes and used as a control measure for appetite. This test was performed as previously described [20].

Forced swimming test (FST) (8-9 weeks old; GF, n=8; CGF, n=8; SPF, n=8): Each mouse was placed in a plexiglas cylinder (height, 30cm; diameter, 15cm) filled with 15cm water ($24 \pm 1^{\circ}\text{C}$) and forced to swim for six minutes. The video tracking system only recorded the immobility time in the last five minutes. The immobility time in the FST was widely used as an index of depression-like behavior [9, 13].

LC-MS/MS analysis

All mice (8-9 weeks old; GF, n=8; CGF, n=8; SPF, n=8) were euthanized by chloral hydrate (4%) at dosage of 0.20ml/20g and then sacrificed in random order. The brain tissue was quickly removed from the cranium. We used the mouse brain anatomical atlas to collect the prefrontal cortex, and carefully dissected out the prefrontal cortex on an ice-cold plate and then weighed it. The obtained prefrontal cortex was rapidly frozen with liquid nitrogen and then stored at -80°C for later analysis.

Briefly, the procedure of LC-MS/MS analysis was: 1) added 200ul water into the samples, and homogenized after freezing in liquid nitrogen first (FLASH); 2) added 240ul MeOH and 800ul MTBE, then the obtained mixture was sonicated for 20 minutes in low-temperature water bath and subsequently centrifuged (14,000rpm x 10 minutes, 10°C); 3) dried the obtained supernatant with nitrogen, then added 200ul of 90% isopropanol/acetonitrile solution into the dried extract for reconstitution during mass spectrometry analysis; 4) after centrifugation (14,000rpm x 10 minutes, 10°C), the supernatant was obtained for mass spectrometry analysis. The mass spectrometry analysis was conducted using the UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600) in Shanghai Applied Protein Technology Co., Ltd).

Statistical analysis

The data were normally distributed and homoscedastic. One-way analysis of variance (one-way ANOVA) was used to identify the significantly changed ($p < 0.05$) lipid metabolites among the three groups. If a significant difference was observed, we used the Bonferroni post-hoc test to check which two groups differed significantly. The orthogonal partial least-squares discriminant analysis (OPLS-DA) model was performed to visualize the discrimination between the different groups. The lipid metabolites with variable importance in projection (VIP) value > 1 were viewed as the differential lipid metabolites. The pronouncedly changed lipid metabolites (GF vs. SPF) identified by both one-way ANOVA and OPLS-DA model were viewed as the key differential lipid metabolites. Meanwhile, we used hierarchical clustering

algorithm to investigate whether these key differential lipid metabolites could correctly cluster the samples. Moreover, if one key lipid metabolite was also significantly changed between GF mice and CGF mice ($p < 0.05$, one-way ANOVA; $VIP > 1$, OPLS-DA), then this metabolite could be restored by gut microbiota colonization.

Results

Altered behavioral phenotypes

The results of OFT showed that the total distances were similar between SPF mice and GF mice ($p = 0.87$) (Figure 1A), which demonstrated a similar locomotor activity in GF mice. But, compared to SPF mice, GF mice showed significantly increased center distance ($p = 0.004$) (Figure 1B) and center time ($p = 0.016$) (Figure 1C). Meanwhile, compared to SPF mice, GF mice showed significantly decreased immobility time ($p = 0.014$) (Figure 1D). These results might suggest a potential link between the anxiety/depression-like behaviors and the 'microbiota-gut-brain axis'.

The NSFT was carried out to assess the stress-induced anxiety of mice in the new environment. Both the latency to eat familiar food and the consumption of familiar food were viewed as the two evaluation indexes. As shown in Figure 1, there was significant difference on the latency to food between SPF mice and GF mice ($p = 0.005$) (Figure 1E), but no significant difference on the food consumption between SPF mice and GF mice ($p = 0.73$) was observed (Figure 1F). These results suggested that the abovementioned anxiolytic- and antidepressant-like behaviors in GF mice did not result from the general increase in feeding.

To check whether or not the changed behavioral phenotypes could be reversed after colonization with gut microbiota, CGF mice were used here. As shown in Figure 1, compared to SPF mice, CGF mice still showed similar locomotor activity (Figure 1A), and significantly increased center distance ($p = 0.018$) (Figure 1B), increased center time ($p = 0.030$) (Figure 1C), and decreased immobility time ($p = 0.037$) (Figure 1D). Meanwhile, compared to SPF mice, CGF mice still showed significantly less latency to food ($p = 0.003$) (Figure 1E) and similar food consumption ($p = 0.68$) (Figure 1F). These results demonstrated that the colonization of gut microbiota to the adolescent GF mice was not sufficient to reverse the changed behavioral phenotypes.

Key differential lipid metabolites

In the positive ion mode, the score plot of the OPLS-DA model showed that GF mice could be obviously separated from SPF mice with no overlap (Figure 2A). Analysis of the corresponding OPLS-DA loading plot resulted in the identification of 186 differential lipid metabolites ($VIP > 1.0$) responsible for the sample separation. Meanwhile, the results of one-way ANOVA showed that only 74 of the 186 differential lipid metabolites had p -value < 0.05 . Thus, in positive ion model, we identified 74 key differential lipid metabolites. Using the same methods, 68 key differential lipid metabolites in negative ion mode were identified (Figure 2B). These key differential lipid metabolites mainly belonged to glycerophospholipids,

glycerolipids, sphingolipids, and saccharolipids. Further analysis showed that the 74 key differential lipid metabolites from positive ion mode could effectively cluster the samples with only one GF mice wrongly clustered into the SPF group (Figure 3A), and the 68 key differential lipid metabolites from negative ion mode could also effectively cluster the samples with 100% accuracy (Figure 3B).

Most affected metabolites

To find out the most affected lipid metabolites by the absence of gut microbiota, the weighted correlation network analysis (WGCNA) was used here. As shown in Figure 4, we identified five clusters (modules) of highly correlated lipid metabolites, and found that the green cluster was significantly related to the absence of gut microbiota. Module-trait analysis identified that the following 13 lipid metabolites from the green cluster were the most affected lipid metabolites: ChE(22:6)+NH₄, TG(58:5)+NH₄, PE(36:5)+H, PE(32:1)-H, PE(34:2)-H, PE(34:3)-H, PG(34:2)-H, PG(38:7)-H, PE(36:5p)-H, PG(42:9)-H, CL(72:5)-H, PE(36:5)-H and PE(32:0)-H. Among these metabolites, 10 metabolites were belonging to Glycerophospholipids.

Restored key lipid metabolites

Among the 74 key differential lipid metabolites from positive ion mode, eight metabolites were also significantly changed between GF mice and CGF mice ($p < 0.05$, one-way ANOVA; VIP > 1, OPLS-DA); among the 68 key differential lipid metabolites from negative ion mode, 17 metabolites were also significantly changed between GF mice and CGF mice ($p < 0.05$, one-way ANOVA; VIP > 1, OPLS-DA). Therefore, in total, 25 key differential lipid metabolites could be restored after colonization with gut microbiota (Figure 5). The levels of these restored key differential lipid metabolites were similar between SPF mice and CGF mice. These restored key differential lipid metabolites belonged to glycerophospholipids, glycerolipids, and coenzyme (Co).

Discussion

In this study, we found reduced anxiety- and depression-like behaviors in GF mice. The changed behavioral phenotypes in GF mice were not restored after colonization with gut microbiota, which demonstrated that the colonization of gut microbiota to the adolescent GF mice was not sufficient to reverse the changed behavioral phenotypes. Meanwhile, we found that the absence of gut microbiota could mainly affect the levels of glycerophospholipids, glycerolipids, sphingolipids, and saccharolipids in the prefrontal cortex of mice. Among the most affected metabolites by the absence of gut microbiota, most of the metabolites belonged to Glycerophospholipids. After colonization with gut microbiota, we found that the levels of 25 key differential lipid metabolites were restored. These reversed lipid metabolites belonged to glycerophospholipids, glycerolipids, and Co. Our results demonstrated that the gut microbiota could affect the lipid metabolism in the prefrontal cortex of mice. Our findings could provide original and valuable data for future studies to further investigate the microbiota-gut-brain axis.

Previous studies reported that compared to SPF mice, GF mice displayed increased motor activity and reduced anxiety-like behavior [12, 21]. Our previous studies also found reduced anxiety-like behavior in GF mice [9, 13]. In this study, the results of OFT also showed significantly reduced anxiety-like behavior in GF mice. The immobility time in the FST was widely used as an index of depression-like behavior in previous studies [9, 13]. Our previous study reported a significantly decreased immobility time in GF mice relative to SPF mice [13]. Here, we also found that GF mice had a significantly decreased immobility time. Gut microbiota had a close relationship with hippocampal gene expression of the serotonin receptor 1A (5HT1A) [22], which was one of the most common serotonin receptors related to emotional behavior and anxiety [23, 24]. Thus, the reduced anxiety- and depression-like behavior in GF mice might indicate that the absence of gut microbiota affected the level of hippocampal gene expression of 5HT1A. The abnormal high or low levels of anxiety and fear response have indicated the disturbed emotional regulation, but we did not study whether it was beneficial or not; then future studies should further investigate this question.

Some works have been done to study whether these changed behavioral phenotypes in GF mice could be restored by colonizing with gut microbiota [12]. Diaz et al. found that the early colonization with gut microbiota could restore several behavioral patterns of GF mice, but the changed behavioral phenotypes in the OFT were not successfully restored after colonization with gut microbiota to the adolescent GF mice [12]. Both our previous study [13] and the present study also obtained a similar conclusion. Therefore, future studies were needed to find out whether there was a sensitive/critical period for the normal gut microbiota to affect the host behaviors.

Many studies have found that the gut microbiota could play an important role in the development of host behaviors, such as social interaction-related behaviors and anxiety [21, 25]. These behaviors are commonly viewed as the hall mark of mental disorders. Hsiao et al. reported that the gut microbiota could modulate the physiological and behavioral abnormalities, which were associated with neurodevelopment disorders [26]. Our previous study found that the relative abundance of Actinobacteria and Bacteroidetes were significantly changed in MDD patients [9]. However, the underlying mechanisms of the interaction between gut microbiota and mental disorders are still unclear. Using the social defeat animal model of depression, we found that the lipid metabolism in the prefrontal cortex of rats was significantly perturbed [27]. Meanwhile, our previous work showed that the antidepressants could also influence the lipid metabolism in the prefrontal cortex of rats and mice [28, 29]. Therefore, the combination of these previous findings with our present results suggested that the disturbed gut microbiota might play an important role in the development of depression by affecting lipid metabolism in the prefrontal cortex.

Several limitations should be mentioned here. Firstly, the untargeted LC-MS/MS approach was used alone here to identify the changes in lipid metabolism in the prefrontal cortex; then future studies should further use targeted approaches to study the specific relationship between these key differential lipid metabolites and gut microbiota. Secondly, we did not conduct repeated tests on each sample; the samples were tested one by one. More stable results might be obtained if the repeated independent tests on the same

samples were used. Thirdly, we only have known that the absence of gut microbiota could influence the lipid metabolism in the prefrontal cortex of mice, but the contribution of each member of the gut microbiota was unclear. Fourthly, we only investigated the effects of gut microbiota on lipid metabolism; therefore future studies were needed to identify other factors responsible for the re-equilibration of (some) lipid levels. Fifthly, although GF mice were key to answer to the question “is microbiota relevant in a given pathology?”, a comparison with other methods of microbiota ablation in wild-type mice might also be helpful for this question (i.e, broad-spectrum antibiotics), because the complete absence of the gut microbiota during the whole adolescence of the mouse could importantly impact on several physiological functions, such as immune system development and serotonin levels [30]. Sixthly, the SPF and CGF mice did not have exactly the same gut microbiota composition, which might have light negative effects on our results. Seventhly, the complete absence of the gut microbiota during the whole adolescence of the mouse could importantly impact on the serotonin levels, but in this study, we did not measure the serotonin levels in the different groups. Eighthly, the 15 minutes of pretest 24 hours before the FST test session was not conducted here; then its effect on the results was needed future studies to explore. Ninth, we did not study how the lipid metabolism alterations caused by gut microbiota could have implications for the development of depression. Finally, we did not analyze how the altered lipid metabolism changed the host behaviors; future studies could treat mice with inhibitors of lipid synthesis, (ie of sphingolipids, ceramids, etc) in the presence or absence of microbiota to find it out. Exploring their relationship will have important meaning for explaining the pathogenesis of depression.

Conclusions

In conclusion, this study found the reduced depression- and anxiety-like behaviors in GF mice, and 142 key differential lipid metabolites between SPF mice and GF mice. After colonization with gut microbiota, 25 key differential lipid metabolites that belonged to glycerophospholipids, glycerolipids, and Co could be restored. Our results showed that the absence of gut microbiota could alter the host behaviors, and affect the lipid metabolism in the prefrontal cortex of mice. Our findings could provide original and valuable data for future studies to further investigate the effects of gut microbiota on brain functions.

Declarations

Acknowledgements

This work was supported by the National Key R&D Program of China (2017YFA0505700), the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2019PT320002300), the Natural Science Foundation Project of China (81820108015, 81701360, 81601208), the Chongqing Science & Technology Commission (cstc2017jcyjAX0377), and the Special Project on Natural Chronic Non-infectious Diseases (2016YFC1307200).

Disclosures

The authors declare that they have no conflict of interest.

References

- [1] Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell*. 2016; 164(3): 337-40.
- [2] Zhan G, Yang N, Li S, et al. Abnormal gut microbiota composition contributes to cognitive dysfunction in SAMP8 mice. *Aging (Albany NY)*. 2018; 10(6): 1257-1267.
- [3] Uzbay T. Germ-free animal experiments in the gut microbiota studies. *Curr Opin Pharmacol*. 2019; 49: 6-10.
- [4] Chen J, Zheng P, Liu Y, et al. Sex differences in gut microbiota in patients with major depressive disorder. *Neuropsychiatr Dis Treat*. 2018; 14: 647-655.
- [5] van der Lugt B, Rusli F, Lute C, et al. Integrative analysis of gut microbiota composition, host colonic gene expression and intraluminal metabolites in aging C57BL/6J mice. *Aging (Albany NY)*. 2018; 10(5): 930-950.
- [6] Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell*. 2012; 148: 1258–1270.
- [7] Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*. 2011; 141: 599–609.
- [8] Clarke G, Grenham S, Scully P, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry*. 2013; 18: 666–673.
- [9] Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry*. 2016; 21(6):786-796.
- [10] Ghazalpour A, Cespedes I, Bennett BJ, et al. Expanding role of gut microbiota in lipid metabolism. *Curr Opin Lipidol*. 2016; 27(2): 141-7.
- [11] Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science*. 2012; 336 (6086):1262-1267.
- [12] Diaz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *PNAS*. 2011; 108(7):3047-52.
- [13] Chen JJ, Zeng BH, Li WW, et al. Effects of gut microbiota on the microRNA and mRNA expression in the hippocampus of mice. *Behav Brain Res*. 2017; 322(Pt A):34-41.

- [14] Chen JJ, Xie J, Zeng L, et al. Urinary metabolite signature in bipolar disorder patients during depressive episode. *Aging (Albany NY)*. 2019; 11(3):1008-1018.
- [15] Ancelin ML, Carrière I, Boulenger JP, et al. Gender and genotype modulation of the association between lipid levels and depressive symptomatology in community-dwelling elderly (the ESPRIT study). *Biol Psychiatry*. 2010; 68(2): 125-132.
- [16] Hou L, Wei X, Zhuo Y, et al. GC-MS-based metabolomics approach to diagnose depression in hepatitis B virus-infected patients with middle or old age. *Aging (Albany NY)*. 2018; 10(9): 2252-2265.
- [17] Chen JJ, Bai SJ, et al. Urinary biomarker panel for diagnosing patients with depression and anxiety disorders. *Transl Psychiatry*. 2018; 8(1):192.
- [18] Chen JJ, Xie J, Li WW, et al. Age-specific urinary metabolite signatures and functions in patients with major depressive disorder. *Aging (Albany NY)*. 2019; Accepted.
- [19] Berer K, Mues M, Koutrolos M, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature*. 2011; 479(7374):538-541.
- [20] Li N, Liu RJ, Dwyer JM, et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol Psychiatry*. 2011; 69(8):754-761.
- [21] Neufeld KM, Kang N, Bienenstock J, et al. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil*. 2011; 23(3):255-264.
- [22] Beilharz J E, Kaakoush N O, Maniam J, et al. Cafeteria diet and probiotic therapy: cross talk among memory, neuroplasticity, serotonin receptors and gut microbiota in the rat. *Mol psychiatry*. 2018; 23(2): 351-361.
- [23] Kalynchuk L E, Pinel J P J, Meaney M J. Serotonin receptor binding and mRNA expression in the hippocampus of fearful amygdala-kindled rats. *Neurosci Lett*. 2006; 396(1): 38-43.
- [24] Menard J, Treit D. Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev*. 1999; 23(4): 591-613.
- [25] Desbonnet L, Clarke G, Shanahan F, et al. Microbiota is essential for social development in the mouse. *Mol Psychiatry*. 2014; 19(2):146-8.
- [26] Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. 2013; 155(7):1451-1463.
- [27] Liu YY, Zhou XY, Yang LN, et al. Social defeat stress causes depression-like behavior with metabolite changes in the prefrontal cortex of rats. *Plos One*. 2017; 12(4):e0176725.

[28] Bai SJ, Zhang X, Chen Z, et al. Insight into the metabolic mechanism of Diterpene Ginkgolides on antidepressant effects for attenuating behavioural deficits compared with venlafaxine. *Sci Rep.* 2017; 7(1): 9591.

[29]. Bai SJ, Hu Q, Chen Z, et al. Brain region-specific metabolite networks regulate antidepressant effects of venlafaxine. *RSC Adv.* 2017; 7:46358-46369.

[30] Luo Y, Zeng B, Zeng L, et al. Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. *Transl Psychiatry.* 2018; 8(1): 187.

Table

Table 1 Reversed key differential metabolites (GF vs. SPF) after colonizing with gut microbiota

LipidIon	CalcMz	IonFormula	RT min	SPF vs. GF			CGF vs. GF			Ion-Model
				FC	p-value	VIP	FC	p-value	VIP	
PE(32:1)-H	688.4922805	C37 H71 O8 N1 P1	11.303	0.41	1.66E-05	1.83	0.45	4.27E-05	2.19	negative
PG(34:2)-H	745.5025115	C40 H74 O10 N0 P1	10.313	0.31	0.011	1.41	0.14	0.001	1.89	negative
PE(36:5p)-H	720.4973655	C41 H71 O7 N1 P1	10.058	0.24	0.001	1.50	0.44	0.020	1.63	negative
PE(34:2)-H	714.5079305	C39 H73 O8 N1 P1	11.401	0.26	5.54E-05	1.83	0.30	0.0001	2.17	negative
PG(42:10)-H	841.5025115	C48 H74 O10 N0 P1	8.764	0.71	0.003	1.75	0.76	0.015	1.63	negative
LPG(20:4)-H	531.2728465	C26 H44 O9 N0 P1	2.063	1.74	0.001	1.68	1.47	0.032	1.56	negative
LPE(18:0)-H	480.3095655	C23 H47 O7 N1 P1	4.253	0.75	0.021	1.31	0.79	0.046	1.37	negative
PC(38:7)+HCOO	848.5447105	C47 H79 O10 N1 P1	10.213	0.24	0.008	1.40	0.34	0.022	1.46	negative
CL(70:3)-H	1430.011905	C79 H147 O17 P2	20.996	0.42	0.009	1.47	0.51	0.027	1.63	negative
PE(34:3)-H	712.4922805	C39 H71 O8 N1 P1	10.542	0.10	3.52E-06	1.94	0.21	2.02E-05	2.25	negative
PE(36:5)-H	736.4922805	C41 H71 O8 N1 P1	10.347	0.64	0.028	1.20	0.57	0.009	1.69	negative
CL(72:5)-H	1454.011905	C81 H147 O17 P2	21.139	0.40	0.015	1.31	0.41	0.018	1.69	negative
PE(36:5)-H	736.4922805	C41 H71 O8 N1 P1	10.615	0.44	0.009	1.32	0.51	0.020	1.56	negative
PG(34:2)-H	745.5025115	C40 H74 O10 N0 P1	10.093	0.37	0.0002	1.68	0.35	0.0001	2.09	negative
PG(38:7)-H	791.4868615	C44 H72 O10 N0 P1	8.7621	0.73	0.0006	1.86	0.67	6.62E-05	2.15	negative
PI(36:4e)-H	843.5392915	C45 H80 O12 N0 P1	10.371	0.62	0.002	1.44	0.73	0.026	2.03	negative
PE(32:0)-H	690.5079305	C37 H73 O8 N1 P1	11.238	0.43	0.009	1.34	0.45	0.012	1.60	negative
PE(36:5)+H	738.5068335	C41 H73 O8 N1 P1	10.347	0.69	0.0003	1.71	0.59	1.04E-05	2.58	positive
DG(40:4)+NH4	690.6031005	C43 H80 O5 N1	14.977	0.11	0.0003	1.62	0.23	0.001	2.04	positive
PC(52:5)+H	1004.804183	C60 H111 O8 N1 P1	18.035	0.40	0.005	1.46	0.55	0.036	1.96	positive
TG(60:11)+NH4	970.7858155	C63 H104 O6 N1	19.336	0.29	0.005	1.40	0.45	0.030	1.61	positive
PE(38:7p)+H	746.5119185	C43 H73 O7 N1 P1	10.549	0.28	0.004	1.35	0.46	0.031	1.55	positive
PG(36:2)+Na	797.5303095	C42 H79 O10 N0 P1 Na1	10.219	0.64	0.010	1.77	0.65	0.013	1.73	positive
LPC(16:0)+H	496.3397685	C24 H51 O7 N1 P1	3.048	0.80	0.022	1.37	0.77	0.010	1.91	positive
Co(Q8)+NH4	744.5925355	C49 H78 O4 N1	15.413	0.54	0.027	1.21	0.45	0.008	2.05	positive

^aP-values were derived from one-way ANOVA test. ^bvariable importance in the projection (VIP) was obtained from OPLS-DA with a threshold of 1.0.

Figures

Figure 1

Effect of gut microbiota on mood-related behavior: A) Open-field test (OFT): total distance was used to assess the locomotor activity. No difference in total distance was observed among GF (n=8), SPF (n=8) and CGF (n=8) mice. B, C) OFT: center distance and time spent in center were used to assess anxiety-like behavior. GF and CGF mice displayed an increased proportion of center distance and time spent in center relative to SPF mice. D) Forced swimming test (FST): immobility time was used to assess depression-like behavior. GF (n=8) and CGF (n=8) mice displayed an decreased proportion of immobility time relative to SPF (n=8) mice. E, F) Novelty suppressed feeding test (NSFT): latency to food and food consumption were used to assess the stress-induced anxiety of mice in the new environment. No difference in food consumption was observed among GF (n=8), SPF (n=8) and CGF (n=8) mice, but the latency to food was decreased in GF and CGF mice compared to SPF mice. Data presented as means ± standard errors of the mean. *P<0.05, **P<0.01.

Figure 2

Metabolomic analysis of prefrontal cortex from GF (n=8) and SPF (n=8) mice: A) orthogonal partial least-squares discriminant analysis (OPLS-DA) model showed an obvious difference in lipid metabolites obtained in positive ion model between GF and SPF mice. B) OPLS-DA model showed an obvious difference in lipid metabolites obtained in negative ion model between GF and SPF mice.

Figure 3

Hierarchical clustering heatmap constructed using key metabolites: A) 74 key metabolites identified in positive ion model could effectively discriminate GF (n=8) and SPF (n=8) mice. B) 68 key metabolites identified in negative ion model could effectively discriminate GF (n=8) and SPF (n=8) mice.

Figure 4

Most affected lipid metabolites by the absence of gut microbiota identified using weighted correlation network analysis

Figure 5

Reversed key metabolites after colonizing with gut microbiota. GF (n=8), SPF (n=8) and CGF (n=8) mice were used. Data presented as means $\pm$ standard errors of the mean.