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Fecal Microbiota Transplantation from Healthy Donors Reduced Alcohol-induced Anxiety and Depression in an Animal Model of Chronic Alcohol Exposure

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Abstract

Alcohol addiction can cause brain dysfunction and threatens both individuals and society. Recently, emerging studies have suggested the dysbiosis of gut microbiota induced by alcohol exposure contributed to the reward-seeking behaviors as well as anxiety, depression. In the current study, animal model of chronic alcohol exposure was established by providing mice with gradient concentrations of alcohol from 2%, 4%, and 6% to 8% for 21 days. Moreover, three fecal microbiota transplantation (FMT) plans were innovatively designed to explore the potential effects of FMT from 3 healthy donors on alcohol-induced neuropsychic behaviors. To our knowledge, for the first time, we found that anxiety and depression after alcohol intake were gradually relieved with the extension of transplantation. Although the two-week FMT starting at the end of alcohol treatment had few effects, the transplantation started at 8% ethanol exposure alleviated alcohol-induced depression in tail suspension test. More importantly, accompanied by three-week exposure, the five-week FMT significantly decreased anxiety-like behaviors in open field test and depression in tail suspension test. These data validated the role of gut microbiota in alcohol addiction and indicated the modulation of healthy donor FMT on alcohol-related anxiety and depression, providing a new target for treating alcohol addiction by targeting microbiota.

Key Words: alcohol addiction, anxiety, depression, fecal microbiota transplantation, gut microbiota

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Introduction

Alcohol addiction, owing to excessive alcohol intake, is one of the most prevalent neuropsychiatric diseases and afflicts our society. According to the World Health Organization, harmful use of alcohol occupied 3 million deaths every year[1]. On the one hand, alcohol exerts its major adverse effects on nervous, gastrointestinal and cardiovascular systems, resulting in insomnia, alcoholic hepatitis, and congestive heart failure (11). On the other hand, alcohol also plays a key role in a high proportion of suicides, vehicle accidents and violent crimes (37). Genetically, alcohol addiction is considered as a relapsing neuropsychiatric disorder characterized by compulsive behaviors to seek and intake alcohol, losses of self-disciplined ethanol drinking, and the development of anxiety and depression (15). As a result, the neuropsychic behaviors are critical criterions and priorities to alleviate in alcohol addiction intervention and treatment. Unfortunately, the underlying mechanisms of alcohol addiction remain largely unknown, which makes it challenging to find effective therapeutic targets to alleviate neuropsychic symptoms and clinically manage addiction.

The human body is a complex ecosystem, including more than 10^{13}-10^{14} bacteria and 10 million microbial genes (22). The most abundant area of microflora is the gut, where the phyla *Firmicutes* (species such as *Clostridium*) and *Bacteroidetes* (species such as *Bacteroides*) account for the most (28). The gut microbiota not only participates in intestinal digestion, nutrition and innate immunity, but also plays a key role in obesity, diabetes, and cardiovascular diseases (20). Recently, expanding evidences revealed the crucial role of gut microbiota played in regulating neuropsychic behaviors. For example, reduced anxiety-like behavior and decreased serotonin receptor 1A expression were found in germ-free mice (21). In animal models of depression and chronic stress, mice exhibited a distinct composition of gut microflora community (5). Some modulations of the microbiome, like the Mediterranean diet, suggested its protective effects on depression by maintaining beneficial microbiota profiles (3). Although there is paucity information of exact mechanisms through which this impact can be exerted, the production of short-chain fatty acids (SCFAs) (34), the regulation of tryptophan metabolism and cytokine expression (23), and immune activation (7) are potential pathways of brain-microbe interactions.

Several distinct but complementary methods, such as germ-free mice, antibiotics, probiotics/prebiotics treatment and fecal microbiota transplantation (FMT), have revealed the particular contributions of gut microbiota homeostasis in regulating behaviors and emotion (13). Targeting the gut microbiota to modulate brain dysfunction and neuropsychic behaviors has shown a promising future. By restructuring the beneficial bacteria community and reinforcing the gut barrier defense, the FMT received widely attentions in its clinical application. In preclinical studies, FMT from depressed patient to microbes-depleted rats induced depression-like behaviors and altered tryptophan metabolism (12). The oral intake of probiotics *bifidobacterium* and prebiotic fructo-oligosaccharides and galacto-oligosaccharides exhibited anti-depressant effects in chronic stress models (1, 39). Clinical research also showed that one week of FMT relieved disease severity and prolonged survival among severe alcoholic hepatitis patients, demonstrating the efficacy of healthy donor FMT therapy (26). A growing number of evidences have revealed the gut microbiota dysbiosis during alcohol exposure (6, 25, 30). Especially, the transplantation of enteric bacteria from alcohol-treated mice to healthy controls elicited withdrawal-induced anxiety and depression (38). Based on the facts above, we hypothesized that the fecal microbiota transplantation from healthy donors could alleviate alcohol-induced anxiety/depression-like behaviors in mice.

To this end, we colonized C57BL/6 mice with gradient concentrations of alcohol for three weeks, establishing the model of chronic alcohol exposure. Moreover, three different FMT ways were innovatively designed and started at the end of exposure, 6% full dose alcohol treatment and the beginning of the whole exposure period. The open field test, tail suspension test, forced swim test and alcohol preference test were used to assess the adverse effects of chronic alcohol intake and protective efficacy of FMT. To our knowledge, this is the first study to explore the effects of healthy donor FMT on alcohol-induced neuropsychic changes, which might provide a potential approach for clinically managing alcohol addiction treatment.

Materials and Methods

Study Design

To explore whether the FMT had the ability to modulate alcohol-induced anxiety, we established the model of chronic alcohol exposure. The chronic

[1] World Health Organization. Global status report on Alcohol and health 2018. http://www.who.int/substance_abuse/publications/global_alcohol_report/gsr_2018/en (9, December 2018, data last assessed).

alcohol intake lasted for three weeks followed by two-week tap water drinking. To assess the validity of the model, the open field test, tail suspension test, forced swim test and alcohol preference test were performed after three-week chronic alcohol exposure. Behavioral assessments were also performed after 5 weeks. Mice received FMT were defined as the FMT group. Alcohol-treated mice with no bacteria transplantation were defined as the alcohol group. Mice drinking tap water during the whole study were regarded as control group.

Animals

In this study, 110 male C57BL/6 mice aged 4 to 5 weeks were bought from the Laboratory Animal Center, Shandong University. All mice were kept under controlled light/dark (12:12 hours, lights on 07.00-19.00 h), temperature and humidity conditions. Water and food were available to all mice except in alcohol preference test. All experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Shandong University. All efforts we made were to minimize mice suffering and reduce the number of animals used.

Chronic Ethanol Exposure

After three days facility adaption, mice were exposed with gradient concentration of ethanol in drinking water for three weeks. Here, mice were treated with ethanol solution at low concentrations initially (36). The concentration (v/v) of alcohol was progressively increased (2%, 4% to 6% every 3 days) and reached 8% for 12 days. To avoid the disturbance of uncomfortable environment, anorexia or thirsty, the average daily liquid intake and food consumption were measured every three days. After the three-week alcohol exposure, open field test, tail suspension test, forced swim test and alcohol preference test were conducted to evaluate the validity of this model. The chronic alcohol exposure lasted for 21 days followed by two-week tap water drinking.

Open Field Test

The anxiety-like behaviors and locomotor activity were measured by open field test. When exposed to a novel stressful environment, anxiety mice spent less time and traveled less distance in inner zone. The open field chambers (25 cm × 25 cm × 45 cm, length × width × height) were connected to an automated video-tracking system (DigBehav, Jiliang Software Technology, Shanghai, PRC). The performance of each animal within the area was recorded for 5 min and translated by the DigBehav software to the total distance, mean speed, inner zone distance and time, which all served as dependent variables. The apparatus was cleaned with 75% ethanol between each test.

Tail Suspension Test

The tail suspension test is valuable to evaluate depression-related behaviors. When mice were suspended, the inescapable condition led to immobility, which is a reflection of despair. In detail, mice were individually hung by the tail, with an adhesive tape about 2 cm from the tail tip. Each mouse was placed to a steel platform 35 cm above the ground. The performances of each mouse were videotaped by a video camera for 6 min. The immobility time was manually recorded and analyzed. In this study, mice were considered as immobility when they hung still above the ground, including the small front legs movements and complete motionlessness.

Forced Swim Test

The forced swim test is used to assess depression-like behaviors by recording mice immobility time and increased immobility time was regarded as an index of depression. Mice were placed individually in a clear open glass cylinder (25 cm × 15 cm, height × diameter), with water depth of 15 cm and temperature of 25°C. Water was changed among each mouse to remove remaining odors. The test was lasted for 6 min and the immobility time within the last 4 min was scored by the same observer blind to each group. During the 6 min interval, mice were regarded as immobility when stopped struggling except for slight movements to keep the head above the water.

Alcohol Preference Test

We also performed alcohol preference test as previously described (19). Mice were individually housed and deprived water for 24 h before the test. At the first day, two bottles were simultaneously provided for each home cage while one was water filled; the other was filled with ethanol solution. We also changed the bottles' position at the second day to prevent place preference formation. The weight losses (grams) of each bottle were recorded after the test, and the relative consumption (%) of ethanol was used to show the preference to alcohol.

Fecal Microbiota Transplantation

In this study, 3 young male volunteers (aged from 20 to 30) were chosen with vigorous screening. All volunteers agreed for the feces collection and signed consent forms. The volunteers were physically and mentally healthy, without alcohol drinking and antibiotics treatment for at least one year. The volunteers were kept in a balanced diet and healthy lifestyle during the whole experiment. Stool samples from one person with a weight of ~5 g were considered adequate. Fresh fecal samples were collected two hours before each mice oral gavage. The protocol was conducted as previously described (33). Briefly, the material was weighted and homogenized in a mixed preparation under sterile conditions, which aimed at eliminating the discrepancies of the fecal samples from volunteers, removed the unsolvable particles using stainless steel sieves with a final pore size of 0.40 mm, and centrifuged at 6000 × g for 15 min at 4°C. After centrifugation, the supernatant was discarded and the remaining material was resuspended in sterile phosphate buffer saline (PBS) solution, amended with 10% glycerol and frozen at -80°C until used. Microbial concentrations were determined microscopically by a Petroff-Hauser counting chamber. Here, to make the bacteria visible under microscope, the Gram staining was conducted under the instructions of the Gram Staining Kit (BIO-KONT, Shenzhen Kangtai Biological Products, Guangdong, PRC). Mice in FMT group received 200 μL suspensions with a minimum dose of approximately 10^{10} bacteria at each oral gavage. Alcohol group received equivalent volume of PBS. The FMT was conducted three times a week and started at the end of alcohol treatment period (FMT_1), 6% alcohol treatment (FMT_2) and the beginning of whole exposure period (FMT_3), respectively.

Statistics

Statistical analysis was performed using GraphPad Prism 5 Software. The data are presented as means ± standard error of the means (SEMs). Statistical comparisons were analyzed by one-way analysis of variance (ANOVA) followed with Bonferroni post hoc analysis. $P < 0.05$ was considered as statistically significant.

Results

Establish Animal Model of Chronic Alcohol Exposure

To simulate the process of chronic alcohol consumption, mice were treated with gradient concentration of alcohol in their drinking water for three weeks (Fig. 1A). Specifically, the alcohol solution concentration (v/v) was progressively increased from 2%, 4%, and 6% to 8% every three days. Mice were continuously received 8% full dose alcohol for 12 days. The average food and liquid intake recorded every three days showed no significant difference between alcohol-treated and control group (data not shown), which eliminated the biases that stem from anorexia/thirsty-induced depression. To evaluate the validity of this model, behavioral tests at the end of exposure session were conducted. The response to a stressful environment and locomotor activity were assessed by open field test (Fig. 1B). Mice in alcohol group spent significantly less time ($t_{(9)} = 3.410$, $P = 0.0078$) and traveled less distance in inner zone ($t_{(9)} = 4.848$, $P = 0.0009$) compared to the control group. Besides, there was no difference on the total distance ($t_{(13)} = 0.6034$, $P = 0.5566$) or mean speed ($t_{(13)} = 0.6036$, $P = 0.5565$) between two groups, suggesting few effects of alcohol drinking on locomotor activity. In tail suspension test (Fig. 1C), there was a significant decrease mobility time in the alcohol group compared to the control group ($t_{(9)} = 2.764$, $P = 0.0220$). Similarly, increased mobility time ($t_{(27)} = 2.207$, $P = 0.0360$) in forced swim test was observed in the control group (Fig. 1D). These data showed the alcohol-induced anxiety/depression-like behaviors and also indicated the feasibility of this alcohol exposure method. In alcohol preference test, a significant higher preference was observed in alcohol-treated mice under 4% ethanol solution ($t_{(29)} = 3.324$, $P = 0.0024$). Similar trends were also observed in 2% or 8% ethanol concentration solutions but no statistically significant difference was found (Fig. 1E).

Two-Week FMT Had Few Effects on Alcohol-Induced Anxiety or Depression

To explore whether the FMT would attenuate the alcohol-induced negative emotions, we firstly conducted the FMT at the end of three-week exposure (FMT_1) and lasted for 2 weeks. In open field test, the time spent in center field was measured firstly. There was an overall significant difference among each group ($F_{(2,18)} = 5.414$, $P = 0.0160$) (Fig. 2A). Post hoc analysis revealed that both FMT and alcohol groups spent remarkable less time in inner zone compared to the control group ($P < 0.05$). Similarly, there were also a significant effect of alcohol in center distance percentages ($F_{(2,18)} = 9.460$, $P = 0.0019$) (Fig. 2B), total distances ($F_{(2,18)} = 7.378$, $P = 0.0054$) (Fig. 2C) and mean speed ($F_{(2,18)} = 7.378$, $P = 0.0054$) (Fig. 2D). However, no difference was

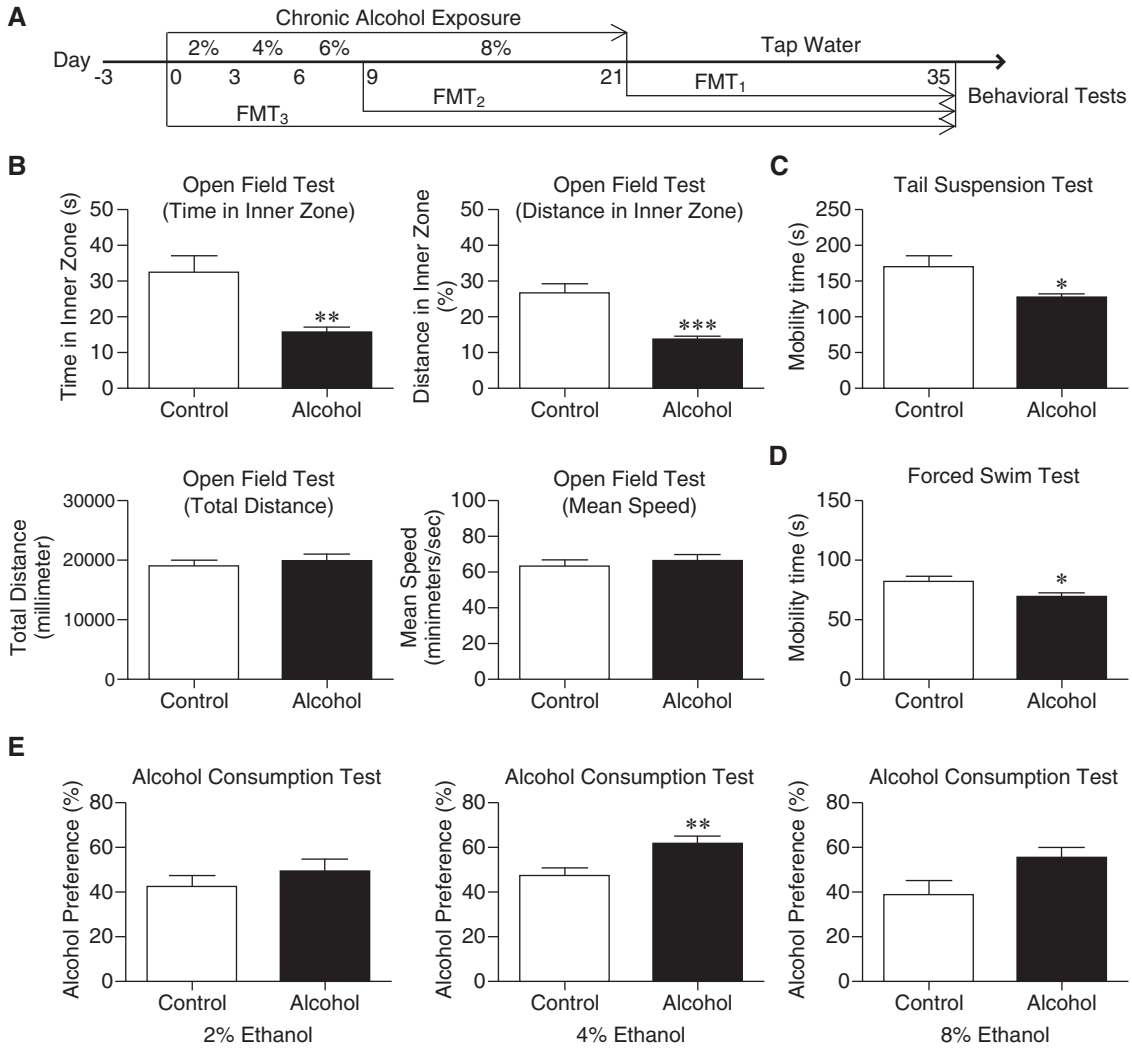

Fig. 1. Establish the model of chronic alcohol exposure and fecal microbiota transplantation (FMT). (A) Mice were treated with gradient concentration of alcohol for three weeks. The water drinking period was lasted for two weeks. The FMT was conducted three times a week and started at the end of alcohol intake period (FMT_1), 6% alcohol treatment (FMT_2) and the beginning of the whole exposure period (FMT_3) respectively. After the three-week alcohol exposure, behavioral tests were conducted to evaluate the validity of this model. (B) In open field test, alcohol-treated mice spent significantly less time and traveled less distance in inner zone compared to control. No difference on the total distance or mean speed was found between two groups. Besides, chronic ethanol exposure also elicited significantly decreased mobility in tail suspension test (C) and forced swim test (alcohol group, n = 23; control group, n = 13) (D). Furthermore, (E) alcohol group had a significant preference to 4% alcohol solution (alcohol group, n = 20; control group, n = 15). Similar trends were also observed in 2% (alcohol group, n = 8; control group, n = 6) or 8% (each group, n = 6) concentration solutions. Results are displayed as means ± SEMs. Significant results were determined by Student's t tests when $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

found between FMT and alcohol group in the four behavioral indices. In open field test, there was no significant difference from FMT and alcohol group, so the tail suspension test was performed. A significant difference ($F_{(2,17)} = 9.047$, $P = 0.0026$) in mobility time was observed among each group (Fig. 2E). Expectedly, there was a tendency of FMT to reduce the depression-like behaviors as a significant decrease in mobility time was only observed in the alcohol group ($P < 0.05$). In forced swim test (Fig. 2F), however, there was no difference among the three groups ($F_{(2, 19)} = 1.277$, $P = 0.3044$).

FMT_2 Alleviated Alcohol-Induced Depression in Tail Suspension Test

Since the FMT_1 did not show expected effects, we then started the transplantation at the begin-

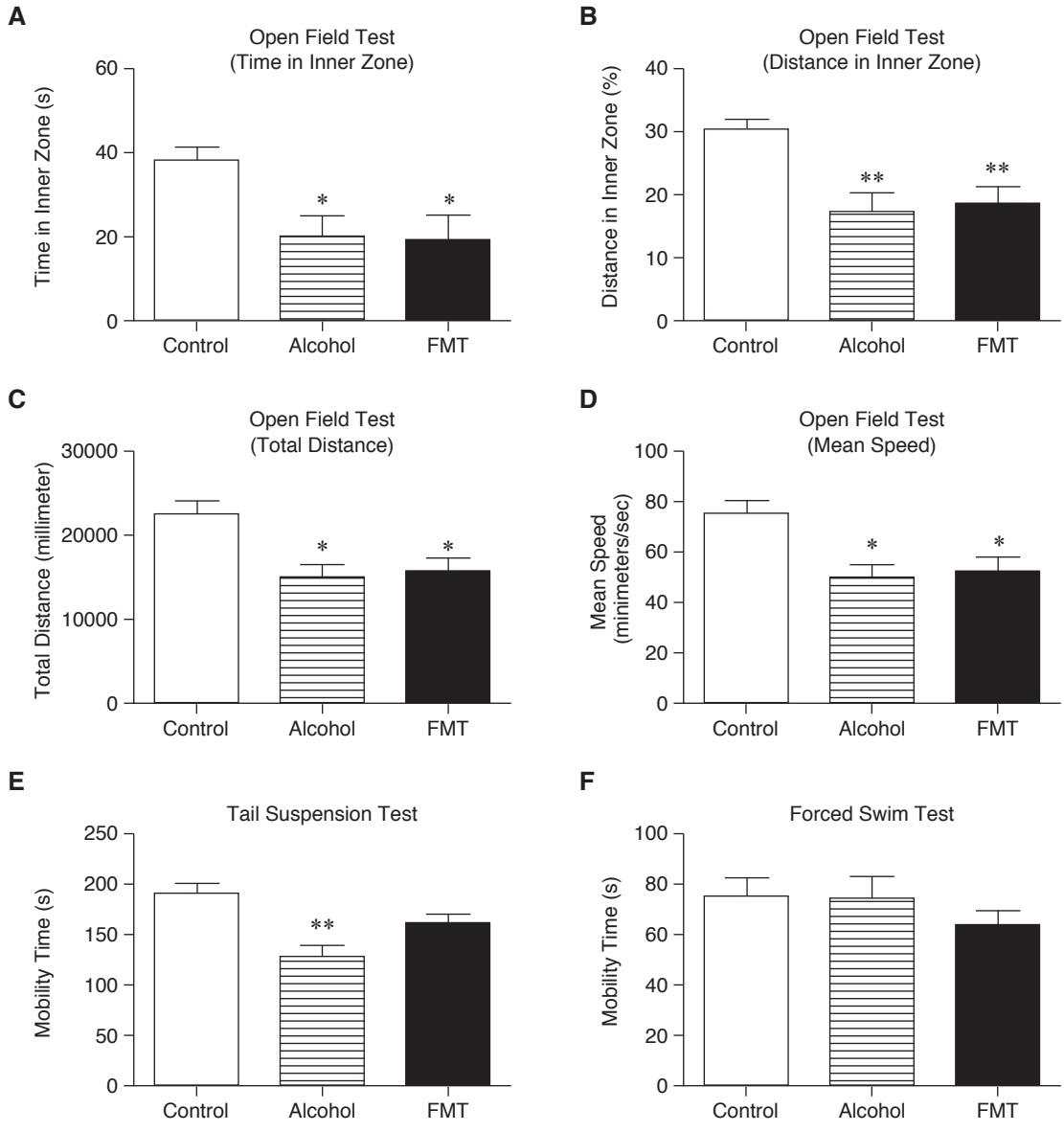

Fig. 2. FMT_1 could not alleviate alcohol-induced anxiety or depression. We firstly conducted the FMT at the end of three-week exposure (FMT_1). In open field test, both FMT and alcohol group had decreased inner zone time (A). Similarly, the center distance percentage (B), total distance (C) and mean speed (D) in FMT and alcohol group were significantly less than control group. However, no difference was found between FMT and alcohol group in the four behavioral variables. (E) In tail suspension test, only the alcohol group showed a significant decrease in mobility time. No difference among groups was found in forced swim test (F). Results are displayed as means ± SEMs. Data were analyzed by one-way ANOVA with Bonferroni post hoc analysis. *$P < 0.05$ (A, C, D *vs*. Control), **$P < 0.05$ (B, E *vs.* Control). n = 6-7 per group.

ning of 8% alcohol exposure (FMT_2). In open field test, consistent with FMT_1, the time spent ($F_{(2,17)} = 4.274$, $P = 0.0340$) (Fig. 3A) and distance travelled in inner zone ($F_{(2,17)} = 12.48$, $P = 0.0006$) (Fig. 3B), total distance ($F_{(2,17)} = 10.20$, $P = 0.0016$) (Fig. 3C) and mean speed ($F_{(2,17)} = 10.20$, $P = 0.0016$) (Fig. 3D) among control, alcohol, and FMT group, differed significantly from one another. Importantly, although no statistical change was found, FMT reduced anxiety-like behaviors and only the alcohol mice showed lower time ($P < 0.05$) and distance ($P < 0.05$) in inner zone. However, similar with the performance in FMT_1, the follow-up analyses revealed that both alcohol and FMT mice showed significant decreases in total distance ($P < 0.05$) and mean speed ($P < 0.05$) compared to the control

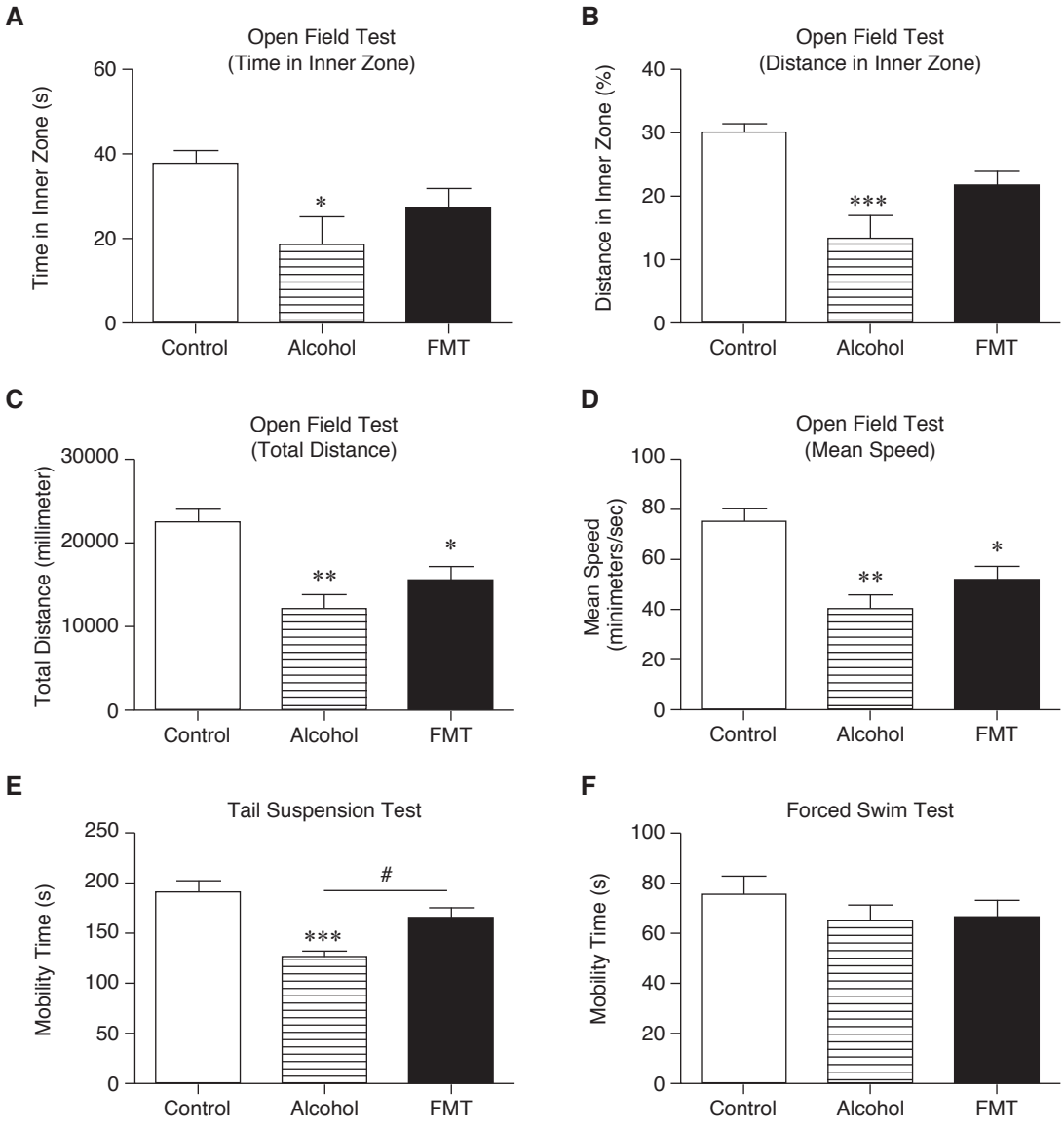

Fig. 3. FMT_2 decreased alcohol-induced depression in tail suspension test. We also started the transplantation at the beginning of 8% alcohol exposure (FMT_2). In open field test, alcohol group showed remarkable reduction in the time spent (A) and distance travelled in inner zone (B). However, both the alcohol and FMT mice exhibited decreased total distance (C) and mean speed (D) compared to control. In tail suspension test (E), alcohol mice had less mobility time compared with FMT mice, indicated the decrease of depression after transplantation. (F) The forced swim test revealed no significant difference among the groups. Results are displayed as means ± SEMs. Data were analyzed by one-way ANOVA with Bonferroni *post hoc* analysis. $^{*}P < 0.05$ (A, C, D *vs*. Control), $^{**}P < 0.05$ (C, D vs. Control), $^{***}P < 0.001$ (B, E *vs*. Control), $^{\#}P < 0.05$ (E *vs*. FMT). n = 6-7 per group.

group, whereas no difference was found between alcohol and FMT group. In conclusion, FMT_2 exhibited its anti-anxiety functions in open field test, which lead us to further explore its availability in alcohol-induced depression. As expected, in tail suspension test, alcohol mice had less mobility time compared with the control group ($P < 0.001$) (Fig. 3E). Moreover, locomotor activity was significantly improved in FMT mice compared with the alcohol group ($P < 0.05$), which indicated that FMT_2 alleviated alcohol-induced depression. Once again, the forced swim test revealed no significant difference among FMT, alcohol and control group ($F_{(2,19)} = 0.6166$, $P = 0.5514$) (Fig. 3F).

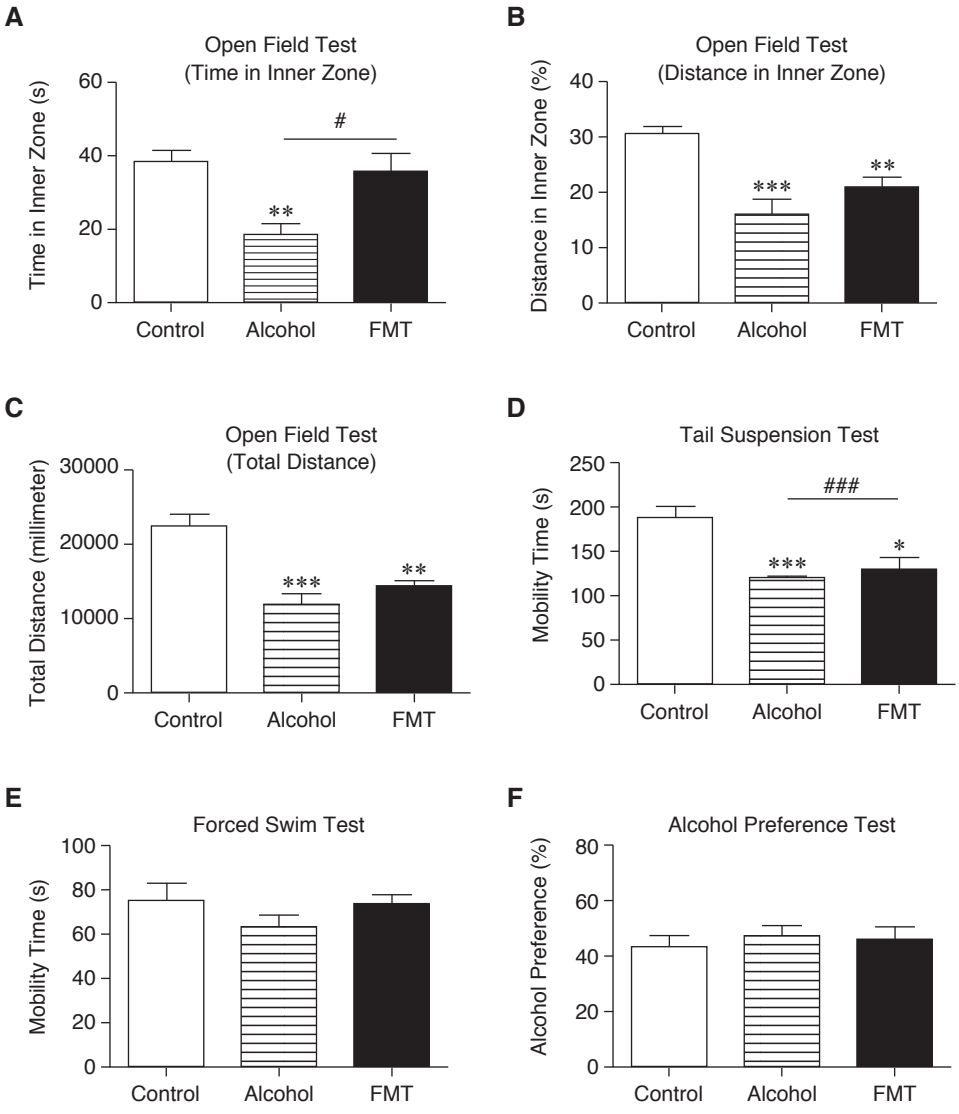

Fig. 4. Five-week FMT (FMT_3) modulated anxiety and significantly improved depression. (A) FMT-treated mice spent significant more time in inner zone compared with alcohol group. No significant difference was found in inner zone distance (B), and total distance (C) between alcohol and FMT group. The FMT mice also significantly increased their mobility time in tail suspension test compared to alcohol group (D). However, in forced swim test (E), no difference in mobility time was found among all groups. (F) The preference to alcohol was not altered in alcohol-treated mice after FMT_3, either. Results are displayed as means ± SEMs. Data were analyzed by one-way ANOVA with Bonferroni post hoc analysis. $^{**}P < 0.05$ (A, B, C, D *vs*. Control), $^{***}P < 0.001$ (B, C, D *vs*. Control), $^{\#}P < 0.05$ (A *vs*. FMT), $^{\#\#\#}P < 0.001$ (D *vs*. FMT). n = 6-7 per group.

Five Weeks FMT Decreased Anxiety in Open Field Test and Significantly Redeemed Depression in Tail Suspension Test

The transplantation of gut microbiota from the beginning of 8% alcohol exposure exhibited antidepressive effects in alcohol-treated mice, which drove us to perform the FMT and alcohol exposure simultaneously (FMT_3) to explore the potential effects of prolonged FMT in emotion regulation. Expectedly, the FMT group spent significant more time in inner zone compared with alcohol group in open field test ($P < 0.05$) (Fig. 4A), illustrating a reduction in anxiety-like symptoms after transplantation. Although there was an overall difference among groups in inner zone distance ($F_{(2,18)} = 14.01$, $P = 0.0003$) (Fig. 4B), and total distance ($F_{(2,18)} = 17.94$, $P < 0.0001$) (Fig. 4C), no significant difference was found between alcohol and FMT group in followed Bonferroni analyses. Notably, we also

found that FMT group had significantly increased time in tail suspension test compared to the alcohol group ($P < 0.001$) (Fig. 4D), whereas only the alcohol group displayed less mobility time compared with control mice ($P < 0.001$). These results revealed that FMT_3 significantly redeemed alcohol-induced depression in tail suspension test. However, in forced swim test, there was no difference among control, alcohol and FMT groups in mobility time ($F_{(2,19)} = 1.264$, $P = 0.3078$) (Fig. 4E). Besides, no matter when the FMT started and how long the transplantation lasted, no significant preference difference was found among each group (Fig. 4F), even though there was a tendency of FMT to reduce the preference. These evidences indicated few effects of FMT on reversing the 4% ethanol likeness.

Discussion

In the current study, chronic alcohol exposure induced anxiety/depression-like behaviors and alcohol preference. Moreover, three different fecal microbiota transplantation ways were innovatively designed to explore the potential effects of FMT from healthy donors on alcohol-induced neuropsychic behaviors. To our best knowledge, for the first time, we found that anxiety and depression were gradually relieved with the prolongation of FMT. Compared with two-week FMT, five weeks transplantation remarkably increased time spent in inner open field and locomotor activity in tail suspension test. However, there was no significant alcohol preference alternation in FMT-treated mice. These data demonstrated the modulation of FMT on alcohol-related anxiety and depression, providing a potential target for treating alcohol addiction.

FMT was in a significant effect in increasing the time in center field and mobility time in tail suspension test. With the prolongation of transplantation, the anxiety and depression-like behaviors induced by alcohol were gradually corrected. Accumulative evidences had suggested a causal relationship between gut microbiome transplantation and behavioral changes such as anxiety and depression, which were both involved in the negative reinforcement of alcohol addiction (10). One of the direct evidences was reported by Xiao and his colleagues (38). The authors found the FMT from alcohol-treated mice to healthy controls significantly altered intestinal microflora composition and facilitated depressive behavior in forced swim test and tail suspension test (38). Interestingly, psychiatric disorders such as major depressive disorder (MMD) are often comorbid with alcohol dependence, and the FMT from MDD patients to germ-free mice resulted in depression-like behaviors compared with transplantation "healthy microbiota" derived from healthy individuals (40). Notably, the time spent in center open field was also increased after transplantation, which indicated the potential role of FMT in attenuating anxiety. The deficit of brain stress system serves as a critical element in alcohol addiction initiation and maintenance, and a growing of evidences suggested its relationship with intestinal bacteria. In rats, absence of the gut microbiota exacerbated anxiety-like behavior and responses to acute stress (2). However, the early-life disturbance of the enteric microbiome did not impact anxiety-related behaviors but selectively affected visceral pain in adulthood (24). In the model of chronic restraint stress, administration of probiotics *Lactobacillus helveticus NS8* improved both anxiety/depression-like aberrations (17). Although the current study cannot give an exact interpretation, these findings along with ours suggested the correction of dysbiotic microbiota might decrease the risk of anxiety after chronic alcohol exposure.

The immune activation serves as a critical pathway in brain-gut communication. Chronic alcohol consumption leads to disrupted gut barrier and dislocation of microbiota. Then, the leaky gut will activate immune cells and increase the expression of cytokines, permit and exacerbate a neuroinflammatory response, and finally lead to brain inflammation, which acts as an inducer in alcohol-seeking behavior (16, 31). Although there was little information on how FMT interacts with the immune system, in this study, the FMT might attenuate alcohol-related neuropsychic behaviors by suppression of the inflammation and down-regulation of cytokines expressions. Ferrere and his colleagues found that the FMT could prevent gut microbiota dysbiosis and alcohol-induced liver injury, with the decreases of IL-1β, IL-6, and IL-10 (8). When researchers transplanted the microbiota from depression rats to control, the pro-inflammatory profile was also partially transferred, with an increase of plasma C-reactive protein levels (12). In a model of ulcerative colitis, accompanied by attenuated inflammation, decreased Bifidobacterium was also recovered after FMT treatment (35). The expression of IL-1b and IL-10 was also down-regulated. Additionally, administration of VSL#3 (a mixture of 8 different strains of bacteria) also significantly reduced the levels of plasma cytokines TNF-α, IL-6, and IL-10 in patients with alcoholic cirrhosis (18). Taken together, these findings indicated the potential anti-inflammatory properties of FMT.

Depression and stress are two vital risk factors for the development and maintenance of addiction (27, 32). But the correction of compulsive and ex-

cessive alcohol drinking behaviors had more clinical significance. To direct assess whether the FMT could attenuate the motivation of alcohol drinking, we performed alcohol preference test. However, we found few effects of FMT on reversing alcohol likeness. It has been reported that antibiotics-treated mice enhanced their sensitivity to cocaine reward and cocaine-induced locomotor sensitization, which provided the first evidence that the alternation of gut microbiota affected drug-seeking behaviors (14). In clinical researches, alcohol-dependent subjects accompanied with gut microbiota dysbiosis had a more severe profile of alcohol-dependence syndromes (like craving, depression) than the other non-dysbiotic subpopulation (4), suggesting the role of gut dysbiosis in maintaining addition and difficulties in addiction treatment. In line with this view, the non-alcoholic minimal hepatic encephalopathy patients responded better to the treatment of rifaximin plus probiotics, as they exhibited a consistent decline in certain ammonia-producing bacteria genera like *Clostridium* and *Streptococcus* (41). Although no significant change was observed in the alcohol preference test among each group, we cannot deny the potential efficacy of FMT, especially as anxiety and depression were significantly reduced with the prolongation of transplantation. A refined FMT method with longer duration and larger amount of bacteria in each gavage might have expected effects. So far, microbiota-targeted therapy such as FMT, antibiotics and probiotics treatment in drug addiction needs systematic research. Further studies are also necessary to examine which method or combination thereof is most efficacious and acceptable.

In this study, we established the model of chronic alcohol exposure by providing gradient concentration of alcohol in mice drinking water. The average food and liquid intake were recorded every three days to eliminate the apparent negative emotions because of anorexia or thirsty. Based on the fact that alcohol-treated mice exhibited significant anxiety and depression, this inexpensive, technically simple and time-saving method presents its validity as a model of chronic alcohol exposure. Interestingly, the tail suspension test (TST) showed expected effects of FMT but the forced swim test (FST) failed. The two current tests involve different neuronal mechanisms. Long term alcohol exposure led to the deficiency of dopamine systems, which might make FST difficult to reveal the behavior changes, as the FST is involved in variations of the dopamine concentration (9, 29). In contrast, the adrenergic systems were less influenced by alcohol, and the TST showed the transplantation had expected effects in attenuating depression-like behaviors. Certainly, one can argue that the spontaneous regression of addiction during two-week water drinking period could affect the alcohol preference. This, however, could not be avoided and was also present in both FMT and alcohol group.

In summary, we performed three distinct FMT plans to investigate its potential in improvement of alcohol-induced neuropsychic symptoms. For the first time, we found the prolonged FMT decreased anxiety in open field test and redeemed depression in TST. However, we did not find its significant effect on attenuating alcohol preference. Our study highlighted the striking effect of gut microbiota in alcohol use, suggesting the FMT from healthy donors might be a potential treatment for alcohol-induced negative emotions and providing preliminarily data for further animal and clinical experiments.

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Disclosure of Interest

No competing financial interests exist.

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