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Ingestion of *Faecalibaculum rodentium* causes depression-like phenotypes in resilient *Ephx2* knock-out mice: A role of brain–gut–microbiota axis via the subdiaphragmatic vagus nerve

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Abstract

Background: The brain–gut–microbiota axis plays a crucial role in the bidirectional interactions between the brain and the gut. Soluble epoxide hydrolase (coded by the *Ephx2* gene) plays an important role in inflammation, which has been implicated in stress-related depression. *Ephx2* knock-out (KO) mice exposed to chronic social defeat stress (CSDS) did not show depression-like behaviors, indicating stress resilience. Here we examined whether the brain–gut–microbiota axis influences the resilience in *Ephx2* KO mice.

Methods: Effects of fecal microbiota transplantation (FMT) from CSDS-susceptible (or control) mice in wild-type (WT) mice and *Ephx2* KO mice treated with an antibiotic cocktail (ABX) were investigated. Behavioral, biochemical tests and 16S ribosome RNA analysis were performed.

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Author Statement

Contributors

K.H. designed the study. SW, TI, YQ, JS, LC, YW, JZ, YP, YF, YT, XW, LM, and XW performed the experiments. BDH provided *Ephx2* KO mice. SW analyzed the data. SW and KH wrote the draft, and all authors approved the final version. We declare that all authors contributed and have approved the final manuscript.

Statement of Interest

All authors report no biomedical financial interests or potential conflicts of interest.

Results: FMT from CSDS-susceptible mice produced anhedonia-like behavior in ABX-treated WT and *Ephx2* KO mice. The 16S ribosome RNA analysis showed that *Faecalibaculum rodentium (F. rodentium)* may be responsible for the observed anhedonia-like behavior following FMT from CSDS-susceptible mice. Ingestion of *F. rodentium* for 14 days produced depression- and anhedonia-like behaviors, higher blood levels of interleukin-6, and reduced expression of synaptic proteins in the prefrontal cortex of ABX-treated *Ephx2* KO mice. Furthermore, subdiaphragmatic vagotomy blocked the development of these behavioral abnormalities after ingestion of *F. rodentium*.

Limitations: Detailed mechanisms are unclear.

Conclusions: These findings suggest that *F. rodentium* might contribute to the conversion of resilient *Ephx2* KO mice into KO mice with depression-like phenotypes. The brain–gut–microbiota axis via the subdiaphragmatic vagus nerve plays a crucial role in susceptibility and resilience to stress.

Keywords

Depression; Microbiome; Resilience; Susceptibility; Vagus nerve

1. Introduction

Humans vary widely in their response to stress. Multiple lines of evidence have suggested that resilience is the ability of several neural circuits that are involved in the interface between the brain and the periphery to adapt to changing stress levels (Anacker et al., 2018; Cathomas et al., 2019; Dantzer et al., 2018; Feder et al., 2009; Franklin et al., 2012; Russo et al., 2012; Southwick et al., 2005). However, the precise mechanisms underlying resilience and vulnerability to stress remain poorly understood.

The brain–gut–microbiota axis is a complex multi-organ bidirectional signaling system between the brain and the gastrointestinal microbiota and plays an important role in host homeostasis (Cryan et al., 2019; Cussotto et al., 2018; Dinan and Cryan, 2017; Fung et al., 2017; Long-Smith et al., 2020). Numerous preclinical studies have demonstrated that an abnormal composition of gut microbiota may contribute to the resilience or susceptibility in rodents exposed to stress (Bear et al., 2021; Jianguo et al., 2019; Szyszkowicz et al., 2017; Wang et al., 2020a; Wang et al., 2020b; Yang et al., 2019; Yang et al., 2017; Zhang et al., 2019; Zhang et al., 2020).

Epoxy fatty acids, which are produced from polyunsaturated fatty acids through the action of cytochrome P450s, have shown to possess potent anti-inflammatory actions. These epoxy fatty acids are metabolized into the corresponding diols by soluble epoxide hydrolase (sEH; coded by the *Ephx2* gene), and inhibition of sEH can enhance the beneficial effects of epoxy fatty acids (Atone et al., 2020; Hashimoto, 2019; Imig et al., 2009; Morisseau and Hammock, 2005, 2013; Swardfager et al., 2018; Wagner et al., 2017). We previously reported that sEH inhibitors have potent beneficial effects in animal models of depression, Parkinson's disease, schizophrenia, and autism spectrum disorder (Hashimoto, 2016; Ma et al., 2019; Pu et al., 2020; Ren et al., 2016; Ren et al., 2018). Interesting, *Ephx2* KO did not

show anhedonia- and depression-like phenotypes after exposure to chronic social defeat stress (CSDS), which suggested that *Ephx2* KO mice are resilient to stress (Hashimoto, 2016; Ren et al., 2016).Furthermore, we demonstrated that microbiome depletion by treatment with an antibiotic cocktail (ABX) was associated with resilience in mice after CSDS exposure (Wang et al., 2020b), and that fecal microbiome transplantation (FMT) from CSDS-susceptible mice caused anhedonia-like phenotypes in ABX-treated wild-type (WT) mice (Wang et al., 2020a), which suggested a crucial role of the brain–gut– microbiota axis in resilience and susceptibility. However, it remains unclear whether FMT from CSDS-susceptible mice causes depression-related behavioral abnormalities in resilient *Ephx2* KO mice.

The purpose of this study was to examine the role of the brain–gut–microbiota axis in resilience of *Ephx2* KO mice. First, we examined whether FMT from CSDS-susceptible mice causes behavioral and biochemical abnormalities in ABX-treated WT and *Ephx2* KO mice. Furthermore, we determined the blood levels of pro-inflammatory cytokine interleukin-6 (IL-6) and synaptic proteins (i.e., GluA1 [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor A1] and PSD-95 [postsynaptic density 95]) in the prefrontal cortex (PFC) because the expression of these synaptic proteins in the PFC from rodents with depression-like phenotypes was shown to be lower than that of rodents without depression-like phenotypes (Duman and Aghajanian, 2012; Ohgi et al., 2015; Zhang et al., 2017). Second, using 16S ribosome RNA gene sequencing, we examined the diversity and composition of the gut microbiota in fecal samples. In this study, we identified the microbe (*Faecalibaculum rodentium* [*F. rodentium*]) to be potentially responsible for the behavioral and biochemical changes in recipient mice following FMT from CSDS-susceptible mice. Third, we investigated whether ingestion of *F. rodentium* for 14 days causes depression-related behaviors in ABX-treated *Ephx2* KO mice. Finally, because the vagus nerve is known to play a role in the communication between the brain and microbiota (Bonaz et al., 2018; Bravo et al., 2011; Cawthon and de La Serre, 2018; Cryan et al., 2019; Long-Smith et al., 2020; Pu et al., 2021; Wang et al., 2020a; Wang et al., 2020c; Zhang et al., 2020), we examined whether subdiaphragmatic vagotomy (SDV) blocks depression-related behaviors in ABX-treated *Ephx2* KO mice after ingestion of *F. rodentium*.

2. Materials and Methods

2.1. Animals

Male adult C57BL/6 mice (n = 30, 8 weeks old, body weight = 20–25 g, Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice (n = 20, 13–15 weeks old, body weight > 40 g, Japan SLC, Inc.) were used. A colony of *Ephx2* KO mice (provided from UC Davis) with targeted deletion of sEH gene (*Ephx2*) which was backcrossed to C57BL/6 background were used (Sinal et al., 2000). Adult male *Ephx2* KO mice (n = 83) were used in this study. Animals were housed under controlled temperatures and 12 h/12 h light/dark cycles (lights on between 07:00–19:00 h) with *ad libitum* access to food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee (Permission number: 31–149, and 2–409). Animals were deeply

anesthetized with isoflurane before being sacrificed via cervical dislocation. All efforts were made to minimize suffering.

2.2. Chronic social defeat stress (CSDS) model and behavioral tests

Detailed methods of CSDS and behavioral tests were given in Supplemental Information.

2.3. Collection of fecal samples, treatment with an antibiotic cocktail and FMT

Fresh fecal samples were collected from CSDS-susceptible mice and control (no CSDS) mice on days 12–14 in sterilized screw cap microtubes immediately after defecation and stored at −80°C until FMT, as previously reported (Wang et al., 2020b). The fecal samples were collected from each mouse around 9:00 – 10:00 on each day to avoid circadian effects on the microbiome. Totally about 30 collected tubes containing approximately 0.5 g of feces per each tube were used for FMT.

Based on previous reports (Pu et al., 2019; Pu et al., 2021; Wang et al., 2020a; 2020b; Yang et al., 2019; Zhan et al., 2018), broad-spectrum antibiotics (ABX: ampicillin 1 g/L, FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan: neomycin sulfate 1 g/L, Sigma-Aldrich Co. Ltd, St. Louis, MO, USA: metronidazole 1 g/L, FUJIFUILM Wako Pure Chemical Corporation, Tokyo, Japan) dissolved in drinking water were given *ad libitum* to male C57BL/6 mice for 14 consecutive days.

The drinking solution was renewed every 2 days.

Before FMT, fecal samples were removed from the freezer in every morning, and they were allowed to thaw for 10 – 15 min at room temperature. Then drinking water (10 mL/g feces) was added to the tube including the fecal samples. The drinking water including fecal samples (0.2 mL/mouse) was given to the ABX-treated wild-type (WT) and *Ephx2* KO mice using gastric gavage for consecutive 14 days.

2.4. Transplantation of *F. rodentium*

Ephx2 KO mice were given drinking water alone or drinking water containing ABX on days 1–14. Subsequently, mice were divided into three groups: water + water group, ABX + water group, and ABX + microbe (*Faecalibaculum rodentium*) group.

Faecalibaculum rodentium (*F. rodentium*, catalog number: JCM30274) (Chang et al., 2015; Lim et al., 2016) were purchased from RIKEN BioResource Research Center (Tsukuba, Ibaraki, Japan). Mice were orally administered water or water containing the microbes (approximately 1×10^8 CFU/day) for 14 days (days 15–28) using gastric gavage (Figure 4A). The locomotion test and tail suspension test (TST) were performed on day 29. The forced swimming test (FST) and 1% SPT were performed on days 30 and 31, respectively. On day 32, plasma samples and PFC samples were collected as described above, and stored at −80°C until use.

To examine the role of vagus nerve in the behavioral abnormalities in *Ephx2* KO mice after ingestion of *F. rodentium,* we performed subdiaphragmatic vagotomy (SDV), as previously reported (Pu et al., 2021; Wang et al., 2020b; Zhang et al., 2020). Sham surgery or SDV was

performed under anesthesia with 5% isoflurane. Mice were put under a microscope (Leica LEICA S9E, Germany), and hair was removed from the abdomen. The esophagus of each mouse was exposed to the full view. The ventral and dorsal vagus nerves of the esophagus were severed. After muscle and skin were sutured, mice were kept in clean cages until complete recovery from anesthesia. Then, mice were housed in cages for 14 days (days 1–14) (Figure 5A). ABX was given to all KO mice in drinking water for 14 days (from day 15 to day 28) (Figure 5A). Subsequently, mice were divided into four groups: sham + water group, SDV + water group, SDV + microbe (*F. rodentium*) group, and sham + microbe (*F. rodentium*) group. Water alone or water containing the microbes (approximately 1×10^8 CFU/day) was administered orally for 14 days (day 29 to day 42) using gastric gavage (Figure 5A). The locomotion test and TST were performed on day 43. The FST and 1% SPT were performed on days 44 and 45, respectively. On day 46, plasma samples and PFC tissues were collected and stored at −80°C until use.

2.5. Measurement of IL-6 and Western blot analysis

Detailed methods of IL-6 ELISA and Western blot were given in Supplemental Information.

2.6. 16S ribosome RNA analysis and measurement of short-chain fatty acids

Detailed methods of 16S ribosomal RNA analysis and measurement of SCFAs in fecal samples were given in Supplemental Information.

2.7. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). The body weight data were analyzed using repeated measures one-way or two-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test. Comparisons among four groups were performed using two-way ANOVA followed by Fisher's LSD test or the Kruskal–Wallis test, followed by the Mann-Whitney U-test. Data for alpha-diversity of the gut microbiota were analyzed using the Kruskal–Wallis test, followed by the Mann-Whitney U-test. For beta-diversity of the gut microbiota, principal component analysis (PCA) based on the OTU level was performed using analysis of similarities (ANOSIM) by R package vegan (2.5.4) (Xia and Sun, 2017). Correlations between SCFAs and the relative abundance of bacteria were analyzed by Spearman's correlation. The P-values of less than 0.05 were considered statistically significant.

3. Results

3.1. FMT from CSDS-susceptible mice induced an anhedonia-phenotype in antibiotic-treated mice

We used an antibiotic cocktail (ABX: ampicillin 1 g/L, neomycin sulfate 1 g/L, and metronidazole 1 g/L) for microbiome depletion as reported previously (Wang et al., 2020a; Wang et al., 2020b). First, we investigated the effects of FMT from CSDS-susceptible mice in WT mice and *Ephx2* KO mice treated with ABX (Figure 1A). There were no changes in body weight among the four groups (Figure 1B).

In the 1% SPT, FMT from CSDS-susceptible mice produced significant reductions in sucrose preference in ABX-treated WT mice (Figure 1C), which is consistent with a previous report (Wang et al., 2020b). Furthermore, FMT from CSDS-susceptible mice produced the reduced sucrose preference of SPT in ABX-treated *Ephx2* KO mice, which was indicative of an anhedonia-like phenotype in the KO mice. Interestingly, we observed significantly higher sucrose preference in *Ephx2* KO mice compared with WT mice following FMT from CSDS-susceptible mice (Figure 1C), which suggested that KO mice were more resistant than WT mice. In contrast, sucrose preference of WT mice and *Ephx2* KO mice did not differ after FMT from control (no CSDS) mice (Figure 1C).

FMT from CSDS-susceptible mice caused significant increases in blood IL-6 levels in both the *Ephx2* KO and WT mice, although blood IL-6 levels were significantly lower in *Ephx2* KO mice compared with WT mice (Figure 1D). Blood IL-6 levels did not differ significantly in either the WT or *Ephx2* KO mice after FMT from control (no CSDS) mice (Figure 1D).

FMT from CSDS-susceptible mice significantly decreased protein expression of GluA1 and PSD-95 in the PFC of both ABX-treated WT and *Ephx2* KO mice (Figure 1E and 1F). Furthermore, there were no changes between two groups after FMT from CSDS-susceptible mice. There were no significant changes in GluA1 or PSD-95 expression in the PFC of either group after FMT from control mice (Figure 1E and 1F).

3.2. Abnormal diversity of gut microbiota after FMT from CSDS-susceptible mice

The diversity and composition of gut microbiota in the four groups were analyzed using alpha- and beta-diversity. The Kruskal–Wallis test revealed significant differences in the observed species index and ACE index between the four groups (Figure 2A and 2B). *Post-hoc* analyses showed that the alpha-diversity in the WT mice after FMT from CSDS-susceptible mice was significantly higher than that in WT mice after FMT from control mice, whereas alpha-diversity was not altered in the *Ephx2* KO mice after FMT from either CSDS-susceptible or control mice (Figure 2A and 2B). In contrast, there was no difference in Chao 1 index between the four groups (Figure 2C). Regarding beta-diversity, PCA was applied to analyze the bacterial community composition of gut microbiota in the four groups (Figure 2D). PCA revealed significant separation in the community composition, which was evaluated using an analysis of similarities ($R = 0.3241$, $P = 0.001$) (Figure 2D).

3.3. Abnormal composition of gut microbiota at the taxonomic level

At the genus level, five bacteria (*Akkermansia*, *Butyricimonas, Faecalibaculum Parabacteroides,* and *Ruminococcus*) were detected in the four groups (Figure S1A–F). Interestingly, FMT from CSDS-susceptible mice significantly increased the relative abundance of *Faecalibaculum* in both WT and KO groups compared with the two groups that underwent FMT from control mice (Figure S1D).

At the species level, eight bacteria [*Akkermansia muciniphila*, *Bacteroides sp. TP-5*, *Clostridiales bacterium CIEAF 021, Clostridium fusiformis, Clostridum sp. ASF356, Clostridium sp. ASF502, F. rodentium*, and *Lactobacillus johnsonii (L. johnsonii)*] were detected in the four groups (Figure 3A–I). Interestingly, FMT from CSDS-susceptible mice significantly increased the relative abundance of *F. rodentium* in both WT and KO groups

(Figure 3H), which suggested that *F. rodentium* may be responsible for the anhedonia-like behavior observed in both groups after FMT from CSDS-susceptible mice.

3.4. Levels of short chain fatty acids in fecal samples

Short-chain fatty acids (SCFAs) produced by microbiome play a role in brain-gut communication (Dalile et al., 2019; Silva et al., 2020; Wu et al., 2020). There were significant genetic effects in acetic acid, lactic acid, and propionic acid (Figure S2A–C). However, there were no changes in butyric acid and succinic acid in any of the four groups (Figure S2D and S2E). Levels of lactic acid and propionic acid after FMT from CSDS-susceptible mice were significantly higher in WT mice than in *Ephx2* KO mice (Figure S2B and S2C). There was a positive correlation ($r = 0.621$, $P < 0.001$) between the relative abundance of *Lactobacillus johnsonii* and lactic acid levels in all groups (Figure S2F).

3.5. Ingestion of F. rodentium caused depression-like behaviors in antibiotic-treated Ephx2 KO mice

Next, we investigated whether *F. rodentium* causes depression-related behavior phenotypes in ABX-treated *Ephx2 KO* mice (Figure 4A). There were no changes in either body weight (Figure 4B) or locomotion (Figure 4C) in any of the three groups. Immobility time in the tail suspension test (TST) in the ABX + microbe group was significantly higher than that in both the water + water and antibiotic + water groups (Figure 4D). Immobility time in the forced swimming test (FST) in the ABX + microbe group was significantly higher than that in the water + water group, but not in the ABX + water group (Figure 4E). Furthermore, sucrose preference of SPT in the ABX + microbe group was significantly lower than that in both the water + water and ABX + water groups (Figure 4F).

Blood IL-6 levels in the ABX + microbe group were significantly higher than in the other two groups (Figure 4G). Moreover, the expression of synaptic proteins (i.e., GluA1 and PSD-95) in the PFC of the ABX + microbe group was significantly lower than in the other two groups (Figure 4H and 4I). Unexpectedly, we did not detect increases in *F. rodentium* abundance in host intestine after repeated ingestion of *F. rodentium* (Figure S3G). The data suggest that showed that repeated ingestion of *F. rodentium* causes depression-related behaviors, systemic inflammation, and reduction in the expression of synaptic proteins in the PFC in antibiotic-treated *Ephx2* KO mice.

3.6. Blocking of anhedonia- and depression-like behaviors by SDV in antibiotic-treated Ephx2 KO mice after ingestion of F. rodentium

To examine the role of the vagus nerve in depression-related behaviors in antibiotic-treated *Ephx2* KO mice after ingestion of *F. rodentium*, we performed SDV (Figure 5A). There were no changes in body weight (Figure 5B) or locomotion (Figure 5C) in any of the four groups. The immobility times of the TST and FST in the SDV + microbe group were significantly lower than those in the sham + microbe group (Figure 5D and 5E). Furthermore, sucrose preference of SPT in the SDV + microbe group was significantly higher than that in the sham + microbe group (Figure 5F). Moreover, blood IL-6 levels in the SDV + microbe group were significantly lower than in the sham + microbe group (Figure

5G). The expression of GluA1 and PSD-95 in the PFC of the SDV + microbe group was significantly higher than that in the sham + microbe group (Figure 5H and I).

The data show that SDV blocked the development of behavioral abnormalities, increased blood IL-6 levels, and decreased expression of synaptic proteins in the PFC in antibiotic-treated *Ephx2* KO mice after oral ingestion of *F. rodentium*.

4. Discussion

There were several major findings in the current study. First, FMT from CSDS-susceptible mice produced anhedonia-like behavior, systemic inflammation, and downregulation of synaptic proteins in the PFC of WT mice and *Ephx2* KO mice treated with ABX. Sucrose preference of *Ephx2* KO mice after FMT from CSDS-susceptible mice was higher than that of WT mice after FMT from CSDS-susceptible mice, which suggested that KO mice are more resilient than WT mice. In contrast, sucrose preference of both WT and *Ephx2* KO mice after FMT from control (no CSDS) mice was not different. Second, 16S ribosome RNA analysis showed that alpha-diversity in WT mice was increased after FMT from CSDS-susceptible mice, but not in *Ephx2* KO mice. The PCA showed different distributions of bacteria among the four groups. At the species level, the relative abundance of *F. rodentium* was higher in both WT and KO mice after FMT from CSDS-susceptible mice than those mice that underwent FMT from control (no CSDS) mice, which suggested that *F. rodentium* may be responsible for these changes. Third, oral ingestion of *F. rodentium* for 14 days caused depression-related abnormal behaviors, higher blood IL-6 levels, and downregulation of synaptic proteins in the PFC in ABX-treated *Ephx2* KO mice. Finally, SDV blocked these behavioral and biochemical changes in the ABX-treated *Ephx2* KO mice after oral injection of *F. rodentium*. Collectively, it is likely that *F. rodentium* induces depression-related abnormal behavior in ABX-treated *Ephx2* KO mice through the brain–gut–microbiota axis via the vagus nerve. It is noteworthy that ingestion of *F. rodentium* can convert resilient *Ephx2* KO mice into KO mice with depression-like phenotypes via the brain– gut–microbiota axis.

It is known that treatment with ABX can induce dramatic alterations in the diversity and composition of gut microbiota in the host intestine (Becattini et al., 2016). We have previously reported that FMT from CSDS-susceptible mice do not produce anhedonia-like phenotype, higher blood IL-6 levels, or downregulation of synaptic proteins in the PFC in water-treated WT mice but do so in ABX-treated WT mice after FMT from CSDS-susceptible mice (Wang et al., 2020a). Thus, microbiome depletion by ABX is necessary for these changes to occur in recipient WT mice after administration of “anhedonia-related microbes” obtained from CSDS-susceptible mice (Wang et al., 2020a). In this study, we found that FMT from CSDS-susceptible mice produced anhedonia-like behavior, systemic inflammation, and reduced expression of synaptic proteins in the PFC of WT mice and *Ephx2* KO mice treated with ABX. In contrast, we previously reported that *Ephx2* KO mice did not show anhedonia- and depression-like phenotypes after CSDS, which indicated that KO mice are resilient to stress (Ren et al., 2016). Taken together, we demonstrated that FMT in antibiotic-treated *Ephx2* KO mice using “anhedonia-related microbes” obtained from CSDS-susceptible mice causes anhedonia-like behavior through systemic inflammation. The

precise mechanisms underlying the anhedonia-like phenotype in ABX-treated *Ephx2* KO mice caused by FMT of “anhedonia-related microbes” from CSDS-susceptible mice remain unknown. It seems that FMT using “anhedonia-related microbes” from CSDS-susceptible mice may produce systemic inflammation in recipient mice, resulting in an anhedonia-like phenotype.

We found that the relative abundance of *F. rodentium* in mice with FMT from CSDS-susceptible mice was higher than that in mice with FMT from control (no CSDS) mice, which suggested that *F. rodentium* is responsible for the anhedonia-like behavior. Oral ingestion of *F. rodentium* for 14 days produced depression-related behaviors and reduced the expression of synaptic proteins in the PFC in ABX-treated *Ephx2* KO mice through systemic inflammation, even though these KO mice were resilient to CSDS (Ren et al., 2016). However, we could not detect a higher abundance of *F. rodentium* in the ABX + microbiome group although the microbiome were given orally for 14 days. The data suggest that *F. rodentium* did not colonize in the intestine after repeated ingestion. Although the precise mechanisms by which ingestion of *F. rodentium* causes depressive-like behaviors in *Ephx2* KO mice are currently unknown, it seems that metabolites or bacterial products from *F. rodentium* may contribute to systemic inflammation, resulting in depression-like phenotypes in mice. Nonetheless, further study is needed to investigate the mechanisms for *F. rodentium*-induced depression-like phenotypes in ABX-treated *Ephx2* KO mice.

Yang et al. (2019) showed that FMT from “anhedonia susceptible rats” exaggerated depression-like phenotypes, such as anhedonia, in ABX-treated mice. In contrast, FMT from “anhedonia resilient rats” could improve depression-like phenotypes, including anhedonia, in ABX-treated mice (Yang et al., 2019). Furthermore, we previously reported that microbiome depletion by ABX might contribute to stress resilience in mice after exposure to CSDS via the brain–gut– microbiome axis (Wang et al., 2020b). In this study, we found that repeated ingestion of *F. rodentium* produced behavioral and biochemical abnormalities in ABX-treated *Ephx2* KO mice through systemic inflammation. The data suggested that *F. rodentium* facilitates susceptibility in ABX-treated resilient *Ephx2* KO mice, although it remains unclear how *Ephx2* regulates gut microorganisms in the host. A recent study showed that sEH is an endogenous regulator of obesity-induced intestinal barrier dysfunction, which leads to systemic inflammation (Wang et al., 2020c). Although detailed mechanisms underlying depression-like phenotypes by ingestion of *F. rodentium* are unknown, it seems that systemic inflammation may play a role in the conversion from resilience to susceptibility. Increasing evidence suggests that promoting the growth of commensal bacteria is likely to confer some increase in resilience under stress (Bear et al., 2021). Future research is needed to study the relationship between *F. rodentiusm* in the host gastrointestinal tract, susceptibility and resilience.

The fastest and most direct pathway for gut microbiota to influence the brain may be by hijacking vagus nerve signaling (Fulling et al., 2019). The subdiaphragmatic vagus nerve plays a crucial role in the crosstalk between the brain and gut microbiota, and is thought to contribute to depression (Pu et al., 2021; Wan et al., 2021; Wang et al., 2020a; Zhang et al., 2020). We recently reported that SDV blocks depression-like behaviors and altered composition of gut microbiota after a single injection of lipopolysaccharide (Zhang et al.,

2020). It has also been reported that the vagus nerve mediates anti-inflammatory effects of antidepressants (Ondicova et al., 2019). In addition, we have previously reported that SDV significantly blocks depression-related behaviors in ABX-treated mice following repeated ingestion of *Lactobacillus intestinalis* and *Lactobacillus reuteri* (Wang et al., 2020a). In this study, we found that SDV significantly blocked blood IL-6 levels and depression-related behaviors in ABX-treated *Ephx2* KO mice after repeated ingestion of *F. rodentium*. A recent study showed that systemic IL-6 is originated from adipocytes during acute psychological stress (Qing et al., 2020). Collectively, it seems that the subdiaphragmatic vagus nerve plays an important role in systemic inflammation and depression-like behaviors in *Ephx2* KO mice after ingestion of *F. rodentium*.

Interestingly, vagus nerve stimulation has been used in the treatment of treatment-resistant epilepsy and other brain disorders such as depression, anxiety, autism spectrum disorder, and Alzheimer's disease (Mridha et al., 2021). It is suggested that the benefits of vagus nerve stimulation may be at least partially mediated by the recruitment of the acetylcholine system (Mridha et al., 2021). Therefore, it may be interesting to investigate the role of acetylcholine system in the effects of SDV in depression-like behaviors after ingestion of *F. rodentium.*

It has been reported that *F. rodentium* is enriched in mice fed with a diet free comprising aryl hydrocarbon receptor, which leads to inflammatory diseases such as hepatic steatosis (Brawner et al., 2019; Wada et al., 2016) and obesity (Sharma et al., 2018). A recent study showed that *F. rodentium* strongly correlated with phospholipoids such as phosphatidylethanolamine, phosphatidylcholine, and phosphoglyceride (Lee et al., 2020), indicating the important role of *F. rodentium* in the phospholipid synthesis. Furthermore, a recent study using a mouse model of colorectal cancer showed that *F. rodentium* (*F.* PB1), an endogenous strain of the mouse microbiota, is underrepresented in early stage of tumorigenesis (Zagato et al., 2020). Although the detailed functions of *F. rodentium* are currently unknown, it seems that *F. rodentium* may play a role in the phospholipid synthesis which is involved in progression of cancer (Cheng et al., 2016). Here, we found that oral ingestion of *F. rodentium* in antibiotic-treated resilient *Ephx2* KO mice caused depression-like behaviors via the vagus nerve, although the detrimental effects in the gastrointestinal tract of hosts due to *F. rodentium* remains unclear. Future research is needed to examine the effects of *F. rodentium* in the host gastrointestinal tract.

In conclusion, the present data showed that FMT of "depression-related microbes" from CSDS-susceptible mice into antibiotic-treated *Ephx2* KO mice results in anhedonia-like phenotypes. Moreover, repeated oral ingestion of *F. rodentium* causes depression-like behaviors in antibiotic-treated *Ephx2* KO mice via the subdiaphragmatic vagus nerve. Thus, oral ingestion of *F. rodentium* could convert resilient *Ephx2* KO mice into KO mice with depression-like phenotypes. This study provides new avenues for investigating the brain–gut–microbiota axis via the vagus nerve in susceptibility and resilience to stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlight

- *Ephx2* KO mice do not show depression-like phenotypes after chronic social defeat stress (CSDS).
- Fecal microbiota transplantation (FMT) from CSDS-susceptible mice caused depression-like phenotype in antibiotic-treated *Ephx2* KO mice.
- Ingestion of *F. rodentium* caused depression-like phenotypes in antibiotic-treated *Ephx2* KO mice.
- Subdiaphragmatic vagotomy did not show depression-like phenotypes in the antibiotic-treated *Ephx2* mice after ingestion of *F. rodentium.*
- The brain–gut–microbiota axis via the subdiaphragmatic vagus nerve plays an important role in susceptibility and resilience.

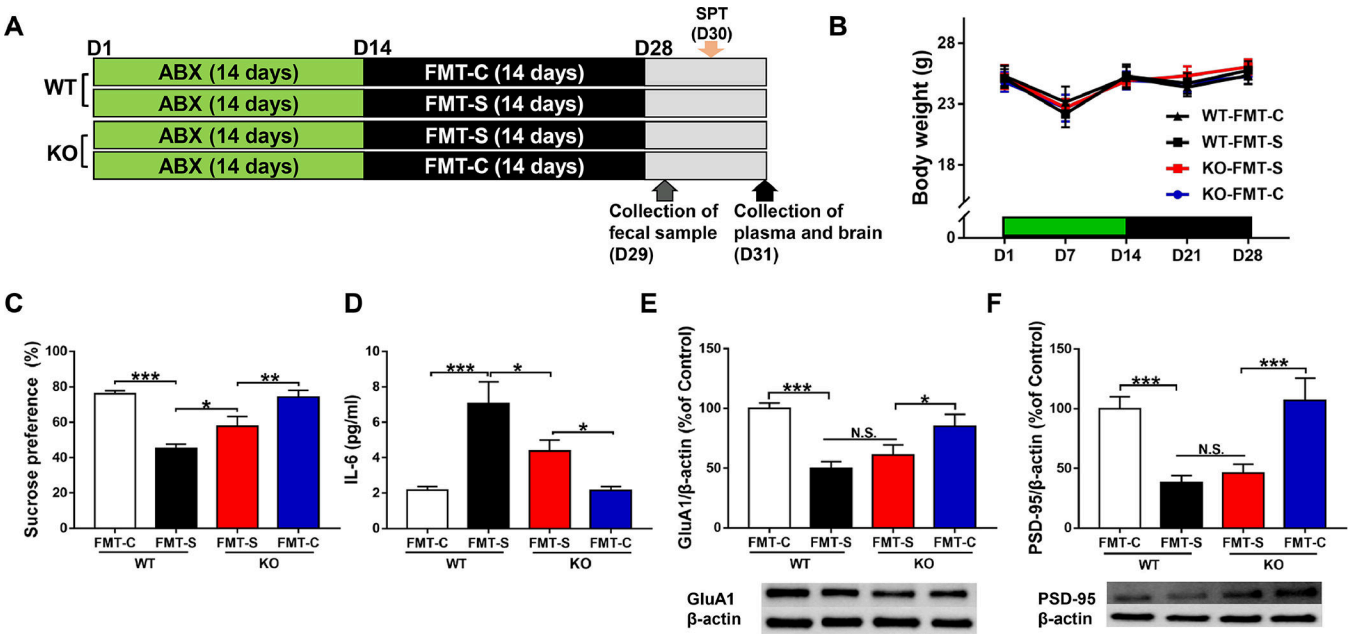

Figure 1. Effects of FMT in WT and *Ephx2* KO mice treated with antibiotic cocktail

(A): The schedule of treatment with antibiotics (ABX), fecal microbiota transplantation (FMT), feces collection, sucrose preference test (SPT), and collection of plasma and brain. Antibiotic cocktail in drinking water was given to adult male WT and KO mice for 14 days (day 1 – day 14). Subsequently, FMT of control (no CSDS) mice or CSDS-susceptible mice was performed for 14 days (day 15 – day 28). On day 29, fecal samples were collected. On day 30, 1% SPT was performed. On day 31, plasma and brain (i.e., PFC) were collected. (B): Body weight (repeated measures two-way ANOVA, genotype: $F_{1,36} = 0.001$, P =0.981, FMT: $F_{1,36} = 0.078$, P = 0.782, interaction: $F_{1,36}= 0.038$, P = 0.846). (C): SPT (two-way ANOVA, genotype: $F_{1,36} = 2.102$, P =0.156, FMT: $F_{1,36}=41.424$, P<0.001, interaction: $F_{1,36} = 3.650$, P = 0.064). (D): Plasma IL-6 (two-way ANOVA, genotype: $F_{1,36} = 3.532$, P = 0.068, FMT: $F_{1,36} = 24.926$, P < 0.001, interaction: $F_{1,36} =3.497$, P = 0.070). (E): GluA1 (two-way ANOVA, genotype: $F_{1,36} = 0.062$, P = 0.804, FMT: $F_{1,36} = 23.814$, P < 0.001, interaction: $F_{1,36} = 2.924$, P = 0.096). (F): PSD-95 (two-way ANOVA, genotype: $F_{1,36} =$ 0.381, P = 0.541, FMT: $F_{1,36} = 27.647$, P < 0.001, interaction: $F_{1,36} =0.001$, P = 0.973). Data are shown as mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001. FMT: fecal microbiota transplantation. NS: not significant. WT + FMT-C: Wild-type mice + FMT from control (no CSDS) mice. WT + FMT-S: Wild-type mice + FMT from CSDS susceptible mice. KO + FMT-S: *Ephx2* KO mice + FMT from CSDS-susceptible mice. KO + FMT-C: *Ephx2* KO mice + FMT from control (no CSDS) mice.

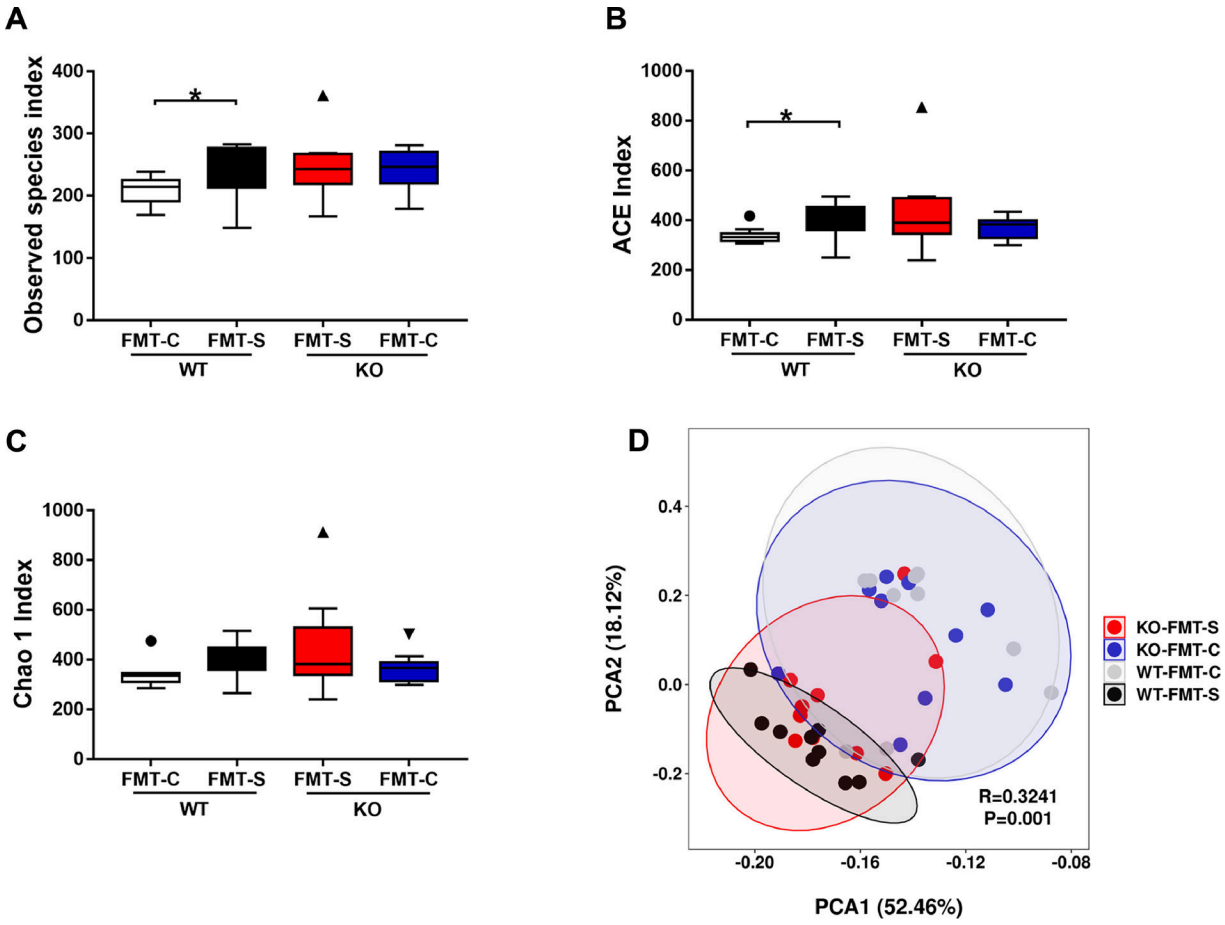

Figure 2. Effects of antibiotic cocktail and different genotype of mice in the diversity of gut microbiota after FMT

(A): Observed species index (Kruskal-Wallis test, $X^2 = 7.861$ P = 0.049) (B): ACE index (Kruskal-Wallis test, $X^2 = 8.537$ P = 0.036). (C): Chao 1 index (Kruskal-Wallis test, antibiotics: $X^2 = 6.631$ P = 0.085). (D): PCA analysis of gut bacteria data R = 0.3241 P = 0.001) Data are shown as mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001. NS: not significant. FMT: fecal microbiota transplantation. NS: not significant. WT + FMT-C: Wild-type mice+ FMT from control (no CSDS) mice. WT + FMT-S: Wild-type mice + FMT from CSDS susceptible mice. KO + FMT-S: *Ephx2* KO mice + FMT from CSDS-susceptible mice. KO + FMT-C: *Ephx2* KO mice + FMT from control (no CSDS) mice.

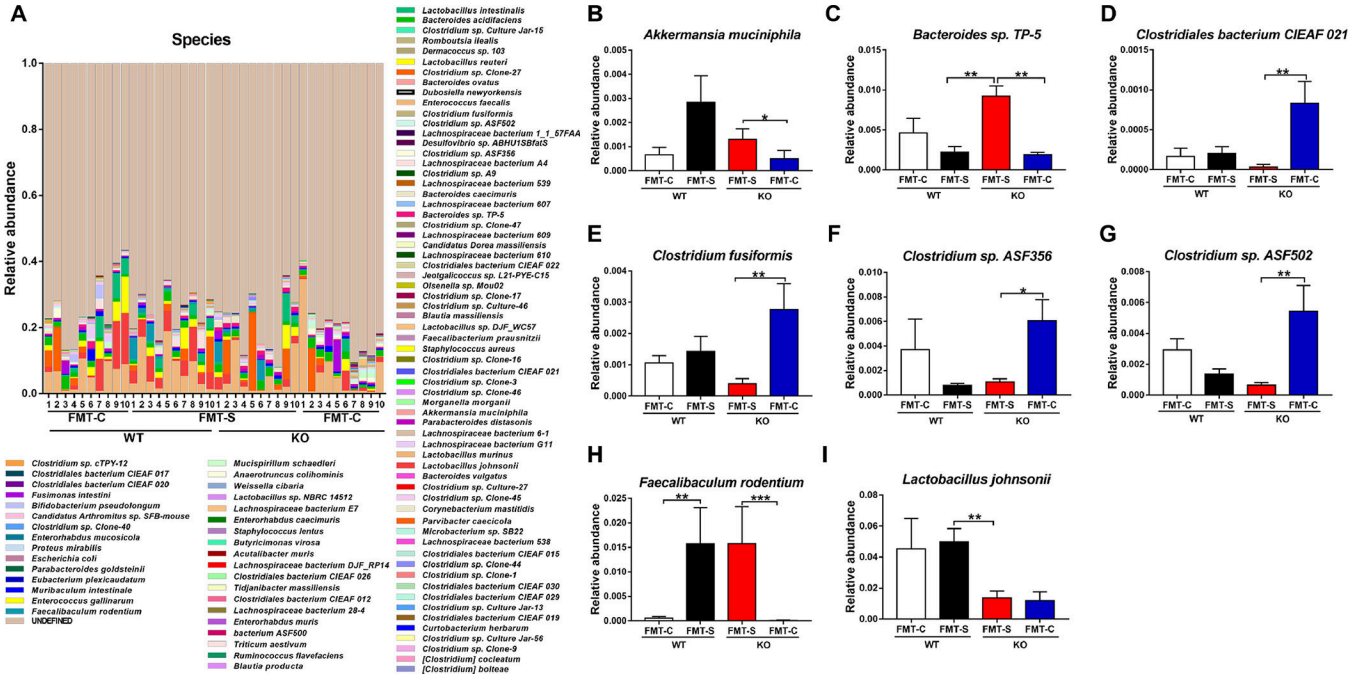

Figure 3. Altered composition in the gut microbiota at the species level

(A): The relative abundances of species in fecal samples of the four groups. (B): *Akkermansia muciniphila* (Kruskal-Wallis test, $X^2 = 8.607$ P = 0.035). (C): *Bacteroides sp. TP-5* (Kruskal-Wallis test, $X^2 = 14.821$ P = 0.002). (D): *Clostridiales bacterium CIEAF 021* (Kruskal-Wallis test, $X^2 = 13.240$ P = 0.004). (E): *Clostridium fusiformis* (Kruskal-Wallis test, $X^2 = 10.053$ P = 0.018). (F): *Clostridium sp. ASF356* (Kruskal-Wallis test, $X^2 = 10.858$ P = 0.013). (G): *Clostridium sp. ASF502* (Kruskal-Wallis test, $X^2 = 11.198$ P = 0.011). (H): *Faecalibaculum rodentium* (Kruskal-Wallis test, $X^2 = 21.971$ P < 0.001). (I): *Lactobacillus johnsonii* (Kruskal-Wallis test, $X^2 = 11.701$ P = 0.008). Data are shown as mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001. FMT: fecal microbiota transplantation. NS: not significant. WT + FMT-C: Wild-type mice+ FMT from control (no CSDS) mice. WT + FMT-S: Wild-type mice + FMT from CSDS-susceptible mice. KO + FMT-S: *Ephx2* KO mice + FMT from CSDS-susceptible mice. KO + FMT-C: *Ephx2* KO mice + FMT from control (no CSDS) mice.

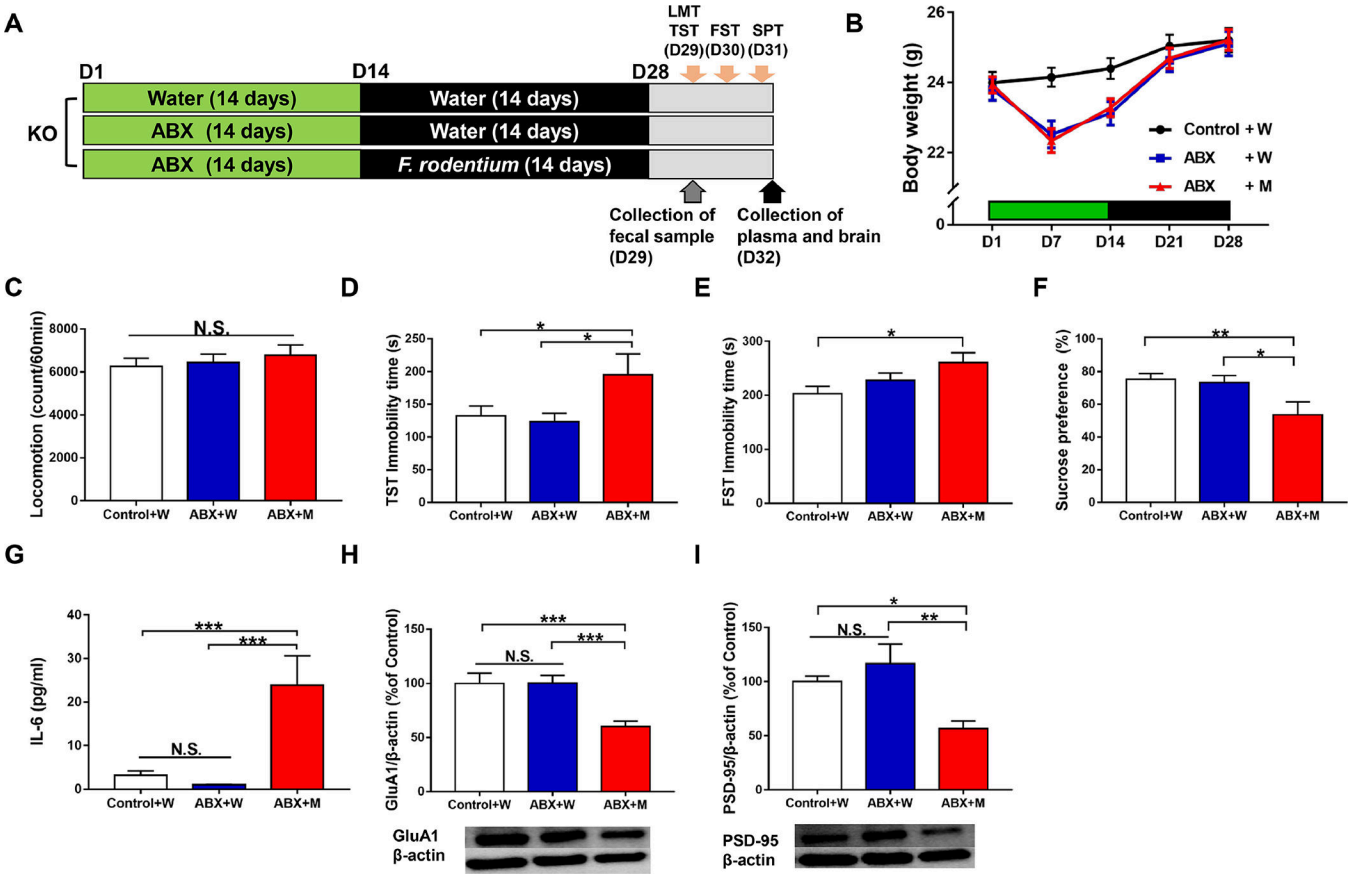

Figure 4. Effects of ingestion of *F. rodentium* in the antibiotic-treated *Ephx2* KO mice

(A): The schedule of treatment of antibiotic cocktail (ABX), microbiota transplantation, collection of fecal samples, behavioral tests and collection of plasma and brain. Antibiotic cocktail (ABX) or water in drinking water was given to adult male *Ephx2* KO mice for 14 days (day 1 – day 14). Subsequently, *F. rodentium* were administered orally for 14 days (day 15 – day 28) using gastric gavage. On day 29, fresh fecal samples were collected before behavioral tests. On day 29, locomotion test (LMT) and tail suspension test (TST) were performed. Forced swimming test (FST) and 1% SPT were performed on day 30 and 31, respectively. On day 32, plasma and brain were collected. (B): Body weight (repeated measures one-way ANOVA, $F_{2,29} = 2.265$, $P = 0.122$). (C): Locomotion (LMT) (one-way ANOVA, $F_{2,29} = 0.380$, $P = 0.687$). (D): TST (one-way ANOVA, $F_{2,29} = 3.364$, $P = 0.049$). (E): FST (one-way ANOVA, $F_{2,29} = 3.545$, $P = 0.042$). (F): SPT (one-way ANOVA, $F_{2,29} = 4.767$, $P = 0.016$). (G): Plasma IL-6 (one-way ANOVA, $F_{2,29} = 11.308$, $P < 0.001$). (H): GluA1 (one-way ANOVA, $F_{2,29} = 9.126$, $P = 0.001$). (I): PSD-95 (one-way ANOVA, $F_{2,29} = 6.630$, $P = 0.004$). Data are shown as mean ± S.E.M. (n = 10 or 11). *P < 0.05, **P < 0.01, ***P < 0.001. NS: not significant. Control + W: water + water treated KO mice. ABX + W: antibiotic + water treated KO mice. ABX + M: antibiotic + microbe treated KO mice.

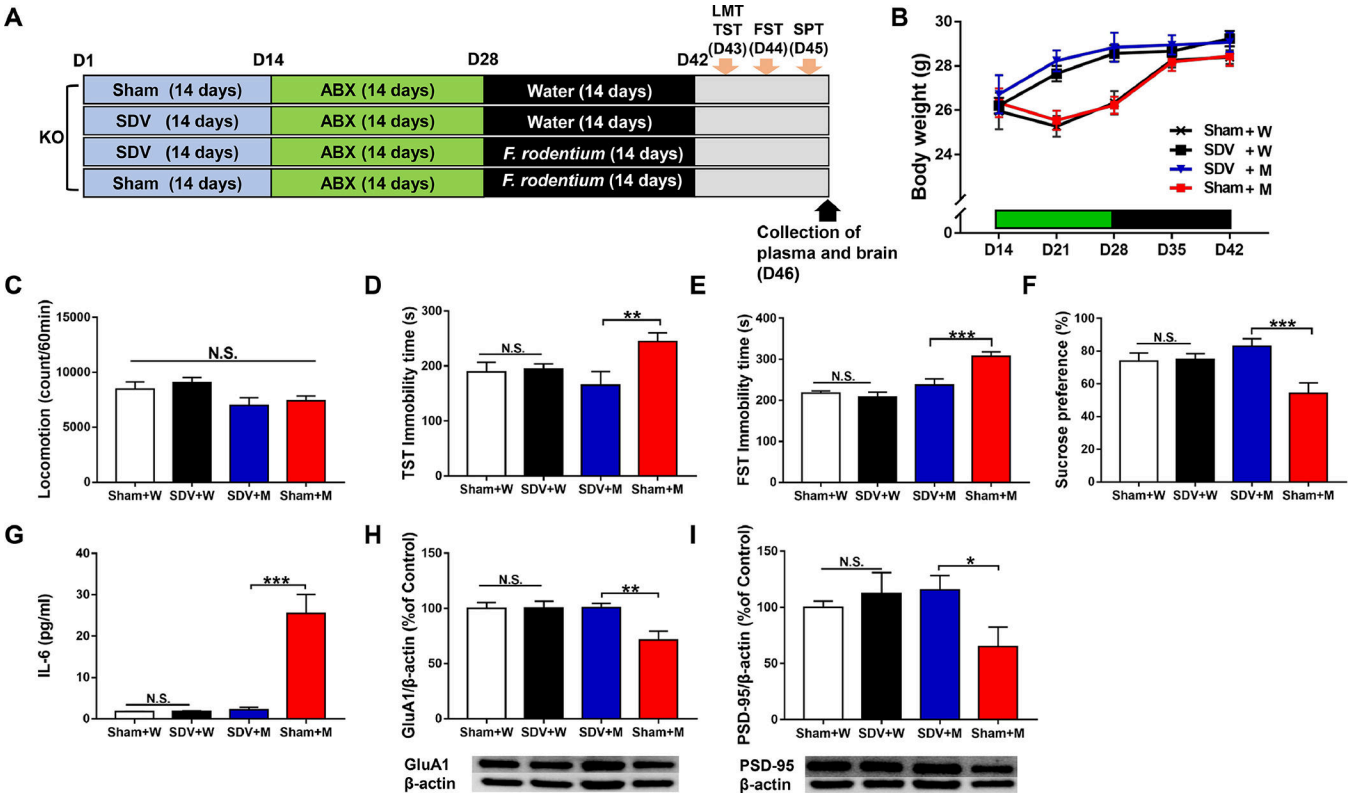

Figure 5. Effects of SDV on behavioral abnormalities after transplantation of *F. rodentium* in the antibiotic-treated *Ephx2* KO mice

(A): The schedule of subdiaphragmatic vagotomy (SDV), treatment of antibiotic cocktail (ABX), *F. rodentium* transplantation, behavioral tests, and collection of plasma and brain. SDV or sham was performed in adult male *Ephx2* KO mice, and mice were recovered 14 days after surgery (day 1 – day 14). Subsequently, antibiotic cocktail (ABX) in drinking water was given to all mice for 14 days (day 15 – day 28). Subsequently, transplantation of *F. rodentium* was performed for 14 days (day 29 – day 42). LMT and TST were performed on day 43. FST and 1% SPT were performed on day 44 and day 45, respectively. On day 46, plasma and brain were collected. (B): Body weight (repeated measures two-way ANOVA, SDV: $F_{1,27} = 0.323$, P = 0.575, microbe transplantation: $F_{1,27} = 2.012$, P = 0.168, interaction: $F_{1,27} = 0.163$, P = 0.689). (C): Locomotion (two-way ANOVA, SDV: $F_{1,27} = 0.024$, P = 0.878, microbe transplantation: $F_{1,27} = 7.632$, P = 0.010, interaction: $F_{1,27} = 0.763$, P = 0.390). (D): TST (two-way ANOVA, SDV: $F_{1,27} = 4.645$, P = 0.040, microbe transplantation: $F_{1,27} = 0.594$, P =0.448, interaction: $F_{1,27}$= 6.088, P = 0.020). (E): FST (two-way ANOVA, SDV: $F_{1,27} = 13.367$, P = 0.001, microbe transplantation: $F_{1,27} = 30.209$, P <0.001, interaction: $F_{1,27}$= 7.604, P = 0.010). (F): SPT (two-way ANOVA, SDV: $F_{1,27} = 8.593$, P = 0.007, microbe transplantation: $F_{1,27}$ =1.349, P = 0.256, interaction: $F_{1,27} = 7.482$, P = 0.011). (G): Plasma IL-6 (two-way ANOVA, SDV: $F_{1,27} = 23.632$, P < 0.001, microbe transplantation: $F_{1,27} = 25.599$, P < 0.001, interaction: $F_{1,27} = 24.023$, P < 0.001). (H): GluA1 (two-way ANOVA, SDV: $F_{1,27} = 5.622$, P = 0.025, microbe transplantation: $F_{1,27}$ =5.073, P = 0.033, interaction: $F_{1,27} = 5.447$, P = 0.027). (I): PSD-95 (two-way ANOVA, SDV: $F_{1,27} = 4.564$, P = 0.042, microbe transplantation: $F_{1,27} = 1.172$, P = 0.288, interaction:

$F_{1,27} = 1.684$, P = 0.205). Data are shown as mean ± S.E.M. (n = 7 or 8). *P < 0.05, **P < 0.01, ***P < 0.001. NS: not significant. Sham + W: sham + water treated KO mice. SDV + W: SDV + water treated KO mice. SDV + M: SDV + microbe transplantation treated KO mice. Sham + M: sham + microbe transplantation treated KO mice.