# The Gut Microbiota Mediates the Inhibition of Seizure-induced Respiratory Arrest in DBA/1 Mice fed a High Tryptophan Diet

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**Research Article**

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# Abstract

**Background:** Central 5-hydroxytryptamine (5-HT) defects are responsible for the occurrence of audiogenic seizure-induced respiratory arrest (S-IRA) in DBA/1 mice, an animal model of sudden unexpected death in epilepsy (SUDEP). We aimed to explore the effect of a high tryptophan diet (HTD) on SUDEP and its possible mechanism involving 5-HT metabolism via gut microbiota.

**Methods:** Primed animals were randomly assigned to the normal diet (ND) group or HTD group. Before and after diet interventions, 1) S-IRA rates were evaluated, 2) the concentrations of upstream-to-downstream 5-HT metabolites in the plasma and brain were detected by ultra-high-pressure liquid chromatography, and 3) the fecal flora biodiversity and species composition were analysed by 16S rDNA microbiota profiling. Second, antibiotics or probiotics combined with the HTD and S-IRA rates were reassessed.

**Results:** The S-IRA rate in DBA/1 mice was significantly reduced in the HTD group compared with that in control. The HTD obviously increased the levels of tryptophan in the telencephalon and 5-HT and 5-hydroxyindoleacetic acid levels in both the telencephalon and midbrain. The HTD significantly increased the species richness and diversity of gut microbiota. Moreover, there was a significant difference in the gut microbiota composition between the HTD and ND groups. However, antibiotics or probiotics had no synergistic or antagonistic effects on S-IRA reduction mediated by the HTD.

**Conclusions:** An HTD is efficient in lowering S-IRA rates in DBA/1 mice, possibly via modulation of 5-HT metabolism and gut microbiota. Our findings shed light on dietary prevention of SUDEP.


# 1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is one of the main causes of death in patients with epilepsy [1–3]. Therefore, it has become a common public health burden among neurological diseases [4]. However, the pathogenesis of SUDEP remains elusive. It is generally believed that seizure-induced arousal and respiratory and cardiac dysfunction are major causes of SUDEP [5, 6]. DBA/1 mice have been regarded as appropriate animal models for studying SUDEP. The mice can present generalized audiogenic seizures (AGSz), followed by seizure-induced respiratory arrest (S-IRA) and sudden death induced by acoustic stimulation, consistent with the respiratory dysfunction most often witnessed in SUDEP patients [7, 8]. Studies showed that cardiac dysfunction in DBA/1 mice lagged behind S-IRA, and dying animals could be resuscitated by assisted ventilation [8], suggesting that S-IRA may be the main cause of death in this SUDEP model.

5-Hydroxytryptamine (5-HT) is an important neurotransmitter mainly synthesized in the nuclei of the midbrain and medullary raphe of the brainstem [9]. Evidence has shown that 5-HT plays a key role in modulating arousal, respiratory and cardiac functions by corresponding control centres in the upper and lower brainstems [10, 9, 11]. In the brain, tryptophan hydroxylase 2 (TPH2) catalyses tryptophan (TRP) to synthesize 5-hydroxytryptophan (5-HTP), which is then decarboxylated by aromatic L-amino acid

decarboxylase to form 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) is the end product of 5-HT metabolism [12]. Our previous research found that the 5-HTP, 5-HT, and 5-HIAA contents and TPH2 activity in the brainstem of DBA/1 mice were significantly decreased compared with those in the brainstem of C57BL/6 mice. Intraperitoneal injection of fluoxetine, a selective 5-HT reuptake inhibitor (SSRI), or 5-HTP, the precursor of 5-HT, significantly reduced the incidence of S-IRA in DBA/1 mice [13–15]. In contrast, the incidence of S-IRA in DBA/1 mice markedly increased after pretreatment with 5-HT antagonists [16, 17]. The above evidence suggests that 5-HT deficiency is probably the underlying reason for SUDEP in DBA/1 mice.

The metabolism of 5-HT is closely related to gut microbiota. Almost 90% of 5-HT in the human body is produced in the gut by enterochromaffin cells [18, 19]. Furthermore, the intestinal flora may be connected with the central nervous system (CNS) through the dynamic two-way "gut-brain axis" [20] to affect brain function [21] and central neurotransmitters such as 5-HT [22], which are therefore involved in neurological diseases [23]. A previous study demonstrated that the concentrations of 5-HT and 5-HIAA in the hippocampus of male germ-free (GF) animals were significantly higher than those of control animals [24]. Supplementation with probiotics or prebiotics largely increased the level of central 5-HT in animals [25, 26]. The above evidence indicates that gut microbiota mediates the synthesis and metabolism of peripheral and central 5-HT.

Mounting evidence suggests that the gut microbiota mediates epileptogenesis. Studies have demonstrated that probiotics have a protective effect on the sensitivity to antiepileptic drugs in patients with drug-resistant epilepsy [27, 28]. Moreover, a retrospective study reported that six patients with drug-resistant epilepsy achieved temporary seizure freedom during antibiotic treatment [29]. An animal study also found that a ketogenic diet (KD) inhibited acute electrically stimulated seizures and spontaneous tonic-clonic seizures by altering the intestinal flora composition of mice [30]. The above findings suggest that the function or composition of the gut microbiota is closely related to seizures or susceptibility to epilepsy.

As an important essential amino acid for 5-HT synthesis, TRP is mainly obtained from the diet [31]. Oral administration of TRP or chronic high tryptophan diet (HTD) intervention in rats can obviously increase 5-HT levels in the CNS [32, 33]. We hypothesized that HTD could reduce the occurrence of SUDEP in DBA/1 mice. Moreover, our study aimed to explore how upstream-to-downstream 5-HT metabolites changed in the plasma and brain and whether gut microbiota were involved in mediating the effect of HTD on SUDEP.

# 2. Materials And Methods

## 2.1. Animals

Wild-type male DBA/1 mice were obtained from the Hunan SJA Laboratory Animal Co., Changsha, Hunan, China. All DBA/1 mice were housed 5–6/cage in a standard animal facility under controlled conditions

(temperature 22 ± 3°C, humidity 55%±5%) with a 12 h light-dark cycle and had free access to food and water.

## 2.2. Seizure induction and resuscitation

All DBA/1 mice (from postnatal days 26–28) were subjected to an acoustic stimulation paradigm and induced daily for three consecutive days (each interval was more than 24 hours) to evoke AGSz and S-IRA. Each mouse was placed in a transparent plastic chamber and stimulated continuously with a 110 dB electric bell (Zhejiang People's Electronics, Zhejiang, China) for 60 seconds or until the mouse exhibited tonic seizures. DBA/1 mice with S-IRA were resuscitated by an animal ventilator within 5 seconds of their last breath as previously reported [7]. DBA/1 mice with at least one S-IRA were considered successfully primed. Only primed DBA/1 mice were used in subsequent experiments.

## 2.3. Experimental design and grouping

As shown in Fig. 1a, primed animals were randomly assigned to the normal diet (ND) group or HTD group for a one-month dietary intervention. Animals in the ND group were fed a normal diet with a TRP level of 2.1 g/kg, while the TRP level of the HTD group was increased to 4 g/kg, as previously reported [34] (**Supplementary Tables 1 and 2**). Both diets were prepared by Beijing Keao Xieli Feed Co. (Beijing, China). Acoustic stimulation, examination of 5-HT and related metabolites, and determination of the species composition and diversity of intestinal flora were performed before and after dietary intervention.

Next, the animals were randomly divided into four groups: HTD with a vehicle for seven days (HTD + V7), HTD with antibiotics (HTD + Ab), HTD with a vehicle for 21 days (HTD + V21) and HTD with probiotics (HTD + Pb) (Fig. 1b). Animals in the HTD + Ab group were treated with the HTD and antibiotics for 7 consecutive days after one month of the HTD. The antibiotic mixture was prepared as previously reported [30]. Animals in the HTD + V7 group were given an equal volume of saline by intragastric administration for seven days. Animals in the HTD + Pb group were treated with the HTD and probiotics for 21 consecutive days immediately after one month of the HTD. A mixture of *Lactobacillus helveticus* (*L. helveticus*) (Taiwan Yaxin Biotechnology Co., Beijing, China) with a live bacteria content of $10^9$ CFU/mL was obtained as previously described [25]. The probiotic suspension was changed daily and shaken regularly to prevent precipitation. Animals in the HTD + V21 group did not have probiotic powder added to their drinking water. Acoustic stimulation was performed in each group before and after different interventions.

## 2.4. 16S rDNA microbiota profiling

Animals were placed into a clean cage lined with sterile filter paper. Fecal samples were collected immediately after defecation, quick-frozen in liquid nitrogen and stored at -80°C. Microbial community genomic DNA was extracted from fecal samples following the standard protocol. The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified with primer pairs 338 F (5'-ACT CCT ACG GGA GGC AGC AG-3') and 806 R (5'-GGA CTA CHV GGG TWT CTA AT-3') by a PCR thermocycler (ABI GeneAmp® 9700, CA, USA). Purified amplicons were pooled in equimolar amounts and paired-end

sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA). Operational taxonomic units (OTUs) with 97% similarity cut-off were clustered using UPARSE software (Uparse v7.0.1001), and chimaeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analysed by RDP Classifier against the 16S rRNA database using a confidence threshold of 0.7. The metagenomic analysis of intestinal flora was analysed on the Majorbio I-Sanger Cloud Platform (www.i-sanger.com). Additionally, alpha diversity was analysed using the Chao estimator (an index of species relative abundance), the observed richness (Sobs) and the Shannon diversity index (an index of the complexity of species diversity). Beta diversity analysis was calculated through cluster tree analysis to study the similarities or differences in community structures among different samples, and principal coordinate analysis (PCoA) was used to compare group differences in the overall microbiota profile.

## 2.5. UHPLC (Ultra High-Pressure Liquid Chromatography)

Animals were intraperitoneally injected with 1% chloral hydrate (400 mg/kg) for deep anaesthesia. Cardiac blood (0.5-1.0 mL) was carefully extracted from each mouse. Then, the mouse was killed by decapitation for collection of brain tissue. A mark was made in the anterior fontanelle as the bregma point. The brain tissue was cut at bregma − 3 mm, -5.5 mm, and − 9 mm and carefully separated on ice. Blood and brain samples were rapidly frozen in liquid nitrogen and stored at -80°C away from light. The contents of TRP and 5-HT metabolites of each sample were quantified on a UHPLC-MS/MS platform. The compounds of these metabolites were labelled by benzoyl-13C6 chloride and used as internal standards for quantification. All analytical standards and internal standards were prepared individually at a concentration of 1 mg/mL as a stock solution. The samples of calibration curves were finally obtained by mixing the calibration curve solution with internal standard solution (benzoyl-13C6 chloride-derivatized standard mixture) to generate calibration levels covering a range of 0.008-40 μM for TRP or 0.0016-8 μM for other metabolites. UHPLC-MS/MS analysis was performed on an Agilent 1290 Infinity II UHPLC system (Santa Clara, CA, United States). Raw data were processed by Agilent MassHunter Workstation Software (version B.08.00) using the default parameters. The peak areas of the target compounds were integrated, and the output was used for quantitative calculation.

## 2.6. Statistics

Statistical analysis was performed using SPSS 19.0 software. The incidence of S-IRA between the two groups was compared using the chi-square test, and the split chi-square test was used in pairwise comparisons among multiple groups. The concentrations of TRP- and 5-HT-related metabolites between two groups were compared using independent sample t-tests and expressed as the mean ± SD. For gut microbiota analysis, normally distributed data were compared using Student's t-test, and nonparametric data were compared using the Wilcoxon rank-sum test. Statistical significance was inferred if $p < 0.05$, and pairwise comparisons between multiple groups in the incidence of S-IRA were statistically significant if $p < 0.05/3$.

# 3. Results

## 3.1. The HTD reduced S-IRA susceptibility and modulated 5-HT metabolism in DBA mice

The incidence of S-IRA was significantly lower in the HTD group than in the ND group (50.94% vs. 71.79%, $p < 0.05$) (Fig. 2).

DBA/1 mice from the HTD group exhibited higher TRP levels in the telencephalon ($p < 0.05$), lower 5-HTP levels in the pons and medulla ($p < 0.05$), higher 5-HT levels in the plasma ($p < 0.05$), telencephalon ($p < 0.05$) and midbrain ($p < 0.01$), and higher 5-HIAA levels in the telencephalon ($p < 0.01$) and midbrain ($p < 0.001$) than those in the ND group (Fig. 3).

## 3.2. The HTD altered the abundance, diversity and distribution of gut microbiota

The alpha diversity analysis showed obviously higher Chao (479.29 ± 18.02 vs. 341.95 ± 110.18, $p < 0.001$), Sobs (412.00 ± 17.39 vs. 300.20 ± 99.27, $p < 0.001$) and Shannon indexes (4.36 ± 0.21 vs. 3.91 ± 0.56, $p < 0.05$) in the HTD group than in the ND group (Fig. 4a), suggesting that HTD treatment significantly increased the number of observed OTU sequence tags and the observed richness and species diversity. Hierarchical cluster analysis showed that all samples were divided into three distinct subgroups based on fecal bacterial community composition (Fig. 4b), indicating that the composition of the bacterial community in the HTD group was obviously different from that of the ND group. PCoA of sequencing data showed significantly separate clustering of the gut microbiota structure between the ND and HTD groups, and PC1, PC2 and PC3 accounted for 25.09%, 17.75% and 9.63% of the variation, respectively (Fig. 4c, 4d). The microbial composition of the samples from individuals fed the same diet was similar.

As shown in Fig. 5a and **b**, *Bacteroidetes* and *Firmicutes* were the most abundant phyla observed in all samples at the phylum level. The relative abundances of *Proteobacteria* ($p < 0.01$) and *Actinobacteria* ($p < 0.05$) were significantly increased and that of *Cyanobacteria* ($p < 0.05$) was strikingly decreased in the HTD group compared with the ND group (Fig. 5c). In addition, at the order level, there was an increase in the relative abundance of *Campylobacterales* ($p < 0.05$), *Desulfovibrionales* ($p < 0.05$) and *Burkholderiales* ($p < 0.01$) and a decrease in the relative abundance of *Gastranaerophilalesa* ($p < 0.05$) and *Anaeroplasmatales* ($p < 0.05$) in the HTD group compared with the ND group (Fig. 5d).

## 3.3. Effect of antibiotics or probiotics on the S-IRA rate in HTD-fed DBA/1 mice

Compared to the HTD group, there was no significant alteration in the S-IRA rate by HTD + Ab or HTD + Pb intervention (Fig. 6a). There was also no difference in S-IRA % between HTD + Ab or HTD + Pb and their respective vehicles (Fig. 6b, 6c).

# 4. Discussion

Diet therapy as a treatment strategy for epilepsy has a long history. For instance, KD, as a well-known low-carb, high-fat diet, is widely used in the treatment of epilepsy, autism spectrum disorders and Alzheimer's disease [35, 36]. However, to date, there is no effective diet intervention for SUDEP prevention. Alteration of dietary TRP is often used as a noninvasive method to manipulate the TRP levels of the body, thereby affecting 5-HT neurotransmission in the CNS [37]. HTD has been applied in the treatment of fatty liver disease [38], diabetes [39], Alzheimer's disease [40] and so on, which indicates that HTD is safe and feasible as an adjunctive therapy. Our study is the first to demonstrate that HTD is an effective diet intervention in preventing SUDEP in DBA/1 mice.

In this study, we found that an HTD significantly increased 5-HT levels in the telencephalon. Previous studies showed that SSRIs significantly increased the 5-HT content in the frontal cortex of rats [41]. Extensive synaptic connections were found between the cortex of the telencephalon and 5-HT neurons in the dorsal raphe of the midbrain sublattice [42]. In addition, some scholars found that telencephalon was also involved in the arousal mechanism of consciousness disorders [43]. Therefore, we hypothesized that the increase in telencephalon 5-HT levels may affect S-IRA occurrence in DBA/1 mice through the neural network between the telencephalon and midbrain. We also found that the HTD significantly increased 5-HT levels in the midbrain. Our previous study showed that the occurrence of S-IRA was significantly inhibited by selectively activating 5-HT neurons in the midbrain through optogenetic technology in transgenic DBA/1 mice [44]. We speculated that the HTD reduced S-IRA in this SUDEP model due to the elevation of the 5-HT concentration in the midbrain. Interestingly, the 5-HT level was not significantly altered in the pons and medulla of DBA/1 mice after the HTD, which differs partially from the results of Zhan's study in which multiunit recordings showed decreased firing of neuron populations both in the medullary and midbrain raphe, and single-unit recordings of serotonergic neurons revealed consistently decreased firing in the medullary raphe but a mixture of increased and decreased firing in the midbrain raphe during the ictal and postictal periods of an established Sprague Dawley (SD) rat seizure model [45]. We conjectured that the inconsistency may be due to strain differences and seizure triggering methods between DBA/1 mice and SD rats. As the literature stated, the midbrain raphe may be more likely involved in the mechanism of unconsciousness, the medullary raphe may be more involved in cardiorespiratory dysfunction during and after epileptic seizures in SD rats [45], while the midbrain may be more critical in S-IRA in this SUDEP model. In the future, the specific roles of these two nuclei and whether selectively activated 5-HT neurons in the medulla are associated with a reduced incidence of SUDEP should be studied further.

We found that the species abundance and diversity of the gut microbial community of animals in the ND group was less than that of animals in the HTD group. There was an obvious difference in the intestinal flora composition between the HTD and ND groups, and the gut microbiota relative abundance of the HTD-treated mice was dominated by *Proteobacteria* and *Actinobacteria*. The mechanisms underlying bacterial-induced 5-HT signalling are not well understood. Studies proved that some *Proteobacteria* and *Actinobacteria* species were closely related to the increase in short-chain fatty acids (SCFAs) [46], which

were reported to be capable of promoting 5-HT production in peripheral blood [47]. Since we did not test the SCFA differences in the HTD and ND groups, we did not know if the HTD reduced the S-IRA rate by affecting the gut microbiota and then elevating SCFAs and eventually peripheral and central 5-HT. In addition, some studies found that local stimulation of gut metabolites mediated by certain intestinal flora can regulate brain activity through the autonomic nervous system [48], and the stimulation of peripheral vagal nerves could modulate the regulation of central 5-HT [49]. Other bacterial species have also been reported to be capable of modulating 5-HT metabolism. For example, the administration of lipopolysaccharide, a cytoderm component of gram-negative bacteria, significantly increased the production of 5-HT in the prefrontal cortex, striatum and midbrain of animals [50, 51], possibly via the modulation of TPH activity [51]. In addition, some bacterial metabolites, such as acetic acid (an SCFA), can regulate the expression of serotonin receptors in the gut and brain, as well as change behaviours in animals [52]. Generally, the exact mechanism by which the gut microbiota mediates the changes in 5-HT levels in the CNS through the "gut-brain axis" is relatively complicated and still needs further exploration.

A previous study found that the levels of 5-HT and 5-HIAA in the hippocampus of male GF animals were significantly higher than those in the corresponding controls [24]. It is therefore natural to speculate that antibiotics would further elevate brain 5-HT levels in DBA/1 mice, which would accordingly enhance the inhibitory effect of HTD on SUDEP. Moreover, antibiotics did not exert synergistic effects with the HTD for S-IRA suppression. This finding should not be ascribed to the difference in the intestinal environment between GF animals and antibiotic-treated animals because first, in our study, there were also elevated levels of 5-HT and 5-HIAA in the midbrain of the antibiotic treatment group compared with the control group (as shown in **Supplementary Fig. 1**), and second, intestinal environment testing showed that antibiotics inhibited most of the intestinal flora of DBA/1 mice (as shown in **Supplementary Fig. 2**), which was consistent with previous reports [53, 30]. These observations somehow equated antibiotic-treated animals to GF animals. It is more likely that the production of central 5-HT in DBA/1 mice reached saturation after administration of the HTD, thus, the combination of the HTD and antibiotics could not further increase the concentration of 5-HT in the CNS (see **Supplementary Fig. 3**).

Probiotics or prebiotics intervention is closely related to peripheral and central 5-HT metabolism in animals. For instance, *Lactobacillus plantarum* PS128 (PS128) significantly augmented striatal 5-HT levels in GF mice [54]. Oral *Lactobacillus reuteri 3* (*L. reuteri 3*) notably elevated 5-HT levels in the periphery and colon of depressed mice [55]. At present, it is believed that the possible mechanism by which probiotics or prebiotics affect central 5-HT is related to their effect on the reconstruction of TRP metabolism and 5-HT transformation [26], but the exact mechanism is unclear. In our study, the HTD combined with probiotics was not superior to the HTD alone in SUDEP prevention. This combination did not significantly change the metabolism of TRP and 5-HT in plasma or the CNS (as shown in **Supplementary Fig. 4**). We deduced that, on the one hand, supplementation with probiotics may not produce a superimposition effect on the HTD-mediated 5-HT increase in the CNS. On the other hand, the HTD combined with probiotics may alter the regulation of central 5-HT by directly changing the intestinal flora spectrum or indirectly affecting certain metabolites of the "gut-brain axis". Another possible explanation is that Lactobacillus, Proteobacteria and Actinobacteria modulated 5-HT metabolism through

the same pathway in the "gut-brain axis", but this was not revealed by our current study and still needs further research.

Taking into consideration the important role of central 5-HT synthesis in SUDEP, it is meaningful to detect 5-HT deficiency in patients with epilepsy, which is helpful in differentiating those who are at high risk for SUDEP. Recent studies found that PET/SPECT could monitor alterations of the 5-HT receptor/5-HT transporter in associated brain regions by serotonergic probes [56] or regional blood flow [57] and had been applied in neuropsychiatric and neurodegenerative disorders [58, 59]. Therefore, screening patients with serotonin-targeted PET/SPECT is a promising prospect for the prevention and treatment of SUDEP in the future.

In conclusion, our research was the first to demonstrate that an HTD significantly reduced the incidence of S-IRA in DBA/1 mice, possibly mediated by gut microbiota, which affected the synthesis and metabolism of 5-HT, primarily in the CNS. However, the specific molecular mechanism remains to be further clarified. Our findings may open another window for the pathogenesis of SUDEP, and an HTD is expected to be a promising candidate for the prevention of SUDEP in clinical practice, especially for patients with central serotonin deficiency.

# Declarations


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### Availability of data and material

All source data supporting the findings of this manuscript are available from the corresponding authors upon request.

**Code Availability:** Not applicable

### Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Qiang Yue and Mingfei Cai. The first draft of the manuscript was written by Qiang Yue. The manuscript were revised by Bo Xiao, Chang Zeng, and Qiong Zhan. All the authors critically read and approved the final manuscript.

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## Compliance with Ethical Standards

## Conflicts of interest/Competing interests

The authors have no conflicts of interest/competing interests to declare that are relevant to the content of this article.

## Ethical approval

The study were complied with the guidelines of the Care and Use of Laboratory Animals (NIH USA), and experimental protocols were approved by the Animal Ethical and Welfare Committee and the Institutional Animal Care and Use Committee, Xiangya Hospital, Central South University, China (No. 202009559). All efforts were made to reduce the number of animals used and their suffering.

**Consent to Participate:** Not applicable

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# Figures

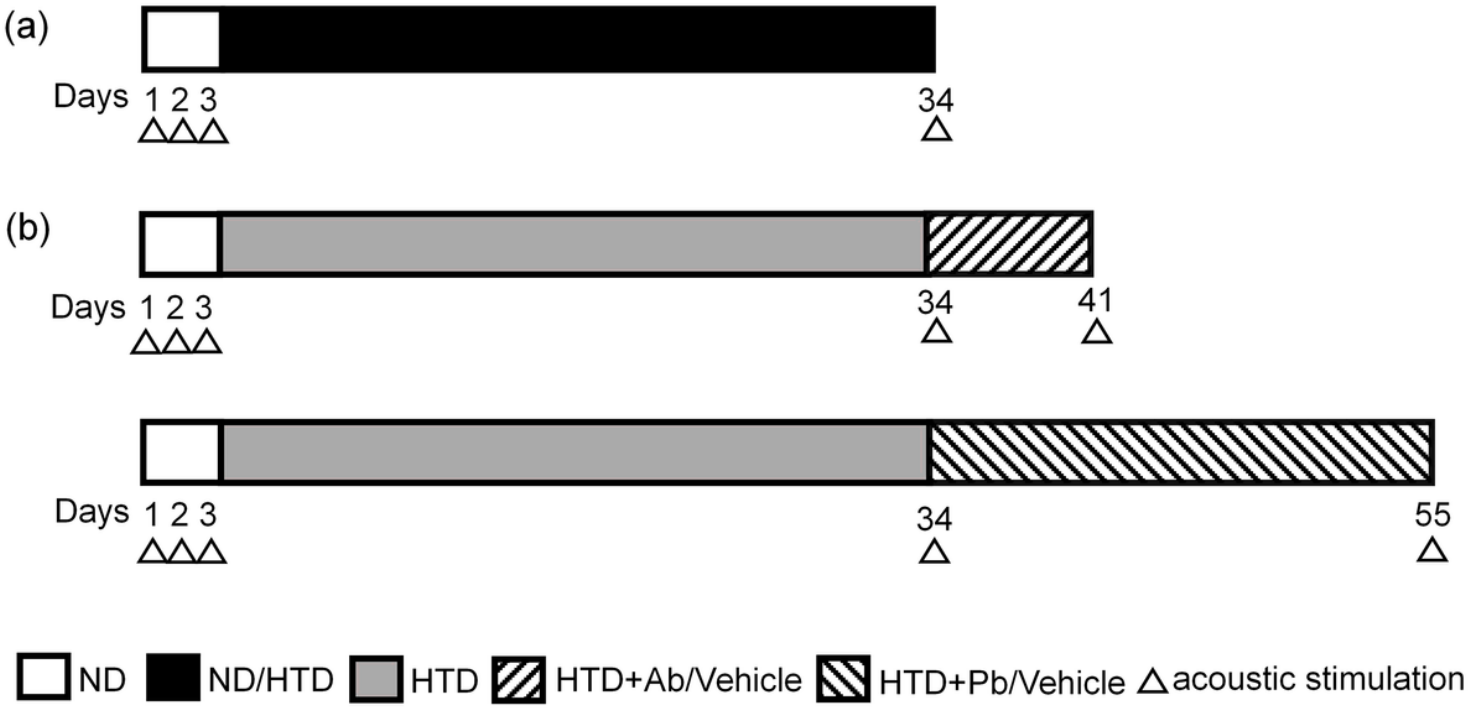



**Figure 1**

Schematic timeline of seizure induction, diet intervention, and acoustic stimulation at various time points in the experiment. ND, normal diet; HTD, high tryptophan diet; Ab, antibiotics; Pb, probiotics

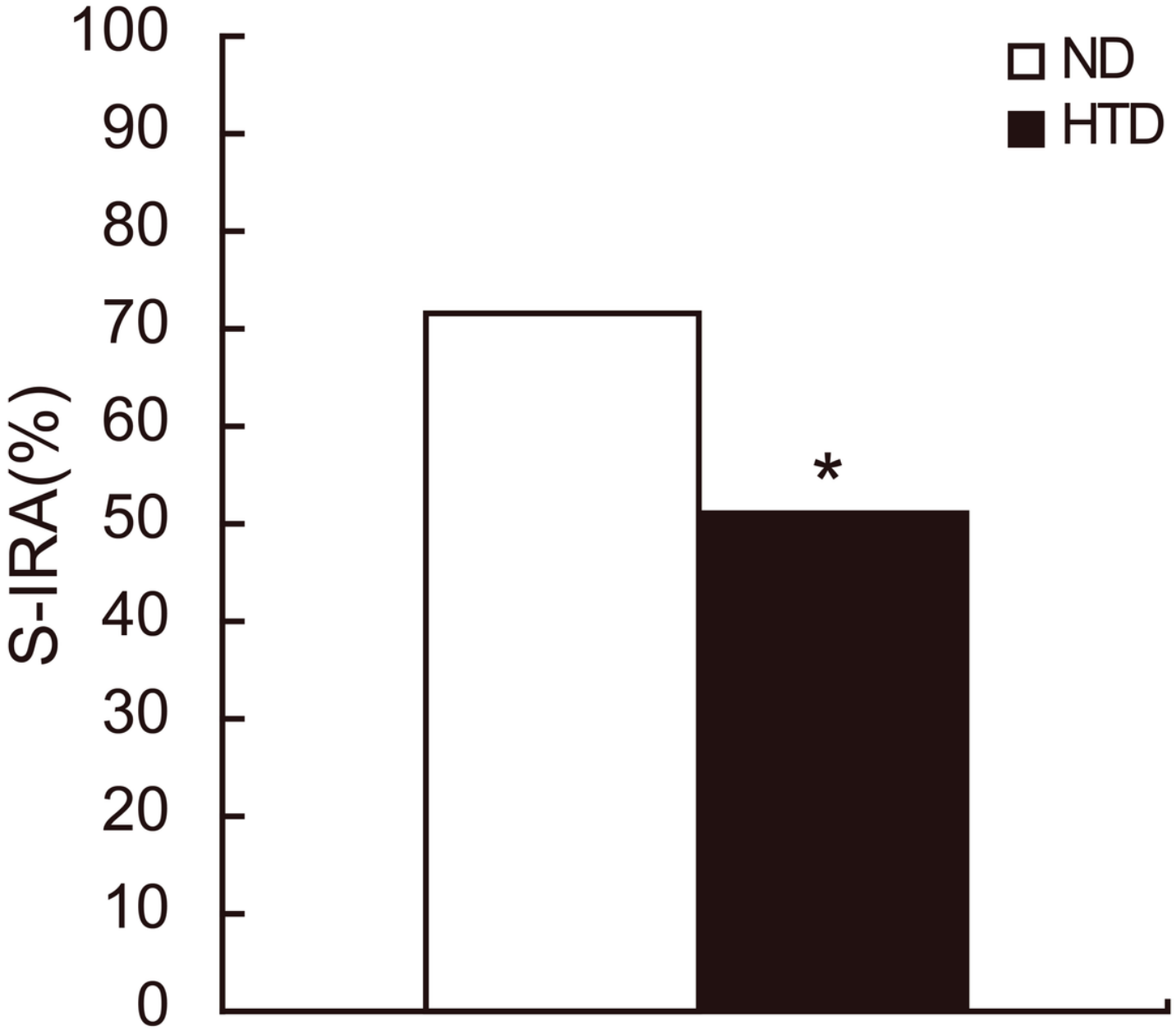
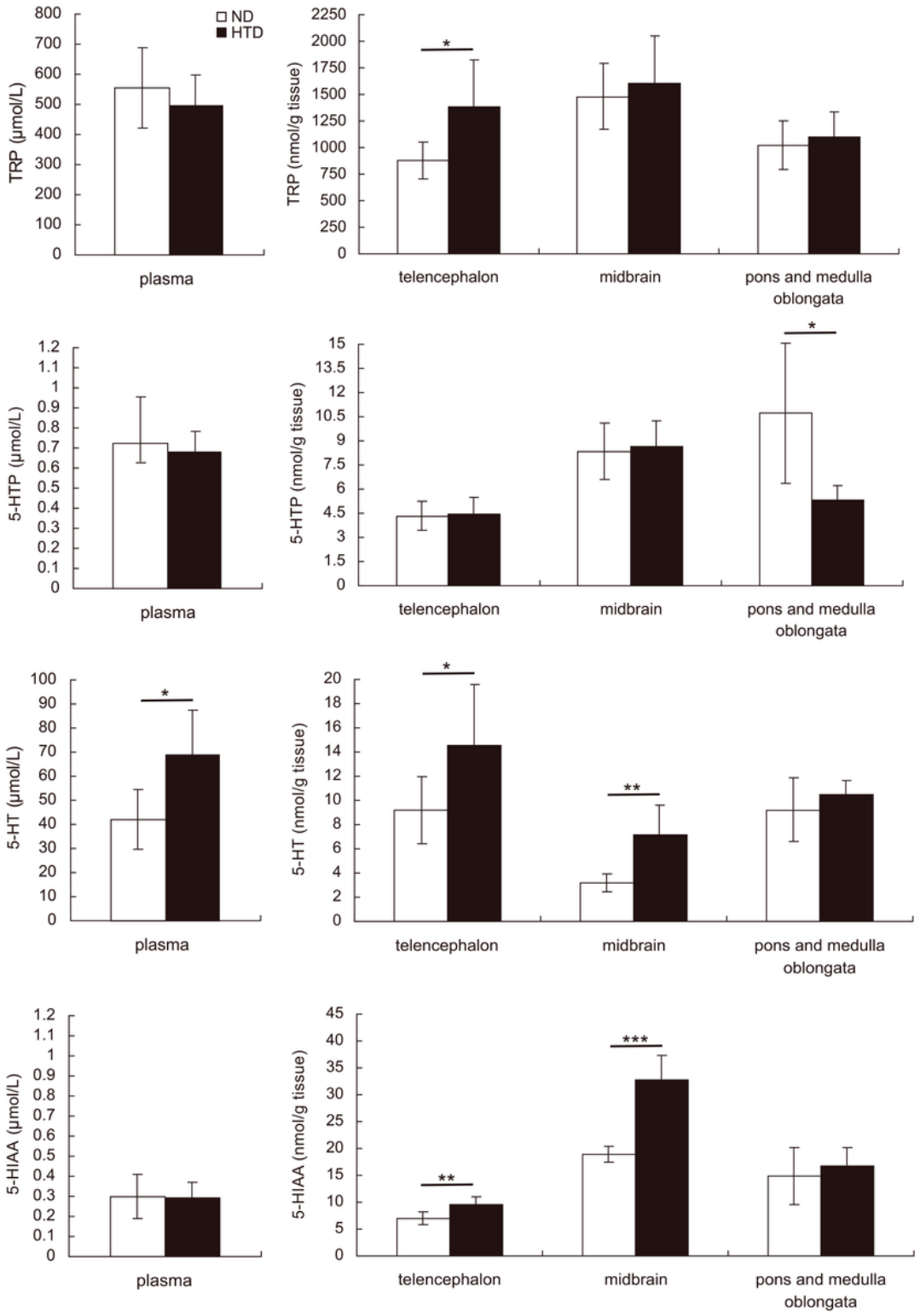


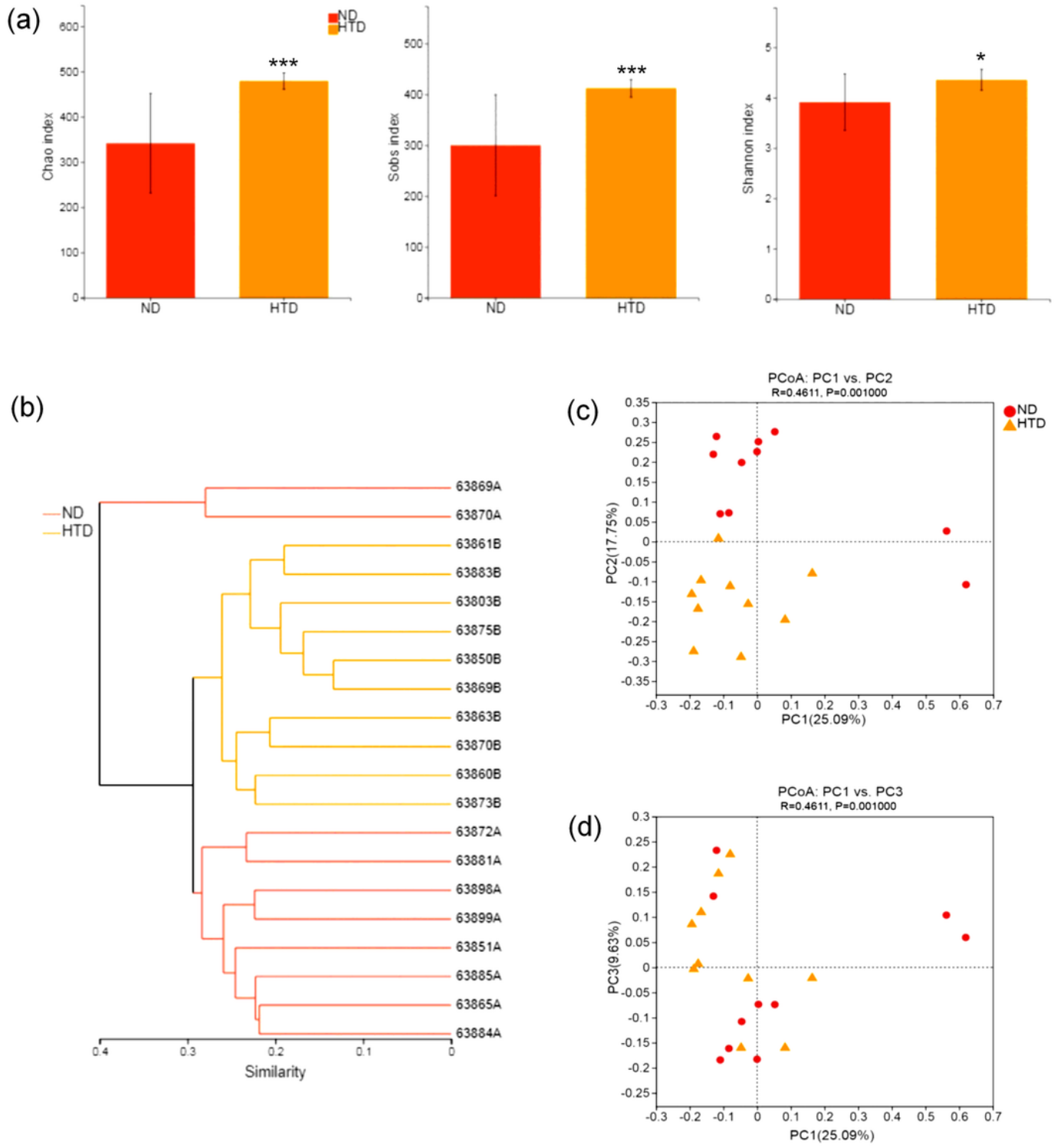

**Figure 2**

The S-IRA rate in DBA/1 mice was significantly decreased in the HTD group. ND, n=39. HTD, n=53. Statistical analysis was performed by the Chi-square test. * $p < 0.05$. S-IRA, seizure-induced respiratory arrest; ND, normal diet; HTD, high tryptophan diet




## Figure 3

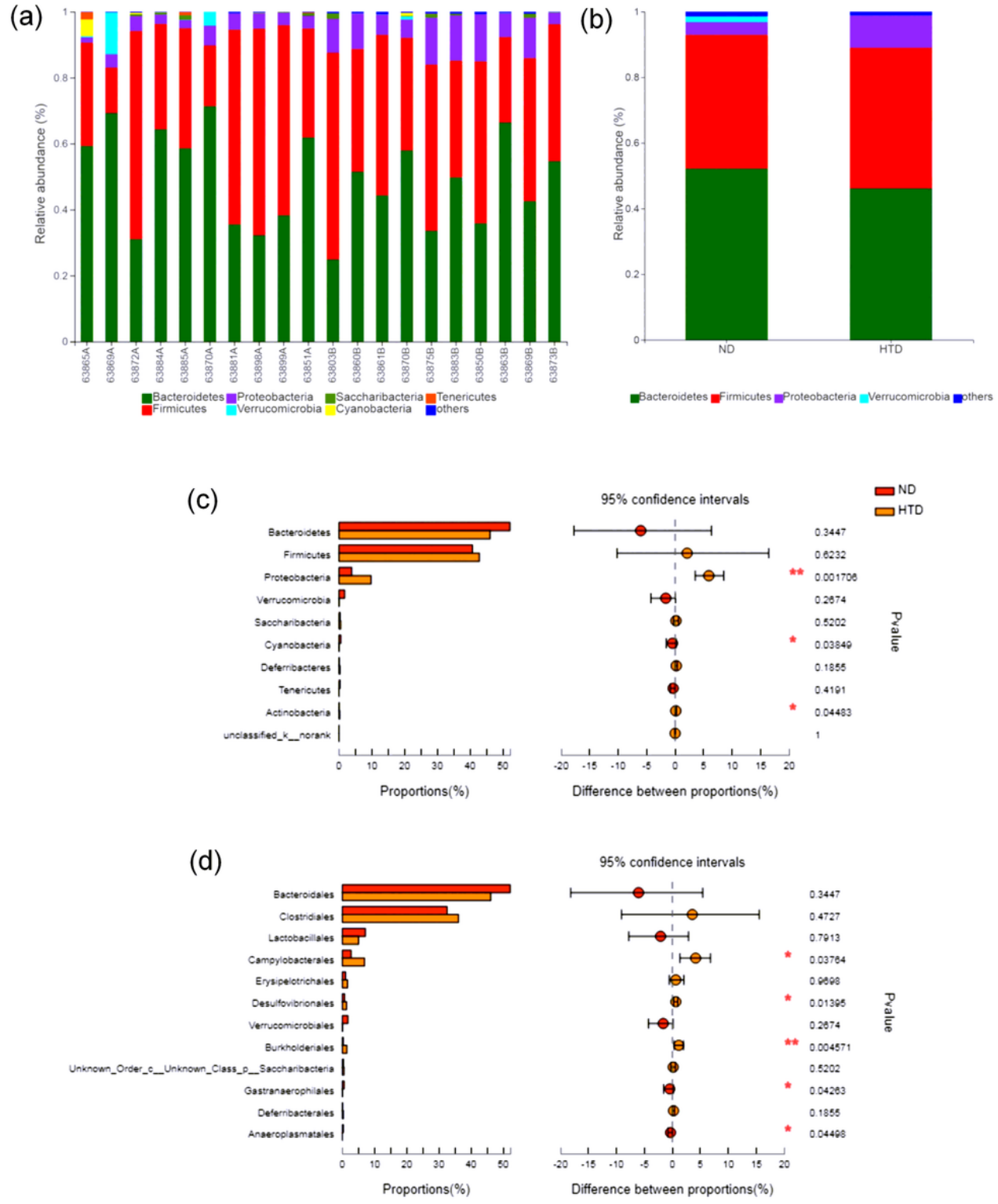
Comparison of the concentrations of TRP- and 5-HT-related metabolites in plasma and brain regions of DBA/1 mice between the HTD and ND groups. n=6 in each group. Statistical analysis was performed by the independent samples t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data represents as the mean ± SD. ND, normal diet; HTD, high tryptophan diet; TRP, tryptophan; 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid




## Figure 4

Alpha and beta diversity analyses between the HTD and ND groups. (a) Alpha diversity analysis of gut microbiota was performed with the Wilcoxon rank-sum test between the two groups. Data represents as the mean ± SD. (b) Hierarchical cluster analysis using Bray-Curtis distances. Each sample was marked with a branch of different colours and divided into diverse cohesive groups according to their distance thresholds. (c),(d) PCoA based on Bray-Curtis dissimilarity. The microbiota of each sample from the ND

(red circle) and HTD groups (orange triangle) is represented by different points. n=10 in each group. * p < 0.05, ** p < 0.01, *** p < 0.001. ND, normal diet; HTD, high tryptophan diet; Sobs, observed richness; OTUs, operational taxonomic units; PCoA, principal coordinate analysis




## Figure 5

Microbial composition and group differences of gut microbiota between the ND and HTD groups. Vertical bar charts depict the various species compositions of different samples (a) and groups (b) at the phylum

taxonomic level. The horizontal bar charts depict the taxonomic differences between the two groups at the phylum (c) and order (d) levels. n=10 in each group. Statistical analysis was performed by the Wilcoxon rank-sum test. * p □ 0.05, ** p □ 0.01. ND, normal diet; HTD, high tryptophan diet

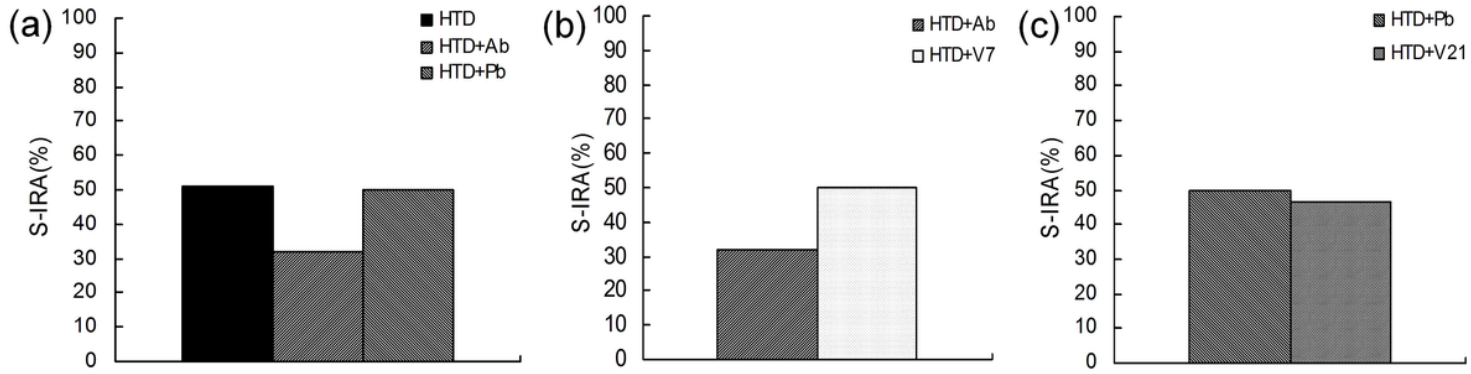



**Figure 6**

Effect of antibiotics or probiotics on the S-IRA rate of HTD-fed DBA/1 mice. HTD, n=53. HTD+Ab, n=22. HTD+Pb, n=20. HTD+V7, n=16. HTD+V21, n=15. Statistical analysis was performed by the split chi-square test. S-IRA, seizure-induced respiratory arrest; HTD, high tryptophan diet; HTD+Ab, HTD with antibiotics; HTD+Pb, HTD with probiotics; HTD +V7, HTD with vehicle for seven days; HTD +V21, HTD with vehicle for 21 days

# Supplementary Files

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