Supplementary Methods

1. Modified multiple platform water bath method (MMPM)

Each MMPM device consisted of a large tank (40×30×20 cm) and eight platforms (3 cm in diameter, 4 cm in height, 3 cm interval between platform) situated within the tank, surrounded by water up to 1-2 cm beneath the platform surface. SD-treated mice were placed in this device, which allowed them to move easily between the platforms, and permitted them to eat and drink freely. When the mice entered rapid eye movement sleep, muscle atonia could cause them to fall into the water and wake up. The mice would then try to climb up quickly to prevent drowning. During the SD period, the water at the bottom of the tank was kept clear, and the temperature was maintained at 20-25°C. In the CON group, mice were placed in a water tank with four large platforms (12 cm in diameter, 4 cm in height)[1-3].

2. Behavioral tests

All mice were transferred to the behavior testing room at least 30 minutes prior to test commencement to habituate to the conditions. All tests were conducted between 8:00 and 17:00, during the light phase of the cycle, in the following sequence.

2.1. Y-maze test

The mouse was placed in the center of the Y-maze (comprising 3 arms, each 30 cm in length and spaced 120° apart) and allowed to explore the arms freely for 5 min. The number of all arm entries and alternations was recorded by the software. An arm entry was only recorded if the mouse entered the arm completely[4].

2.2. Elevated plus maze test

The elevated plus maze has a "+" configuration and consists of two open arms (25×5×0.5 cm) and two closed arms (25×5×16 cm) with a center platform (5×5×0.5 cm). Each mouse was placed in the central square facing one of the open arms and allowed to move freely for 5 minutes. The number of entries (defined as the mouse's nose tip entering the arm) into each arm and time spent in the open arms were recorded and used to calculate the mice's anxiety-like behavior[4].

2.3. Open-field test

The open-field test was used to assess exploratory activities and anxiety-like behavior. The apparatus was a 50×50 cm open arena with 30 cm high walls. The mouse was placed in the center of the open field and allowed to explore freely for 8 min. The number of explorations and time spent in the inner area (33×33 cm), as well as the mouse's path throughout the apparatus, were recorded by software[4].

2.4. Morris water maze test

The Morris water maze was used to evaluate the spatial learning and memory capabilities of mice. It was conducted in a circular tank divided into four quadrants. A platform was hidden 1 cm below the surface of the milky water and placed in the center of one quadrant. During the training trial, mice were allowed to swim for 60 s to locate the hidden platform and stay on it for 10 s after researching it. If a mouse could not find the platform within 60 s, it was guided to the submerged platform. The mice were trained twice per day over five consecutive days, with an inter-trial interval of 2-3 h. On the last day, the mice were tested for memory retention within 60 s in the absence of the platform. The movement path and number of times crossing the original platform location were recorded[5].

3. 16S rDNA amplicon sequencing

Genomic DNA was extracted from fecal samples using a FastDNA Spin Kit for Feces (MP Biomedicals, USA). The V3-V4 region of the 16S rDNA gene was amplified from microbial genomic DNA using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'). A special barcode was added to the 5' end of the forward primer to identify each sample. The PCR system and program were as described previously[6, 7]. The products were excised from a 1.5% agarose gel, purified using DNA Gel/PCR Purification Miniprep Kit (Hangzhou Beiwo Medical Technology Co., Ltd., China), and quantified using a NanoDrop microvolume spectrophotometer (Thermo Fisher Scientific, USA). Libraries were prepared using the TruSeq DNA LT Sample Preparation Kit (Illumina, San Diego, CA, USA) and sequenced for 500 + 7 cycles on a NestSeq 2000 using a MiSeq Reagent Kit. Sequence analysis was performed using the Quantitative Insights into Microbial Ecology 2 (QIIME2) with the DADA 2 package. QIIME2 was used to calculate the Shannon index and the Bray-Curtis distance (as a metric of beta diversity). Principal coordinates analysis was performed on a website (https://www.omicstudio.cn/tool) to

obtain principal coordinates and visualize complex, multidimensional data. To confirm differences in the abundances of individual taxa between groups, the Linear Discriminant Analysis Effect Size method was used on a website (https://www.bic.ac.cn/BIC/#/) for quantitative biomarker analysis (LDA > 3).

4. Metabolites extraction and non-targeted metabolome analysis

Fecal samples (60 mg, ±1%) were collected from the four groups of mice. A 600 μL ice-cold mixture solution (methanol:acetonitrile:water = 2:2:1, v/v/v, -20°C) and three steel beads were added to each fecal sample. The samples were vortexed for 30 s, homogenized at 60 Hz for 45 s for 8 cycles, and then ultrasonicated in an ice bath for 10 minutes. All samples were incubated at -20°C for 1 h to precipitate proteins and centrifuged at 15,000 rpm for 15 min at 4°C to collect the supernatant. Aliquots of each sample (5 μL) were taken out and mixed to serve as quality control sample[8].

For LC-MS analysis, a UPLC BEH Amide column (1.7 μm, 2.1 mm × 100 mm, Waters) was used. Mobile phase A was water containing 25 mM CH3COONH4 and 25 mM NH4OH, while mobile phase B was 100% acetonitrile. The spectrum signal was acquired by electrospray ionization using positive and negative ionization modes. The resulting LC-MS data were processed using Compound Discoverer 3.3 (Thermo Fisher Scientific, USA), and all features from positive and negative modes were combined into a single feature table for analysis. Partial least squares discriminant analysis and volcano plots were performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca). Differential metabolites were selected based on fold change (FC) and significance from Student's t-test (FC > 1.5 or FC < 0.67, $p < 0.05$)[9, 10]. Metabolic pathway enrichment analysis based on differential metabolites with known KEGG IDs was performed using MetaboAnalyst 6.0.

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