

METHODS

Behavioral tests

Sucrose preference test (SPT) and body weight (BW)

All animals were first trained to consume a palatable (1%) sucrose solution before the test. Then mice were deprived of water and food for 24 h. Subsequently, mice were exposed to one bottle of 1% sucrose solution and one bottle of water for 60 min, and the positions of the bottles were changed every 30 min. Before and after SPT, every bottle was weighed. The calculation formula was sucrose preference (%) = sucrose intake/ (sucrose intake + water intake) ×100%. Moreover, body weight is another important index for evaluating the depressive state of mice.

Open field test (OFT)

The OFT was performed to measure the spontaneous activity and anxiety-like behavior of the rodents. The mice were kept in the room for >30 min before the test to allow them to adapt to the new environment, and they were tested in a completely random order. The activities of the animals were recorded and analyzed by a computer-automated animal activity video tracking system (Ethovision XT 11.5, Noldus, the Netherlands). The rectangular cage (50×50×35 cm) used for the test was divided into 9 square areas, and the center square area was defined as the center zone. The mice were gently placed in the central area of the cage when the test started, and then the video tracking system recorded and quantified the behaviors of the mice for 5 min. The floor of the apparatus was thoroughly cleaned with 75% ethanol and dried before the next animal was tested.

Elevated plus-maze (EPM) test

The elevated plus-maze was to test the anxiety-like behaviors and to track the spontaneous behaviors of mice. The maze consisted of two perpendicular intersecting runways (5 cm width × 35 cm length).

One runway had tall walls (closed arms; 15 cm height), and the other one had no walls (open arms).

The arms were connected by a central area (5×5 cm). At the beginning of the test, rodents were placed in the central area, facing one of the open arms, and the cumulative time (s) spent in the open and closed arms were recorded. In addition, the total distance (cm) traveled was recorded throughout the 5 min test.

Tail suspension test (TST)

Mice were individually hung by the tail from a perpendicular rod on the top of a box (30×30×30 cm), and medical adhesive tape was stuck to 2 cm from the tip of the tail. The immobility time was recorded for 5 min/test session.

Supplementary Table.1. The RT-PCR primer sequences of AKT, GSK-3 β , CRMP2, Tubulin, IBA1, Caspase-1, and GAPDH we assessed in our study.

Primer name	Primer sequence (5'-3')
CRMP2-S	TGGCAAGATTGTGAATGATGACC
CRMP2-A	GTCAGGCATCTGGAAGCGAGTA
Tubulin-S	GTATGCCAAGCGTGCCTTTG
Tubulin-A	AGCAGCACCTTGTGACATTT
IBA1-S	AAGCAGGGATTTGCAGGGAG
IBA1-A	CTTCAAGTTTGGACGGCAGAT
Caspase1-S	TGCCTGGTCTTGTGACTTGGAG
Caspase1-A	TGTCCTGGGAAGAGGTAGAAACG
GAPDH-S	CCTCGTCCCGTAGACAAAATG
GAPDH-A	TGAGGTCAATGAAGGGGTCGT

Supplementary Table.2. The antibody information.

Western Blot			
Antibody	Dilution	Source	Identifier
CRMP2	1:20,000	Abcam	#ab129082
p-CRMP2 (phosphoT514)	1:1,000	Abcam	#ab129066
α -tubulin	1:5,000	Abcam	#ab52866
IBA1	1:1,000	Abcam	#ab178846
Caspase-1	1:1,000	Abcam	#179515
GAPDH	1:10,000	Abcam	#ab9485

1. Kraeuter AK, Guest PC, Sarnyai Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. *Methods in Molecular Biology*. 2019;1916:99–103. Epub 2018/12/12. doi: 10.1007/978-1-4939-8994-2_9. PubMed PMID: 30535687.
2. Rodgers RJ, Dalvi A. Anxiety, Defence and the Elevated Plus-maze. *Neuroscience and Biobehavioral Reviews*. 1997;21(6):801–10. Epub 1998/01/07. doi: 10.1016/s0149-7634(96)00058-9. PubMed PMID: 9415905.