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Research paper

Neonatal maternal separation impairs cognitive function and synaptic plasticity in adult male CD-1 mice

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ABSTRACT

Maternal separation (MS) increases the risk of occurrence of anxiety, depression, and learning and memory impairment in offspring. However, the underlying molecular biological mechanisms remain unclear. In the current study, offspring CD-1 mice were separated from their mothers from postnatal day 4 to postnatal day 21. At 3 months of age, the male offspring were selected for the evaluation of anxiety- and depression-like behaviors and learning and memory function. Western blotting and RT-PCR were used to examine the expression levels of brain-derived neurotrophic factor, tyrosine kinase receptor B, postsynaptic density-95, and synaptophysin. Long-term potentiation (LTP) and long-term depression (LTD) were recorded at Schaffer collateral/CA1 synapses. Furthermore, basal synaptic transmission was evaluated via the recording of the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs). The results showed that adult offspring CD-1 mice displayed anxiety- and depressive-like behaviors as well as impaired spatial learning and memory abilities. Electrophysiological analysis indicated that MS impaired LTP, enhanced LTD, and reduced the frequency of mEPSCs in pyramidal neurons in the CA1 region. Our findings suggested that MS can lead to anxiety, depression, and cognitive deficits, and these effects are associated with alterations in the levels of synaptic plasticity-associated proteins, consequently, also synaptic plasticity.

1. Introduction

People who experience adverse environmental factors during early life often exhibit mental disorders, such as anxiety, depression, and impaired learning and memory ability (Strathearn et al., 2020; Herzberg and Gunnar, 2020). Disruption of the mother–neonate relationship represents a chronic stress event with lasting consequences for brain functions (Joushi et al., 2021a, 2021b). Maternal separation (MS) manipulation, a protocol used to mimic an impaired mother–child relationship, exerts prominent adverse influences on the developing brain, frequently accompanied by mental disorders, including cognitive dysfunction (Pusceddu et al., 2015; Bianco et al., 2021; Yang et al., 2023). However, the cellular mechanisms responsible for these effects are unclear.

It is well-documented that hippocampal synaptic plasticity underlies information processing and memory formation (Mazzocchi-Jones et al., 2011; Yang et al., 2021a, 2021b). MS-induced learning and memory impairments are closely associated with altered long-term potentiation (LTP) and the occurrence of long-term depression (LTD). Studies have shown that Wistar offspring rats that underwent MS during the lactation period displayed impaired LTP induction in hippocampal CA3-CA1 synapses both as juveniles and in old age (Sousa et al., 2014; Joushi et al., 2021a, 2021b). Similarly, elevated platform stress-induced LTP reinforcement in the dentate gyrus of the hippocampus was impaired in adult Wistar offspring rats that had been subjected to MS (Wang et al., 2013). Meanwhile, C57BL/6 J offspring mice exposed to MS during early life (from postnatal day PND2 to PND20) showed a lower magnitude of LTP in mossy fiber-CA3 synapses in adolescence relative to their control littermates (Shin et al., 2016). However, synaptic plasticity, including LTP and LTD, at CA3-CA1 synapses of mice from outbred colonies, such as CD-1 mice that have more similar genetic heritability to humans, has not been explored at any age.

Cognitive dysfunction and altered synaptic plasticity induced by MS have been linked to alterations in the brain-derived neurotrophic factor

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(BDNF) signaling pathway. BDNF, which is highly expressed in the hippocampus, plays an important role in neural differentiation and survival (Zhao et al., 2019). BDNF can improve Ca^{2+} influx through N-methyl-D-aspartic acid receptor (NMDAR) and the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor to the postsynaptic membrane, both of which are necessary for LTP formation, by binding to tyrosine kinase receptor B (TrkB) (Wu et al., 2016). Previous study found that the BDNF/TrkB signaling pathway was altered in MS-treated adult Wistar rats who showed spatial learning and memory deficits in the Barnes maze test (Cordier et al., 2021). Meanwhile, another study showed that the levels of BDNF mRNA were significantly decreased in the hippocampus of adult Wistar rats that had undergone MS in the lactation period (Aisa et al., 2009). Environmental enrichment, a simple and effective method for improving cognitive deficits, was reported to ameliorate MS-induced LTP impairment and cognitive dysfunction by restoring BDNF protein levels in the hippocampus of adolescent Wistar rats (Joushi et al., 2021a, 2021b; Dandi et al., 2018). Synaptic plasticity is regulated by a wide range of synaptic plasticity-associated proteins, such as postsynaptic density-95 (PSD-95) and synaptophysin (SYN), both of which control the release of synaptic vesicles and glutamate receptor density in the postsynaptic membrane (Ding et al., 2020; Van Westen et al., 2021). However, the changes in synaptic plasticity-associated proteins induced by MS are poorly characterized.

In this study, we investigated whether alterations in synaptic plasticity and synaptic plasticity-associated proteins in the hippocampus of adult CD-1 offspring mice are involved in the cognitive impairment resulting from MS. For this, we first assessed changes in behavior, including the occurrence of anxiety, depression, and hippocampus-dependent spatial learning and memory deficits, in offspring mice exposed to MS. Subsequently, we determined the expression levels of synaptic plasticity-associated proteins, including BDNF, TrkB, PSD-95, and SYN. Finally, we recorded LTP and LTD at CA3/CA1 synapses and miniature excitatory postsynaptic currents (mEPSCs) in CA1 neurons.

2. Materials and methods

2.1. Animals

CD-1 mice (6–8 weeks of age, specific-pathogen-free grade) were purchased from Beijing Vital River Laboratory Animal Device Co., Ltd. The mice were maintained at a humidity of 55±5 % and a temperature of 22–25°C under a 12-h-light/12-h-dark cycle (lights on at 07:00 h). Food and water were available ad libitum. After 2 weeks of acclimatization, male and female mice were paired at a ratio of 1:2. When the vaginal plug was detected in the perineum, the female mice were housed individually in a standard cage until delivery. The male offspring mice were randomly assigned to a control (Control) group and a maternal separation (MS) group, eight in each group. The day of birth was recorded as PND0. Pups assigned to the MS group were separated from their mothers for 3 h per day (from 09:00–12:00 h from PND4 until weaning at PND21), while those in the Control group underwent no intervention. Subsequently, male mice from both groups were separated from their mothers and raised in separate cages, with 2 mice per cage. The experiments were performed when the male offspring mice reached 3 months of age. A total of 16 pregnant female mice and 16 male offspring mice were used in this experiment based on the previous studies (Zhao et al., 2019) and no animals were excluded. All experimental procedures involving animals were approved by the Experimental Animal Committee of Anhui Medical University and complied with the guidelines for humane treatment established by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences of the Anhui Medical University and in compliance with ARRIVE guidelines.

2.2. Open field test

The open field consisted of a black square arena (50 cm × 50 cm) with 25 cm high walls. During the 5-minute experiment, the mice were placed into the apparatus facing the wall from one of the four corners. After the 5-minute test, the mice were returned to their own cages. The open field was wiped with 75 % alcohol and left to dry. The time and number of entries in the center area and total distance were recorded by ANY-Maze software.

2.3. Elevated plus maze

The elevated plus maze apparatus consisted of a cross-shaped platform comprising two open arms (30 × 6 cm), two closed arms (30 × 6 × 15 cm), and a central arena (6 × 6 cm) that was placed 80 cm above the ground. Mice were individually placed in the central area facing one of the open arms and allowed to freely explore the maze for 6 min. The number of entries into and the time spent in the open arms were analyzed using ANY-Maze software (Stoeling). To eliminate any influence of olfactory cues from the previous mouse, the device was cleaned with 75 % alcohol and dried thoroughly after each mouse.

2.4. Forced swimming test

Mice were individually placed in a glass cylinder (28 cm in height, 18 cm in diameter) filled with water to a height of 15 cm. The temperature of the water was maintained at 22 ± 1°C. Mice were placed in the cylinder for 6 min and the immobility time of the mice was recorded during the last 4 min of the test. Mice were defined as immobile when they did not struggle in the water, made only the necessary movements to keep their heads above the surface of the water, and did not attempt to escape. The water in the tank was replaced at the end of each trial.

2.5. Tail suspension test

The tail suspension test was performed as previously reported (Ueno et al., 2022). Briefly, mice were suspended 35 cm above a table with tape placed 1 cm from the tip of the tail. The entire experiment, which lasted for 6-min, was video-recorded, and the immobility time during the last 4 min was quantified.

2.6. Morris water maze test

The Morris water maze (MWM) test was used to evaluate the spatial learning and memory abilities of the mice. The water maze consisted of a circular black water tank (100 cm in diameter and 30 cm in height; divided into four quadrants) containing opaque water (21–22°C and 25 cm in depth). An escape platform (10 cm in diameter and 24 cm in height) was placed in one of the quadrants (considered the target quadrant). The test was divided into two parts, namely, a learning period and a memory period. During the learning period, mice were randomly placed in the water in any of the four quadrants with their heads facing the wall of the pool during each test. If the mice climbed onto the platform within the prescribed time (60 s), they were allowed to rest on the platform for 30 s. If the mice failed to find the platform within 60 s, they were guided to the platform and allowed to rest on it for 30 s. Each mouse performed the test four times a day at 15-min intervals for 7 days. During the memory period, the platform was removed and the mice were placed in the water in the quadrant opposite the target quadrant and allowed to explore freely for 60 s. ANY-Maze software (Stoeling) was used to analyze the escape latency, distance swam, and swimming velocity of the mice in the learning period, as well as the percentage of time spent and distance swam in the target quadrant in the memory period.

Table 1
Primer sequence.

Gene	Amplicon Size (bp)	Forward primer (5′→3′)	Reverse primer (5′→3′)
β-actin	120	AGTGTGACGTTGACATCCGT	TGCTAGGAGCCAGAGCAGTA
PSD−95	110	GCTCCCTGGAGAATGTGCTA	TGAGAAGCACTCCGTGAACT
SYN	124	GCCTACCTTCTCCACCCTTT	GCACTACCAACGTCACAGAC
BDNF	94	TTACTCTCCTGGGTTCCTGA	ACGTCCACTTCTGTTTCCTT
TrkB	104	TCTGGAGGGTGCTATGCTAT	GGGGCAGAAACTCCAGAAAA

2.7. Tissue preparation

Mice were anesthetized with 2 % sodium pentobarbital and euthanized. Brain tissue was quickly obtained from the executed mice on ice, and brain slices containing the hippocampus were cut with a vibrating microtome for electrophysiological experiments. Alternatively, hippocampal tissue was taken out on ice, snap-frozen in liquid nitrogen, and subsequently placed in a −80 ℃ refrigerator for western blotting and Reverse transcription-quantitative polymerase chain reaction tests.

2.8. Western blotting

After euthanasia, hippocampal tissue was dissected and stored at −80°C until analysis. Hippocampal tissue was lysed in RIPA lysis buffer (Beyotime; P0013B) and centrifuged at 12,000 × g for 15 min. Equal amounts of protein were separated by SDS–PAGE and then transferred to PVDF membranes (Millipore; IPVH00010). After blocking at room temperature for 2 h, the membranes were incubated first with primary antibodies—rabbit anti-PSD-95 (1:2000; Abcam), rabbit anti-SYN (1:1000; Bioss), rabbit anti-BDNF (1:1000; Boster), rabbit anti-TrkB (1:1000; Abcam), and mouse anti-GAPDH (1:1000; Zsbio)—overnight at 4°C and then with the corresponding secondary antibody (horseradish peroxidase [HRP]-labeled goat anti-mouse or anti-rabbit IgG (1:20,000; Zsbio) for 2 h. An ECL kit (Thermo; 340958) was used for protein detection and ImageJ software was used for quantification.

2.9. Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from hippocampal tissue using Trizol reagent (Life Technologies; 15596018) and tested for purity using a spectrophotometer. The extracted RNA was reverse transcribed to cDNA using an RT Kit from TaKaRa (RR047A), and the cDNA was then used as a template for fluorescence qPCR. The reaction system comprised 5 μL of 2× SYBR Green Mix, 1 μL of each forward and reverse primer, 1 μL of cDNA, and 2 μL of RNase-free water. The reaction conditions were as follows: one cycle of pre-denaturation at 95°C for 1 min, followed by 40 cycles of 95°C for 20 s and 60°C for 1 min. The primer sequences are shown in Table 1.

2.10. Electrophysiology

The mice were anesthetized by an intraperitoneal injection of sodium pentobarbital and euthanized. The 15–20 ml slice solution of pre-cooled and saturated mixed oxygen (95 % O_2 +5 % CO_2) was used for cardiac perfusion. The brain tissue was then removed from the cranial cavity and placed in the slicing tray of the vibrating microtome. Then the brain tissue is quickly immersed in the slice solution and serially sectioned into 400-μm-thick slices containing the dorsal hippocampus using a vibrating microtome. Before recording, the sections were incubated for 1 h in artificial cerebrospinal fluid (aCSF) consisting of 130 mM NaCl, 25 mM NaHCO3, 10 mM D-glucose, 4.4 mM KCl, 2 mM MgCl2, 1.25 mM KH2PO4•H2O, and 2 mM CaCl2•6H2O, with 95 % O_2 and 5 % CO_2. A tungsten wire stimulating electrode was placed in the hippocampal CA3 area and a glass recording electrode was placed in the CA1 area for the recording of field excitatory postsynaptic potentials (fEPSPs). Once the field potential was amplified, the output signal was processed. After identifying the smallest stimulus that could induce a fEPSP, the stimulus intensity was gradually increased, and the amplitude of the presynaptic action potential and post-synaptic fEPSP caused by each stimulus was

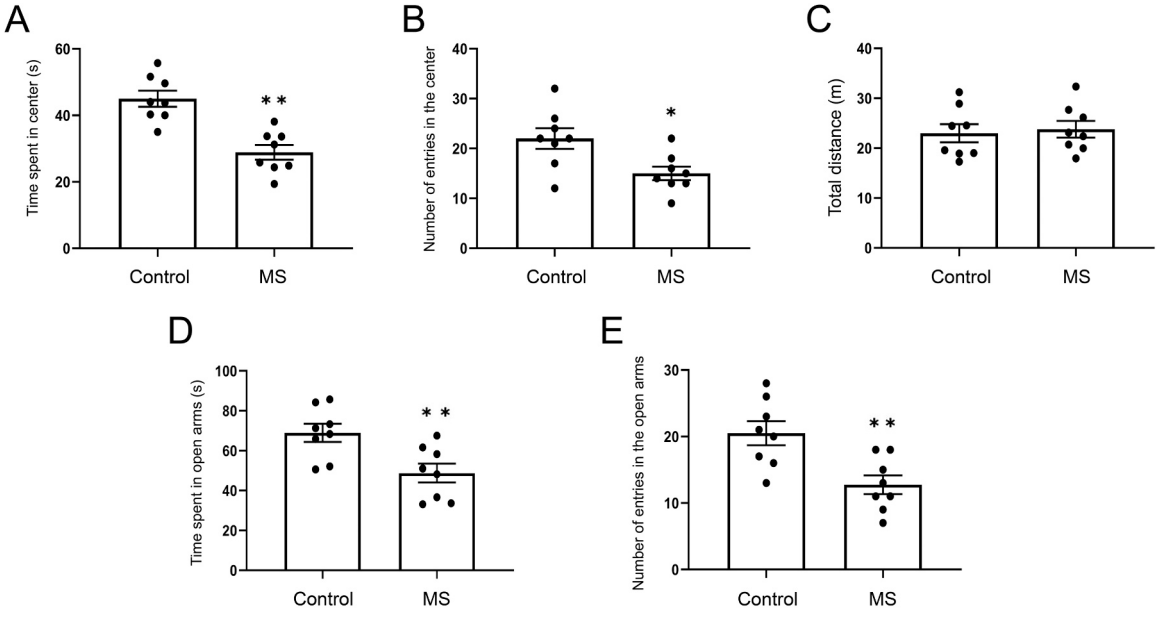

Fig. 1. Determination of the effects of maternal separation on anxiety-like behaviors. (A) The time spent in and (B) the number of entries into the central area in the open field test. (C) The total distance moved in the whole arena in the open field test. (D) The time spent in and (E) the number of entries into the open arms in the elevated plus maze test. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Control group, n = 8 mice.

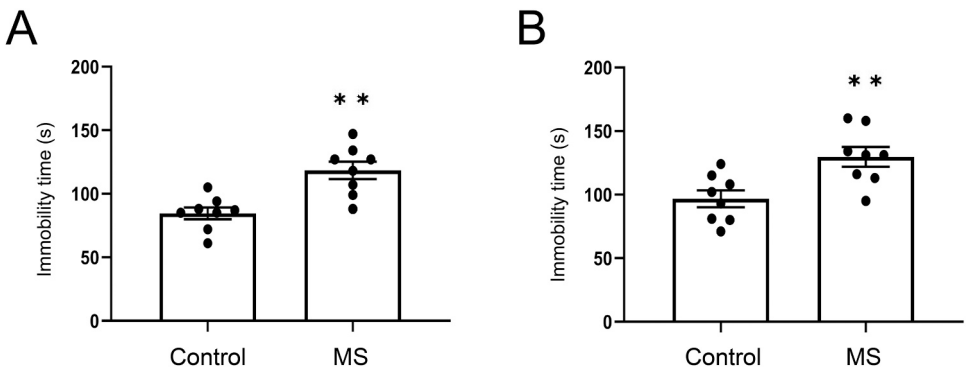

Fig. 2. Maternal separation (MS) increased depression-like behaviors. (A) The immobility time in the forced swimming test. (B) The immobility time in the tail suspension test. $^{**}P < 0.01$ vs. the Control group, n = 8 mice.

recorded. The width of the square wave stimulus was 0.1 ms and the stimulus frequency was 0.033 Hz. A stimulus intensity that could induce 50 % of the maximal fEPSP amplitude was used as the test stimulus intensity and stable baseline fEPSPs were recorded for 30 min. A double pulse stimulation with time intervals of 20, 40, 60, 80, 100, 150, 200, 400, 600, 800, and 1000 ms was applied, following which the ratio of the amplitude of the second fEPSP to that of the first fEPSP was calculated. LTP was induced by high-frequency electrical stimulation (100 Hz, 1000 ms ×2, 30-s interval) and LTD by low-frequency electrical stimulation (1–2 Hz, 1000 m s ×2, 30-s interval), and both were recorded for 90 min after induction. mEPSCs of hippocampal CA1 pyramidal neurons were recorded using a pipette filled with an internal solution consisting of 140 mM CsCl, 2 mM MgCl, 2 mM CaCl, 10 mM EGTA, 10 mM HEPES, 2 mM ATP-Na, pH7.3 (with 5 N CsOH). The resistance of the micropipettes ranged from 3 to 5 MΩ. Bicuculline methiodide (10 μM) and TTX (1 μM) were added to the aCSF to block GABA receptors and Na^+ channels, respectively. Data acquisition was performed with PatchMaster v2.73 with holding at −65 mV. Mini Analysis Program v6.0.3 was used to automatically detect mEPSCs. The average value from 40 to 120 min to calculate the fEPSP slop.

2.11. Data analysis

All data were analyzed using GraphPad Prism 8.0 and are expressed as means ± standard error of the mean. Behavioral data were analyzed using two-tailed, unpaired t-tests, except for the training in the MWM

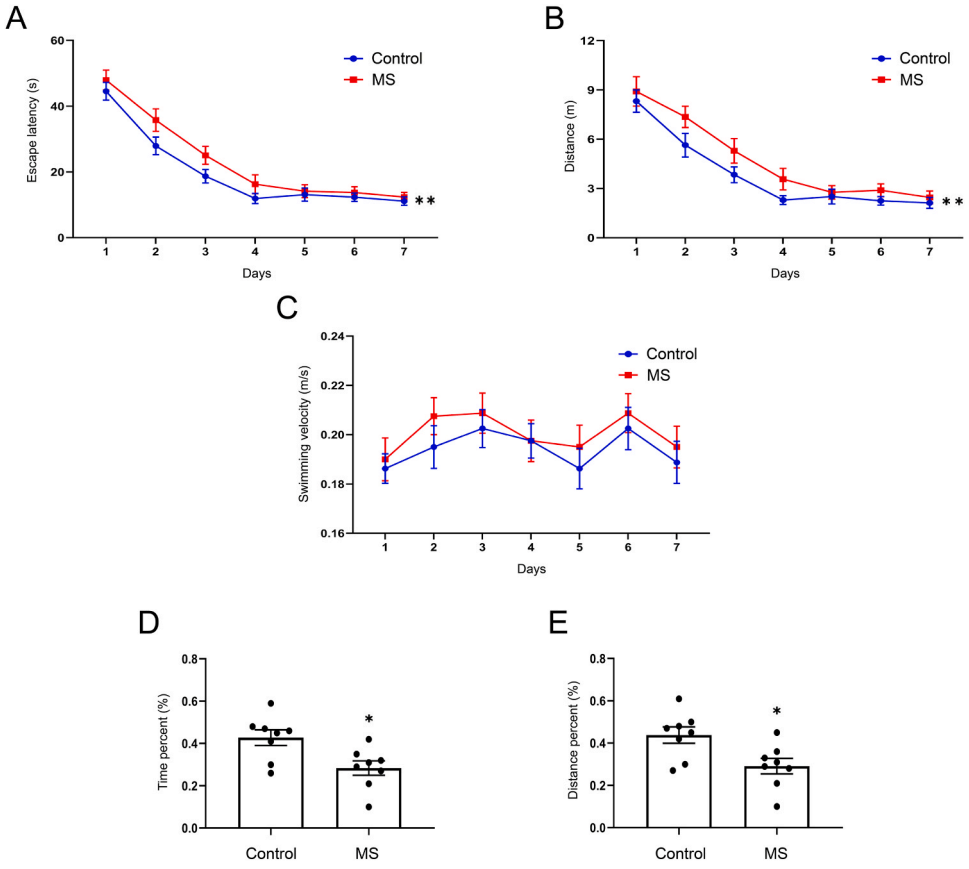

Fig. 3. Maternal separation (MS) impaired spatial learning and memory function. (A) The escape latency, (B) distance swam, and (C) swimming velocity in the learning phase of the Morris water maze test. (D) The percent time and (E) the percent distance swam in the target quadrant in the memory phase of the Morris water maze test. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Control group, n = 8 mice.

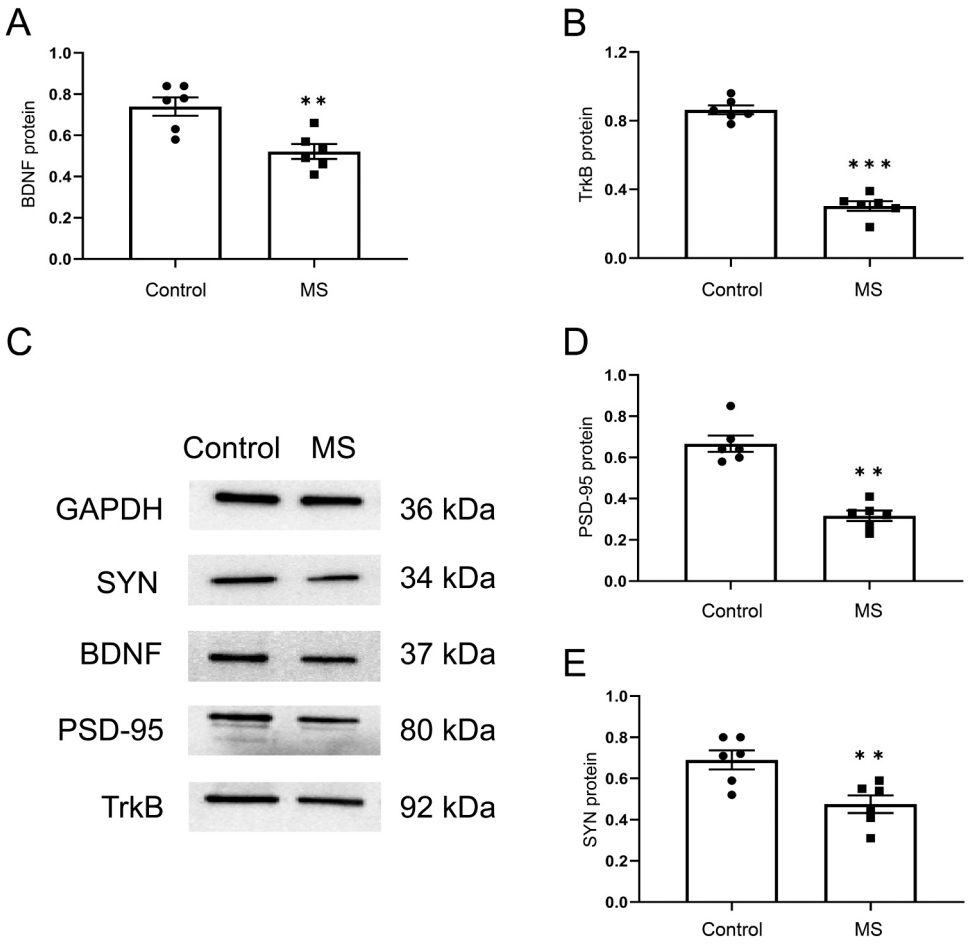

Fig. 4. Maternal separation (MS) decreased the protein levels of synaptic plasticity-associated proteins in the hippocampus **(full-length blots/gels are presented in Additional** Fig. 1). (A) The protein levels of BDNF and (B) TrkB in the hippocampus. (C) Representative western blot for BDNF, TrkB, PSD-95, and SYN in the hippocampus. (D) The protein levels of PSD-95 and (E) SYN in the hippocampus. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the Control group, n=6 hippocampus from 6 mice.

test, where escape latency, distance swam, and swimming velocity were analyzed using two-way repeated-measures ANOVA. Western blotting, RT-PCR, and electrophysiological recording data were also compared using two-tailed, unpaired t-tests. P-values <0.05 were considered significant.

3. Results

3.1. MS increased anxiety-like behaviors in offspring mice

We first evaluated the effects of MS on anxiety-like behaviors in offspring mice. The results showed that the time spent in and the number of entries into the central area in the open field test were significantly reduced in offspring mice of the MS group compared with that in animals in the Control group (Time: Control: 45±2.42 s; MS: 28.89±2.20 s; $t = 4.92$, $P < 0.05$; Entries: Control: 22±2; MS: 15±1; $t = 2.80$, $P < 0.05$; Fig. 1A, B). No difference in total distance moved in the open field test was observed between the two groups (Fig. 1C). In order to further assess the influence of MS on anxiety levels, we introduced another behavioral model of anxiety, the elevated plus maze test. The results showed that the offspring mice in the MS group spent less time in and had fewer entries into the open arms when compared with control animals (Time: Control: 68.94±4.57 s; MS: 48.74±4.70 s; $t = 3.08$, $P < 0.05$; Entries: Control: 20±2; MS: 12±1; $t = 3.37$, $P < 0.05$; Fig. 1D, E). These results suggest that MS increases anxiety-like behaviors in offspring mice.

3.2. MS increased depression-like behaviors in offspring mice

Next, we wanted to determine the influences of MS on depression-like behaviors in offspring mice. The results showed that the offspring mice subjected to MS displayed longer immobility time when compared with animals of the Control group in the forced swimming test (Control: 84.63±4.70 s; MS: 118.40±6.87 s; $t = 4.06$, $P < 0.05$; Fig. 2A). To further identify the increase in depression, we also evaluated the immobility time in the tail suspension test. Similarly, immobility time was significantly increased in offspring mice of the MS group compared with that in mice of the Control group in the tail suspension test (Control: 96.75±6.60 s; MS: 129.80±7.81 s; $t = 3.23$, $P < 0.05$; Fig. 2B). These results suggest that MS increases depression-like behaviors in offspring mice.

3.3. MS impaired hippocampal-dependent learning and memory in offspring mice

To determine the effect of MS on spatial learning and memory, we subsequently subjected the offspring mice to the MWM test. In the learning phase of the test, for all the offspring mice, the escape latency and distance swam gradually declined with an increasing number of training days (Escape latency: $F_{(6,84)} = 62.00$, $P < 0.01$; Distance: $F_{(6,84)} = 36.21$, $P < 0.01$; Fig. 3A, B). The offspring mice of the MS group spent more time and swam longer distances locating the hidden platform when compared with offspring mice of the Control group (Escape latency: $F_{(1,14)} = 10.77$, $P < 0.01$; Distance: $F_{(1,14)} = 12.61$, $P < 0.01$;

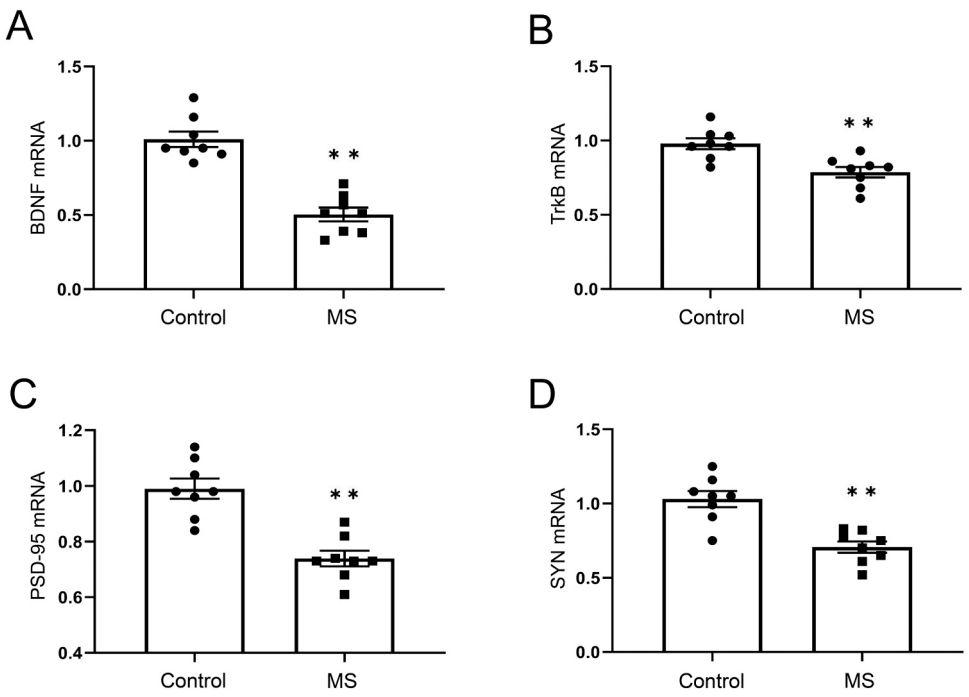

Fig. 5. Maternal separation (MS) decreased the relative mRNA levels of synaptic plasticity-associated proteins in the hippocampus. (A) The relative mRNA levels of BDNF and (B) TrkB in the hippocampus. (C) The relative mRNA levels of PSD-95 and (D) SYN in the hippocampus. $^{**}P < 0.01$ vs. the Control group, n=8 hippocampus from 8 mice.

Fig. 3A, B). No difference in swimming velocity was detected between the two groups (Fig. 3C).

In the memory phase, during which the hidden platform was removed, offspring mice of the MS group spent significantly less time and swam significantly shorter distances in the target quadrant than offspring animals in the Control group. (Time percent: Control: 0.44 ±0.04; MS: 0.29±0.04; $t = 2.86$, $P < 0.05$; Distance percent: Control: 0.44±0.04; MS: 0.29±0.04; $t = 2.74$, $P < 0.05$; Fig. 3D, E). These results suggest that MS significantly impaired spatial learning and memory function in offspring mice.

3.4. MS decreased the mRNA and protein levels of BDNF, TrkB, PSD-95, and SYN

It is well known that the levels of synaptic plasticity-associated proteins in the hippocampus were associated with synaptic plasticity and cognitive function. So. we measured the mRNA and protein levels of BDNF, TrkB, PSD-95, and SYN in the hippocampus by RT-PCR and western blotting, respectively. The results showed that the mRNA and protein levels of BDNF, TrkB, PSD-95, and SYN were lower in the MS group than in the Control group (protein: BDNF: Control: 0.74±0.04; MS: 0.52±0.04; $t = 3.80$, $P < 0.01$; TrkB: Control: 0.86±0.03; MS: 0.30 ±0.03; $t = 14.61$, $P < 0.01$; PSD-95: Control: 0.67±0.04; MS: 0.32±0.03; $t = 7.43$, $P < 0.01$; SYN: Control: 0.69±0.05; MS: 0.48±0.04; $t = 3.41$, $P < 0.01$; Fig. 4A–E, Additional Fig. 1**;** mRNA: BDNF: Control: 1.01±0.05; MS: 0.50±0.05; $t = 7.26$, $P < 0.01$; TrkB: Control: 0.99±0.04; MS: 0.79 ±0.04; $t = 3.74$, $P < 0.01$; PSD-95: Control: 0.99±0.04; MS: 0.74±0.03; $t = 5.50$, $P < 0.01$; SYN: Control: 1.03±0.05; MS: 0.71±0.04; $t = 4.90$, $P < 0.01$; Fig. 5A–D).

3.5. MS impaired LTP, excitatory synaptic transmission and increased LTD in the hippocampus

Growing evidence indicates that hippocampal synaptic plasticity including LTP and LTD plays an important role in regulating learning and memory, and MS impairs hippocampal synaptic plasticity. Thus, were recorded LTP and LTD at Schaffer collateral/CA1 synapses. The results showed that high-/low-frequency stimulation induced normal LTP/LTD in offspring mice of the Control group. Furthermore, hippocampal-dependent LTP was impaired in animals of the MS group, namely, the mean fEPSP slope was significantly lower in the MS group (Control: 1.77±0.09; MS: 1.27±0.06; $t = 4.66$, $P < 0.01$; Fig. 6A, B). Regarding LTD, the mean fEPSP slope was lower in the MS group than in the Control group, indicating the LTD was enhanced by MS (Control: 0.76±0.04; MS: 0.47±0.01; $t = 6.96$, $P < 0.01$; Fig. 6C, D , E). At last, we further investigated the effect of maternal separation on excitatory synaptic transmission in the CA1 pyramidal neurons from hippocampal slices. The results showed that the frequency of mEPSCs was markedly reduced in the offspring of the MS group compared with that in offspring of the Control group (Control: 3.07±0.22; MS: 0.95±0.17; $t = 7.54$, $P < 0.01$; Fig. 7A, B). No difference in mEPSC magnitude was observed between the two groups (Control: 13.73±0.89 pA; MS: 13.97±1.08 pA; $t = 0.17$, $P = 0.87$; Fig. 7C). Collectively, these results suggest that maternal separation disrupts hippocampal LTP and LTD, and impaired excitatory synaptic transmission, thereby may contribute to learning and memory deficits in the offspring mice.

4. Discussion

In this study, we found that offspring male CD-1 mice subjected to MS exhibited anxiety- and depression-like behaviors as well as spatial learning and memory deficits. Furthermore, MS led to a significant decrease in the expression levels of BDNF, TrkB, PSD-95, and SYN in the hippocampus and impaired synaptic function, as determined through the recording of LTP/LTD and mEPSCs in the hippocampus of offspring mice that experienced MS.

4.1. MS increased anxiety- and depression-like behaviors and promoted cognitive impairment in offspring mice

Brain structure and behavioral phenotypes can be modified by the external environment (Cavalcanti et al., 2020; Mooldijk et al., 2021).

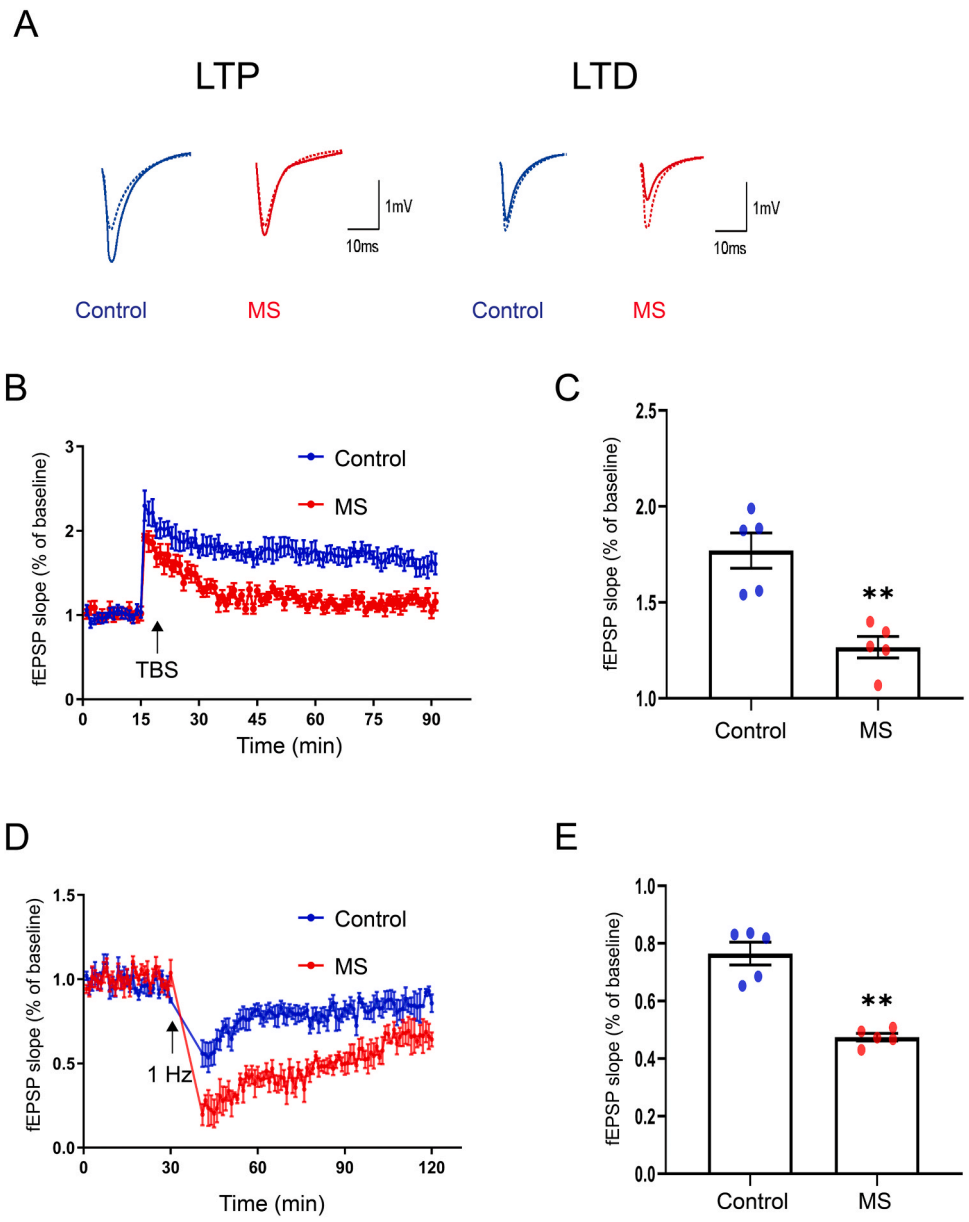

Fig. 6. Maternal separation (MS) altered hippocampal long-term potentiation (LTP) and long-term depression (LTD) at Schaffer collateral/CA1 synapses. (A) Sample records of field excitatory post-synaptic potentials (fEPSP) recording between two groups. Traces were recorded before (dotted line) and after (full line) LTP and LTD conditioning, scale bar 1 mV/10 ms. (B) Plots of normalized slopes of field excitatory post-synaptic potentials showing that theta-burst stimulation (TBS) induced significantly smaller LTPs in male offspring in the MS group. (C) Percent change in LTP magnitude. (D) Plots of normalized slopes of field excitatory post-synaptic potentials showing that low-frequency stimulation (LFS) induced a significantly higher LTD in male offspring of the MS group than in those of the Control group. (E) Percent change in LTD magnitude. $^{**}P < 0.01$ vs. the Control group, n = 2 slices from 5 per mice.

The lactation period is critical for brain development, and, during this period, the brain is highly susceptible to interference from environmental factors (Miyazaki et al., 2020; Gawlińska et al., 2021). Clinical studies have reported that adults who lose a parent in childhood are at increased risk of a range of psychiatric disorders, including anxiety, depression, and cognitive decline (Harris et al., 1986; Zhao et al., 2022). The results of preclinical studies showed that adolescent Wistar rats exposed to MS-induced stress display anxiety-like behavior in the open field test (Joushi et al., 2021a, 2021b). Consistent with this observation, we found that adult male CD-1 mice subjected to MS exhibited anxiety-like behavior in the open field test (a decrease in the time spent in the central area) and the elevated plus maze test (a decrease in the time spent exploring the open arms) relative to that seen in control animals. In addition, based on the results of the forced swimming test and the tail suspension test, MS increased depressive-like behavior in offspring mice, which was consistent with a previous study showing that MS increased depression-like behavior in adult c57BL/6 J offspring mice as evaluated using the sucrose preference test (Wu et al., 2023). However, we also found that offspring mice that experienced MS showed hippocampal-dependent learning and memory impairment in the MWM test. These results are inconsistent with those of previous studies in which MS was reported to ameliorate cognitive impairment in offspring Wistar rats during the partially baited radial-arm maze task (Kambali et al., 2019). The contradictory results might be explained by differences in the MS protocol employed, the time points examined, and/or the animal strains used, a possibility that requires further investigation.

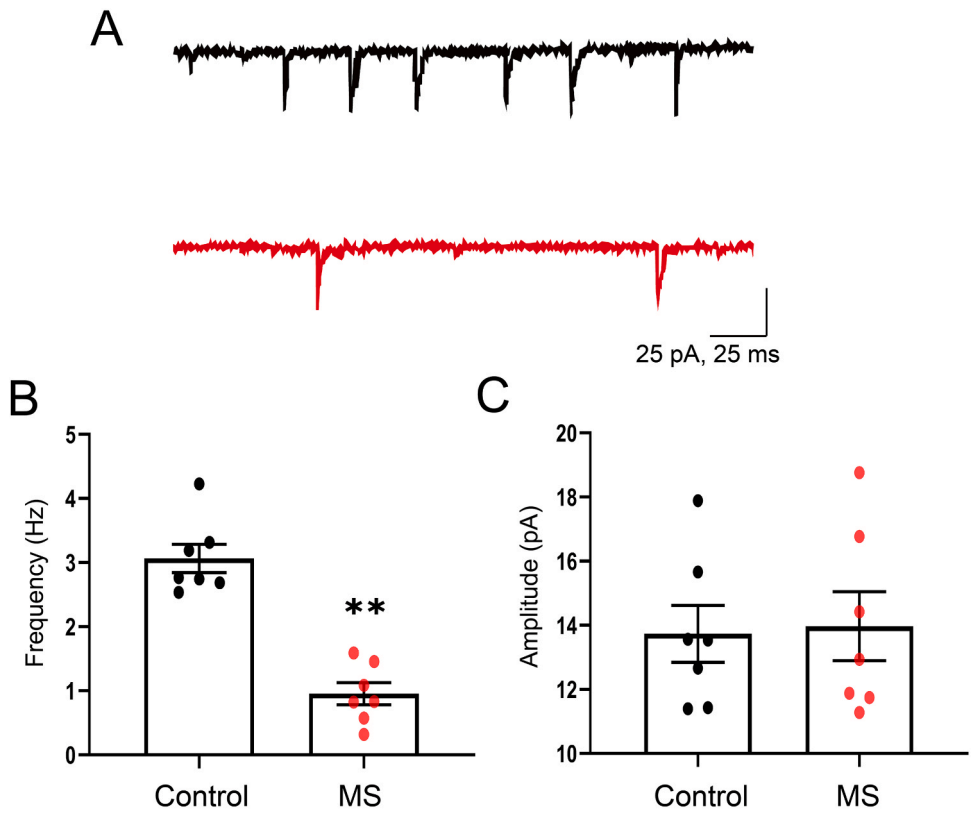

Fig. 7. Maternal separation (MS) impaired excitatory synaptic transmission. (A) Offspring in the MS group showed significantly reduced miniature excitatory postsynaptic current (mEPSC) amplitude compared with the Control group. (B) No difference in mEPSC amplitude was observed between the MS group and the Control group. $^{**}P < 0.01$ vs. the Control group, n = 7 neurons from 3 mice.

4.2. MS altered synaptic plasticity and synaptic transmission in the hippocampus

It is widely acknowledged that early life stress has profound effects on hippocampal neural circuits, dendritic remodeling, synaptic transmission, and synaptic plasticity (Gruss et al., 2008; Solarz et al., 2021). Induced LTP and LTD are cellular processes that can be used to model synaptic plasticity and are closely associated with cognitive function. LTP is believed to be the cellular mechanism underlying the acquisition of new memories, while LTD is involved in suppressing previously acquired memories (Sun et al., 2022). One study showed that early life stress-induced learning and memory dysfunction are associated with impaired LTP and enhanced LTD (Kim et al., 1996). Offspring Sprague–Dawley rats separated from their mothers from PND14 to PND21 showed impaired LTP and cognitive impairment (Cao et al., 2014). Meanwhile, offspring C57BL/6 J mice exposed to MS from PND2 to PND17 displayed a marked increase in LTD at CA3-CA1 synapses and impaired spatial memory (Talani et al., 2023). Furthermore, environmental enrichment and pharmacological intervention mitigated MS-induced cognitive decline in offspring mice by reversing LTP impairment (Joushi et al., 2021a, 2021b; Shahraki et al., 2022). In the present study, our results showed that MS from PND4 to PND21 exerted significant negative effects on LTP and enhanced LTD in hippocampal CA3-CA1 synapses of adult male offspring CD-1 mice, which contributed to the learning and memory impairment induced by MS. Neuronal activity also plays an important role in the regulation of learning and memory function. Yang et al. (2021) reported that neuronal activity was decreased in the prelimbic cortex of mice following anesthesia combined with surgery, concomitant with cognitive dysfunction (Yang et al., 2021a, 2021b). MS can decrease neuronal activity by regulating the balance between excitatory and inhibitory synaptic transmission. An enhancement of GABAergic miniature inhibitory postsynaptic current (mIPSC) frequency and a decrease in glutamatergic mEPSC amplitude were observed in male mice exposed to repeated MS (Talani et al., 2023). However, our results suggested that MS significantly reduced the frequency of glutamatergic mEPSCs in hippocampal CA1 neurons without changing their amplitude. We think that the differences in the electrophysiological characteristics of hippocampal neurons between the two studies might be due to the different MS protocols used.

4.3. MS decreased the levels of synaptic plasticity-associated proteins in the hippocampus

BDNF is the best-characterized neurotrophic factor and is highly expressed in the hippocampus, where it regulates synaptic plasticity, synaptic transmission, and cognitive function by binding to its receptor TrkB (Burnouf et al., 2013; Ma et al., 2014; Xu et al., 2021). Treatment of hippocampal slice with BDNF significantly increased the number of docked synaptic vesicles in excitatory synapses and increased the expression levels of synaptotagmin, synaptophysin, and synaptobrevin proteins, which are closely related to synaptic vesicular release. BDNF/TrkB signaling activates the PI3K/AKT and PLC-γ pathways, thereby inducing LTP and increasing synaptic transmission (Numakawa et al., 2018; Zheng et al., 2019). Furthermore, hippocampus-specific BDNF knockout mice showed deficits in LTP and cognitive impairment (Patterson et al., 1996; Heldt et al., 2007), while the BDNF/TrkB signaling pathway was reported to be inhibited in nerve-injured mice that displayed impaired synaptic plasticity and long-term memory deficits (Wang et al., 2019). Similarly, we found that the expression levels of BDNF and TrkB were significantly decreased in the hippocampus of offspring mice subjected to MS, implying that the BDNF/TrkB signaling pathway was inhibited in the hippocampus of these mice. The down-regulation of the BDNF/TrkB signaling pathway in the hippocampus may have contributed to the impaired synaptic plasticity and

transmission, and cognitive impairment induced by MS in offspring CD-1 mice. SYN is a presynaptic vesicle transmembrane protein associated with the release of synaptic vesicles, while PSD-95 is a scaffolding protein essential for glutamate clustering in the postsynaptic membrane. The expression levels of these two proteins are closely related to synaptic plasticity and cognitive function (Liu et al., 2018; Jia et al., 2023). Importantly, the frequency of glutamatergic mEPSCs is associated with the probability of presynaptic synaptic vesicle release and the amplitude of glutamatergic mEPSCs is related to glutamate receptor density in the postsynaptic membrane, which are closed related the levels of SYN and PSD-95 (Levine et al., 1995; Xiao et al., 2001; Huang and Moser, 2018). In the current study, we found that MS decreased SYN expression levels in the hippocampus, explaining the observed decrease in glutamatergic mEPSC frequency. However, the detected decrease in PSD-95 expression levels did not alter the amplitude of glutamatergic mEPSCs, perhaps through a compensatory increase in the expression of other postsynaptic proteins. Future research should delve deeper into the molecular mechanisms that underlie compensation of postsynaptic current amplitudes.

Despite the importance of our findings, our study had several limitations. First, the association between MS-induced learning and memory impairment and synaptic dysfunction was only detected at the phenomenon level. We did not seek to improve MS-induced cognitive deficits by ameliorating synaptic dysfunction. Secondly, we did not evaluate changes in behavior or molecular biological indicators in female offspring mice because sex-specific differences have been reported among offspring of MS-exposed mice (Bachiller et al., 2020; Grochecki et al., 2022). Finally, we did not examine the effect of MS on markers of synaptic plasticity in other regions associated with cognitive function.

4.4. Conclusion

Combined, our findings showed that MS increased the levels of anxiety and depression and impaired learning and memory function in adult offspring CD-1 mice. Additionally, MS impaired LTP, enhanced LTD induction, and decreased synaptic transmission in hippocampal slices of offspring mice. These results suggested that synaptic dysfunction may be a possible underlying mechanism for MS-induced cognitive impairment.

Ethics approval

All animal experiments complied with the ARRIVE guidelines, the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996, and the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences of the Anhui Medical University (NO.LLSC20190710).

Consent to publish

All authors reviewed and approved the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Data availability

Data will be made available on request.

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