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Cognitive impairment following maternal separation in rats mediated by the NAD^+/SIRT3 axis via modulation of hippocampal synaptic plasticity

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Maternal separation (MS) during early life can induce behaviors in adult animals that resemble those seen in schizophrenia, manifesting cognitive deficits. These cognitive deficits may be indicative of oxidative stress linked to mitochondrial dysfunction. However, there is limited understanding of the molecular mechanisms regulating mitochondria in neural circuits that govern cognitive impairment relevant to schizophrenia, and their impact on neuronal structure and function. A 24-h MS rat model was utilized to simulate features associated with schizophrenia. Schizophrenia-associated behaviors and cognitive impairment were assessed using the open field test, pre-pulse inhibition, novel object recognition test, and Barnes maze test. The levels of mitochondrial proteins were measured using western blot analysis. Additionally, alterations in mitochondrial morphology, reduced hippocampal neuronal spine density, and impaired LTP in the hippocampus were observed. Nicotinamide (NAM) supplementation, administration of honokiol (HNK) (a SIRT3 activator), or overexpression of SIRT3 could inhibit cognitive deficits and cellular dysfunction. Conversely, administration of 3-TYP (a SIRT3 inhibitor) or knocking down SIRT3 expression in control rats led to deficits in behavioral and hippocampal neuronal phenotype. Our results suggest a causal role for the NAD+/SIRT3 axis in modulating cognitive behaviors via effects on hippocampal neuronal synaptic plasticity. The NAD+/SIRT3 axis could be a promising therapeutic target for addressing cognitive dysfunctions, such as those seen in schizophrenia.

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INTRODUCTION

Cognitive impairment is a common feature of severe mental illness. Maternal separation (MS) is an early life intervention that can produce behaviors of adult animals reminiscent of schizophrenia, including cognitive deficits [1]. Cognitive impairment has been considered as a core symptom domain of schizophrenia [2] in which the hippocampus has been strongly implicated [3–6]. In animal models of schizophrenia, hippocampal neuronal dysfunction has also been associated with cognitive deficits. In particular, the impairment of hippocampal neuronal dendritic complexity has been shown to contribute causally to stress-induced cognitive deficits [7–9]. Given that neuronal morphology is a strong determinant of synaptic connectivity and strength, understanding the factors that control hippocampal neuronal dendritic regulation may help in developing treatments to ameliorate cognitive deficits.

Mitochondria appear to be ideally suited to contribute. Growing evidence points to a central role of mitochondria in the etiology of psychiatric disorders [10]. In neurons, mitochondria support metabolic demands through energy supply [11, 12]. Cell cultures and neurodevelopmental studies have implicated mitochondria in the regulation of both dendritic arborization [13–17] and spine and synapse formation [18, 19]. However, whether mitochondrial dysfunction impairs hippocampal neuronal dendritic complexity and consequently contributes to cognitive deficits associated with schizophrenia remains unknown.

Our previous studies have suggested that degradation of nicotinamide adenine dinucleotide (NAD+) and associated bioenergetics failure of cellular metabolism may be one of the major factors leading to neuronal damage [20, 21]. NAD+ is an essential cofactor in most enzymatic reactions supporting fundamental mitochondrial functions including oxidative phosphorylation and enzymatic reactions of the tricarboxylic acid cycle [22–24]. When NAD+ is degraded, mitochondria become incapable of ATP synthesis. Previous study has demonstrated that when naïve mice were treated with an NAD+ precursor, neuronal mitochondrial function recovers [25], and an increase in mitochondrial NAD+ will reduce acetylation of mitochondrial proteins and reactive oxygen species (ROS) generation in hippocampal tissue [26]. Furthermore, a high NAD+ level facilitates the activation of proteins involved in mitochondrial quality control, such as Sirtuin3 (SIRT3). SIRT3 reinforces mitochondrial antioxidant

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defense by deacetylating and increasing the activity of superoxide dismutase 2 (SOD2) [27, 28]. However, it is not clear whether the mitochondria-related NAD+/SIRT3 axis is involved in hippocampal neuronal synaptic plasticity and the schizophrenia associated cognitive impairment.

Mother-infant interaction may be a key factor in brain maturation, and stress associated with MS may induce the development of psychosis or susceptibility to psychotic disorders [20, 21]. The abnormal behaviors and molecular changes taking place after a single 24 h period of MS on a postnatal day (PND) 9 in rats successfully recapitulate several features of schizophrenia [29–31]. Therefore, the MS rat model has become a powerful tool for exploring the neurobiological bases of schizophrenia.

MATERIALS AND METHODS

Animals and maternal separation

Forty nulliparous female and forty male eight-week-old Wistar rats were obtained from Beijing Vital Rival Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats of the same sex were caged together, with 3 or 4 per cage. The animals were mated at the age of 3 months and the males were removed one week later. The mated female rats were housed individually in ventilated plastic cages in a temperature- and humidity-controlled (22 ± 2 °C, 50 ± 10%) holding facility with a constant 12 h day-night cycle (lights: 08:00 – 20:00). All animals had free access to food and tap water. The MS protocols were performed according to previous studies [30, 32]. Females were checked twice daily for delivery (08:00 and 17:00). The day of delivery was considered as PND 0. Each pregnant rat delivered an average of 10 ± 2 offspring. On PND9, litters were randomly assigned to one of the two groups: the MS or control groups. In brief, the mothers were removed at 10:00. The pups remained in their home cages with the heated mat for 24 h, after which the mothers were returned to their cages. The control groups grew naturally to adulthood. All the litters were otherwise left undisturbed except for the routine cleaning of the cages. On PND 21, the MS and the control groups of pups were weaned, and then group-housed by sex (3–4 per cage). All subsequent assessments were carried out only on male offspring to avoid the effects of estrogen on the regulation of neuronal activity and animal behavior [33, 34]. All procedures involving animals were approved and carried out according to the guidelines of the Institutional Animal Care Committee of Renmin Hospital of Wuhan University.

Experimental design

Experiment 1: On PND9, the dams and their pups were randomly assigned to either the control group or the MS group. On PND10, after MS, each infant group was further divided into several subgroups with approximately 20 pups in each subgroup. The four groups were as follows: control group receiving vehicle (Control+Vehicle), MS group receiving vehicle (MS +Vehicle), control group receiving nicotinamide (NAM) (Control+NAM), and MS group receiving NAM (MS+NAM). The NAM groups received oral gavage of NAM (100 mg/kg/d, diluted in vehicle) for 30 days from PND56 to PND85. The dosage and treatment protocol for NAM were based on previous studies [35, 36]. The vehicle groups received daily saline (1 ml/kg) from PND56 to PND85 (see supplementary Fig. 1).

Experiment 2: Pups from both the MS and control groups were randomly divided into two subgroups of 15 each and were intraperitoneally injected with 3-TYP (a SIRT3 inhibitor; 10 mg/kg/d; Cayman Chemical Company, Ann Arbor, MI; CAS: 120241-79-4) [37], honokiol (HNK) (a SIRT3 activator, 10 mg/kg/d; Cayman Chemical Company; CAS: 35354-74-6) [38] or vehicle for 15 days. The vehicle solution consisted of 90% saline and 10% DMSO (1 ml/kg/d) (see supplementary Fig. 1).

Experiment 3: The 36 MS pups were divided into three groups: MS +Vehicle, MS+NAM, and MS+NAM+3-TYP. The MS+NAM+3-TYP group received NAM by gavage for 30 days and intraperitoneal injections of 3-TYP (10 mg/kg) during the last 15 days. The MS+NAM group received NAM by gavage for 30 days. The MS+Vehicle group received saline (1 ml/kg/d) by gavage for 30 days and the vehicle solution (90% saline and 10% DMSO at 1 ml/kg/d i.p.) at the last 15 days (see supplementary Fig. 1).

Experiment 4: AAV9-SIRT3 and AAV9-si SIRT3 were stereotactically injected into rats under isoflurane anesthesia. The bilateral hippocampal CA1 region injections were performed at the following coordinates: −2.4 mm anteroposterior, ±3.75 mm mediolateral from the bregma, and −2.6 mm dorsoventral from the dural surface. A viral suspension (1 µl) containing 2×10^9 vector genomes per µl was infused into each site at a rate of 0.25 µl/min using a 10 µl glass syringe with a fixed needle. After the injection, the needle was left in place for 10 minutes and then slowly removed over 2 minutes. Rats were kept on a heating pad until they fully recovered from anesthesia. Three weeks after the stereotactic injection, a multiple behavioral test was conducted on the rats (see supplementary Fig. 1).

For the rest of the detailed methods, please refer to the supplemental information of this paper, which covers the following aspects:

Behavioral testing of animals
- Open field test (OFT)
- Novel object recognition test
- Barnes maze test
- Elevated-Plus Maze (EPM)
- Sucrose preference test (SPT)
- PPI test

NAD^+ quantification
Protein extraction and western blot analysis
Electron microscopy
Immunofluorescent staining assay
Golgi-cox staining
Electrophysiology

DATA ANALYSIS AND STATISTICS

The data are reported as means ± standard error of the mean (SEM) and were analyzed using SPSS Statistics version 20.0 (SPSS Inc.). Significance in the datasets was assessed through Student's unpaired two-tailed t-tests for comparisons between two groups, while one-way or two-way analysis of variance (ANOVA) was utilized for comparisons involving three or more groups. The results of the Barnes maze test were analyzed by repeated measures two-way ANOVA. Statistical significance was considered at a p-value below 0.05.

RESULTS

Reduced SIRT3 expression was observed in the individuals with schizophrenia

To investigate changes in SIRT3 gene expression in the postmortem hippocampus of schizophrenia patients, an analysis was conducted using the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The database GSE53987 included gene expression data from 15 schizophrenia patients and 18 matched healthy controls. In comparison to age- and sex-matched controls, a significant decrease in SIRT3 mRNA levels was observed in the hippocampus (Supplementary Fig. 2A), but not in the prefrontal cortex (Supplementary Fig. 2B), suggesting potential involvement of hippocampal SIRT3 in schizophrenia pathology. Furthermore, a clinical study revealed reduced SIRT3 protein expression in peripheral blood mononuclear cells (PBMCs) among schizophrenia patients as compared to the healthy controls (Supplementary Fig. 2C, D).

NAM administration restored LTP and hippocampal neuronal dendritic complexity in MS rats

In terms of the synaptic transmission and plasticity characteristics of CA1 synapses, MS rats displayed impaired LTP (Fig. 1A, B) and elevated paired-pulse ratio (PPR) (Fig. 1C). However, NAM treatment effectively reversed the LTP impairment (Fig. 1A, B) and normalized the PPR (Fig. 1C). Consistent with these results, neuronal structure analysis revealed a reduced spine density in MS rats compared to control rats (Fig. 1D, E), with spine density recovering after NAM treatment in MS+NAM rats (Fig. 1D, E).

NAM administration normalized microglial engulfment of hippocampal neuronal spines in MS rats

Microglia play a critical role in the synaptic pruning process [39]. To investigate the underlying cause of reduced hippocampal neuronal spines, we assessed the phagocytic function of microglial

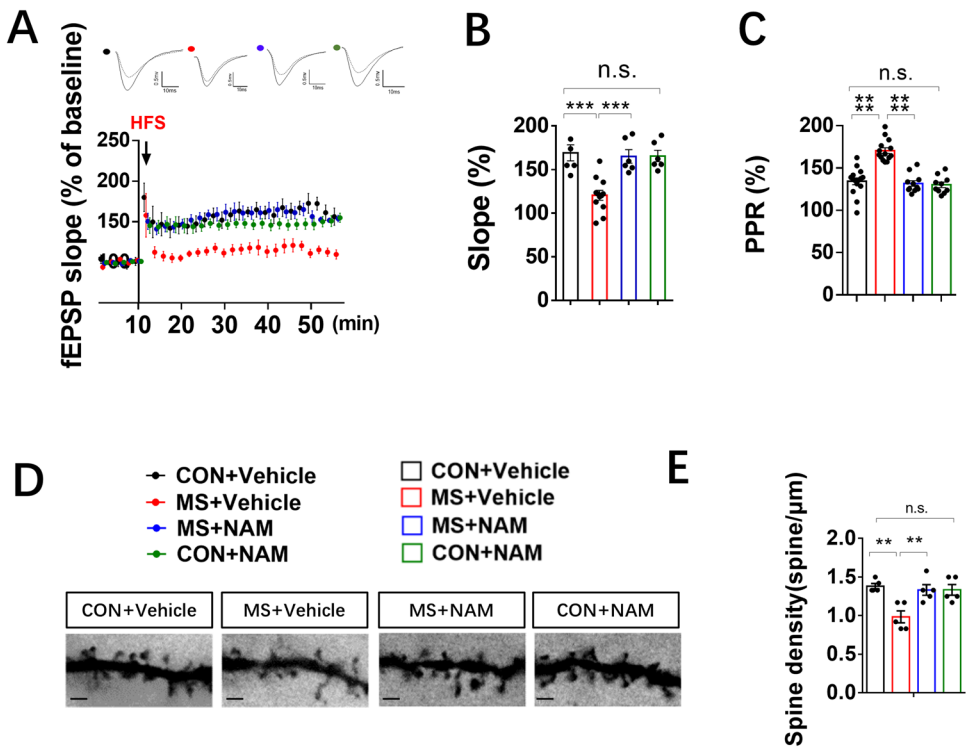

Fig. 1 Administration of NAM restored behavioral and hippocampal cellular phenotypes affected by MS. A NAM administration reversed MS-induced LTP impairment. And NAM administration did not change LTP in group CON+NAM compared with control. Arrows indicate LTP induction. **B** Quantitative analysis of data in (A) (n = 4 per group). **C** NAM administration reversed MS-induced PPR impairment (n = 4 per group). **D** The representative micrographs of hippocampal dendrites in the CA1 region. Scale bar, 2 μm. **E** NAM administration reversed MS-induced spine density decrease (n = 5, per group). The data are presented as mean ± SEM for each group. n.s., not significant; **p < 0.01, ***p < 0.001, ****p < 0.000.

cells through immunohistochemical co-labeling of CD68 and SYN in the hippocampus. Our results demonstrated an elevated co-labeled area of SYN+ and CD68+ in the MS+Vehicle group (Supplementary Fig. 4A–D). However, daily administration of NAM via gavage (from PND56 to PND85) in MS+NAM rats significantly reduced the co-labeled area of SYN+ and CD68+ in the hippocampus compared to the MS+Vehicle group (Supplementary Fig. 4A–D).

MS rats displayed lower SIRT3 expression in the hippocampus, which was normalized by NAM administration

To assess the role of SIRT3 in hippocampal neuronal phenotypes, we conducted co-labeling of SIRT3 and NeuN. Our results revealed a notable decrease in the co-stained area in the MS+Vehicle rats, and which was mitigated by NAM treatment (Fig. 2A–D).

NAM administration normalized mitochondrial morphology in the hippocampal neurons in MS rats

Subsequently, we investigated the impact of early-life stress on mitochondrial morphology in hippocampal neurons. No significant differences in mitochondrial density were observed among the four groups (Fig. 2E, F). However, the mitochondrial area was notably increased in MS rats (Fig. 2E, J). Remarkably, NAM administration attenuated mitochondrial swelling (Fig. 2E–G).

SIRT3 regulated microglial engulfment of hippocampal neuronal spines

In Experiment 2, our results revealed that HNK administration increased the proportion of co-stained SIRT3 and NeuN-positive cells compared to the MS+Vehicle group (Fig. 3A–D). No significant differences in mitochondrial density were observed among the four groups (Fig. 3E, F). However, the mitochondrial area was notably reduced following HNK administration (Fig. 3E, G). Conversely, 3-TYP administration elevated the mitochondrial area in Control+3-TYP rats (Fig. 3E, G). Moreover, 3-TYP administration elevated the co-labeled area of SYN+ and CD68+ in the Control+3-TYP group compared to Control+Vehicle rats (Supplementary Fig. 5A–D). HNK administration suppressed the elevated co-labeled area of SYN+ and CD68+ compared to the MS+Vehicle group in the hippocampus (Supplementary Fig. 5A–D).

SIRT3 mediated NAD+ stabilization and influenced hippocampal neuronal dendritic complexity and LTP

Neuronal analysis revealed spine restoration following HNK administration in MS+HNK rats (Fig. 4A, B), while Control+3-TYP rats exhibited reduced spine density and less intricate dendritic arborization after 15 days of 3-TYP administration (Fig. 4A, B). Impaired LTP and elevated PPR were observed in Control+3-TYP rats (Fig. 4C–E), both of which were fully rescued by HNK administration (Fig. 4C, D) with subsequent PPR restoration in MS +HNK rats (Fig. 4E).

SIRT3 inhibitor blocked NAM-induced restoration of microglial engulfment of hippocampal neuronal spines

In experiment 3, we investigated the crucial role of SIRT3 in enhancing hippocampal neuronal dendritic complexity and cognitive function associated with schizophrenia in NAM-induced MS rats. Our results revealed that no significant differences in mitochondrial density were observed among the three groups in hippocampal neurons (Supplementary Fig. 6E, F). However, mitochondria in MS+NAM+3-TYP rats exhibited swelling compared to those in MS+NAM rats (Supplementary Fig. 6E, G). Furthermore, 3-TYP administration led to an elevated co-labeled area of SYN+ and CD68+ in MS+NAM+3-TYP rats compared to the MS+NAM group (Supplementary Fig. 7A–D).

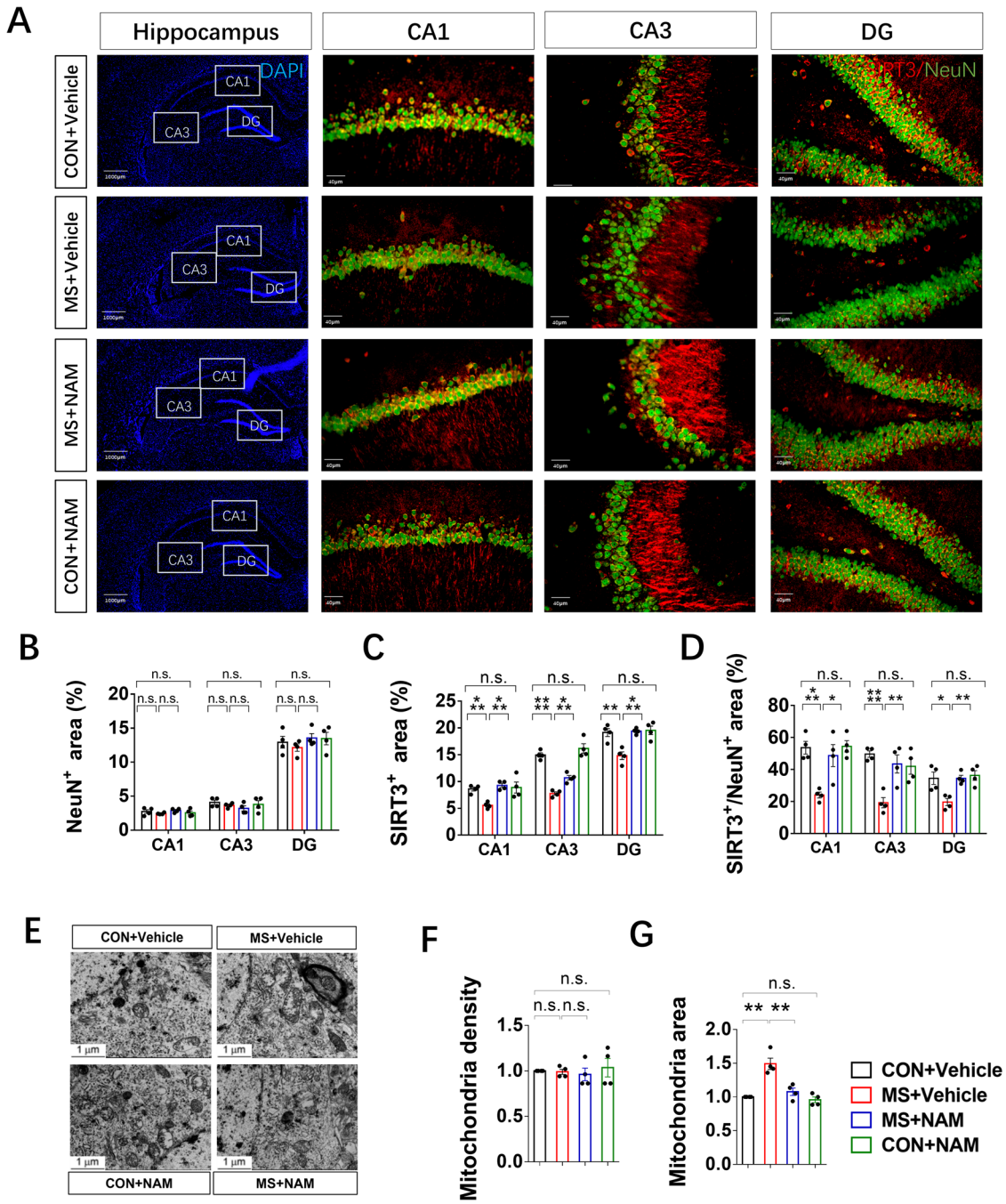

Fig. 2 NAM administration restored SIRT3 expression in neuronal cells and mitochondrial morphology in the hippocampus. **A** Representative immunofluorescence images show the expression of NeuN+ (green pixels), SIRT3+ (red pixels), and DAPI (blue) in the hippocampus of the four groups. **B** NAM administration didn't affect the percentage of neuronal cell area in the hippocampus (n = 4, per group). **C** NAM administration normalized the percentage of SIRT3+ area in the hippocampus induced by MS (n = 4, per group). **D** NAM administration normalized the percentage of NeuN+ and SIRT3+ co-labeled area in the hippocampus induced by MS (n = 4, per group). **E** Representative electron micrographs from the hippocampal neurons in CON+Vehicle, MS+Vehicle, MS+NAM, and CON+NAM rats. **F** Mitochondrial density was comparable in the four groups. Results were normalized to the control group (n = 4, per group). **G** Mitochondrial area was comparable among the four groups. Results were normalized to the control group (n = 4, per group). The data are presented as mean ± SEM for each group. n.s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

SIRT3 inhibitor effectively blocked the NAM-induced restoration of hippocampal neuronal dendritic complexity and LTP

Our results demonstrated that 3-TYP administration in the MS +NAM+3-TYP group hindered the restoration of spine density (Supplementary Fig. 8A, B), LTP (Supplementary Fig. 8C, D), and PPR (Supplementary Fig. 8E).

SIRT3 signaling had an impact on neuronal plasticity and cognitive behavior

Knocking down SIRT3 in the hippocampal CA1 region reduced spine density (Fig. 5C, D). This intervention also led to a reduction in latency in reaching the target hole during the Barnes maze test (Fig. 5E). Moreover, SIRT3 knockdown elevated the time spent in investigating the novel object in the novel object recognition test (Fig. 5F).

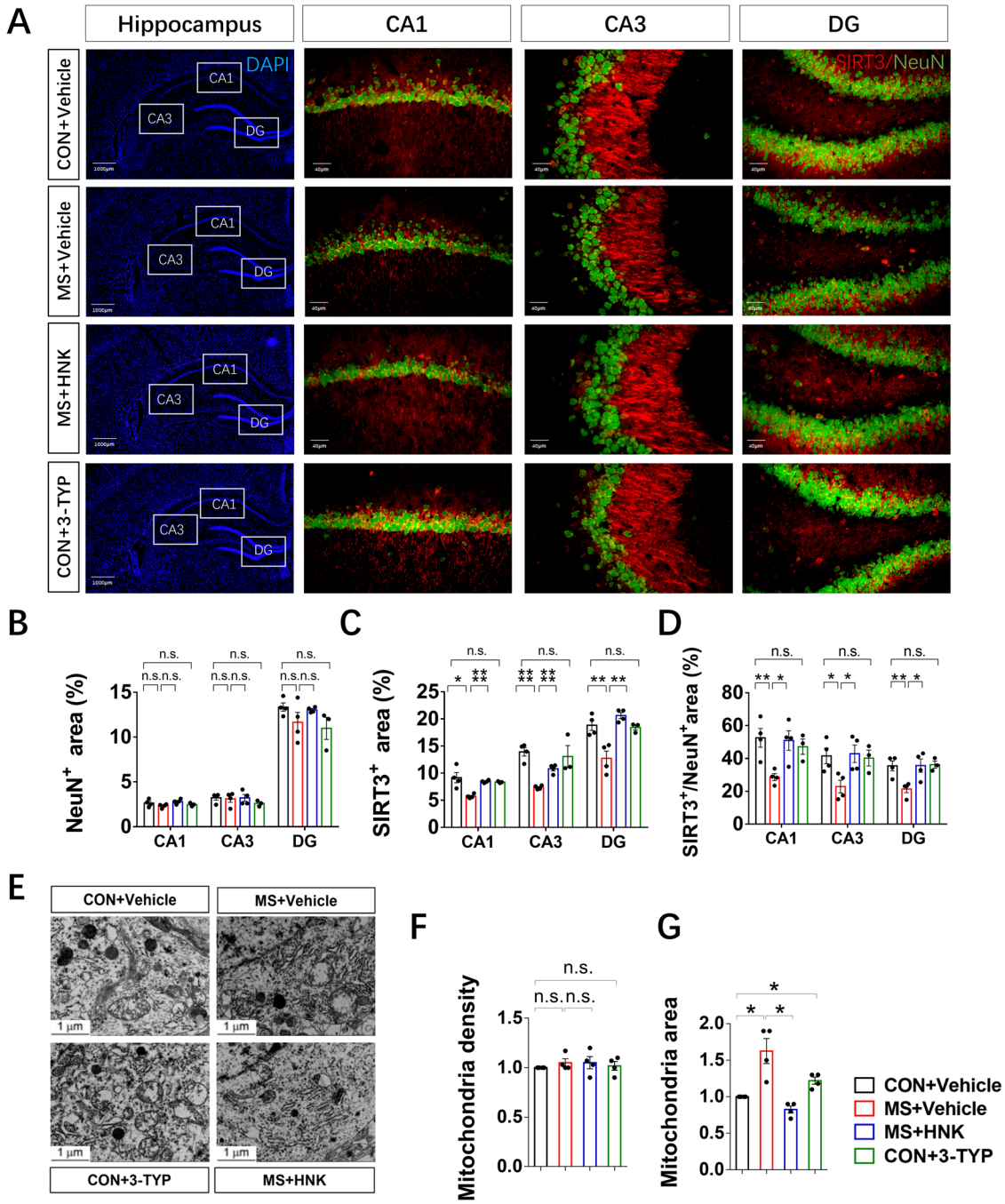

Fig. 3 Impact of SIRT3 activation and inhibition on neuronal cells and mitochondrial morphology in the hippocampus. A Representative immunofluorescence images showing the expression of NeuN+ (green pixels) and SIRT3+ (red pixels) in the hippocampus. **B** HNK and 3-TYP administration had no effect on the percentage of neuronal cell area in the hippocampus (n = 4, per group). **C** Elevated percentage of SIRT3+ area in the hippocampus after HNK administration in MS rats (n = 4, per group). **D** Elevated percentage of NeuN+ and SIRT3+ co-labeled area in the hippocampus after HNK administration in MS rats (n = 4, per group). **E** Representative electron micrographs of the hippocampal neurons in CON+Vehicle, MS+Vehicle, MS+HNK, and CON+3-TYP rats. **F** Mitochondrial density was comparable in the four groups. Results were normalized to the control group (n = 4, per group). **G** Mitochondrial area was comparable among the four groups. Results were normalized to the control group (n = 4, per group). The data are presented as mean ± SEM for each group. n.s., not significant; *p < 0.05, **p < 0.01, and ****p < 0.0001.

Additionally, the OFT indicated an increased distance following SIRT3 knocked down (Fig. 5G). PPI testing revealed impaired prepulse inhibition after SIRT3 knocked down, and with no difference at basic startle reactivity (Fig. 5H). Conversely, neuronal analysis demonstrated a restoration in spine density upon SIRT3 overexpressed in MS rats (Fig. 6C, D). Furthermore, overexpressing SIRT3 in MS rats reduced latency in reaching the target hole during the Barnes maze test (Fig. 6E) and elevated exploration time of the novel object in the

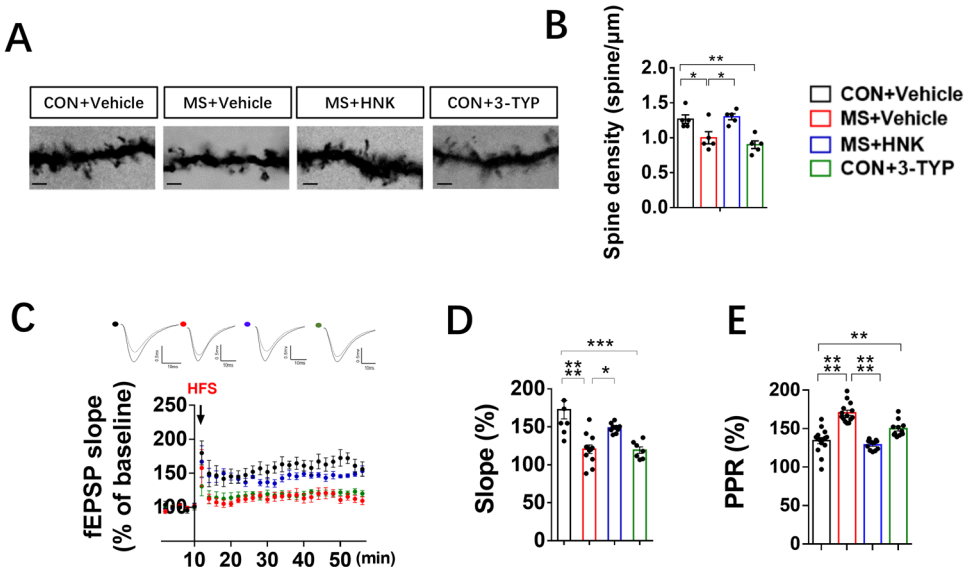

Fig. 4 Impact of SIRT3 activation and inhibition on cellular and behavioral phenotypes. A The representative micrographs of hippocampal dendrites in the CA1 region. Scale bar, 2 μm. **B** Restoration of spine density after HNK administration in MS rats. And reduced spine density after 3-TYP administration on control rats (n = 5, per group). **C** Restoration of LTP after HNK administration in MS rats. And impairment of LTP after 3-TYP administration on control rats. Arrows indicate LTP induction. **D** Quantitative analysis of data in (**C**). **E** Effects of HNK and 3-TYP administration on the PPR (n = 4 per group). The data are presented as the mean ± SEM. n.s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

novel object recognition test (Fig. 6F). Overexpression SIRT3 also reversed the MS-induced increased distance in the OFT (Fig. 6G) and partially ameliorated impaired prepulse inhibition in the PPI test in MS rats (Fig. 6H).

DISCUSSION

This study identified the mitochondrial NAD+/SIRT3 axis as a critical mediator in the pathophysiology of schizophrenia, providing a mechanistic framework linking mitochondrial function to synaptic plasticity and cognitive outcomes. We observed alterations in the mitochondrial NAD+/SIRT3 axis in the hippocampus, reduced hippocampal neuronal spine density, impaired LTP in the CA1 region, and cognitive behavior deficits in the MS rat model. Furthermore, our results demonstrated that supplementing NAD+ or activating/overexpressing SIRT3 restored synaptic plasticity in hippocampal neurons and rescue cognitive impairments associated with schizophrenia in MS rats. In contrast, inhibiting SIRT3 activity or knocking down SIRT3 resulted in deficits in both hippocampal neuronal function and behavioral phenotypes. Our study strongly suggests the NAD+/SIRT3 axis might represent a crucial therapeutic target for schizophrenia and its associated cognitive deficits.

Mitochondrial dysfunction is commonly reported in schizophrenia [40, 41]. Early-life stress has been shown to induce alterations in mitochondrial gene expression and mitochondrial dysfunction [42, 43]. Spine remodeling, a crucial biological process that shapes brain connectivity, has been implicated in the regulation of complex behaviors and cognitive functions, such as learning and memory [44]. Reduced spine density and increased immature spines in the hippocampus have been observed in schizophrenia patients [45, 46]. The role of mitochondrial function in dendritic and spine complexity has been well-documented in both early brain development [13, 14, 17–19] and neurodegeneration [47, 48]. Mitochondria are not only essential for providing the energy required to sustain synaptic activity but also play a key role in calcium buffering and redox signaling, both of which are crucial for spine stabilization and plasticity [49]. In our study, we observed that early-life stress induced both mitochondrial swelling and a reduction in dendritic spine density in hippocampal neurons at adulthood. A previous study suggests that reduced dendritic spine numbers likely impair postsynaptic transmission, which in turn compromises synaptic efficacy, leading to significant reductions in both the induction and maintenance of LTP [50]. Hippocampal LTP and long-term depression (LTD) are Hebbian forms of synaptic plasticity that are widely believed to comprise the physiological correlates of associative learning and memory, and their impairment could account for the cognitive deficits [51]. In line with previous studies, our results showed that early-life stress induced impaired LTP in hippocampal neurons. However, the mitochondrial contributions to the molecular mechanisms regulating spine stabilization and maturation remain incompletely understood.

SIRT3, the primary mitochondrial NAD+-dependent protein deacetylase, plays a crucial role in maintaining mitochondrial redox homeostasis by regulating the function of electron transport chain complexes I and III, thereby preventing excessive ROS generation within the mitochondria [52, 53]. Our previous study showed that inhibition of SIRT3 led to increased acetylation of SOD2, elevated ROS levels, and mitochondrial damage in HT22 cells [21]. In the present animal study, MS significantly reduced SIRT3 levels in hippocampal neurons, which was associated with mitochondrial swelling, indicative of impaired bioenergetic efficiency [54, 55] and reduced ATP production [56]. These mitochondrial abnormalities likely exacerbated oxidative stress, disrupting energy-dependent neuronal processes such as dendritic spine maintenance and LTP. Consistent with previous studies, our results suggests that SIRT3 dysregulation amplifies oxidative damage, contributing to synaptic dysfunction and cognitive deficits.

We also identified a potential role for microglia in mediating stress-induced synaptic pruning. Microglia, which are highly dynamic and phagocytic during brain development, are known to modulate synaptic connectivity based on neuronal activity

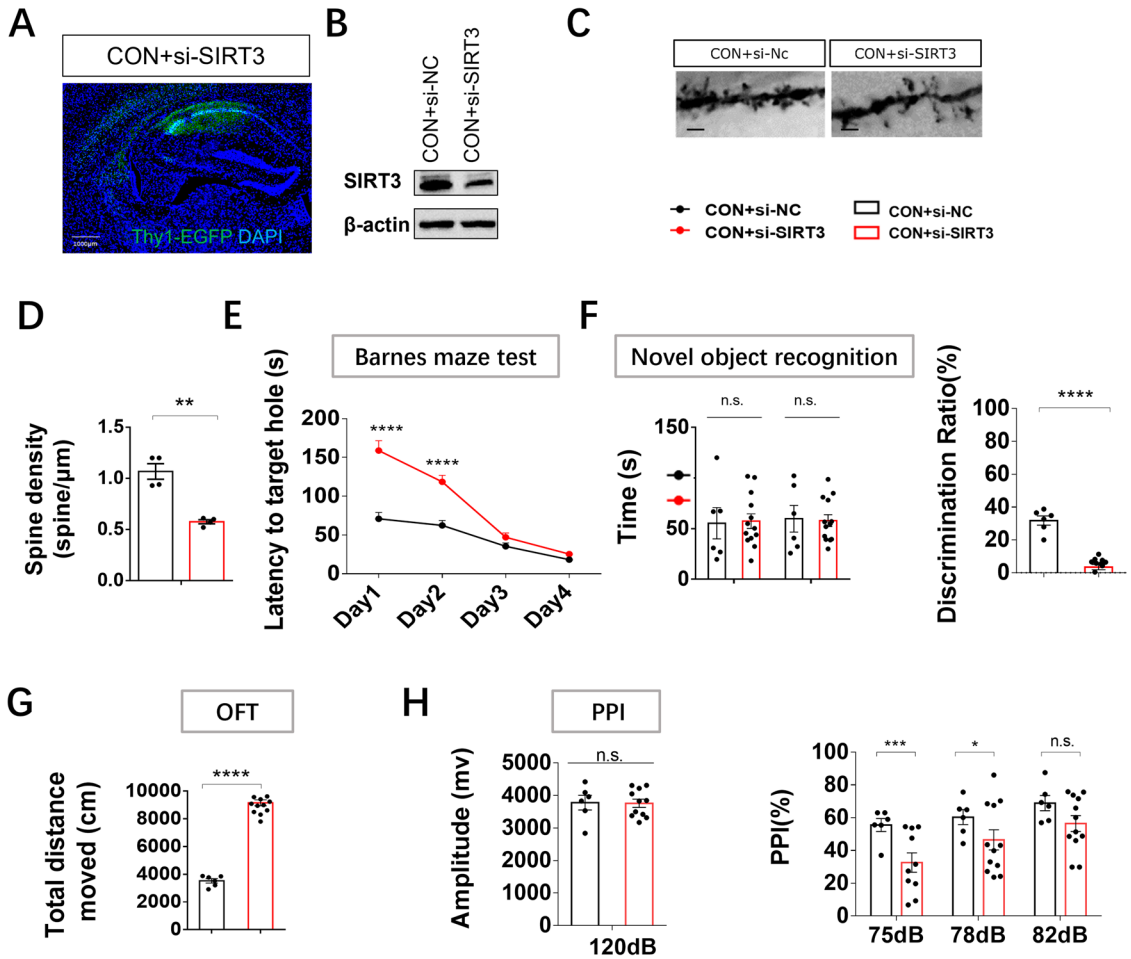

Fig. 5 Knocking down SIRT3 in the hippocampal CA1 region induced deficits in neuronal plasticity and cognitive behavior. A AAV carrying EGFP was injected into the CA1 region of 8-wk-old rats and was examined after 3 weeks. The EGFP was expressed in different coronal sections of the hippocampus and was restricted to the CA1 region. **B** Representative immunoblots of SIRT3 protein expression in the hippocampus from rats after AAV-Nc or AAV-SIRT3 injection. **C** The representative micrographs of hippocampal dendrites in the CA1 region. Scale bar, 2 μm. **D** Quantitative analysis of the spine density about hippocampal dendrites in the CA1 region (n = 5, per group). **E** Knocking down SIRT3 in the hippocampal CA1 region of CON rats elevated latency to the target hole in the Barnes maze test (CON+si-Nc n = 6, CON +si-SIRT3 n = 13). **F** Knocking down SIRT3 in the hippocampal CA1 region of CON rats reduced the discrimination ratio in the novel object recognition test (CON+si-Nc n = 6, CON+si-SIRT3 n = 13). **G** Knocking down SIRT3 in the hippocampal CA1 region of CON rats elevated total distance traveled during the OFT (CON+si-Nc n = 6, CON+si-SIRT3 n = 13). **H** Knocking down SIRT3 in the hippocampal CA1 region of CON rats induced PPI deficits (CON+si-Nc n = 6, CON+si-SIRT3 n = 13). The data are presented as the mean ± SEM. n.s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

[57–59]. We proposed that disruptions in the NAD+/SIRT3 axis contribute to mitochondrial dysfunction, which could amplify oxidative stress and alter neuronal signaling pathways. These changes might indirectly affect microglial activity by promoting the release of damage-associated molecular patterns or modulating neuronal signaling molecules such as ATP or fractalkine, which are key regulators of microglial behavior [60, 61]. Furthermore, specific molecular pathways may mediate the excessive microglial engulfment of dendritic spines in MS rats. These include complement cascade (C1q and C3)-dependent phagocytic signaling, transforming growth factor β (TGF-β), and chemokine signaling pathways, all of which have been implicated in microglial-mediated synaptic remodeling [39, 62–66]. Together, these mechanisms converge to impair LTP in the CA1 region, contributing to cognitive deficits observed in MS rats. Future studies should explore these pathways in greater detail to delineate how the NAD+/SIRT3 axis orchestrates mitochondrial and microglial interactions, ultimately shaping synaptic plasticity and cognitive outcomes.

Despite these insights, our study has some limitations. While pharmacological and viral-mediated manipulations provided valuable evidence for SIRT3's role, cell-type-specific approaches would better delineate its functions in distinct neuronal populations. Additionally, although the MS model effectively mimics many schizophrenia-related features, it may not fully capture the complexity of human schizophrenia. Expanding these findings to other models or human studies is crucial for generalizability. Lastly, while NAD+ supplementation shows potential, its clinical translation requires further investigation into feasibility, safety, and long-term efficacy.

CONCLUSIONS

In conclusion, our results established the role of the NAD+/SIRT3 axis in regulating mitochondrial function and synaptic plasticity under early-life stress, with significant implications for cognitive behavior. The NAD+/SIRT3 axis might be a promising therapeutic target for addressing cognitive deficits associated with schizophrenia.

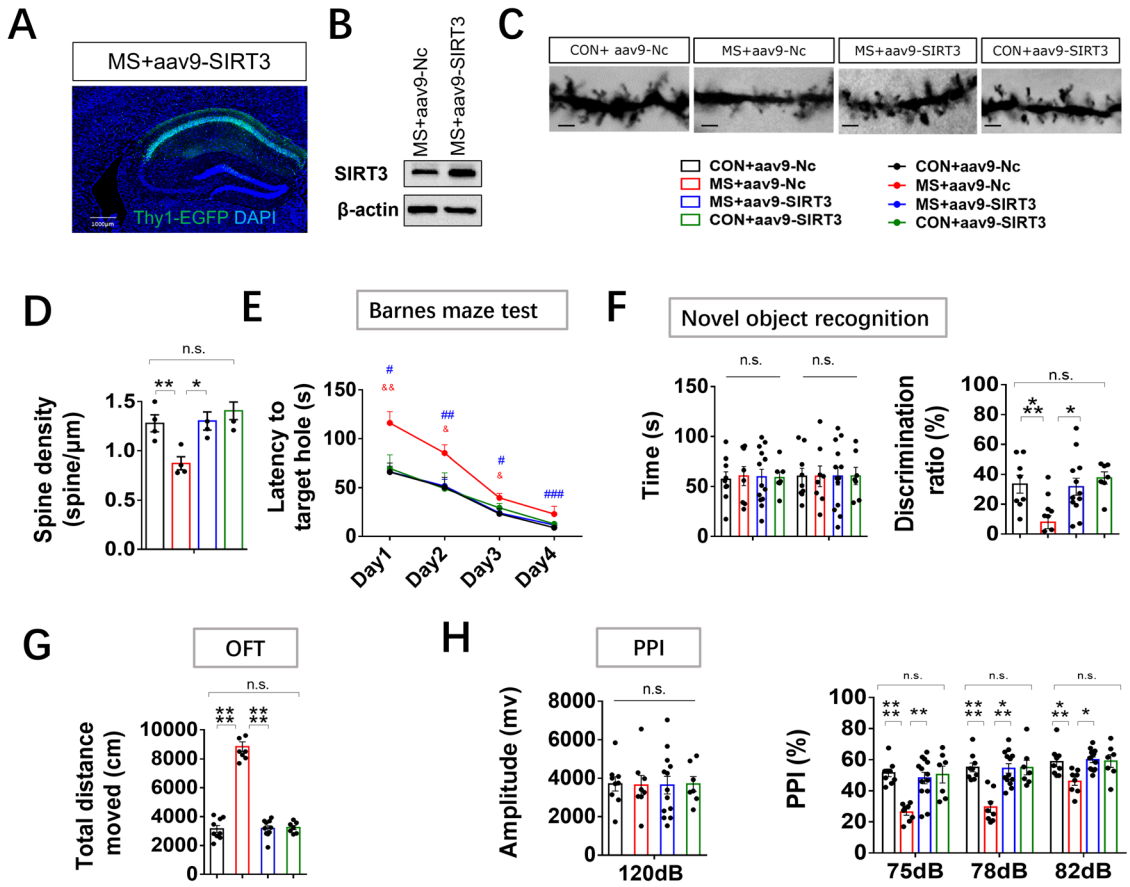

Fig. 6 Overexpressing SIRT3 in the hippocampal CA1 region alleviated ELS-induced deficits in neuronal plasticity and cognitive behavior in MS rats. A AAV carrying EGFP was injected into the CA1 region of 8-week-old rats and was examined after 3 weeks. The EGFP was expressed in different coronal sections of the hippocampus and was restricted to the CA1 region. **B** Representative immunoblots of SIRT3 protein expression in the hippocampus from rats after AAV-Nc or AAV-SIRT3 injection. **C** The representative micrographs of hippocampal dendrites in the CA1 region. Scale bar, 2 μm. **D** Quantitative analysis of the spine density about hippocampal dendrites in the CA1 region (n = 5, per group). **E** Overexpressing SIRT3 in the hippocampal CA1 region of MS rats reduced the latency to the target hole in the Barnes maze test (CON+aav9-Nc n = 9, MS+aav9-Nc n = 8, MS+aav9-SIRT3 n = 13, CON+aav9-SIRT3 n = 7). $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ as MS+aav9-Nc rats compared to the CON+aav9-Nc rats, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#\#}p < 0.001$ as MS+aav9-SIRT3 rats compared to the MS+aav9-Nc rats. **F** Overexpressing SIRT3 in the hippocampal CA1 region of MS rats elevated discrimination ratio in the novel object recognition test (CON+aav9-Nc n = 9, MS+aav9-Nc n = 8, MS+aav9-SIRT3 n = 13, CON+aav9-SIRT3 n = 7). **G** Overexpressing SIRT3 in the hippocampal CA1 region of MS rats reduced the total distances traveled during the OFT (CON+aav9-Nc n = 9, MS+aav9-Nc n = 8, MS+aav9-SIRT3 n = 13, CON+aav9-SIRT3 n = 7). **H** PPI deficits were rescued after overexpressing SIRT3 in the hippocampal CA1 region of MS rats (CON+aav9-Nc n = 9, MS+aav9-Nc n = 8, MS+aav9-SIRT3 n = 13, CON+aav9-SIRT3 n = 7). The data are presented as the mean ± SEM. n.s., not significant; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

DATA AVAILABILITY

Data generated during the current study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

G-H W, H-L W, Y-S L, F-S C and K-K H designed the study and wrote the protocol. K-K H and F-S C performed the experiments and analyzed the data. K-K H, H-H, R-X, Y-X, C-S, and S-L X performed literature searches and drew the figures. F-S C and K-K H wrote the manuscript. G-H W, H-L W, F-S C revised the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the Renmin Hospital Affiliated to Wuhan University (Wuhan, China) approved the study protocol.

ADDITIONAL INFORMATION

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