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Melanin concentrating hormone regulates the JNK/ERK signaling pathway to alleviate influenza A virus infection-induced neuroinflammation

Qianlin Zhang[1], Xiaoyang Liu[1], Qiankun Ma[1] and Jiewen Zhang[1*]

Abstract

Melanin concentrating hormone (MCH) controls many brain functions, such as sleep/wake cycle and memory, and modulates the inflammation response. Previous studies have shown that influenza A virus (IAV) infection-induced neuroinflammation leads to central nervous damage. This study investigated the potential effects of MCH against neuroinflammation induced by IAV infection and its mechanism. MCH (1 and 2 mg/ml) was administrated for 5 consecutive days before IAV infection. Pentobarbital-induced sleep tests, an open-field test, and a Morris water maze were performed to measure sleep quality, spatial learning and memory ability. Neuronal loss and microglial activation were observed with Nissl staining and immunofluorescence assay. The levels of inflammatory cytokines and the expression of the JNK/ERK signaling pathway were examined by ELISA and western blot. IAV infection led to poor sleep quality, impaired the ability of spatial learning and memory, caused neuronal loss and microglial activation in mice's hippocampus and cortex. Meanwhile the level of inflammatory cytokines increased, and the JNK/ERK signaling pathway was activated after IAV infection. MCH administration significantly alleviated IAV-induced neuroinflammation, cognitive impairment, and sleep disorder, decreased the levels of inflammatory cytokines, and inhibited neuronal loss and microglial activation in the hippocampus and cortex by regulating the JNK/ERK signaling pathway. Therefore, MCH alleviated the neuroinflammation, spatial learning and memory impairment, and sleep disorder in IAV-infected mice by regulating the JNK/ERK signaling pathway.

Highlights

- IAV infection could cause the sleep disorder, impairment of the spatial learning and memory.
- IAV infection could cause the neuronal loss and activation of microglia, increase the inflammatory cytokines to result in neuroinflammation.
- MCH alleviated the cognitive impairment and sleep disorder in IAV-infected mice.
- MCH inhibited the neuron damage, microglia activation, and inflammatory cytokines expression, to alleviate the neuroinflammation in IAV-infected mice.
- MCH alleviated the neruoinflammation in IAV-infected mice by regulating the JNK/ERK signaling pathway.

Keywords Melanin-concentrating hormone, Influenza A virus, Neuroinflammation, Cognitive, Sleep, Microglia

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Introduction

Neuroinflammation is an immunological cascade reaction in the central nervous system (CNS) and is a key factor in the pathology of various neurological diseases, such as vascular dementia, sleep deprivation, and neurodegenerative diseases [1]. The research shows that microglia is involved in the occurrence of neuroinflammation by promoting the activation of mitogen-activated protein kinase (MAPK), Janus kinase (JAK) /signal transduction and activator of transcription (STAT), nuclear factor κB (NF-κB), or other signaling pathways [2]. It has been found that microglia is a main type of glial cell and the first line of immune defense in the CNS. However, abnormal activation of microglia can stimulate the overproduction of tumor necrosis factor (TNF) -α, interleukin (IL) -1β, IL-6, and other proinflammatory factors that cause neuronal damage, which ultimately lead to learning and memory dysfunction [3, 4]. Neuroinflammation is a complex and orderly process, closely related to cognitive dysfunction and sleep disorders [5–8].

Melanin concentrating hormone (MCH) is predominantly expressed in the lateral hypothalamic area and is pivotal in regulating energy homeostasis [9]. Recent evidence has highlighted that MCH neurons play an important role in regulating rapid eye movement, sleep, feeding behavior, and memory [10–12]. Previous studies have pointed out that MCH may regulate the memory process and muscle tone during REM sleep [13]. The earlier study has revealed that MCH mediates proinflammatory effects in the intestine and other systems [14]. However, whether MCH is involved in the occurrence and development of neuroinflammation has not been found.

H7N7, as a neurotropic influenza A virus (IAV), is capable of invading and infecting neurons and adjacent glial cells in the CNS to release proinflammatory cytokines and chemokines, inducing neuroinflammation and long-term impairments, accompanied by disorders in the sleep and memory and other neurological symptoms [15, 16]. Previous studies have verified that IAV infection-induced neuroinflammation can induce cognitive deficits, sleep disorders, and many neurological disorders, such as meningitis and neurodegenerative diseases [15, 17]. So, based on previous studies, intranasally injection of H7N7 was used to induce neuroinflammation in mice in this study. The role of MCH in neuroinflammation was investigated by detecting the spatial learning and memory, cognitive function, sleep, inflammatory cytokines level, and activation of microglia in the brain tissues after MCH administration, and its mechanism: whether it is related to JNK/ERK signaling pathway were also explored.

Materials and methods

Animals and viruses

Sixty female C57BL/6J mice (8–10 weeks) were purchased from the animal experimental center of Zhengzhou University (SCXK (Yu) 2021-0009). Compared with male mice, female mice were more susceptible and produced higher levels of chemokines and cytokines during infection with influenza viruses [18]. Mice were maintained under specific pathogen-free conditions on a 12-h light/dark cycle with free access to food and water.

Stocks of influenza A Virus (H7N7) was obtained from Biobw (Beijing, China). Virus stocks were propagated by infection of 10-d-old embryonated chicken eggs to obtain the H7N7 virus mother liquor, and the titers of H7N7 virus were determined by standard plaque assays [15].

All experiment procedures were reviewed and approved by the Ethics Committee of the Henan Province People's Hospital.

Experimental design

Sixty mice were randomly divided into five groups ($n=12$): control group, mice were given saline (intra-BLA injection) and PBS (intranasal). H7N7 group, mice were given saline (intra-BLA injection) and H7N7 (intranasal). MCH low dose+H7N7 group, mice were given 1.5 μl MCH a dose of 1 mg/ml (intra-BLA injection) and H7N7 (intranasal). MCH high dose+H7N7 group, mice were given 1.5 μl MCH a dose of 2 mg/ml (intra-BLA injection) and H7N7 (intranasal). MCH+H7N7+NSC95397 group, mice were given 1.5 μl MCH a dose of 2 mg/ml and the MKP-1 inhibitor NSC95397 at a dose of 4 mg/kg (intra-BLA injection) and H7N7 (intranasal).

The mice received intranasally infection of the H7N7 virus to establish the animal model of neuroinflammation, while the mice in the control group were intranasally inoculated with sterile PBS. Before the virus infection, 1.5 μl MCH (ChinaPeptides, Co., Ltd, China) was administered to mice for 5 consecutive days at a dose of 1 and 2 mg/ml into the basolateral amygdala (BLA) respectively, as described previously [19]. To regulate the JNK/ERK pathways, the MKP-1 inhibitor NSC95397 (HY-108543, MedChemExpress, USA) was microinjected at a dose of 4 mg/kg into the BLA. In contrast, mice in the H7N7 group and the control group received saline instead.

Drug microinjection and influenza infection

Mice were anesthetized by intraperitoneal injection of ketamine-xylazine solution (10 ml/kg) and fixed in the stereotaxic frame. As described previously [19], the location of bregma was determined according to the stereotaxic technique, and the location of BLA (posterior 1.4 mm, lateral±3.0 mm, and depth of 4.8 mm relative to the bregma) were determined according to the brain

atlases. After drilling into the skull, a guide cannula was implanted vertically into the BLA. The roxithromycin ointment applied before suture and the mice were injected with 80, 000 U of penicillin (5 mg/kg) for three consecutive days after the operation to prevent infection. After 7 days of recovery, mice were administered MCH or saline (1.5 μl) in the BLA through the injection cannula for 5 min.

On the third day of microinjection administration, mice were anesthetized by ketamine-xylazine solution (10 ml/kg) and infected intranasally with 10 focus-forming units of H7N7 virus in 20 μl sterile PBS. Control mice were infected intranasally with 20 μl sterile PBS. Following the influenza infection, the mice's physical appearance and food intake were monitored, and the body weight of the mice was measured daily to calculate the percentage change from the initial body.

Sleep monitoring test

After 24 h influenza infection, mice were intraperitoneally injected with sodium pentobarbital (30 mg/kg, in saline) to detect the sleep condition [20]. Sleep occurred with the righting reflex disappearance (the mice could not be righted for more than 30 s), and awakening occurred with the righting reflex recovery. The time of sleep latency and sleep duration were recorded with stopwatches.

Open-field test

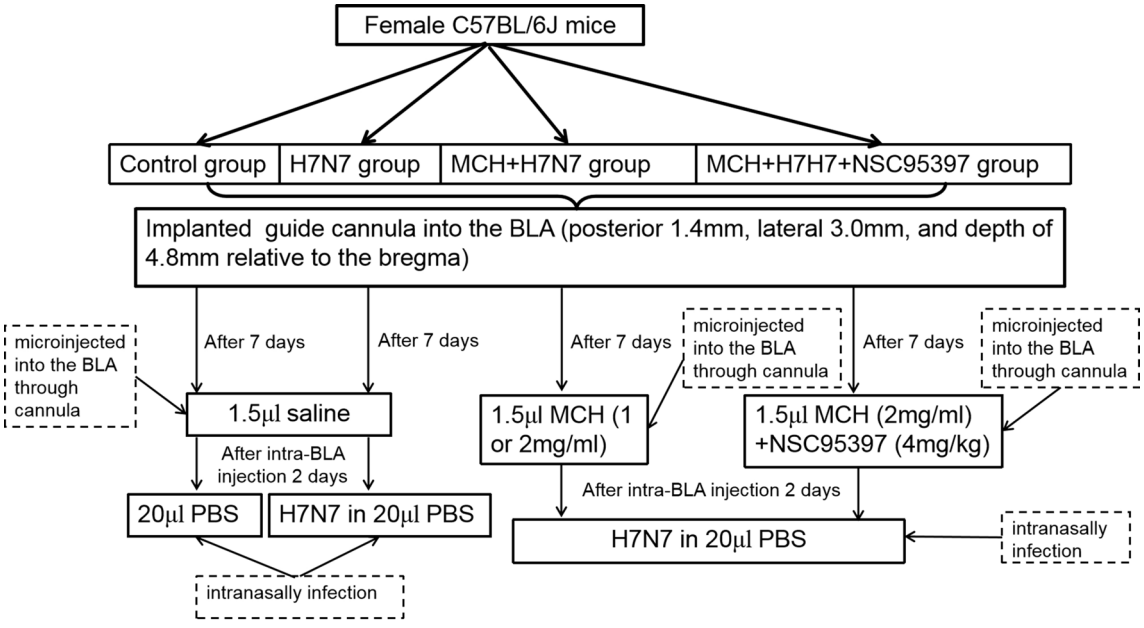
To investigate the exploratory or anxiety like behavior of the mice in each group, the open-field test was performed [15] at 8 d after infection. A white PVD open-field apparatus (40×40×40 cm) was used, and the mice were placed along one side of the apparatus for 5 min. Total distance traveled, and average speed were obtained from ANY-maze behavioral tracking software (Stoelting, USA).

Morris water maze

The effect of MCH on spatial learning and memory was assessed using the Morris water maze (ZS-001, Beijing, China) with a circular pool (diameter, 120 cm and height of 40 cm) at 8 d after infection, lasted 5 d. As described previously [15, 17], a platform (8 cm in diameter) was hidden 1 cm underneath the surface of the water in the pool (filled with water). Before the training, a visible platform task was performed. Mice were placed on the platform for 30 s before each test started. Then, the mice were placed in the water to find the platforms. After the pretraining, the platform was removed, and the mice were allowed to swim for 30 s to assess spatial memory for the platform location. A reversal test was performed: the platform was moved to the opposite quadrant of the pool. The mice were placed on the platform for 30 s and given three trials to locate the platform in the new target quadrant. 24 h later, the mice were allowed to swim to assess the platform. All data, including the escape latency (time to reach the platform), path length to the platform, swimming speed and percentage time spent in the target quadrant, were analyzed and recorded by MT-200 image analysis software (Taimeng, Chengdu, China).

Nissl staining

The mice were deeply anesthetized and sacrificed within 24 h of completing the behavioral test. Then, the brain



Time line schematic

tissues were quickly removed and fixed in 4% paraformaldehyde at 4℃ for more than 72 h. The portion of the brain tissue that included the whole hippocampus was embedded in paraffin and cut into 5 μm sections. After being dewaxed with xylene, the sections were stained with methyl violet staining solution for 10 min. The sections were differentiated with Nissl differentiation solution (Beyotime, Beijing, China) for 5 s after rinsed with water. Finally, the sections were dehydrated, cleared, and sealed with neutral gum. The number of Nissl+cells was observed by an optical microscope using × 20 objective with the same condition at a resolution of 1024×1024 pixels and the numbers of Nissl+cells were automatically count by Image J software: ① Open the picture with Image J, ② Image>Type>8-bit, ③ Image>Adjust>Threshold, ④ Process>Binary>Watershed, ⑤ Analyze>Analyze Particles, click OK. ⑥ The number of Nissl+cells counted were observed directly in the Summary window.

Immunofluorescence staining

After dewaxed and rehydrated, the paraffin section was incubated with 0.3% Triton X-100. Then, the sections were blocked with 1% BSA for 2 h at room temperature and incubated with primary antibody (Rabbit anti-Iba1, 1:1000, PA5-27436, Thermo Fisher, USA) overnight at 4℃. On the second day, the sections were incubated with secondary antibody: AlexaFluor 488 anti-rabbit IgG (1:1000, A-11008, Thermo Fisher, USA) for 2 h at room temperature after washed with PBS. Finally, the sections were counterstained with DAPI and sealed with an antifade mounting medium. The green fluorescence was represented the Iba-1 positive cell. The fluorescence signals were detected using a fluorescence microscope using × 20 objective with the same condition at a resolution of 1024×1024 pixels. The numbers of Iab-1 positive cells in the hippocampus and cortex was calculated by Image J software (NIH, MD, USA). Iba-1$^+$ cells were counted in a blinded manner and the mean values were calculated by 3 randomly selected microscopic fields from each section. The data were expressed as the mean number of cells per square millimeter.

Enzyme-linked immune sorbet assay (ELISA)

The mice were sacrificed by cervical dislocation within 24 h of completing the behavioral test. The hippocampus were harvested and homogenized with RIPA lysis buffer. After centrifugation at 4℃ for 10 min at 12,000 × g, the IL-6, IL-1β, and TNF-α ELISA kit (SEKM-0007, SEKM-0002, and SEKM-0034, Solarbio, Beijing, China) were used to determine the levels of inflammatory cytokines according to the manufacturer's protocols. Absorbance at 450 nm was measured with a microplate reader, and the concentration of the cytokines was calculated according to the measured optical density and the standard curve.

Western blot

Hippocampus were homogenized using RIPA lysis buffer. After centrifugation, the protein concentration was determined using the bicinchoninic acid (BCA) method. 40 μg proteins were separated via SDS-PAGE and transferred onto the polyvinylidene fluoride membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween (TBST) for 2 h at room temperature. The primary antibodies (Cell Signaling Technology, USA): JNK (1:1000, Cat. #67096S), phosphorylated JNK (p-JNK) (1:1000, Cat. #4668T), ERK (1:1000, Cat. #9102S), p-ERK (1:1000, Cat. #4377T), and MKP-1 (1:1000, Cat. #35217S) were incubated overnight at 4℃. After washing with TBST for 3 times, the membranes were incubated with secondary antibodies (1:2000, Cat. #5127S) for 2 h at room temperature. Finally, the bands were visualized via chemiluminescence, and the density of the bands was calculated using Image J software.

Statistical analysis

Data were analyzed and plotted by GraphPad Prism 8.0 and were presented as mean±SD. Differences among groups were analyzed via a two-way analysis of variance, followed by Tukey's post hoc test. $P<0.05$ was considered statistically significant.

Results

MCH improved sleep disorder of IAV-infected mice

Body weight changes of mice in each group after infection with IAV were shown in Fig. 1A. Infection with IAV resulted in body weight loss. While compared with the H7N7 group, MCH inhibited the weight loss induced by IAV.

Sleep latency and sleep duration of mice in each group were shown in Fig. 1B and C. Compared with the control group, the sleep latency was prolonged, and the sleep duration was shortened in the H7N7 group. MCH could reduce sleep latency and increase sleep duration in IAV-infected mice, suggesting that MCH could improve the sleep disorders induced by IAV infection.

MCH alleviated spatial learning and memory impairment of IAV-infected mice

The locomotor activity and exploratory behavior of mice were tested in the open-field test (Fig. 2A and B). Compared with the control group, the total distance traveled was shorter, and the average speed of mice in the H7N7 group was slower, suggesting that infection with IAV reduced mice's exploratory behavior and locomotor activity. The total distance traveled was longer, and the average speed was faster after MCH administration, indicating that MCH improved the exploratory and locomotor activity of IAV-infected mice to a certain extent.

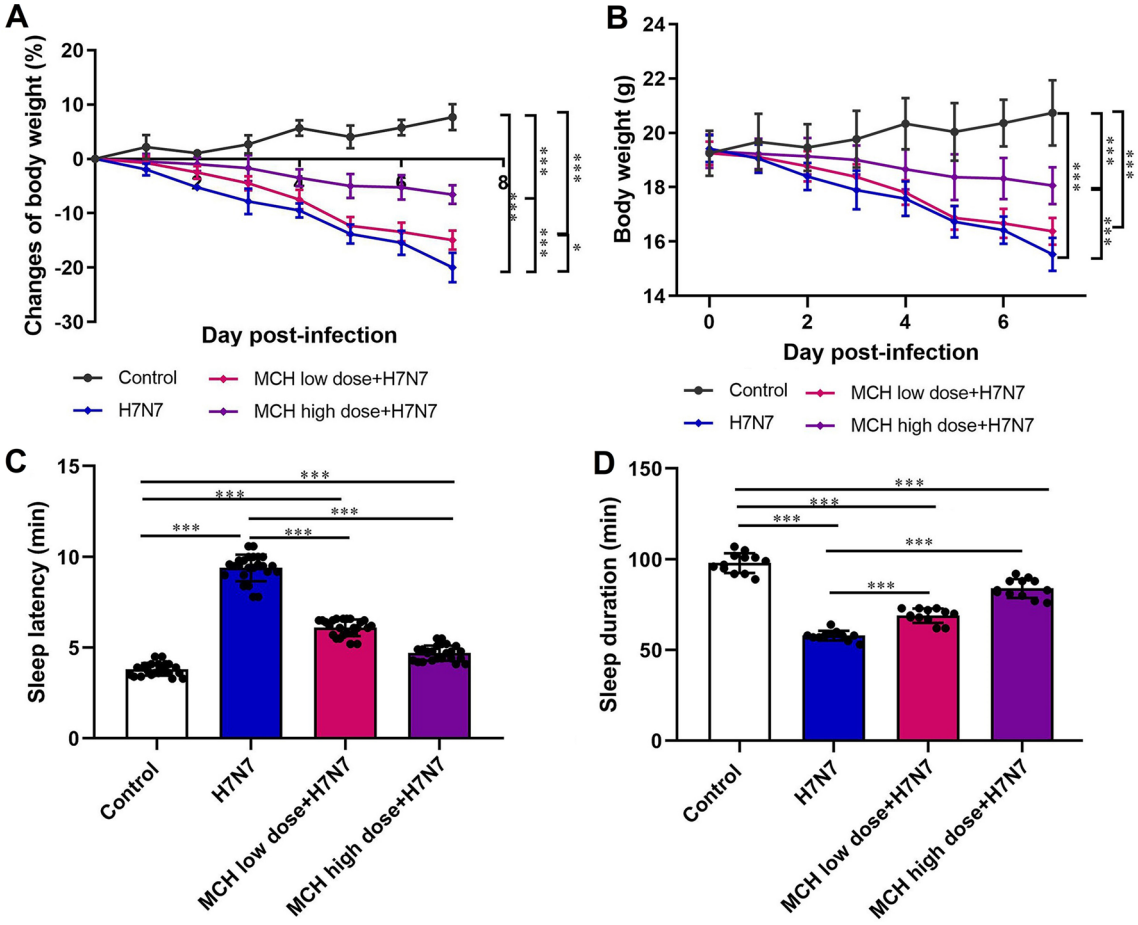


Fig. 1 Effect of MCH on the weight and sleep of IAV-infected mice. (**A** and **B**) The percent changes of body weight loss and the absolute body weights of mice post-infection. (**C**) Sleep latency. (**D**) Sleep duration. Data were expressed as mean ± SEM, $n = 12$ for body weight tests and sleep monitoring test. $^{*}P < 0.05$, $^{***}P < 0.001$

Training in the Morris water maze task was performed to investigate the effects of MCH on spatial learning and memory function (Fig. 2C-F). The escape latency changed with training time, and the effect of the groups was significant. Compared with the control group, the swimming velocity in the H7N7 group was slower, and the duration of escape latency and the total distance movement of mice in the H7N7 group was longer, while the time spent in the target quadrant was shorter, suggesting that infection with IAV impaired memory and cognitive function. MCH administration significantly reduced the duration of escape latency to reach the platform and the total distance movement, and increased the swimming velocity and the time spent in the target quadrant of mice, indicating that MCH alleviated the impairment of spatial learning and memory function of IAV-infected mice.

MCH alleviated the loss of neurons damage of IAV-infected mice

To explore the neuroprotective effect of MCH, the morphology, and number of neurons in the DG and CA1 domain of the hippocampus and cortex of mice in each group were detected using a Nissl staining assay (Fig. 3A). In the control group, Nissl bodies were distributed in the DG and CA1 regions of the hippocampus and cortex. The Nissl bodies were markedly decreased in the hippocampus and cortex in the H7N7 group, compared to the control group. Following MCH administration, the Nissl bodies were increased in the hippocampus and cortex of MCH+H7N7 groups. The results of quantitative analysis of the number of neurons are shown in Fig. 3B. Compared with the control group, the number of neurons was significantly reduced in the H7NH7 group. MCH administration inhibited the loss of neurons to a certain extent. The results suggested that MCH effectively alleviated the loss of neurons induced by IAV infection.

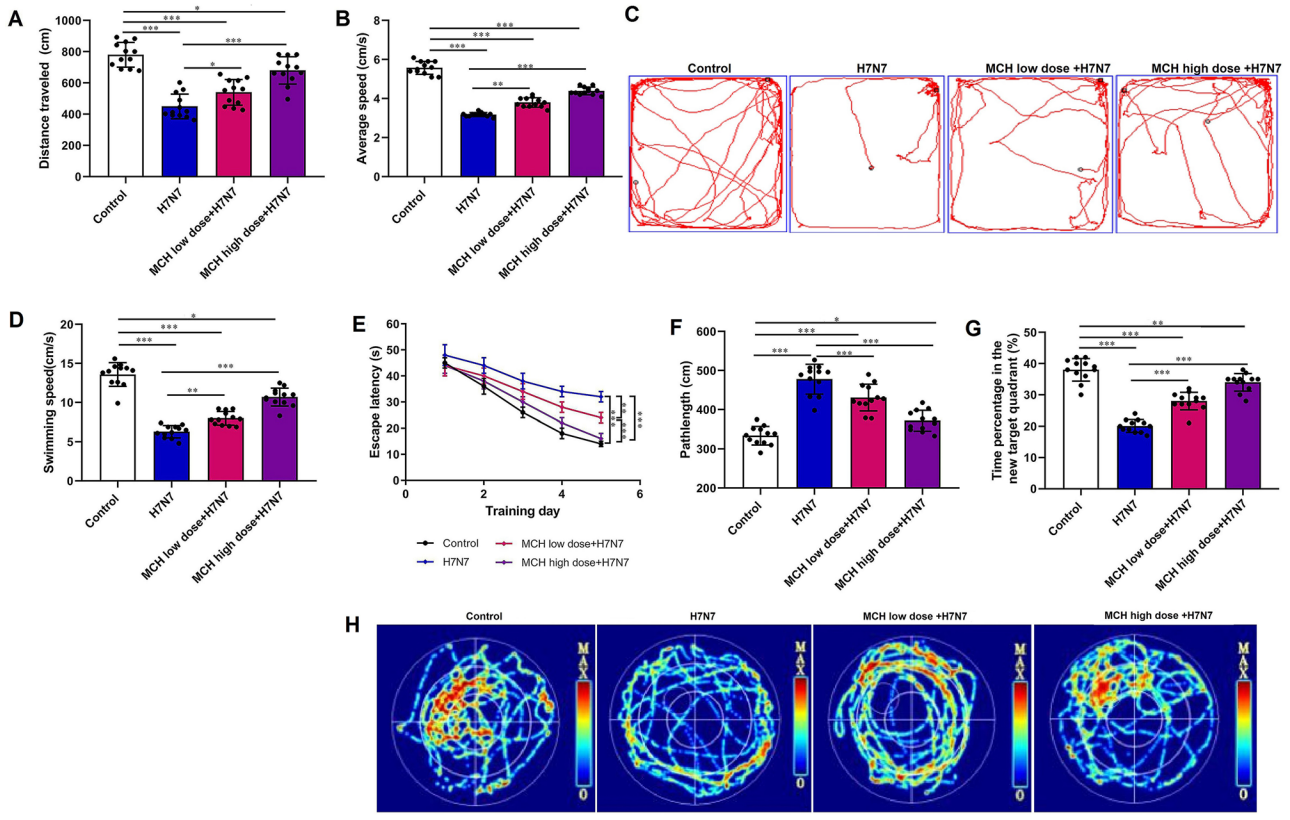


Fig. 2 Effect of MCH on locomotion, spatial learning and memory of IAV-infected mice. (**A**) The total distance traveled in the open-field test. (**B**) The average speed in open-field test. (**C**) The trackplots of mice from the open-field test. (**D**) The average swimming velocity during a visible platform task. (**E**) Changes in the escape latency during the Morris water maze test training period. (**F**) The path length of swimming in Morris water maze test. (**G**) Time spent in the target quadrant in the Morris water maze test. (**G**) The movement trail map of mice in the Morris water maze test. Data were expressed as mean ± SEM, $n = 12$ for open field test and Morris water maze test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

MCH inhibited the activation of microglia

One of the typical features of neuroinflammation is the abnormal activation of glial cells. As a main type of glial cell, microglia plays an important role in the occurrence and development of neuroinflammation [21]. Iba1 participates in morphological changes associated with activated microglia/macrophages state and is widely used as a marker for microglia/macrophages in the brain tissues. And the activation of microglia (Iba-1^{+}) may lead to the overproduction of proinflammatory mediators, which could induce neuroinflammationc [22, 23]. Iba-1 staining was performed to investigate the effect of MCH on microglial activation status in the hippocampus and cortex (Fig. 4). Compared with the control group, the numbers of Iba-1^{+} cells were increased in the H7NH group, suggesting that infection with IAV induced microglial activation, resulting in neuroinflammation in mice. And the Iba-1^{+} cells were decreased after MCH administration, indicating that MCH could inhibit the activation of microglia induced by IAV infection.

MCH suppressed the level of inflammatory cytokines in IAV-infected mice

Activation of microglia triggers the release of various inflammatory markers, such as IL-1β and TNF-α, under the pathological state [24]. The concentration of IL-1β and TNF-α in the brain tissues was measured by ELISA to explore the effect of MCH on neuroinflammation. As shown in Fig. 5A, the levels of IL-1β and TNF-α in the hippocampus of the H7N7 group were significantly increased. The effect was significantly suppressed by pretreatment with the MCH. These results indicated that the MCH inhibited the levels of inflammatory cytokines to alleviate the neuroinflammation.

MCH inhibited the activation of the JNK/ERK signaling pathway

The effect of MCH on the JNK/ERK signaling pathway was examined by western blot (Fig. 5B-D). The results indicated that the ratio of p-JNK/JNK and p-ERK/ERK were strikingly increased in the H7N7 group compared to the control group. Whereas MCH administration decreased the p-JNK/JNK and p-ERK/ERK ratios,

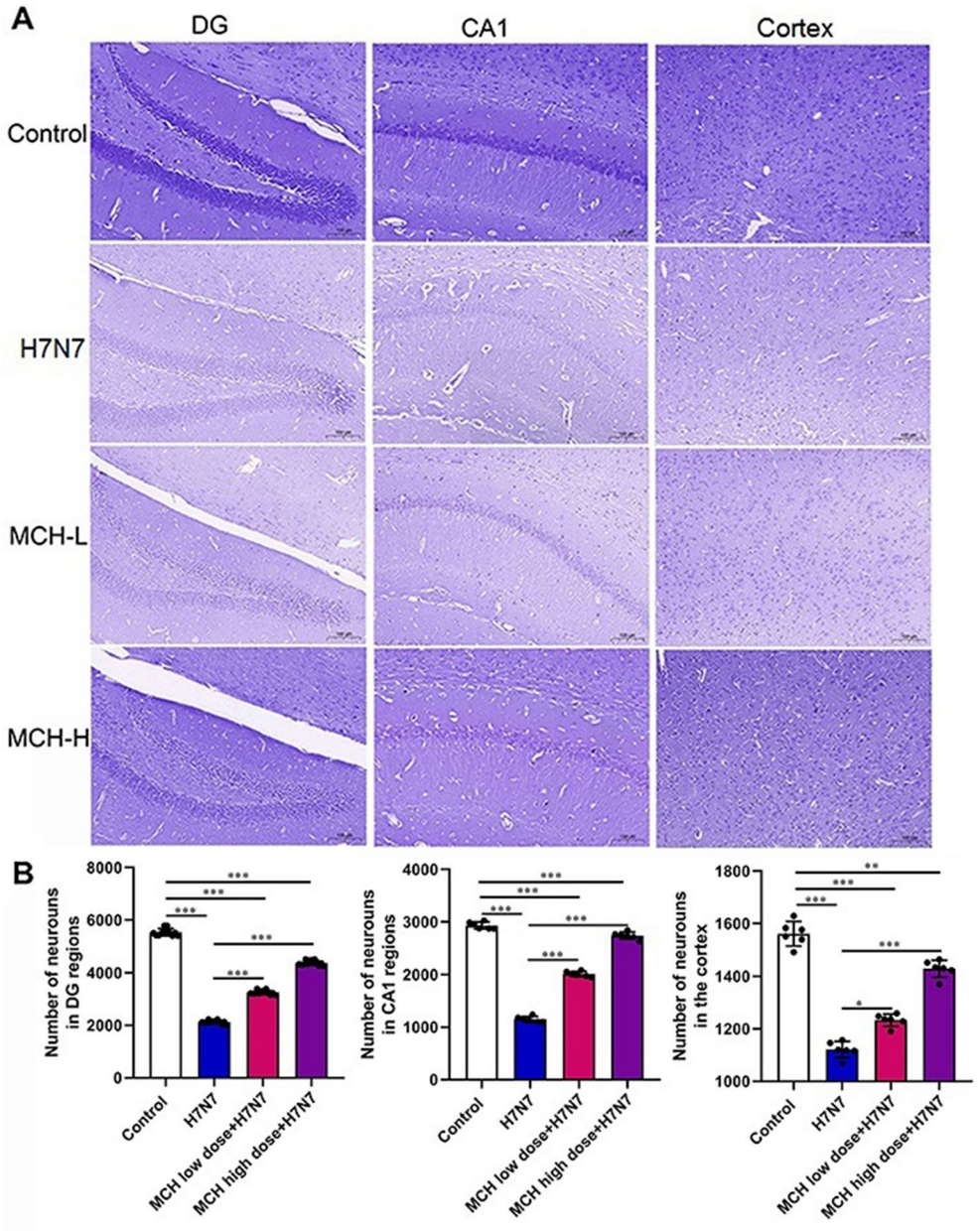


Fig. 3 Effect of MCH on the loss of neurons of IAV-infected mice. (**A**) Representative Nissl staining in the hippocampal DG and CA1 regions and the cortex. Magnification: 100×, bar = 100 μm, MCH-L, MCH low dose + H7N7, MCH-H, MCH high dose + H7N7. (**B**) The number of neurons in the hippocampal DG and CA1 regions and the cortex. Data were expressed as mean ± SEM, $n = 6$, 5 slices for each mouse. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

indicating that MCH markedly inhibited the activation of the JNK/ERK pathway in the IAV-infected mice.

MCH alleviated the neuroinflammation in IAV-infected mice by inhibiting the JNK/ERK pathway

To further explore the mechanism underlying the effect of MCH on JNK/ERK dephosphorylation, the MKP-1 inhibitor NSC95397 was administrated to the IAV-infected mice treated with MCH to promote JNK/ERK phosphorylation. The decrease in p-JNK/JNK and p-ERK/ERK ratios and the increase in MKP-1 expression in IAV-infected mice treated with MCH were inhibited by NSC95397 (Fig. 6A). As shown in Fig. 6B-F, NSC95397 significantly reversed the effect of MCH on cognitive impairment, sleep, expression of inflammatory cytokines, loss of neurons, and microglia activation in IAV-infected mice, suggesting that NSC95397 markedly attenuated the neuroprotective effect of MCH in IAV-infected mice. These results indicated that MCH could alleviate the neuroinflammation induced by IAV infection and improve cognitive impairment and sleep disorders by inhibiting the activation of JNK/ERK signaling pathway.

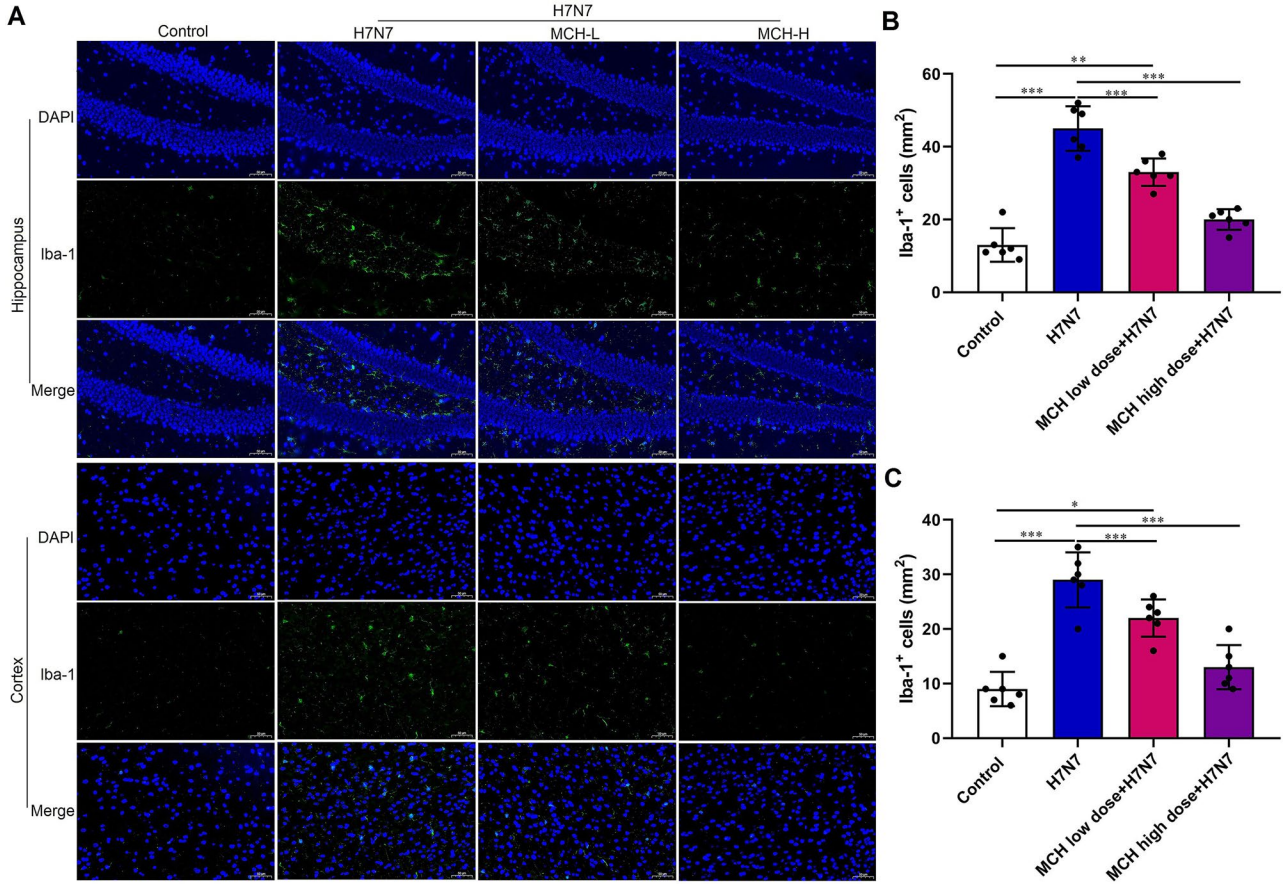


Fig. 4 Effect of MCH on microglia activation in the hippocampus and cortex of IAV-infected mice. (**A**) Representative immunofluorescence staining in the hippocampus and cortex of IAV-infected mice Magnification: 200×, bar = 50 μm, MCH-L, MCH low dose + H7N7, MCH-H, MCH high dose + H7N7. (**B**-**C**) The numbers of Iba-1$^+$ cells in the hippocampus and cortex. Data were expressed as mean ± SEM, $n = 6$, 5 slices for each mouse. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Discussion

The present study revealed that MCH effectively relieved the neuroinflammation induced by IAV infection, improved cognitive impairment and sleep disorders, inhibited the neruonal loss and microglial activation, down-regulated the expression of inflammatory cytokines, and the mechanism might be related to MCH inhibition of the phosphorylation process of the JNK/ERK signaling pathway. This study was among the first to demonstrate the alleviation effect of MCH on IAV-induced neuroinflammation involving in the regulation of the JNK/ERK signaling pathway.

Infection with neurotropic IAV strains can enter the CNS through the blood-brain barrier and subsequently lead to neuronal cell damage or death induced by neuroinflammation through the activation of microglia and the alteration of inflammatory cytokines expression [17, 25]. When IAV infection triggers neuroinflammation, the inflammatory cytokines can impair hippocampal long-term potentiation, impairing spatial learning and cognitive [15]. Notably, abnormal activation of glial cells (astrocytes and microglia) could mediate neuroinflammation. In turn, the physiological function of glial cell, which modulates sleep, is influenced by neuroinflammation [21, 26]. In the present study, it was found that mice had shown spatial learning and memory impairment and sleep disorders, and the activated microglia and the level of inflammatory cytokines increased in the hippocampus after H7N7 infection, suggesting that infection with IAV induced spatial learning and memory impairment, sleep disorder, and neuroinflammation of the mice.

MCH controls many brain functions in mammals, such as food intake, metabolism, stress response, anxiety, sleep/wake cycle, and memory [27, 28]. For example, MCH could regulate anxiety-like behavior and intestinal dysfunction by regulating intestinal permeability and inflammation in chronic acute combining stress-stimulated mice [19]. Substantive experimental data suggested that the MCH played a role in the pathophysiology of depression, in addition, in the control of REM sleep [29]. A study reported that MCH might have neuroprotective effects via downstream pathways associated

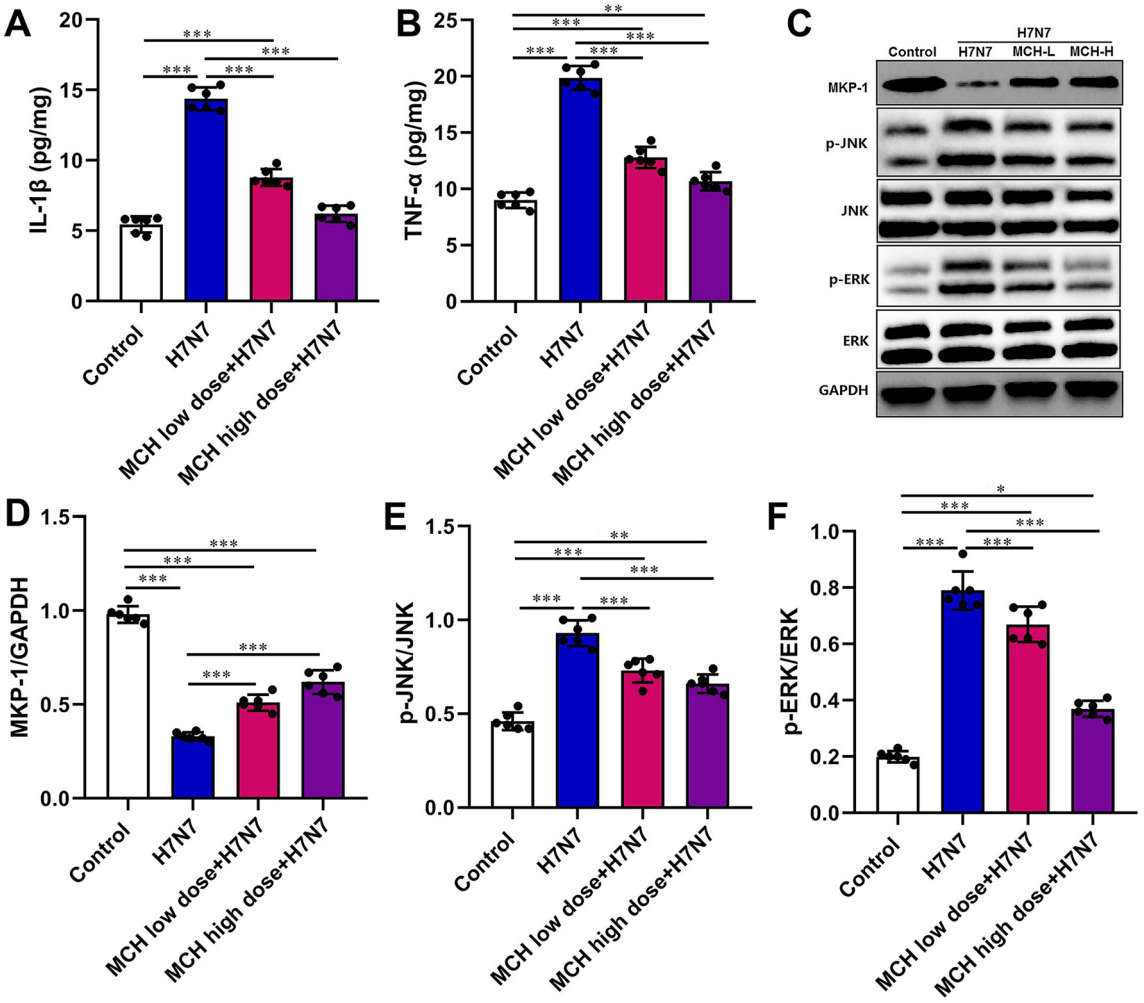


Fig. 5 Effect of MCH on the level of inflammatory cytokines and JNK/ERK signaling pathway in influenza-induced mice. (**A**-**B**) The levels of IL-1β and TNF-α detected by ELISA, $n=6$. (**C**) Expression of MKP-1, p-JNK, JNK, p-ERK, and ERK proteins in the hippocampous detected by Western blot. (**D**-**F**) Quantitative analysis of the ratio of p-JNK/JNK and p-ERK/ERK. Data were expressed as mean ± SEM, $n=6$ for ELISA assay and Western blot assay. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

with enhancing neuronal synapses to improve memory impairment in the therapy of neurodegenerative diseases [30]. The preliminary study found that the serum MCH was significantly reduced in patients with cerebral ischemic stroke and was closely related to sleep disorders and memory dysfunction [31]. In early Alzheimer's disease, impaired MCH-system function contributed to aberrant excitatory drive and sleep defects, which could compromise hippocampus-dependent function [32]. Besides, MCH also mediated the modulation of inflammation [33]. However, there was no report on the role of MCH in IAV infection-induced neuroinflammation yet. In this study, we demonstrated that exogenous MCH alleviated the impairments in spatial learning and memory, and sleep disorders in IAV-infected mice, consistent with the literature. Moreover, the MCH also decreased inflammatory cytokine levels and inhibited microglia's over-activation in brain tissues to alleviate neuroinflammation.

It is generally accepted that as the members of MAPKs, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK) play a crucial role in neuroinflammatory [34, 35]. *In vivo and in vitro* studies demonstrated that LPS initiated an inflammatory reaction by activating the MAPK family, including JNK, ERK1/2, and p38 [36, 37]. The Japanese encephalitis virus could induce neuroinflammation in cell and animal experiments through the proven downstream MAPK, ERK, and JNK pathways [38]. In our study, the phosphorylation levels of JNK and ERK in the IAV-infected mice were significantly increased, suggesting that IAV infection-induced neuroinflammation was related to the activation of the JNK/ERK signaling pathway. Exogenous MCH dramatically inhibited the activation of the JNK/ERK signaling pathway in the IAV-infected mice. Meanwhile, NSC95397, inhibitor of mitogen-activated protein kinase phosphatase-1 (MKP-1), reversed the

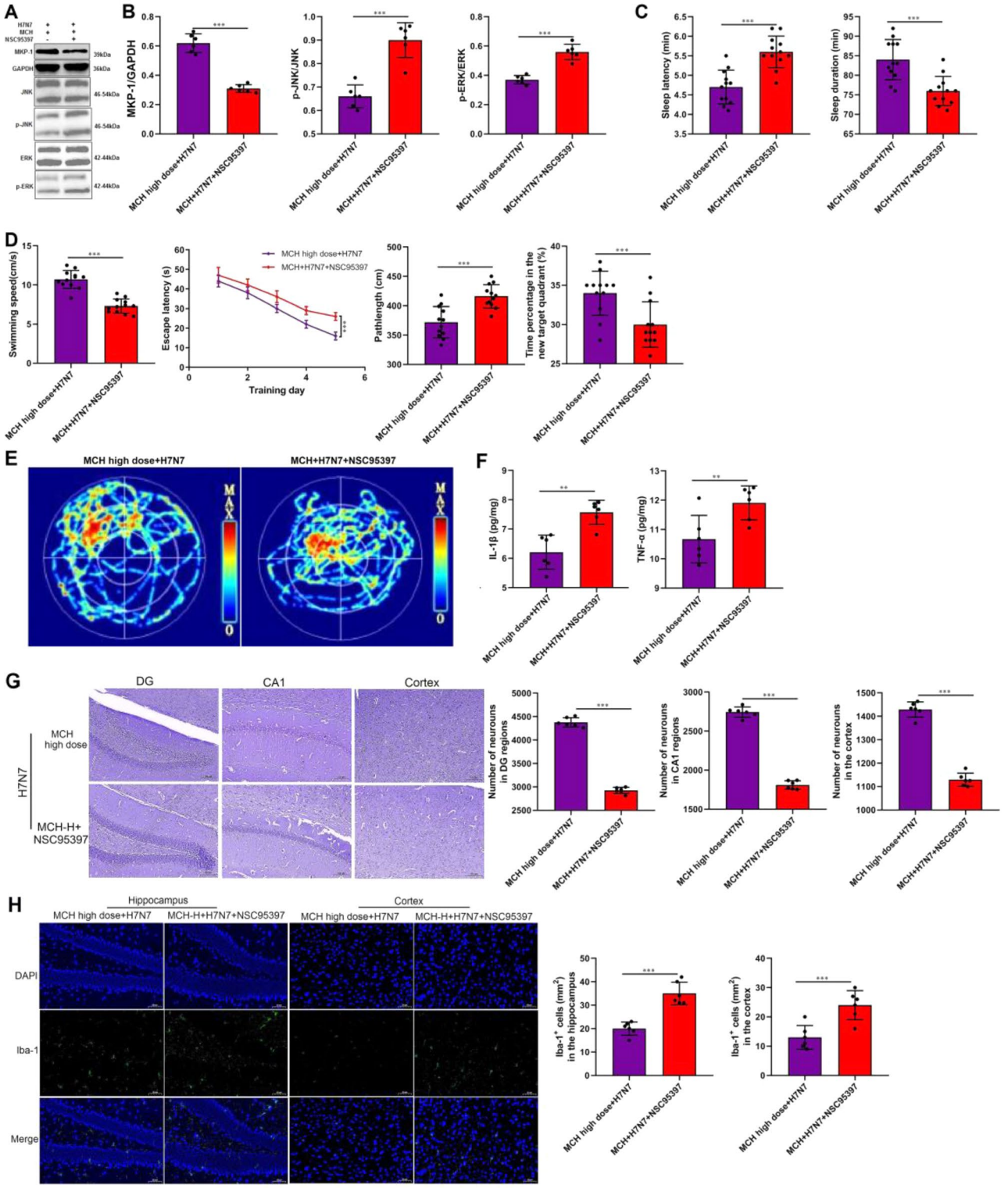


Fig. 6 The mechanism of MCH in neuroinflammation induced by IAV infection. (**A**-**B**) Expression of MKP-1, p-JNK, JNK, p-ERK, and ERK proteins detected by western blot and the quantitative analysis, $n=6$. (**C**) Sleep latency and sleep duration, $n=12$. (**D**) The swimming velocity, escape latency, path length, and time spent in the target quadrant in the Morris water maze test, $n=12$. (**E**) The movement trail map of mice in the Morris water maze test, $n=12$. (**F**) The levels of IL-1β and TNF-α detected by ELISA, $n=6$. (**G**) Representative Nissl staining in the hippocampal DG and CA1 regions and cortex. Bar = 100 μm, $n=6$, 5 slices for each mouse. (**H**) Representative immunofluorescence staining of Iba-1^+ cells in the hippocampus and cortex. Bar = 50 μm, MCH-H, high dose MCH, $n=6$, 5 slices for each mouse. Data were expressed as mean ± SEM, $^{**}P<0.01$, $^{***}P<0.001$

MCH-elicited changes in p-JNK/JNK, p-ERK/ERK, IL-6 and TNF-α levels, and cognitive impairment, sleep disorders, neuronal damage and microglia activation in IAV-infected mice, indicating that JNK/ERK signaling pathway was involved in the effect of MCH on neuroinflammation. For the first time, our study demonstrated that MCH alleviated neuroinflammation induced by IAV infection, improved cognitive and sleep disorders, and inhibitd the phosphorylation of the JNK/ERK signaling pathway. The results may add to our understanding of how MCH exerts its neuroprotective effects on brain functions, including cognitive and sleep/wake cycles. In addition, Studies showed that the MCH played a critical role in the development of Meth-induced locomotor sensitization in rats by inhibiting the phosphorylation of ERK [39]. On the contrary, MCH-treated rats had increased hepatic triglyceride storage along with specific up-regulating of activity of JNK1 [40]. So, we speculated that the effect of MCH on JNK/ERK phosphorylation might depend on tissue type and inflammatory environment, which should be addressed in further study.

Nevertheless, the study possesses certain limitations. Firstly, the experiments were only conducted in the female mice, further studies are needed to determine whether MCH was able to exert the same effect in H7N7-infected male mice. Secondly, the physiological levels of MCH and the correlation between MCH expression and inflammation, sleep, and memory in influenza patients was not assessed. More and more clinical evidence should be collected to address the effect of IAV on the level of MCH and the correlation among them. Then, the effect of MCH on the function of other brain regions, and the side effects of MCH still need to be in-depth discussion. And the effect of MCH on microglia had yet to be verified in the in-vitro cell culture experiments. Finally, how to increase MCH levels in the human brain during IAV infection was unsolved. There was still a long way to go regarding the clinical application of MCH in the treatment of neuroinflammation. In addition, there was a evidence showed that exogenous MCH microinjected into the BLA induced anxiety-like behaviors in mice [19]. However, the anxiety-like behavior of mice had not been evaluated in this study, so it was unknown whether neuroinflammation induced by IAV was associated with anxiety-like behavior in mice. And there was not designed two additional control groups of mice injected with low or high dose of MCH to evaluate the effect of MCH on the anxiety-like behaviors in mice in this study, which was also one of the limitations of the study.

Conclusion

MCH alleviated neuroinflammation and improved spatial learning and memory impairment and sleep disorder in IAV-infected mice. The mechanism, at least in part, by inhibiting the JNK/ERK signaling pathway. The findings provided a basis for the use of MCH in neuroinflammation-related diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12974-024-03251-z.

Supplementary Material 1

Author contributions

QZ designed the experiments, performed the experiments, and wrote the manuscript. XL and QM performed the experiments, finished the data analysis, and wrote the manuscript. QZ and JZ interpreted the results wrote and revised the manuscript. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

All experiment procedures were conducted in accordance with the "Guiding Principles in the Care and Use of Animals" (China) and were approved by the Laboratory Animal Ethics Committee of the Henan Province People's Hospital.

Competing interests

The authors declare no competing interests.

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