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Cognitive impairment in a classical rat model of chronic migraine may be due to alterations in hippocampal synaptic plasticity and N-methyl-D-aspartate receptor subunits

Mingjie Zhang[1,*], Yufei Liu[2,*], Guanqun Hu[3], Li Kang[1], Ye Ran[1], Min Su[1], and Shengyuan Yu[1]

Abstract

Although migraine is a major global public health problem, its impact on cognitive abilities remains controversial. Thus, the present study investigated the effects of repeated administration of inflammatory soup to the dura of rats, over three weeks, on spatial cognition, hippocampal synaptic plasticity, and the expression of N-methyl-D-aspartate receptor subunits. Additionally, low doses of amitriptyline (5 mg/kg) were applied to assess its therapeutic effects. The inflammatory soup group exhibited significant reductions in the cutaneous stimulation threshold, presence of mild cognitive impairment, and decreased long-term potentiation in right hippocampus. However, amitriptyline improved pain behaviors, enhanced cognitive function, and increased synaptic plasticity in the inflammatory soup rats. On the other hand, the administration of amitriptyline to normal rats negatively influenced synaptic plasticity and reduced the expression of N-methyl-D-aspartate receptor subunits. The present results indicate that inflammatory soup-induced dural nociception led to impairments in spatial cognition that could be attributed to reductions in hippocampal long-term potentiation and the decreased expression of N-methyl-D-aspartate receptor subunits.

Keywords

Chronic migraine, cognitive impairment, synaptic plasticity, hippocampus

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Introduction

Migraine is a major global public health problem that dramatically reduces quality of life[1,2] and impacts patients' work, rest, and cognitive function. The onset of migraine is associated with a significant decline in global cognitive efficiency, but this can be addressed via administration of sumatriptan-naproxen.[3] During migraine attacks, patients experience cognitive symptoms that include feeling distracted, an inability to concentrate, and difficulties performing mental tasks such as retrieving names from memory.[4] In particular, young adults with migraine are at higher risk of dementia[5] and cognitive impairment is more common in chronic migraineurs, especially those who overuse medications. However, other studies have reported that cognitive decline is not associated with migraine and nonmigraine headaches, especially in older subjects.[6] For example, a longitudinal study investigating a population-based sample reported no clear influence of migraine on cognitive function over time.[7] Some researchers have

[1]Department of Neurology, the First Medical Centre, Chinese PLA General Hospital, PR China
[2]Department of Neurology, Tianjin Third Central Hospital, PR China
[3]Department of Neurology, Tianjin Union Medicine Center, PR China

*These authors contributed equally to this work.

Corresponding Author:
Shengyuan Yu, Department of Neurology, Chinese PLA General Hospital, Beijing 100853, China.
Email: yusy1963@126.com

proposed that the mixed results of previous studies may be due to differences in the duration, frequency, and intensity of migraines among the populations included therein.[8]

Functional magnetic resonance imaging studies of migraineurs have revealed structural and functional changes in brain areas associated with cognitive processing, such as the hippocampus, parahippocampal gyrus, and orbitofrontal cortex,[9] where hippocampal impairments are related to the frequency of migraine.[10] Similarly, transgenic mice with familial hemiplegic migraine type 1 (FHM1), a monogenic form of migraine with aura, exhibit learning and memory impairments but show significantly stronger long-term potentiation (LTP) in the hippocampus.[11] These authors suggested that this phenomenon is related to the upregulation of glutamate transporters but did not test glutamate transporter levels. Because transgenic mice models cannot provide a full picture of migraine, the present study employed a rat model of migraine involving the repeated application of inflammatory soup (IS) to the dura over 21 days. Previous studies have reported that the nociceptive behaviors and cutaneous allodynia observed in this rat model are similar to the behaviors displayed by migraineurs.[12–14]

Amitriptyline (AMI) is a member of the tricyclic antidepressant family but is also widely used for chronic pain prevention.[15] The present study evaluated hippocampus-related spatial cognition, hippocampal plasticity, and synapse-related receptor expression in a rat model of chronic migraine, as well as the therapeutic effects of AMI in this model.

Experimental procedures

Ethical concerns

All experimental procedures were approved by the Committee on Animal Use for Research and Education of the Laboratory Animals Centre, General Hospital of Chinese People's Liberation Army (Beijing, PR China), and were consistent with the ethical guidelines of the International Association for the Study of Pain in conscious animals.[16] All efforts were made to minimize the suffering of the animals.

Habituation

The present study included 76 male Wistar rats (180–220 g) purchased from the Laboratory Animal Center, Academy of Military Medical Science of the People's Liberation Army. The rats were housed separately in a temperature-controlled environment (22–24°C) under a 12-h light/dark cycle and were gently handled for 2 to 3 min per day.

Study groups

The IS used in the present study consisted of 0.2 mM prostaglandin E_2 and 2 mM each of histamine, serotonin (5-HT), and bradykinin. The rats were divided into the following four groups: IS ($n = 19$), IS + AMI ($n = 20$), AMI ($n = 19$), and control (CON; $n = 18$). The IS (2 μL) was administered to both the IS and IS + AMI groups, whereas the AMI and CON groups received normal saline (NS). As a therapeutic measure, intragastric (ig) AMI (5 mg/kg) dissolved in water was given to the IS + AMI and AMI groups, whereas the IS and CON groups received water. During the 21-day experiment, pain thresholds were tested every day prior to the IS or NS administration, and weight was measured once per week. The Morris water maze (MWM) behavioral test was carried out after 21 days.

Surgical procedures

The cannula implantation protocol used in the present study was similar to those previously described by Zhang et al.[17] and Su et al.[18] Briefly, the rats were anesthetized with 10% chloral hydrate (4 mL/kg, intraperitoneal (ip)) and placed into a stereotactic frame. Then, a lengthwise incision was made on the head of each rat, and all connective tissues were removed using 3% hydrogen peroxide to expose the bregma. Next, a craniotomy was performed using a drill (1 mm in diameter; +1.5 mm after and +1.5 mm lateral to the bregma) to prevent damage to the dura. A plastic cannula with a stainless steel needle extending 1 mm from the bottom of the tube was inserted into the hole and fixated using 502 glue; a cap was used to close the cannula and thus prevent clogging. Dental cement was used for further fixation, and the incision was sutured. All rats received prophylactic treatment with an antibiotic (cefdinir 10 mg/kg, ig) for three days after surgery. All experiments were conducted four days after the operation.

Pain threshold examination

To assess pain thresholds, the rats were placed in a $45 \times 30 \times 15\,cm^3$ cage that allowed for free movement, and pressure thresholds were obtained using von Frey filaments with manufacturer-assigned force values (15, 10, 8, 6, 4, and 2 g) that were applied perpendicularly to the periorbital region. A positive response to the von Frey test was indicated by a rat stroking its face using the ipsilateral forepaw, with the head quickly recoiling to the other side or vocalization after the stimulus.[14] Facial allodynia was assessed using the von Frey test, and the 50% positive response threshold was calculated.

MWM experiment

The MWM test is designed to measure spatial learning and memory abilities. In the present study, the tank used for the MWM test was 150 cm in diameter and 60 cm in height and was divided into four quadrants. This tank was filled to a depth of 45 cm with water colored with nontoxic black ink and contained a movable black platform (10 cm in diameter, 2 cm beneath the water surface) placed in the third quadrant as the target. A camera was located above the center of the maze and was connected to a computer for recording and analysis. The environment was kept under dark and quiet conditions, and the water temperature was maintained at $23 \pm 1^{\circ}C$.

The experiment consisted of four stages: the acquisition phase (AP), space exploring test (SET), reversal phase (RP), and reversal exploring test (RET). During the AP, each rat performed two sets of eight trials per day for four days. In each trial, the rats were randomly placed into the water facing the pool wall at one of the four starting points (one in each quadrant) and were allowed to swim freely for 60 s until they reached the platform; if a rat did not reach the platform in 60 s, it was guided to the platform and allowed to remain thereon for 10 s. The intervals between two trials were at least 5 min in duration, and there was an 8-h interval between the two sessions in a single day. The time required to locate the platform (escape latency) and swimming speed were recorded by the camera connected to the computer. During the SET, the platform was removed from the pool, and the rats were released from the starting point in the fourth quadrant. The time spent swimming in the target quadrant and the number of platform crossings within 60 s were recorded. During the RP, the platform was moved to the opposite quadrant (fourth quadrant), and the rats were trained to locate the platform in the same manner as in the AP stage over a two-day period. During the RET, the platform was removed from the pool, and the rats were released from the first quadrant. The methods used and data recorded were the same as in the SET.

LTP recordings

The electrophysiological experiments were performed 1 to 3 days after the 21-day IS (or NS) administration period. Following anesthetization with 10% urethane (4 mL/kg, ip), each rat was placed into a stereotaxic frame (Narishige, Tokyo, Japan), and a hole large enough to accommodate the recording and stimulating electrodes was drilled into the skull. A monopolar copper recording electrode (0.18 mm in diameter) was placed in the dorsal CA1 region (3.5 mm posterior to the bregma, 2.5 mm lateral to midline, and 2.0 mm ventral to the dura), and a concentric bipolar copper stimulating electrode with a tip separation of 0.2 mm was placed in the dorsal CA3 region (4.2 mm posterior to the bregma, 3.5 mm lateral to midline, and 2.0 mm ventral below the dura). Stimuli ranging from 0.1 to 1.0 mA were applied every 10 s at various intensities to obtain the input/output (I/O) curve. As a baseline, a stimulus (range: 0.3–0.5 mA) that could evoke 50% to 70% of its maximum effect was selected for sampling, with single-pulse stimulations delivered every 60 s for 30 min. After recording the baseline, high-frequency stimulation (HFS; 12 pulses at 100 Hz for 6 s, repeated 30 times) was administered to induce LTP. Field excitatory postsynaptic potentials (fEPSPs) were recorded following single-pulse stimulations delivered every 60 s for 60 min.[19,20]

Western blot assays

The hippocampal tissue samples were homogenized in ice-cold lysis buffer (P0013B; Beyotime Biotechnology, Haimen, China) containing 0.1% phenylmethylsulfonyl fluoride (Beyotime Biotechnology). Next, the homogenates were centrifuged at 12,000*g* for 20 min at 4°C, the dissolved proteins were collected from the supernatant, and the protein concentrations were determined using a bicinchoninic acid protein assay kit (CW0014; Beyotime Biotechnology). Identical amounts of protein (15 μg) were separated using 6% to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and were then transferred onto polyvinylidene fluoride membranes (Millipore, Burlington, MA, USA).

After blocking the membranes with 5% skim milk for 1 h at room temperature, they were incubated overnight at 4°C with the primary antibodies for anti-N-methyl-D-aspartate receptor subunit 2A (NMDAR2A; 1:3,000, Millipore), anti-NMDAR2B (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), synaptophysin (1:10,000, Millipore), anti-beta-catenin (1:5,000, Millipore), anti-active-beta-catenin (1:5,000, Millipore), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:6,000, Millipore). After three washes, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (1:5,000, ZSGB-BIO, Beijing, China) for 1 h at room temperature. Finally, the antibody-reactive bands were visualized using enhanced chemiluminescence detection reagents (Millipore) and a gel imaging system (Tanon 5200; Tanon Science & Technology Co Ltd., Shanghai, China).

Immunofluorescence staining

After perfusion with 0.1 mol/l phosphate buffer (pH 7.4) and 4% paraformaldehyde, the brains were removed from the rats and dehydrated with graded sucrose solutions. The brain slices were sectioned at a thickness of 10 μm and then washed three times in phosphate-buffered saline. The slices were blocked with 10% goat

serum for 1 h at room temperature, incubated with NR2A and NR2B (1:1,000; Abcam, Cambridge, UK) for 18 h at 4°C, and then incubated with Alexa 488-conjugated anti-rabbit IgG (1:1000; Thermo Fisher, USA) for 6 h at room temperature. Finally, the slices were stained with 4,6-diamidino-2-phenylindole for 3 min at room temperature, and the fluorescent signals were detected with a laser-scanning confocal microscope.

Statistical analyses

SPSS 22 software (SPSS Inc., Chicago, IL, USA) was used for all data analyses, and all data are presented as mean ± standard error of the mean. Abnormally distributed data were analyzed using the Kruskal–Wallis test to determine differences among the groups. Other data were analyzed using either one-way analysis of variance (ANOVA) or two-way repeated-measures ANOVA. A least significant differences *t*-test (when the variance was regular) or Dunnett's T3 test (when the variance was irregular) was used to compare the differences among the groups. In addition, p values <0.05 were considered to indicate statistical difference.

Results

Facial withdrawal

The time to facial withdrawal was measured to confirm the presence of cutaneous allodynia. Facial withdrawal threshold had significant differences among four groups ($F = 34.620$, $p < 0.0001$, two-way repeated-measures ANOVA). There were no significant differences ($p > 0.05$, one-way ANOVA) among the four groups on Day 1. However, after the application of IS or NS for 21 days, the time to facial withdrawal of the IS group was significantly reduced compared to the CON group ($p < 0.001$, one-way ANOVA) from Day 7 to Day 21. There was no significant difference between the IS + AMI and IS groups ($p = 0.657$, one-way ANOVA) on Day 7, but difference was obvious from Day 14 ($p = 0.038$, one-way ANOVA) to Day 21 ($p = 0.038$, one-way ANOVA) (Figure 1).

Effects of chronic IS on the MWM test

The MWM test was conducted to ascertain the effects of IS on spatial cognition, spatial learning, and memory. The swimming speeds of the four groups did not have any differences ($F = 2.592$, $p = 0.055$, two-way repeated-measures ANOVA). The latencies of all four groups significantly decreased over time in each quadrant during the AP. However, the IS group spent more time finding the platform ($F = 4.845$, $p = 0.002$, two-way repeated-measures ANOVA), especially in the first and second quadrants on Day 4 compared to the other three groups (first quadrant, $p = 0.03$; second quadrant, $p = 0.011$; one-way ANOVA; Figure 2(a)). During the SET, the target quadrant dwell time ($p = 0.035$, one-way ANOVA) and platform crossings ($p < 0.001$, one-way ANOVA; Figure 2(d) and (e)) both significantly decreased in the IS group compared to CON group.

Reversal learning analyses were also conducted. The latencies were significantly increased in IS group compared to CON group ($p = 0.041$, two-way repeated-measures ANOVA) and AMI group ($p = 0.032$, two-way repeated-measures ANOVA). Meanwhile, the IS group exhibited a significant increase in latency compared to the CON group on the first day of the RP in the second quadrant ($p = 0.026$, one-way ANOVA; Figure 2(b)). During the RET, there was no significant difference in the target quadrant among the four groups, but significantly fewer platform crossings were seen in the IS group than the CON group ($p = 0.004$, one-way ANOVA; Figure 2(f) and (g)). Additionally, there were significantly fewer platform crossings in the IS group

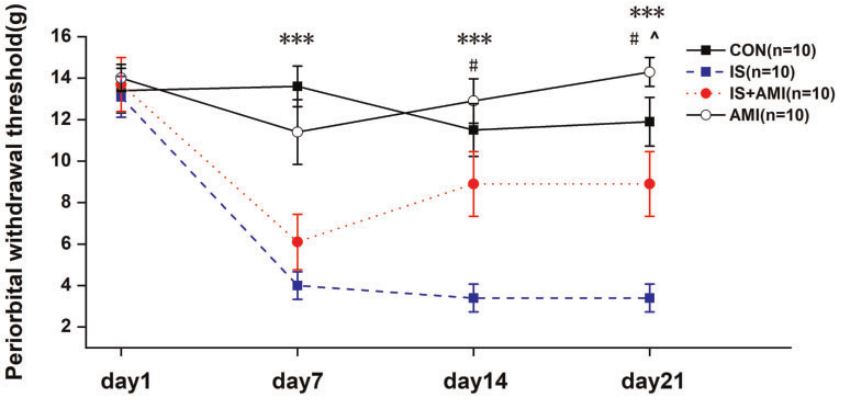

Figure 1. Threshold changes in cutaneous allodynia in the four experimental rat groups. All data are presented as mean ± standard error of the mean (SEM); ***$p < 0.001$, IS versus CON; #$p < 0.05$, IS versus IS+AMI; ^$p < 0.05$, IS + AMI versus AMI.
CON: control; IS: inflammatory soup; AMI: amitriptyline.

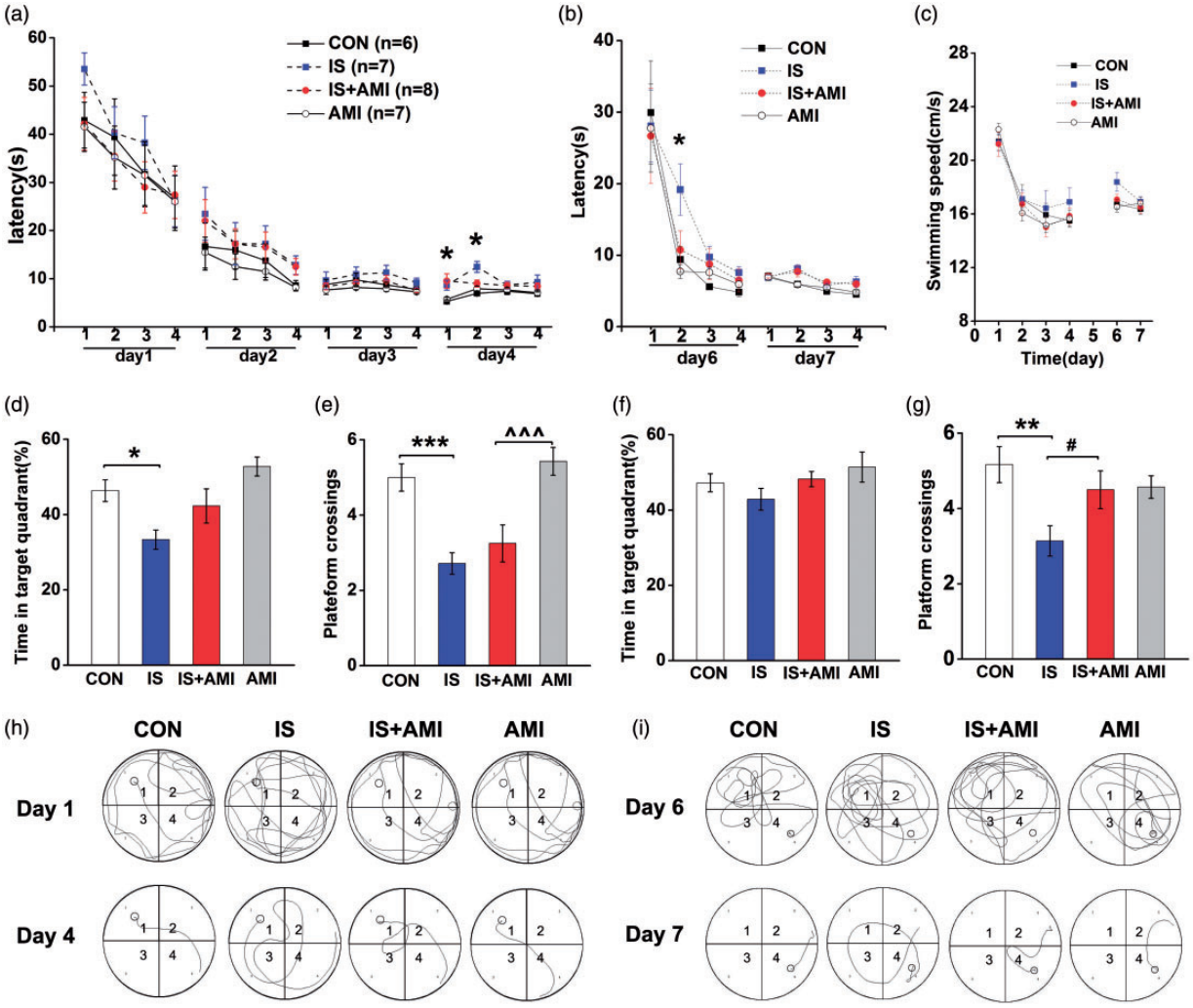

Figure 2. Performance in the Morris water maze (MWM) test. (a) Mean escape latencies for each quadrant for the four groups during the acquisition phase (AP), (b) comparison of time spent in the target quadrant among the four groups in the space exploring test (SET), (c) swimming speeds for each training session in the AP and reversal phase (RP), (d) target quadrant dwell time during the SET, (e) number of platform crossings during the SET, (f) target quadrant dwell time during the RET, (g) grdNumber of platform crossings during the reversal exploring test (RET), (h) representative swim traces of rats for Day 1 and Day 4 during the AP, and (i) representative swim traces of rats for Day 6 and Day 7 during the RP. All data are presented as mean ± SEM; *$p < 0.05$, **$p < 0.01$,***$p < 0.001$, IS versus CON; #$p < 0.05$, IS versus IS+AMI; ^^^$p < 0.001$, IS+AMI versus AMI.
CON: control; IS: inflammatory soup; AMI: amitriptyline.

than the IS + AMI group ($p = 0.031$, one-way ANOVA; Figure 2(g)).

Effects of chronic IS on LTP in the hippocampus

LTP recordings were performed to assess the effects of chronic IS and AMI on synaptic plasticity in the hippocampus. As shown in Figure 3, after HFS, the fEPSP slope increased in all groups. There was no difference among the four groups in terms of the fEPSP in the left hippocampus after HFS ($p > 0.05$, Figure 3(a) and (b)). However, in the right hippocampus, the fEPSP in the IS group was significantly lower than in the CON group ($p < 0.05$, Figure 3(c) and (d)). There was no difference between the CON and IS + AMI groups in the right hippocampus ($p < 0.05$, Figure 3(c) and (d)), which is indicative of the effectiveness of AMI. Interestingly, the fEPSP was significantly lower in the AMI group than in the CON group ($p < 0.01$, Figure 3 (c) and (d)).

Effects of chronic IS on NMDAR subtypes and synaptophysin expression

Proteins related to cognitive function were examined with Western blot assays (Figures 4 and 5) and immunofluorescence staining (Figure 6). There was no

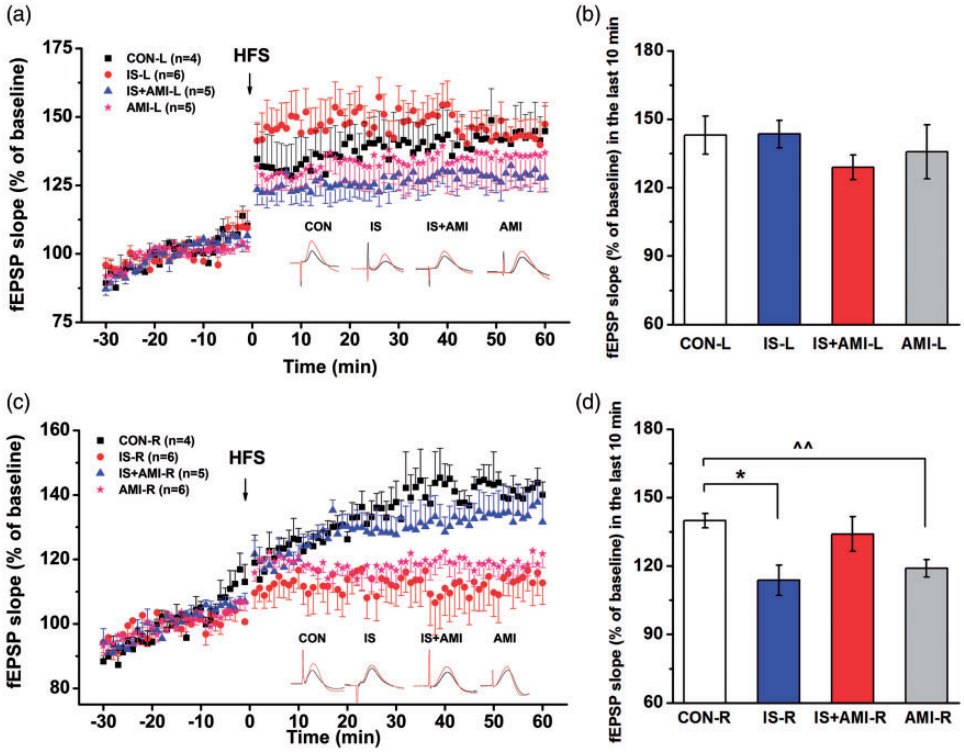

Figure 3. Changes in synaptic function in the CA3–CA1 regions. (a) fEPSP slopes in the left hippocampus, (b) mean normalized fEPSP slopes over the last 10 min in the left hippocampus, (c) fEPSP slopes in the right hippocampus, and (d) mean normalized fEPSP slopes over the last 10 min in the right hippocampus. All data are presented as mean ± SEM; *$p < 0.05$,IS versus CON; ^^$p<0.01$, AMI versus CON. fEPSP: field excitatory postsynaptic potential; HFS: high-frequency stimulation; CON: control; IS: inflammatory soup; AMI: amitriptyline.

difference in the expression of synaptophysin or NR2B among the four groups on either side of the hippocampus (Figures 4(b) and (d) and 5(b) and (d)). However, NR2A expression was markedly downregulated in the IS + AMI ($p < 0.05$ vs. CON group, Figures 4(c) and 5(c)) and AMI ($p < 0.001$ on the left side, $p < 0.01$ on the right side vs. CON group, Figures 4(c) and 5(c)) groups on both sides of the hippocampus and in the IS group in the right hippocampus ($p < 0.001$ vs. CON group, Figure 5(c)). Interestingly, the NR2A/2B ratio decreased in both the IS + AMI and AMI groups ($p < 0.01$ vs. CON group, $p < 0.05$ vs. IS group, Figure 5(e)). Additionally, the NR2A/2B ratio decreased in both the IS ($p < 0.01$ vs. CON group, Figure 5(e)) and IS + AMI ($p < 0.05$ vs. CON group, Figure 5(e)) groups.

As shown in Figure 6, NR2A and NR2B were both stained with green fluorescence, and their nuclei were stained blue. As membrane proteins, NR2A and NR2B are represented by either the distribution of green points or green rings around nuclei. NR2A expression obviously decreased in the IS, IS + AMI, and AMI groups compared to the CON group (Figure 5(a)), and NR2B expression obviously decreased in the AMI group compared to the CON group (Figure 5(b)). These results were similar to those of the Western blot assays.

Discussion

The present study assessed whether repeated application of IS leads to cognitive impairment and whether a small dose of AMI can effectively treat any such cognitive impairment (and pain). The IS group of rats exhibited cutaneous allodynia and cognitive impairment that may have been due to impaired hippocampal LTP and reduced expression of NMDAR subunits. Although a small AMI dose improved the IS-induced allodynia and cognitive impairment, the same dose of AMI resulted in impaired hippocampal LTP and lower expression of NMDAR subunits in normal rats.

Cognitive impairment

Many studies have demonstrated a relationship between migraine and cognitive impairment, and most observed poor cognition during migraine attacks.[21] Other studies have reported that episodic migraine patients do not show abnormalities in cognitive evaluations during interictal periods,[22,23] which suggests that cognitive

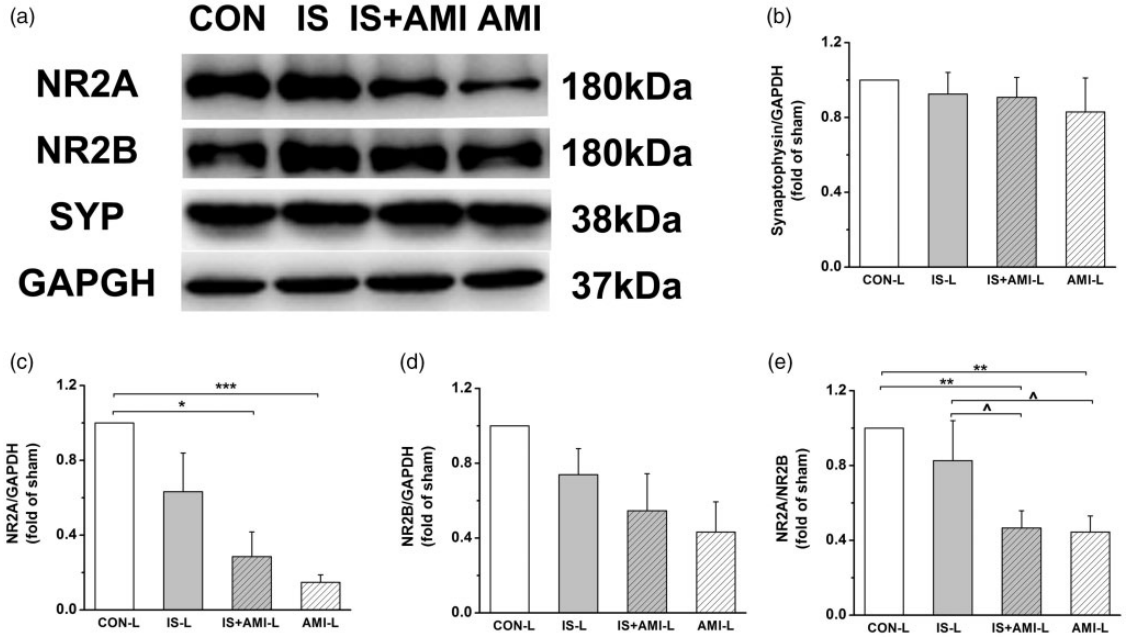

Figure 4. Western blot analyses of NR2A, NR2B, and synaptophysin protein levels in the left hippocampus. (a) Western blot assay results, (b) synaptophysin/GAPDH ratio, (c) NR2A/GAPDH ratio, (d) NR2B/GAPDH ratio, and (e) NR2A/NR2B ratio. All data are presented as mean ± SEM; *$p < 0.05$, **$p<0.01$, ***$p < 0.001$, CON versus other groups; ^$p<0.05$, IS versus IS+AMI or AMI.
GAPDH: glyceraldehyde 3-phosphate dehydrogenase; CON: control; IS: inflammatory soup; AMI: amitriptyline.

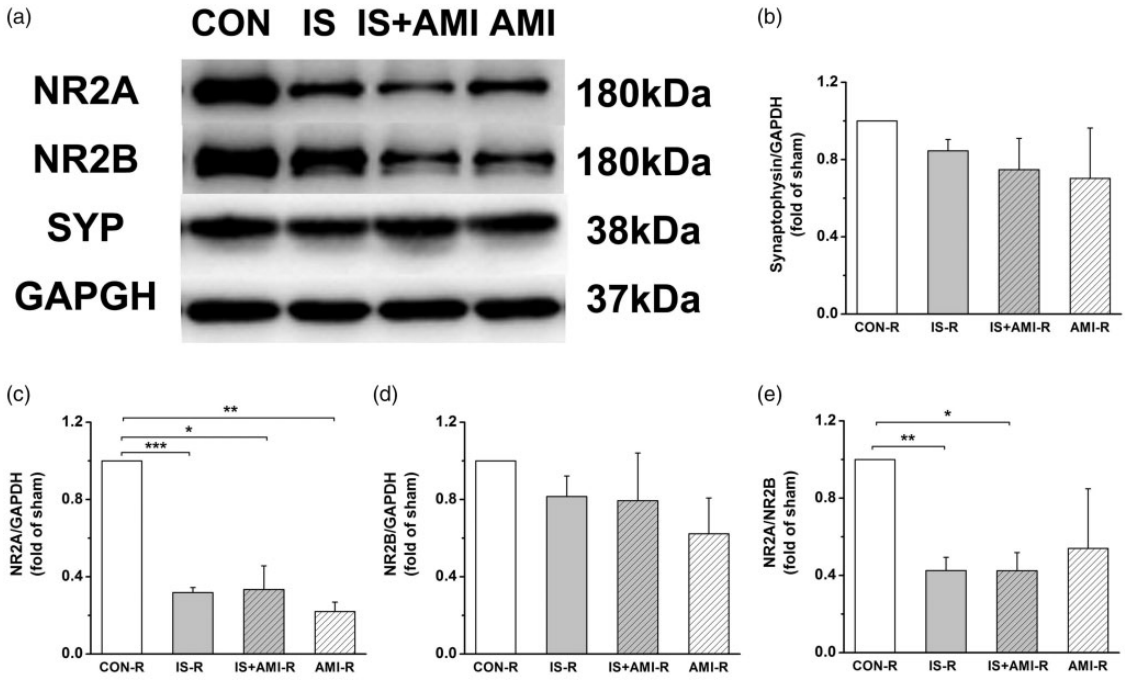

Figure 5. Western blot analyses of NR2A, NR2B, and synaptophysin protein levels in the right hippocampus. (a) Western blot assay results, (b) synaptophysin/GAPDH ratio, (c) NR2A/GAPDH ratio, (d) NR2B/GAPDH ratio, and (e) NR2A/NR2B ratio. All data are presented as mean ± SEM; *$p < 0.05$, **$p<0.01$, ***$p < 0.001$, CON versus other groups.
GAPDH: glyceraldehyde 3-phosphate dehydrogenase; CON: control; IS: inflammatory soup; AMI: amitriptyline.

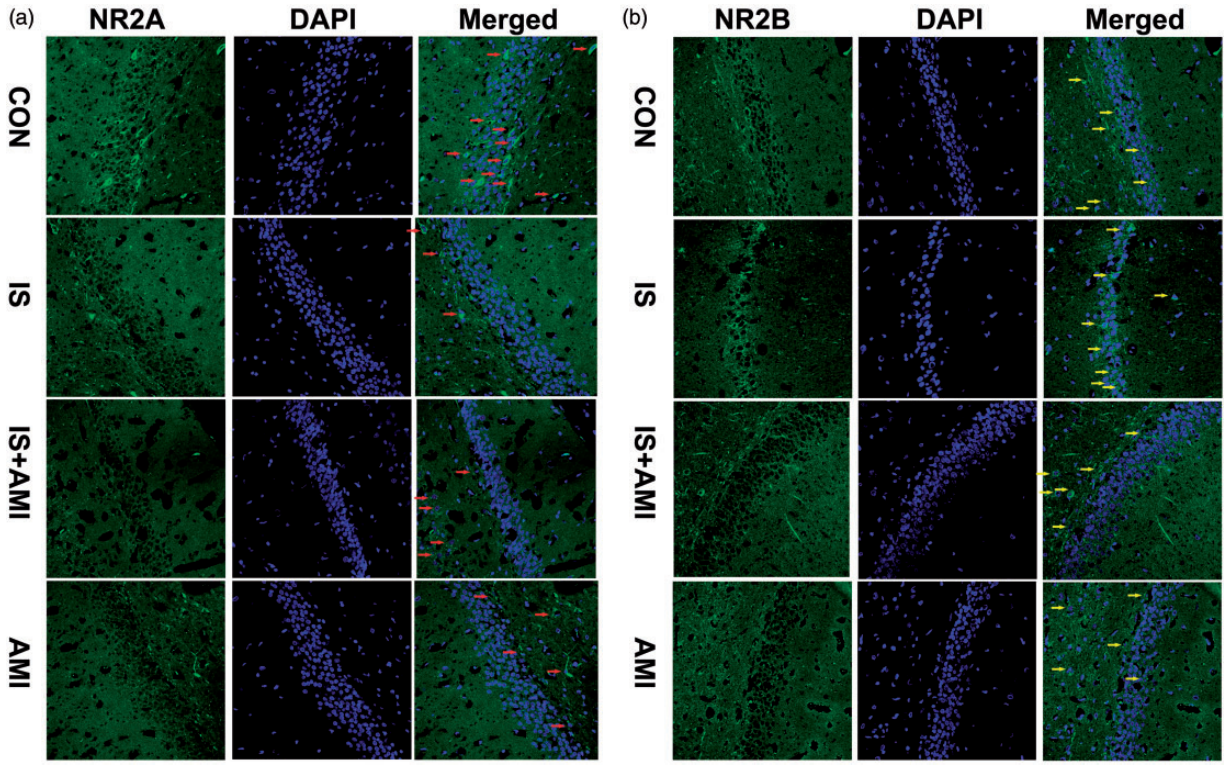

Figure 6. Immunofluorescence staining of NR2A and NR2B expression. (a) NR2A immunofluorescence staining results and (b) NR2B immunofluorescence staining results.
CON: control; IS: inflammatory soup; AMI: amitriptyline; DAPI: 4,6-diamidino-2-phenylindole.

dysfunction during migraine attacks is reversible. In the present study, additional analgesic drugs were not administered to the rats because this parallels the overuse of medications in humans.

The repeated administration of IS to the dura of the rats resulted in spatial memory impairments during both the SET and the RET of the MWM. Additionally, the reduced rate of learning observed in the IS group during the AP was in accordance with previous findings showing that spinal cord injury (SCI) in FHM1 transgenic mice causes chronic neuropathic pain.[11,24,25] Moreover, reference memory is determined based on the preference of an animal for the platform area when the platform is absent during the SET.[26] In the present study, the IS rats displayed poor performance in terms of identifying the target quadrant, because their dwell time decreased and they also showed fewer platform crossings (Figure 2(b) and (c)). SCI mice also spend significantly less time in the target quadrant and rely on looping search strategies during this test.[24]

The RP and RET are typically used to detect spatial impairments[26]; such impairments were seen in the AP and SET in the present study. Also, the IS rats exhibited an increased latency in the RP, which indicates that they did not have the cognitive flexibility required to learn about new objects. In a spared nerve injury (SNI) rat model, the animals exhibited poor performance during a reversal task.[27] In the present study, the time spent in the target quadrant during the RET did not significantly differ among groups; the number of platform crossings better reflected memory accuracy. During repeated trials, tests with a higher degree of difficulty can more accurately reflect differences between IS and CON groups. SCI mice,[24] SNI rats,[27] and the present IS-induced headache rats all represent models of chronic pain resulting in impaired spatial learning and memory. However, it is possible that the cognitive deficits observed were induced by the chronic pain and were not attributable specifically to headache.

Hippocampal impairments

The hippocampus is a very important brain region with respect to cognitive behaviors that involve memory, and measurement of LTP in this area is important for understanding the synaptic basis of learning, memory, and information storage.[28] The present study showed that IS-induced headache significantly decreased synaptic LTP in the right CA3–CA1 region, which is consistent with the behavioral test (MWM) results. Chronic social defeat also suppresses LTP in CA3–CA1 region synapses, as shown by hippocampal slices,[29] which is in

accordance with the findings of the present study. Furthermore, other researchers found that synaptic transmission in the hippocampal CA1 region was impaired by chronic pain induced via partial ligation of the sciatic nerve,[30] as well as by chronic stress.[31,32] However, contrasting results were observed in a chronic visceral pain FHM1 mouse model, wherein CA1 hippocampal LTP was increased.[11,33] It is possible that neuropathic pain and visceral pain are fundamentally different; the increased LTP in the latter studies may have been due to enhanced memory of visceral pain, while the suppression of LTP in the former studies may have led to impaired cognition, as observed herein.

NMDARs play an important role in synaptic plasticity. As important subunits of NMDARs, NR2A and NR2B are primarily located in the adult cortex and hippocampus and promote LTP and long-term depression (LTD). However, these subunits have different functions; moreover, NR2A is predominantly confined to the synapses of mature neurons, whereas NR2B is mainly distributed extrasynaptically.[34,35] Liu et al.[35] showed that the expression of NR2A, but not NR2B, was markedly decreased in the hippocampus in a rat model of vascular dementia, which indicates that weakened synapses and a reduced capacity for neuronal survival are more related to NR2A than NR2B. It has also been shown that selective inhibition of NR2A prevents the induction of LTP without affecting LTD production.[35] In the present study, NR2A levels and the NR2A/NR2B ratio decreased in the IS group, whereas there were no significant difference in NR2B level between the IS and CON groups. These findings are consistent with the results of previous studies.

In the present study, the deficits in LTP in the hippocampus of IS rats may have been due to the downregulation of NR2A over the 21 days of IS administration. Synaptophysin is a major presynaptic membrane protein associated with synaptic vesicles[36] that plays a role in synaptic plasticity in the central nervous system.[37] Hung et al.[37] observed upregulation of presynaptic proteins in the medial prefrontal cortex of rats with neuropathic pain, whereas Moriarty et al.[38] reported the absence of changes in presynaptic protein levels in both the medial prefrontal cortex and hippocampal CA1 region in a rat model of neuropathic pain. In the present study, there was no significant group difference in synaptophysin level, which indicates that synaptophysin is not involved in the suppression of hippocampal LTP.

In humans, the right hippocampus is primarily involved in spatial navigation, while hippocampal dominance has yet to be reported in rodents. Liu et al.[39] found that right hippocampal volume was positively associated with positive migraine outcomes, and a cross-sectional study showed that migraineurs have lower left and total (right plus left) hippocampal volume.[40] Chen et al.[41] reported that the volumes of the hippocampal subfields were lower in medication-overuse headache (MOH) patients than those in normal controls, except for the right hippocampus tail, bilateral parasubiculum, and hippocampal fissure. In the rats in the present study, there were more group differences in the right versus left hippocampus. Klur et al.[42] observed greater number of differentially expressed genes in the right hippocampus than the left hippocampus (623 vs. 74), as well as functions specific to one or other side of the hippocampus. The present results suggested that the right hippocampus is more associated with pain and spatial memory.

Effects of low-dosage AMI

AMI is one of the most commonly used medications for migraine and has well-documented efficacy as a migraine prophylactic.[15,43] However, determining the exact mechanisms underlying the effects of AMI has proven difficult due to its inhibitory effects on 5-HT and noradrenaline reuptake, and its blockade of ion channels, where these phenomena appear to be unrelated to its antidepressant effects.[44] AMI is a tricyclic antidepressant administered in doses of 100–300 mg/d that has an onset time of approximately 1 month. However, because AMI is an antagonist of tetrodotoxin (TTX)-sensitive[45] and TTX-resistant[46,47] sodium channels, its effects onset relatively quickly and necessitate a lower dosage. In the present study, AMI treatment significantly improved cutaneous allodynia compared to the IS group beginning on Day 7 and was associated with a 66.7% recovery of the withdrawal threshold compared to the CON group on the last day of testing. Also, AMI improved performance during the SET and RET of the MWM, increased LTP, and ameliorated the expression of NR2A at the end of the third week.

A study using 36TgAD mice to investigate the potential of AMI as a therapy for Alzheimer's disease (AD) found that AMI altered Aβ and tau deposition, enhanced the expression of synaptic factors in the hippocampus (i.e. synaptophysin, synapsin I, postsynaptic density-95 protein (PSD-95), and spinophilin), and facilitated learning and memory.[48] Although AMI may also influence cognitive impairment, it remains unclear whether these effects occur directly or via improvements in pain.

Interestingly, in the present study, AMI monotherapy impaired LTP in the right CA3–CA1 hippocampal synapses and also reduced NR2A expression without affecting the MWM results. Although AMI has positive effects on chronic pain and depression, it is also associated with adverse effects such as somnolence and dry mouth. Thus, even when a patient or rat does not exhibit discomfort, the administration of AMI may lead to harmful effects.

Conclusions

The present study employed a rat model of chronic headache involving repeated infusion of IS to the dura and found that the rats exhibited learning and memory deficits in the MWM test. Furthermore, the electrophysiological results indicated reduced-strength baseline synaptic transmission and decreased levels of synaptic plasticity in the CA1 region. However, AMI, which is used as a prophylactic drug, effectively reduced pain sensitivity in the rat model and slightly ameliorated the abovementioned deficits. On the other hand, a single administration of AMI in the absence of IS impaired synaptic plasticity and reduced the expression of NMDAR subunits. The present findings emphasize the importance of treating chronic headache due to its possible impact on cognitive ability.

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Author Contributions

SY and MZ conceived of and designed the study. MZ, YL, GH, LK, YR, and MS were responsible for the acquisition of data. MZ and YL analyzed and interpreted the data and drafted the article, and SY revised it for intellectual content. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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ORCID iD

Shengyuan Yu https://orcid.org/0000-0001-8933-088X

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