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Epileptic Disorders

ORIGINAL ARTICLE

Nucleus accumbens shell electrical lesion attenuates seizures and gliosis in chronic temporal lobe epilepsy rats

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Abstract

Objective: Temporal lobe epilepsy (TLE) is the most prevalent form of epilepsy. Prior research has indicated the involvement of the nucleus accumbens shell (NAcSh) in the process of epileptogenesis, thereby implying its potential as a therapeutic target for TLE. In the present study, we investigated the antiepileptic effect of the NAcSh electrical lesion.

Methods: Chronic TLE was induced by stereotactic injection of kainic acid (KA) into the hippocampus 3 weeks after KA administration, and NAcSh electrical lesions were performed. Seizures in rats were monitored by video electroencephalogram (EEG) 1 week following the NAcSh electrical lesion. Besides, the spatial memory function assessment in rats was conducted using the Morris water maze (MWM) test in the final week of the experiment. Later, hippocampal glial cell activation and neuron loss in rats were evaluated through immunohistochemistry.

Results: TLE rats subjected to NAcSh electrical lesion exhibited a significant reduction in the frequency of seizures compared to untreated TLE rats. Furthermore, NAcSh electrical lesion led to less activation of hippocampal glial cells and fewer neuronal loss in TLE rats. It is worth noting that the NAcSh electrical lesion did not cause additional memory impairment.

Significance: In the present study, the NAcSh electrical lesion exhibited a definitive therapeutic effect on the chronic TLE rat model, potentially due to decreased hippocampal TLE-induced activation of glial cells and neuron loss. In conclusion, our results indicated that the NAcSh is a promising therapeutic target for TLE and possesses high potential for clinical application.

KEYWORDS
gliosis, hippocampus, lesion, nucleus accumbens shell, temporal lobe epilepsy

Shuaishuai Xue and Peiyao Yi contributed equally.

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1 | INTRODUCTION

Epilepsy is a prevalent neurological disorder, characterized by synchronized electrical discharges and neuronal hyperexcitability, and culminating in seizures.[1,2] The number of epilepsy patients worldwide is estimated to be over 70 million.[1] Epilepsy encompasses various types, with temporal lobe epilepsy (TLE) emerging as the most common one.[3,4] TLE is featured by recurrent spontaneous seizures (SRSs) and is often accompanied by cognitive and psychological deficits, which impose significant burdens on affected individuals, their families, and society as a whole.[5–7]

For the majority of TLE patients, the primary treatment modality involves the administration of anti-seizure medication (ASM). Although more than 25 ASMs are currently available, approximately only 66% of TLE patients respond to ASMs.[1] Presently, while surgical therapy is considered to be the most effective treatment for patients with refractory TLE, challenges persist for patients with multiple foci or foci located in functional brain areas, as well as cases with unidentified foci.[8] Therefore, the identification of novel therapeutic targets for TLE is urgently needed.

The nucleus accumbens (NAc), a crucial ventral striatum region, is traditionally thought to be associated with emotional processes and limbic–motor interfaces.[7] However, recent studies have also shown that NAc is associated with TLE.[7,9–13] In TLE mice, c-fos expression was significantly higher in the NAcSh than in the nucleus accumbens core (NAcC), and inhibition of medium spiny neurons in NAcSh effectively reduced TLE propagation.[11] In addition, several clinical researches have also proven that NAc deep brain stimulation (DBS) effectively reduces seizures.[12,13] Nonetheless, the therapeutic efficacy of targeting the NAc to treat TLE is controversial, and its underlying mechanisms remain unclear.

The NAc is often subdivided into NAcC and nucleus accumbens shell (NAcSh) based on fiber attachment patterns and electrophysiological and histochemical features.[14] And the NAcSh is more tightly related to the limbic system than the NAcC. Moreover, NAcSh has a higher density of medium spiny neurons than NAcC. Hence, the NAcSh may contribute to TLE propagation. At the molecular level, the distribution of NAcC neurotransmitters and receptors is uniform, whereas the distribution of NAcSh neurotransmitters and receptors is highly heterogeneous.[7] Our previous imaging studies identified significant structural alterations in the NAcSh compared to the NAcC in patients with TLE. Further analysis revealed an enhanced functional connectivity between the hippocampus and NAc in TLE patients.[9,10] Although the specific involvement of these structural and functional alterations in seizures remains unknown, these intriguing findings strongly suggested the deep involvement of the NAc, especially the NAcSh subregion, in TLE. Notably, research by Schmitt and colleagues demonstrated that NAc-DBS resulted in a median reduction of 37.5% in the incidence of disabling seizures among patients without any alteration in neuropsychological and psychiatric evaluations.[12] Additionally, the NAc-DBS treatment resulted in a 50% reduction in the frequency of disabling seizures for 75% of epilepsy patients, according to Kowski et al. Consistent with Schmitt et al.'s findings, there were no significant changes in psychiatric or neuropsychological assessments among patients with epilepsy.[13] Our previous clinical investigation has demonstrated that NAcSh-DBS significantly reduced the frequency of seizures in patients with TLE and concurrently ameliorated psychiatric and neuropsychological symptoms (unpublished data). Hence, it is reasonable to speculate that the lack of differentiation between NAc subregions in previous clinical studies may have contributed to the unsatisfactory efficacy of NAc-DBS.

Based on previous research, as well as our prior studies, we implemented NAcSh electrical lesion on a chronic TLE rat model to investigate potential TLE therapeutic effects of targeting NAcSh. Excitingly, our results demonstrated that NAcSh electrical lesion effectively and significantly attenuated seizures in TLE rats, thereby highlighting NAcSh as a promising surgical target for TLE treatment.

Key points

- NAcSh electrical lesion reduced seizure frequency in TLE rats.
- NAcSh electrical lesion did not result in any additional impairment of memory function.
- NAcSh electrical lesion decreased hippocampal glial cell activation and neuron loss in TLE rats.

2 | MATERIALS AND METHODS

2.1 | Animals

In this experiment, 200–250 g adult male Sprague-Dawley rats (Beijing SPF Biotechnology Co., Ltd.) were used. Rats were kept in SPF-class animal facilities (22°C–25°C) and ad libitum access to water and food. The rats were housed in a 12 h day–night cycle. Prior to the experiment, all rats rested for at least 1 week. Rats were randomly assigned to control, kainic acid (KA), and lesion groups. Southern Medical University's Animal Experimentation Committee has approved the experimental protocol of this study

(NFYY-2020-1037). Every effort was made to minimize the number of animals used as well as the animal suffering in this study.

2.2 | Chronic TLE rat model building

The KA-induced TLE model was constructed according to the methods described in previous articles.[15–18] Briefly, KA (Sigma) was dissolved in saline (1 μg/μl) and injected slowly (0.1 μL/min) into the right hippocampus (anteroposterior (AP), −5.6 mm; mediolateral (ML), −4.5 mm; dorsoventral (DV), −5.5 mm) under anesthesia (isoflurane, RWD). KA solutions were replaced with saline injections for the control group (Figure 1B). The severity of seizures was evaluated using the modified Racine scale.[19] Diazepam (10 mg/kg i.p., King York) was given to rats 2 h after the first generalized seizure to reduce mortality. Only rats that developed grade IV and higher seizures were taken into the subsequent study. In the KA group, two rats died, while the remaining six survived and were subsequently utilized in further experiments. In the lesion group, two rats were excluded due to improper localization of the electrical lesion, leaving six rats for subsequent experimentation. The control group consisted of six rats.

2.3 | NAcSh stereotactic electrical lesion and electrodes implantation surgery procedure

The NAcSh coordinates (AP: +1.6 mm, ML: −1.0 mm, and DV: −7.7 mm) were determined according to the Paxinos and Watson Rat Brain Atlas (6th edition). Considering that the latency of the KA-induced TLE rat model is approximately 14 days [37], a homemade stainless-steel double-stranded electrode was implanted into the rat NAcSh under isoflurane

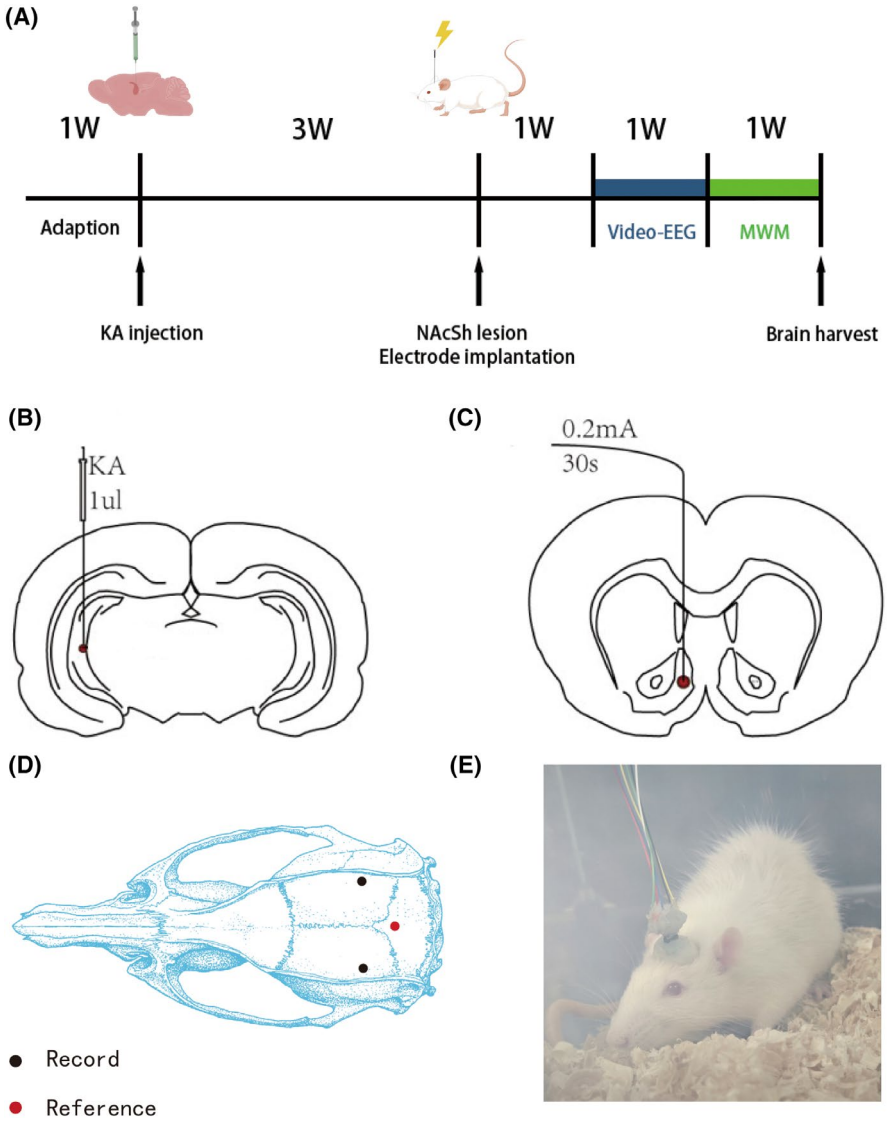

FIGURE 1 Experimental design schematic. (A) Schematic diagram of the experimental scheme (by Figdraw). (B) Diagram of KA injection site. (C) Diagram of the electrical lesion site. (D) Schematic diagram of electrode placement (the size of the dots is not to scale). The image was modified from Paxinos and Watson Rat Brain Atlas (6th edition). (E) Photograph of a rat that underwent EEG examination.

(RWD) anesthesia 14 days after KA injection. The electrode was wrapped with insulating material, and the insulating layer was scraped 0.2 mm from the tip to ensure that the tip was conductive. The stimulator (Ugo Basile) was connected to the electrodes via wires. The lesion current was 0.2 mA and the duration was 30 s (Figure 1C). Lesion and injection sites were histologically confirmed, and the rats with incorrect sites were excluded at the end of the study (Figure 2A,B). Subsequently, three stainless-steel screws were screwed into the rat skull as electroencephalogram (EEG) electrodes. The recording and reference electrodes were placed as shown in Figure 1D. The recording electrodes were located above the hippocampus, and the reference electrode was placed above the cerebellum. And then, the screws were connected to the micro-plugs by a wire. Finally, the screws were fixed with dental cement.

2.4 | Video-EEG recording

The EEG signal cable was connected to the micro-plug via a reversing device to ensure the rat's movement was not restricted (Figure 1E). Rat seizures were recorded (8 h per day, 9:00 a.m.–5:00 p.m.) by video EEG during the 5th week after KA injection. Seizures of grades III–V are observed and recorded. The EEG was recorded through the BL420F system (1–100 Hz low- and high-frequency filter, sampling rate: 500 Hz, TaiMeng). All behavioral observations and EEG were analyzed offline by trained professionals who had no prior knowledge of the grouping of the rats.

2.5 | Histological procedures

2.5.1 | Tissue processing

Six weeks after KA injection, rats were deeply anesthetized with isoflurane (RWD) and intracardially perfused with phosphate-buffered saline and 10% neutral paraformaldehyde (PFA). The brains were harvested and fixed in 10% PFA for 48 h. Then, the brains were dehydrated and paraffin embedded. Coronal sections of 3 μm were prepared with a microtome (Leica).

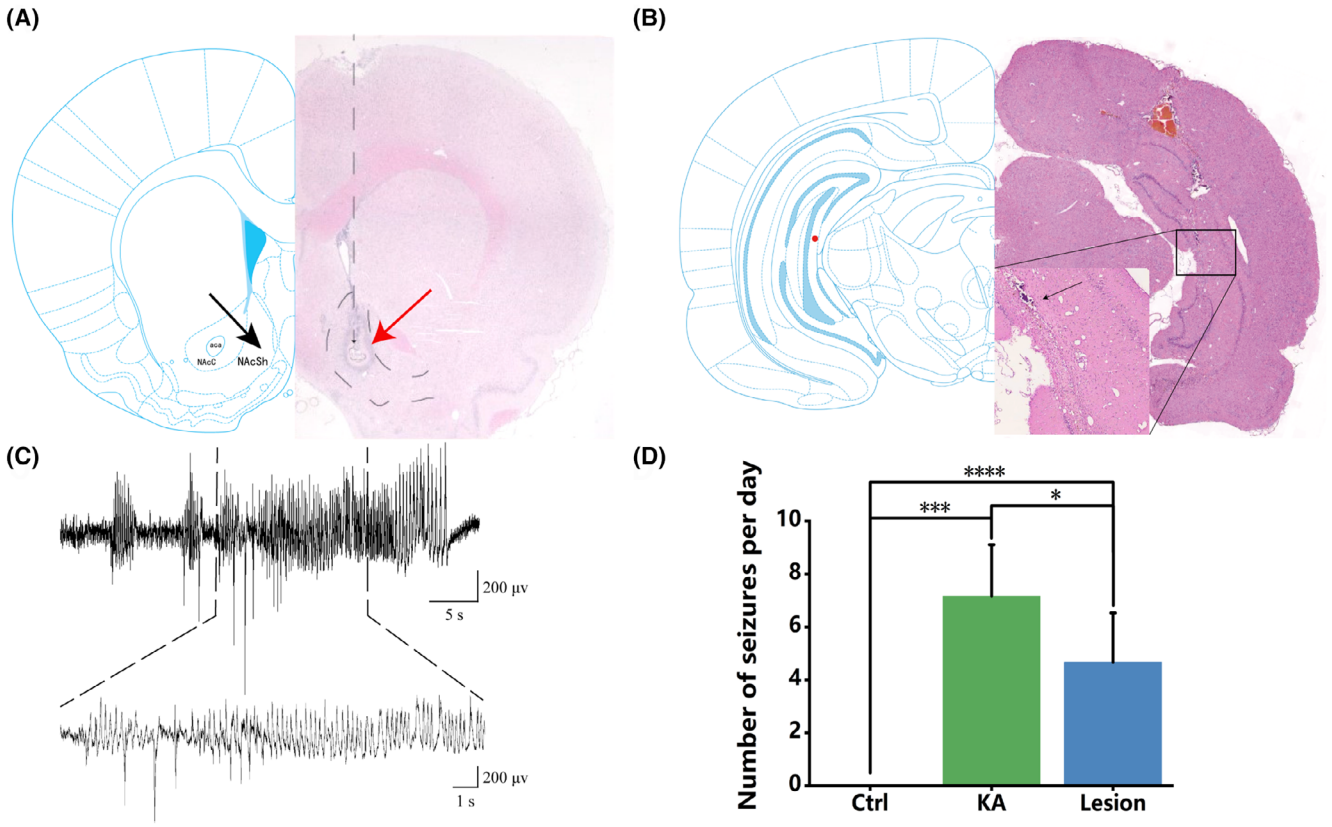

FIGURE 2 NAcSh electrical lesion significantly reduces seizure frequency in TLE rats. (A) HE staining image showed the location of electrodes in the NAcSh. The left half image was modified from Paxinos and Watson Rat Brain Atlas (6th edition). (B) HE staining image showed the KA injection site in the hippocampus. The black arrow indicates the position of the syringe needle tip. The left half image was modified from Paxinos and Watson Rat Brain Atlas (6th edition). (C) Representative epileptiform EEG patterns. (D) Quantification of the average number of seizures per day in each group of rats ($n = 6$, $^*p < .05$, one-way ANOVA).

2.5.2 | Hematoxylin and eosin (HE) staining

HE staining was conducted according to routine protocols. Briefly, the tissue sections were initially dewaxed in xylene for two cycles of 30 min each. Subsequently, they were rehydrated through a series of graded alcohol concentrations (100%, 100%, 95%, 90%, 80%, and 70%), with each step lasting 5 min. The sections were then stained with hematoxylin solution (ZSGB) for 2 min, followed by immersion in 1% acid ethanol (1% hydrogen chloride in 75% ethanol) for 1 s, and subsequently rinsed in distilled water. Thereafter, the sections underwent staining with eosin solution (ZSGB) for 20 s. The sections were then thoroughly washed with distilled water, dried, and sealed with neutral resin before being photographed using an optical microscope (Olympus).

2.5.3 | Immunohistochemistry

Following the dewaxing and rehydration process described earlier, sections were transferred to boiling sodium citrate buffer (pH 6.0, 15 min) for antigen retrieval. After inactivating endogenous peroxidase in a 10% hydrogen peroxide solution for 10 min, the sections were washed twice in double-distilled water. For half an hour, the sections were incubated with 5% bovine serum albumin (BSA) to block non-specific antibody binding. Primary antibodies (Iba-1, Abcam, 1:1000; NeuN, 1:500, HuaAn; GFAP, 1:500, Abcam) were incubated 12 h at 4°C. After that, sections were treated with goat anti-rabbit IgG (ZSGB) and horseradish peroxidase-streptavidin (ZSGB) for 30 min at 37°C. Diaminobenzidine (DAB) hydrochloride-H_2O_2 solution was used to visualize antibody binding sites. At last, the sections were sealed after hematoxylin counterstaining. Images were captured with an optical microscope (Olympus) and processed with Image J software.

2.6 | Morris water maze (MWM)

MWM experiments were started in 5th week after the KA injection. The water maze consists of a circular water tank, a tracking camera, and a behavior analysis system. The circular tank was placed on a platform. The water tank was filled with water and blackened with black dye to facilitate the tracking camera to recognize the rat's movement trajectory. The escape platform was located in the northeast quadrant, about 2 cm below the water surface. Days 1–6 were the training phase, and the rats were given 90 s to find the platform for each training session and then allowed to rest on the platform for 20 s for the next training session. Each rat received four training sessions per day. On day 7, the platform was removed, and the rats were subjected to a test lasting 90 s. The behavior of each rat during the training and test phases was recorded by a tracking camera and analyzed using a behavioral analysis system (Topview).

2.7 | Statistical analysis

Data are presented as mean ± standard deviation. SPSS software (Ver. 11.5) processed and analyzed the data. Statistical significance was determined with one-way ANOVA. Tests were two sided, and $p < .05$ was considered a significant difference for all analyses.

3 | RESULTS

3.1 | Chronic epilepsy model is successfully established

The flow chart of the experimental design is shown in Figure 1A. Briefly, after an acclimatization period, the KA and lesion groups were given intrahippocampal KA injections. Following 3 weeks of KA injection, the lesion group received NAcSh lesion. Video-EEG monitoring was initiated 4 weeks after the KA injection. Subsequently, 1-week MWM testing was performed in the 5th week after the KA injection. The video demonstrates that the rats in the KA group exhibited seizure activity, whereas the control rats did not display any seizure-related behavior (Ctrl 0 vs. KA 7.16667 ± 1.94079, $p = .325*10^{-5}$, one-way ANOVA, Tukey's post-hoc test, Figure 2D). In addition, we recorded typical epileptiform discharge patterns in the KA group of rats (Figure 2C). Collectively, these results indicated that the chronic TLE rat model was successfully established.

3.2 | NAcSh electrical lesion alleviates seizure symptoms

In order to evaluate the potential impact of NAcSh electrical lesion on seizure frequency in TLE rats, video-EEG monitoring was conducted to monitor seizures. Video-EEG monitoring results revealed reduced seizures in the lesion group compared to the KA group (KA 7.16667 ± 1.94079 vs. lesion 4.66667 ± 1.8619, $p = .035$, one-way ANOVA, Tukey's post-hoc test). Meanwhile, no seizures were observed in the control group (Figure 2D). Thus, these results demonstrated that the NAcSh electrical lesion effectively reduced seizures in TLE rats.

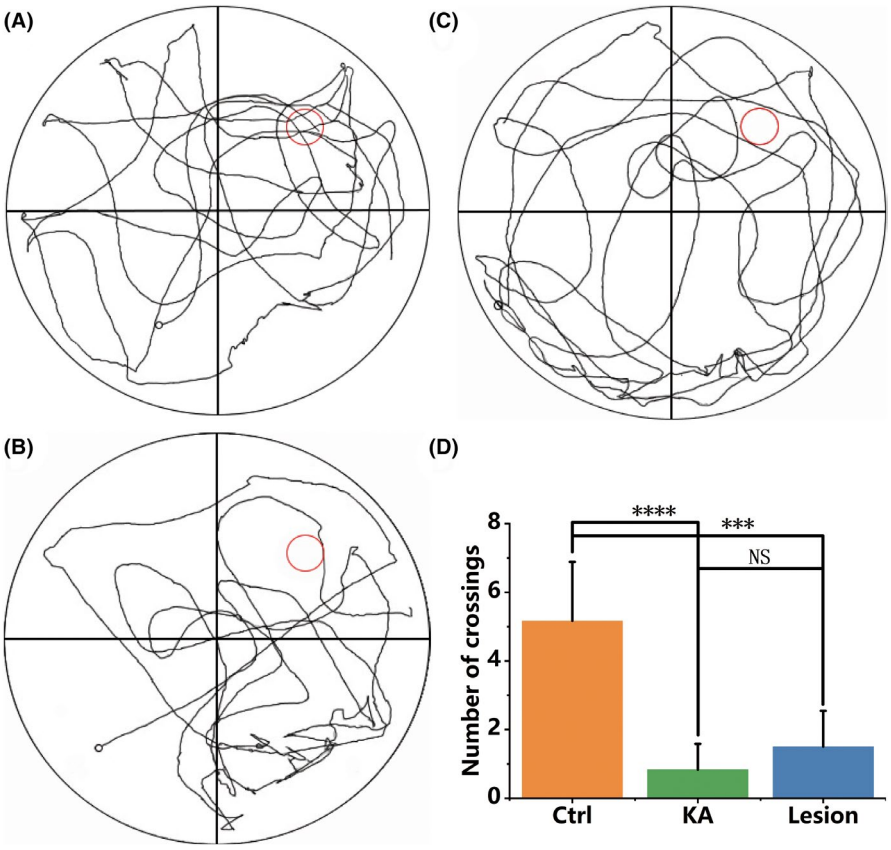

FIGURE 3 NAcSh electrical lesion does not result in additional memory function impairment in TLE rats. (A–C) Representative movement trajectory of TLE rats in Morris water maze. Red circles represent escape platforms. (D) Statistical analysis of the number of times rats crossed the platform on the last day of the Morris water maze experiment ($n = 6$, ns > 0.05, ****$p < .0001$, ***$p < .001$, one-way ANOVA).

3.3 | NAcSh electrical lesion does not exacerbate TLE-related memory impairment

Basically, TLE is often accompanied by memory function impairment.[20] Therefore, the potential impact of NAcSh electrical lesion on TLE-related memory impairment was analyzed through MWM.

During the MWM test phase, the control group exhibited concentrated trajectory movements in the quadrant housing the escape platform. Conversely, the KA and lesion groups showed disorganized trajectories and aimless search (Figure 3A–C). In comparison to the control group, the number of times the KA group crossed the platform dramatically decreased (Ctrl 5.16667 ± 1.7224 vs. KA 0.83333 ± 0.75277, $p = .00006$, one-way ANOVA, Tukey's post-hoc test), suggesting that chronic TLE is associated with impaired spatial memory function in rats. In contrast, the lesion group only showed a slight increase in the number of traverses which was not statistically significant when compared to the KA group (KA 0.83333 ± 0.75277 vs. lesion 1.5 ± 1.04881, $p = .63$, one-way ANOVA, Tukey's post-hoc test, Figure 3D). While these findings did not clarify whether the NAcSh electrical lesion ameliorated TLE-related memory impairment, they suggested that the NAcSh electrical lesion did not result in additional memory impairment.

3.4 | NAcSh electrical lesion reduces the number of neurons and causes gliosis in the NAcSh

In order to investigate the potential mechanism of NAcSh electrical lesion to modulate epileptic seizures, we first investigated the effect of NAcSh electrical lesion on NAcSh. It is well known that the brain is mainly composed of neuronal cells and glial cells. Therefore, we quantified the number of neuronal cells as well as glial cells in each group of NAcSh by means of immunohistochemistry. Specifically, we used NeuN antibody to label neuronal cells in NAcSh. Through statistical analysis, we found that there was no statistically significant difference in the number of neuronal cells in the NAcSh of the KA group compared to the control group (KA 425.33333 ± 83.64847 vs. Ctrl 455.5 ± 173.10893; $p = .89477$, one-way ANOVA, Tukey's post-hoc test). However, the neuronal cell count in the NAcSh of rats in the lesion group was significantly less than that in the control group (lesion 213.33333 ± 57.69806 vs. Ctrl 455.5 ± 173.10893 $p = .89477$, one-way ANOVA, Tukey's post-hoc test) versus the KA group (lesion 455.5 ± 173.10893 $p = .89477$ vs. KA 425.33333 ± 83.64847, one-way ANOVA, Tukey's post-hoc test; Figure 4A upper panel, B). Therefore, we conclude that TLE does not affect neurons in NAcSh. Instead, NAcSh electrical lesions can effectively damage neuronal cells in NAcSh.

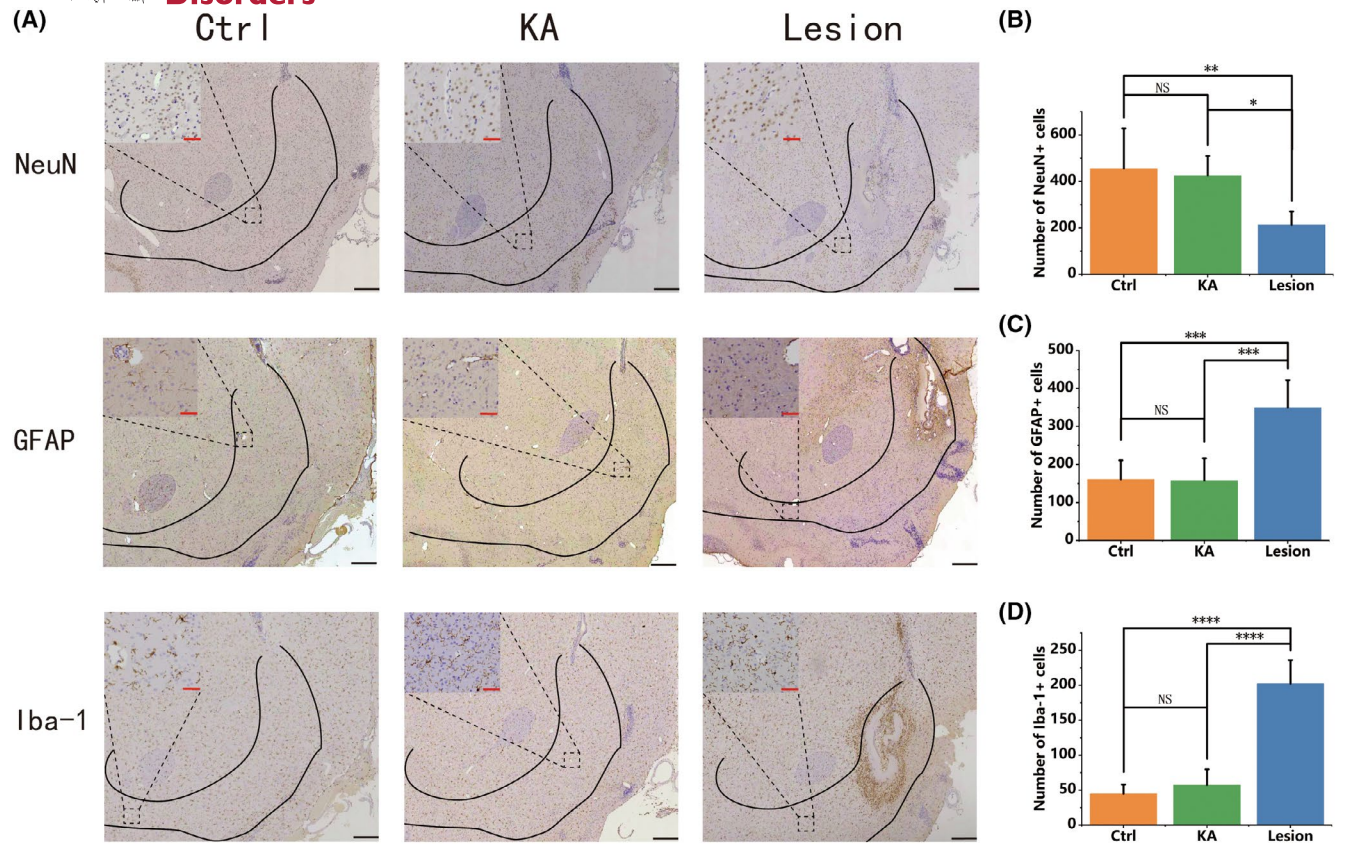

FIGURE 4 The effects of NAcSh lesion on the NAcSh in TLE rats. (A) Representative immunohistochemistry images showed the NeuN, GFAP, and Iba-1 expression in the NAcSh in each group (scale bar: red line: 50 μm, black line: 250 μm). The region between the two solid black lines is the NAcSh. (B–D) Quantitative analysis of NeuN, GFAP, and Iba-1 expression in various subregions of the NAcSh in each group ($n = 6$, ns > 0.05, ****$p < .0001$, ***$p < .001$, one-way ANOVA).

Subsequently, we labeled astrocytes using GFAP antibody and found that there was no statistically significant difference in the number of astrocytes in the NAcSh of the two groups of rats in the KA group compared to the control group (KA 157.66667 ± 58.18476 vs. Ctrl 160.5 ± 50.27425 $p = .99643$, one-way ANOVA, Tukey's post-hoc test). However, the number of astrocytes in the NAcSh of rats in the lesion group was significantly higher than that in the control group (lesion 213.33333 ± 57.69806 vs. Ctrl 455.5 ± 173.10893 $p = .0002$, one-way ANOVA, Tukey's post-hoc test) versus KA group (lesion 213.33333 ± 57.69806 vs. KA 157.66667 ± 58.18476 $p = .0002$, one-way ANOVA, Tukey's post-hoc test; Figure 4A middle panel, C). Consistent with the findings regarding alterations in astrocyte numbers, there was no statistically significant difference in the number of microglia in the NAcSh of rats between the KA group and the control group (KA 57.33333 ± 22.43806 vs. Ctrl 44.66667 ± 13.35165 $p = .65368$, one-way ANOVA, Tukey's post-hoc test). However, the number of microglial in the NAcSh of rats in the lesion group was significantly greater than that in the control group (lesion 202.33333 ± 33.69075 vs. Ctrl 44.66667 ± 13.35165 $p = .00000001$, one-way ANOVA, Tukey's post-hoc test) versus the KA group (lesion 202.33333 ± 33.69075 vs. KA 57.33333 ± 22.43806 $p = .00000001$, one-way ANOVA, Tukey's post-hoc test; Figure 4A upper panel, D). Therefore, we suggest that NAcSh electrical lesion may treat TLE by damaging neuronal cells in NAcSh rather than modulating the status of glial cells in NAcSh.

3.5 | NAcSh electrical lesion alleviates hippocampal neuronal loss in TLE rats

The hippocampus is a common foci of TLE, and one of its essential pathological features is neuronal loss.[21] To quantitatively assess the impact of NAcSh electrical lesion on TLE-induced neuronal loss, we conducted immunohistochemical staining for the mature neuron marker NeuN in each group (Figure 5A). The results showed that the KA group exhibited significantly fewer neuron numbers in the CA1 (KA 60.33333 ± 7.44759 vs. Ctrl 96.66667 ± 7.36659, $p = .000005$, one-way ANOVA, Tukey's post-hoc test), CA3(KA 41.83333 ± 14.70261 vs. Ctrl 125 ± 17.19302 $p = .00000002$, one-way ANOVA,

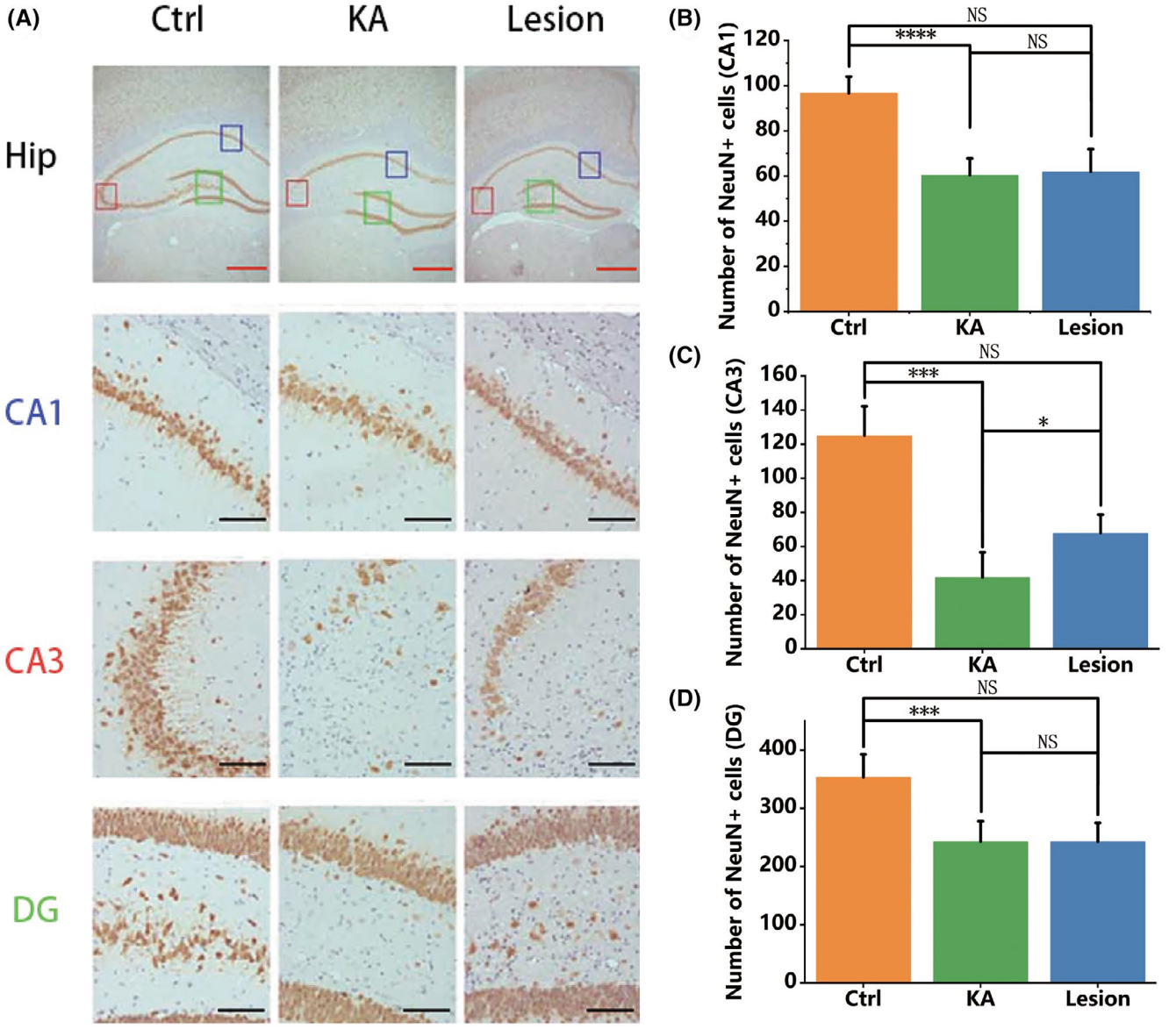

FIGURE 5 NAcSh lesion diminishes CA3 region neuronal loss in TLE rats. (A) Representative immunohistochemistry images showed the NeuN expression in the hippocampus in each group (scale bar: red line: 50 μm, black line: 10 μm). (B–D) Quantitative analysis of NeuN expression in various subregions of the hippocampus in each group ($n = 6$, ns > 0.05, ****$p < .0001$, ***$p < .001$, one-way ANOVA).

Tukey's post-hoc test), and DG (KA 242.5 ± 35.24628 vs. Ctrl 353.33333 ± 39.39374, $p = .0002$, one-way ANOVA, Tukey's post-hoc test) regions compared to the control group. Notably, the lesion group displayed a higher number of neurons in the CA3 region relative to the KA group (KA 41.83333 ± 14.70261 vs. lesion 67.83333 ± 10.90718, $p = .0019$, one-way ANOVA, Tukey's post-hoc test), underscoring the reversal of hippocampal CA3 neuronal loss in TLE rats following NAcSh electrical lesion (Figure 5B–D). These findings collectively emphasize the potential of NAcSh electrical lesion as a mitigating factor against hippocampal neuronal loss in TLE.

3.6 | NAcSh electrical lesion reduces astrocytes and microglia activation in TLE rats

Glial cells, including microglia and astrocytes, play a crucial role in the pathogenesis of TLE.[22–26] Thus, immunohistochemical staining of Iba-1 and GFAP were performed to explore the effect of NAcSh electrical lesion on hippocampal gliosis.

As shown in Figure 6A, enlarged and deeply stained microglia cell bodies were seen in the hippocampus of rats in the KA group. Some of these cells became elongated or spindle shaped. Relative to the control group, the KA group showed a

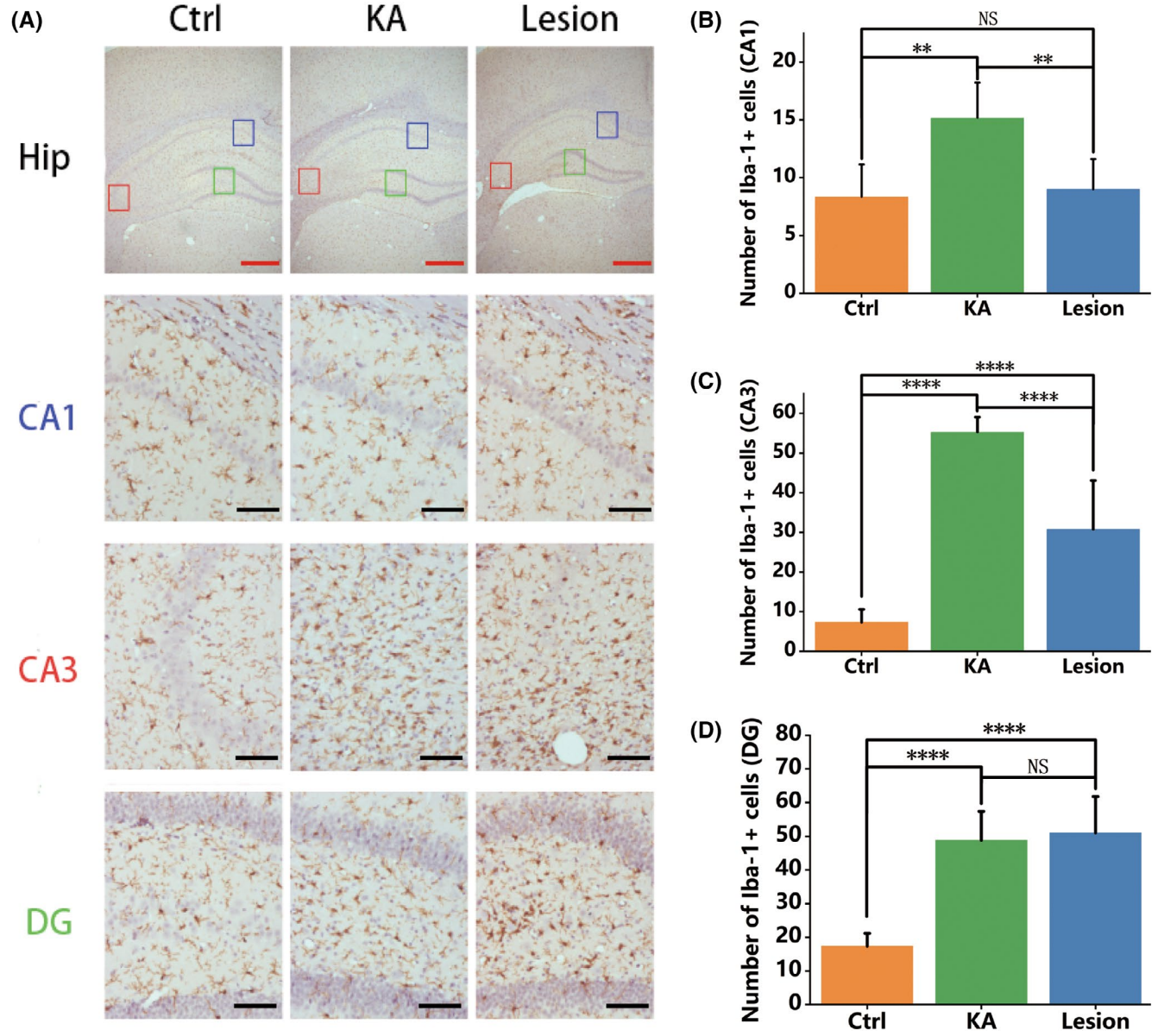

FIGURE 6 NAcSh electrical lesion reduces microglia activation in TLE rats' CA1 and CA3 regions. (A) Representative immunohistochemistry images displayed microglia in CA1, CA3, and DG regions of different group rats (scale bar: red line: 50 μm, black line: 10 μm). (B–D) Quantification of the number of activated microglia in CA1, CA3, and DG regions of different group rats ($n = 6$, ns > 0.05, ****$p < .0001$, one-way ANOVA).

significant increase in the number of Iba-1-positive cells in all subregions of the hippocampus (CA1: Ctrl 8.33333 ± 2.80476 vs. KA 15.16667 ± 3.0605, $p = .002$; CA3: Ctrl 7.3 ± 3.3 vs. KA 55.3 ± 3.8 $p = 4.06*10^{-13}$; DG: Ctrl 17.33333 ± 3.88158 vs. KA 48.83333 ± 8.61201, $p = .00002$, one-way ANOVA, Tukey's post-hoc test). These results reflected extensive glial cell activation in all subregions of the hippocampus of TLE rats. Additionally, the lesion group of rats exhibited a significant decrease in microglia activation in the CA1 (KA 15.16667 ± 3.0605 vs. Lesion 9 ± 2.60768, $p = .005$, one-way ANOVA, Tukey's post-hoc test) and CA3 (KA 55.3 ± 3.8 vs. Lesion 30.8 ± 12.3, $p = 6.6*10^{-9}$, one-way ANOVA, Tukey's post-hoc test) regions, but not in the DG region (KA 48.83333 ± 8.61201 vs. Lesion 51 ± 10.77033, $p = .89$, one-way ANOVA, Tukey's post-hoc test), when compared to the KA group (Figure 6B–D). The above results suggested that NAcSh electrical lesion can effectively inhibit microglia activation in the CA1 and CA3 regions of the hippocampus of TLE rats.

Similarly, GFAP expression was also detected to assess reactive astrogliosis in the hippocampus. Following recurrent seizures, astrocytes in the hippocampus displayed features of activated astrocytes, such as enlarged cell bodies and intense staining in the KA group (Figure 7A). The quantity of GFAP-positive cells in the CA1 regions of the hippocampus exhibited significantly higher amounts in the KA group than it is in the control group (Ctrl 25.16667 ± 3.65605 vs. KA

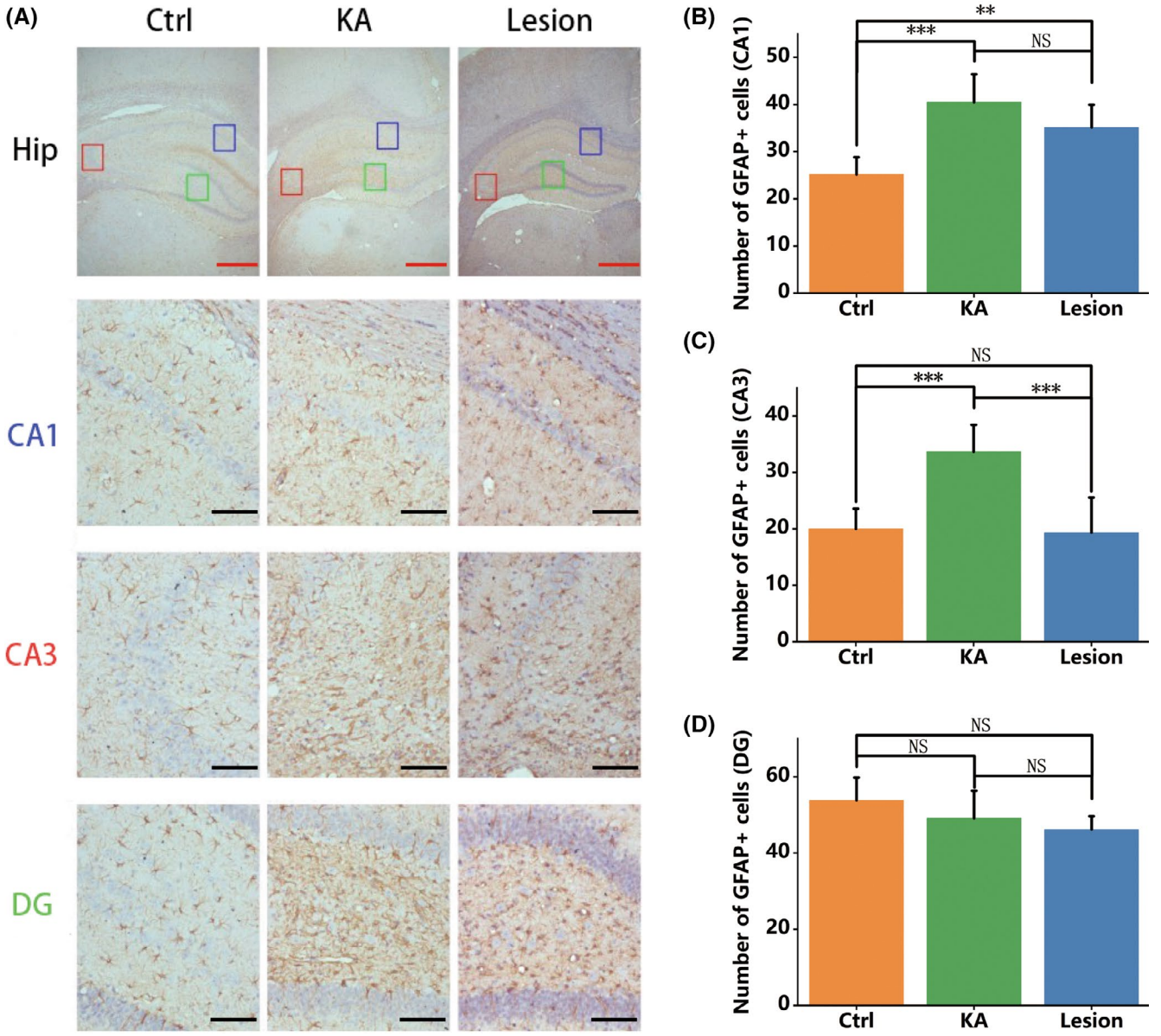

FIGURE 7 NAcSh lesion reduces astrocyte activation in the CA3 region of TLE rats. (A) Representative immunohistochemical images showing GFAP expression in different hippocampal subregions (scale bar: red line: 50 μm, black line: 10 μm). (B–D) Quantification of GFAP-positive astrocytes in the CA1, CA3, and DG regions, respectively ($n = 6$, ns > 0.05, ***$p < .001$, **$p < .01$, one-way ANOVA).

40.5 ± 5.92453, $p = .00019$, one-way ANOVA, Tukey's post-hoc test). Consistent with GFAP expression in the CA1 region, the number of GFAP-positive cells in the CA3 region in the KA group was likewise markedly elevated than that in the control group (Ctrl 20 ± 3.57771 vs. KA 33.66667 ± 4.76095, $p = .00069$, one-way ANOVA, Tukey's post-hoc test). In comparison, the number of GFAP-positive cells in the CA3 (KA 33.66667 ± 4.76095 vs. lesion 19.33333 ± 6.21825, $p = .00044$, one-way ANOVA, Tukey's post-hoc test) region of the hippocampus significantly diminished in the lesion group, compared to the KA group. Nonetheless, there was no statistically significant difference between the lesion group and the KA group in terms of the number of GFAP-positive cells in the CA1 region (KA 40.5 ± 5.92453 vs. lesion 35.16667 ± 4.79236, $p = .17$, one-way ANOVA, Tukey's post-hoc test, Figure 7B,C). Interestingly, there were no statistically significant differences in the number of GFAP-positive cells in the DG region among the three groups, indicating that neither TLE nor NAcSh electrical lesion induced astrocyte activation in the DG region (Ctrl = 53.83333 ± 5.98052 vs. KA = 49.16667 ± 7.19491 vs. lesion = 46.16667 ± 3.48807, $p = .1$, one-way ANOVA, Figure 7D).

4 | DISCUSSION

In the present study, we investigated the potential efficacy of NAcSh electrical lesion in reducing seizures in TLE rats

based on previous research and our earlier findings.[7,9–11] Our results demonstrate that the NAcSh electrical lesion significantly reduces seizure frequency. In addition, we further show a significant decrease in gliosis and neuronal loss in the hippocampus of TLE rats following the NAcSh electrical lesion. These findings firmly suggest that targeting NAcSh may represent a promising therapeutic strategy for the management of TLE.

As an essential structure within the ventral striatum, NAc is mainly considered to be involved in learning and motivated behavior.[14] However, accumulating studies indicate that NAc also plays a key role in TLE. For example, multiple research groups have consistently reported a substantial increase in c-fos expression within the NAc across various TLE models.[11,27,28] Although substantial evidence demonstrated various changes in NAc in TLE conditions, the functional significance of such changes, especially whether and how NAc participates in TLE, is still unknown. In imaging studies, radial diffusion (RD) and fractional anisotropy (FA) values are commonly used to assess structural integrity. Previous imaging studies have demonstrated reduced FA and increased RD values in the bilateral NAcSh of patients with left mesial TLE (mTLE) compared to healthy controls.[9,10] In addition, left mTLE patients have reduced functional connectivity of the left NAcSh to the whole brain. In contrast, the functional connectivity of the right NAcSh to the whole brain is increased in contrast to the control. Right-sided mTLE patients had lower FA and higher RD values confined to the left NAcSh relative to the control group. However, when compared to healthy controls, the FA and RD values of NAcC in left- or right-sided TLE patients showed no significant difference.[9] Similarly, the functional connectivity of NAcC to the whole brain in left-sided mTLE patients was not significantly different compared with controls.[10] The above studies suggested that TLE-induced pathological and functional alterations predominantly existed in the NAcSh. Furthermore, our previous study showed that inhibition of NAcSh GABAergic neurons attenuated epilepsy propagation.[11] Based on these previous data, we speculated that NAc, especially the NAcSh, may play an important role in epileptogenesis, but the underlying mechanisms still need to be elucidated. Therefore, in the present study, we performed NAcSh electrical lesions in TLE rats to clarify the potential relationships between NAcSh and TLE. Our results demonstrate a significant reduction in seizures following the NAcSh electrical lesion. In alignment with previous research, we chose to electrical lesion on the affected side rather than the bilateral NAcSh.[29,30] Considering the extensive connectivity between the bilateral hippocampus, it is likely that the so-called mirror image of the epileptic focus appears in the contralateral hippocampus.[31] The presence of mirror epileptic foci may affect the efficacy of unilateral treatment. However, a previous study reported that unilateral electrical lesions in the bilateral hypothalamocortical kindling rat model did not alter contralateral seizure onset or generalization.[32] Furthermore, the anatomical differences between rodents and primates may not be ignored, as in primates, the connections between the cerebral hemispheres are not as dense as in rats.[33–35] Considering the above facts, a bilateral NAcSh electrical lesion may not necessarily be more effective than a unilateral one. The risk versus benefit of unilateral and bilateral treatment for the NAc is currently unknown, and further experiments are needed to verify it. However, only the unilateral NAcSh electrical lesion, at least in the present study, is safe and effective enough to reduce seizures in TLE rats.

Numerous existing studies indicated that there was a specific epilepsy regulatory loop that regulated the onset and propagation of seizures in the subcortical regions and cortex.[36,37] In the context of the TLE, the regulatory loop pertains to the hippocampus, frontal cortex, entorhinal cortex, amygdala, anterior cingulate, and thalamus.[38–40] The NAc, an essential ventral striatum region, receives inputs from both the prefrontal cortex and ventral hippocampus. It also projects to the basal ganglia, thereby playing a vital role in the reward and modulation of cortical and brainstem motor centers.[41–43] Several studies have indicated that the activation of delta opioid receptors, which are extensively present in reward circuits, exhibits proconvulsant characteristics. Moreover, it has been proposed that the proconvulsive properties of delta opioid receptors may be associated with the neurocircuitry underlying the absence of epilepsy.[44] Therefore, it is reasonable to speculate that reward circuits may also be involved in epilepsy propagation. Given the NAc integral function within reward circuits and its significant interactions with the hippocampus and frontal cortex, it is considered a promising target for seizure intervention. Intriguingly, our data suggest that the pathogenesis of TLE does not affect the number of neurons as well as glial cells in NAcSh. Conversely, epileptic rats subjected to NAcSh electrical lesion exhibited a marked decrease in neuronal count, along with significant astrogliosis and microglial activation in the NAcSh. Consequently, we propose that the NAcSh electrical lesion may have effectively mitigated seizures, neuronal loss, and glial cell activation in the hippocampus of epileptic rats by disrupting the reward neural circuitry involving the NAc. Furthermore, the observed gliosis in the NAcSh is likely attributable to local inflammatory responses following the electrical lesion procedure.

Loss of hippocampal neurons and memory dysfunction are significant characteristics of TLE.[45,46] The CA3 region of the hippocampus is critical for information encoding and spatial memory.[47] Consequently, neuronal loss in the CA3

area can profoundly impair spatial memory function. In the current study, we observed that the NAcSh electrical lesion effectively mitigated neuronal loss in the CA3 region of the hippocampus. Additionally, there was a tendency for spatial memory to improve in epileptic rats receiving NAcSh electrical lesions, although there was no statistical difference. This finding indicates that NAcSh electrical lesion may exert a neuroprotective effect on neurons within the CA3 region of epileptic rats. Second, the enhancement of spatial memory ability further substantiates the notion that NAcSh electrical lesion may protect neurons in the CA3 region of epileptic rats. Because the preservation of more neurons in the CA3 area may directly enhance the performance of rats in spatial navigation tasks. We hypothesize that the NAcSh electrical lesion ameliorates spatial memory dysfunction in epileptic rats by mitigating neuronal loss in the CA3 region. In conclusion, NAcSh electrical lesion may not only contribute to the attenuation of seizures but also hold the potential for enhancing cognitive function in individuals with TLE. While our study demonstrated the potential of NAcSh electrical lesion to mitigate neuronal loss in the CA3 region and cognitive dysfunction in epileptic rats, further research is required to elucidate the underlying mechanisms. For instance, it remains to be elucidated whether NAcSh electrical lesion confers neuroprotection through direct modulation of specific molecular pathways or via indirect mechanisms, such as the modulation of particular neural circuits. Furthermore, validating the efficacy of NAcSh electrical lesion across various TLE models will enhance our comprehensive understanding of its potential therapeutic application in TLE treatment. Although the detailed mechanisms of how the NAcSh modulates TLE seizures are still unknown, our results provide direct evidence that NAcSh is indeed involved in seizures of TLE. Anyway, based on previous studies and our current findings, we believe that NAcSh is closely related to TLE and also a promising therapeutic target for TLE.

Glia cell proliferation has been observed in several TLE animal models,[26,48,49] and glia cells are thought to play an essential role in epileptogenesis.[50–53] Consistent with previous studies, we observed reactive proliferation of astrocytes and microglia in the hippocampus of TLE rats.[49,54] However, the pathophysiological and functional implications of microglia proliferation are not clear yet. Previous research has demonstrated that reactively activated microglia secrete a range of pro-inflammatory factors and chemokines, which are crucial for glial cell proliferation. Additionally, various antiepileptic drugs have been shown to mitigate seizures by attenuating glial cell activation and the subsequent release of inflammatory mediators.[55,56] In this study, reduced hippocampal astrocyte activation was observed in the lesion group, suggesting that NAcSh electrical lesion may reduce hippocampal glial cell activation and thus seizures through an unidentified mechanism. Nevertheless, the possibility that the observed decrease in hippocampal glial cell activation is secondary to a reduction in seizure frequency cannot be excluded. Further research is necessary to determine whether the observed reduction in glial cell activation is a direct effect of the NAcSh electrical lesion or an indirect consequence of diminished seizure activity.

TLE treatment is a tough challenge faced by doctors and patients. Here, we demonstrated a durable antiepileptic effect of NAcSh electrical lesion on chronic TLE rats induced by KA. Although the electrophysiological and neurochemical mechanisms underlying this effective antiepileptic effect still need additional investigation, the findings from this research may advance our understanding of TLE pathogenesis and facilitate the identification of novel therapeutic targets for TLE management.

5 | CONCLUSION

Our study showed that NAcSh electrical lesion reduced seizure frequency in TLE rats. It also alleviated neuronal loss and glial cell activation in the hippocampus. Besides, the NAcSh electrical lesion did not cause additional memory impairment. These findings suggest that targeting the NAcSh may represent a safe and promising therapeutic strategy for the treatment of TLE.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Test yourself

1. Status epilepticus is defined as a seizure lasting for:
   A. >30 minutes
   B. >50 minutes
   C. >20 minutes
   D. >10 minutes
   E. >40 minutes
2. The main clinical manifestation of epilepsy is:
   A. Convulsions
   B. Intellectual developmental regression
   C. Ataxia
   D. Dystonia
   E. Upper motor neuron paralysis
3. The main clinical distinction between epileptic seizures and psychogenic nonepileptic seizures is the presence of:
   A. Generalized convulsions
   B. Sudden collapse
   C. Rapid breathing with vocalizations
   D. Clenched hands and rigid lower limbs
   E. Pupil dilation with loss of light reflex

Answers may be found in the supporting information.