Nucleus accumbens shell lesions alleviate symptoms in kainic acid-induced epileptic rats

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Abstract

Epilepsy is a recurrent neurological disease caused by hypersynchronous firing of neurons in the brain. Neuronal apoptosis, microgliosis, or astrogliosis in hippocampus are considered to be important features of temporal lobe epilepsy. As an important part of the ventral striatum, the nucleus accumbens is closely connected to the hippocampus. As the findings of the reviewed articles indicated, the nuleus accumbens is divided into the shell and the core. The nucleus accumbens shell is a relevant brain region to process reward-related and motivated behaviours, emotional process and social information. Nucleus accumbens shell has a good application effect in the field of drug addiction and mania. Our previous studies have shown that the nucleus accumbens shows abnormal excitation after seizures. In our previous experiments, the number of seizures decreased when this excitation was disrupted. However, the mechanisms of action of Nucleus accumbens shell lesions remain unclear. In this paper, we explored the hippocampal changes inhibited by nucleus accumbens lesions on the basis of the kainic acid animal model of epilepsy. We explored the anti-epileptic effect and mechanism of action of electrical lesions in nucleus accumbens, and found that the mechanism may be to reduce the toxic effects caused by the proliferation of astrocytes and microglia in the hippocampus. It can also inhibit the reduction of neurons and thus play a role in controlling seizures. These results suggest that the nucleus accumbens plays an important role in inhibiting seizures.

Nucleus accumbens shell lesions alleviate symptoms in

kainic acid-induced epileptic rats

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Abstract

Epilepsy is a recurrent neurological disease caused by hypersynchronous firing of neurons in the brain. Neuronal apoptosis, microgliosis, or astrogliosis in hippocampus are considered to be important features of temporal lobe epilepsy. As an important part of the ventral striatum, the nucleus accumbens is closely connected to the hippocampus. As the findings of the reviewed articles indicated, the nuleus accumbens is divided into the shell and the core. The nucleus accumbens shell is a relevant brain region to process reward-related and motivated behaviours, emotional process and social information. Nucleus accumbens shell has a good application effect in the field of drug addiction and mania. Our previous studies have

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Keywords: Epilepsy; Nucleus accumbens shell; Lesion; Kainic acid; Hippocampus

1 INTRODUCTION

Epilepsy is a common serious brain condition, affecting over 70 million people worldwide, characterized by neuronal hyperexcitability and synchronized electrical discharges, leading to seizures [1, 2]. Temporal lobe epilepsy (TLE) is the most common type of epilepsy and is frequently resistant to drug treatment[3].

In the seizure onset zone, seizures are generated by propagating epileptic activity, while seizures are maintained by perpetuating it[4-6]. As an important part of the ventral striatum, nucleus accumbens (NAc) involves motivational and emotional processes and limbic-motor interfaces. It is divided into two distinct areas, core (NAcc) and shell (NAcSh)[7, 8]. In addition to the extended amygdala, lateral hypothalamic area, and ventral tegmental area(VTA), the NAc projects to other related regions [9, 10]. TLE has recently been shown to be mediated by the NAc, particularly the NAcSh [6, 11]. A high density of medium spiny neurons in the NAcSh suggests an important role for epileptiform activity expansion[12, 13].

TLE is induced by inttra-hippocampal injection of Kainic acid(KA) ,which ultimately results in chronic epilepsy a pattern of hippocampal sclerosis similar to that found in patients with TLE[14, 15]. Previous studies have indicated that NAcSh is an effective target for drug addiction and significantly alleviates seizures[13]. However, the concrete mechanism of lesions in TLE rats remains unknown. Therefore, we aimed to further investigate the suppression of spontaneous recurrent seizures following NAcSh lesion development in rats with KA-induced TLE.

In this study, we investigated the impact of NAcSh lesions on electroencephalogram (EEG), pathological damage of the hippocampus, and neuronal density of KA-induced epileptic rats. Here, we investigated whether NAcSh lesions are efficacious in a KA-induced rat model of TLE with spontaneous seizures.

2 MATERIALS AND METHODS:

2.1 Animals

Adult male Sprague–Dawley rats weighing 200–250 g were used in this experiment. Rats were housed four per cage at an ambient temperature of 22°C–25°C and under a 12-h day-night cycle, with free access to food and water. Each group has 6 rats(Control ,sham-NAcSh lesion ,Epilepsy and NAcSh lesion), and following an experimental design of randomized blocks.We used a protocol approved by the Southern Medical University Animal Experimentation Committee(NFYY-2020-1037). Efforts were made to minimize animal suffering and reduce the number of animals used.

2.2 Establishment of the epileptic model and behavior monitoring

The rat epilepsy model was established as described previously paper(Fig. 1A). After the administration of anesthesia(Isoflurane), KA (Sigma) was dissolved in sterile saline at a concentration of 1 μg/μL, and 1 μL was slowly infused (0.1 μL/min) using a microsyringe (RWD, China) into the right hippocampus (AP: –5.6 mm, ML: –4.5 mm, DV: –5.5 mm) to induce the epilepsy model. The control group received saline injections instead of KA(Fig. 1B). A modified Racine scale was used to evaluate seizure severity after 1 h, with a 5-stage rating scale based on behavioral changes. To reduce mortality, rats were treated with diazepam (10 mg/kg i.p., King York, China) 2 h after the first generalized seizure. The incubation period of the KA-induced epileptic rats was within approximately 14 days after the acute phase. We used a camera to

continuously monitor rats for seizure occurrence. The hair on the backs of the rats was shaved off to easily differentiate between them.

2.3 Stereotactic electrical lesion of the NAcSh

Two weeks after KA injection, a lesion electrode was implanted into the NAcSh (AP: +1.6 mm, ML: –1.0 mm, DV: –7.7 mm) of rats after anesthesia(Isoflurane), the stimulator connects electrode and the half of the control and epilepsy group was given an electrical lesion (0.2 mA; 30 s) (Fig. 1C). The behavior of the rats and severity of seizures was continuously monitored using a camera after the lesion.

2.4 EEG recording

EEG recordings were taken during the chronic period for 1 h every day post-SE. Rats were anesthetized with chloral hydrate (400 mg/kg, Sigma, USA). EEG was monitored with the BL420F system and analyzed with the same software (TaiMeng, China). The biopotential amplifier modules used a three-electrode arrangement (red, black, and white)(Fig. 1D).

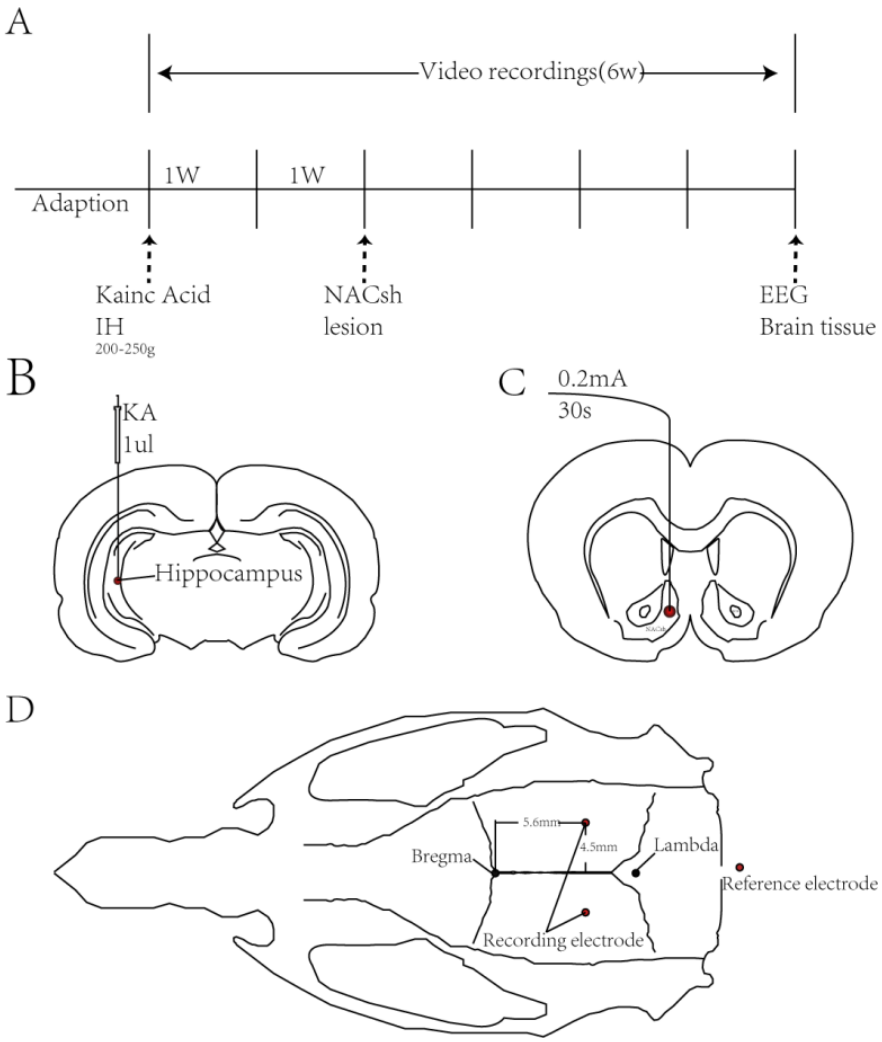

Figure 1: The experimental design was as follows: (A) Research roadmap. (B) KA (1 μg) was injected into the right hippocampus (AP: –5.6 mm, ML: –4.5 mm, DV: –5.5 mm) to induce TLE conditions (schematic

adapted from Paxinos and Franklin, 2001). Saline was injected into the same area to serve as the control group. (C) Stereotactic electrical lesion of the NAcSh site at 2 W. (D) Placement of the subcutaneous neck region and the hippocampal electrodes.

2.5 HE staining

After 6 weeks, rats were anesthetized with 4% chloral hydrate (100 g/ml) and intracardially perfused with 0.9% saline, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4). The brain was removed and post-fixed in 4% PFA at 4°C overnight, and dehydration and paraffin embedding were performed according to routine procedures. Sections were sectioned into frontal sections for histological analysis. Subsequently, the sections were deparaffinized in xylene for 15 min and rehydrated in descending grades of ethyl alcohol (100%, 95%, 85%, and 70%) and distilled water for 5 min each. Then, the sections were stained with 0.1% hematoxylin-eosin solution until the desired staining was achieved. The slides were then dehydrated and cover-slipped. Images were obtained using a light microscope (Olympus, Tokyo, Japan). The brain regions mainly included the hippocampal CA1 and CA3 pyramidal cell layers and the dentate gyrus (DG) (n = 6 per group)

2.6 Immunohistochemistry

Briefly, sections were deparaffinized, rehydrated, and then moved to 10 nM citrate buffer (pH 6.0) in a microwave oven for 15 min for antigen retrieval. Sections were then subjected to peroxidase to quench endogenous activity with 3%H_2O_2 for 10 min and washed twice with double-distilled water. Next, after the non-specific binding site was blocked with 5% bovine serum albumin for 30 min, the sections were incubated overnight at 4degC with rabbit-IBA1 (1:1000, Abcam, USA), GFAP (1:500, Abcam, USA), and NEUN (1:500, HuaAn, China) for staining of microglia, astrocytes, and neurons, respectively. Following incubation, the sections were then incubated with goat anti-rabbit IgG (ZSGB, China) for 30 min, followed by horseradish peroxidase-streptavidin (ZSGB, China) for another 30 min. The antibody binding sites were visualized by reaction with DAB-H_2O_2 solution. Images were obtained using a digital camera (Olympus, USA) attached to a light microscope and processed using image analysis software (Image J, 1.46a, NIH, USA).

2.7 Statistical analysis

All data are presented as mean +- S.E.M. Statistical analysis was carried out by SPSS11.5 for Windows. One-way ANOVA followed T's test was used to calculate statistical significance. For all analyses, the tests were two-sided and a $P < 0.05$ was considered significant.

3 RESULTS

3.1 NAcSh lesion decreased epileptic discharge events

EEG examination was performed to assess the effects of the NAcSh lesions on epileptic discharge. Compared to the Epilepsy group(rats subjected to KA with no lesioning), NAcSh lesioning alleviated epileptic discharge symptoms, as verified by the duration and amplitude of seizure spikes(Fig. 2A).

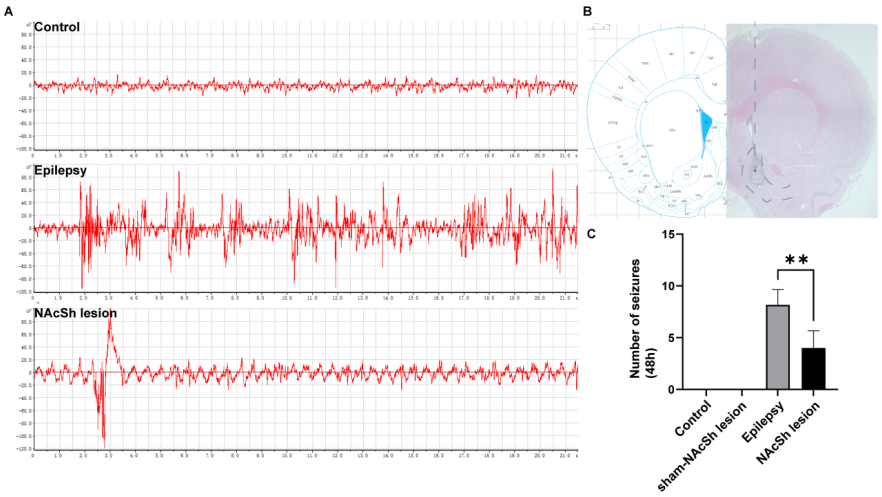

Figure 2:Effect of NAcSh lesions on seizures. (A) Brain waves in rats. (B) Stereotactic electrical lesion of the NAcSh site. (C) The number of seizures in epilepsy and NAcSh lesion rats. (n=6/group).***P <0.0001.

3.2 NAcSh lesions play protective effect in hippocampal neurons of TLE rats

Cell loss in hippocampal CA3 region is important in the pathophysiology of TLE .The survival of neurons in the CA3 region of the hippocampus was detected by Neun-marked neurons(Fig. 3A). The surviving neurons were significantly lower in the epilepsy group than in the control group. Obviously, the number of surviving neurons in the NAcSh lesion group was higher than that in the epilepsy group but lower than that in the control group(Fig. 3B).

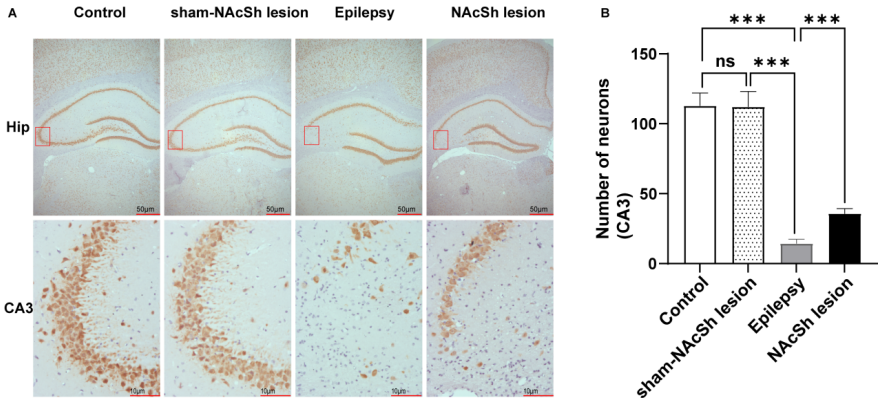

Figure 3:Effect of NAcSh lesioning on hippocampal neurons in epilepsy rats. (A) NeuN expression in the hippocampal tissue of rats in each group. (B) Quantification of NeuN-positive neurons in the CA3 region. Data are presented as the mean ± SEM. NAcSh: Nucleus accumbens shell. (ns > 0.05 , ****P < 0.0001).

3.3 NAcSh lesions reduced the number of activated microglia and astrocytes induced by seizure in TLE rats.

A pan-specific marker of microglia, Iba-1, was stained on the tissues of rats with ionized calcium (2+)-binding adaptor molecules (Fig. 4A), and these microglia were round, ellipsoidal, and had few processes. The epilepsy group rats had enlarged and intensely stained Iba-1-positive cell bodies, with some of the cells becoming long and narrow or fusiform in shape. Those with NAcSh lesions had significantly fewer activated microglia than those with epilepsy (Fig. 4B). Microglial responses to seizures are reduced in hippocampal CA3 after treatment with NAcSH lesion and seizure-induced activation of microglia.

Reactive astrogliosis in the hippocampus was assessed by GFAP staining. The morphology of the astrocytes in each group was observed microscopically. The astrocytes displayed altered size and morphology after seizures (Fig. 5A). After NAcSh lesioning, GFAP-positive astrocytes were significantly decreased in the CA3 region (Fig. 5B).

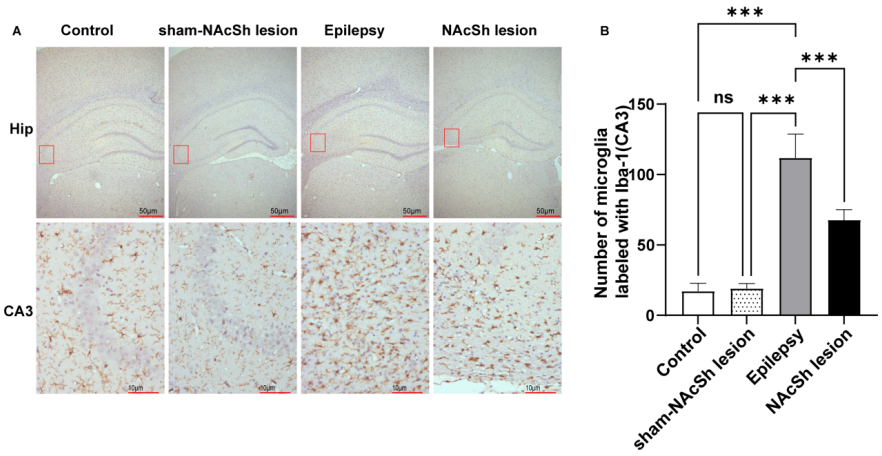

Figure 4: Fewer seizure-induced Iba-1-positive cells were present in the CA3 areas of the hippocampus in epileptic rats treated with NAcSh lesioning. (A) Microglia were stained with an antibody against Iba-1 (a microglial marker). (B) Quantification of the number of activated microglia showed a significant difference in the CA3 areas of the hippocampus.Iba-1: Ionized calcium-binding adaptor molecule 1, NAcSh: Nucleus accumbens shell. Data are presented as mean ± standard error of measurement, n = 6 per group. (ns > 0.05 , ***P < 0.001).

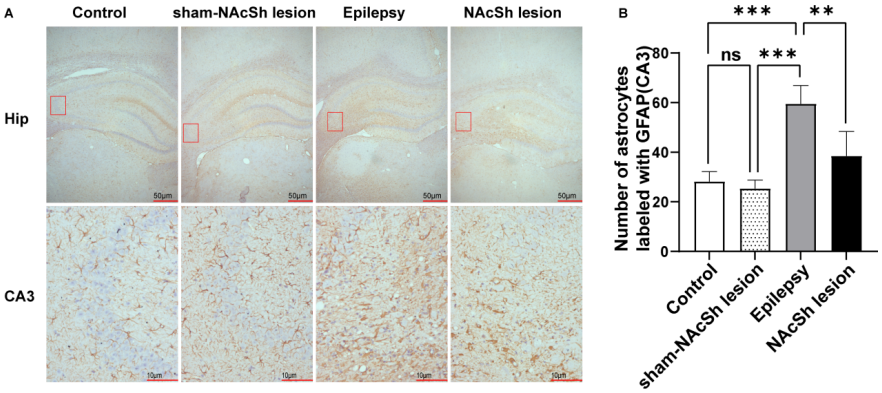

Figure 5: Effect of NAcSh lesioning on hippocampal astrocytes in epilepsy rats. (A) GFAP expression in the hippocampal tissue of rats in each group. (B) Quantification of GFAP-positive astrocytes in the CA3 region of the hippocampus. Data are presented as the mean ± SEM. NAcSh: Nucleus accumbens shell, GFAP: Glial fibrillary acidic protein,(ns > 0.05, ***P < 0.001).

4 DISCUSSION

In the present study, we found that NAcSh lesions decreased epileptic discharge, and the number of seizures was reduced after NAcSh lesions. And it also reduced the number of activated microglia and astrocytes induced by seizures in TLE rats and attenuated neuronal injury in KA-induced epileptic rats. The above content showed that seizures were alleviated, which may be of great therapeutic significance for clinical deep brain stimulation (DBS) treatment of epilepsy[6].

Regarding the regulatory loop of epilepsy, some scholars believe that it regulates the origin and spread of epileptic seizures in the cortex and subcortical structures[16, 17]. The limbic system, which includes the hippocampus, amygdala, entorhinal cortex, anterior cingulate, frontal cortex, and thalamus, is related to the TLE regulatory loop[18-20]. The sclerotic hippocampus has an abnormal neuronal activity initiation region, which spreads to the frontal cortex[21].Some projections from the ventral hippocampus and ventral cingulate to the NAcSh, while efferent fibers mainly project to the VTA and frontal cortex[22, 23]. Stereotactic ablations of the NAcSh have been applied to treat drug addiction, and refractory psychiatric disorders have also been identified as effective targets[24, 25].When treatment of drug refractory epilepsy (DRE) with neuropsychological disorders, seizures were significantly alleviated[6, 26]. In some studies, in which the NAc was electrically stimulated in a rat model combined with pilocarpine to initiate seizures, high-frequency stimulation of the NAc significantly prolonged the incubation period of seizures and inhibited their severity[13, 27]. Moreover, the second phase and total duration of seizures were also reduced in this model.

The activation of dopamine D2 receptors in the NAc, as well as a pathway through which postictal locomotor activity is mediated, have recently been highlighted in several reports[18, 28]. Several inputs pass through the NAc, including those from the ventral hippocampus, amygdala, and prefrontal cortex, and are projected to the basal ganglia. Thus, the brainstem, cortical ,and glutamatergic afferents terminated in the NAc, which are responsible for reward, modulate cortical and brainstem motor centers[7, 9, 28]. Recently, reward signal propagation and seizure discharge have been shown to have a similar neural network pattern[29, 30]. Interestingly, in NAcSh lesions, alterations in the hippocampus are primarily located in CA3. One probable reason is that the P13K/AKT mTOR signaling pathway associated with neural plasticity and hippocampal neurogenesis is compensatory activated[31]. Moreover, as there is a high density of GABAergic medium spiny neurons expressing D2 receptors in the NAcSh, another possible contributing factor is that NAcSh lesions reduce the inhibition downstream which includes the midbrain and hypothalamus, and additionally improves hippocampal function[32]. However, the detailed mechanisms underlying this effect remain unclear.

Microglia and astrocytes collaboratively contributed to epileptogenesis in the epileptic rat model. And reactive microglia appeared first, followed by reactive astrocytes and increased susceptibility to seizures[33, 34]. Considering the results of this study, the stereotactic electrical lesion of the nucleus accumbens shell may be through the regulation of pathways, which further reduces the activation of microglia, hinders the progressive activation, increase astrocytes, and plays an anti-epileptic role[35, 36]. This change is most pronounced, especially in the CA3 areas of the hippocampus. Further investigations using more interventions are required to clarify the precise mechanisms underlying the interaction between glial cells and NAcSh lesions.

We demonstrated a long-lasting antiepileptogenic effect of NAcSh lesions on KA-induced seizures in rats. To block seizures, the lesion needs to be blocked in the vital phases of the epileptic network and the varied of abnormal neuronal discharges after hippocampal injury must be addressed. The neurochemical and electrophysiological mechanisms underlying such an effective antiepileptogenic effect merit further study. Information obtained from these studies may help us to explore the clinical treatment of patients with TLE. The regret of this study is that we did not find a better way to continuously monitor the EEG of epileptic seizures in animals.

List of abbreviations

Nucleus accumbens shell	NAcSh
Kainic acid	KA
Temporal lobe epilepsy	TLE
Ventral tegmental area	VTA
Electroencephalogram	EEG
Paraformaldehyde	PFA
Nucleus accumbens	NAc
Drug refractory epilepsy	DRE

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CONFLICT OF INTEREST

The authors report no conflict of interest

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