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Original Article

Chronic anterior thalamic nucleus deep brain stimulation reduces gliosis and neuronal loss in hippocampal CA2 and CA3 regions of epileptic rhesus monkeys

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Abstract: Background and objective: Anterior thalamic nucleus deep brain stimulation (ATN-DBS) has been shown to be an effective method in seizure reduction. However, its underlying mechanisms remain unclear. This study aimed to determine the potential beneficial effects of ATN-DBS on hippocampus in epileptic rhesus monkeys. Methods: Eighteen rhesus monkeys were randomly divided into three groups: control group (n=6), sham-DBS group (n=6) and DBS group (n=6). ATN-DBS was applied to kainic acid (KA)-induced epileptic monkeys. Behavioral seizures were continuously recorded. Neuronal nuclei (NeuN), hematoxylin and eosin (H&E) and glial fibrillary acidic protein (GFAP) staining were used to assess hippocampal injury and the effects of chronic ATN-DBS. NeuN and caspase-3 levels were evaluated by western blot. Results: Seizure frequency was 45.1% lower in the DBS group compared with the sham-DBS group (P<0.05). In the DBS group, H&E and NeuN staining revealed more cells in the CA3 region (P<0.05), while GFAP staining showed less GFAP-positive cells in both CA2 (P<0.01) and CA3 (P<0.05) regions, compared with the sham-DBS group. Furthermore, caspase-3 levels decreased (P<0.05, *vs.* the sham-DBS group) and NeuN levels increased (P<0.05, *vs.* the sham-DBS group) in the DBS group. Conclusion: These results suggest that ATN-DBS can reduce gliosis and neuronal loss in hippocampal CA2 and CA3 regions in epileptic rhesus monkeys, which may be one of the most important mechanisms in treating epilepsy with ATN-DBS.

Keywords: Anterior thalamic nucleus, deep brain stimulation, epilepsy, anti-gliosis, neuronal loss reduction

Introduction

Temporal lobe epilepsy (TLE) is considered as the most common refractory epilepsies. Over two-third of the TLE patients becomes seizure-free after surgery [1, 2]. Nevertheless, approximately one-third of these patients could not be terminated by traditional surgery. Multiple reasons are responsible for this unpleasant result such as incomplete resection of mesial temporal lobe structures, multiple epileptic foci and bilateral temporal lobe epilepsy [3, 4]. Various treatments have been used in clinic to obtain better seizure control such as stem cell, microRNA, deep brain stimulation (DBS), and vagus nerve stimulation [5].

DBS is a pulsing neurostimulation technique against epilepsy [6]. Various DBS targets (including the fornix, subthalamic nucleus, cerebellum and the anterior thalamic nucleus [ATN]) have been evaluated [7]. ATN-DBS has been shown to be an effective method of decreasing seizure frequency in both clinic trails and animal studies [8, 9]. Recently, a multicenter, double-blinded and randomized trial conducted by Fisher *et al.* revealed a significant decrease in seizure frequency (40.4%) in patients with ATN-DBS [10].

Despite the pleasant outcome, the mechanisms of ATN-DBS remain unclear. Many animal studies have already been conducted to reveal the mechanisms of ATN-DBS. However, pathological and molecular alternations in epileptic animals receiving chronic ATN-DBS have not been extensively addressed. Epileptic animal models and patients have shown that a number of cellular and molecular events including alteration in receptors and neurotransmitters, aber-

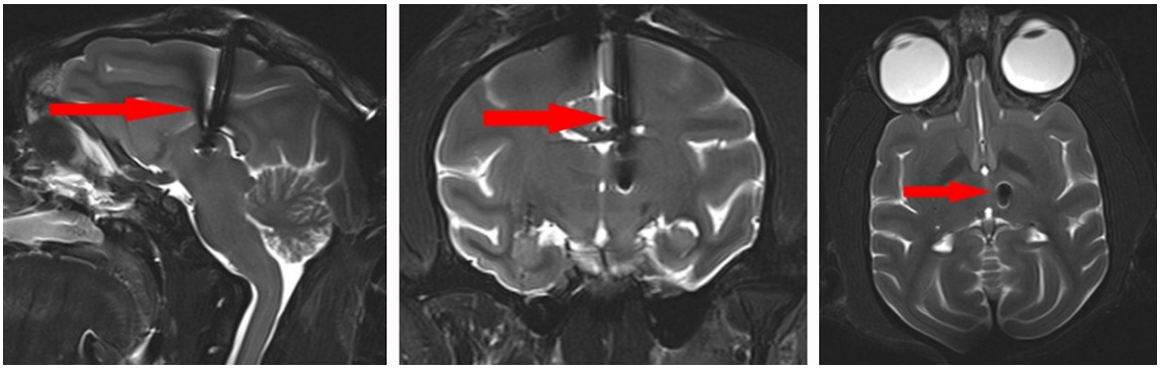

Figure 1. MRI scans of the sagittal, coronal and axial sections. Arrows showed the position of the leads in the monkey's brain.

Table 1. The detail of stimulation parameters

Stimulation parameters	
Stimulation mode	Constant voltage
Polarity	Bipolar (1-, 4+)
Amplitude (V)	1.5
Rate (Hz)	130
Pulse width (μs)	60
Electrode impedance (Ω) (between electrodes 1 and 4)	1.360-1.842
Current density (mA/cm^2)	6.6-9.0

Different from Medtronic product, the electrodes from the Pins are numbered 1-4.

rant neurogenesis, gliosis and extended neuronal death are involved in epileptogenesis [11, 12]. Hippocampal specimens from TLE patients who underwent epilepsy surgery revealed the characteristic pattern of segmental neuronal cell loss and concomitant astrogliosis. Furthermore, similar results were found in epileptic animal models [13]. According to previous studies, ATN-DBS is relative to modify neuron transmitter levels (such as glutamine, GABA and aspartate) and change gene expression (such as the BDNF gene) [7, 14]. Nevertheless, few studies have focused on hippocampal gliosis and neuronal loss with ATN-DBS. Furthermore, compared with rodents, large primate animals are more similar with human beings. Therefore, we designed a randomized controlled study to determine the effects of chronic ATN-DBS on hippocampus in epileptic monkeys induced by KA hippocampal injection.

Material and methods

Ethics and animals

This study (Permit number: SYXK 2012-0240) was approved by the Ethics Committee of the Beijing Neurosurgical Institute, and was performed in accordance with recommendations from the Guidelines for the Use and Care of Experimental Animals. Every effort was made to minimize suffering. Eighteen male rhesus monkeys (4-6 years old, 5.6-6.9 kg) were provided by the Laboratory Animal Center of the Military Medical Science Academy of China. These monkeys were divided into three groups: control group (n=6), sham-DBS group (n=6), and DBS group (n=6). All researchers involved in this experiment were completely blinded to the assignment of the group division.

The monkeys were bred in separate organic glass cages (150 × 100 × 90 cm), lived in a standardized environment (temperature: 24°C, humidity: 50-70%), and had free access to food and water.

DBS implantation

Ketamine hydrochloride (10 mg/kg, intramuscular; Hengrui Pharmaceutical, Nanchang, Jiangxi, China) and fentanyl (1.5 μg/kg, intravenous; Renfu Pharmaceutical, Yichang, Hubei, China) were injected to anesthetize the monkeys. During anesthesia, vital signs of the animals were monitored. After endotracheal intubation, the monkeys were positioned on a stereotaxic device (David Kopf Instrument, Tujunga CA, USA), kept the orbitomeatal line parallel to the table surface.

The DBS system (Model G101; Pins Medical Co. Ltd, Beijing, China) was implanted stereotacti-

Table 2. Seizure number in monkeys induced by KA

Group	Number of seizures			Latency (day)	Seizure interval (day)	Monthly frequency	Score
	Partial seizure	Generalized seizure	Total				
Control	-	-	-	-	-	-	-
Sham-DBS	20.7±5.6	3.9±2.1	24.6±7.7	6.2±2.4	4.2±1.8	3.4±1.5	52.2±13.8
DBS	12.3±2.9*	1.2±1.0*	13.5±3.9*	9.8±4.9*	7.9±2.1*	2.1±0.6*	29.7±7.8*

*, P<0.05; -, no result.

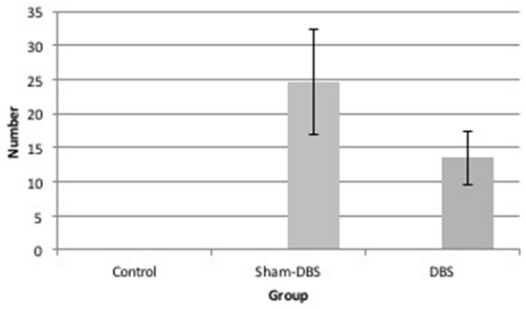

Figure 2. Statistical bar graphs of the total number of seizures among the three groups. Total seizures significantly decreased in the DBS group (P<0.05, vs. the sham-DBS group).

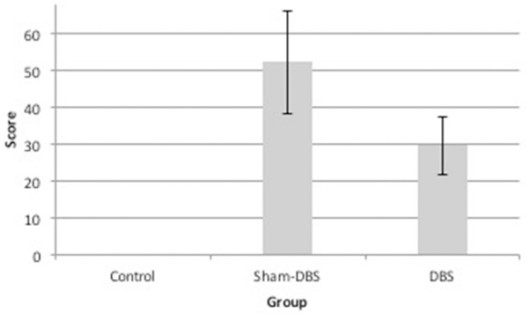

Figure 3. Statistical bar graphs of scores of seizures among the three groups. The scores of seizures in the DBS group obviously decreased (P<0.05, vs. sham-DBS group).

cally by an experienced team, including three neurosurgeons and one anesthetist. The exact position of the left ATN is 8.5 mm posterior to the bregma, 2.0 mm lateral to midline and 20.0 mm from the dura, which refer to the Rhesus Monkey Brain in Stereotaxic Coordinates [15]. The stimulator was located 2-4 cm below the left scapula and 2-4 cm lateral to the spine. The extension was tunneled subcutaneously through the neck to the back, which was the only difference from regular DBS surgery. The above DBS implantation was designed to simulate the relative bearing in the human body and keep the device beyond the monkey's reach. The wound was sutured in the end. After implantation, the entire system was tested to ensure well-connection.

With the stimulator off, magnetic resonance imaging (MRI, 7.0T; Clinscan, Bruker, Ettlingen, Germany) was used to confirm the accuracy of the electrodes location (**Figure 1**). Considering a previous study, the 7.0T MRI scan is unlikely to induce a severe heating injury [16]. Antibiotics (ceftriaxone, 30 mg/kg, Roche, Shanghai, China) were continuously injected twice per day for three days. One week after KA injection, stimulators were activated in the DBS group to ensure that the animal models were comparable at the outset. The control group did not undergo DBS implantation. Stimulation parameter details are presented in **Table 1**.

Establishment of the KA-TLE model and video-electrocorticography (ECoG) monitoring

After two-week recovery, the monkeys were anesthetized with the same method mentioned above. KA (2 μg/μL/kg; Sigma, St. Louis, MO, USA), wildly used to establish TLE animal models, was injected into the left hippocampus with stereotactic instrument both in the DBS and sham-DBS groups [17]. According to the Rhesus Monkey Brain in Stereotactic Coordinates, the position for the injection is 8.5 mm posterior to the bregma, 13 mm lateral to midline and 32.0 mm from the pia mater [15]. The same volume of normal saline was injected into the same position in the control group. All animals were resuscitated within two hours after the injection.

Continuous video recording was conducted to monitor behavioral seizures for 18 hours each day for six months. EcoG monitoring was conducted according to the previous study [18]. Seizure activity was evaluated by the Raine scale, which is listed as follows: Stage 0, no detectable motor manifestation; Stage I, facial automatisms including salivation, "mouth cleaning-like" behavior and tongue automatisms; Stage II, facial movement, head clonus and

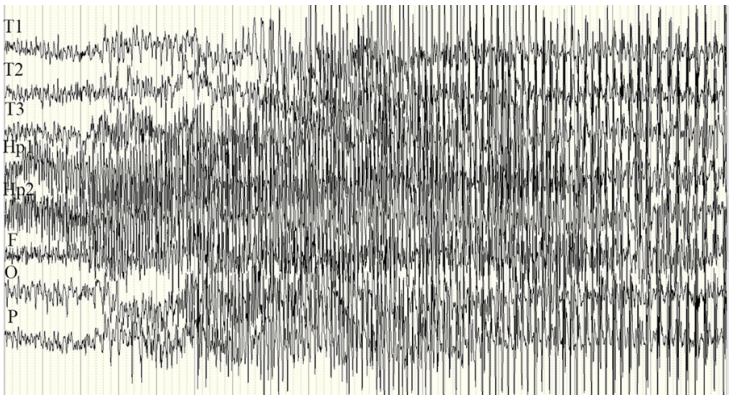

Figure 4. ECoG of KA-TLE monkey models. Spike discharges were first found in Hp1 and Hp2 electrodes and later were speared to other regions. Hp1 and Hp2 represented the left hippocampus; T1, T2, T3 represented the left temporal lobe. F represented the frontal lobe. O represented the occipital lobe. P represented the partial lobe.

head shakes; Stage III, forelimb clonus; Stage IV, bilateral forelimb clonus, rearing, arched body posture and straub tail; Stage V, generalized clonic seizures and postural impairment [19]. All seizures were counted by the video recording.

Pathological examinations

After six months of observation, monkeys were anesthetized and decapitated. The left hippocampus was dissected and thoroughly rinsed with saline. The hippocampus was divided into two parts: anterior part was performed for western blot, and posterior part was managed for pathological examinations. Before excising the hippocampus into 5-mm-thick histological samples, the hippocampus was fixed in 4% paraformaldehyde for 24 hours, dehydrated in alcohol, cleared with xylene and embedded in paraffin. Then, paraffin-embedded brain sections were sliced into 4-μm-thick sections and stained.

In order to investigate hippocampal injury and gliosis, slices were stained by hematoxylin and eosin (H&E), neuronal nuclei (NeuN) and glial fibrillary acidic protein (GFAP).

Immunohistochemistry was carried out according to previous method [20]. Briefly, sections were rinsed in 0.1 M PBS (PH = 7.4) 3 times and incubated in 3% H_2O_2 in methanol for 10 min and then blocked for nonspecific antigen binding using 4% BSA/0.1% Triton-100/PBS (BP+) for 1 h at room temperature, followed by an overnight incubation with primary antibody: mouse monoclonal anti-NeuN (1:500, Millipore, Billerica, MA, USA); rabbit polyclonal anti-GFAP (1:200, Abcam, Cambridge, MA, USA) at 4°C. Sections were then rinsed in 0.1 M PBS three times and incubated with biotinylated anti-rabbit IgG (1:500, Abcam, Cambridge, MA, USA); anti-mouse IgG (1:500, Abcam, Cambridge, MA, USA) for 1 h at room temperature, and then treated with Elite avidin-biotin enzyme complex (ABC; Vector Laboratories) for 1 h. Visualization of labeling was achieved using 3,3'-diaminobenzidine (DAB, Vector Laboratories, SK-4100).

Morphological examinations of the hippocampal cornu ammonis (CA) sub-regions were independently blinded conducted by two pathologists with a light microscope (Axio imager A2; Carl Zeiss, Gottingen, Germany). Histological injuries in the hippocampus were assessed, and the numbers of apoptotic neurons were counted in 10 random views at 400×. Furthermore, the number of surviving intact pyramidal cells of the 10^{th}, 30^{th} and 50^{th} NeuN-stained slice was counted in 10 random high-powered views (400×) in the hippocampus. The same method was used in counting GFAP-positive neurons (used 11^{th}, 31^{th} and 51^{th} GFAP-stained slice) in the hippocampus.

Caspase-3 and NeuN

As mentioned above, the anterior part of the hippocampus was obtained to measure the level of caspase-3 and NeuN by western blot. Western blot was carried out according to previous method [21]. Briefly, tissues were lysed in a buffer containing a protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) and homogenated. The homogenate was centrifuged and the supernatant was collected for further analysis. Protein concentrations were measured by BCA Protein Assay Kit (Novagen, San Diego, CA, USA). Equal amounts of protein were boiled in loading buffer (Beyotime, Beijing, China) and separated by 10% polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose, and the blots were probed overnight with rabbit anti-caspase-3 (1:500, Abcam,

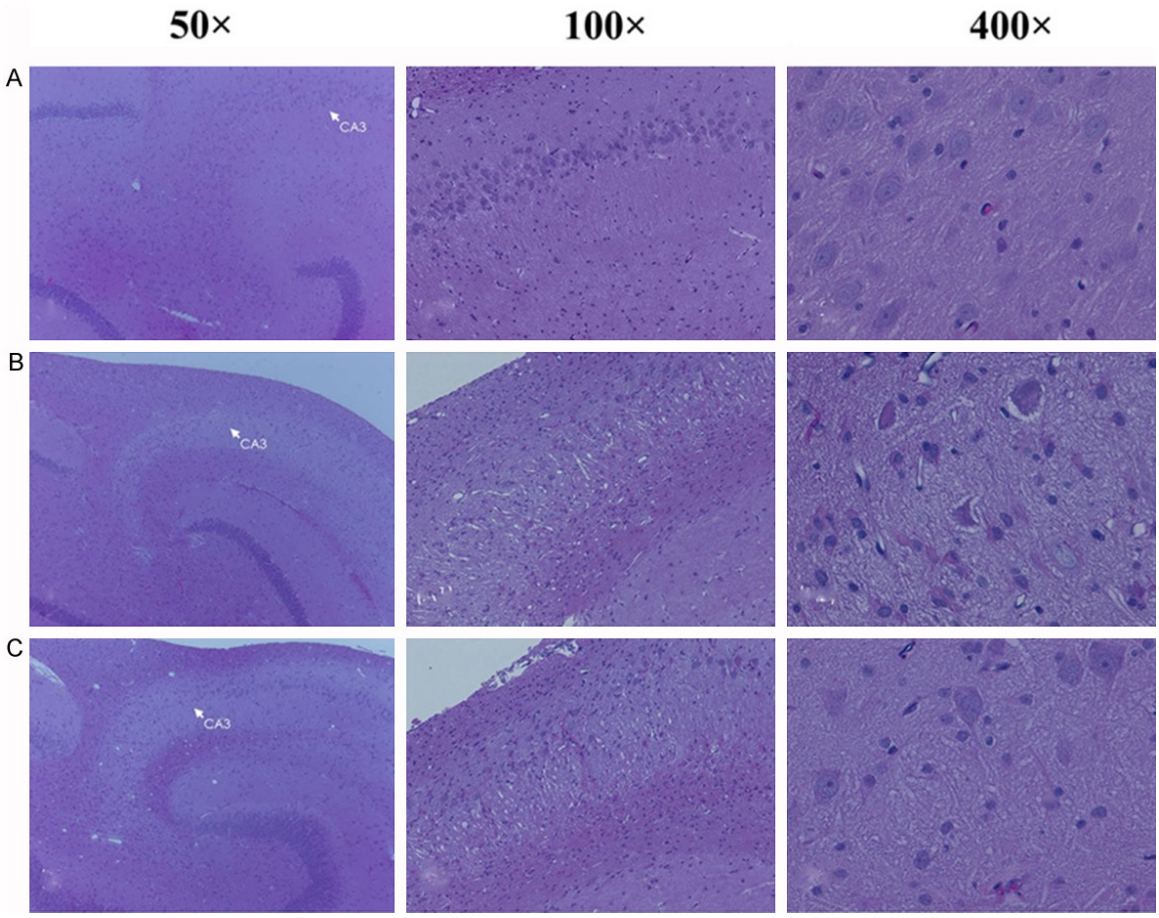

Figure 5. Images of H-E staining in hippocampal CA3 region, magnification x10, x100, x400. A. Control group. B. Sham-DBS group. C. DBS group. The condition of neuronal injury in DBS group is between control group and sham-DBS group.

Cambridge, MA, USA), mouse anti-NeuN (1:500, Millipore, Billerica, MA, USA) and β-actin antibodies (1:500, internal standard, Santa Cruz, San Diego, CA, USA) at 4°C. Primary antibodies were visualized using secondary antibodies conjugated to horseradish peroxidase (Santa Cruz, San Diego, CA, USA) and ECL reagent (Pierce, Rockford, IL, USA). Furthermore, β-actin was treated as a standardized protein level.

Statistical analysis

All data are presented as mean ± standard deviation (SD) and analyzed by using one-way ANOVA, followed by least significant difference post-hoc tests. All statistics were calculated using SPSS 22.0 software (SPSS Inc, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

Epileptic seizures

All monkeys were monitored by continuous video recording in separate cages after KA injection. There was no seizure in the control group (saline injection). All animals in sham-DBS and DBS groups successfully developed seizures. More detailed information of seizures was demonstrated as follows (**Table 2**). Mean total seizures in the DBS group was 13.5±3.9, which was 45.1% lower than in the sham-DBS group ($P<0.05$, **Figure 2**). Mean seizure intervals in the DBS and sham-DBS groups were separately 7.9±2.1 and 4.2±1.8. Monthly seizure frequency in the DBS group was 2.1±0.6, which was 38.2% lower than in the sham-DBS group ($P<0.05$). Compared with the sham-DBS group (52.2±13.8), mean seizure score in the DBS group was 29.7±7.8 ($P<0.01$, **Figure 3**).

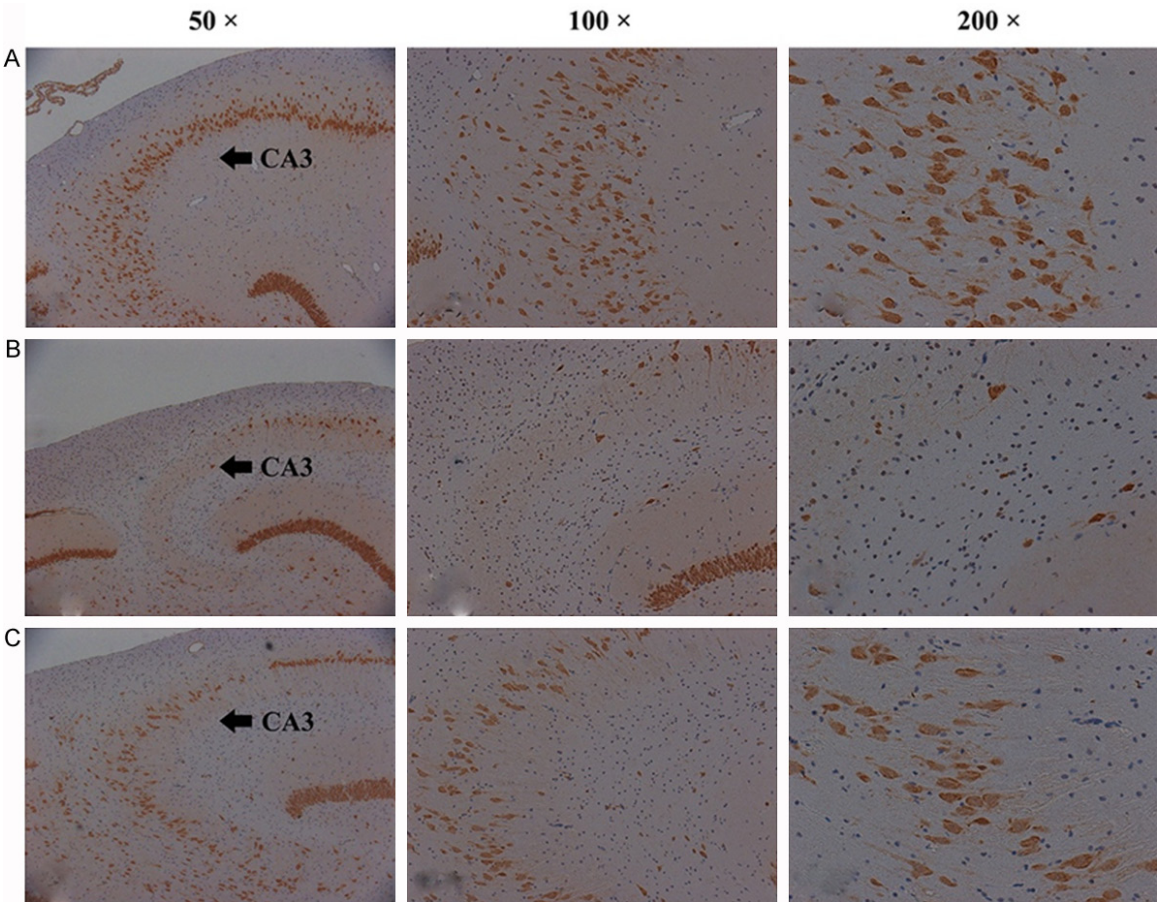

Figure 6. Images of NeuN staining in hippocampal CA3 region magnification x10, x100, x400. A. Control group. B. Sham-DBS group. C. DBS group. The condition of neuronal loss in DBS group was between control group and sham-DBS group.

Table 3. The number of NeuN-positive cells in the CA3 region

Control group	35.3±5.2
Sham-DBS group	2.7±2.3*,^
DBS group	15.6±4.9*

*, $P<0.05$ *vs.* the control group; #, $0.01<P<0.05$ sham-DBS *vs.* DBS group; ^, $P<0.01$ sham-DBS *vs.* DBS group.

Our results revealed ATN-DBS can obviously reduce seizures in KA-induced epileptic monkeys. The results of ECoG was showed in **Figure 4**.

Neuronal loss and apoptosis in the hippocampal CA3 region

In order to determine the potential effect of ATN-DBS on the hippocampus, pathological and molecular examinations were conducted on samples from the left hippocampus. H&E and NeuN staining results revealed that only

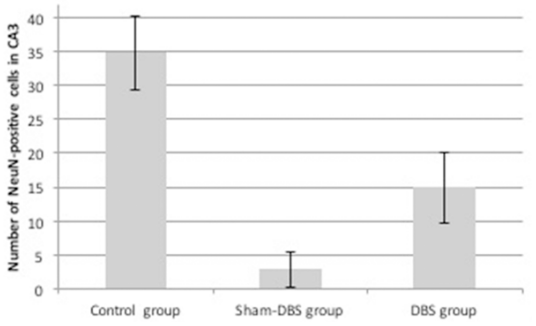

Figure 7. Statistical bar graphs of number of NeuN-positive cells in hippocampal CA3 region. There was a significant difference between sham-DBS group and DBS group.

mild differences were observed in the hippocampal CA1, CA2 and CA4 regions. Thus, we counted the number of cells to estimate injury in the hippocampal CA3 region. In the sham-DBS group, the pyramidal layer shown by H&E staining was almost completely lost in the CA3

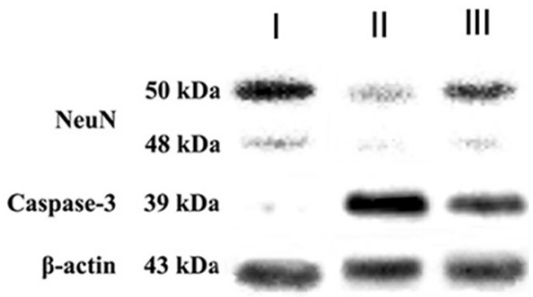

Figure 8. Western-blot analysis of NeuN and caspase-3. β-actin was measure as a marker for protein levels standardization. A. Control group. B. Sham-DBS group. C. DBS group.

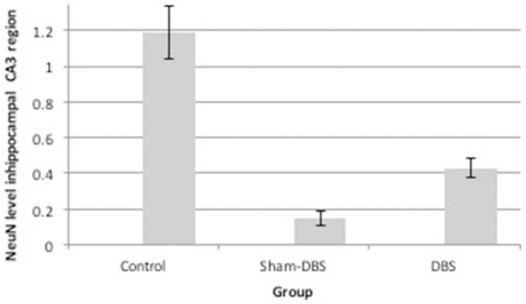

Figure 9. Statistical bar graphs of NeuN level in hippocampal CA3 region among three groups. The NeuN level in DBS group increased obviously (P<0.05, vs. sham-DBS group).

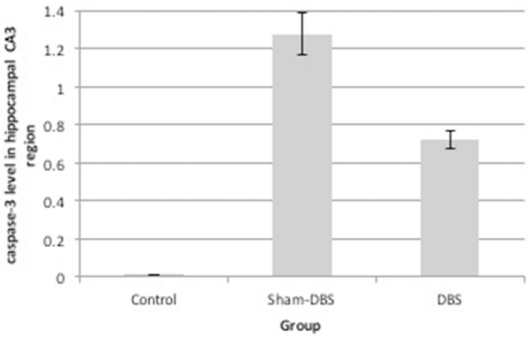

Figure 10. Statistical bar graphs of caspase-3 level in hippocampal CA3 region among three groups. The caspase-3 level in DBS group decreased obviously (P<0.05, vs sham-DBS group.

region. On the contrary, in the DBS group, the loss of pyramidal cells in the CA3 region was also apparent but less severe. Great differences were observed in the control group, pyramidal neurons were normal stained and had regularly shaped cell bodies in the CA3 region of the hippocampus. This result is shown in **Figure 5**.

In the 10th, 30th and 50th NeuN-stained slice, pyramidal cells were counted at 10 random subfields with high-power views (400×) using the method mentioned above. As shown in **Figure 6**, sparsely distributed neurons were observed in the sham-DBS group. However, the number of neurons in control group was much higher than that in the sham-DBS group. Furthermore, the condition of the DBS group is between the conditions of the other two groups. These data were further confirmed by counting the NeuN-positive cells (**Table 3** and **Figure 7**). In the CA3 region, the number of NeuN-positive cells in the DBS group was higher than that in the sham-DBS group and lower than in the control group ($P<0.05$). Based on western blot analyses in the CA3 region, NeuN level in the DBS group (0.43±0.05) was higher than that in the sham-DBS group (0.15±0.04) and lower than that in the control group (1.19±0.15) (all $P<0.05$, **Figures 8** and **9**). These results suggest that chronic ATN-DBS can protect neurons and reduce neuronal loss in hippocampal CA3 region.

The level of caspase-3 in the CA3 region was evaluated to measure neuronal apoptosis and injury in the hippocampal CA3 region [21]. As shown in **Figures 8** and **10**, caspase-3 levels increased in both the DBS (0.72±0.05) and sham-DBS (1.28±0.11) groups (all $P<0.05$, *vs.* the control group [0.01±0.00]). Compared with the sham-DBS group, chronic ATN-DBS reduced the level of caspase-3 ($P<0.05$). The results above suggest that chronic ATN-DBS can reduce neuronal injury and has an anti-apoptosis effect on hippocampal CA3 region.

Gliosis in the hippocampal CA2 and CA3 regions

GFAP expression in every hippocampal sample was evaluated by immunohistochemical staining and GFAP-positive cell counting. Moderate differences were observed in the hippocampal CA1 and CA4 regions among the three groups. As shown in **Figure 11**, GFAP expression was not active in the control group, though some GFAP-positive cells could be found in the hippocampal CA2 and CA3 regions. GFAP expression in the sham-DBS group was remarkable, especially in CA2 and CA3 regions. Many GFAP-positive cells can be detected in these regions. However, the number of GFAP-positive cells in

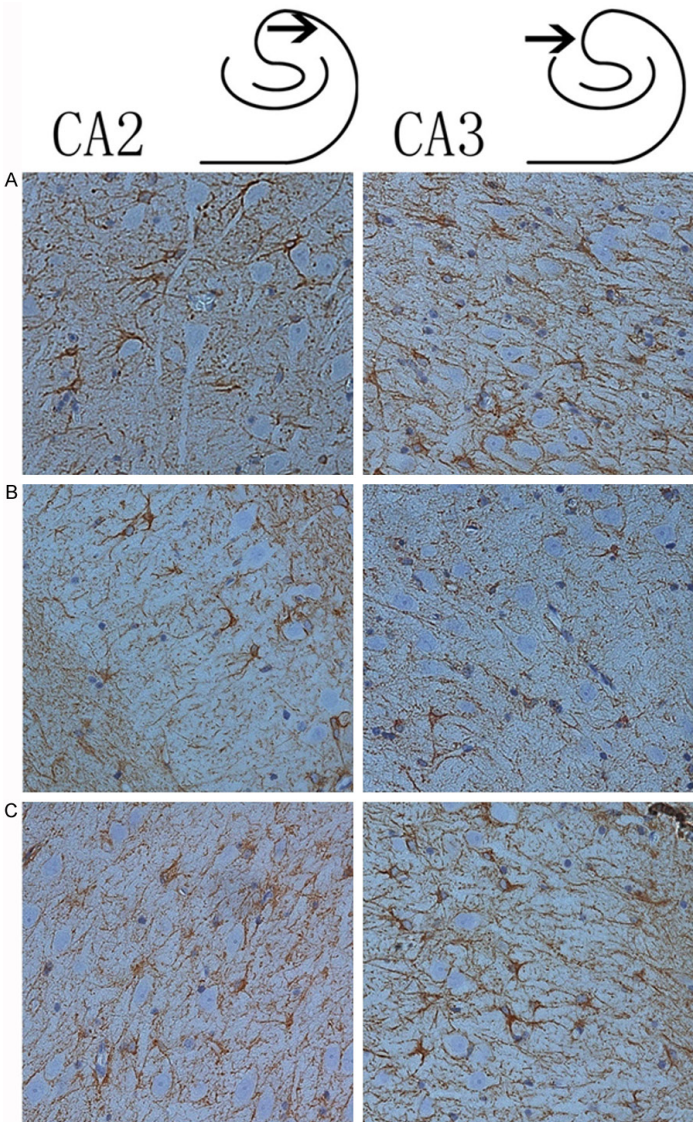

Figure 11. Images of GFAP staining in hippocampal CA2 and CA3 region. A. Control group. B. Sham-DBS group. C. DBS group. The condition of gliosis in DBS group is between control group and sham-DBS group.

Table 4. The number of GFAP-positive cells in CA2 and CA3 regions

Group	CA2	CA3
Control group	10.9±1.7*	7.9±2.7*
Sham-DBS group	15.8±2.3	20.6±2.8
DBS group	10.8±2.5*	18.1±2.3^

*, P<0.01 vs. the sham-DBS group; ^, P<0.05vs. sham-DBS group.

DBS group decreased in the CA2 and CA3 regions, compared with the sham-DBS group. In CA2 region, more GFAP-positive cells were observed in the sham-DBS group than that in the other two groups (all $P<0.01$). A significant difference in hippocampal CA3 region between the sham-DBS and control groups was explored ($P<0.05$). The number of GFAP-positive cells in the DBS group was higher than that in the control group ($P<0.01$) and lower than in the sham-DBS group ($P<0.05$) (**Table 4** and **Figure 12**).

Discussion

In this study, we implanted the DBS system into the left ATN and established epileptic monkey models by KA hippocampal CA3 region injection in both DBS and sham-DBS groups. Monkeys in both DBS and sham-DBS groups successfully developed spontaneous seizures and were confirmed by continuous video-ECoG monitoring. Hippocampus can be divided into different regions: CA1, CA2, CA3 and CA4 region, the overall hippocampus cannot represent each hippocampal subfield. In addition, the roles of different hippocampal subfields in epileptogenesis are not identical [22]. In this study, histopathological examination of each subfield was performed to detect the effect of ATN-DBS on epileptogenesis. Epileptogenesis a process of converting a normal into an epileptic brain [23]. And many researchers emphasized the important of inhibition of epileptogenesis. The anti-epileptogenic therapeutic window may only remain open during several weeks after injury (DBS was activated one week after epileptic model establishment in our study), when some delayed cell death may still be preventable [23, 24]. Unlike the many study that DBS implantation on the chronic stage of epileptic animal model, in our study, the DBS was conducted before establishing KA-TLE model and activated in acute stage, in order to reduce the mortality and demonstrate the effect of ATN on epileptogenesis.

Our data revealed that mean total seizures in the DBS group reduced by 42.8% compared with the sham-DBS group, sharing similar results with previous clinic and animal studies.

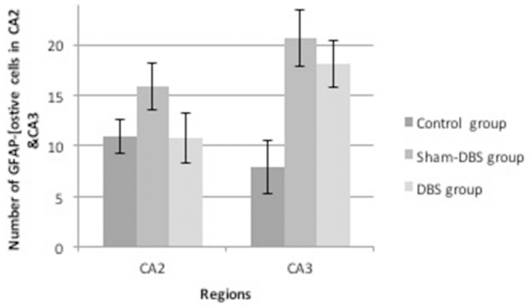

Figure 12. Statistical bar graphs of number of GFAP-positive cells in CA2 and CA3 regions among three groups. The GFAP-positive cells in DBS group decreased significantly ($P<0.05$, vs sham-DBS group.

Qun Zhang *et al.* found that ATN-DBS significantly decreased the incidence of generalized seizures by 21.3% compared with the control group [8]. Shuo-Bin Jou *et al.* observed that 20% of rats with ATN-DBS developed seizures, while 87.5% of the same was observed in the control group [9]. Fisher *et al.* conducted a randomized controlled study, which revealed a 41% median seizure reduction in the first year and 69% median seizure reduction in the fifth year with ATN-DBS treatment [10, 25]. All these studies implied the association between seizure reduction and ATN-DBS. However, the rates of reduction were different. Different stimulation parameters may be responsible for these results. Fisher's trail applied 5 V, 90 μs and 145 Hz [10]. The reasons why we used this parameter (amplitude: 1.5 V, rate: 130 Hz, and pulse width: 60 μs) are listed as follows: though monkeys share many common points with humans, there are still certain differences between the human brain and monkey brain, especially the dimension of the nucleus. The electrodes used in the monkeys were similar with that used in humans. As we all know, the monkey's ATN is smaller than a human's ATN; thus, these basic parameter was used to avoid side effects.

Multiple planes were performed with H&E, NeuN staining and western blot. The samples from DBS group presented less apoptosis and more surviving neurons, and lower caspase-3 levels and higher NeuN levels in hippocampal CA3 region, compared with the sham-DBS group. In addition, less NeuN-positive cells and higher caspase-3 levels were observed in the sham-DBS group when compared with the control group. Previous studies have confirmed that the hippocampal CA3 region obtains the most serious injury with KA injection [26]. Two types of neuronal death including apoptosis and necrosis have been proposed to occur in KA models. A number of studies have reported that apoptosis was the major mode of cell death in KA-TLE models [27]. Our data indicated that neuronal cells in the hippocampal CA3 region with KA injection have a tendency to undergo apoptosis. However, with ATN-DBS, caspase-3 levels were reduced and NeuN-positive cells remained elevated, which suggest ATN-DBS relieves survival stress, has an anti-apoptosis effect and reduces neuronal loss in the CA3 region.

Gliosis is one of the most common pathological changes after injury in the central nervous system (CNS). Since at least half of glial cells are astrocytes, gliosis is generally considered as the proliferation of astrocytes. In the classical view of gliosis, it has an adaptive reaction to seizure and a significant role in epileptogenesis [28]. GFAP, an astrocyte-specific cytoskeletal protein, has been supposed to induce astrogliosis reaction during epilepsy by previous studies [29]. In addition, the enhancement of GFAP-positive cells in the hippocampus in epileptic animals and patients have been confirmed by previous studies [30, 31]. Our result is highly consistent with these studies. Moreover, the DBS group with chronic ATN-DBS revealed a significant decrease in GFAP level in the CA2 and CA3 regions. These results suggest that chronic ATN-DBS can reduce gliosis in the hippocampal CA2 and CA3 regions. Gliosis triggers glutamine-reversible hyperexcitability in the hippocampal circuit, which could be associated with epilepsy [32]. With ATN-DBS, the reduction of gliosis in the hippocampus may be one of the reasons for seizure reduction.

We can conclude from the results above that chronic ATN-DBS induced an anti-apoptotic effect and reduced gliosis and neuronal loss in the hippocampal CA2 and CA3 regions and lower seizures. Our findings may closely relate to the therapeutic effects of ATN-DBS in treating refractory epilepsy, while other pathways may perform important roles in controlling seizures. For instance, a previous study has confirmed that ATN-DBS can change neurotransmitter levels [33]. Further research needs to be conducted to determine the connection between our findings and these previous studies.

There are some limitations in our study. Eighteen rhesus monkeys were divided into three groups (six monkeys per group). DBS system implantation and KA injection were employed in the sham-DBS and DBS groups. Various methods were used to measure samples. Compared with the rodent trail, the number of animals in our investigation was small (six monkeys per group). However, due to strict restrictions regarding the experimental use of primates, the small number of animals is unavoidable. Considering that previous studies even used a smaller amount of monkeys, we think this investigation can reveal the effect of ATN-DBS [34, 35]. Furthermore, the size of ATN between monkeys and humans are different. Thus, employing the DBS system to stimulate ATN and other nuclei could induce side effects. However, a previous clinical study defined ATN as an unspecific area that includes ATN, the medial aspect of the ventral anterior nucleus, the anterior aspect of the dorsomedial nucleus, and white matter between these nucleuses [36]. Hence, the difference in ATN size can be tolerated. The procedure (DBS implantation before KA-TLE model establishment) we used in this study is not completely consistent with clinical practice. Nevertheless, previous studies have used the same procedure [37]. In order to get the monkey recovery after DBS implantation surgery and achieve the aim of this study (detect the effect of ATN-DBS on epileptogenesis), we had to implant a DBS system before KA injection.

In summary, the present study used KA-induced epileptic monkeys to assess the effect of chronic ATN-DBS on the concentrations of hippocampal neurons. Our findings indicated that chronic ATN-DBS can reduce seizure frequency in epileptic monkeys induced by KA hippocampal injection; KA hippocampus injection can lead to neuronal apoptosis and loss in the CA3 region; chronic ATN-DBS has an anti-apoptotic effect and can reduce neuronal loss in a KA-TLE model in the CA3 region; and chronic ATN-DBS can reduce gliosis in hippocampal CA2 and CA3 regions in a KA-TLE model. Our preliminary data furthers our understanding of the mechanisms of chronic ATN-DBS in epileptic treatment.

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Disclosure of conflict of interest

None.

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