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Potential Protective Effects of Chronic Anterior Thalamic Nucleus Stimulation on Hippocampal Neurons in Epileptic Monkeys

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ABSTRACT

Background: Stimulation of the anterior nucleus of the thalamus (ANT) is effective in seizure reduction, but the mechanisms underlying the beneficial effects of ANT stimulation are unclear.
Objective: To assess the beneficial effects of ANT stimulation on hippocampal neurons of epileptic monkeys.
Methods: Chronic ANT stimulation was applied to kainic acid-induced epileptic monkeys. Behavioral seizures were continuously monitored. Immunohistochemical staining and western blot assays were performed to assess the hippocampal injury and the effects of ANT stimulation.
Results: The frequency of seizures was 42.8% lower in the stimulation group compared with the sham-stimulation group. Immunohistochemical staining and western blot analyses indicated that neuronal loss and apoptosis were less severe and that neurofilament synthesis was enhanced in the stimulation monkeys compared with the sham-stimulation group. These data showed that the hippocampal injury was less severe in monkeys in the stimulation group than in those in the sham-stimulation group.
Conclusions: Our data suggest that chronic ANT stimulation may exert protective effects on hippocampal neurons and boost the regeneration of neuronal fibers. These effects may be closely related to the mechanisms of ANT stimulation in epilepsy treatment.

Introduction

More than 50% of all seizures are partial seizures [1], and among seizure disorders, temporal lobe epilepsy (TLE) is the most common and has the best surgical outcomes [2]. Nearly 70% of TLE patients who undergo resective surgery achieve seizure freedom [3,4]. However, the remaining patients and those who are not candidates for surgery respond less positively to traditional therapy [4]. Alternative methods have been developed to achieve better seizure control in these patients. Deep brain stimulation (DBS), a novel neurostimulation technique that was developed decades ago [5], is attracting increasing interest. Various DBS targets have been evaluated, including the subthalamic nucleus, fornix, cerebellum and the anterior nucleus of the thalamus (ANT). ANT stimulation effectively suppresses seizures [5–7], and a recent large randomized controlled study demonstrated that chronic ANT stimulation

Abbreviations: ANT, anterior nucleus of the thalamus; CA, cornu ammonis; Ctl, control group; DBS, deep brain stimulation; ECoG, electrocorticography; GS, generalized seizure; H-E staining, hematoxylin-eosin staining; HSP-70, heat shock protein-70; KA, kainic acid; MAP2, microtubule-associated protein-2; MRI, magnetic resonance imaging; NeuN, neuronal nuclei; PS, partial seizure; SD, standard deviation; Shm, sham-stimulation group; Stm, stimulation group; TLE, temporal lobe epilepsy.

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decreases seizure frequency [8]. Additionally, ANT stimulation has been accepted as an alternative therapy in clinical practice in Europe, and approval is pending in the United States [9]. Animal studies have been conducted to uncover the underlying mechanisms through which ANT stimulation exerts its anti-convulsant effects [10,11]. However, the pathological and molecular alterations in epileptic animals receiving chronic ANT stimulation have not been extensively addressed. Moreover, the effects of chronic ANT stimulation on large primate animals are not clear. Therefore, we designed this randomized controlled study to investigate the effects of chronic ANT stimulation on hippocampal neurons of kainic acid-induced (KA) epileptic monkeys.

Materials and methods

Ethics and animals

The Beijing Neurosurgical Institute ethics committee approved this study (Permit number: SYXK 2012-0240). We conducted this study in accordance with the Guidelines for the Use and Care of Experimental Animals. Efforts were made to minimize animal suffering during the procedures.

Eighteen male rhesus monkeys (*Macaca mulatta*, Laboratory Animal Center, Military Medicine Academy, Beijing, China) weighing 6.2 ± 0.4 kg were assigned to a stimulation group (Stm, $n = 6$), a sham-stimulation group (Shm, $n = 6$) or a control group (Ctl, $n = 6$). Previous studies have verified the sufficiency of the animal numbers in each group [12]. All experimenters involved in the study were completely blinded to the animal group assignment. The monkeys were bred in a standardized environment (cages, 150 cm × 100 cm × 90 cm; temperature, 24 °C; humidity, 50–70%, free access to food and water).

DBS implantation

Anesthesia was achieved by injections of ketamine hydrochloride (10 mg/kg, intramuscular; Hengrui Pharmaceutical, Nanchang, Jiangxi, China) and fentanyl (1.5 μg/kg, intravenous; Renfu Pharmaceutical, Yichang, Hubei, China), and vital signs were monitored. The monkeys were mounted onto a stereotaxic device (David Kopf Instruments, Tujunga, CA, USA), and DBS systems (Model G101, Pins Medical Co. Ltd., Beijing, China) were stereotactically implanted by an experienced team. The left ATN was targeted according to *The Rhesus Monkey Brain in Stereotaxic Coordinates* (Fig. 1A) [13], with the stereotactic coordinates 8.5 mm posterior to the bregma, 2.0 mm lateral to the midline, and 20.0 mm from the dura. These were routine DBS surgery procedures, except that the extension was tunneled subcutaneously through the neck to the back, 2–4 cm below the left scapula and 2–4 cm lateral to the spine, where the stimulator was located. Sufficient wire was reserved on the head to ensure adequate range of motion. The connector was fixed at mid-neck. The placement of the DBS stimulator and the extension was to ensure that the DBS device was beyond the monkey's reach and to simulate the relative bearings in the human body. The wounds were carefully sutured. The entire system was tested to ensure correct connections and the "off" status of the stimulator. Magnetic resonance imaging (MRI, 7.0T, ClinScan, Bruker, Ettlingen, Germany) was performed to verify the accurate placement of the DBS lead in the ANT, as shown in Fig. 1B and Fig. 1C. We previously determined that the 7.0T MRI system was unlikely to induce severe heating injury around the electrodes [14]. In 2 monkeys, the lead positions were 4–6 mm posterior to the ideal ANT position; therefore, these monkeys underwent lead replacements. Subsequent MRI scans failed to show any thalamic lesion in these monkeys. Antibiotics (ceftriaxone, 30 mg/kg, Roche, Shanghai, China) were injected twice per day for three days. The monkeys were fed normally for two weeks to minimize the effects of microthalamotomy [15]. The stimulators in the Stm group were activated 7 d after KA injection (see KA Administration and Behavioral Seizure Monitoring Section) to ensure that the animal models were comparable at the outset. The parameters of the DBS system are provided in Table 1. The Ctl group monkeys did not undergo DBS surgery.

KA administration

Two weeks after DBS implantations, the KA-induced epileptic animal model was established using methods described previously [16,17]. The monkeys were anesthetized as described above. KA (2 μg/μL/kg, Sigma, St. Louis, MO, USA) was stereotactically injected into the left hippocampus of monkeys in the Stm and Shm groups (coordinates: 8.5 mm posterior to the bregma, 13.0 mm lateral to the midline, and 32.0 mm from the pia mater) [13]; in the Ctl group monkeys, an equivalent amount of saline was injected in a similar manner. The monkeys were fed normally for 6 m after the modeling.

Video recordings and electrocorticography (ECoG) monitoring

After KA/saline injection, the monkeys were continuously monitored for behavioral seizures with video recording for 12 h/day

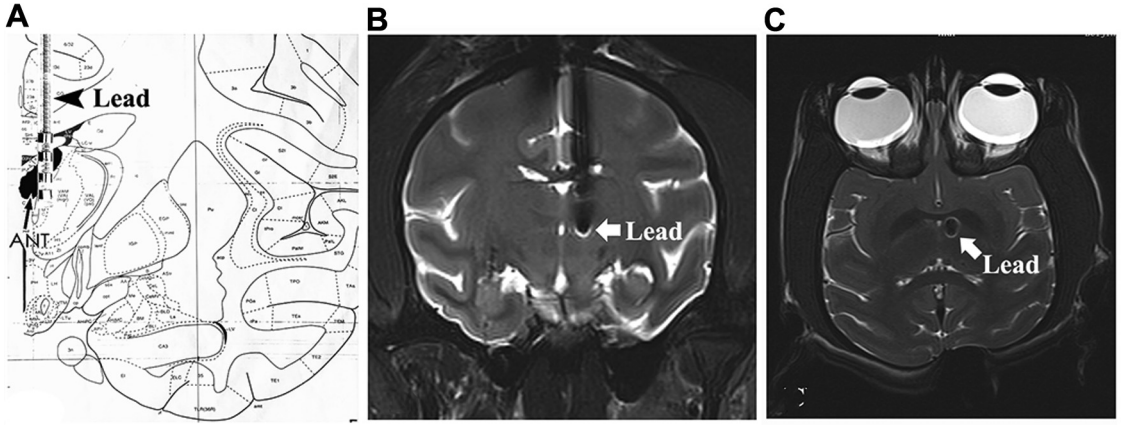

Figure 1. Monkey brain atlas and lead position verification by MRI. (A) An atlas figure showing the ANT position and ideal DBS lead placement. Arrow, ANT; arrowhead, DBS lead. (B and C) Representative MRI figures showing the DBS lead positions. Arrow, DBS lead.