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Deep Brain Stimulation of the Ventral Pallidum Attenuates Epileptiform Activity and Seizing Behavior in Pilocarpine-Treated Rats

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ABSTRACT

Background: Brain stimulation is effective for people with intractable epilepsy. However, modulating neural targets that provide greater efficacy to more individuals is still needed.
Objective/hypothesis: We investigate whether bilateral deep brain stimulation of the ventral pallidum (VP-DBS) has potent seizure control in pilocarpine-treated rats.
Methods: VP-DBS (50 Hz) was applied prior to generalized forebrain seizures or after generalized brainstem seizures manifested. Behavioral seizures were assessed using a modified Racine scale. *In vitro* and *in vivo* electrophysiological techniques were employed to identify how VP-DBS affects proximal and distal neuronal activity. The open field test was used to see if acute and chronic VP-DBS affected gross motor function or arousal state. Parametric and non-parametric statistics with post-hoc analysis were performed.
Results: VP-DBS prior to pilocarpine prevented behavioral forebrain and brainstem seizures in most animals (n = 15). VP-DBS after brainstem seizures emerged prevented or reduced the appearance of subsequent behavioral brainstem seizures (n = 11). VP-DBS attenuated epileptiform activity in the hippocampus (n = 5), but not in the primary somatosensory cortex (S1) (n = 4) *in vivo*. Electrical stimulation in the VP increased VP GABAergic neuronal firing activity from 3.1 ± 1.4 Hz to 7.6 ± 1.7 Hz (n = 8) *in vitro* and reduced substantia nigra reticulata and superior colliculus neuronal spiking activity from 25.4 ± 3.3 Hz to 18.2 ± 1.4 Hz (n = 6) and 18.2 ± 1.4 Hz to 11.0 ± 1.1 Hz (n = 18), respectively, *in vivo*.
Conclusion: VP-DBS can be a novel and potent therapeutic approach for individuals with intractable epilepsy.

Introduction

Antiepileptic drugs (AEDs) are the first line of treatment for individuals with epilepsies, but 30% are considered intractable [1]. The most common intractable form of epilepsy is temporal lobe epilepsy (TLE), which can secondarily generalize to other brain areas to manifest generalized tonic–clonic seizures (GTCSs). Notably, GTCSs pose the greatest risk for seizure-related injuries and are accompanied by pronounced cardio-respiratory dysfunction, which are contributors for sudden unexpected death in epilepsy (SUDEP) [2]. One option for intractable patients is resective surgery, which can reduce seizures with low morbidity in adults with TLE [3]. However, some still have seizures despite this approach [4,5], while others are not candidates for surgery [3]. Alternatively, deep brain stimulation (DBS) is a promising therapeutic for seizure control. At present, only vagus nerve stimulation (VNS) and Neuropace responsive neurostimulation® (RNS) are FDA-approved for epilepsy.

The VP is a basal ganglia (BG) nucleus that serves an important role for limbic and affective function and receives GABAergic input from the nucleus accumbens [6], glutamatergic input from the subthalamic nucleus (STN) and basolateral amygdala [7] and dopaminergic input from the midbrain [8]. The VP projects to BG nuclei such as the substantia nigra reticulata (SNR) and STN, to limbic-related areas such as the lateral septum, amygdala, lateral hypothalamic area and ventral tegmental area (VTA) [9]. Finally, VP has poly-synaptic projections to brainstem structures such as reticular formation [10], raphe nuclei [11], medulla, superior colliculus (SC) and pedunculopontine nucleus (PPN) [12].

Despite extensive connections to BG nuclei, brainstem and limbic structures, only two studies examined the role of the VP in seizures.

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First, epileptiform activity in the pilocarpine rat model of TLE was noted in the VP prior to epileptiform activity in the hippocampus [13], which posits that the VP may be upstream of the hippocampus and a possible modulator of TLE. Second, increasing or decreasing VP neuronal activity with pharmacology *in vivo* attenuated or aggravated generalized absence seizures, respectively [14]. No other studies elaborated on these limited, but cogent, findings. Here, we investigated whether VP-DBS can be efficacious in the pilocarpine rat model of TLE with secondarily generalized seizures.

Materials and methods

Animals and surgery

All animal use was in compliance with the guidelines of the National Institutes of Health and Albany Medical College (AMC) Animal Care Committee. Animals were purchased from Taconic (Germantown, NY), and all procedures were performed during the light phase of the light–dark cycle (7:00 AM–7:00 PM, lights on).

Male Sprague Dawley rats weighing 225–350 g were anesthetized with 2% isoflurane using an inhalant system (Harvard Apparatus, MA, USA) in a stereotaxic frame (David Kopf Instruments, CA, USA). Body temperature was maintained at 37 °C throughout the surgery (Harvard Apparatus, MA, USA). After, burr holes were made in the cranium according to the target brain coordinates. Stainless steel twisted wire electrodes (125 μm diameter, Plastics One, VA, USA) were implanted bilaterally in the VP (from bregma: 0.3 mm posterior, 2.5 mm lateral, and 7.0 mm ventral from dura) or the STN (from bregma: 4.16 mm posterior, 2.4 mm lateral, and 8.0 mm ventral from dura). A screw electrode was implanted in the primary somatosensory cortex (S1) (from bregma: 4.3 mm posterior, 5.0 mm lateral, and 2.0 mm ventral from dura) to obtain electrocorticograms (eCoGs) with a reference screw electrode placed in the midline, anterior of bregma. A twisted wire bipolar electrode was implanted in the CA1 hippocampus (from bregma: 4.0 mm posterior, 2.4 mm lateral, and 3.2 mm ventral from dura) to obtain hippocampal local field potentials (LFPs). Dental cement (Duralay Reliance Dental, IL, USA) was used to fasten recording electrodes, anchor screws and pedestal in place. Post-operatively, topical bacitracin was applied on the scalp, and animals were given penicillin (80 μg/kg) subcutaneously (subQ), and buprenorphine (subQ: 0.12g/kg) was administered every 12 hours for 72 hours post-surgery for pain management.

Stimulation and LFPs

At least one week after electrode implantation surgeries, rats were divided into two groups: a stimulation pre-pilocarpine group and a stimulation post-brainstem seizure group. Initially, we applied bilateral VP-DBS at 150 Hz, 300 μA, 90 μsec pulse width in the cathodal bipolar configuration which are similar settings used in rodent preclinical studies [15]. However, VP-DBS did not alter generalized forebrain or brainstem seizures when applied 1 hour prior to pilocarpine and throughout the monitoring period (n = 3, data not shown). Therefore, we pursued another stimulation frequency. Since increasing VP neuronal activity with pharmacology could attenuate generalized behavioral seizures [14], we applied 50 Hz VP stimulation based on a premise that low to intermediate stimulation frequencies can increase proximal neuronal activity [16]. Moreover, this frequency attenuated TLE with cortical stimulation in a clinical study [17] and diminished hippocampal epileptiform activity *in vitro* [18]. Therefore, rats in both groups were continuously stimulated bilaterally in the VP at 50 Hz, 300 μA, 90 μs duration in the cathodal bipolar configuration using a Grass S88X dual stimulator (Natus Neurology Inc, RI, USA) coupled to current isolation units (Natus Neurology Inc, RI, USA). In the first "pre-pilo" group, stimulation was on for 1 hour prior to 40 mg/kg pilocarpine injection intraperitoneal (IP). LiCl (3 mEq) and 2 mg/kg scopolamine/terbutaline were IP-injected 12–18 hours and 30 min, respectively, prior to pilocarpine. After administration, VP-DBS continued for the 4 hour behavioral monitoring period. In the second "post brainstem-generalization" group of animals, rats were IP-administered pilocarpine, and electrophysiological recordings were performed in either the S1 or hippocampus concurrently with behavioral monitoring. LFPs were obtained in the differential configuration (Model 3000, A-M Systems, WA, USA), sampled at 1 kHz, high- and low-passed at 1 Hz and 300 Hz, respectively, and digitized (MiniDigi 1B, Molecular Devices, CA, USA). After the first behavioral brainstem seizure, VP-DBS was turned on with the stimulation parameters described above.

Since inhibiting the STN with high frequency stimulation (STN-DBS) is a proposed neuromodulatory therapy for epilepsy [19,20] and the VP directly inhibits the STN [12], we also tested whether efficacy from VP-DBS may be due to this inhibitory action on the STN by comparing STN-DBS and VP-DBS effects on hippocampal epileptiform activity and on behavioral generalized brainstem seizures in pilocarpine-treated rats. Rats were implanted with twisted wires in the hippocampus to record LFPs and stimulating twisted wires bilaterally in the STN to deliver constant STN-DBS at 130 Hz, 300 μA, 60 μs duration in the cathodal bipolar configuration [20] once the first brainstem seizure manifested. Stimulation and recording continued for another hour before animals were sacrificed.

Behavioral testing

Behavioral seizures were scored by blinded reviewers using a Racine scale that was modified to include brainstem seizure phenotypes [21]: 1: staring and mouth clonus; 2: head nodding; 3: unilateral forelimb clonus; 4: rearing and bilateral forelimb clonus; 5: rearing and falling; 6: wild-running and jumping; 7: wild-running and jumping followed by tonic-clonic seizures. Stages 1–3 represent partial forebrain seizures, stages 4–5 are generalized forebrain seizures, and stages 6–7 are generalized brainstem seizures.

We used the open field test (OFT) to examine whether VP-DBS could affect gross locomotor activity and arousal since the VP is part of the BG and also has connections to limbic and brainstem structures. Animals were placed in a custom-made plexiglass open field apparatus (80 × 80 × 40: width × length × height in cm) with lighting for 10 min, and their behavior was recorded with a video camera. Total distance traveled and percent time immobile were analyzed with Any-maze software (Stoelting Co., IL, USA).

In vitro whole-cell recordings

Rat pups at postnatal days 12–18 were anesthetized with 1.2 g/kg urethane and then transcardiac-perfused in dissecting solution containing (in mM): 87 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 24 $NaHCO_3$, 25 glucose and 75 sucrose (oxygenated with 95% O_2/5% CO_2). Brains were sliced 400 μm thick in an angled parasagittal orientation on a vibratome VT 1200S (Leica, IL, USA) and incubated for 1 hour prior at room temperature in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 25 $NaHCO_3$, and 10 glucose at pH 7.4, heated to 34 °C. VP neurons were visualized with an Olympus BX51WI upright microscope (Olympus Optical, NY, USA) equipped with a 40× water immersion lens with differential interference contract optics with infrared (DIC-IR). Whole-cell patch-clamp electrodes were pulled from borosilicate capillaries (World Precision Instruments, FL, USA) and filled with intracellular solution containing (in