ARTICLE IN PRESS

Brain Stimulation ■■ (2015) ■■–■■

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Asynchronous Distributed Multielectrode Microstimulation Reduces Seizures in the Dorsal Tetanus Toxin Model of Temporal Lobe Epilepsy

Sharanya Arcot Desai [a,b], John D. Rolston [c], Courtney E. McCracken [d], Steve M. Potter [a,b], Robert E. Gross [a,e,*]

[a] The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, USA
[b] Laboratory for Neuroengineering, Georgia Institute of Technology, Atlanta, USA
[c] Department of Neurological Surgery, University of California, San Francisco, USA
[d] Department of Pediatrics, Emory University School of Medicine, Atlanta, USA
[e] Department of Neurosurgery, Emory University School of Medicine, Atlanta, USA

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 9 August 2015
Accepted 14 August 2015
Available online

Keywords:
Epilepsy
Microstimulation
Theta oscillations
Memory
Multielectrode array

ABSTRACT

Background: Electrical brain stimulation has shown promise for reducing seizures in drug-resistant epilepsy, but the electrical stimulation parameter space remains largely unexplored. New stimulation parameters, electrode types, and stimulation targets may be more effective in controlling seizures compared to currently available options.
Hypothesis: We hypothesized that a novel electrical stimulation approach involving distributed multielectrode microstimulation at the epileptic focus would reduce seizure frequency in the tetanus toxin model of temporal lobe epilepsy.
Methods: We explored a distributed multielectrode microstimulation (DMM) approach in which electrical stimulation was delivered through 15 33-μm-diameter electrodes implanted at the epileptic focus (dorsal hippocampus) in the rat tetanus toxin model of temporal lobe epilepsy.
Results: We show that hippocampal theta (6–12 Hz brain oscillations) is decreased in this animal model during awake behaving conditions compared to control animals ($p < 10^{-4}$). DMM with biphasic, theta-range (6–12 Hz/electrode) pulses delivered asynchronously on the 15 microelectrodes was effective in reducing seizures by 46% ($p < 0.05$). When theta pulses or sinusoidal stimulation was delivered synchronously and continuously on the 15 microelectrodes, or through a single macroelectrode, no effects on seizure frequency were observed. High frequency stimulation (>16.66 Hz/per electrode), in contrast, had a tendency to increase seizure frequency.
Conclusions: These results indicate that DMM could be a new effective approach to therapeutic brain stimulation for reducing seizures in epilepsy.

Introduction

Among the different epilepsy syndromes, mesial temporal lobe epilepsy (MTLE) is the most drug resistant [1]. Electrical stimulation has shown promising but limited results for controlling seizures in cases where drugs have proven ineffective [2–4]. However, the electrical stimulation parameter space, including different spatio-temporal stimulation patterns, remains largely unexplored.

Microelectrode arrays (MEA) have been used extensively for single/multi-unit recording and stimulation in the field of brain machine interfaces [5–7]. With microelectrode arrays, several spatio-temporal patterns of stimulation can be delivered, which are not possible with the traditional deep brain stimulation macroelectrodes [8,9]. While this technique has not been tested for controlling seizures in epilepsy, multielectrode arrays have provided several insights into the generation and propagation of seizures. For example, Stead et al. [10] have used high density microelectrodes to record microseizures that occur more frequently at the epileptic focus and are not picked up on macroelectrodes or even on adjacent microelectrodes spaced less than 1 mm away. These microseizures will occasionally evolve into large-scale clinical seizures. Stimulation through MEAs may have the advantage of interacting with the epileptic network at such micro scales, preventing microseizures from maturing into disabling clinical seizures.

* Corresponding author. Tel.: +404 727 2354; fax: +404 712 8576.
E-mail address: rgross@emory.edu (R.E. Gross).

1935-861X/© 2015 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.brs.2015.08.008

In support of this hypothesis, it was shown by Wagenaar et al. [9] in cultures of cortical neurons that distributed microstimulation through 25 microelectrodes on 64-electrode MEAs is capable of completely eliminating spontaneous culture-wide, seizure-like bursting events. In contrast, stimulation through a single microelectrode, even at high frequencies (~50 Hz) analogous to contemporary deep brain stimulation, failed to stop the bursting events [9]. Single unit recording revealed that the distributed microstimulation approach, in which stimulation pulses were delivered asynchronously on the 25 microelectrodes, increased tonic background firing rate of the culture, which prevented the bursts from occurring. Adjusting the stimulation rate in a closed-loop fashion based on ongoing culture-wide firing rate achieved better burst control at lower stimulation frequencies [9]. The effectiveness of the distributed microstimulation approach in reducing spontaneous seizures *in vivo* has not heretofore been tested.

Another aspect of electrical stimulation that is crucial for determining therapeutic success is stimulation parameter selection, including stimulation frequency, waveform, amplitude and pulse-width. In clinical deep brain stimulation parameter selection is often done empirically, based on trial and error [11]. While this empirical technique has produced reasonably good disease and symptom control for Parkinson's disease and other disorders, an approach based on hypothesis testing has yielded improved control of symptoms [12]. Stimulation parameter selection based on an understanding of the pathophysiology of the disease state and the mechanism of action of brain stimulation may be crucial for achieving complete disease control with minimal side effects.

For applications in epilepsy, one such parameter space that deserves attention is the theta frequency range. Hippocampal theta oscillations [13] have been associated with decreased seizures in several animal models of epilepsy. For example, in the pilocarpine model of epilepsy hippocampal theta is reduced in amplitude and power and is shifted toward higher frequencies [14]. When hippocampal theta was induced, either through injection of the muscarinic agonist carbachol into the medial septum or through tail pinch, the number of epileptic spikes was drastically reduced. In another study [15] it was shown that 4–8 Hz electrical stimulation or injection of carbachol at the medial septum stopped pentylenetetrazol-induced facial-forelimb seizures within 5 s and stopped ictal activity during electrically induced status epilepticus within 10 s. Yet a few other recent studies in the pilocarpine and ventral tetanus toxin models of epilepsy in rats have shown that hippocampal theta activity precedes seizures perhaps suggesting that hippocampal theta may represent a pro-seizure state. For instance, the 2014 paper on the rat pilocarpine model of epilepsy showed that much of the increased preictal neuronal activity correlated with preictal theta activity in the CA1 and subiculum hippocampal theta preceded seizures in the CA1 and subiculum, whereas preictal firing of neurons in the dentate gyrus was independent of theta [16]. Another 2014 paper showed that in the ventral tetanus toxin model of epilepsy, hippocampal theta preceded seizure onsets and more seizures were observed during REM sleep, a condition where theta is prevalent in rats [17]. These seemingly conflicting relationships between hippocampal theta and seizures make this a particularly interesting frequency parameter space to further explore.

In this report, we explore the effects of multimicroelectrode theta stimulation in the dorsal intrahippocampal tetanus toxin model of epilepsy, a non-lesional model of mesial temporal lobe epilepsy exhibiting spontaneous seizures [18]. Additionally, the model produces interictal spikes and high frequency oscillations similar to those seen in human epilepsy [19,20]. Given the high number of spontaneous seizures (about 30 per day), low mortality rate and focal onset of seizures, this is an excellent model for studying the effects of electrical stimulation on focal spontaneous Racine scale 5 seizures [21].

Materials and methods

All animal procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Emory University Institutional Animal Care and Use Committee. In all, 30 male Sprague–Dawley rats (300–350 g at the time of surgery) were used in these studies. Out of these, 25 rats received distributed stimulation through microelectrode arrays and 5 rats received single point stimulation through macroelectrodes. Fig. 1 and Table 1 provide an outline of experimental design with allocation details of the 30 rats in the different stimulation protocols tested in this study. The below paragraphs describe the microelectrode array and macroelectrode implantation surgeries in detail.

(A) Tetanus toxin/saline injection and microelectrode array implantation (n = 25): Twenty-five rats were anesthetized with 1.5–3% inhaled isofluorane before receiving a craniectomy over the right dorsal hippocampus. Five smaller craniectomies, including one over the cerebellum, were made for skull screws (Plastics One, Roanoke, VA). In 17 rats, 25 ng of tetanus toxin in 0.5 µl of sterile PBS was injected into the right dorsal hippocampus at co-ordinates 3.3 mm AP (antero-posterior), 3.2 mm ML (medio-lateral), 3.1 mm DV (dorso-ventral) with respect to bregma. In 8 rats (controls), 0.5 µl of sterile PBS was injected at the same coordinates. A freshly pulled glass pipette was used to deliver the micro-injections with the Nanoject microinjection device (Drummond, Broomall, PA). Five minutes after the pipette was lowered into the brain, seven injections of 69 nl tetanus toxin or saline solution were made spaced 30 s apart.

These 25 rats were implanted with a sonicoplated MEA (Tucker-Davis Technologies, Alachua, FL) [22] in the right dorsal hippocampus, ipsilateral to the injection site. Sonicoplating (DC electroplating with platinum black under ultrasonic vibration) was done to reduce impedance of MEAs by an order of magnitude [22]. MEAs consisted of 2 rows of 8 electrodes (33 µm diameter) each, with electrodes in the outer row measuring 4 mm in length and electrodes on the inner row measuring 3 mm in length. Distance between the two rows was 1 mm and electrodes within the same row were separated by 175 µm.

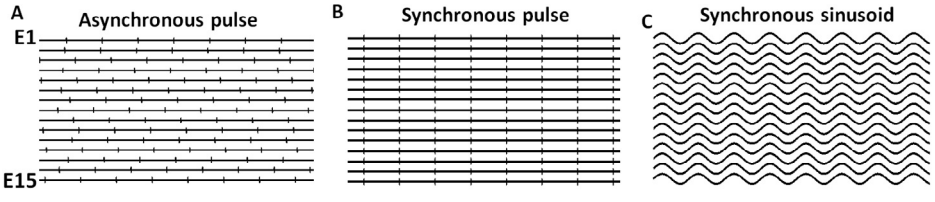

Figure 1. Outline of the experimental design. The different stimulation protocols tested and the number of rats and number of sessions in each of the stimulation protocols is showed in the flowchart.