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PRINCIPAL INVESTIGATOR: Alexander Rotenberg

CONTRACTING ORGANIZATION: Children's Hospital Corporation
Boston, MA 02115

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Alexander Rotenberg

E-Mail: alexander.rotenberg@childrens.harvard.edu

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CHILDREN'S HOSPITAL CORPORATION,
THE OFFICE OF SPONSORED PROGRAMS
300 LONGWOOD AVE
BOSTON MA 02115-5724 CODE11.

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14. ABSTRACT

We are employing the rat lateral fluid percussion injury (LFPI) model of severe traumatic brain injury (TBI) to test the antiepileptogenic and neuroprotective capacity of cathodal transcranial direct current stimulation (tDCS). tDCS is a well-tolerated and portable technique for focal suppression of cortical activity. We hypothesize that cathodal tDCS will mitigate excitotoxic neuronal injury acutely after TBI to by reducing neuronal firing, interfering with excitatory synaptic transmission and limiting neuronal exposure to excess glutamate. We hypothesize further that these effects of tDCS will reduce the risk of epileptogenesis after TBI. We aim to test whether acute tDCS in the LFPI model of TBI (1) reduces excess glutamate release, (2) reduces the extent of apoptotic cell death, and (3) improves the neurologic outcome as measured by severity of post-traumatic epilepsy. We will test such neurprotective and antiepileptogenic tDCS potential with stimulation delivered at specified timings after TBI. tDCS is already in limited human clinical use and there are established safety guidelines. Therefore, if our hypotheses are confirmed, this work could be promptly translated to human trials and lead to the development of a practical rapidly-deployed intervention for prevention of TBI-induced epilepsy.

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TABLE OF CONTENTS

1.	**INTRODUCTION**	**3**
2.	**BODY (EXPERIMENTAL SUMMARY AND RESULTS)**	**3**
3.	**KEY RESEARCH ACOMPLISHMENTS (SUMMARY)**	**6**
4.	**REPORTABLE OUTCOMES**	**6**
5.	**CONCLUSIONS**	**6**

1. INTRODUCTION

Cerebral injury can lead to epileptogenesis, the process by which the brain is transformed into an enduring pathological state (epilepsy) characterized by repeated unprovoked seizures. Notably, despite the availability of >30 antiepileptic drugs, there is presently no anti-epiletogenic agent that can prevent the onset of epilepsy after brain injury.

Severe traumatic brain injury (TBI) is among the most commons epileptogenic cerebral insults. In humans, TBI leads to epilepsy in 20-50% of instances and is the most common cause of acquired epilepsy in adults. Notably, epilepsy does not start immediately after TBI or other forms of brain injury. Rather, spontaneous seizures follow a post-injury latent period of weeks to months during which epileptogenesis takes place. The development of post-traumatic epilepsy after a latent period has been an intriguing observation and challenge for more than half a century. The time window between insult and the clinical manifestation of post-traumatic epilepsy offers a unique opportunity to implement antiepileptic therapeutic interventions that may be able to prevent the development of the disease. However, such approaches to date have shown limited success.

Excitotoxicity following a primary insult is a widely-recognized mechanism by which TBI leads to cell death, neuronal injury, and, ultimately, to epileptogenesis. Human and animal data indicate that TBI results in an immediate increase in glutamate in the injured area, and thereby to excessive excitation of neurons. In the long-term, TBI induced -related hyperexcitation of surviving neurons contributes to lasting neurologic symptoms such as post-TBI seizures which affect up to 50% of patients with severe military head wounds.

We **hypothesize** that focal electrical suppression of excessive neuronal activation (and thereby excitotoxic cell injury) following TBI will mitigate posttraumatic epilepsy. Current methods in trials aimed to modify TBI-triggered excitotoxicity include hypothermia (Faridar, et al. 2011) and pharmacologic glutamate receptor antagonism. However, precise and focal cerebral hypothermia is difficult to achieve, especially outside of the hospital settings. Similarly, the efficacy of glutamate receptor antagonists also has not been proven, and these agents affect the brain globally rather than focally, and are potentially associated with toxicity. Accordingly, there remains **an unmet need** for a technique to rapidly and focally suppress excessive neuronal firing in order to reduce regional excitotoxicity after TBI. Particularly, there is a need for a method that is easy to apply acutely after TBI, even in special circumstances such as those of the battle field, during a therapeutic time window when rapid suppression neuronal activity may reduce TBI-induced excitotoxic damage. We therefore propose to test the neuroprotective and antiepileptogenic potential of one such method: transcranial direct current stimulation (tDCS), a noninvasive technique for reduction of neuronal excitability by low amplitude direct electrical currents. Our **rationale** for implementing cathodal tDCS in the proposed experiments is the technique's excellent safety profile and decades-old demonstrated capacity for rapid and reversible suppression of spontaneous and evoked cortical activity.

To test whether cathodal tDCS, delivered immediately or after a short delay following TBI, can suppress post-TBI excitotoxic neuronal injury and prevent posttraumatic epilepsy, we propose to apply it in the rat lateral fluid percussion injury (LFPI) posttraumatic epilepsy model. The LFPI model has the advantage of well-described pathologic changes and predictable posttraumatic epilepsy which develops 4-6 weeks after cortical injury, and will enable the use of published data toward hypothesis development and experimental design. Our overall goal is to test whether the severity of TBI-induced neurologic injury and likelihood of posttraumatic epilepsy can be reduced by cathodal tDCS applied as a neuroprotective measure acutely after injury.

2. BODY (Experimental summary and Results)

2.1 Rapid lateral fluid percussion TBI model. To meet our objectives, at the start of the budget period, we previously modified the established LFPI posttraumatic epilepsy model to allow high throughput generation of animals with posttraumatic seizures and other sequelae. Briefly, the conventional LFPI model is a 2-step procedure, requiring one surgery for craniectomy and secure of a cannula for fluid percussion, and a second surgery for the epidural fluid percussion itself. As an improvement on the classic LFPI procedure, we designed and validated a new protocol, termed rapid fluid percussion (rLFPI), where the procedure is completed in <2 hours, thus enabling high throughput experiments, and also limiting the exposure of each animal to anesthetics and analgesics which can confound the interpretation of experimental results (Hameed et al., Neuroreport 2014).

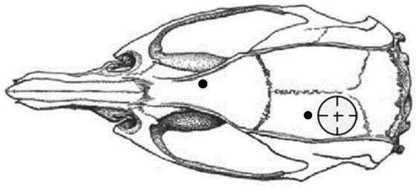

Figure 1. Location of injury site and skull screw electrodes. A 4 mm craniectomy is represented by the circle with crosshair. Solid black circles indicate the EEG recording electrode positions over the right olfactory bulb (reference electrode) and over the anterior perilesional cortex.

2.2 Experimental overview. We used adult male Long-Evans rats for all experiments. Briefly, each rat underwent craniectomy followed by rLFPI over the left parietal cortex (Figure 1). Following injury, rats were then treated with either verum cathodal tDCS (100 μA for 30 minutes) or sham tDCS (100 μA for 5 seconds). Rats were then humanely sacrificed and brains were harvested at intervals after injury. We hen evaluated, by real-time polymerase chain reaction (RT-PCR) expression of c-fos and related immediate early genes that mark

excitotoxic neuronal injury. For experimental purposes, ipsi-lesional and contra-lesional neocortical tissue was analyzed separately. With these experiments, contrary to our expectations, we found no support for our hypothesis that cathodal tDCS suppresses acute hyperixcitation or injury markers in the cortex which we previously identified in the rLFPI rat brain injury model (Wang et al., 2014).

2.3 Preliminary results [SOW Aim 1]: For experimental purposes, ipsi-lesional and contra-lesional neocortical tissue was analyzed separately. With these experiments, contrary to our expectations, we found no support for our hypothesis that cathodal tDCS suppresses acute hyperixcitation or injury markers in the cortex (Fig 2).

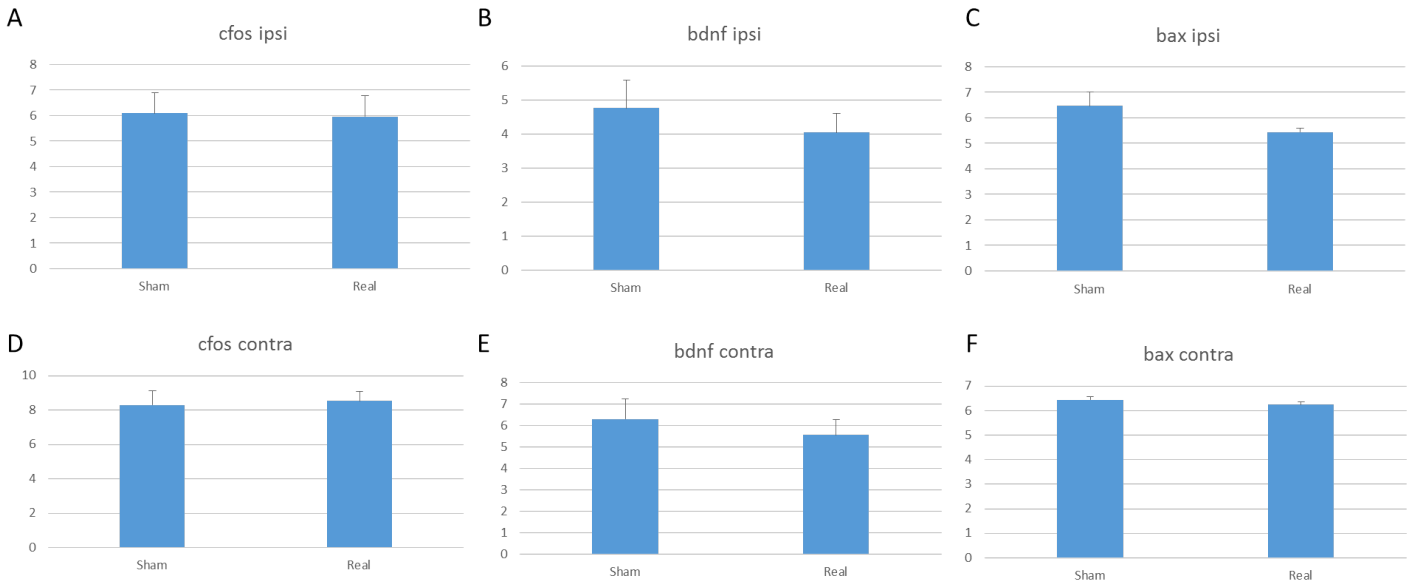

Figure 2. IEG expression 4 hours after TBI. (A) C-fos, BDNF and BAX expression in the ipsi-lesional and contra-lesional cortex homogenates rats treated with verum (Real) or sham cathodal tDCS. Y-values reflect fold-change in gene expression relative to uninjured control. No significant difference between sham and verum tDCS groups were identified for any of the three gene markers. (n=6 per group)

Following the findings in these initial experiments, we reasoned that perhaps we are not testing for correct injury markers. We therefore repeated the studies, but rather than measuring immediate early gene markers, we measured markers of oxidative stress as reflected in the ratio of glutathione to oxidized glutathione (a reliable rLFPI injury marker that that we recently identified). Here, as with immediate early gene's, we found reproducible elevation of the biochemical injury marker, but no significant effect of cathodal tDCS, either in the acute or subacute (as in SOW Aim 2) period (Fig 32).

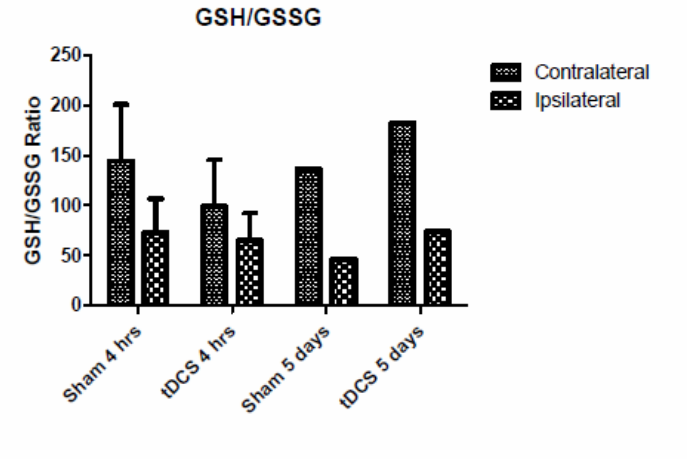

Figure 3. GSH/GSSH ratio after TBI and tDCS treatment. Following absence of data indicating protection from neurotoxicity with IEG markers, we expanded analysis to include measures of regional oxidative stress, a downstream consequence of glutamate-mediated excitotoxicity. Tissue was harvested after TBI and verum or sham tDCS as above, and analyzed by high performace liquird chromatography (with funds separate from those used for our award). Here, we identified depressed GSH/GSSH ratio after injury in the ipsi-lesional cortex, but no improvement in this measure fillowing tDCS (n = 6/group at 48 hrs post-TBI, and 2/group at 5 days post-TBI). As above, this underscored the absecnce of therapeutic efficacy with low-amplitude, 100 µA, tDCS.

2.4 Investigation of Pitfall in Aim 1: Given the absence of therapeutic effect in Aim 1, we conducted a series of experiments (with funds separate from the present award) to test whether cathodal tDCS suppression of cortical excitability was uniform throughout the cortex. Our rational was prior observation in our laboratory that cortical cell elements which are oriented in different directions, have orientation-0specific response to tDCS. Thus, we hypothesized that while some portions of the cortex may experience depression and reduction of posttraumatic excitability by tDCS, other excitation in other parts of the cortex may be facilitate, rather than depressed. To test the hypothesis, we reduce the tDCS preparation to the isolated neocortical slice setup where direct current could be conducted across the cortical thickness via Ag/AgCl electrodes. With this preparation, we identified that indeed some portions of the slice experienced suppression of excitability, while other portions were potentiated.

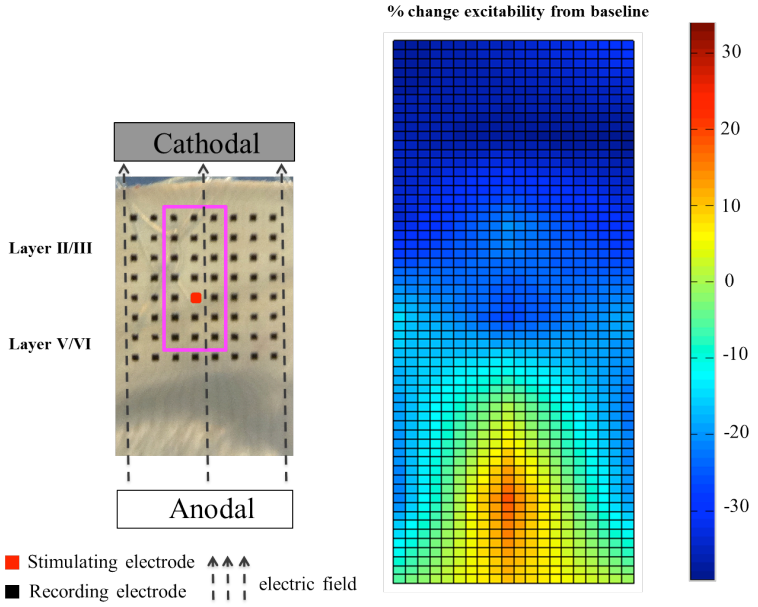

Figure 4. Variable degree of long-term depression (LTD) of cortical excitability as a function of cortical layer. Beyond the scope of the parent proposal, and with separate funds, we conducted experiments in isolated mouse neocortex. Mice we chosen for this portion of the experiment to conserve laboratory funds. Briefly, P15-20 mice were sacrificed and brains were extracted for in vitro slice preparation, which mimics acute brain injury in vivo. 400µM slices from the sensorimotor cortex were plated on an 8X8 micoelectrode array (left) and bathed in oxygenated artificial cerebrospinal fluid. Cortical excitability was measured as the magnitude change in the slope of the field excitatory postsynaptic potential generated by stimulation of Layer V cortical electrodes (left). With this protocol, we identified that while cortical excitability was depressed in most of the cortex (blue, right), cortical excitability was enhanced in appreciable portions of the deep layers (yellow/red, right). (N=9 slices) These data raise the possibility of incomplete suppression of cortical excitability by low-amplitude tDCS.

2.5 Resolution of Pitfall in Aim 1: One logical explanation for the incomplete depotentiation of excitability throughout the cortex after injury is insufficient tDCS dose, and we had anticipated this possibility in the parent proposal, and were prepared to test a range of stimulation intensities. Accordingly, after completing the isolated brain slice recordings, we returned to the original experiments and collected cortical tissue from another cohort of rats treated with verum or sham tDCS (2 groups, N=5 per group). Analysis of this tissue is pending at the time of this writing. In parallel, we are preparing animals for rLFPI, followed by tDCS and then EEG recordings as in SOW Aim 3. In anticipation

2.6 Conclusion: 100 µA cathode tDCS does not ameliorate TBI-related upregulation of acute neuronal injury markers. However, we recognize that if a treatment is ineffective, then dose may be an issue. Thus, after a pause to research the mechanisms that may explain the asence of a therapeutic effect of low-amplitude tDCS, we are resuming the experiments, now with increased strength of direct current.

3. KEY RESEARCH ACOMPLISHMENTS (SUMMARY)

1. We validated the traditional lateral fluid percussion rodent posttraumatic epilepsy protocol to improve efficiency and reduce animal anesthesia exposure (Hameed et al., Neuroreport, 2014; Wang et al., Neuroreport 2014)
2. We developed a novel method for measutring the effects of tDCS on cortical excitability in isolated neocortical slices, in vitro.

4. REPORTABLE OUTCOMES

Please see sections 2.1 - 2.5, above.

5. CONCLUSIONS

We conclude that 100 μA cathodal tDCS is unlikely to protect injured cortex from excitotoxic brain injury. However, we recognize that with any treatment, absence of efficacy may related to mechanism or to dose. We therefore are repeating the experiments with a higher-amplitude tDCS current, as we anticipated in the Pitfalls sections of our proposal. While we introduced a delay into the experimental sequence, the additional studies that we conducted were funded by mechanisms that were separate from this award, and we have been very economical with the funds. A formal request for a no-cost extension will follow.

6. REFERENCES

Hameed MQ, Goodrich GS, Dhamne SC, Hsieh TH, Wang YP, Rotenberg A. Rapid Lateral Fluid Percussion Injury (rLFPI) rodent model of traumatic brain injury and post-traumatic epilepsy. Neuroreport, 2014 Feb 17 PubMed PMID: 24553065

Sun Y, Lipton J, Sahin M, Rotenberg A. Mechanism of metabotropic glutamate receptor 5-dependent long-term depression induced by cathodal direct-current stimulation in mouse primary motor cortex. Society for Neuroscience Annual Meeting Abstr2014: 601.01. (Poster)

Wang Y, Hameed MQ, Rakhade SN, Iglesias AH, Muller PA, Mou DL, Rotenberg A. Hippocampal immediate early gene transcription in the rat fluid percussion traumatic brain injury model. Neuroreport. 2014 Aug 20;25(12):954-9