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CO-INVESTIGATOR(S) AND THEIR HOME DEPARTMENTS: N/A

PROJECT TITLE: Transcranial Direct Current Stimulation to Strengthen Corticospinal Connections and Promote Recovery After Injury

SYNOPSIS OF PROPOSAL: (use only space provided below; minimum 11 point font)

We have been able to strengthen connections between the brain and the spinal cord using invasive electrical stimulation of the corticospinal tract (CST) in a rat model of hemiplegia. Here we propose a preclinical study of electrical stimulation to restore motor function in our rat model using transcranial direct current stimulation (TDCS) of the motor cortex. TDCS increases neuronal excitability when a current is past with the anode placed over cortex. The technique is safe and non-invasive and can be administered during performance of behavioral tasks, both in rats and humans. We propose to compare three groups of rats with hemiplegic CST injury—TDCS, sham TDCS, and TDCS paired with behavior training—using two outcome measures—strength of CST connectivity and performance of skilled motor tasks. We hypothesize that TDCS will strengthen the sparse CST connections spared by injury and improve performance of skilled motor tasks. We anticipate that the effects of stimulation will be augmented by behavior training during stimulation. Thus, we hope to restore CST circuitry in our defined anatomical rat model using an intervention that can be easily translated to human trial.

CURRENT SOURCES OF RESEARCH FUNDING (include begin/end dates and total direct costs)

Title: Neuroscientist Academic Development Award K12 NS001698-09
Source of Funding: NIH-NINDS
Role on Project: Trainee
Dates of project: 7/1/04-6/30/05, 7/1/08-6/30/10
Annual direct costs: $125,000
Total direct costs: $375,000

Title: Harnessing corticospinal activity to promote motor function
Source of funding: New York State Spinal Cord Injury Board
Role on project: Co-PI (with Dr. John H. Martin)
Dates of entire project: 4/1/07 – 3/31/09
Annual direct costs: $110,693
Total direct costs: $221,387

PENDING APPLICATIONS FOR RESEARCH FUNDING (include proposed begin/end dates and total direct costs)
None.

12 MONTH BUDGET (July 1, 2009 to June 30, 2010)	
SALARIES with FRINGE:	
PI (portion salary + fringe)	**$**
Technician (%)	**$**
SUB-TOTAL	**$**
EQUIPMENT:	
None	
SUB-TOTAL	**$**
PATIENT CARE COSTS:	
None	
SUB-TOTAL	**$**
ALL OTHER EXPENSES:	
Supplies	**$**
Animal purchase	**$**
Animal care costs	**$**
SUB-TOTAL	**$**
TOTAL PROPOSED BUDGET	**$50,000**

DETAILED BUDGET JUSTIFICATION: (use only space provided; minimum 11 point)

Jason B. Carmel, MD, PhD. I will be working in the Laboratory of Dr. John Martin, in the Department of Neuroscience. We request funds to cover (%) of my salary plus benefits. Additional salary support is provided by a Neuroscientist Academic Development Award (NSADA), a K-12 training grant awarded to the Division of Child Neurology. I will be responsible for experimental design, in collaboration with Dr. Martin, and will conduct the surgeries, training, electrophysiology experiment, and necessary analyses. I will oversee the research technician who will be working on the project.

Research technician We request funds for a part-time research technician (%). The technician will assist during surgeries, train animals, prepare reagents, monitor the animals during stimulation, and conduct routine analyses on behavioral and morphological data. Dr. Carmel will train him or her.

Supplies. We request funds to purchase electrodes, laboratory reagents, neuroanatomical tracers, and other chemical supplies. We also request funds to purchase videotapes and computer supplies for the behavioral analyses.

Animal costs. We request funds to purchase Sprague-Dawley rats needed for our experiments ($). We estimate needing 30 rats for conducting the proposed experiments. We request funds to cover per diem care charges in the NYS Psychiatric Institute animal care facility ($; average time in facility is XX days; per diem rate of $0.xx).

SIGNATURES OF APPROVAL

A. I certify that the information presented in this proposal is, to the best of my knowledge, complete, accurate, and developed according to practices commonly accepted within the scientific community.

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Signature of Principal Investigator *Date*

B. I have reviewed this application and take responsibility for ensuring that the necessary space, personnel, and facilities which are mentioned in the application which pertain to my Department will be available for this project should it be funded. I recommend that this application be submitted.

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Signature of Department Chairman *Date*

REMINDERS

DO NOT INCLUDE LETTERS OF SUPPORT.

REVIEW APPLICATION WITH SENIOR FACULTY.

Study Protocol: Transcranial Direct Current Stimulation to Strengthen Corticospinal Connections and Promote Recovery After Injury

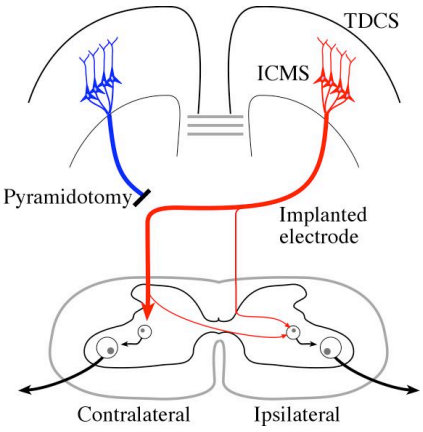
A) Goals

We have been able to restore connections between the cerebral cortex and the spinal cord in a rat model of hemiplegia using repetitive electrical stimulation of the corticospinal tract (CST)[1]. Stimulation augments neuronal activity, which leads to strengthening of synaptic connections by activity-dependent competition. Here we propose to promote CST connections after injury using a different stimulation technique, transcranial direct current stimulation (TDCS), which is routinely used in humans to modulate cortical neuronal activity. Employing our rat model of hemiplegia, we propose to determine if TDCS can strengthen CST connections and restore skilled motor function. We will also determine if pairing TDCS with behavior training further enhances CST connections. The ultimate goal of the work is to develop a preclinical model that can be used to plan human trials of TDCS to improve motor recovery in people with hemiplegia.

B) Rationale

The proposed experiments will be performed in Dr. John Martin's laboratory in the Department of Neuroscience. Previous work in Dr. Martin's laboratory demonstrates that activity is vital to the proper development of the CST, the direct connection between motor cortex and the spinal cord[2]. Likewise, in maturity physical therapy can be used to restore function to an impaired limb, in part, by increasing activity in the neural circuits used to control it. The purpose of electrical stimulation is to augment neural activity of CST motor neurons. Thus, we harness a basic mechanism for establishing synaptic connections in the CST as therapy to help rebuild it.

The CST constitutes the principal pathway for skilled voluntary movement in humans. Injury of the CST causes paralysis because motor control centers in the cerebral cortex are disconnected from spinal motor circuits. The CST is largely crossed, so injury to the tract emanating from one hemisphere causes paralysis on the opposite side of the body. However, a sparse ipsilateral CST projection from the uninjured side of the brain to the impaired side of the spinal cord remains after injury. (Figure 1, thin red lines). Our strategy is to strengthen the synaptic connections of this sparse ipsilateral projection using TDCS to help restore CST control of skilled movement.



← *Figure 1. Schematic of proposed experiments and preliminary results. After unilateral pyramidotomy the spinal cord ipsilateral to the intact hemisphere receives sparse CST input. In our previous study electrical stimulation was delivered to the intact CST by an implanted electrode. This caused greater CST connectivity to ipsilateral spinal motor circuits. We propose a similar effect using TDCS of the uninjured CST. Intracortical microstimulation (ICMS) will be used as a physiological outcome measure, as in our recent work (see below).*

In our published study, we used repetitive electrical stimulation of the CST in the rat to boost its activity and promote function[1]. In rats with injury to one pyramid, where all of the CST axons from one hemisphere are tightly bundled, we implanted an electrode over the intact pyramid, and used it to deliver trains of stimulation (Figure 1; stimulation parameters 333 Hz, 45msec, every 2 seconds for 6 hours a day over 10 days). This electrical stimulation caused the sparse ipsilateral CST axons that were spared after the lesion to form significantly more and stronger synaptic connections in the cervical spinal cord than those in animals with injury alone. In a preliminary behavioral experiment, electrical stimulation after injury caused significant improvement in a complex walking task that otherwise shows permanent impairments without intervention. Thus,

selective electrical stimulation of the CST can restore lost connections and improve motor function after unilateral injury in the rat.

Direct electrical stimulation of the CST, as effective as it is in strengthening spared connections, requires invasive surgery, and therefore would not be feasible as a therapy in people with stroke or spinal injury. Two tools have recently become available to deliver noninvasive electrical stimulation to the brain, and the CST in particular. Transcranial magnetic stimulation (TMS) uses current generated by changes in a magnetic field delivered by a powerful electromagnet held over the area of cortex to be stimulated. TMS could more closely mimic the phasic depolarizing stimulation we performed using implantable electrodes. However it cannot be scaled for selective CST stimulation in rats, and it has several practical limitations for use in humans. First, in order to mimic the kind of stimulation we used, repetitive TMS (rTMS) must be given. rTMS boosts the firing rate of the CST, but it also predisposes the subject to seizures, a risk that is amplified in people with previous brain injury, who would be candidates for such therapy. Second, rTMS has several practical impediments to use, particularly in children, including the need for head immobilization and scalp irritation underneath the magnet.

Instead, we intend to use the second noninvasive stimulation technique, transcranial direct current stimulation (TDCS). TDCS can be applied with almost no risk of seizures, no discomfort, and no limitation on movement, allowing the stimulation to be paired with behavioral training. And TDCS can be applied to rats. Several studies have used TDCS to alter rodent cortical excitability in freely-moving rats[3,4]. Although likely beneficial on its own, TDCS paired with behavior training may selectively strengthen those circuits necessary to perform a motor task. TDCS also improves motor learning, which may enhance relearning of skills after injury[5]. Human studies have successfully combined TDCS with occupational therapy[6] and robot-assisted arm training[7] to promote recovery after stroke.

TDCS uses a shift in resting potential to alter excitability. When a low amplitude current is passed with the anode over the cortex, it causes a slight depolarization of the cortical neurons, but without generating an action potential. The neurons, however, increase their firing rate. If the cathode is placed over the motor cortex the effect is opposite—the motor cortex is hyperpolarized, and activity is diminished. The mechanism of TDCS-induced excitability or depression may depend on long-term potentiation or depression of cortical synapses[8].

The use of TDCS after stroke has largely targeted rebalancing of the two hemispheres to promote motor function. When one hemisphere is injured, not only does this hemisphere lose corticospinal control of the spinal cord because the motor cortical areas are damaged, but the damage affects excitability in the other hemisphere as well. Normally, the motor cortex in one hemisphere inhibits the motor cortex in the other through their connections across the corpus callosum. After stroke, the uninjured hemisphere becomes more active because it has lost constitutive inhibitory drive from the injured hemisphere. Conversely, the injured hemisphere may be further weakened by the inhibitory drive of the unaffected hemisphere, now unchecked because of the loss of inhibition from the damaged side. The human studies that use TDCS to improve motor function after stroke have used either inhibitory (cathodal) TDCS over the unaffected hemisphere[6,9] or excitatory (anodal) TDCS over the injured hemisphere[7,10].

We propose a novel function for tDCS, but one firmly grounded in the biophysics of its actions. Instead of balancing the CST output from each hemisphere, we intend to strengthen the output selectively from the unaffected hemisphere to the ipsilateral, and impaired side of the spinal cord, similar to our invasive stimulation study (Figure 1). The ipsilateral component comprises approximately 10% of the CST descending axons in primates. However, the functional importance of the ipsilateral CST may outstrip the proportion of descending axons. Ipsilateral corticospinal innervation may be denser than previously thought as many collateral branches from the crossed projection end up recrossing in the cervical spinal [11]. After stroke, the hand ipsilateral to injury shows significant and lasting impairments[12]. Perhaps most importantly, both children and adults have used one functional hemisphere to recover

individuated finger movements in their impaired hand[13,14]. Thus, the ipsilateral CST is capable of supporting fine motor control.

However, this evidence begs the question why the majority of people with severe unilateral brain injury do not recover significant motor function. We have developed a hypothesis, based on our animal studies, why this might be the case. Using intracortical microstimulation in the rat, a refined method for mapping the cortical motor representation, we addressed the function of the ipsilateral CST and its dependence on a functional CST emanating from the other hemisphere (Figure 1, ICMS)[15]. Stimulation of the motor cortex in naïve rats elicits contralateral movements at low stimulus current and both contralateral and ipsilateral movements at higher stimulus intensity. After unilateral injury, however, even high intensity stimulation of the intact hemisphere fails to elicit ipsilateral movements. This indicates that the sparse ipsilateral CST connections from the motor cortex on one side depend on the function of the denser CST connections from motor cortex on the other side. Importantly, we have been able to restore ipsilateral responses after injury in this model acutely by systemically raising neuronal excitability with the potassium channel blocker 4-aminopyridine. This suggests that the ipsilateral axons of the intact CST need greater excitatory drive, a drive usually provided by the CST from the other hemisphere. We propose that TDCS will strengthen the ipsilateral CST sufficiently to allow it to function independently. We expect to see this manifested with electrophysiological testing and with improvement in motor behavior.

C) Methods

Overview: The study will compare three treatment groups—TDCS, sham TDCS, and TDCS paired with behavior training—using two outcome measures—strength of CST connectivity and performance of skilled motor tasks. All animals will receive pre-training on skilled motor tasks until they meet performance criteria. All will receive electrode implantation over one motor cortex and cut injury of the other CST at the medullary pyramid. TDCS or sham TDCS will be delivered to the motor cortex of the intact CST for 2 hours a day over 10 days after injury. Rats in the TDCS and training group will receive training while the TDCS is active. Behavior testing will occur every 5 days, twice during the stimulation period and twice in a 10-day follow-up period. Although three tasks will be used to assess motor function, the tests will be short to limit the training effect of testing. Twenty days after injury, animals will have a terminal intracortical microstimulation mapping of the intact motor cortex. Our hypothesis is that rats receiving 10 days of TDCS will demonstrate stronger spinal connections and improved performance on skilled motor tasks compared to sham TDCS animals. We predict that behavior training during the TDCS will further strengthen these effects versus TDCS alone.

General Surgical methods: Anesthesia is induced with ketamine (80 mg/kg intraperitoneal (IP)) and xylazine (10 mg/kg IP) and maintained using IP ketamine injections to render the animal unresponsive to paw pinch. Body temperature is maintained at 39° C by a heating pad. Post-surgical pain control is maintained with buprenorphine 0.05 mg/kg IP every 12 hours for 48 hours.

Electrode implantation and stimulation: Although TDCS is performed through scalp electrodes in humans, we will attach the electrode directly to the skull of the rats to ensure consistent placement and reliable stimulation. Following published studies, we will use an electrode that is fitted inside a plastic jacket, and the jacket filled with saline[3,4]. This electrode will be attached over the forelimb representation of the motor cortex at bregma +2mm and lateral 2.5mm. An electrode is then placed over the thorax to serve as a counter electrode. Stimulation will be delivered for 2 hours a day over ten days at 0.2 mamp intensity using a constant current stimulator (A-M Systems).

CST injury: Animals will have one pyramid cut, injuring all of the CST axons emanating from one hemisphere. Through a ventral approach, a small craniotomy is made in the occipital bone and a microknife used to cut the medullary pyramid as in our previous studies[1,15].

Intracortical microstimulation: Twenty days after injury, animals will be subjected to a terminal electrophysiology experiment. Animals are placed in a stereotaxic frame and a craniotomy made over the forelimb area of M1 of the intact hemisphere. Electrode

penetrations, perpendicular to the pial surface and approximately 0.5 mm apart, are made into M1, from 1.5mm to 4.5mm lateral to bregma and –0.5mm to 5.5mm rostral to bregma. We use low impedance tungsten microelectrodes (Microprobe, Inc.; 0.5 MOhm impedance). Stimuli (45 ms duration train, 330 Hz, 0.2 ms biphasic; every 2 sec) are delivered using a constant current stimulator (A-M Systems). Motor effects from both contralateral and ipsilateral forelimbs and the threshold current needed to provoke them are recorded. The threshold is defined as the lowest current that consistently produced a motor effect (>50% of trials).

Skilled Motor Tasks: All animals will have pre-training on 4 skilled motor tasks. Three motor tasks are used for testing of skilled motor function before and every 5 days after injury, using videotape analysis of the movements. One task, pellet reaching, will be used in the TDCS and behavior-training group as the training component. Two reaching tasks will be used. Both employ a plexiglass box with a slit that allows only one forelimb access to a platform with food. In a *pellet-reaching task* rats must grasp a round pellet while withdrawing the forelimb through the aperture in the box. Although this task will be used by the TDCS and behavior-training group as the training component, all animals will be pre-trained on it to ensure equal treatment across groups. We have also developed a novel *pasta-reaching task* which presents a piece of dry pasta oriented either vertically or horizontally. In addition to testing for success rate the pasta-reaching task allows measurement of the paw angle just before it contacts the pasta. Matching the paw orientation to the orientation of the pasta, known as preshaping, likely relies heavily on the CST. A *horizontal ladder-walking task* tests visually guided locomotion, which is sensitive to CST injury and recovery. Rats are introduced to one end of a 60cm horizontal ladder with plexiglass side barriers and irregularly spaced rungs. Proper paw placement on the rungs while the rat traverses the ladder is scored using defined criteria. Finally, a *pasta manipulation task* tests for grasp and digit movements while eating a 7cm piece of dry pasta. Such fine motor manipulations are seen when rats eat seeds, insects, and other natural food sources, and they depend on CST function. After CST injury, the number of manipulations using the forepaw contralateral to injury drops; we expect that this number will increase with TDCS and to a greater degree with TDCS and behavior training together.

Statistical analysis: We will use 5 rats for each of the three groups. Although the expected effect size and group size has not been subject to formal power analysis, we have shown significant effects in our previous study of invasive electrical stimulation using this number[1]. We will compare electrophysiological data using standard analysis of variation (ANOVA). Behavioral data will be compared across the three groups and the various times after injury using repeated measures ANOVA.

Conclusions: The effect of the best current treatment for hemiplegia, physical therapy, is modest and relies on patients who can actively participate. Such therapy is difficult to apply to young children, who may most benefit from activity-based treatments, and to people with near complete paralysis. Using TDCS on our anatomical models will allow us to test its effects on the ipsilateral CST and the strength of it connections. Knowing how TDCS promotes recovery in our model system will be critical for its safe and effective use in humans.

D) Future Plans

I plan to use the pilot data generated by the proposed study to prepare a translational study using TDCS to improve motor function in children with severe unilateral injury. To this end, I will apply for a K99/R00 grant no later than July 12, 2010. Initially, I plan to study TDCS in the course run by Sarah Lisanby, MD, Professor of Clinical Psychiatry and Chief, Brain Stimulation and Therapeutic Modulation Division, Columbia University and New York State Psychiatric Institute. I then plan to spend the mentored portion of the award with someone who specializes in brain stimulation for motor recovery—Dr. Leonardo Cohen at the NIH, for example. I would then conduct the human trials within the Division of Pediatric Neurology at Columbia, possibly in collaboration with the Department of Rehabilitation Medicine. Patient recruitment would be through the clinical service, the NICU follow-up clinic (headed by Jane Lee, MD), and the spasticity clinic (headed by Drs. Richard Anderson and Claudia Chiriboga).

Eventually, I hope to both run an animal-based laboratory investigating the basic mechanisms of motor recovery and direct a multi-disciplinary clinic specializing in children with motor dysfunction due to brain and spinal cord injuries. The clinic would also serve as the base for future clinical trials.

E) References

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