**INTRODUCTION**

I thank the reviewers of my first application for their extremely thoughtful, helpful comments to my proposal. I have given great thought to each comment from each reviewer and have incorporated all suggested changes into my revised proposal. I have provided detailed responses to the reviewers' comments below. Reviewers' comments addressed 1) career development, 2) the research plan and 3) methodological issues.

Since the initial submission, I published a paper in the *Journal of Neuroscience* demonstrating that in a ct model of hemiplegia, rebalancing activity in motor cortex after unilateral activity blockade led to anatomical recovery of corticospinal connections and motor skill recovery. This finding lends even greater support to the hypotheses in this proposal that an imbalance in motor cortex activity of the two sides produces hemiparetic symptoms, and rebalancing motor cortex activity promotes recovery. I also engaged in several training opportunities to prepare me for the experiments planned in Aim 2 of this proposal. I worked as an interventionist in Dr. Gordon's intensive training day camp with children with hemiplegia, gaining experience in conducting the intervention, working with children with hemiplegia and performing motor skill assessments. I also began training in transcranial magnetic stimulation (TMS) methods, under the direction of Dr. Lisanby. I took a CME course in TMS, which covered topics of methodology, applicability, safety and efficacy. I also became a certified in TMS motor threshold determination by the Division of Brain Stimulation and Therapeutic Modulation (BSTM), by passing a training program. These training activities have given me experience in the metholodologies to be used in my proposed studies.

**GENERAL RECOMMENDATIONS**

**1) CAREER DEVELOPMENT**

**A. Expanded training plan in neurology, rehabilitation and working with children:** All reviewers indicated a need for greater training in clinical neurology and rehabilitation.

**1.** Increased didactic training: In addition to the coursework previously proposed, I will take courses at Columbia University in **child neurology and rehabilitation**. I will take **Management of Pediatric Conditions I and II** from the Department of Physical Therapy at Columbia University. I will also attend a CME conference, the **Hershey Conference on Developmental Brain Injury**, held biannually, which covers prenatal neurodevelopment; injury, neurogenesis and repair; hypoxia-ischemia, inflammation and neuroprotection; neuroimaging; and seizure disorders. I will also participate in a **TMS Mini-Fellowship during Year 1**, offered by Dr. Alvaro Pascual-Leone's laboratory at Harvard Medical School. The mini-fellowship provides hands-on learning of basic principles, use and clinical applications of TMS. The fellowship provides training in determining motor thresholds, recording motor evoked responses, cortical mapping and paired-pulse TMS. The fellowship cover TMS safety, writing TMS protocols and TMS study design.

**2.** Increased practical training: I have planned many training opportunities for working with human subjects, **particularly children and persons with neurological disorders**. To this end, I have secured the support of several new Research Consultants who will guide my training in clinical aspects of my proposed project—Leonardo Cohen, Ph.D., a TMS pioneer at the National Institutes of Health; Eugene Tunik, Ph.D., P.T., New York University, who studies the motor system of normal subjects and is planning studies in stroke patients; Claudia Chiriboga, M.D., a pediatric neurologist in the Department of Neurology at Columbia University Medical Center (CUMC) and Jason Carmel, M.D., Ph.D., a pediatric neurologist at CUMC. Training is explained in greater detail on pp. xx. **1)** I will visit the laboratory of Dr. Eyre annually to observe TMS studies in children with CP; additionally, Dr. Eyre will come to my laboratory during my first bout of data collection to assist and advise. **2)** I am volunteering in Dr. Gordon's laboratory as an interventionist in his intensive training day camp, gaining experience working with children with hemiplegia, conducting intensive training studies and performing motor skill assessments. **3)** I will observe TMS experiments on patients with stroke in the laboratory of Dr. Cohen. **4)** I will observe TMS experiments on normal subjects and stroke patients in the laboratory of Dr. Tunik. **5)** I will shadow a clinician in the Spasticity Clinic of CUMC, under the supervision of Dr. Chiriboga, to gain experience in working with children with CP. **6)** I will observe TMS studies in children with autism in the BSTM under the supervision of Dr. Alexandra Sporn. Although I recognize that CP and autism are very different conditions, there is much to be gained by working with children with autism. I will learn ways of negotiating potential confounds to my TMS study, including subject distractibility and challenges keeping the child still and calm during the procedure.

**3.** Additional training in clinical research design and interpretation**.** I will enroll in two courses offered in the Department of Psychiatry at Columbia University Medical Center. **Research Design and Methodology**

will cover principles of clinical research design. Since I am not yet experienced in clinical research, this course will provide excellent training in design and methodological issues from the perspective of clinical research. **I will take this course before the fellowship begins.** I will also take a two-semester course in clinical research-oriented **Statistics** during **Year 2** of my fellowship. This course will cover parametric and nonparametric statistics, regression analyses and population analyses. This course will train me in the analysis of clinical research data.

**B. Long-term career development:** Reviewers requested that I provide a more explicit plan to achieve academic independence, both in my personal statement and in my letters of support from my mentors and department chair. I have detailed my plans on pp. xx in a new section, Plans for Academic Independence. Upon funding of this proposal, or the obtainment of other funding, I will be given a tenure-track appointment in the BSTM Division at CUMC. [I am also a finalist candidate for a tenure-track position in the Department of Biobehavioral Sciences at Teacher's College; the outcome of the search will be known before this grant is due, and if I am given the position, I certainly will mention it here. If I get the TC job I believe I will get a dual appt. with the BSTM.] My letters of support from my mentors and department chair [will] include more explicit plans for my promotion and career development. I have also addressed future research directions on pp. xx in my plan for academic independence.

**2) RESEARCH PLAN**

**A. Heterogeneity of hemiplegic CP population:** There was a concern, particularly by Reviewer 3, that since CP has multiple causes, the heterogeneity of the population would introduce variability to the data. I agree with this concern, and have therefore chosen to limit my study to children with perinatal stroke that occurred at age < 1 yr. Diagnosis of perinatal stroke will be verified by medical records and reviews of CT or MRI scans from the age of diagnosis. Since activity-dependent competition in early life is believed to drive the emergence of CP symptoms, brain injury occurring at different ages (perinatal vs. older children) would likely very different effects on motor system development.

**B. Efficacy of intensive training of the affected arm in CP rehabilitation:** Several reviewers expressed concern about the efficacy of the proposed intensive training protocol on promoting motor still recovery in children with hemiplegia. My original application did not adequately emphasize the evidence that this therapy has been shown to be effective in improving motor function in children with hemiplegia. Dr. Gordon has performed this therapy in approximately **90 children** and has shown statistically powerful improvements in motor skill of the affected arm after the training. Most of these children received ten days of therapy; the current proposal will employ a fifteen day training period, which we predict to be even more successful than a ten day period. Preliminary data on four children in the high skill training group are presented in this proposal; even with an n of 4, a statistically significant recovery was found.

The reviewer cited the recent Cochrane review of intensive training in CP rehabilitation. Certainly, there is variability in methodology and extent of recovery among different laboratories. When studies are analyzed as a group, as in this review, methodological differences among the studies tend to mitigate the positive results of the studies. Dr. Gordon's work, using standard methods for over 90 children, has reliably shown significant recovery after training. The inconsistency in the studies in the Cochrane review points to the importance of developing well-tested, rigorously structured rehabilitation methods, as this study proposes to determine.

**C. Training effectiveness at ages 8-13:** Reviewer 3 questioned the training efficacy for children ages 8-13, beyond the period of early developmental plasticity. A study by Dr. Gordon has directly addressed this issue and showed that intensive training of the impaired arm produces the same degree of recovery whether children are younger (4-8) or older (9-13)(Gordon 2006). Moreover, a similar training paradigm has been shown to produce significant, long-lasting recovery in stroke patients, many of whom are age 50 and older. The remarkable plasticity of the brain over the entire lifespan of a human or animal has been further emphasized in the introduction (p. x).

**D. Cortical excitability and feasibility of TMS in children with CP:** Reviewers, particularly #5, raised questions about the use of TMS in children with CP; whether TMS could adequately excite cortex in these children and whether the procedure was feasible. Two laboratories in particular, those of Dr. Janet Eyre (a consultant on this project) and Dr. Martin Staudt, have used TMS to measure cortical excitability in children with CP. Dr. Staudt's group has tracked patients during an intensive training protocol similar to that proposed

here, and has preliminary data that show expansion of motor areas after training. Thus, the TMS protocol is feasible in children with hemiplegia (note, I hope to have prelim data from Janet Eyre for the submission).

**3) METHODOLOGICAL ISSUES**

**A. No account for dropouts:** The power analysis has been modified to account for dropouts. The largest dropout rate from Dr. Gordon's studies has been 15%; since the proposed studies are more intensive (including TMS), I have allowed for a 25% dropout rate. (p. xx)

**B. Low proposed number of subjects:** There was concern (reviewer 3) that the proposed number of subjects (n=15/group) was too low to show meaningful correlations between map changes and motor improvement. I increased the n to 20 per group to increase the chances of finding meaningful correlations. With a 25% dropout rate, the total proposed n is 50.

**C. Recruitment of minority and female subjects:** The proposed ethnic and gender breakdown of subjects was in line with the previously published studies of Dr. Gordon. The studies will take place in New York, NY, a highly racially diverse community. The neighborhoods of Columbia University Medical Center and Teachers College are very diverse. The school systems and medical facilities where recruitment will take place are also very diverse. Finding adequate numbers of all races and genders has not been a problem in other studies of Dr. Gordon. (note, the human subjects tally form has been corrected)

## 1. The Candidate

### A. Candidate's Background

I am currently an Associate Research Scientist in the Department of Neuroscience (formerly the Center for Neurobiology and Behavior) at Columbia University, New York. My career objective is to build an independent research program in cerebral palsy (CP) neurorehabilitation as an academic scientist. My research program will be built upon a solid foundation of basic neurophysiology, neuroanatomy and motor behavior. Neurorehabilitation strategies for CP and other disorders have proven to be most effective when based on the knowledge of the mechanisms of disease development and progression. Often, human neurorehabilitation studies are not driven by specific hypotheses or an understanding of the mechanisms of disease. My training using animal models to study the mechanisms of motor development and motor control after stroke will provide a solid framework to study neurorehabilitation, with the goal of developing effective treatments for CP.

Understanding the neural mechanisms of brain dysfunction and rehabilitation has been an abiding research interest of mine. In the fall of 1995, I entered the University of Texas Health Science Center at Houston Graduate School of Biomedical Sciences. I joined the laboratory of Randolph Nudo, Ph.D. Dr. Nudo's research examines plasticity of primary motor cortex (M1) following motor learning and vascular cortical injury. In his laboratory, I studied behavioral and neurophysiological recovery in squirrel monkeys after a strokelike cortical injury to M1. During the 1996-1997 academic year, I completed a research project to fulfill the requirements for a Master of Science degree. Normal squirrel monkeys were trained on a pellet retrieval task that requires skilled finger movements. After training, a small vascular infarct was made to digit representations within M1, destroying approximately 30% of the hand area. Following the infarct, animals were trained on the pellet retrieval task using their impaired hand for approximately one month. Then, another map of M1 was derived. These animals that had received daily rehabilitative training retained more hand area than animals that had not received postinfarct training, pointing to **a critical role of skilled use of the affected arm in rehabilitation**. For my M.S. thesis project, I determined the sequence of arm movements that monkeys used to retrieve food pellets before vs. after an M1 lesion. Typical arm movements that monkeys used to extract pellets from the well were digit flexion + wrist extension, digit flexion + wrist ulnar deviation, and digit flexion + forearm supination. Some monkeys used different movement patterns to retrieve pellets after infarct, indicating that **behavioral compensation plays an important role in recovery of motor skill**[1].

Upon my completion of a Master of Science degree, Dr. Nudo moved the laboratory to the University of Kansas Medical Center. I continued working in Dr. Nudo's laboratory toward the degree of Doctor of Philosophy. I was awarded a Ph.D. with Honors in August 2002. In my doctoral work, I completed two projects: 1) Interplay between constraint of the unaffected arm, training of the affected arm and cortical reorganization after M1 injury; and 2) Sensorimotor deficits caused by lesions to rostral vs. caudal sectors of M1. I received training in intracortical microstimulation, quantitative motor skill assessment, and animal behavioral training. My doctoral work was funded by an Individual National Research Service Award.

In the first project, I looked at differences in map reorganization after M1 injury following three types of post-injury interventions: no intervention, restraint of the unaffected arm, and restraint of the unaffected arm **plus** daily training of the affected arm in a pellet retrieval task. Previous studied had shown that restraint plus daily training of the affected arm resulted in maximal retention of the M1 hand representational area. The goal of my work was to determine if training or restraint alone produced the adaptive changes in M1 physiology. In my study, animals that received restraint of the unaffected arm without training were no different than the controls that had not received any intervention. Since squirrel monkeys do not use their forelimbs for continual postural support or fine motor skills in normal cage behavior, I concluded that normal cage use of the affected forelimb without training was not sufficient to drive changes in the M1 motor map. This study pointed to **a critical role of skilled use of the affected arm in adaptive cortical reorganization** [2]**.**

My second project examined sensory-related deficits in pellet retrieval after focal lesions to the rostral or the caudal sectors of M1. Many previous studies have established that M1 receives direct and indirect inputs from somatosensory cortex, and that these inputs are important in sensorimotor control. Inputs are functionally segregated, with rostral M1 receiving proprioceptive sensory inputs and caudal M1 receiving cutaneous sensory inputs. My study showed that **these sensory inputs are behaviorally relevant** [3]. Before a lesion to rostral or caudal M1, monkeys retrieved pellets by directing the hand to the well, inserting fingers directly into it, and extracting the pellet. Lesion to rostral M1 produced an aiming defect, in which monkeys frequently failed to accurately direct the hand to the well. Instead, fingers touched the surface of the board outside the well

before entering the well. We postulated that this aiming defect could be due to an impairment in the sensorimotor integration of limb proprioceptive information in movement control. In contrast, after a lesion to caudal M1, responses were well aimed but monkeys showed an impairment in object manipulation. They frequently examined their palm visually for the presence of the pellet after an attempted retrieval, possibly indicative of a defecit in the ability of M1 to use cutaneous information in controlling finger movements.

**Rostral and caudal M1 lesions result in different deficits in sensory-dependent motor control that correlate with segregation of sensory inputs to M1.** In the same study, I examined M1 maps before and after rostral or caudal M1 lesions. Monkeys were trained during the one-month postlesion period, using their impaired arm to retrieve food pellets. After either rostral or caudal lesions, postlesion training caused a retention of the M1 motor representation, compared with untrained, lesioned controls, in which the representation shrinks. This result indicates that **postlesion training of the impaired arm facilitates recovery regardless of the exact lesion location within M1** [4]**.**

Since my training in Dr. Nudo's laboratory provided me with expertise in examining the recovering motor system from neurophysiological and motor behavioral perspectives, I sought a postdoctoral fellowship that would teach me different perspectives for looking at the motor system, and especially development. I joined the laboratory of John Martin, Ph.D. in the Center for Neurobiology and Behavior at Columbia University. In his laboratory, I have been studying development of the corticospinal (CS) system in cats, response to motor cortex activity blockade, and strategies for promoting recovery. In his laboratory, I learned neuroanatomical techniques, including tract tracing, quantitative anatomical analysis of connectivity, immunohistochemistry, in situ hybridization, and light microscopy. I have combined these anatomical techniques with kinematic analyses of visually-guided motor behaviors, especially during locomotion, and intracortical microstimulation. Using these new approaches have enabled me to examine the developing and recovering motor system from many angles. An Individual National Research Service Award funded my postdoctoral work.

Motor cortical activity during early postnatal development in cats and in humans is essential for the proper development of motor circuits. My work has examined the effects of unilateral M1 activity blockade on CS system development. Unilateral activity blockade during a critical period in development results in aberrant organization of CS terminations in the spinal cord. Additionally, unilateral activity blockade causes permanent deficits in visuomotor control and prevents formation of the motor cortical map. Unilateral inactivation serves as an experimental model for key CS circuit changes that occur in hemiplegic cerebral palsy (CP).

In my first project in Dr. Martin's laboratory, my research determined the timing of the developmental period(s) in which activity is important in shaping the CS system. Unilateral inactivation during postnatal weeks **(PW) 5-7** led to most profound, permanent aberrant organization of CS connections with spinal motor circuits, indicating that this is a period in which **M1 activity is instrumental in development of CS system organization**. Inactivation at earlier **(PW 3-5)** or later **(PW 8-12)** times did not disrupt CS the pattern of connectivity, but did result in decreased local branching and presynaptic bouton density at axon terminals. Thus, M1 activity is important in **shaping CS organization during a discreet developmental period**, while M1 activity is important in **the refinement of CS terminals during a protracted period** [5].

In my second project in Dr. Martin's laboratory, I studied visuomotor deficits induced by unilateral M1 activity blockade during the critical period for CS organizational development, PW 5-7. This work was completed in collaboration with Dr. Trevor Drew at the University of Montreal. Dr. Drew's laboratory is the foremost group studying cortical control of locomotion in animals. I examined visually-guided stepping behavior on a horizontal ladder and overstepping obstacles on a treadmill. After unilateral M1 activity blockade during PW 5-7, cats consistently overstepped rungs on the ladder and obstacles on the treadmill. However, cats did not show aberrance in trajectories used in stepping movements. This study points to the importance of **M1 activity in the development of endpoint control but not trajectory control** [6].

My first two studies demonstrated the importance of M1 activity in the development of CS system anatomy and visuomotor skill. Unilateral activity loss provides a competitive disadvantage to the inactivated side; the active side is able to secure and maintain synaptic space that is normally occupied by the inactivated side. Ipsilateral projections are normally pruned back during development because of activity-dependent synaptic competition, but after unilateral activity blockade, the active side maintains ipsilateral CS projections. I hypothesized that **rebalancing activity between the two M1 hemispheres** would promote recovery.

I next completed an experiment in which I inactivated one M1 hemisphere during the critical period for establishing CS tract connections, PW 5-7, and then inactivated the contralateral, previously active M1

hemisphere from PW 7-11. The goal of this second inactivation, termed "alternate inactivation," was to remove the competitive advantage of the previously active side, thus allowing the first inactivated side to recover. Alternate inactivation resulted in a **redistribution of CS terminations in the spinal cord to appropriate spinal motor laminae** and **recovery of endpoint control**, as measured on the horizontal ladder-walking task[6]. An additional recent study, in collaboration with Samit Chakrabarty, a research scientist in the laboratory, has shown that while unilateral M1 inactivation results in a loss of the motor map, alternate inactivation reconstitutes the normal motor map [7]. Thus, rebalancing activity after unilateral activity blockade produces CS anatomical recovery, motor skill recovery, and restitution of the motor map.

My predoctoral and postdoctoral training has given me expertise in **studying neurological disorders and rehabilitation from a mechanistic perspective**. I have looked at stroke and CP recovery through anatomical, physiological, and behavioral lenses, all pointing toward understanding the neural bases of recovery. My work has revealed the importance of **balancing motor cortex activity**, through training of the affected side or inactivation of the intact side, in promoting recovery. The proposed project will enable me to translate my basic science knowledge to of the important clinical condition, hemiplegic cerebral palsy (CP). I firmly believe that effective therapies for neurological disorders can best be developed from a solid understanding of the underlying neural circuitry, the mechanisms involved in recovery of the disorder. The proposed project will tackle CP rehabilitation from two unique perspectives: examining mechanisms of recovery after unilateral M1 activity loss/injury in both an animal model and in children with CP. Addressing the important clinical issue of CP rehabilitation *concurrently* in the animal and the human provides an exciting opportunity to test specific hypotheses in both models. While some hypotheses are able to be safely tested in humans, in research that will provide direct therapeutic insights, other hypotheses are not yet ready to be tested in humans, but nonetheless are extremely important for understanding the mechanisms of brain recovery in hemiplegic CP. My unique training opportunity and background enable me to examine these important clinical problems mechanistically in both animals and humans.

In the proposed project, I will gain experience in translational research. I will translate my findings in the cat to the human. The cat is the *ideal model* because development of the motor system has extremely close parallels with the human. In other species, including non-human primates, neonates possess moderately to highly developed CS circuitry and refined motor skills (e.g. ability to climb and hold on its to mother's back immediately after birth). However, in both the cat and the human, the CS system is poorly developed at birth, and neonates possess little or no skilled motor abilities. Development of CS circuits and motor skill occur during maturation of the animal or human, and is a protracted process extending to sexual maturity (3-4 months) in the cat and through adolescence in the human. Thus, I have chosen the cat to study activity-dependent competition in the motor system. Additionally, since the issues I am addressing focus on physical rehabilitation, rather than genetic manipulations, I have chosen to translate my animal work and expertise into the human rather than into a mouse model.

**B. Career Goals and Objectives**

My **long-term career objectives** are to:

- Become an expert in human motor control and rehabilitation.
- Develop a successful CP rehabilitation research program.
- Translate knowledge of the mechanisms of recovery to practical CP rehabilitation strategies.
- Become an instructor and mentor to scientists and clinicians performing mechanism-driven neurorehabilitation research.

My **immediate objectives** over the course of the award are to:

- Gain expertise in transcranial magnetic stimulation experimental design, data collection and interpretation.
- Gain expertise in human subject study design and human motor behavior analysis.
- Gain expertise in translating clinically relevant information from animal models to patients.
- Gain experience working directly with children with CP.
- Become an independent investigator studying neural mechanisms of recovery in children with CP.

The proposed studies will give me an extraordinary training opportunity in which I will translate my solid basic science background to the important clinical problem of CP. Since my predoctoral and postdoctoral studies have both examined the role of balancing motor cortex activity in restoring function after inactivation or

stroke, the proposed project is an ideal mechanism by which I can apply my well-reasoned perspectives on motor system recovery to a clinical model and condition. Although I have experience with the techniques proposed in Aim 1, the approach of the current proposal is novel to me. I will be approaching both experiments of Aim 1 with the intention of testing hypotheses that will also be directly tested by me in patients. Since I will have access to both humans and the animal model, it will also be possible for me to develop new hypotheses in the human study that I can test in the animal model, if safety precludes immediate testing in patients. Gaining expertise in such a translational strategy is an important training plan that will shape my development as an independent rehabilitation neuroscientist.

**Intensive practice with the affected side**, paired with **restraint of the unaffected side** has shown great promise in the treatment of stroke and hemiplegic CP. In the proposed project, I will study the use of intensive practice in children with hemiplegic CP. My primary mentor, Dr. Andrew Gordon, has extensive training in human motor psychophysics and has a world-renowned treatment research program in place, using intensive practice as therapy for children with hemiplegia. By working in his laboratory, I have access to a large patient population, a fully-equipped laboratory in which to conduct my research, and ample opportunity for training by Dr. Gordon and his experienced research team on methodology, analyses, and data interpretation. Thus, his resources and expertise will provide me a stellar environment in which to conduct my research and learn new experimental skills. I do not yet have experience working with human subjects. Since my career goal is to develop a research program studying CP rehabilitation in humans, it is essential for me to gain experience in human subject work. Dr. Gordon's lab is the ideal environment for me to obtain this experience.

I will use transcranial magnetic stimulation (TMS) to uncover changes in the excitability of motor cortex caused by intensive practice. I will examine changes in the size and threshold of the arm representation in the motor map in both hemispheres. This knowledge will lead to a greater understanding of the **motor cortical mechanisms of skill recovery**. Such knowledge is critical for the further development of effective CP therapies. TMS is an important tool for probing the human motor system in a non-invasive way. My training under the proposed project will give me valuable training in TMS; this training will enable me to incorporate the very important tool of TMS into my future, independent research program. Dr. Sarah Lisanby, an expert in TMS, will serve as a co-mentor to this project, providing me with training in the TMS data collection and interpretation. **This project will provide me with the expertise I need to become an independent investigator in the field of neurorehabilitation**.

**C. Career Development and Training Activities**

**Sponsors and consultants:**

The following three mentors will supervise my training activities and career development:

Primary Sponsor: **Andrew M. Gordon, Ph.D.** Professor of Movement Sciences, Department of Biobehavioral Sciences, Teachers College, Columbia University. Dr. Gordon is the author of more than 70 peer reviewed research articles on motor control and movement disorders (especially CP). Dr. Gordon is an expert in the psychophysical analysis of prehension in normal individuals and subjects with disabilities and movement disorders, such as cerebral palsy, Parkinson's disease, and Huntington's disease. His research program has the goal of understanding the neural mechanisms behind these motor control deficits. I choose Dr. Gordon as a mentor because his laboratory examines movement control and rehabilitation in a mechanistic fashion. He has an established research program studying intensive training of the affected arm in children with hemiplegic CP. More than 35 children have completed his laboratory's rehabilitation program. His expertise in motor control in children with CP will provide me with an exceptional training experience. I am confident that my training experience in his lab will give me a solid foundation from which to build an independent research program in neurorehabilitation. Dr. Gordon has mentored many Ph.D. candidates and postdoctoral fellows. Several of Dr. Gordon's students are now independent investigators in motor control as faculty in rehabilitation departments. Three former post-doctoral students are also independent faculty members. Dr. Gordon will enable me to conduct research in his laboratory and will advise me on a regular basis on human motor control theory, psychophysics and biomechanics of grasping, research design, data analysis, and presentation and publication.

Co-sponsor: **Sarah H. Lisanby, M.D.** Associate Professor of Clinical Psychiatry; Chief, Brain Stimulation and Therapeutic Modulation (BSTM) Division, Columbia University and New York State Psychiatric Institute. Dr. Lisanby in an internationally recognized leader in the field of TMS. She is an expert in use of TMS as both an

assessment of brain function and as a therapeutic intervention. Dr. Lisanby and her research group will train me in TMS methodology, analysis and interpretation of data, and study design. Dr. Lisanby has built a translational research program that studies, in parallel, animal models and patient populations, with the goal of developing effective treatments for medication resistant disorders. For example, her R01 funded work developed magnetic seizure therapy in a monkey model and translated those results into the first human trials with this novel experimental treatment for depression. She will provide me with important guidance on how to approach the study of a disease from both an animal model and a clinical perspective. I will meet with her weekly to discuss experimental progress, data analysis and publication, and career development.

Co-sponsor: **John H. Martin, Ph.D.** Professor of Clinical Psychiatry and Neurosurgery, Center for Neurobiology and Behavior, Columbia University. Dr. Martin is an expert in corticospinal development, neuroanatomy, motor system physiology (EMG, ICMS), motor control, and motor psychophysical analysis in the cat. He has published more than 30 articles and a neuroanatomy textbook, now in its third edition. Dr. Martin's laboratory is the only laboratory in the world studying activity-dependent development of the cat motor system. Dr. Martin's laboratory is well-equipped with microscopes, microscopy analysis systems, electrophysiology equipment, surgical tools, histology tools and reagents, and computers. My previous work in Dr. Martin's lab has given me expertise in tract tracing, light microscopy, motor skill analysis and ICMS. Dr. Martin will continue to mentor me in the experimental design of the cat model. In the proposed project, I will build upon my expertise in animal research to implement a translational research program. Additionally, since Dr. Martin has experience in EMG signal analysis and extracellular field potential analysis, he will be a valuable consultant on my TMS aim. I will meet with him regularly to discuss experiments, data analysis and publication, and career development.

Research Consultant: **Janet Eyre, M.D.** Professor of Paediatric Neuroscience, Department of Clinical Medical Sciences, University of Newcastle upon Tyne. Dr. Eyre is the leader of an active cerebral palsy research program. Her work is among the broadest, most in-depth characterizations of motor system physiology in children with hemiplegia. Dr. Eyre has tracked children who have, or were at risk of developing, hemiplegic CP from birth through young adulthood. She has studied changes in TMS excitability of motor cortex in these subjects, work that has greatly enhanced our understanding of the development of the damaged motor system. Dr. Eyre is proficient at performing TMS on children of all ages, from premature neonates to young adults. Dr. Eyre will serve as a research consultant, assisting me in data interpretation and methodological troubleshooting. She will be a valuable resource to me as I develop my research program. I will communicate with her frequently by phone and email, and I will visit her lab once a year to observe her patient studies and discuss data, interpretation, future directions, and career development. I will allocate travel money for her to visit Columbia University during the first TMS testing sessions in CP patients, so that she may provide additional guidance, and as needed thereafter.

Research Consultant: Eugene Tunik, Ph.D., P.T. Assistant Professor, Department of Physical Therapy, New York University. Dr. Tunik is an expert in the use of TMS to gauge the function of the motor system. His use of TMS has provided valuable insights into the neural bases of motor control and learning. Dr. Tunik's research elegantly combines TMS and kinematic analyses of movement. I will visit his lab regularly to observe and participate in TMS studies in normal subjects and stroke patients. This will provide me valuable training in performing and interpreting TMS studies of the motor system. Dr. Tunik also uses TMS to make "virtual lesions" to the motor system during performance of a movement. This technique will be of great value in my future directions and therefore will be extremely beneficial to learn.

Research Consultant: Leonardo Cohen, Ph.D., National Institutes of Health. Dr. Cohen is a pioneer in the use of TMS to study the motor system in normal subjects and in patients with neurological disorders. By visiting Dr. Cohen's laboratory to observe and participate in TMS studies, I will receive excellent training in the use and interpretation of TMS. Dr. Cohen will also be an extremely valuable source of direction in the analysis and interpretation of my own TMS data. (need to solidify his involvement/schedule)

Research Consultant: Claudia Chiriboga, M.D., M.P.H. Associate Professor and Interim Director, Division of Pediatric Neurology, Department of Neurology, Columbia University Medical Center. Dr. Chiriboga is a board-certified pediatric neurologist specializing in neurodevelopmental disorders and spasticity. She leads the Spasticity Clinic at Columbia University Medical Center, which sees patients for spasticity diagnoses and monitoring, botulinum toxin injections and consultations with physical therapists and neurosurgeons for further treatments. Dr. Chiriboga will oversee my volunteer experience in the Spasticity Clinic, where I will gain

experience working with patients with spasticity (including patients with CP), measuring spasticity and learning about spasticity treatments.

Research Consultant: Jason Carmel, M.D., Ph.D. Resident in pediatric neurology, Department of Neurology, Columbia University Medical Center. Dr. Carmel has extensive experience with children with neurological disorders, including cerebral palsy. Dr. Carmel will provide me with assistance in subject recruitment and in performing clinical examinations in potential research subjects. Dr. Carmel will help in verifying the cause of CP in potential research subjects and will assist me in screening subjects for participation.

**Training Activities**

**1. Training in Transcranial Magnetic Stimulation**

**A. Training in the use of TMS to study the motor system:** Drs. Eugene Tunik and Leonardo Cohen will provide training in the use of TMS to study the motor system in normal subjects and in subjects with stroke. Dr. Eugene Tunik will provide me regular, practical training in TMS performance and data interpretation. Dr. Tunik combines the use of TMS and kinematic analysis of movement to examine the motor system. Therefore, I will receive extensive training in the motor aspects of TMS and in kinematics in his laboratory. Since his lab is located in close proximity to my home institution, frequent visit and communication will be quite feasible. I will also visit the laboratory of Dr. Leonardo Cohen for X period annually to observe and participate in TMS studies of adult stroke patients and to discuss my own data and interpretation during the later years of my fellowship. I will participate in a **TMS Mini-Fellowship during Year 1**, offered by Dr. Alvaro Pascual-Leone's laboratory at Harvard Medical School. The fellowship provides hands-on learning of basic principles, use and clinical applications of TMS. The fellowship also provides training in determining motor thresholds, recording motor evoked responses, cortical mapping and paired-pulse TMS. The fellowship will also cover TMS safety, writing TMS protocols and TMS study design.

**B. Training in TMS Methodology:** Dr. Lisanby will direct my training in methodology and interpretation of TMS. The BSTM Division holds weekly TMS training sessions. Three-times annually, the BSTM Division offers a three-day intensive Continuing Medical Education (CME) course on TMS. **I have already taken the CME course and have been certified as a TMS practitioner by the BSTM.** Dr. Lisanby oversees all training and will ensure that I receive extensive training in TMS methodology, data analysis and interpretation. I will continue building my expertise in TMS coil placement, EMG recording, MEP data collection and analysis, and management of artifacts by participating in ongoing training and refresher sessions. Dr. Lisanby and her research staff will participate in and supervise data collection and will ensure that all protocols are followed accurately and completely. I will also receive training in the use of structural MRI and co-registration of TMS coordinates to MRI landmarks using the frameless stereotaxic system.

**2. Training in Human Study Implementation and Human Subjects Research**

I will take courses at Columbia University to gain didactic training in child neurology and rehabilitation. I will take **Management of Pediatric Conditions I and II** from the Department of Physical Therapy, in which I will learn about physical therapy approaches to treating child neurological disorders such as CP.

I have planned many training opportunities for working with human subjects, particularly children and persons with neurological disorders. **1)** I will visit the laboratory of Dr. Eyre annually to observe TMS studies in children with CP; additionally, Dr. Eyre will come to my laboratory during my first bout of data collection to assist and advise. **2)** I am volunteering in Dr. Gordon's laboratory as an interventionist in his intensive training day camp, gaining experience working with children with hemiplegia, conducting intensive training studies and performing motor skill assessments. **3)** I will observe TMS experiments on patients with stroke in the laboratory of Dr. Cohen. **4)** I will observe TMS experiments on normal subjects and stroke patients in the laboratory of Dr. Tunik. **5)** I will volunteer in the Spasticity Clinic of Columbia University Medical Center, under the supervision of Dr. Chiriboga, to gain experience in working with children with CP. The spasticity clinic sees outpatients one day every other week. I will observe and assist in measuring spasticity in children; I will also observe botulinum toxin injections and will sit in on consultations between neurologists, physical therapists, neurosurgeons and patients' families in discussing treatment options for the children. This training opportunity promises to also serve as an opportunity for me to inform clinicians about my study and to recruit subjects. **6)** I will observe TMS studies in children with autism in the BSTM under the supervision of Dr. Lisanby. Although I clearly recognize that CP and autism are very different conditions, there is much to be gained by working with

children with autism. I will learn ways of negotiating potential confounds to my TMS study, including subject distractibility and challenges keeping the child still and calm during the procedure.

**3. Training in Motor Skill Assessment and Kinematics**

Dr. Andrew Gordon will direct my training in human motor skill assessment and kinematics. Dr. Gordon is one of the foremost experts in the field of motor control and rehabilitation in children with CP. Under Dr. Gordon's guidance, I will learn skills regarding clinical assessment and will gain experience in kinematic analysis of movement coordination to quantify quality of movement (an area largely ignored). **I will take Biomechanical Analysis of Human Movement and Kinematic Lab,** both offered at Teachers College to deepen my training in biomechanics and kinematics. Additionally, my training in Dr. Tunik's laboratory, described above, will give me further experience in the kinematic assessment of movement.

**4. Training in Clinical Research Design and Statistics**

I will enroll in two courses offered in the Department of Psychiatry at Columbia University Medical Center. **Research Design and Methodology** will cover principles of clinical research design. Since I am not experienced yet in clinical research, this course will provide excellent training in design and methodological issues from the perspective of clinical research. **I will take this course before the fellowship begins.** I will also take a two-semester course in clinical research-oriented **Statistics** during **Year 2** of my fellowship. This course will cover parametric and nonparametric statistics, regression analyses and population analyses. This course will train me in the analysis of clinical research data.

**D. Training in Responsible Conduct of Research**

During the first year of training, I will take **Responsible Conduct of Research (G6001)** at the Columbia University Graduate School of Arts and Sciences**.** The topics covered in this course will be scientific objectivity, research design, scientific misconduct, mentoring, record-keeping and ownership of data, authorship and peer review, use of human subjects or tissue in biomedical research, use of animals in biomedical experimentation and use of hazardous materials in research, conflict of interest and issues of sharing data, and grants.

Dr. Martin will aid me in fulfilling the requirements of the Institutional Animal Care and Use Committee at Columbia University Medical Center. He will continue to guide me in the ethical principles of animal research, including issues of animal health, pain management, social/emotional health, minimization of number of animals and number of invasive procedures used, and euthanasia protocols. Drs. Gordon and Lisanby will aid me in fulfilling the requirements of the Institutional Review Board at Columbia University Center, and will guide me on the ethical principles of research in the in children with CP. Dr. Gordon will supervise the responsible conduct of research in the Center for Cerebral Palsy Research at Teachers College. Dr. Lisanby will supervise the responsible conduct of research in the Transcranial Magnetic Stimulation Laboratory at the New York State Psychiatric Institute.

**Timeline for the proposed career development activities**

The table below lists training and experimental activities by year. The amount of shading in each bin represents the relative amount of time to be spent on each activity each year. For training activities, the bulk of the learning will be initiated in the first and second year. By the end of Year 2, I will have a firm grasp on TMS and motor assessment training, as well as having gained substantial experience working with children with CP. I will continue to deepen my knowledge as the years proceed and I obtain data to analyze and interpret.

Since I am familiar with protocols to be used in the cat experiments (Aim 1), I will initiate those experiments during the first year of the grant. I will complete the animal work by the end of Year 2, and will spend the remaining time analyzing data and publishing findings. Since I am less experienced in the protocols to be used in Aim 2, I will spend Year 1 focusing on training. I will begin my first cohort of children (n=3) during the middle or end of Year 1. Since the rehabilitation program for the children runs seasonally (three weeks in July and three weeks in December, during the children's school vacations), it will be feasible to work on the cat project and the human project during the same year. I will work on the cat project outside the months of July and December. The first cohort will require 1- and 6-month follow-up testing and analysis, and my cat experiment schedule will be adjusted to allow ample time for follow-up activities with the subjects. During the later years of the grant, I will have more time to devote to human subjects; this time will allow me to sufficiently follow up with

each cohort, complete and publish the results of Aim 2, as well as provide time for exploration of future directions. This will allow for compilation of plans and preliminary data for an R-series grant application.

Since courses 1 and 5 are one week or less in duration, the proposed schedule of coursework is feasible.

Timeline of Proposed Activities

| Activity | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|
| Training: | | | | | |
| TMS methodology, analysis, interpretation | Course 1 | | | | |
| Human study implementation; human subjects research | Course 4 | Course 5 | | | |
| Human motor assessment, analysis, interpretation | | Courses 2-3 | | | |
| Statistics | | | Course 6 | | |
| Research Design, Responsible conduct of research | Course 7 | | | | |
| Experimentation: | | | | | |
| Aim 1A | | | | | |
| Aim 1B | | | | | |
| Aim 2 | | | | | |

**COURSES:**

Course 1: Transcranial Magnetic Stimulation Mini-Fellowship, Dept. of Neurology, Harvard U. Medical School
Course 2: Biomechanical Analysis of Human Movement, Teachers College
Course 3: Kinematic Lab, Teachers College
Course 4: Management of Pediatric Conditions (I and II), Dept. of Physical Therapy, Columbia University
Course 5: Hershey Conference on Developmental Brain Injury
Course 6: Statistics, Department of Psychiatry, Columbia University Medical Center
Course 7: Responsible Conduct of Research, Columbia University Graduate School of Arts and Sciences

**PATH TO ACADEMIC INDEPENDENCE**

This fellowship will provide me the training and experience I need to become an independent academic scientist in the field of neurorehabilitation. Upon funding of this fellowship, or other grants to cover my salary and research expenses, I will be promoted to Assistant Professor of Clincal Psychiatry in the Department of Psychiatry at Columbia University Medical Center. A faculty position at Columbia will provide me an optimal environment for continuing my research career. During completion of my K award experiments, I will be building strong professional relationships among different departments (Neuroscience, Neurology, Biobehavioral Sciences and Psychiatry), setting the stage for a career of interdisciplinary collaboration in neurorehabilitation. The institutional environment is exceptional, as described in this application, with outstanding resources, equipment and available patients for study recruitment.

This fellowship will also enable me to collect preliminary data for submission of a strong R-series grant application. Although this fellowship will answer important questions about the mechanisms of CP recovery, it will certainly uncover many more. One exciting use of TMS is the ability to make *transient*, non-damaging "virtual lesions" to a part of the motor system by temporarily inactivating a region of the nervous system. Virtual lesion experimentation could be used to further refine hypotheses about mechanisms of recovery. Virtual lesions could be made to motor areas hypothesized to mediate recovery; if lesions temporarily (on the order of seconds to minutes) worsen motor performance on a hand task, which would provide additional evidence that the area in question is indeed involved in recovery. Dr. Tunik, a consultant on this fellowship, is a leading researcher in the area of virtual lesions of the motor system.

Other important questions about the use of intensive training in CP rehabilitation remain, such as the optimal age of the child for therapy, the optimal duration and intensity of the therapy, and the optimal means of retaining motor skill improvements long-term. These important issues will be topics of future research.

Moreover, there is dire need for the development of novel, effective therapies for CP. The use of TMS not only as a mapping tool but also as a therapy is exciting. TMS is currently used to effectively treat a variety of neurological and psychiatric disorders. Additional, recent studies in the treatment of stroke show that pairing cortical stimulation with physical rehabilitation enhances the therapeutic effect of either method independently.

**2. STATEMENTS BY SPONSOR AND CO-SPONSORS (NOTE THESE LETTERS WILL BE EDITED TO INCORPORATE REVIEWERS' SUGGESTIONS)**

**STATEMENT OF SPONSOR, Andrew M. Gordon, Ph.D.**

I am pleased to agree to work with Dr. Kathleen Friel to transition her research skills from animal studies to humans. Dr. Friel maintains a steadfast desire to help people clinically and to conduct clinically relevant research which will lead to the improved treatment in patients with cerebral palsy (CP). I have mentored Dr. Friel in the formulation of her research proposal along with Drs. John Martin and Sarah Lisanby, and she has demonstrated excellent potential as a clinical scientist. If funded, I will host her in my laboratory for a period of 5 years.

Dr. Friel is in a unique position to study movement disorders associated with CP. She has been conducting cutting edge research in the area of plasticity in animal models for the last 12 years. Her graduate studies with Dr. Randolph Nudo yielded some of the most innovative work to date on the topic. Specifically her work was among the first to show the importance of "skilled" training in the recovery of motor function and M1 maps. She also showed that rostral and caudal M1 lesions result in different deficits in sensory-dependent motor control that appear to correlate with segregation of sensory inputs to M1.

For her postdoctoral work she has been working with Dr. John Martin in the Center for Neurobiology and Behavior at Columbia University, where she has been studying development of the corticospinal (CS) system in cats, response to motor cortex activity blockade, and strategies for promoting recovery. There she has expanded her repertoire of experimental and analytical skills, and the work has led to some profound findings. She has shown that M1 activity is instrumental in development of CS system organization. This finding is profound as it implies that the lack of M1 activity associated with non-use of the involved upper extremity in hemiplegic CP may result in permanent aberrant organization of CS connections with spinal motor circuits. This may also have implication for potential adverse affects of casting the "non-involved" upper extremity in humans during constraint-induced movement therapy if this is done too early as it could result in permanent damage of the circuitry underlying control of this extremity.

Her work to date, resulting in 17 peer-reviewed papers in excellent journals, alone make an outstanding contribution to the area of plasticity. However, what is particularly impressive is that Dr. Friel is committed to translating this work to the study of CP. She is thus in a unique position of having critical knowledge and experience that will allow her to directly address the hypotheses derived from this work to intensive training protocols in children with CP. To date, the efficacy of most treatment approaches for hemiplegic CP is not known. Emerging evidence from our lab and that of others is suggesting a benefit of intensive use of the involved extremity along with restraint of the non-involved extremity (i.e., constraint-induced movement therapy, CIMT). This area of work is promising, however, there is much more knowledge required before

CIMT can be applied more broadly. In particular, beyond the intensity of treatment, it is not known whether some attributes are more beneficial than others (i.e., the key ingredients). In fact, the only connection to animal models to date is "shaping", and the relevance of this component is not known. Thus, Dr. Friel is in the unique position of being able to directly test some of the components of training specifically shown to be most beneficial in animal models (i.e., progressively skilled behaviors) and apply them to the study of humans. Furthermore, the neural basis for improvements in function in children with hemiplegia are largely unknown. Thus the proposed TMS studies will help elucidate the changes in M1 representations to determine the extent to which changes in function are driven by the contralateral or ipsilateral pathways. This knowledge would be profound, and the conduct of this work would transition her to become an independent scientist with a focus on CP. Dr. Friel has chosen a very exciting research topic as a basis of the didactic and experiential research training.

Columbia University has a long standing reputation for research in systems neuroscience. Columbia has a large group of active investigators in the area of motor control and movement disorders (A.M. Gordon, A.M. Gentile, T.R. Kaminski at Teachers College and C. Ghez, J. Krakauer P. Mazzioni and J. Martin at Physicians and Surgeons) and 5-8 post-doctoral fellows and 15-20 pre-doctoral students whose research interests largely overlap. We have collaborated closely in developing a training program for students and postdocs interested in motor control. We offer a weekly research training seminar at Teachers College in which all faculty, students and post-doctoral fellows present their work. Postdoctoral fellows are expected to present their work twice per year in this setting. We also offer a monthly forum where faculty, students and postdocs present their work or discuss recent papers at Physicians and Surgeons. We have regular guest lectures and Dr. Friel will continue to interact with visiting scientists. We also have collaborations in the area of movement disorders with faculty at Physicians and Surgeons (L. Cote and K. Marder) with who she will have access to. I will introduce her to prominent people in the rehabilitation field, and provide her with further opportunities for collaboration. We will meet individually each week and I expect she will be collecting/analyzing data under my direct supervision, as well as learning about responsible conduct of clinical trials. I will provide Dr. Friel with pertinent articles and books to read and will discuss them with her. I will assist her in preparing manuscripts for publication and scientific presentations. As Dr. Friel's skills further develop, she will assume increasing responsibility for research projects. I have a steadfast commitment to the training in the area of rehabilitation research (nearly all of my graduate students are physical and occupational therapists) and I maintain a personal interest in every trainee. I will assist in coordinating the efforts of all her mentors and consultants to ensure that her training is progressing as planned. To facilitate this I will have periodic meetings with the co-mentors, Drs. John Martin and Sarah Lisanby. I expect the training to lead to her development into a principal investigator capable of leading a multi-disciplinary research team and running her own laboratory.

I have made all state-of-the-art laboratory resources available to Dr. Friel. These include a 3-D optoelectronic (8 camera) device with real time video processing capability (Vicon), housed in 42 Thompson Hall. We also have a Cyberglove (Virtual Technologies) consisting of 18 sensors embedded in a lycra/nylon glove for precise measurement of hand and finger kinematics (1055 Thorndike Hall) should we deem that necessary. We have a grip instrument with up to 5 force-torque sensors (Nano F/T, ATI Industrial Automation) which measure forces in 6-directions at each opposition (located in 1055 Thorndike Hall) should she wish to study pehension in more detail. The instrument is interfaced to a PC through the serial port and acquisition and data analysis is performed with custom written software (SC/Zoom, Umeå, Sweden and LabView). We also have a Grass stimulator, two electromagnetic position-angle sensing systems (Polhemus Fastrack) for measuring movement of the object (1055 Thorndike Hall), We have an eye-tracker (A-S-L) should we need to examine eye-hand coordination. I will provide Dr. Friel with office space and a computer. We have full-time secretarial support, as well as access to both electrical and machine shops. We recently obtained an Major Instrumentation Grant from the National Science Foundation to further upgrade these facilities as needed.

I have mentored four predoctoral students (S. Duff, postdoc at Penn State, L. Muratori and E. Lamberg, Associate Professors of PT at SUNY Stony Brook and J. Charles, Assistant Professor of PT at Emory University). I have also mentored four postdocs (A. Rao, Assistant Professor of PT, Columbia University College of Physicians and Surgeons, R. Reilmann, Assistant Professor of Neurology, University of Munster, I. Salimi, Research Scientist, Columbia University College of Physicians and Surgeons and E. Rabin, Assistant Professor, New York Institute of Technology). Presently I have two postdocs in my lab (T. McIsaac and F. Albert), one physician scientist working with me on a K award (P. Raghaven) and three doctoral students. I have high expectations that the training will also be successful for Dr. Friel and I am very excited about the proposed studies and the support of Dr. Friel's scientific career.

Sincerely,

Andrew M. Gordon, Ph.D.
Professor
Biobehavioral Sciences
Teachers College

**Statement of Co-Sponsor, Sarah H. Lisanby, M.D.:**

I am delighted to serve as a co-sponsor of Dr. Kathleen Friel's proposed research project. I am an Associate Professor of Clinical Psychiatry and the Chief of Brain Stimulation and Therapeutic Modulation (BSTM) Division at Columbia University and the New York State Psychiatric Institute. I have previously served as mentor to successful K-awardees on applications of transcranial magnetic stimulation (TMS). My role as co-sponsor on her K will be to provide mentoring and support for the TMS portions of the project. This will include extensive training in theoretical and methodological aspects of TMS. She will participate in our weekly hands-on TMS training sessions, and our three-day, in-depth course on TMS as part of Columbia University's Continuing Medical Education program. I will ensure that Dr. Friel takes full advantage of these and other training opportunities offered by my Division. Additionally, I will meet with Dr. Friel weekly during the course of her K award, providing one-on-one supervision of her training and progress of her study. I will make available to Dr. Friel our considerable resources and expertise in TMS, including our human TMS labs where the subjects will be studied, equipment and software to collect and analyze TMS measures of motor cortex excitability, and our frameless stereotaxic equipment, which she will need for detailed motor cortex mapping. I will also ensure the availability of the medical supervision for the TMS sessions, including ensuring adequate medical screening for seizure risks factors and proper monitoring during TMS sessions.

Dr. Friel is an outstanding candidate for this career development award. She received excellent training in the world-renowned stroke model laboratory of Randolph Nudo, Ph.D. at the University of Kansas Medical Center. In Dr. Nudo's laboratory, she mastered motor mapping (intracortical microstimulation) and primate motor behavioral training techniques. She published four first-author manuscripts in high-quality journals from her doctoral work, an exceptional number of publications to arise from a doctoral thesis in primate research. She was a co-author on an additional seven articles during her graduate career, demonstrating her finesse for collaborative research. She presented her work at national conferences each year of her graduate studies. She received a predoctoral NRSA from NINDS to fund her graduate work.

In 2002, after receiving her Ph.D., she began a postdoctoral fellowship in the laboratory of John Martin, Ph.D. in the Center for Neurobiology and Behavior at Columbia University. Dr. Martin is a recognized leader in the study of motor control and motor development. His laboratory is the foremost lab for the study of corticospinal development in an animal model. His work is consistently published in top scientific journals. Dr. Friel has been highly productive in Dr. Martin's lab, earning both an institutional and an individual NRSA to fund her work. She has published two first-author papers in top journals and has submitted a third. She has presented her work each year at national conferences.

Dr. Friel demonstrates an outstanding potential for scientific excellence. Working closely with her on this submission, I have been very impressed with her productivity and her drive. Her proposed K award project is creative and scientifically important. She has built a solid transition plan to carry her superb basic science skills toward understanding a clinical issue. Her proposed plan to study CP mechanistically in both an animal model and in patients is highly likely to yield valuable insights into treatment of CP. She has demonstrated the ability to carry out thoughtful experiments in animals, and I am confident that she will be very successful in applying her expertise to the clinical issue of CP rehabilitation. She has shown a great capacity for quick learning and insightful interpretation of results. I am excited to serve as her co-sponsor and confident in her ability to complete a successful research plan. I am equally eager to mentor her continued career development, as I am confident that she will be a leader in neurorehabilitation research.

Sincerely,

Sarah H. Lisanby, M.D.
Associate Professor of Clinical Psychiatry
Chief, Brain Stimulation and Therapeutic Modulation (BSTM) Division,
Columbia University and New York State Psychiatric Institute.

**Statement of Co-Sponsor, John H. Martin, Ph.D.:**

I am writing as a co-sponsor for Dr. Kathleen Friel, who is applying for a K-01 award. Kathleen joined my lab in 2002 as a postdoctoral fellow; she is currently a research scientist. Kathleen has an enduring interest in the functions of the motor cortex and the corticospinal system in health and disease. Kathleen is a unique researcher because she has expertise at both a cellular and a systems level. Thus, she is well-poised embark on her own research program that takes advantage of the best that several labs have to offer. She is also unique in that she has cerebral palsy. Her decision to pursue a translational research program matches well with her rigorous training in the neurobiology of normal and impaired motor systems function, her remarkable publication record for this stage of her career, and her disability.

My laboratory studies development of the corticospinal (CS) system from a systems perspective. According to my NIH program director, my research is the only NIH-funded program studying this important topic. Our focus over the past 10 years has been to elucidate the role of motor experience and other activity-dependent processes in shaping postnatal CS system development. Our experiments are concentrated primarily at two levels of the system: the primary motor cortex (M1), where the CS system originates, and the spinal motor circuits, where CS tract axons terminate. We take a multidisciplinary approach—using neuroanatomical, electrophysiological, and behavioral techniques—to achieve an understanding of the basic questions of CS system development. One of our principal findings is that activity-dependent competition between the CS systems on each side is a major determinant in establishing the pattern of CS terminations in the spinal gray matter and in early development of motor skills. Recently, one of Kathleen's research consultants, Janet Eyre and her colleagues, has used our model of activity-dependent competition in the cat to explain development of hemiparetic signs in cerebral palsy (Eyre et al, Annals of Neurology, 2007). Thus, our findings not only explain key features of normal development of the CS system in animals, but also have helped to elucidate the mechanisms of development of cerebral palsy.

In addition to studying CS system development, my lab has begun to examine the response of the mature motor systems to spinal and brain injury. While we maintain a strong basic science program, a key thrust of our these projects is devising ways to promote motor function in humans after injury. To this end, I collaborate with several clinical departments. I currently have a neurosurgical, a pediatric neurology, and a rehabilitation medicine resident in the lab. An attending neurosurgeon is collaborating on one of our spinal injury projects, as is a visiting scientist from Japan who received his PHD in developmental neuroscience and an MD in rehabilitation medicine. Thus, the laboratory environment fosters a unique translational perspective in the context of understanding the basic science of motor systems function and repair. I fully expect that Kathleen will take advantage of the diverse clinical expertise in the lab, not only while she is conducting the cat experiments, but during the human studies as well.

Kathleen and I have worked closely over the past few years and we will continue to do so during the cat experiments. We will meet several times each week to discuss the experiments

informally, and weekly, on a more formal basis with our collaborators. When she completes the proposed cat experiments, we will continue to meet weekly with Drs. Gordon and Lisanby. While we have not yet formally collaborated with Dr. Janet Eyre and her colleagues in Newcastle, I have discussed our research program and findings with her on several occasions, including a visit to her laboratory several years ago. If Kathleen's application is funded, we plan an initial visit by Dr. Eyre at Columbia as we set up to use TMS in children. We hope to have Dr. Eyre return yearly as her schedule permits. Kathleen will visit Dr. Eyre's laboratory yearly to gain experience with TMS experimental design, implementation, and analysis. The findings of Eyre and colleagues in humans have be important in shaping how we design and interpret our cat experiments. Conversely, our findings in the cat have been critical for interpretation of Dr. Eyre's findings in the human.

I have mentored two MD-PHD students, one is on the neurology faculty at the Cleveland Clinic/Case Western University Medical School (Dr. Scott Cooper) and the other one has just started his residency (Dr. Lucas Campos). I am currently mentoring a third MD-PHD student. In addition, I am mentoring a third year pediatric neurology resident who is supported by a K-12 Neurological Sciences Academic Development Award). I have mentored an additional seven postdoctoral fellows.

My research program in CS system development has received continuous NIH funding since 1997. I am beginning the third year of my 5 year RO1. All of our developmental studies have been published in major general neural science journals. As the field is recognizing the importance of our work, I have been invited to write several review articles on CS system development and spinal injury repair. In addition to NIH funding, I have two spinal/brain injury-related grants from the New York State Spinal Cord Injury Research Board. With this funding, my laboratory has more than an adequate support staff (research technicians, histologist) and all of the necessary equipment to conduct and analyze the proposed neuroanatomical and neurophysiological studies.

Sincerely,

John H. Martin, Ph.D.
Professor
Center for Neurobiology and Behavior
Columbia University

**Statement of Research Consultant, Janet Eyre, M.D.:**

I am writing to express my eagerness to serve as a Research Consultant for Dr. Kathleen Friel's proposed research project. I am a Professor of Paediatric Neuroscience at the University of Newcastle upon Tyne in England. I am a pediatric neurologist and am Head of a Research Laboratory in Newcastle upon Tyne which investigates the development of the corticospinal system in man and its plasticity after perinatal brain injury. I have 25 years experience working with children with CP in clinical and research capacities. Dr. Friel's research plan presents an exciting opportunity to study plasticity of the motor system in children with CP after rehabilitation. This research matches my own research and clinical interests, and therefore I am excited to be involved in this project.

In my laboratory, I have made extensive use of TMS and electrophysiological recordings to study motor system development in neurologically healthy children, in children with a CP diagnosis, and in children at risk for CP because of neonatal trauma. I have also studied corticospinal function and axon guidance in humans with a mutation in L1 cell adhesion molecule (L1CAM), a mutation that causes leg spasticity and other motor deficits. I have published more than 50 articles about development and function of the corticospinal system in humans. My work parallels the work of Dr. Martin quite well. Our work in the human has uncovered developmental principles that Dr. Martin has also shown in the cat.

I am extremely experienced in conduction of TMS studies in children, from neonates up to young adults. Performing TMS on children, particularly children with CP, requires sensitivity toward issues of spasticity, cooperativity, anxiety, and boredom. Although Dr. Lisanby, a co-sponsor of this grant, and her research group is also sensitive to the procedures required for TMS in children, my expertise will be extremely helpful to Dr. Friel, especially as she begins her TMS work. I will travel to New York during the first TMS sessions for the first cohort of children in the proposed project, to be present as an advisor during the experimentation. Additionally, I will host Dr. Friel in my laboratory for one week each year of the project. Dr. Friel's visits will be coordinated with my research schedule so that she will be present to observe and assist in TMS studies in my laboratory. Since I work with a very large number of patients, Dr. Friel will have the opportunity to observe studies on children of varying age and severity of disability. During her annual visit, Dr. Friel and I will also discuss her progress, data analysis, and study design. In addition, we will be in contact throughout the year by phone and electronic mail to discuss data, analysis, methodological issues, and career development.

Sincerely,

Janet Eyre, M.D.
Professor
Paediatric Neuroscience
University of Newcastle upon Tyne in England.

## 3. ENVIRONMENTAL AND INSTITUTIONAL COMMITMENT TO CANDIDATE

### A. Description of Institutional Environment

See letter below (B).

### B. Institutional Commitment to Candidate's Research Career Development

I am writing to describe the institutional support behind Dr. Kathleen Friel's application for a Career Development Award to Promote Diversity in Neuroscience Research. Along with other faculty of the Center for Neurobiology and Behavior, I have absolutely no reservation that Dr. Friel can successfully complete the proposed project. She will benefit from a first-class training experience. Having had excellent training and experience in mechanisms of post-stroke recovery in motor cortex, Dr. Friel came to Columbia with superb credentials. She applied her skills to study the effect of neural activity on plasticity of corticospinal projections in an animal model of cerebral palsy (CP). However, she realized that to become a versatile scientist in translational CP research, she needs training in non-invasive cortical stimulation and in human subjects research. She was promoted to Associate Research Scientist and encouraged to apply for a K01 award.

Dr. Friel's training will be based in three exceptional research environments: the Center for Neurobiology and Behavior: the Brain Stimulation and Therapeutic Modulation (BSTM) Division of the New York State Psychiatric Institute (NPYSI) both at the at the Columbia University Medical Center (CUMC); and the Movement Sciences Laboratory and Center for Cerebral Palsy Research at Teachers College (TC). CUMC is one of the world's foremost centers for patient care, research and health sciences education. The Center for Neurobiology and Behavior is widely recognized as a first rate basic neuroscience research organization. The NYSPI has been one of the leading institutions for studies of the biological basis of brain function for over 100 years. TC has been a pioneer in research related to physical activity. It is ranked # 1 in U.S. News & World Report's latest ranking of U.S. graduate schools in education. The Movement Science and Education/Kinesiology program at TC has a tradition of excellence, focusing on behavioral, biomechanical and neural bases of development, posture, locomotion, reaching and prehension, with emphasis on elucidating the neural basis of movement disorders. The BSTM Division focuses on use of emerging electromagnetic approaches to modulating brain function. Under the dynamic leadership of Dr. Lisanby the division has an international reputation in excellence in non-invasive brain stimulation. Dr. Friel's research will bridge these centers, a unique opportunity to study CP rehabilitation from mechanistic and clinical angles.

Dr. Friel's mentors, Drs. Gordon, Lisanby and Martin, are regarded as experts in their research areas. Each has an outstanding record of publications, grant support and mentoring. Dr. Friel's training under their guidance will greatly enhance her ability to become competitive for future positions and grant support and to successfully pursue a career in translational research. Dr. Friel's mentors each provide a unique and critically important component of her training. Dr. Martin is a superb neuroscientist, with an impressive history of research in motor control mechanisms and plasticity in animal models. He is adept in neuroanatomical, electrophysiological and behavioral techniques. Dr. Lisanby will provide state-of the–art expertise in brain-stimulation techniques. She is the President of the International Society for Transcranial Stimulation. Dr. Gordon has extensive experience in human studies of CP and has an emphasis on arm/hand function in CP with the goal of developing the next generation of rehabilitation practices. He received the 2007 Gayle Arnold Award for Best Scientific Paper from the American Academy for Cerebral Palsy and Developmental Medicine.

Dr. Friel's research home will continue to be based in Dr. Martin's lab in our Center for Neurobiology and Behavior, where she will benefit from interactions with Claude Ghez, a motor control expert. Over the past six years we have recruited many faculty in systems neuroscience. We recruited Mickey Goldberg, who heads the Mahoney-Keck Center for Brain and Behavior, devoted to the systems-level study of mammalian cortex. We have a new Center for Theoretical Neuroscience, co-led by Larry Abbott and Ken Miller. Miller has made pioneering contributions to the field of activity-dependent cortical development. Other members of the Center of Neurobiology and Behavior with strong interests in systems neuroscience include Eric Kandel, Richard Axel and Joy Hirsch. Our Center has a strong tradition in developmental neuroscience, with Tom Jessell, Jane Dodd, Carol Mason, Peter Scheiffele and others. This aggregation of talented, collegial investigators provides a rich environment for Dr. Friel's further growth as a neuroscientist.

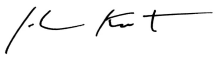
Dr. Friel has proposed several training opportunities, particularly related to the human subjects work of Aim 2. She will deepen her training in biomechanics and kinematics by taking two courses at TC: *Biomechanical Analysis of Human Movement* and *Kinematic Lab*. Dr. Friel will also be trained in transcranial magnetic stimulation (TMS) by Dr. Lisanby and her staff. She is enrolled for a CME course in TMS, offered by the BSTM Division in November 2007. These training activities will broaden her knowledge base and enable her to effectively apply her expertise to patient populations.

Dr. Friel currently holds the position of Associate Research Scientist, a title that allows her to apply for R-series grants. Her mentors and I will encourage and assist her in applying for an R grant during her K award. Dr. Friel will spend 100% of her time performing research and training activities. I commit to providing Dr. Friel this protected time. She will be exempt from teaching during her award.

The requirement for the Career Development Award to Promote Diversity in Neuroscience Research is that the candidate has a disability. Dr. Friel has CP, which seriously impairs motor skills. No physical accommodations are needed. While she cannot independently perform surgeries proposed in Aim 1, the lab staff provides assistance. Her CP will not impair her ability to perform human research (Aim 2) independently. We have no other scientists with disabilities in our center; the number within the university is extremely low. The number of scientists with disabilities nationally is also low. Award of this grant will promote diversity within our center, our university, and nationally.

Dr. Friel is bright, energetic, and brimming with creative ideas. She clearly has the potential to make major contributions to the study of the etiology of, and therapeutic approaches to, CP. I urge you to help her take the next step in fulfilling her potential by honoring her with a Career Development Award to Promote Diversity in Neuroscience Research.

Sincerely,



John Koester, Ph.D., Professor of Clinical Neurobiology and Behavior (in Psychiatry), Acting Director, Center for Neurobiology and Behavior

Caring
Healing
Educating
Student
Health Service
at Columbia University Medical Center

60 Haven Avenue New York, NY 10032
Phone (212) 305-3400 Fax (212) 342 3955

February 6, 2006

To Whom It May Concern:

Kathleen Friel is currently under my care. She has been diagnosed with cerebral palsy.
If you any questions please feel free to contact me.

Sincerely,

Sarah A. Werbel, M.D.
Primary Care Physician
NYS license # 222783

## 4. RESEARCH PLAN

### A. SPECIFIC AIMS

Activity-dependent competition drives development of the corticospinal (CS) system during early postnatal life. Animal studies have demonstrated that imbalance of activity between the motor cortex (M1) in the two hemispheres causes aberrant CS circuit formation and motor impairments[5, 6, 8, 9]. Rebalancing M1 activity on the two sides later in development restores normal CS connections and motor function[10]. In human **hemiplegic CP**, a neurological disorder characterized by poor motor control, motor areas become damaged during perinatal development. The damage tends to have a unilateral predominance. Decreased CS system activity on the affected side is thought to be crucial to development of hemiplegic CP. I hypothesize that restoring balance between the activity of M1 on each side is essential for restitution of normal CS circuitry and motor skill.

Treatments for CP are few in number and limited in efficacy. One treatment effective in improving motor function in children with hemiplegic CP is **training of the affected side with concurrent restraint of the unaffected limb**. During treatment, children engage in skilled, repetitive movements with the affective side, while the unaffected side is restrained with a sling. This behavioral treatment **balances M1 activity** by increasing activity of the affected side through training while decreasing activity of the unaffected side through restraint. The proposed work will also examine how different intensities of skill training influence recovery. The important issue of whether training of movements focusing on precise distal control and shaping of increasing skill difficulty is more effective than repetitive task performance without increasing skill difficulty has not been addressed. Understanding the mechanisms underlying behavioral improvements is an important step towards developing new therapies that can be applied to a broader population of children with hemiplegia, especially those with more debilitating impairments who have the greatest need for an effective treatment. This proposal takes a **translational approach** to the study of CP rehabilitation, built upon an understanding of the development of the cat motor system. The applicant will obtain training in TMS, human motor skill assessment and analysis, and translating hypotheses between the animal and the human. By understanding the mechanisms of recovery, **particularly the interplay between training and CS system plasticity in recovery**, the applicant will be in the unique position to translate these mechanisms to improve CP treatment.

This proposal has two specific aims.

Specific Aim 1: **To determine in a cat model the effectiveness of balancing interhemispheric motor cortical activity in reinstating normal anatomy, physiology, and behavior of the motor system after unilateral activity blockade during development.** I will use neuronal tract tracing, kinematic motor skill assessment, and intracortical microstimulation to determine changes in the structure and function of the cat CS system after unilateral M1 activity blockade during a critical period in CS development (PW 5-7) (to model CP) and subsequent intensive use of the affected limb with restraint of the unaffected limb (to model CP rehabilitation). I will determine the effectiveness of **restraint of the unaffected limb** from PW 7-11 or **training of the affected arm plus restraint of the unaffected limb** from PW 7-11 on driving restoration of motor skill. I hypothesize that concurrent increased use of the affected forelimb and restraint of the unaffected forelimb after the activity blockade will drive recovery of stepping and reaching behavior, CS connectivity, and the M1 motor map. I hypothesize that training of the affected arm will produce more robust restorative changes in motor skill, CS connectivity, and the motor map than restraint alone.

Specific Aim 2: **To determine in children with hemiplegic CP the effectiveness of training of the impaired arm at varying skill intensities, along with concurrent restraint of the unimpaired arm, in reshaping the cortical motor map and improving motor skill.** I will use transcranial magnetic stimulation (TMS) to determine changes in the excitability of the CS system after a 3-week period of unimanual practice. Two training protocols will be compared to understand the influence of skill intensity on recovery: 1) progressive increases in task difficulty, and 2) repetitive task performance without skill progression. Aim 2A: I will determine the size of the arm representation in ipsilateral and contralateral M1. Aim 2B: I will establish if improved motor outcome correlates with changes in activation thresholds. I hypothesize that the TMS map in the contralateral motor cortex will expand and that motor response thresholds contralateral to the affected limb will decrease, indicating a key role for the contralateral motor cortex and its descending projections in recovery.

I predict that these TMS changes will correlate with improvements in manual skill. I expect TMS changes and motor skill improvements to be more robust in the group trained with progressive increases in task difficulty.

## B. BACKGROUND AND SIGNIFICANCE

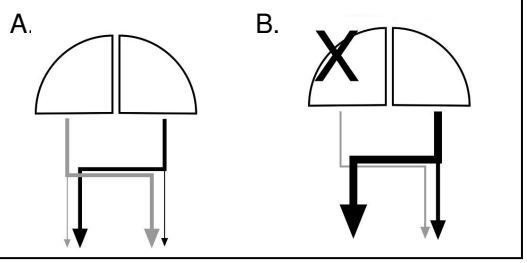
Rehabilitation for people with CP is limited. Efficacy is modest, at most. There is a critical need for more effective treatment options. The goals of the proposed research are to determine the mechanisms of CS system plasticity and recovery in an animal model of CP and in children with CP. We seek to identify the sites of plasticity in the human brain in response to intensive training of the impaired arm, as well as to determine the mechanisms of CS system plasticity and recovery after unilateral loss of activity in an animal model and in children with CP. Understanding the neurobiological bases of improvement in motor outcomes after rehabilitation and the age at which it is most effectively administered will guide the development of future therapies.

*Development of the corticospinal system*

The CS system is the principal system for controlling skilled voluntary movements. Remarkably, CS system development in the cat closely matches that in the human, making the cat an excellent model for studying CS development (for review, see [11]). The CS system in both species is the last pathway to develop, and develops postnatally. In humans, the CS system develops over the first several years of postnatal life, while in cats, the time course is on the order of 2-3 months[11, 12]. Understanding the development of the CS system is essential for devising effective interventions for CP.

Prenatally, in cats and humans, CS projections grow to their targets in the spinal cord. At that time, CS axons have a broad distribution in the grey matter of the spinal cord. At this time in the neonatal human, unilateral TMS evokes bilateral motor responses[12]. As cat development proceeds postnatally, the distribution of CS tract axon projections is pruned to produce the mature, predominantly contralateral distribution of CS axons. At the same time, axon terminals branch and form functional synapses with spinal neurons. In the human, bilateral TMS responses are also shaped into mostly contralateral effects beyond age 1 year[12].

Figure 1. **Competition model of CS circuit formation.** A) Normal CS development: bilateral M1 activity drives formation of a predominantly contralateral CS projection, elimination of most ipsilateral projections. B) Loss of M1 activity on one side results in a weak CS projection from the inactivated side and a dense *bilateral* projection from the active side.




The processes of axon pruning, terminal branch proliferation, and synapse formation are dependent on **activity in motor cortex** (M1) in the cat. Unilateral silencing of M1 activity during a critical period in postnatal development markedly affects the structure and function of the CS system[8, 9, 13, 14]. After M1 inactivation during a 2-week critical period, the density of CS projections from the inactivated M1 to the normal target region in the spinal cord is substantially reduced[14]. These axons have fewer branches and presynaptic sites than controls[14]. Unilateral M1 inactivation also causes permanent deficits in motor skill[6]. In hemiplegic CP, refinement of CS connections does not occur normally; TMS weakly activates the damaged side while the undamaged side maintains bilateral terminations[15].

CS axons from the active M1, in contrast, maintain dense *bilateral* projections. **Competition between the two sides** appears to drive the development of the CS system in the cat and the human. In the cat, when both sides of M1 have normal activity levels, or, interestingly, when activity is blocked on both sides, the normal, predominantly contralateral projection pattern develops[9]. However, if activity is lost on one side during development, the transient ipsilateral terminations from the active side are maintained into maturity.

Recent studies in the cat have revealed the remarkable possibility that the anatomical and motor behavioral consequences of perinatal M1 activity blockade can be ameliorated. Development of the CS system, as described above, appears to be driven by **activity-dependent competition** between the two hemispheres. If activity is lost on one side during a critical period, the active side takes over and maintains synaptic space of the inactive side. If activity of the two sides is **rebalanced**, either by inactivating the active side later in development[10], or electrically stimulating the inactivated side[16], CS axons from the initially inactivated side regain a **normal topographic distribution** and **normal number of synaptic contacts**. Importantly, rebalancing activity on the two sides

**restores motor skill** and **restores the cortical motor representation of the affected limb**. This has important implications for the treatment of hemiplegic CP; increasing the activity of the damaged motor system may promote recovery of CS system structure.

Rebalancing activity on the two sides in the cat led to recovery via reorganization of the *initially* inactivated side. Unilateral cortical inactivation resulted in a permanent exuberance of ipsilateral (double-crossed) CS projections from the active side. However, without intervention, there was a permanent behavioral deficit[6], despite the long-lasting presence of ipsilateral projections from the active side. This suggests that ipsilateral projections from the unaffected cortex **do not mediate recovery**. When motor skill was restored via rebalancing M1 activity, ipsilateral projections from the active cortex were pruned back, indicating that recovery is mediated through projections from the initially inactivated cortex rather than ipsilateral control by the active side.

*Use of transcranial magnetic stimulation to non-invasively probe aberrant motor system development in CP*

TMS is a non-invasive method of stimulating the brain as a probe to study connectivity or as a putative therapeutic agent[17, 18]. Pulsed magnetic fields applied to the head with a stimulating coil induce eddy currents in underlying cortex, which at sufficient strength induce neuronal depolarization and induce action potentials. TMS applied to primary motor cortex can activate the cortical spinal tract and induce motor evoked potentials in the target muscle represented by the region of the motor strip stimulated. TMS has been used extensively to study plasticity in the motor system in healthy subjects after motor learning[19-22], after central nervous system injury and after rehabilitation[19, 23-26].

In human **hemiplegic CP**, motor areas become damaged during perinatal development. The damage tends to have a unilateral predominance. Effects of hemiplegic CP on the functional anatomy of the motor system appear to be similar to what is seen after unilateral M1 inactivation in the developing kitten using axon tracing techniques. Perinatal brain damage, as occurs in hemiplegic CP, results in disuse of the side of the body contralateral to the damage. A maladaptive circular pattern is set into motion: asymmetry in limb use causes malformation of CS circuitry, which further debilitates the affected limb. Appropriate connections may never develop and aberrant connections may form.

Eyre and colleagues have used TMS to examine the functional organization of the motor system in patients with hemiplegic CP[12, 15]. TMS of the undamaged M1 resulted in bilateral muscle activation, suggesting that the **undamaged M1 maintains bilateral projections**. The strength of the ipsilateral response from the undamaged side increases with age, rather than showing the normal age-dependent decrease, and are strong in maturity. TMS responses from the damaged M1, alternatively, are unilateral and weaker than normal in young children and decrease further with age[12]. Commonly, TMS of the damaged side fails to evoke any response in maturity[12].

The differential contributions of contralateral projections from the damaged side vs. ipsilateral projections from the undamaged side in recovery are not known. Data from Janet Eyre's laboratory have used TMS to show a concurrent development of hemiplegic symptoms and short latency ipsilateral projections from the undamaged side, suggesting that the ipsilateral projections do not mediate recovery[12]. Evidence from the cat model shows that recovery mediated by rebalancing activity is accompanied by restoration of the CS projection from the initially inactivated side and a decreased size of the ipsilateral projection from the initially active side, indicating that the ipsilateral projection may not mediate recovery[10].

Upper *extremity function in children with hemiplegia*

The paucity of contralateral TMS evoked responses from the damaged cortex and the bilateral responses from the undamaged side point to aberrant CS tract connectivity in the spinal cord that could underlie aspects of the motor impairment in CP patients. Fine motor control of the fingers and hand is controlled by the motor cortex and CS pathways[27, 28], which are often disrupted in individuals with CP[29-31]. As a result, skilled independent finger movements do not develop normally. There is often abnormal hand posturing and a reduction in distal strength and dexterity[32].

People with hemiplegic CP tend to use their non-affected arm and hand for most motor tasks, and, consequently, tend to underutilize their impaired extremity. Similar behaviors are seen in people who have had unilateral strokes or sensory loss. The impaired extremity tends to be underused long-term. This phenomenon has been termed **learned nonuse**[33]. Persistent neglect of one limb and concurrent overuse of

the other arm creates an imbalance in activity between the two sides. The more-active side may gain a competitive advantage over the neglected side during development. This competition can drive the CS tract of the less-used side into *permanent* dysfunctional organization and, consequently, produce *permanent motor impairments*. Children with hemiplegic CP tend not to use their impaired arm and hand throughout life; disuse of a limb during postnatal development can stunt the activity-dependent structural and functional development of the motor system[34]. Enabling young children to more readily use their affected extremity is likely to increase the competitive ability of the affected side, thus improving the functional and anatomical integrity of the motor system.

*Plasticity of motor cortex in response to injury and rehabilitation*

Although it was once believed that brain plasticity only occurred during development, it is now well-established that the motor representation in M1 is plastic throughout life, and plasticity can be driven by learning or injury to the brain or the periphery (review[46]). Initially after brain injury, cortical area inside and immediately surrounding a lesion loses the ability to drive voluntary movements. **Intensive practice with an affected extremity** causes adaptive changes in the motor map, restoring some function to the cortex. This has been studied in animal models of stroke and in human stroke patients. Glees and Cole (1950) first hypothesized that in the adult, cortex surrounding a lesion takes over function that was lost by the lesion[47]. When this issue was reexamined using more precise mapping procedures in monkeys, it was found that movement representations lost by the lesion did not reappear in adjacent cortical tissue[48] unless subjects received daily motor skill training using the *impaired* limb[49]. Restraint of the unimpaired arm without concurrent training of the impaired hand *failed to restore the motor map*[2]. These studies point to the role of **intensive practice with the affected extremity** in driving restorative changes in the mature motor system.

Motor cortex reorganization occurs after stroke in adult humans[23, 24, 26]. Cortical stroke typically results in a 50% shrinkage of the motor map in the affected hemisphere[48]. Intensive training of the affected extremity produced an expansion of the motor cortical representation contralateral to the affected hand, as shown by TMS [23, 24, 26]. Training-induced recovery in **hemiplegic adult stroke** appears to be mediated by cortical areas on the same side as the stroke. After a period of intensive training of the affected arm, there is an expansion of motor cortical territory contralateral to the trained hand; that is, in the same hemisphere as the stroke, as revealed by TMS[23, 24, 26]. Human and animal studies suggest that secondary motor areas, such as premotor cortex and supplementary motor cortex, in the **affected hemisphere**, are important in mediating recovery[50-52].

Few studies have examined plastic changes in motor areas following rehabilitative interventions in hemiplegic CP. In contrast to adult stroke, which occurs after the CS system has developed into its predominantly contralateral pattern, CP occurs during CS system development, when the CS system still maintains a bilateral projection pattern. Thus, there exists the capacity of ipsilateral connections from the undamaged M1 to remain and mediate recovery. As described above, TMS studies indicate a persistence of ipsilateral projections from the undamaged side in hemiplegic CP. An important, unresolved issue is whether these connections mediate recovery, or if the CS projections from the damaged side mediate recovery.

*Intensive practice in the treatment of CP and stroke*

Stemming from the observation that people with unilateral neurological damage tend to underuse their affected extremity, an effective therapeutic intervention encourages repetitive, skilled use of the affected extremity. Intensive practice with the affected extremity is encouraged by restraint of the less- or non-affected extremity. While the purpose of the restraint is to prevent the patient from using the unaffected or less-affected extremity, thereby focusing on intense practice with the affected arm, the restraint likely leads to less activation of motor centers controlling the unaffected limb. Importantly, in hemiplegic CP patients, this could reduce activity in the aberrant ipsilateral CS projection.

Intensive practice has produced substantial, long-lasting recovery of function in adult stroke patients. Significant improvements in motor function were seen after a 2-week period of training. Improvements were maintained throughout a two-year follow-up period[35-37]. This is a remarkable finding, that two weeks of intensive training is sufficient to produce significant, long-lasting improvements in motor skill[38-41]. This points to the tremendous plasticity of the nervous system, even in older adults.

Intensive practice also improves functional use of the impaired upper extremity in children with hemiplegic CP. One training paradigm, unilateral training of the affected arm, focuses on extensive practice with the

affected arm[42-44]. Children visited the clinic for six hours per day over ten consecutive weekdays (60 hours total). During the 60 hours, children wore a soft sling over the less-affected arm, thereby discouraging use of that arm. Concurrently, children engaged in intensive practice with the affected arm. Children played games and performed tasks that incorporated shaping and repetitive practice into the activities. Motor skill assessments were taken before and one week, one month and six months after the ten-day intervention period. Intensive unimanual training improved motor performance, and this improvement was maintained six months after training. This improvement is maintained 12 months later, and further improvement occurs with subsequent additional training[45]. As described above, the nervous system is plastic throughout life. It has been shown that intensive training of the impaired arm produces the same degree of recovery whether children are younger (4-8) or older (9-13)(Gordon 2006). Training of the impaired arm improves motor skill in patients with stroke or CP, regardless of the age of the patient.

*Translational approach of the proposed work*

This project will examine the role of balancing interhemispheric motor activity in recovery after unilateral motor cortex activity blockade in an animal model and in children with hemiplegic CP. The two aims of this project explore parallel issues in the animal vs. the patient. Both aims will examine recovery-induced motor cortical plasticity. This important knowledge will help guide the development and targeting of hemiplegic CP therapies. Both aims will also investigate the power of skill training in promoting recovery. In both aims, animals or subjects will use their affected arm in tasks (postural support in the cat; repetitive practice in the child) or higher skill (reaching and grasping in the cat and human) tasks. Understanding the effect of skill on recovery is highly clinically relevant— if, in the animal or the human, skill is found to produce more robust recovery, it would suggest that future therapies for hemiplegic CP should involve as high-skilled training as patients can tolerate. This project will give me the exciting opportunity to mechanistically understand recovery in an animal model, which lends itself to neuroanatomical analysis and behavioral regimens beyond what is feasible in a human. This project also allows the direct application of hypotheses to a patient population. Together, the experiments proposed will give me a unique mechanistic and clinically relevant foundation in CP rehabilitation.

*Potential clinical significance of this work*

The importance of determining the location of plastic changes produced by intensive practice is that future therapies could be applied selectively to brain regions most important in mediating recovery. Studying neurophysiological correlates of both unimanual and bimanual therapies is a **key step in understanding the interplay of behavior and cortical plasticity in recovery**. Identifying key loci of plasticity is also important for the development of new therapies. For example, electrical stimulation of motor cortex has promoted recovery in several animal stroke models. Electrical stimulation of CS axons in the cat during normal development and after unilateral inactivation augments CS terminations in the spinal cord. Brain-derived neurotrophic factor (BDNF), which facilitates recovery of motor function after stroke, could be applied locally to the key circuits. TMS could be targeted to the key circuits and applied therapeutically; repetitive TMS has been shown promise in restoring motor function after stroke[53]. Additionally, inhibitory TMS can be applied to the undamaged cortex to balance activity and promote recovery. Such **treatments would be extremely valuable in the treatment of CP**, particularly because they could be applied to patients who otherwise could not participate in intensive-practice rehabilitation, due to young age, severity of deficits, or lower intellectual ability.

## C. PRELIMINARY STUDIES

*Balancing M1 activity of the two hemispheres after unilateral inactivation (PW 5-7) by blocking activity on the active side (PW 7-11)*

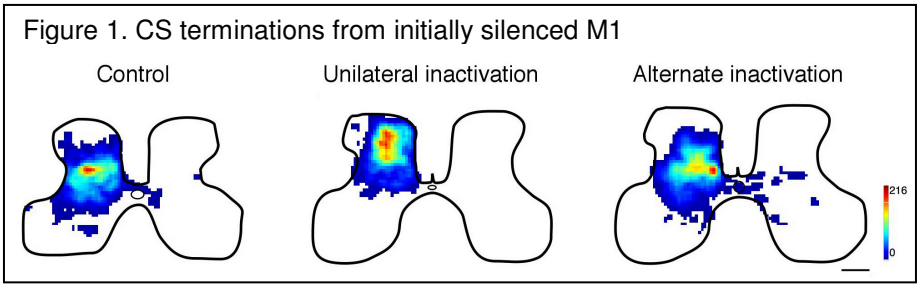
To test the hypothesis that restoration of M1 activity balance plays a vital role in recovery after unilateral activity loss, I first blocked M1 activity unilaterally during the critical period for establishing CS axon terminations in the spinal cord (PW 5-7). Then, I blocked M1 activity on the other side from PW 7-11, which is when CS axon terminations are getter stronger in the cord[54, 55] and the M1 cortical map is developing[56]. I hypothesized that the second inactivation would prevent the second side from securing a competitive advantage over the first side. This study provides the underlying mechanism for the experiment proposed in

**Aim 1.** Constraint of the unimpaired forelimb after unilateral M1 activity blockade, as proposed in Aim 1A, is a behavioral mechanism for decreasing activity of the unimpaired side. Infusing an activity-blocking drug into M1 on the good side produced a similar, albeit more effective, result via a pharmacological means.

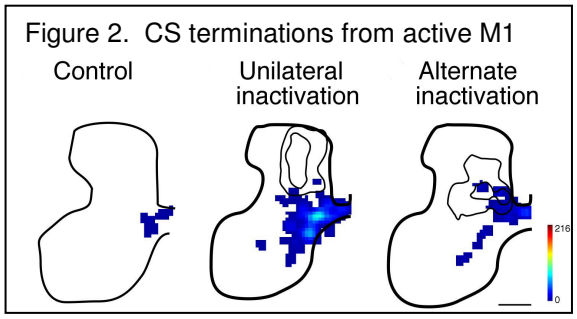
Pharmalogical blockade of the unaffected side is not feasible in humans. Although pharmalogical blockade is not directly translatable to human patients, it uncovered a mechanism of recovery that can serve as a basis for future therapies. Physical restraint of the unaffected arm is one such therapy that applies the principle of *activity balance* to rehabilitation.

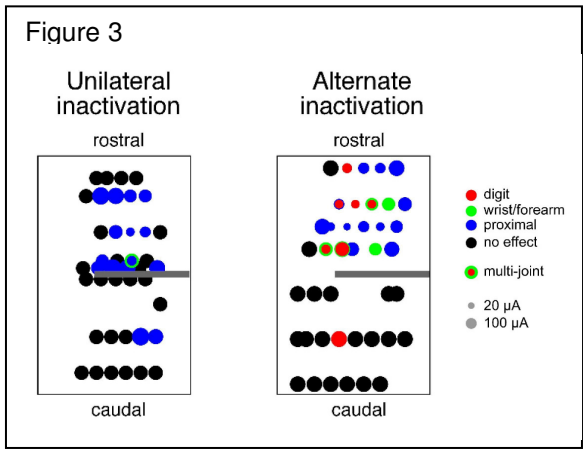
Balancing M1 activity of the two hemispheres restores normal CS circuitry on the initially inactivated side: The topographic distribution of CS axon terminals was measured by tracing label on spinal sections under a light microscope at 200x, using Neurolucida software (Microbrightfield). I tested the hypothesis that balancing M1 activity redistributes CS axon terminals from the initially inactivated side to a normal topographic pattern. Figure 1 shows the distribution of CS terminations in the cervical spinal cord in a control animal, after unilateral inactivation from PW 5-7, and after inactivation of one side from PW 5-7 and the second side from PW 7-11 ("alternate inactivation"). Amounts of axon label have been converted to a color scale (axon label density per $\mu m^2$; scale bar 500µm). In control animals, axon label density is greatest in the motor (intermediate and ventral) laminae (5-7) of the spinal cord. Unilateral activity blockade from PW 5-7 shifts axon label to more dorsal, or somatic sensory, laminae (2-4). Alternate inactivation restores axon label distribution to motor spinal laminae, similar to controls. Axon label density in dorsal (2-4) vs. medial (5-7) spinal laminae was determined and compared among groups.

There was significantly more label in dorsal laminae and less label in intermediate and ventral laminae in the unilateral inactivation group compared to the control group and the alternate inactivation group. There were no statistical differences in axonal distribution between the alternate inactivation and the control groups. Balancing M1 activity on the two sides restored a normal pattern of CS terminations in the spinal cord.




Balancing M1 activity of the two hemispheres reduces the ipsilateral projection from the active side: The topographic distribution of CS axon terminals was measured as described above. I tested the hypothesis that balancing M1 activity reduces the amount of ipsilateral CS axon terminals from the initially active side. Figure 2 shows the distribution of CS terminations in the cervical spinal cord in a control animal (replotted from Figure 1), after unilateral inactivation from PW 5-7, and after alternate inactivation. After the alternate inactivation, the extent of ipsilateral labeling was less than after unilateral inactivation alone. Contours of averaged contralateral axon terminations from the initially inactivated side are superimposed on the regional distribution maps (inner contour: region of densest label; outer contour: region of most labeling). As a percentage of the total area of the ipsilateral gray matter, ipsilateral labeling was significantly less after alternate inactivation, indicating that balancing M1 activity of the two hemispheres reduces the ipsilateral projection from the active side.



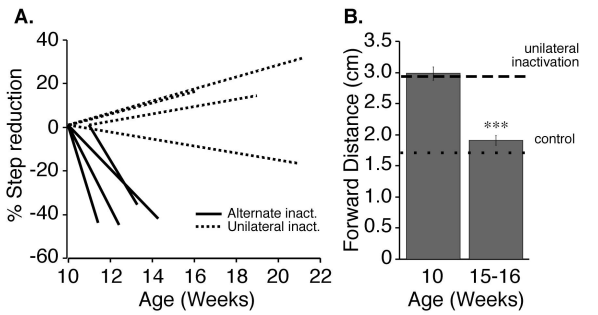




Balancing M1 activity of the two hemispheres restores the M1 motor map: M1 motor representational maps were derived

using intracortical microstimulation (ICMS) as described in the general methods for Aim 1. I tested the hypothesis that balancing M1 activity could restore the motor map to a normal pattern. Figure 3 shows maps of a unilateral inactivation and an alternate inactivation cat, each at age PW 15. Unilateral inactivation abolishes all distal (digit, wrist) responses and decreases the number of proximal (elbow, shoulder) responses. Thresholds were significantly higher than controls.

After alternate inactivation, there was an emergence of digit and wrist representations, particularly in the rostral half of the map. There was an emergence of sites at which movement of multiple joints are represented. Thresholds decreased significantly. Similar results were observed in three other cats. The alternate inactivation map is similar to age-matched controls[56]. Balancing M1 activity restores the motor map.

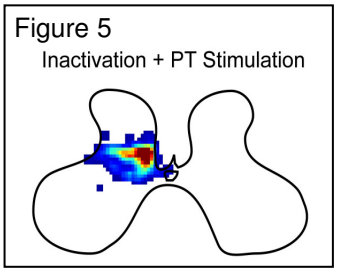
Figure 4




Balancing M1 activity of the two hemispheres restores normal visuomotor skill: Visuomotor skill was tested on a horizontal ladder, using methods identical to those proposed in the present application. After unilateral inactivation, cats show a persistent overstepping on the ladder. Cats stepped by placing the proximal end of the paw (near the wrist) on the rung, rather than placing the paw squarely on the rung like controls. The distance from the forward edge of the rung and the tip of the cat's paw was measured ("forward distance"). To calculate percent step reduction, forward distance at the time of recovery or perfusion was divided by the forward distance at PW 10. Increased step reduction corresponds to a worsening of endpoint control on the ladder task. If cats did not receive intervention after the initial inactivation, overstepping never recovered spontaneously. Figure 4A shows a plot of overstepping after unilateral inactivation (dashed lines; each line represents one cat). Overstepping was tracked many weeks after inactivation (up to week 22). In three of the four cats, overstepping became larger (worse) as a function of time. One cat showed modest reduction in overstepping, but did not return to control levels.

I tested the hypothesis that balancing M1 activity restores normal stepping behavior on the ladder task. Cats readily performed the task after the inactivation. After alternate inactivation (Figure 4A; solid lines), overstepping recovered between PW 11-14. Quantification of forward distance for four alternate inactivation cats is shown in Figure 4B. At PW 10, forward distance of the right paw (contralateral to the first inactivation) was similar to unilateral inactivation (dashed line). By PW 15 or 16, forward distance had significantly improved and was not different from non-inactivated controls (dotted line). Thus, balancing M1 activity on the two sides restored visuomotor skill.

Figure 5



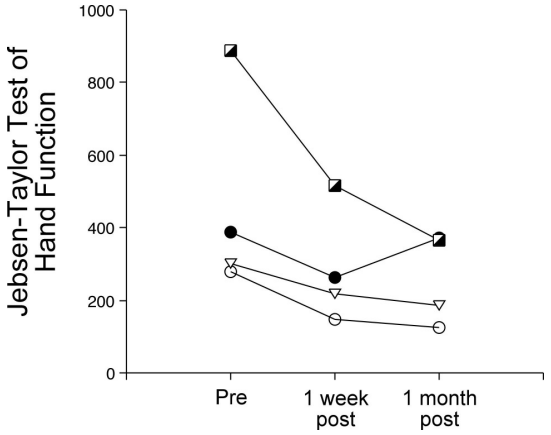

Balancing M1 activity of the two hemispheres by electrical stimulation of the initially silenced M1 restores normal CS circuitry: The topographic distribution of CS axon terminals was measured as described above. In collaboration with Dr. Iran Salimi, a research scientist in the lab, I tested the hypothesis that balancing M1 activity by stimulating descending CS axons in the pyramidal tract (PT) redistributes CS axon terminals to a normal topographic pattern. Figure 4 shows distribution of CS terminations in an animal after M1 activity blockade from PW 5-7 and PT stimulation from PW 7-10. Control and unilateral inactivation animals are shown in Figure 5. PT stimulation results in a shift of axon distribution into more motor laminae, showing that balancing M1 activity on the two sides can shift CS terminations toward a more normal distribution.

*Improvements in hand function after intensive training of the affected arm in children with hemiplegic CP.*

A training paradigm similar to that proposed here has been shown to improve motor performance in children with hemiplegic CP[34-36]. These previous studies have used a 60-hour training period; I expect that the 90-hour training period proposed in this study will produce even greater improvements in motor skill. Preliminary results support the hypothesis that 90 hours of training improves motor performance. Figure 6 shows Jebsen-Taylor Test of Hand Function scores for the affected hand before intervention, one week and one month after intervention. There was a significant effect of time on the Jebsen score, indicating a

significant, lasting improvement in function of the affected hand after intervention (Friedman; $X^2$= 6.5, p = 0.039). Thus, unimanual skill improved during the 90-hour intervention.

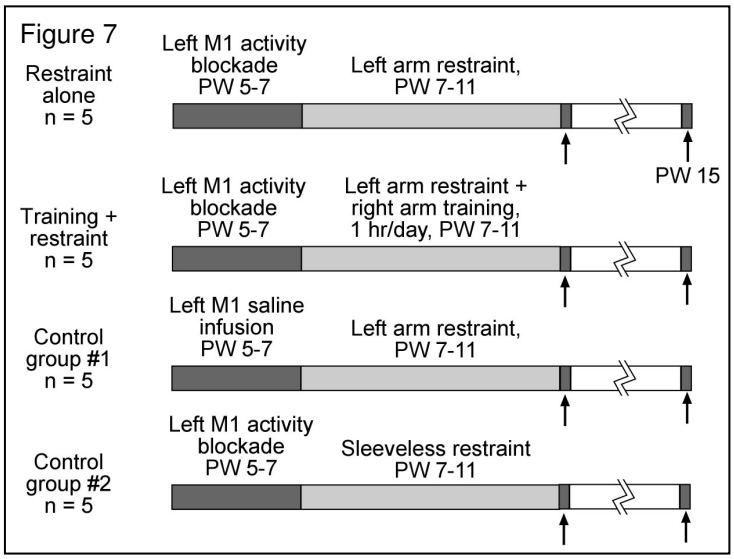
Figure 6




## D. RESEARCH DESIGN AND METHODS

Specific Aim 1: **To determine in a cat model the effectiveness of balancing motor cortical activity in reinstating normal anatomy, physiology, and behavior of the motor system after unilateral activity blockade during development.** This aim will examine recovery after balancing M1 activity via restraint of the unimpaired forelimb or combined restraint of the unimpaired limb and intensive training of the impaired limb. This experiment will test the hypothesis that **increased use of the affected forelimb combined with disuse of the unaffected forelimb enables the previously silenced CS system to increase the extent of terminations and regain normal function**. This aim will also test the hypothesis that training of the affected limb in a skilled motor task will promote more robust recovery than restraint of the unaffected limb alone. An experimental timeline is shown in Figure 7. Activity in M1 will be blocked from PW 5-7 by muscimol infusion. Then, use of the affected limb will be increased either by restraint of the unaffected limb ("restraint alone") or training of the affected limb + restraint of the unaffected limb ("training + restraint"). Two animals in each group will be perfused at PW 11 (Figure 7, arrow 1), to examine the immediate effects of restraint or training on the motor map and CS terminations, while three animals will be perfused at PW 15 (Figure 7, arrow 2), for assessment of the persistence of behavioral, anatomical, and physiological effects. This aim parallels my doctoral research, where I examined the role of unskilled vs. skilled use of the affected arm in recovery in a primate stroke model[2].

Figure 7




In both experimental groups, an osmotic minipump loaded with 10 mM muscimol in saline will be attached to a cannula that will be inserted into M1 of kittens at PW 5. After the pump is implanted, the kitten will be returned to its home cage to develop with its littermates. Muscimol will be infused (0.5 µl/hr, 14 days). The osmotic minipump will be removed at PW 7. Four days after pump removai, a mesh jacket will be placed on the cat. This delay will serve to protect against possible early-overuse exacerbation of deficits (Jones). The cat's left arm will be placed in a sleeve which will be secured to the jacket at the cat's torso. The cat will wear the jacket 24 hours per day from PW 7-11, except during behavioral testing or training (see below). The purpose of the jacket is to require the cat to use the arm contralateral to the previously silenced cortex exclusively.

In the restraint alone group, cats will wear the jacket 24 hr/day aside from behavioral tesitng. The cats will receive no training of the impaired hand. For one hour per day, two consecutive days per week, the jacket will be removed and the animal will be tested on a horizontal ladder-walking task (see General Methods). Cats achieve stable performance after 2-3 sessions of exposure to the task. When cats traverse the ladder, all four limbs are used equally. Thus, testing does not involve the preferential use of one limb vs. another. In the training + restraint group, cats will be trained on a plantar reaching task for 1 hr/day, in two 30-minute sessions separated by at least 4 hr (see General Methods). Cats will be trained to retrieve cubes of meat from a horizontal surface using the affected arm. The jacket will be removed during training so that the cat can use the unaffected arm for postural support during reaching. Cats can be readily trained to reach with the affected

arm by physically blocking access of the unaffected arm to the food reward. Cats will also be tested on the ladder task two consecutive days per week.

Two groups of animals will serve as controls: 1) M1 saline infusion from PW 5-7, left arm restraint from PW 7-11, to control for inactivation, and 2) M1 activity blockade from PW 5-7, sleeveless restraint (which will not restrict use of either limb), to control for limb use.

Anterograde anatomical tracers will be used to label CS axons from each hemisphere. Biotinylated dextran amine (BDA) will be injected into the left M1 and Lucifer Yellow (LY) will be injected into right M1 four weeks prior to the end of the experiment. Two animals in each group will be perfused at PW 11, while three animals will be perfused at PW 15. At the end of the experiment, immediately before perfusion, intracortical microstimulation (ICMS) will be used to derive motor maps of the M1 forelimb representation in Ketamine-anesthetized animals. ICMS maps will be derived bilaterally (similar to Figure 3). Motor response categories (i.e. digit, wrist, elbow, shoulder) and motor thresholds will be noted and compared across groups.

After ICMS mapping is complete, each cat will be deeply anesthetized and perfused with 4% paraformaldehyde. The brain and spinal cord will be removed and processed for BDA and LY on alternate sections. Parasagittal sections through the muscimol infusion site in motor cortex will be stained for nonphosphorylated neurofilament protein SMI-32 to mark pyramidal neurons and parvalbumin (PV), which is a marker for activity [57]. Sections from the inactivated M1 will be compared to homotopic sections from the contralateral M1. Sections will be compared to control animals. SMI staining will determine if muscimol produced a lesion, and PV staining will serve as an assay for activity.

Hypotheses and Expected Results

*Hypothesis 1: Increased use of the affected arm restores CS axon topography and morphology, motor representational maps in M1, and stepping behavior.* These predictions of Aim 1A are rooted in the knowledge that increased use and/or balancing activity has been shown to drive adaptive changes in motor system anatomy, physiology, and behavior[10, 49, 56, 57]. Thus, I expect the proposed experiment to result in a restoration of normal CS terminal distribution and morphology, and a recovery of visuomotor skill. I predict finding g morphological changes in the CS tract on the same side as the inactivation. Results from cat studies in which activity was rebalanced after inactivation by either inactivation of the contralateral M1 or stimulation of the inactivated M1 have shown that balancing activity produces changes in the CS tract from the inactivated side (see Preliminary Data). Ipsilateral projections from the active side do not appear to mediate recovery, as behavioral and motor map recovery are accompanied by a *loss* of ipsilateral projections from the active side.

*Hypothesis 2: Intensive training of the affected arm produces greater improvements in CS axon topography and morphology, motor representational maps in M1, and stepping behavior than restraint alone.* The efficacy of skilled use of the impaired limb in recovery after M1 injury has been extensively documented. In my own doctoral work, I demonstrated that after an M1 lesion in an adult squirrel monkey, restraint of the unimpaired hand alone, without training of the impaired hand, did not promote recovery of the motor map[2]. Since cats, unlike monkeys, are quadapedal animals that will use the affected limb during all moving hours for postural and gait support, I do expect restraint alone to promote recovery (see Hypothesis 1). However, I expect training to provide an additional restorative benefit. Rat studies have shown that after strokelike injury to M1, restraint of the good arm alone provides some rehabilitative benefit, but training increases the recovery[58, 59]. Rats, like cats, are quadrapedal animals that use the unrestrained arm for continual postural and gait support. Although many animal studies have shown that training of the impaired forelimb promotes recovery after stroke, no animal study has yet examine.

***Alternative results and implications:***

No effect of restriction of the unimpaired forelimb and/or training of the impaired forelimb on behavioral recovery, restitution of anatomical connectivity, or motor map restoration. If no recovery is seen during or after treatment in Aim 1, it would indicate that behavioral therapy is highly limited. While this is unlikely, given prior studies in rats and monkeys[4, 49, 58, 59], it would suggest that during development behavior alone may not sufficiently modulate activity-dependent developmental processes. This would suggest two directions. **First**, I could increase behavioral demand by training a more complex task, such as retrieving meat cubes from a 2cm

deep tube, as done previously[60]. **Second**, I could regulate CS system activity by electrical stimulation of the initially inactivated side, as in other experiments in the laboratory (see Figure 5). Pilot experiments show that this normalizes CS axon terminations in the spinal cord, but it is not known if this improves motor performance or the M1 map. Importantly, motor training and CS system stimulation could be concurrent to harness the combined effects of increasing the level of neural activity with stimulation and achieving an appropriate pattern of activity during skilled task performance. If the first 2-3 animals do not show any improvements in these measures, I will re-evaluate the alternatives.

**General Methods for Aim 1**

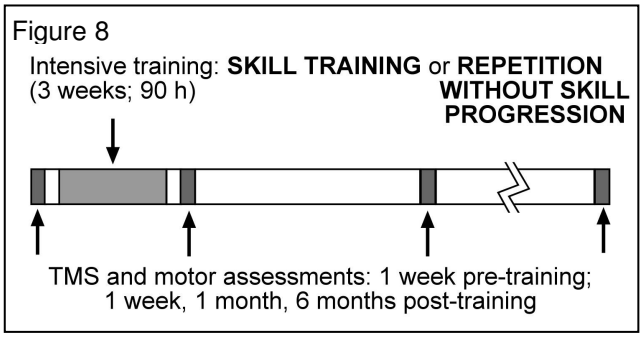
General surgical procedures in kittens: Animals will be administered atropine (0.04 mg IM), tranquilized with Diazepam (0.1 mg/kg IM) and anesthetized with a mixture of Ketamine hydrochloride (25-30 mg/kg IM) and Acepromazine (0.03 mg/kg IM) as needed to abolish reflex withdrawal to pinch of the distal forepaw. Animals greater than 10 postnatal weeks will receive supplemental barbiturate anesthesia, Nembutal (30 mg/kg IV), as needed to abolish reflex withdrawal to pinch of the distal forepaw. Each animal will receive a broad-spectrum antibiotic (i.m.) prior to surgery and will be maintained on a broad-spectrum antibiotic (i.m.) for one week after surgery. For ICMS, anesthesia will be induced with Ketamine IM (25 mg/kg IM) and Xylazine (0.6 mg/kg IM). Ketamine infusion (15 mg/kg i.v.) will maintain anesthesia, supplemented with Xylazine (IM as needed).
Procedures to ensure comfort: The only procedures that cause pain and stress are surgeries. These procedures are unavoidable because the aims of the experiments are to produce changes in the development of the CS system and to monitor these changes using pathway tracing and behavioral techniques. Following surgical procedures, animals do not normally show signs of pain or distress upon awakening from anesthesia. Signs of distress may include weight loss, reduced mobility and lack of grooming. Buprenorphine will be administered (0.005-0.01 mg/kg, SC or IM twice during the 24 hours following surgery and then as needed). The depth of anesthesia will be assessed by absence of withdrawal reflexes, pulse and respiration rate.
Implantation of osmotic minipumps: Animals will be removed from their cage and prepared for sterile surgery to implant an osmotic pump (Alzet, ~200 µl capacity) loaded with a 10 mM solution of muscimol in saline. Pumps will deliver ~0.5 µl/hr for 15-19 days. Exact pump reservoir volume and pumping rate vary between individual pump lots. Following implantation, animals will recover and returned to their cage, where they are allowed to develop in the same cage as their mother and littermates. After the infusion period, the minipump will be removed during a second surgical procedure.
Intracortical tracer injections: Animals will be removed from their cage and prepared for sterile surgery. Tracer will be injected intracerebrally under sterile conditions. Following surgery, animals will recover and are returned to their cage. Animals will be sacrificed four weeks after tracer injections, which will allow sufficient time for transport of tracer to the cord. Biotinylated dextran amine (BDA; Molecular Probes) and Lucifer Yellow (LY) will be pressure injected (300 nl) into motor cortex, one tracer in each hemisphere. Injections are centered within lamina 5, which corresponds approximately to a depth of 1.5 mm from the pial surface. Four weeks after tracer injection, animals will be deeply anesthetized and perfused with 4% paraformaldehyde. Standard histochemical (BDA and LY) protocols will be used[14]. Serial spinal sections will be processed. Differential interference contrast microscopy (i.e., Nomarski) and conventional bright and dark field microscopy will be used to analyze labeled axons. Following transport of tracer to the lower cerivcal spinal cord, animals will be given 20 mg/kg sodium pentobarbital and perfused intracardially with 4% paraformaldehyde to prepare the brain and spinal cord for histological examination.
Analysis of CS axon morphology: To characterize the morphology of CS axon terminals, labeled axons will be traced using Neurolucida (Microbrightfield) and differential interference contrast (i.e. Nomarski) optics at 200x or 400x magnification. Nomarski optics will be used because there is a narrow depth of field that allows the user to distinguish overlaying axons by focusing through the field. Neurolucida Explorer (Microbrightfield) will be used to obtain measures of axon length, branch number, and bouton density.
Behavioral experiments: Cats will be trained to perform two different visuomotor tasks: 1) planar (horizontal) prehension[60], and 2) a horizontal ladder-walking task[6]. Cats will be food-restricted for 24 h before testing. For the planar prehension movements, cats will be trained to reach through a small aperture in the testing cage to grasp a piece of beef (5 mm cube). Movements will be videotaped using a digital video camera. Animals typically learn the task within 1-2 sessions and achieve a criterion performance level after less than 1 week. Measurements of endpoint accuracy and grasp characterisics will be made[60, 61]. ANOVAs will be used to

compare performance during the period of testing. For the ladder-walking task, cats will be trained to walk across a horizontal ladder (88 cm long, 18.4 cm wide, 9mm square rungs). A 5mm cube of meat will be placed at the end of the ladder to encourage the cat to walk across. For testing, rungs will be placed 6 cm apart, although when cats are initially introduced to the task, narrower rung intervals (4 cm, 2 cm) may be used if the cat does not readily step across the 6 cm interval. Animals typically learn the task within 1-2 sessions and achieve a criterion performance level in less than one week. Measurements of the distance from the paw tip to the front edge of the ladder rung (forward distance) will be made[6]. ANOVAs will be used to compare performance during the period of testing.

Intracortical microstimulation: Cats will be anesthetized with a mixture of ketamine (30 mg/kg IM) and Xylazine (0.6 mg/kg IM). Cats will be maintained at an areflexive state by continuous infusion of ketamine (10 mg/kg/h). Two small cranial openings will be made, exposing the lateral portion of the frontal and anterior parietal lobes on each side, being sure to expose forelimb area of M1, which straddles the cruciate sulcus. Microstimulation will be applied through paralene-insulated tungsten microelectrodes (Microprobe; 0.5 M nominal impedance). Stimuli (45 ms duration train, 330-Hz, 0.2-ms biphasic balanced pulses; with a leading cathodic pulse) at a rate of one per 3 s using a commercial constant current stimulator (AM Systems). Since the pericruciate cortex is relatively flat in young animals, one electrode angle (typically 10-15 degrees) can be used for the entire mapping procedure. Stimulation sites will be placed at 1 mm intervals. At each site, the movement evoked at threshold will be defined. Movements of the digits, wrist, forearm, elbow, and shoulder will be documented. Motor effects at 1.5x threshold will be documented. Multijoint response sites will be defined as sites at which responses of two or more different joints can be evoked below 1.5x threshold of the lowest-threshold response. If a response cannot not be evoked with a 100 µA stimulus, the site will be denoted nonresponsive. ICMS will continue until an area 2 mm rostral and 3 mm caudal to the cruciate sulcus (the extent of the M1 forelimb representation) has been mapped. Typically, one map will contain 40-60 sites. To control for anesthetic state of the animal, the side being mapped will be switched every 15-20 sites. ANOVA analyses and nonparametric statistics will be used to compare percent responsive sites and thresholds among groups.

Plans for data analysis: 1) To compare reaching between the unimpaired and impaired limbs, I will measure the distance from the center of the meat cube reward to the tip of digit 3. I will import video into an Apple computer and save each video frame in which the paw makes contact with the meat or the board on which the meat is placed. NIH Image softward will be used to make measurements. Measurements will be averaged for each session, and a repeated-measures ANOVA will be used to make comparisions between limbs and training sessions. 2) To compare stepping between the impaired and unimpaired limbs, measurements of the distance from the paw tip to the front edge of the ladder rung (forward distance) will be made[6]. Repeated-measures ANOVA will be used to compare performance during the period of testing. 3) The percent effective ICMS sites will be compared between hemispheres and across animal groups with a Kruskal Wallis. 4) ICMS thresholds will be compared between hemispheres and across animal groups with ANOVA.

Specific Aim 2: **To determine in children with hemiplegic CP the effectiveness of training of the impaired arm at varying skill intensities, along with concurrent restraint of the unimpaired arm, in reshaping the cortical motor map and improving motor skill.** Children with hemiplegic CP will receive training of the affected arm with concurrent restraint of the unaffected arm for three weeks. In one group, as children improve in task performance, task difficulty will be increased to promote increased skill learning ("skill training" group). A second group of children will be trained on the same tasks, but the task difficulty will not be altered. Children will repetitively perform the tasks at one skill level ("repetition"). This aim parallels Aim 1, in that it examines the role of training in promoting recovery. This aim will test two hypotheses: Aim 2A: Motor skill improvements produced by intensive training of the impaired arm will result in an expansion of the motor map in the damaged hemisphere. The magnitude of the neurophysiological changes will increase as skill training intensity is



increased. Aim 2B: Training of the affected side will result in a decrease in TMS activation thresholds in M1 on the impaired side. I also expect to find an increase in thresholds on the unimpaired side, indicating that the ipsilateral projections from the unimpaired side are not the primary mediators of recovery.

I will determine the efficacy of both training protocols on motor skills, using a kinematic analysis and a battery of clinical tasks. I will use TMS to determine changes in the excitability of the corticospinal system ipsilateral and contralateral to the hemiparetic arm produced by intensive unimanual practice. These data will be used to determine the location and size of the cortical motor representation. Based on animal studies, I propose that this period of training (both skill training and repetition) augments activity of the corticospinal system—and its various downstream targets—to enhance the capacity of the damaged side to activate spinal motor circuits.

I hypothesize that **skill training** will produce greater gains in motor skill and greater improvements in the motor map on the affected hemisphere than **repetition without skill progression**. I hypothesize that the improved motor outcome measures are, in part, mediated by an enhanced capacity of the impaired corticospinal (CS) system to activate contralateral spinal motor circuits. I also hypothesize that changes in TMS-evoked responses will persist six months after unimanual training, since previous studies in children with CP have shown this to be the case[42-44].

The experimental timeline is shown in Figure 8. Fifty children, ages 8-13, with hemiplegic CP will be recruited to participate in the study. The children will all have hemiplegic CP resulting from perinatal stroke. See General Methods for recruitment plans and exclusion criteria, as well as rationale for age selection. Children will participate in a 3-week, intensive day camp training program, held in the Center for Cerebral Palsy Research at Teachers College of Columbia University. Children will be trained in one of two methods: 1) Skill training, in which task difficulty will be increased as task performance improves, and 2) repetition, in which children will repetitively perform tasks without any changes in the skill level of the task over the course of training. One week prior to the start of training, children will be tested on a battery of motor skill tests (Table 1). Results of these test will be used as comparisons with post-training tests. See General Methods for an explanation of each motor test and rationale for use of each test. Behavioral testing will take approximately 2 hours per assessment.

Additionally, kinematic analysis of movement on a reaching and grasping task will be assessed. The task requires children to reach to a target and grasp an object. Reaches with the affected vs. unaffected arms will be compared. Kinematic features of movement such as arm trajectory, grasp aperature, and timing of hand opening during the reach will be analyzed (see below). Kinematic analyses may reveal movement deficits not reflected in other clinical motor assessments. Kinematics of movement will show whether recovery is characterized by normal movement patterns or compensatory movement patterns. I hypothesize that recovery of normal movement patterns will be correlated with greater changes in TMS hand measures, since compensatory movements often involve proximal musculature such as the trunk. Kinematic analysis will be a valuable training experience for me, as I do not currently have experience performing detailed kinematic measurements as proposed here.

Baseline TMS maps of both hemispheres of motor cortex will be obtained using a figure-8 TMS stimulating coil delivering single-pulse stimuli over motor cortex. Motor evoked potentials (MEPs) will be recorded from transdermal EMG electrodes placed bilaterally on following three arm/hand muscles: biceps, abductor pollicis brevis (APB) and extensor carpi radialis (ECR). Motor skill testing and TMS map derivation will take place on different days, as performing both sets of testing on the same day may introduce measurement error due to fatigue or distractibility of the children.

Intensive training of the affected arm will take place on 15 of 21 consecutive days (weekend rest period). Children will be present at the training facility for six hours each day. Each day, children will wear a flexible mesh sleeve over their unaffected arm, which will secure the arm to the torso and render it unusable. Children will be invited to engage in their choice of a variety of motor tasks and games, depending on treatment group (see below). Physical therapists will carefully monitor each child and keep records of time-on-task and task selections. Children will be instructed to practice using their affected arm for one hour per day outside of the day camp, although they will not wear a sling during the hour. Parents/caregivers will keep records of arm use.

One week, one month, and six months after completion of training, motor skills will be reassessed and TMS motor maps will be derived. These evaluation time points have been used in previous studies and have yielded reliable results[42-44]. If I find that my data are different from the previously collected data (that use the same training method as currently proposed), I will assess experimental or patient population differences that may introduce undue variability to my experiment. Table 3 lists the measures that will be obtained. Since pre-training data will be obtained, children will serve as their own controls. To control for TMS variability, five neurologically healthy, age-matched controls will undergo TMS motor map assessment at two time points, one month apart.

Hypotheses and Expected Results

***Expected Results:***

*Hypothesis 1: Greater skill training produces more robust improvements in motor function of the affected arm.* ***Skill training*** *will result in greater motor skill gains than* ***repetition without skill progression****.* This would point to a necessity of skill training in neurorehabilitation. Animal[1, 49, 58, 59] and human[35-38] studies of stroke recovery have demonstrated an importance in skill training of the affected arm in promoting recovery, with functional gains scaled to the amount of skill training received. Several studies in children with hemiplegic CP have shown that intensive training of the impaired hand produces long-lasting (up to 6 months) improvements in skill of the affected arm[43-45]. Balancing motor cortical activity promotes recovery.

*Hypothesis 2: Training-induced motor skill improvements will correlate with an expansion of the motor map in the affected motor cortex, decreased motor thresholds in the affected motor cortex and a decrease in the size of the ipsilateral motor map in the unaffected motor cortex.* **These changes will be more robust in the skill training group.** There have been few studies of training-induced cortical plasticity in children with hemiplegia, which makes the proposed study so important. One study pointed to the importance of balancing M1 activity to promote recovery. After training in a virtual-reality based therapy, in which the child with hemiplegic CP used his impaired arm in skilled, repetitive movements, there was an increase in the fMRI-derived activation signal in the affected cortex, and a concurrent decrease in the signal in the unaffected cortex[62]. This points to a role of the cortex surrounding the lesion in mediating recovery. Similarly, after adult stroke, there is a training-induced expansion of the motor map (as evidenced by TMS) in the affected hemisphere[62]. Moreover, data from our cat model points to plasticity of the affected motor cortex and CS tract in mediating recovery after *balancing of M1 activity*.

I predict that I will find an increase in the size of the motor map in the affected hemisphere. **Improvements will be more robust in the skill training group.** I anticipate that the increase will correlate with one or several behavioral measures. The reason for proposing a large number of subjects (n = 15 per treatment group) is to increase the likelihood that correlations can be found. I hypothesize that the motor tests most sensitive to fine motor skill, the Jebsen-Taylor Test of Hand Function will show the strongest correlations to map changes. Since many human and animal studies have shown that skilled motor learning most robustly modulates brain plasticity[1, 35-38, 49, 58, 59], I expect that changes in fine motor measures, reflective of fine skill learning, will best correlate with plasticity of the cortex on the affected side. Similarly, I expect to see an increase in representational area in joints that are used on the movement coordination task. Kinematic analysis of movements used in the movement coordination task will enable me to test correlations between use of specific joints in task performance and their representational areas in the map.

Motor map thresholds will be correlated to motor recovery measures. I predict that thresholds on the affected side will decrease with training. Improvements will be more robust in the high skill group. Previous studies of normal motor learning and training-induced recovery after stroke in humans and animals[23, 25, 49] have shown that increasing or balancing motor cortex activity with learning and/or post-stroke rehabilitation decreased thresholds in the motor map.

As described in the Background and Significance, after unilateral motor cortex inactivation or damage, the contralateral, unaffected side develops and maintains both contralateral and *ipsilateral* motor pathways. I predict that the ipsilateral response to TMS in the undamaged M1 will decrease after the training period. As

described in Hypothesis 2, a case study in which virtual-reality based therapy of the affected arm was used to treat a boy with hemiplegic CP showed an increase in the fMRI-derived activation signal in the affected cortex, and a concurrent decrease in the signal in the unaffected cortex[62]. This points to a role of the cortex surrounding the lesion—*not the cortex on the unaffected side*— in mediating recovery. Likewise, balancing M1 activity after unilateral activity blockade in the cat showed that recovery was accompanied by a loss of ipsilateral CS projections from the side ipsilateral to the first inactivation, indicating that the active side does not mediate recovery[10].

***Alternative results and implications:***

It is possible that I will not find differences between the skill training and repetition without skill progression groups. If this is found, the implication would be that in CP rehabilitation in this patient population, use of the affected arm is the most important factor in promoting recovery, and skill level is less of a factor. This will be extremely important to know. If low-skill training produces the same level of recovery as high-skill training, future therapeutic interventions could use repetitive training, which would decrease the difficulty and possible frustration of the children.

No increase in size of motor representation or decrease in thresholds on affected side: Recovery could be mediated by the unaffected M1 or by supplementary motor, premotor or subcortical structures. See Hypothesis 4 for a discussion of the role of the unaffected cortex in recovery. To address the issue of the role of supplementary motor, premotor or subcortical structures in recovery, the most effective and feasible method of study would be fMRI. TMS cannot be used to map subcortical structures, and mapping supplementary motor or premotor areas would be a long procedure, too long for a child or teenager to sit still through. Pre- and post-training fMRI studies would allow for visualization of the entire brain. TMS is being proposed in this proposal because TMS is a method of directly activating motor pools. It enables the study of muscle representational area within the motor map and thresholds, which fMRI cannot address. Studies using fMRI require the development of repetitive, simple motor tasks for subjects to perform in the scanner. Children may have difficulty performing such tasks repetitively. Thus, TMS most directly tests our proposed variables and is therefore the most applicable technique. However, fMRI is a feasible avenue if TMS does not yield results.

Increase in size of *ipsilateral* motor representation on unaffected side: In the stroke literature, it has been shown that the damaged hemisphere often mediates recovery, unless the lesion is large and encompasses most of the motor areas in the affected hemisphere[63, 64]. As described above, one case study of hemiplegia rehabilitation pointed to the damaged hemisphere being the site of training-induced plastic changes. However, the role of the damaged vs. undamaged hemisphere in hemiplegic CP recovery is not understood, making the proposed study extremely important. If it is found that the ipsilateral connections from the undamaged cortex become stronger after training, it would suggest that these ipsilateral connections are important in mediating recovery, perhaps working in concert with connections from the damaged side.

No significant correlations between map changes and motor skill improvements: If there are no significant correlations between map changes (percent responsive sites and thresholds) and skill improvements, it is possible that TMS is not sensitive enough to detect training-induced changes. The proposed methods of deriving TMS maps may not be sufficiently sensitive. Although I believe that the muscles from which I will record MEPs are the most likely to change in the map after training, it is possible that map changes of other arm/hand muscles correlate better with recovery. If I fail to see training-dependent changes in maps in the first cohort of children (n=3), I will modify my methodology to include MEP recording of a greater number (or different) muscles.

## General Methods for Aim 2

### Intensive training intervention

General procedures: Subject availability, recruitment, sample size considerations, screening, and inclusion/exclusion criteria are summarized in section E (Human Subjects). The proposed training protocol has been used in several previous studies and is published[42-44]. Intensive therapy is conducted in groups of 2-3 children. This approach allows for social interaction, modeling and encouragement. Each child is assigned to an interventionist to maintain at least a 1:1 ratio. The interventions are provided in a room (located at

Teachers College) arranged so that spaces can be used for children to work individually with their interventionist(s) or in a group with one or two other children. Toys and supplies are openly displayed so that children are free to make choices of activities and interventionists have easy access to them. A two-way mirror is used for parents to observe the intervention without interfering. The interventionists are volunteers from students enrolled in the Teachers College graduate program (licensed physical and occupational therapists), as well as graduate and undergraduate students who complete lab rotations in the program. Typically, there have been 15-20 such individuals per year in the lab. The interventionists are extensively trained (using our training manuals and interactive training sessions) and supervised by the project manager, who is a trained physical therapist and certified social worker with more than 30 years experience working with children and 6 years experience using CI therapy in children with CP. Interventionists will log all activities, participate in daily team meetings to discuss progress, problem solve and plan the next day's activities.

Positive verbal reinforcement will be provided consistently, and the therapist will assist in the activities as necessary to prevent frustration. This assistance will be attenuated as soon as possible. Rest periods will be provided at the child's request to prevent fatigue. An activities log will be kept to note the activities that were performed daily, track difficulties that a child may have with a particular activity, and to note the time of task practice and the number of rests. This will allow us to develop a measure of effort unit (efforts per unit of time) or the total time spent on task, which can provide evidence of "dosage." This can later be related to effect size.

Embedded in the activities program of each intervention will be two tasks, a reaching task and a manipulation task, which will be performed daily and will measure changes in a particular movement pattern during the intervention period. Data recorded from each task will be used to measure changes in the child's ability to perform particular upper extremity movements during the intervention period. The reaching task involves grasp of an object, moving it to a specific spatial area on a template (midline, contralateral and ipsilateral space) and releasing the object. Using a stopwatch, the interventionist will measure start time and stop time for the movement three times to each position on the template.

The manipulative task involves placing 10 coins that decrease in diameter in to a coin bank (2 half-dollars, 2 quarters, 2 nickels, 2 pennies, and 2 dimes). A stopwatch will be used to measure the start time and stop time for each coin. During the CI therapy intervention, the child will use only the involved upper extremity to perform each task. Task completion time will be recorded. The tasks will be video taped to subsequently resolve any ambiguity about a particular movement.

Given the likelihood that children will discontinue use of the more affected extremity following the intervention, children will be asked to practice a set of tasks at home tailored to their specific deficits for 60 minutes each day after the cessation of the intervention. Parents/caregivers will be asked to keep a log provided by the therapist. Since the intervention will be carried out in the home, parents/caregivers will be able to observe the intervention and will be trained by the therapist in the specific practice tasks to be used following completion of the intervention. At the end of the intervention period, parents/caregivers will be given a six-month supply of one-week logs to record the amount of practice carried out and stamped envelopes to send the logs in weekly. If a log sheet is not received within three days of its due date, a phone call will be made to check compliance.

Training of the affected hand**:** The intensive training (also referred to as constraint-induced (CI) therapy) to be employed is the result of extensive piloting and testing in more than 90 children to date[42-44]. Children will wear a sling on their less affected upper extremity to prevent use for six hours per day for 15 out of 21 consecutive days (90 hours). The sling is designed to completely encompass the child's less affected arm and hand (by sewing shut the open end of the sling) and has two straps added that tie around the child's trunk. This procedure prevents use of the hand as an assist in any activities since the hand is completely covered, and the arm is secured to the child's trunk. The sling will be worn at all times during this period except when performing certain agreed upon activities (e.g., toileting or other activities which may compromise balance) or when a break is requested (total time not to exceed one-half hour per six-hour session). The therapist will remain with the child during the six-hour interval of sling use to engage the child in functional and play activities and to monitor compliance. Table 1 presents a list of intensive practice tasks (adapted[43]).

Table 1: Activities used in intensive practice

| Activity Category | Example | # Activities | # Involving Shaping | # Involving Repetitive Practice | Movements Targeted |
|---|---|---|---|---|---|
| Functional tasks | Turning key in lock | 11 | 4 | 8 | Wrist extension, supination pronation |
| Card games | Go Fish | 6 | 6 | 6 | Supination, precision grasp |
| Board games | Life | 14 | 9 | 14 | Supination, wrist extension, precision grasp, maintaining grasp as spatial orientation changes |
| Manipulative games | Connect Four | 21 | 14 | 21 | Digit individuation, precision grasp, wrist extension, modifying grasp to accommodate various objects |
| Puzzles | Jigsaw puzzle | 2 | 0 | 2 | In-hand manipulation, precision grasp, release accuracy |
| Arts and crafts | Coloring | 2 | 0 | 2 | Supination, precision grasp, maintaining grasp through changes in spatial orientation |

Two training protocols will be used. One group of children will receive **skill training** of the affected arm, in which skill demands of motor tasks will be increased as the children gain motor function over the course of the intervention. A second group of children will perform **repetition** of motor tasks with the affected arm, with no increase in difficulty of the task over the period of intervention.

1) **Skill training:** Children will perform a variety of motor tasks (Table 1) repetitively. We present children with choices for each task. Children are typically engaged in each task for at least 20 min.. As children are able to complete motor tasks, the difficulty of the task will be increased. *Shaping* will be embedded in therapy activities. An example of *shaping* using the game Connect Four® involves asking the child to put the game away by picking up disks from the table and placing them back in the game box within defined time intervals. The interventionist records the number of disks the child can pick up and place in the box in 30 seconds. Spatial constraints are added by moving the box to a different position in relation to the disks. Accuracy is manipulated by decreasing the opening in the box into which the disks are placed. Table 2 presents examples of how the skill difficulty of therapy activities will be increased.

Table 2: Examples of Skill Training Tasks

| Activity Category | Example of Activity | Method of Increasing Motor Skill Difficulty |
|---|---|---|
| Functional tasks | Turning key in lock | Decrease size of key and key hole |
| Card games | Go Fish | Use unbeveled cards (more difficult to pick up), place cards on table with lip, requiring cards to be picked up during play rather than slid off edge of table |
| Board games | Life® | Decrease size of playing pieces |
| Manipulative games | Connect Four® | Increase distance between child and game grid; require child to retrieve playing disks from box with small opening |
| Puzzles | Jigsaw puzzle | Decrease size and thickness of pieces |
| Arts and crafts | Coloring | Introduce crayons, markers, and paint brushes of smaller diameter |

2) **Repetition without skill progression**: Children will perform a variety of motor tasks (Table 1) repetitively, and can switch tasks at will, as described for skill training. The key difference between this group and the skill training group is that the skill requirements of the tasks will not be changed during the 3-week intervention. For example, children can select Connect Four® from our available tasks, but rather than having the movement parameters of the task made more difficult over the course of intervention, no changes will be introduced to the movement parameters of the game. Children will be asked to perform the task in a way that is easiest for them.

**Clinical Measures:** Tests were chosen to capture changes in both unimanual and bimanual function and in parent/caregivers' perception of use of the more affected upper extremity as a result of the intervention. All tests will be performed by the same evaluator to reduce measurement error and increase reliability, and will be videotaped for future review. The evaluators will be blinded as to whether children received intensive training. Testing will be performed in the Hand Motor Control Laboratory at Teachers College, Columbia University. Children will be tested immediately before, one week after, one month after, and six months after receiving intensive training. The total time to administer these measures during any one testing session will be no longer than 2 hours. We will also determine how much formal physical and occupational therapy services each child has received so that analyses may be conducted to determine whether rehabilitation history is associated with treatment outcome.

Jebsen-Taylor Test of Hand Function: This is a widely used standardized timed test of hand function[65], which measures the time to complete a battery of manual activities. This test will serve as the primary outcome measure for movement efficiency change. This test is analogous to the Wolf Motor Function Test, but has been standardized for use in children. The timed tasks include card turning; small object placement; simulated eating; stacking checkers; and grasping, transporting, and releasing empty and full cans. The total time to complete all items serves as the score, though individual subtest scores can also be examined. This test was developed to assess broad aspects of hand function commonly used in activities of daily living, to be easily administered in a short period of time, and to utilize materials that are readily available[65]. Reliability and lack of practice effect has been established in typically developing children and in children with stable hand disabilities (including CP). Reliability coefficients ranged from 0.88- 0.97 for dominant hand subtests and from 0.95- 0.99 for non-dominant hand subtests for the children with stable hand disabilities[66]. This test will be performed separately with the involved and non-involved extremity.

Assisting Hand Assessment: The Assisting Hand Assessment (AHA)[67, 68] is a test used to measure and describe the effectiveness with which a child with a unilateral disability makes use of his/her affected hand (assisting hand) in performance of bimanual activities. The AHA is conducted through scoring of observable performance skills exposed during meaningful occupational performance (play). AHA is a standardized and criterion referenced test intended for children with unimanual motor impairments. A rasch analysis of the individual test items has established a test validity for all items with a 99% confidence interval [67] and the reliability is excellent (.97 interrater and .99 intrarater, now specified) [69]. Finally, the AHA is sensitive to change70. The assessment measures how well a child uses his/her arm in a bimanual activity as a sum of scores which may vary from 22 to 88 (a higher score indicating greater ability). The test is video taped and scored by a blind evaluator.

Kinematic analysis of reaching: In this analysis, children will reach toward and grasp a small stationery object from a shelf eye-level to the child. Reflective markers will be placed on the affected and unaffected sides at the shoulder, base of neck (C7), elbow, wrist, metacarpaphalygeal joint (MCP), and tips of each digit. Children will be seated in a chair with both feet resting on the ground, hands placed squarely on the thigh. The experimenter will signal the start of each trial vocally, and the child will reach to the test object, grasp it, and lift it at least 5 cm from the shelf surface. Each testing session will consist of twenty-five trials per arm. Both the affected and unaffected arms will be tested.

Eight infrared cameras will be placed in front of the table to collect the 3-D kinematic data (Vicon). Reflective markers will be placed on the mid-point of the subject's wrist bilaterally. In addition, a marker will be placed on the drawer to indicate its position. Signals will be processed through a digital filter (low pass cutoff frequency 6 Hz).

Plans for data analysis and interpretation: All analyses will be conducted using standard statistical software (primarily SPSS). Comparisons of clinical measures and movement parameters will be made. Children will be

tested prior to the intervention and three times following the intervention (one week after the intervention, one month, and six months after the intervention). For each variable, we will perform a 2 (skill training, repetition without skill progression) group x 4 (test session) ANOVA with repeated measures on the latter factor to assess statistically reliable changes ($p<0.05$). Bonferroni tests will be performed when appropriate. The group-by-test interaction will identify differential effects of the treatment across time.

To determine predictors of severity, we will use regression analysis to determine the relationship between severity of involved hand impairment (determined using the initial scores of the involved hand on the Jebsen-Taylor test), age, asymmetry in cerebral peduncle size, prior treatment and behavior during the intervention session (a surrogate for attention) with the change in score on the Assisting Hand Assessment pre- and post-intervention. Attention to task will be estimated based upon pre-determined definitions by quantifying disruptive, non-complaint, or inattentive behaviors during testing through a review of videotapes of each testing session. The frequency of these behaviors will be used as a surrogate of how well a child attended to task during testing and throughout the intervention. Discrepancies in defining these behaviors will be resolved by discussion during scoring. Both attention to task and initial hand impairment strongly related to outcome during CIMT42.

*Kinematic analysis:* To determine the hand path, the x and y position of the index finger sensor will be plotted. To determine the movement time, we will measure the duration between movement onset (tangential velocity 5 cm/s) and offset (tangential velocity < 5 cm/s). We will also measure the maximum aperture of the hand (defined as the largest distance between the sensors on the tip of the thumb and index finger) and the time (normalized to the reach duration) at which it occurs. In typically developing individuals it occurs around 60-70% of the movement[71]. To determine the continuity of movement, we will count the number of zero crossings in acceleration and normalize them to the movement time. We will also compare movement of the hand to movement of the shoulder to determine the extent of trunk involvement in the movement. Furthermore, we will compare the movement time of the reaching component (time between onset and offset) to the time of the grasping component, and compare the time spent in each component of the movement to the overall movement time. We will determine the straightness of the handpath by calculating a linearity ratio (actual displacement/straight line distance). Finally, we will examine the variability in the handpath (and linearity ration) by taking the root mean square across trials, as well as the variability of each parameter by calculating the coefficient of variation (SD/mean x 100). Based on the results of other studies, we expect that the children will display prolonged movement times, with a greater portion of the time spent in the grasping portion than the reach portion for their more affected extremity compared to their less affected extremity. Their maximum grasp aperture will be smaller and prolonged. They will have low peak velocities that take longer to develop, greater number of zero crossings, more variable movement patterns, and greater contribution of shoulder/trunk motion to the movement.

**Transcranial Magnetic Stimulation**

TMS studies will be conducted in the Brain Stimulation and Therapeutic Modulation (BSTM) Division at the New York State Psychiatric Institute (NYSPI). Dr. Sarah Lisanby, a co-sponsor of the proposed project, is the director of the BSTM Division and will supervise TMS procedures.

Structural Magnetic Resonance Imaging (MRI) for stereotaxic TMS coil-placement: During TMS map derivation, the stimulating coil will be applied to the scalp over the primary motor cortex. To determine the location of the representation in M1 of specific target muscles in each subject, MRI scans will be obtained on a 3 T MRI system at the MR Center in the Department of Radiology. Since there is normal variability in brain topography relative to scalp landmarks, and since this variability may be exacerbated by brain lesions, it is imperative that MRI scans be obtained. The BSTM Division is equipped with two frameless stereotaxic systems (Brainsight Frameless, Rogue Research, Montreal, QC, Canada) that are used to co-register TMS coil position on the scalp with underlying cortical anatomy on the subject's 3-dimensionally rendered MRI scan. This frameless stereotaxic system allows for the targeting of the coil to specific cortical structures.

Electromyography (EMG) to record motor evoked potentials (MEP)

EMG responses to TMS stimulation will be recorded using a bank of 4 custom-modified electrically isolated bioelectric amplifiers (James Long Company, Caroga Lake, NY). These bio-amplifiers are battery powered for AC isolation and customized to reduce artifact induced by magnetic pulses. Features include low distortion and noise. They have especially rapid recovery after saturating signals. The amplified signals will be digitized with

Dell Dimension P200V (200 MHz) PCs using Analogic LS-DAS-12 A/Ds (250 kHz). MEPs will be recorded bilaterally from the following three arm/hand muscles: biceps, abductor pollicis brevis (APB) and extensor carpi radialis (ECR). These muscles were selected because maps from those muscles will give a picture of the motor map in proximal and distal musculature, in muscles that are used in training. The amplified signals will be digitized with Dell Dimension P200V (200 MHz) PCs using Analogic LS-DAS-12 A/Ds (250 kHz).

*Bilateral TMS mapping of motor cortex*

Experimental setup: Subjects will be seated in a chair, wearing earplugs to protect hearing. Subjects will rest both forearms in a fixed position on armrests. Subjects will wear a soft, fitted skull cap onto which the functionally determined position of M1 (confirmed by MRI) and a 1 cm grid will be marked. The will also wear a headband fitted with a position tracker for the frameless stereotaxic system. The pediatric figure-8 stimulating coil (50mm), with a position tracker affixed, will be held to the scalp over each M1 hemisphere, oriented such that induce electrical current will be at a 45° angle with respect to the central sulcus, and in the posterior-to-anterior direction. This is the position optimal for trans-synaptic activation of the CS tract[72]. Consistency of coil positioning will be verified on-line via frameless stereotaxy (Brainsight).

TMS stimulation protocol: Stimulation sites will be spaced at 1 cm intervals. Stimulation will begin in a medial portion of M1; the coil will be progressed laterally until an MEP is obtained. The coil will be moved to adjacent sites within M1 until MEP responses are no longer found for any of the recorded muscles. Both hemispheres of M1 will be mapped. The movement threshold (MT) will be defined as the minimum magnetic flux needed to elicit a threshold EMG response (50 μV in peak to peak amplitude) in a resting target muscle in 5 out of 10 trials using single pulse TMS administered to the contralateral primary motor cortex. Both resting and facilitated MTs for each site will be determined.

Statistical analysis: A repeated-measures study design will be employed. Changes in the percent of responsive sites within a defined area of M1 will be determined in each hemisphere. Changes in MTs in each hemisphere will be calculated. Using a repeated-measures ANOVA, these measures will be compared across times and across subjects as described above for the behavioral measurements. I will perform a Pearson's Correlation analysis on each behavioral variable vs. changes in percent responsive TMS sites and changes in Mts. For the Pearson's Correlation, power analysis indicates that with a sample size n=15, I can expect to find a maximum r=0.67. A Pearson's Correlation tests the linear relationship between variables. If I do not find linear relationships between behavior measurements and TMS measurements, I will employ regression analyses to look for non-linear relationships in the data.

## E. HUMAN SUBJECTS RESEARCH

*Subjects*

Description: A total of 50 children with hemiplegic CP resulting from perinatal stroke that occurred at age < 1 year, will participate in the studies. Age of the subjects will range from 8 to13 years. Informed consent will be obtained from children and their parents. The children will receive $150 for participating in this study. This age group was chosen because the upper extremity control of typically developing children at this age begins to plateau with only subtle improvements occurring during early adolescence (Gordon 2001). Furthermore, they have successfully studied hand function in children with CP and adapted the CI therapy for this age while retaining its central tenants (Gordon).

The proposed study will be conducted within the pre-established intensive training day camp in the Center for Cerebral Palsy Research at Teachers College. The program is open to children ages 4-13, and during each offering of the training program, children ages 4-13 will be included. I will select a subset of children ages 8-13 to include in the proposed study. Children and parents/caregivers will give informed consent to participate. Subject selection will be purely based on age and will be blind to all pre-training clinical measures. I will exclude children ages 4-7 because the TMS procedures require subjects to remain still and to follow instructions for an extended period (1-2 hrs). Such requirements may be too challenging for younger children. In total, six to eight children (ages 4-13) typically enroll in the intensive training program each offering. I propose to include three children per offering for the TMS project that meet the inclusion/exclusion criteria.

Sample size considerations: The sample size calculations assume a significance level ($\alpha$) of 0.01 and power ($1-\beta$)=0.90. In the prior work of the primary mentor[73] they observed a mean change on the Jenson-Taylor Test of hand function from 363 pre-treatment to 278 post-treatment improvement. With $\alpha$=0.01, $1-\beta$=0.90, $\mu_1-\mu_2$=85 and $\sigma$=0.61, 20 subjects are more than sufficient. A second power analysis was performed using TMS response rate data from a study of Janet Eyre, a research consultant to this proposal. Using $\mu_1-\mu_2$=0.3, $\sigma$=0.15, $(1-\beta)$=0.90, $\alpha$=0.01, 20 subjects are more than sufficient. The power calculation allows for a 25% dropout rate for each group. Two previous intensive training studies reported a dropout rate of approximately 15%[48, 49]. The 25% dropout rate planned for in the proposed study is a generous estimate, taking into consideration the intensive nature of both the training and the TMS procedures, which may result in some children dropping out of the study.

Inclusion/exclusion criteria: Children included in the study will have a diagnosis of hemiplegic CP. They will not meet any exclusion criteria listed below. Medical records will be obtained from the children's doctors, or medical history will be provided by a parent/caregiver. The exclusion criteria presented in Table 3 were developed based on previous experience with CI therapy in children with hemiplegic CP and experience with TMS.

Subject screening: Each child will undergo a complete physical examination, blood work, and urine testing to screen for medical problems that may pose a risk for TMS exposure. They will also be screened for other neurological or psychiatric comorbidity that might affect motor cortex excitability (e.g., ADHD, Tourette's Syndrome, Tic Disorder) via IQ testing, the Connors ADHD survey, the Autism Diagnostic Interview, and physician exam.

Table 3

| Exclusion Criteria | Method of Assessment | Rationale |
|---|---|---|
| Age < 8 or > 13 years | Medical records | Sufficient maturity is necessary to comply with protocols; > 13 y may be beyond window of developmental plasticity. |
| CP resulting from perinatal stroke at age < 1 year | Medical records, examination of imaging films at age < 1 yr | Allow for the study of a more homogeneous population, since CP can have many etiologies |
| No cognitive deficits | Kaufman Brief Intelligence Test, score ± 1 SD from normal | Sufficient intelligence is necessary to comply with protocols. |
| Joint mobility: wrist extension, 20º, metacarpophalangeal and proximal interphalangeal joints 10º. | Pre-intervention screening measures | Sufficient flexibility flexible to complete intervention tasks. |
| No heath problems unrelated to CP | Medical records | Sufficient health is necessary to comply with protocols. |
| No seizure history in self or first-degree relatives | Medical records | TMS may evoke seizures in prone children. |
| Visual problems (uncorrected by glasses/contact lenses) | Medical records | Visual acuity is required to comply with protocols. |
| High motor ability in affected arm | Motor activity log, score > 2.5 ( > slight-to-moderate) | If ability of affect arm is high, further training may not provide additional improvements. |
| Severe spasticity | Modified Ashworth test, score > 3 ( > moderate) | Severe spasticity may interfere with task performance and TMS map derivation. |
| Lack of asymmetry in hand function | Jebsen-Taylor score < 50% | If impaired hand is functioning nearly as well as unaffected hand, further training may not provide additional improvements. |
| Orthopedic surgery in affected arm | Medical records | May introduce confounds to data interpretation. |
| Dorsal root rhizotomy | | |
| Botulinum toxin therapy in either upper extremity during last 6 months, or planned during study period | | |
| Currently receiving intrathecal baclofen | | |
| Balance problems | Assessed in lab before intervention by placing sling on child and having child walk 20 feet | Balance problems could pose physical danger to child. |
| Non-removable metal objects in body, other than dental fillings (e.g., orthopedic stabilizing hardware) | Medical records | Poses risk of shifting and injury in MRI scanner. |
| Previous participation in an intervention identical or similar to the proposed study | Medical/Gordon laboratory records | May introduce confounds to data interpretation. |
| Comorbid psychiatric or neurological Disorder | Physician examination and standardized ratings | May introduce confounds to data interpretation. |

Patient availability: Children with hemiplegia will be recruited from the NYC public schools, the Pediatric Orthopedic Department at Overlook Hospital, Summit, NJ, United Cerebral Palsy of New York City, the Spasticity Clinic at Columbia University Medical Center and various rehabilitation clinics in which our graduate students are employed. During the last five years in which we have been doing research on hand function in children with CP in the New York City metropolitan area, Dr. Gordon has tested more than 90 children who meet the criteria of this study. His experience demonstrates feasibility of identifying and recruiting appropriate children. I have estimated a dropout rate of 25%, which is more generous than the 15% dropout rate reported in two earlier trials[48, 49]

Plans for Recruitment: Plans for recruitment and inclusion/exclusion criteria are described in the methods. The children will be recruited from the Pediatric Orthopedic Department at Overlook Hospital, Summit, NJ, United Cerebral Palsy of New York City, the NYC public schools, and various rehabilitation clinics in which graduate

students from Dr. Gordon's lab are employed. Subjects will also be recruited from the Spasticity Clinic at Columbia University Medical Center. All subjects and/or their parents will sign informed consent forms at the time of enrollment which describe the study, purpose, duration, risks and benefits, subject rights, and appropriate contact personnel. The consent form was approved by the Teachers College, Columbia University Institutional Review Board. Information will be treated in strict confidence to the extent provided by law. Subjects' identities will be coded and will not be associated with any published results. The PI will keep codes and identity in a locked file. Videotapes will be used only for research purposes and not be released for any other reason without written consent.

Potential Risks and Procedure for Protection:

Intensive training: As noted above, there are few adverse events associated with the intensive training intervention. The interventions were modified specifically to be as child-friendly as possible. Nevertheless, we approach the treatment with the utmost care. There is a slight psychological risk of frustration associated with lack of progress or failure to accomplish tasks. To minimize this risk, difficult tasks can be substituted with easier ones and positive reinforcement will be continually provided. The therapists will intervene and assist the child as necessary. In addition, there is a risk of transient numbness in the restrained arm during intensive training. If this occurs, subjects will be allowed to temporarily remove the sling. There is also a remote possibility of injury due to loss of balance. However, this is unlikely since stable balance is an inclusion criterion, and the child will be supervised by the therapist at all times during the intervention. Of the 35 children who previously participated in the intensive training intervention, none has experienced adverse events and all but one completed the protocol.

The studies involve the collection of kinetic and kinematic data during behaviors that occur in everyday life. None of the testing procedures are hazardous and therefore carry no obvious physical or psychological risks to the subjects during data collection.

The proposed methods can only be tested in children who are not significantly cognitively impaired and whose motor impairment does not preclude the children from performing rehabilitative tasks. Therefore, this treatment is limited largely to testing patients who have developed some hand function (who may be most receptive to intervention). Nevertheless, it is possible that our results may depend on the severity of hand impairment and specific lesions involved, or even the amount of therapy previously received. Intensive training could be fatiguing and frustrating for the children. It is potentially invasive if not performed with the utmost care. The children will be allowed breaks as necessary, and the activities will be provided based on each child's present capabilities. In previous studies, children were highly cooperative due to the increased attention they received [42, 44, 74].

*Transcranial magnetic stimulation:* Although TMS poses some risk to subjects, we will make every effort to minimize these risks in the subject population. Table 4 summarizes risks and measures I will take to minimize each risk. Since MRI will be used as a component of the TMS data collection, the table also summarizes risks of MRI scan. TMS has been used effectively and safely in studying motor pathways in children (review ref). Eyre and colleagues have used TMS to study the motor system in healthy and CP-affected children, newborn through adolescence[12, 15]. One review examined data from 1036 children who participated in TMS studies (single-pulse, paired-pulse, and repetitive TMS), and seizures were not reported in any child[75]. Even in studies of children with CP and epilepsy, thus prone to seizure activity, there have been no reported cases of seizures in response to single-pulse or paired-pulse TMS[75]. Thus, the literature supports TMS as a safe, effective, non-invasive, important tool for studying motor pathways in healthy children and in children with neurological disorders.

Table 4

| Potential Risk | Explanation/Measure to Minimize Risk |
|---|---|
| Magnetic Resonance Imaging | |
| Metallic objects may shift during scan, causing harm | Children with implanted metal objects are excluded; for other subjects, all metal objects in their possession will be collected before the scan. |
| Claustrophobia due to being confined to small space inside scanner | Scanner bore has mirror that allows subjects to see technician and has been known to help alleviate claustrophobia. Children with intractable claustrophobia will be excluded. |
| Hearing loss due to exposure to loud noises produced by MRI machine during scanning | Sufficiently protective earplugs will be worn by subjects during the scan. |
| Risks of time-varying magnetic fields could induce stimulation of peripheral nerves, cause tingling sensations. | The 3T scanner used in this protocol is considered by the FDA to be too weak to produce peripheral nerve stimulation. Care will be taken to monitor children for symptoms of tingling, and scan will be halted if children report any peripheral nerve sensations. |
| Risks of specific absorption rate (SAR) (heating of body) | All experiments performed on the 3.0 Tesla system will comply with FDA guidelines with regard to SAR. |
| Risks of static magnetic fields | The FDA reports that systems operating at 8.0 Tesla or less do not pose a significant risk. |
| Transcranial Magnetic Stimulation | |
| Potential worsening of symptoms with TMS | Single-pulse TMS is highly unlikely to produce or exacerbate symptoms; no such cases have been reported in TMS studies of children with CP or adults with stroke. |
| Seizure risk | There have been no reported cases of seizures produced by single-pulse TMS in children with CP. Furthermore, children who have a history of seizures will be excluded, even if their seizures are controlled medically. |
| Potential effects of TMS on brain tissue | There have been no reports of TMS-induced brain damage in human or animal studies. |
| Potential hearing loss due to clicking noise of TMS stimulator | Sufficiently protective earplugs will be worn by subjects during the procedure. |
| Potential changes in cognitive function | There have been no reports of long-term changes (> 1 min) in cognitive function in single-pulse or repetitive TMS studies. |

## F. VERTEBRATE ANIMALS

Domestic cats will be used in the proposed experiments. Litters of 4-5 kittens will be obtained at PD 27-29 and will be housed with a lactating female. Specific age, gender and strain of the kittens will depend on availability from the vendor. Kittens will be given at least seven days to acclimate to its new surroundings before experimentation begins on PD 35. Littermates will be group-housed with a lactating female until the cats reach approximately 60 days of age. Cats older that 60 days of age will be housed in individual cages.

The proposed studies investigate the development and function of the CS system. There are no alternatives to experimentation on living animals. The cat was chosen for these studies because much is known about the anatomy and physiology of the nervous system of mature members of this species and the motor skills that mature cats are capable of performing.

All experimental procedures have been approved by the university Institutional Animal Care and Use Committee. A veterinary technician will assist in all surgical procedures, delivering anesthetics and monitoring vital signs, and will assist in post-operative care of the animals. Additionally, a veterinarian, Dr. Mohammed Osman, D.V.M., Ph.D., will monitor the animals daily for the duration of the experiments.

Twenty-five cats will be used in the proposed experiments. Using five cats per group will provide the statistical power necessary to define differences between experimental and control groups. A power analysis using previous stepping data (the most variable of data to be collected in the proposed study) in cats after unilateral inactivation vs. alternate inactivation support the use of n=5 as a sufficient sample size ($\mu_1$-$\mu_2$=0.96, SD=0.38, power=0.9, significance level < 0.05).

## G. SELECTED AGENT RESEARCH

N/A

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