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TITLE: **Harnessing Neuroplasticity to Enhance Functional Recovery in Allogeneic Hand Transplant and Heterotopic Hand Replant Recipients**

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14. ABSTRACT
The overarching hypothesis of this project is that patients recovering from hand transplants, replants and peripheral nerve repairs will exhibit improved hand function resulting from combined transcranial direct current stimulation and behavioral therapies that seek to reverse persistent, amputation-related brain reorganization. This work builds on solid basic findings in neuroscience to develop, implement and evaluate innovative evidence-based rehabilitation in hand transplant, replant and peripheral nerve repair patients. This project will yield effective new therapeutic approaches for improving outcomes of individuals who have undergone hand transplantation, replantation, and peripheral nerve repairs that can be deployed with modest training and minimal cost in a wide variety of settings, including the theatre of engagement. The therapeutic approaches resulting from this work will lead to improved hand function in wounded warriors and civilians, providing them with an improved quality of life through the resumption of meaningful pre-morbid occupational, recreational, familial and social activities.

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Hand transplantation, hand replantation, peripheral nerve injuries, cortical reorganization, neuroplasticity, functional recovery

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1. INTRODUCTION

The overarching hypothesis of this project is that patients recovering from hand transplants, replants and peripheral nerve repairs will exhibit improved hand function resulting from combined transcranial direct current stimulation and behavioral therapies that seek to reverse persistent, amputation-related brain reorganization. This work builds on solid basic findings in neuroscience to develop, implement and evaluate innovative evidence-based rehabilitation in hand transplant, replant and peripheral nerve repair patients. This project will yield effective new therapeutic approaches for improving outcomes of individuals who have undergone hand transplantation, replantation, and peripheral nerve repairs that can be deployed with modest training and minimal cost in a wide variety of settings, including the theatre of engagement. The therapeutic approaches resulting from this work will lead to improved hand function in wounded warriors and civilians, providing them with an improved quality of life through the resumption of meaningful pre-morbid occupational, recreational, familial and social activities.

This annual report will provide a complete summary of the project's research accomplishments to date with respect to our approved Statement of Work.

KEYWORDS: hand transplantation, hand replantation, peripheral nervous system injuries, cortical reorganization, neuroplasticity, functional recovery, rehabilitation.

2. ACCOMPLISHMENTS:

What were the major goals of the project?

The major tasks as listed in the Statement of Work are as follows:

- Major Task 1: Prepare Regulatory Documents and Research Protocol
- Major Task 2: Administrative/Training Procedures
- Major Task 3: Data Collection
- Major Task 4: Data Analysis/Manuscript Preparation
- Major Task 5: Dissemination of Results

What was accomplished under these goals?

Major Activities: During this project year, timely progress on all Major Tasks has been accomplished. Regulatory approvals have been maintained and updated as necessary. Administrative and training procedures have taken place as planned. Data collection for Aim 1 started, and data collection continued under Aim 2. Our research team has refined the data analysis process, which includes the development of new data statistical and visualization techniques that will enable more effective dissemination of complex actigraphy data. Additionally, we have completed extensive analysis of the accelerometer data to account for the sleep and prosthetic device nonwear (in amputees) in order to quantify accurate real-life activity in our subjects. Using this analysis, we are currently preparing a case-study report discussing on a hand transplant recipient before and after the transplanted limb was amputated due to rejection. We are also working on a manuscript detailing differences between groups

using transplanted limbs vs. prostheses. Additionally, dissemination of preliminary data has commenced with the presentation of two abstracts at MU Life Sciences Week in April 2019. Dr. Frey has also given several oral presentations on this work.

Highlights of Preliminary Results and Aim 2 Accelerometry Technique

Below, I illustrate several highlights of our preliminary results from Aim 2, in which wireless accelerometry is used to continuously measure upper limb and/or prosthesis activity continuously during real world activities for three consecutive days.

We are using wireless accelerometers to record activity during everyday life with the intact limb, prosthesis and replanted or transplant hands of current or former amputees. Subjects wore a total of four accelerometers (GT9X Link, ActiGraph Corp, Pensacola, FL) for a period of 3 days (72 hours), see **Figure 1**. The accelerometers were worn on each forearm and each arm. The duration covered was two weekdays and one weekend day (if the participant had an irregular

Figure 1: accelerometers worn on arm and forearm on both sides.

Subjects were shipped a package with instructions on how to use accelerometer and study procedures, along with accelerometers. Subjects returned the accelerometers and activity log after their participation via mail.

Data analysis:

We used the Actilife version 6 to initialize and download sensor data. The sensor data was downloaded using 1s epochs for each sensor. Further data analysis was performed using Matlab R2016a. The data for each sensor was converted in vector magnitude.

$$Vector\ magnitude = \sqrt{x^2+y^2+z^2}.$$

In order to analyze the use of prosthesis, we computed our response variables when the prosthesis was being used and also when the prosthesis was off during waking hours. Thus, our results were not confounded by prosthesis wear pattern.

Nonwear detection:

The time during which prosthesis users did not wear their prosthesis was detected using an algorithm. The vector magnitude was further processed using moving root mean square (RMS) using a moving window of 60 seconds. The nonwear beginning was detected as the time when the mean of the following 30 consecutive minutes in the affected wrist moving RMS value equals to 0. The nonwear end was detected as the mean of the proceeding 30 consecutive minutes in the affected wrist RMS data equaled to 0.

Side contribution:

Reliance:

Rest period was detected for each segment separately. Rest was considered when bilateral sensors had a vector magnitude of 0. When both segments are moving, reliance on the intact side for each segment is computed using the following equation (Chadwell et al., 2018).

$$Reliance\ on\ intact\ segment = \left(\frac{unaffected\ vector\ magnitude}{affected\ vector\ magnitude + unaffected\ vector\ magnitude}\right)$$

The reliance on intact segment was distributed in 13 bins to show reliance on the intact side from 0-100% with 10% increments. 0% reliance on the intact side indicates unilateral activity in the prosthesis forearm or the affected side arm. Similarly, 100% reliance on the intact side suggested unilateral activity in the unaffected forearm or arm. These bins of reliance were plotted in an Archimedean spiral graph to gauge the temporal change in prosthesis use. To simplify the figures, 40-60% reliance on the intact side was collapsed in a single bin signifying bilateral use. **Figure 2** presents an example of the spiral graph for a prosthesis user and a control subject. We also computed how much time was spent in each reliance category to get a better idea of how prosthesis is used.

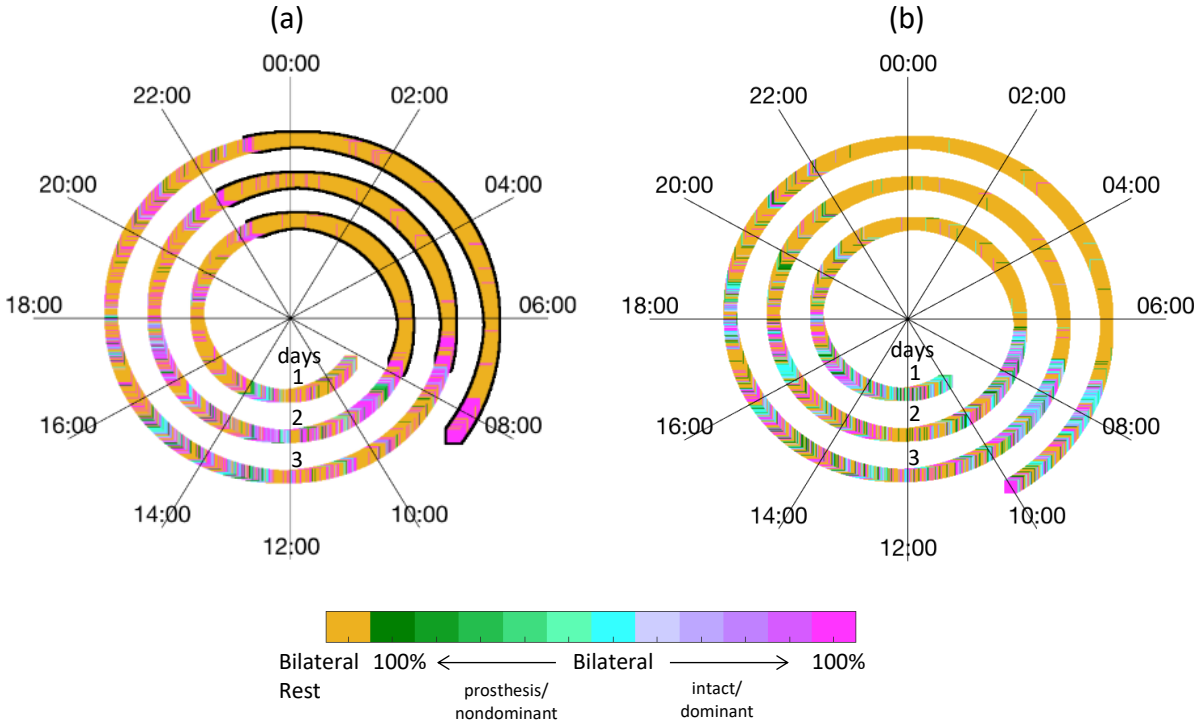


Figure 2: spiral graph showing change in reliance on the intact forearm throughout the day in (a) prosthesis user and (b) control subject, over three days (72 hours). Black outline in prosthesis user represents prosthesis nonwear. The first day of the data collection is plotted in the inner most ring.

Activity ratio:
Activity ratio was computed as the log of affected and unaffected vector magnitude. Activity ratio outside the range of -7 and +7 was assigned values of -7 and +7, respectively. The activity ratio of -7 and +7 indicated 100% unaffected or the affected side respectively.

$$Activity\ ratio = \log\left(\frac{affected\ vector\ magnitude}{unaffected\ vector\ magnitude}\right)$$

Activity magnitude:
Activity magnitude was determined by summation of bilateral vector magnitude per segment. Activity magnitude was 0 when both limbs for the particular segments were at rest.

Relationship between which side contributed more to the overall activity, amplitude of the movement and frequency of the movement was visualized using density plots (Figure 3) (Basso, Lang, Sciences, State, & Therapy, 2018). **Figure 3** shows that the most frequent movements were bilateral and of low magnitude in both prosthesis user and control subjects. Moreover, peak in the center of the graph suggests that the bilateral movements had the highest magnitude in both subjects. In control subjects, shape of the density plot is symmetric, whereas prosthesis user's density plot is skewed towards the unaffected side throughout the day, as was previously shown (Chadwell et al., 2018).

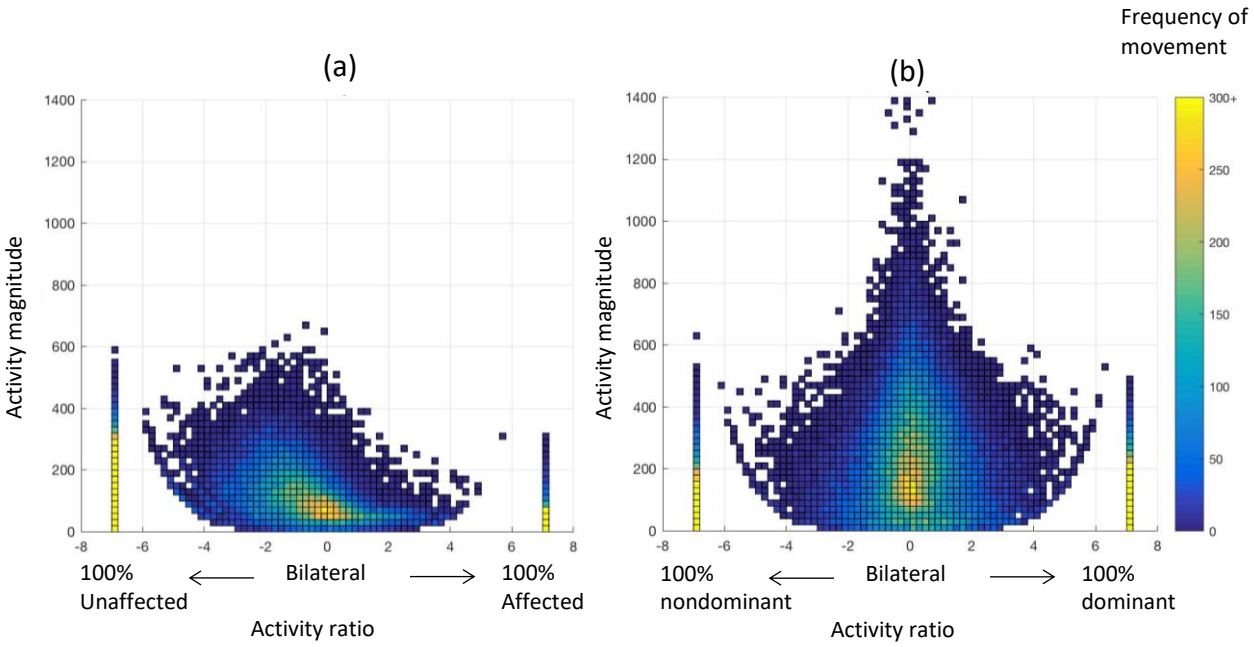
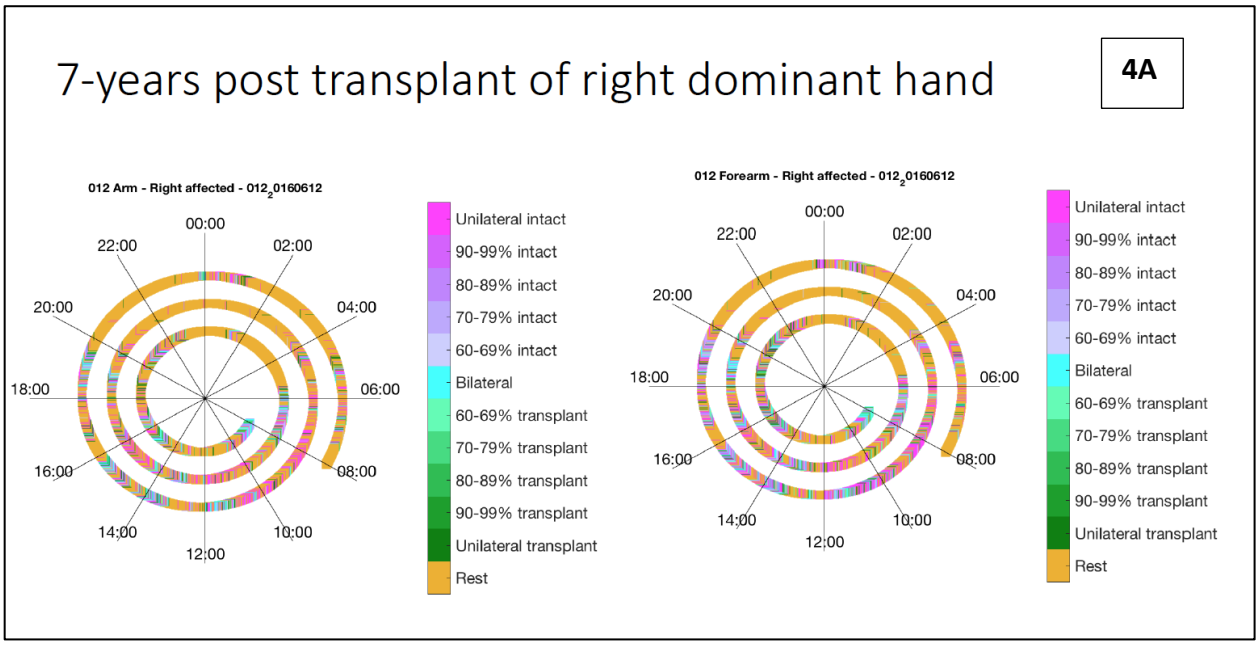


Figure 3: Density plots for forearm activity in (a) prosthesis user and (b) control subject.

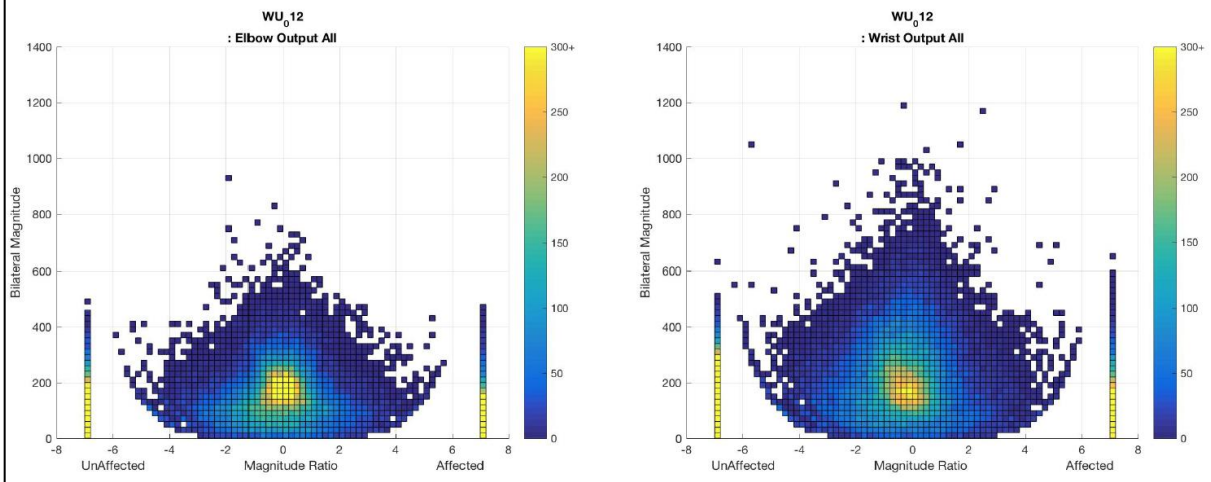
Variability of movement:
Activity variability was defined as the standard deviations of vector magnitude for each segment. Activity variability ratio, defined as the ratio of the activity variability for the affected unaffected side for each segment was also calculated. Bigger activity variability ratio suggested bigger variability in the affected segment's movements. Another parameter to measure variability of movement was activity magnitude variability, defined as the standard deviation of the activity magnitude.

Figures 4A & 4B illustrate the case of EH, a right hand transplant recipient recorded 7 post-surgery. **Figures 4C & 4D** show a comparable set of data from EH after the grafted hand was surgically removed due to complications, during which time he was using a myoelectric prosthesis. Healthy adults exhibit a high degree of bilateral symmetry with the most frequent movements being bilateral. EH shows the same following transplant (**Figs 4A & 4B**). After re-amputation (**Figs 4C & 4D**), he exhibits a pronounced left asymmetry reflecting considerably less use of right (dominant/prosthetic) vs. left (non-dominant/unaffected) limb.



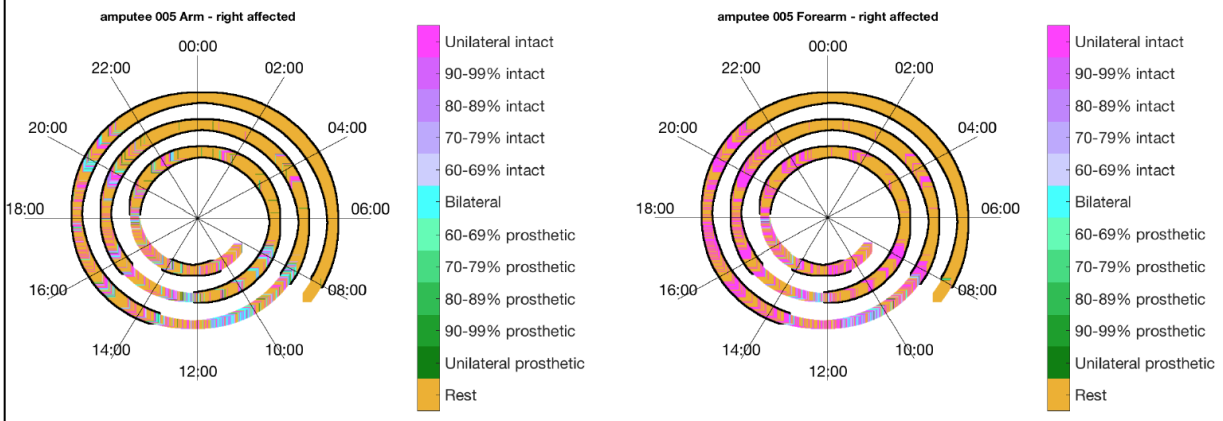
7-years post transplant of right dominant hand

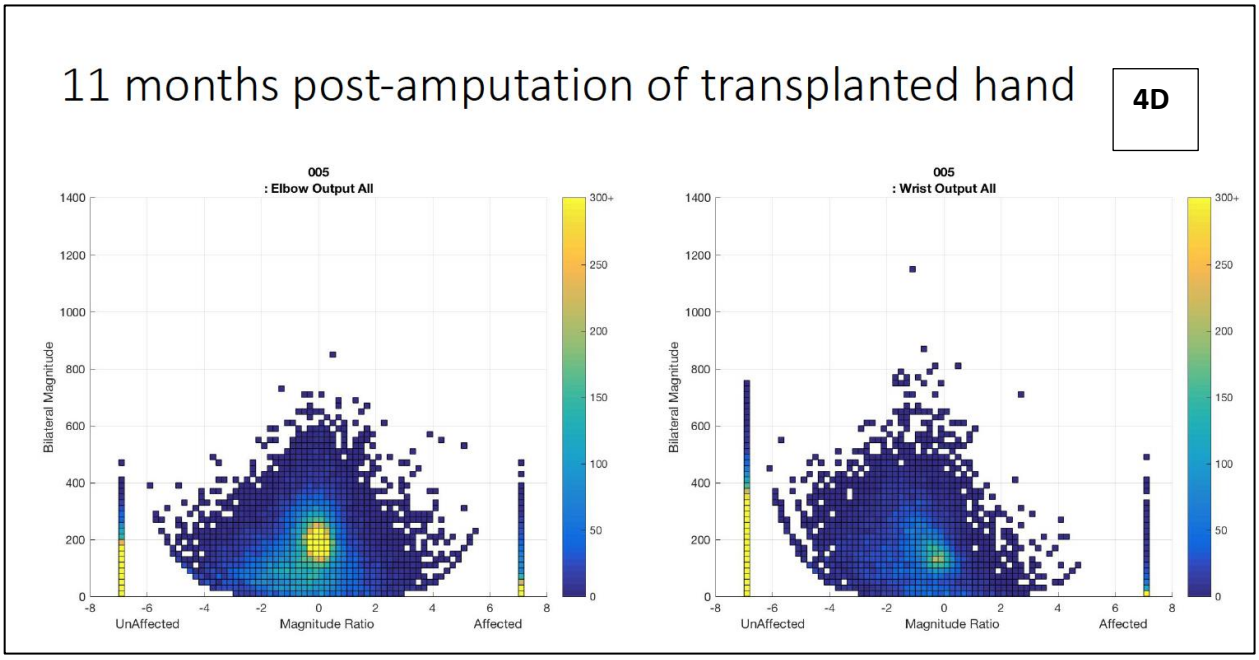
4B



11 months post-amputation of transplanted hand

4C





Sleep/wake detection:

As the next step in our analysis, we are identifying sleep/wake times in order to analyze the characteristics of prosthesis wear only during awake hours. We identified 4 methods of sleep/wake time detection: 1) sleep log, 2) manual eyeballing of the data, 3) sleep/wake algorithm adopted from van Hees et al (2018) (**Figure 5**), and 4) Actilife software's inbuilt sleep/wake detection algorithm. We are currently in the process of assessing which method is optimum in detecting sleep/wake times most reliably (**Figure 6**).

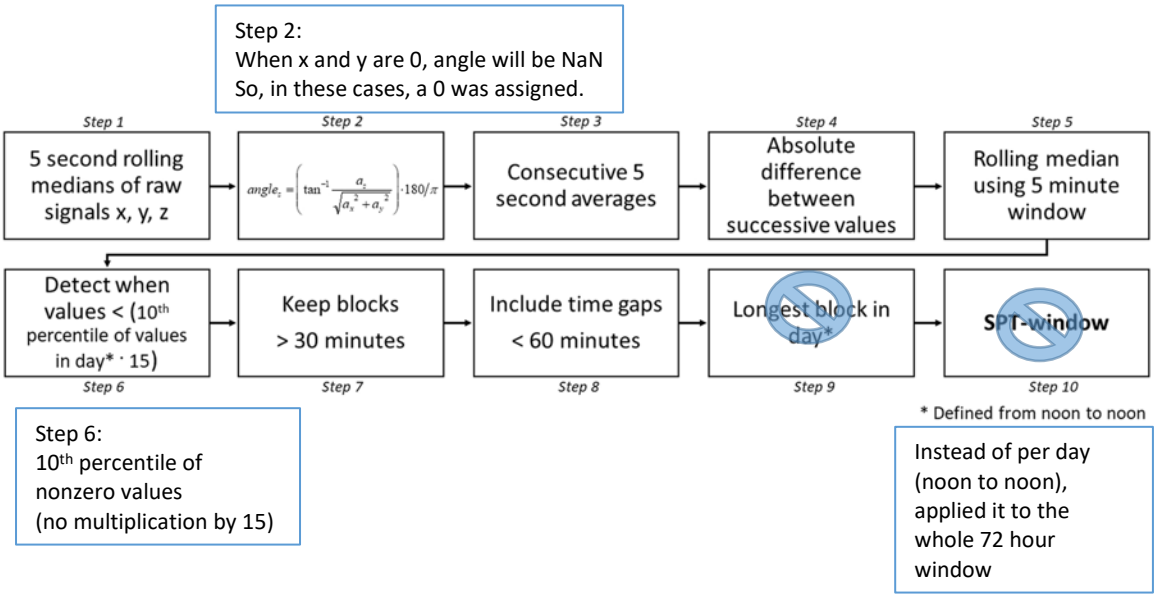


Figure 5: Adoption of the van Hees sleep/wake detection protocol for our study. The main differences were 1) the threshold was set as the 10th percentile of the rolling median using a 5 minute window, and 2) we applied this algorithm to our entire 72 hour period instead of doing a per-day analysis.

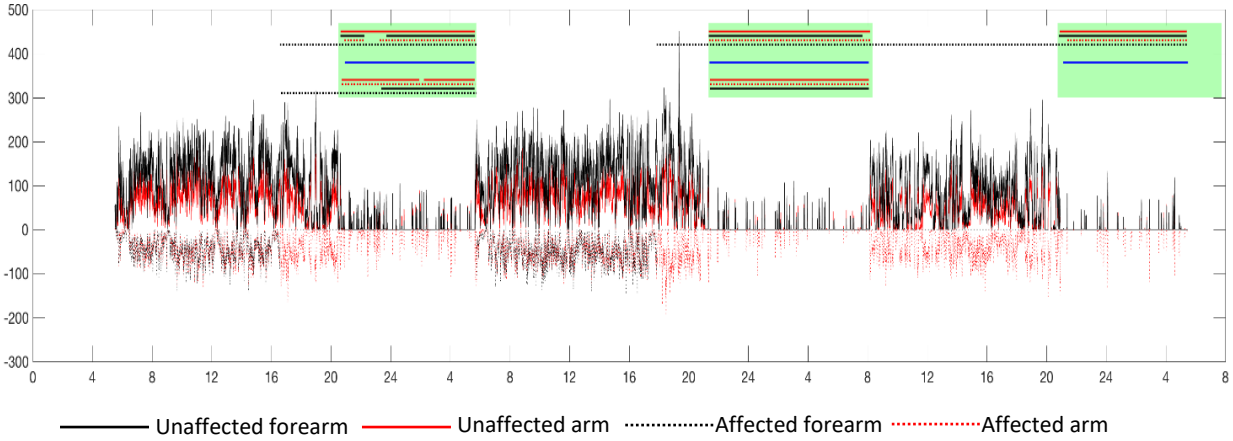


Figure 6: Vector magnitudes for each sensor plotted against time in the lower portion of the figure. In the upper portion, green patch shows sleep log sleep/wake times. Top four horizontal lines show van Hees protocol sleep/wake times, blue line shows eyeballing method sleep/wake times and bottom four horizontal lines represent Actilife sleep/wake times.

No-cost Extension

Importantly, a no-cost time extension was approved to enable the completion of this clinical trial. This included a revised Statement of Work (dated 8/5/19) which eliminated the second hypothesis of this clinical trial. The reasoning behind this change is the difficulty we have experienced recruiting sufficient numbers from our populations of interest. To address and overcome this challenge, we will assign all remaining Aim 1 participants to the active tDCS arm. This will give sufficient power to evaluate our primary hypothesis: that both groups will exhibit improved hand function between the baseline and post-intervention testing (when both standardized tests and accelerometry are repeated) (see page 9-10 for further discussion).

To support this change, MU has revised our regulatory materials and submitted them for approval to our local IRB. After local approval is received, we will submit this substantive change to DoD HRPO for review and approval. MU has shared the revised materials with WU and CMKI to expedite their IRB amendments, which are currently in process.

Specific Objectives: Subtasks are defined in the Statement of Work. Below is a summary of subtask status for the SOW that was approved during the annual project period.

Major Task 1: Prepare Regulatory Documents and Research Protocol

1) ☑ Subtask 1: Collaboration with all sites on development of manuals and training documents for Aim 1.

a. Status: Complete. This work was performed over email, via regular teleconferences between sites, and in-person during site training visits.

2) ☑ Subtask 2: Preparation and submission of human use materials to local IRB and DoD HRPO.
   a. Status: The University of Missouri maintains local IRB approval (1/24/18, CRR 01/09/2019) and DoD HRPO approval (5/25/18, CRR 01/14/19). Washington University maintains local IRB (6/21/18, 05/28/19) and DoD HRPO approval (08/01/18, 06/21/19). The Christine Kleinert Institute maintains local approval (1/14/17, CRRs: 1/11/18, 1/14/19) and DoD HRPO (10/09/18, 01/16/19).
3) ☑ Subtask 3: Quarterly/Annual Report Preparation.
   a. Status: We have submitted all quarterly reports for this year.
4) ☑ Subtask 4: Coordinate with Sites for annual IRB report for continuing review.
   a. Status: Complete.
5) ☑ Subtask 5: Renewal of IRB protocols.
   a. Status: We maintain regulatory approvals as necessary.
6) ☐ Subtask 6: Submit amendments, adverse events and protocol deviations.
   a. Status: No adverse events or protocol deviations to report.

Major Task 2: Administrative and/or Training Procedures

1) ☑ Subtask 1: Assistance with troubleshooting for all research sites.
   Status: MU continues to provide administrative support to WU & CMKI as needed.
2) ☑ Subtask 2: Obtain monthly progress reports from each site and coordinate overall project management across sites.
   Status: in progress, as scheduled.
3) ☑ Subtask 3: Presentation of data at conference.
   Status: in progress. We presented two abstracts related to this project at MU Life Sciences Week 2019.
4) ☑ Subtask 4: Organization, scheduling, and agenda coordination for biweekly teleconference meetings between all research teams.
   Status: in progress, as scheduled. Additionally, we maintain regular correspondence with sites in-between scheduled teleconferences as needed.
5) ☑Subtask 5: Participate in biweekly teleconference meetings between all research teams.
   Status: in progress, as scheduled.
6) ☑Subtask 6: Management and organization of all study records.
   Status: Complete. We maintain complete and organized study records.
7) ☑ Subtask 7: Assess quality of data across sites and provide corrective feedback.
   Status: In progress.
8) ☑Subtask 8: Hire/Identify therapists to administer protocol.
   Status: Complete.
9) ☑ Subtask 9: Hire Research Associate at University of Missouri.

Status: Complete. Dr. Frey hired Dr. Carmen Cirstea, M.D., Ph.D., to contribute 65% FTE to this project.

10) ☑Subtask 10: Travel to CMKI and WU to train research/clinical staff in administration of protocol.

Status: Complete.

11) ☑Subtask 11: Prepare administrative pipeline.

Status: Complete.

Major Task 3: Data Collection

Aim 1

1) ☑ Subtask 1: Gathering of data from medical records

CMKI: As reported in our meeting with the DoD grants team on 3/19/19, CMKI has identified 31 replant recipients from the electronic medical record that have been sent letters of invitation for the study. These letters have had a low response rate, so at CMKI, Dr. Elkin Galvis, a collaborating surgeon at CMKI, has started to call his previous patients directly to speak about potential study participation. This is helpful because Dr. Kaufman, the principal investigator at CMKI, cannot initiate first contact with participants via phone due to their local IRB regulations. Dr. Galvis can contact patients because he is their providing physician. Additionally, Dr. Kaufman has established a referral pipeline with surgical teams in Indianapolis and Cincinnati (both located in close proximity to Louisville). The Cincinnati team has a hand transplant recipient who is interested in learning more about the study and is currently in the recruitment pipeline.

WU: The WU team has gathered a list of potentially eligible participants from the medical record based on relevant medical codes, however, their most successful method of recruitment thus far has been direct referrals from surgeons who treat individuals who may potentially be eligible. WU's team will continue to rely on referrals from physicians to meet their recruitment goals.

MU: As discussed with our grants management team, Dr. Frey's team at MU has experienced difficulty with the recruitment of nerve repair patients. our team is encouraged by recent developments at University of Missouri. Our team has taken advantage of a recently available medical record recruitment service within the School of Medicine that has provided us with a list of 152 potentially eligible participants that have received care at MU within the past ten years.

In this project's most recent quarterly report (06/2019), we discussed our attempts to build a recruitment pipeline with private practice orthopedic surgeons around the Mid-Missouri area. Unfortunately, these attempts have been largely unsuccessful. However, we continue to work closely with the University of Missouri-associated Missouri Orthopedic Institute and the Department of Physical Medicine and Rehabilitation to speak with providers who could potentially provide referrals.

Additionally, we remain open to the possibility that some individuals who receive care at Washington University may actually be located closer to Columbia (a short two-hour drive on the interstate), and encourage participants located in the middle to select the institution that is most convenient for them to travel to during the two weeks of participation.

2) ☐ Subtask 2: Recruitment of patients
   Status: in progress.
3) ☐ Subtask 3: Patient Scheduling
   Status: in progress.
4) ☐ Subtask 4: Protocol Administration
   Status: In progress. Please see below for a summary of completed protocol administration at all sites. As discussed in previous reports and with our grants management team, recruitment has been a challenge for Aim 1 of this project. However, we submitted a revised our Statement of Work with updated recruitment goals with our no-cost time extension, and we are certain that we will successfully complete the revised recruitment goals over the course of the next year.
   WU: 4 (all nerve repairs)
   CMKI: 1 (replant)
   MU: 0

4) ☐ Subtask 5: dEEG protocol administration at MU
   Status: We have not yet completed a participant through Aim 1 at MU. Currently, our priority is the behavioral and tDCS portions of the clinical trial. dEEG administration is currently an opt-out procedure that participants do not have to complete in order to participate in the trial. We will remain focused on developing the novel therapeutic approach for improving outcomes of patients who have undergone hand transplantation, replantation, and peripheral nerve repairs. Although we also hope to administer the dEEG protocol designed for this clinical trial, this portion of the protocol is not necessary to investigate our main hypothesis.
7) ☑ Subtask 7: Coordinate pre/post actigraphy testing on all participants
   Status: In progress as planned. MU continues to successfully coordinate all pre/post actigraphy testing on all Aim 1 participants.

<u>Aim 2</u>

1) ☑ Recruitment of participants and patient scheduling.
   Status: Recruitment for Aim 2 continues as planned. Over the past year, we have developed two fruitful methods of recruitment for Aim 2:
   1. Dr. Frey and Ms. Buchanan remain in regular contact with Hanger Clinic (a national prosthetic device patient care services company that is located in 45 states). In particular, our team works closely with the Director of Scientific Affairs and the Upper Limb Program National Director. These directors are currently working to distribute recruitment materials for Aim 2 through clinics across the United States.

2. Twice weekly, a representative of Frey's laboratory sits in on a 3-hour amputee care clinic in the Physical Medicine and Rehabilitation Department in the University of Missouri School of Medicine. We continually recruit new and interested potential participants into Frey's database of individuals who wish to be contacted about future research opportunities. Although there are a limited number of upper extremity amputees in the clinic (lower extremity patients are by far the majority), we remain diligent in our efforts.

2) ☑ Protocol administration through mail.
Status: In progress as planned. Please see enrollment summary below.
MU:

traumatic amputee	23
congenital amputee	5
amputee due to disease	2
transplant	3
matched controls	8
in progress	6
Withdraw or screen failures	7

Major Task 4: Data Analysis/Manuscript Script

1) ☑ Subtask 1: Communication between research sites and statistical consultant for randomization based on pre-test data.
Status: Complete. The revised Statement of Work (dated 8/5/19) eliminates this subtask because randomization procedures have been eliminated.
2) ☐ Subtask 2: Unblind relevant analyzed data sets.
Status: Not completed. The revised Statement of Work (dated 8/5/19) eliminates this subtask because randomization procedures have been eliminated.
3) ☑ Subtask 3: Lead preparation of manuscripts from all projects.
Status: In progress, as planned.
4) ☑ Subtask 4: Assistance in preparing manuscripts from all projects.
Status: In progress, as planned.
5) ☐ Subtask 5: Final report preparation.
Status: not yet applicable.

Major Task 5: Dissemination of Results

1) ☑ Subtask 1: Presentation of data at conferences.
Status: In progress. Dissemination of data has commenced with the presentation of two abstracts at MU Life Sciences Week in April 2019.
2) ☐ Subtask 2: Attend DoD meeting/PLR.
Status: Not yet complete.
3) ☐ Subtask 3: Coordinate sharing of materials, protocols, and data sets with rehabilitation and scientific communities.
Status: Not yet complete.

What opportunities for training and professional development has the project provided?
All members of the study team have been provided training in the application of transcranial direct current stimulation (tDCS), constraint induced movement therapy (CIMT), and sensory re-education training. Additionally, we expect the work on this project to foster future inter-institutional collaborative projects related to transplantation science.

How were the results disseminated to communities of interest?
Dissemination of data has commenced with the presentation of two abstracts at MU Life Sciences Week in April 2019.

What do you plan to do during the next reporting period to accomplish the goals?
In the next reporting period, we will complete the revised Statement of Work (dated 8/5/19) for this project. This includes finishing data collection on Aim 1 (N=48) and Aim 2 (N=104), completing data analysis, manuscript preparation, and dissemination of results into the scientific community and patient populations. Frey's team will continue to coordinate closely with WU and CMKI to ensure timely completion of study goals and to provide support and feedback.

3. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
The use of constraint-induced movement therapy in the nerve repair population is novel and will make an important contribution to improving rehabilitative care. In addition to pioneering use of constraint-induced movement therapy in nerve repair, the project has already yielded a new set of graduated functional tasks for sensory retraining that will be useful to future research and clinical interventions in this population

What was the impact on other disciplines?
This work may have a broader impact by catalyzing new approaches to harnessing the central nervous system plasticity in recovery from a variety of traumas. Injuries that disrupt the flow of afferent (sensory) and/or efferent (motor) signals between the brain and body – including damage to the limbs (amputation, peripheral nerve injury, brachial plexus lesion) or spinal cord – induce pronounced changes in brain organization that are associated with neuropathic pain and poor outcomes following peripheral nerve repair. The work of this project is broadly relevant to understanding the larger question of whether these reorganizational changes can be reversed in the mature brain, and the functional significance of stimulating such changes. This knowledge is vital to optimizing the long-term impact of surgical and rehabilitative treatments on recovery from the variety of conditions listed above and to understanding their underlying mechanism.

Additionally, the findings of this project may have important implications for current and future efforts in neural engineering, advanced prosthetics and brain-machine interfaces in military and civilian populations.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

4. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change:

Several changes in approach were recently discussed in our NCE application. Firstly, Aim 1 recruitment has been a challenge throughout the course of this award, and across all research sites. In consultation with members of the team including the statistician, we have developed an alternative design that will allow us to address the central question of this project based on a revised enrollment projection (Aim 1: N=48, Aim 2: N=104).

In order to better understand the reasons for our revised enrollment goals and revised statement-of-work, a brief summary of the aims of the project will be helpful. Briefly, our original plan included a recruitment goal of N = 108 Aim 1 clinical trial participants (96 nerve repair patients, 12 transplant or replant recipients) in the chronic stage of recovery from peripheral nerve injuries surgical repairs, and/or hand transplantation and replantation procedures. Prior to the intervention, baseline data is acquired through both standardized tests of hand function and accelerometry recordings of hand use during several days of everyday life. Participants are randomly assigned to one of two groups, both groups undergo "forced" use of the affected hand by placing their good hand in a large mitten that eliminates use of the fingers for grasping and manipulating objects (i.e., constraint-induced movement therapy). Likewise, both groups participate in an intensive and custom-designed sensory retraining therapy, three times per week for two weeks. The difference between groups is that during these therapy sessions, one receives actual transcranial direct current (tDCS) brain stimulation, aimed at enhancing neuroplasticity; the other receives sham tDCS. This manipulation is double-blinded. After the intervention, subjects participate in periodic accelerometry followups to assess long-term changes in hand function during everyday life (within 1 week of the intervention, and at 1 month, 3 months, and 6 months post-intervention). *We are testing two hypotheses: 1) We expect that both groups will exhibit improved hand function between the baseline and post-intervention testing (when both standardized tests and accelerometry are repeated). 2) We predict that those receiving actual tDCS will exhibit overall greater improvements than those receiving sham tDCS.*

Critically, because all participants are 12 or more months post-nerve repair and in all participants Tinel's sign has reached the distal fingertips, we expect that their recovery of hand function will have reached a plateau. Certainly, hand function at the chronic stage of recovery would not be expected to change significantly over the course of two weeks. Therefore, we can be confident that any changes between baseline and post-intervention measures are likely attributable to the intervention.

Based on the literature in stroke patients with hemiplegia, we expect that the effects of intensive therapy and forced use will be large and detectable in our reduced sample (N= 48: 38

nerve repair patients, 10 hand transplant and/or replant recipients). By contrast, any adjuvant effects of tDCS would be expected to be quite small, and thus may be difficult to detect with a reduced sample size. **For this reason, we eliminated the second hypothesis and assign all remaining participants to the active tDCS arm.** This will give sufficient power to evaluate our primary hypothesis: *that both groups will exhibit improved hand function between the baseline and post-intervention testing (when both standardized tests and accelerometry are repeated).* It is true that by eliminating the sham tDCS, we will have sacrificed the ability to make any claims about the contribution of tDCS to such improvements, and this would need to be addressed in follow-up research. We feel that this is a reasonable compromise and are encouraged by the fact that our current Aim 1 participants have already expressed perceptions of improved hand function and satisfaction with the therapy.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  The change in approach discussed above is our solution to the recruitment challenges of Aim 1. The revised Statement of Work (dated 08/05/19) includes updated recruitment goals that are more realistic based on our experiences with the patient population thus far.

  The principal investigator at Washington University School of Medicine, Dr. Amy Moore, is leaving Washington University. Effective 10/16/19, Dr. Moore will transfer her responsibilities as principal investigator to Dr. Mitchell Pet, a plastic and reconstructive surgeon that specializes in wrist, hand and peripheral nerve surgery. We do not expect that this change in PI will cause significant delays in the project because each site is concurrently submitting a substantive amendment to DoD HRPO to approve changes outlined in the revised Statement of Work. Washington University will submit the change in PI in the same amendment.

- **Changes that had a significant impact on expenditures**
  The recently approved NCE application includes a revised budget for which there have been no major changes that have had a significant impact on expenditures.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  Human subjects: nothing to report.
  Animals: not applicable.
  Biohazards/select agents: not applicable.

5. **PRODUCTS**

Publications, conference papers, and presentations:

Morrow, S. (presenter), Cirstea, C., Morrow, S., Koenigsdorf, B., Motawar, B., Buchanan, K., Frey, S. (April 2019). Objective quantification of upper limb activity after reconstructive hand transplant: a case series study. Presented at University of Missouri Life Sciences Week, Columbia MO.

Mann, J. (presenter), Cirstea, C., Morrow, S., Koenigsdorf, B., Motawar, B., Buchanan, K., Frey, S. (April 2019). Monitoring real-world upper limb activity in experienced prosthetic device users. Presented at University of Missouri Life Sciences Week, Columbia MO.

Frey, S.H. (March, 2019). "*And now for something completely different."* University of Missouri Annual Stroke and Rehabilitation Conference.

Journal publications: We are currently preparing a case-study discussing a unique case we have observed: data collected on a transplant recipient before and after the transplanted limb was amputated due to rejection. We are also working on an additional manuscript discussing the real-life activity and limb usage differences between transplant recipients and amputees.

Books or other non-periodical, one-time publications: Nothing to report.
Other publications, conference papers, and presentations: Nothing to report.
Website(s) or other Internet site(s): Nothing to report.
Technologies or techniques: Please see Page 9 under Impact for discussion of novel rehabilitation techniques that have been developed under this award.
Inventions, patent applications, and/or licenses: Nothing to report.
Other Products: Nothing to Report.

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

University of Missouri
Name: Scott H. Frey, PhD, EdM
Project Role: Principal Investigator
Nearest person month worked: 3
Contribution to project: Dr. Frey has overseen all project activities as principal investigator. He developed the change in approach to address recruitment challenges and assessed the feasibility of this change. He provides feedback on all issues related to the project.

Name: Kelli Buchanan, BA
Project Role: Research Coordinator
Nearest person month worked: 9
Contribution to project: Ms. Buchanan acts as overall program coordinator. She serves as liaison between the University of Missouri and subaward sites, leading communication and biweekly meetings. She has managed the regulatory approval process and leads data collection on Aim 2 of the project, including the recruitment and mailing processes.

Name: Carmen Cirstea, PhD, MD
Project Role: Research Associate
Nearest person month worked: 8
Contribution to the project: Dr. Cirstea lends expertise to the analysis of data in Aim 2. Additionally, she has helped to develop the rehabilitation t

Washington University School of Medicine

Name: Amy Moore, MD
Project Role: Principal Investigator
Nearest person month worked: 0.5
Contribution to project: Dr. Moore has overseen all project activities at WU and contributed to protocol development and refinement.

Name: Carie Kennedy, BSN, RN
Project Role: Clinical Research Coordinator
Nearest Person Month Worked: 4
Contribution to Project: Ms. Kennedy has provided work to begin study startup and to obtain local and HRPO approval at WU. Ms. Kennedy attends the biweekly coordinators' teleconference to maintain regular communication, updates and progress reports to Mizzou.

Christine M. Kleinert Institute

Name: Christina Kaufman, PhD
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to project: Dr. Kaufman has overseen all project activities at CMKI. She has contributed to protocol development and refinement, and submission of regulatory and financial documents. She also participated in the site initiation visit/tDCS training provided by the MU team.

Name: Elkin Galvis, MD
Project Role: Co-Investigator
Nearest person month worked: 0.5
Contribution to Project: Dr. Galvis runs the University Hand Service located in a Level I trauma center. He participated in protocol refinement and selection of subject inclusion/exclusion criteria. He also participated in the planning of subject screening and recruitment as well as review of procedures for subject outcomes and follow up.

Name: Ashely Buren-Emrich, MS OTR/L, CHT / Laurie Newsome PT/CHT
Project Role: Therapists at CMKI site
Nearest person month worked: 1
Contribution to project: Ms. Emrich and Ms. Newsome share therapist responsibilities on this project, and provide back up for each other. They have worked together to develop the sensory-reeducation protocol that all centers will use, as well participated in protocol development and refinement. In addition they have participated in tDCS training and functional assessment training provided by the MU team.

Name: Emily Goldman, RN
Project Role: Nurse Coordinator
Nearest person month worked: 0.5

Contribution to project: Ms. Goldman acts as liaison for CMKI and assists with tasks as needed. Additionally, Ms. Goldman attends biweekly coordinators' teleconference to maintain regular communication, updates and progress reports to Mizzou.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

MU: Yes, the following grant ended. This will not impact Dr. Frey's effort on W81XWH1520037.

NS083377 National Institutes of Health/ National Institutes of Neurological Disease and Stroke
Program: Is cortical reorganization following limb amputation functionally relevant and reversible?
Dates: 04/01/2013 – 04/31/2019
Principal Investigator: Scott H. Frey
Direct Funds: $ 1,279,810

CMKI: Nothing to report.

WU: Nothing to report.

- **What other organizations were involved as partners?**
  Washington University School of Medicine and the Christine Kleinert Institute for Hand and Microsurgery are collaborating institutions on this award.

  Organization Name: Washington University School of Medicine
  Location of Organization: St. Louis, Missouri
  Partner's contribution to the project:
  - In-kind support*:*
    - Facilities *(e.g., project staff use the partner's facilities for project activities)*;
    - Collaboration *(e.g., partner's staff work with project staff on the project)*;

  Organization Name: Christine Kleinert Institute for Hand & Microsurgery
  Location of Organization: 225 Abraham Flexner Way, Louisville, Kentucky
  Partner's contribution to the project:
  - In-kind support*:*
    - Facilities *(e.g., project staff use the partner's facilities for project activities)*;
    - Collaboration *(e.g., partner's staff work with project staff on the project)*;

Human Use Regulatory Protocol Information

TOTAL PROTOCOL(S): 3

Protocol 1 of 3 total:

University of Missouri Health Sciences IRB #2008784

Human Research Protection Office (HRPO) assigned A-number: A-18965.2b

Title: Harnessing neuroplasticity to enhance functional recovery in allogeneic hand transplant and heterotopic hand replant recipients

Submitted to and Approved by: University of Missouri Health Sciences IRB, USAMRMC HRPO.

MU IRB

- submitted 12/19/2017
- approved 1/24/18
- CRR approved 01/09/2019

USAMRMC HRPO

- Submitted to USAMRMC HRPO on 3/26/18
- Approved 5/25/18
- Continuing Review Acceptance Memorandum issued 01/14/19

(i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed

Aim 1- nerve repair/transplant/replants

Contacted, not screened: 6

Contacted, screened: 3

Eligible: 1

Aim 1 – accelerometry pre/post testing

Screened: 5

Enrolled: 5

Aim 2

not including participants enrolled at WU while Dr. Frey's team was located there.

Total number of subjects enrolled through 10/14/19: 58

Withdrawals/screen failures through 10/14/19: 9

(ii) amendments submitted to the IRB and USAMRMC HRPO for review:

Approval date	Description

USAMRMC HRPO – pending approval. Submitted Substantive Amendment 253908 for review by USAMRMC HRPO on 10/10/2019. Will not implement changes until USAM USAMRMC HRPO approval is received.

MU IRB- 10/9/19	Review ID 253908 (Full Board; Substantive Amendment). Eliminated the second hypothesis and assign all remaining participants to the active tDCS arm. This will give sufficient power to evaluate our primary hypothesis: that both groups will exhibit improved hand function between the baseline and post-intervention testing (when both standardized tests and accelerometry are repeated).
MU IRB- 10/4/19	Review ID 254332 (expedited). Added dosage questions to the pain medication history questions approved in Review 253494.
MU IRB- 9/17/19	Review ID 253494 (expedited). Revised several study instruments (added questions about pain medication history).
MU IRB- 8/13/19	Review ID 252493 (personnel change form -administrative). Removed a research assistant from the project who is no longer working for the lab.
MU IRB- 7/17/19	Review ID 250193 (expedited). Added a recruitment option (MU Info, a newsletter) and made editorial changes to some recruitment materials.
MU IRB- 6/12/19	Review ID 249292 (expedited). Added the option for participants to remove accelerometers overnight while sleeping due to data analysis issues.
MU IRB- 6/14/19	Review ID 248808 (expedited). Added varied recruitment materials for Aim 2, editorial revisions of study instruments.
MU IRB -6/12/19	Review ID 249292 (expedited). Revised the protocol to state that participants may or may not be asked to remove accelerometers at night.
MU IRB-3/19/19	Review ID 246427 (expedited). Added inclusion question in Aim 2 that ensures participants will have another individual to help them don accelerometers, if they think they will be unable to do it by themselves. Also updated instruments for Aim 1 and made editorial revisions to the consent form to add more precise language about the types of activities that participants will perform (edited for clarity). Also changed

protocol to enable study members to complete therapy tasks during tDCS procedures for time efficiency (this does not change the risk level of the study).

MU IRB- 03/05/19 Review ID 246067 (expedited). Added healthy control participants to Aim 2 in order to compare healthy limb use to individuals who use protheses.

MU IRB- 02/01/19 Review ID 245021 (Expedited). Editorial changes to recruitment materials.

MU IRB- 1/24/19 Review ID 248668 (Administrative). Added research assistant to project.

MU IRB- 1/14/19 Review ID 243893 (expedited). Attached documentation of DoD HRPO approval for substantive amendment 242719 to MU IRB, had to perform an amendment to do this.

MU IRB 1/14/19 Review ID 243840 (expedited) updated study instruments.

MU IRB- 1/9/19 Continuing Review Report
DoD HPRO-1/14/19

MU IRB- 11/13/18 Review ID 242719. Changed consent process for Aim 2 (substantive).
DoD HRPO- 12/31/18

(iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

Nothing to report.

Protocol 2 of 3 total:

University of Louisville Human Subjects Protection Program IRB #15.0950

Human Research Protection Office (HRPO) assigned A-number: A-18965.2

Title: Harnessing neuroplasticity to enhance functional recovery in allogeneic hand transplant and heterotopic hand replant recipients

Submitted to and Approved by: University of Louisville (CMKI utilizes University of Louisville's IRB), USAMRMC HRPO.

Protocol 2 of 3 total:
University of Louisville Human Subjects Protection Program IRB #15.0950
Human Research Protection Office (HRPO) assigned A-number: A-18965.2

Title: Harnessing neuroplasticity to enhance functional recovery in allogeneic hand transplant and heterotopic hand replant recipients
Submitted to and Approved by: University of Louisville (CMKI utilizes University of Louisville's IRB), USAMRMC HRPO.

University of Louisville IRB

- Initial Application Approval: approved 1/14/17
- Continuing Review Report: approved 1/11/19
- Amendment Approvals during this quarter: none

USAMRMC HRPO.

- Approval received: 2/28/19

(i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed

In the last quarter, we contacted an additional 39 eligible patients in addition to the 30 previously identified. Of the 69 letters sent out, only 2 subjects responded, and both were not interested. Our local IRB mandates that we try to contact all subjects by letter first. Subsequently we queried the database for phone numbers, and of the 69 potential subjects 36 were current numbers. We are researching the other 33 subjects to see if we can find current contact information. Of the 36 contacted, only 5 answered the call or returned our message. None of the five were interested.

Our hand transplant patient 7 is very interested in participating in the trial and we are now waiting for him to accrue the vacation time needed to schedule the study. He recently moved to manage a new store in Indiana, which will make scheduling the study much easier. We plan to complete this study in November or early December. Patient 1,2 and 8 are considering participation, and we hope to enroll them at the time of their annual visit, which will be Jan-March of 2020. Hand Tx patient 10 is not eligible as she is a bilateral recipient.

In addition, we have a long standing relationship with the Indiana Hand to Shoulder Center, and the TriHealth Hand Surgery Specialist group in Cincinnati and they are both interested in referring subjects to Louisville for participation. Specifically we have contacted Dr. Tom Kaplan with the Indiana group, and Dr. Peter Stern and Dr. Keifhaber with the Cincinnati group. To date we have not received any referrals, but will continue to contact them.

We have had a subject referred to us by Dr. Frey and his team that lives closer to Louisville. We are planning to enroll this patient in the next quarter.

We have recently strengthened collaborations with the Department of Plastic Surgery at the University of Louisville, and are currently seeking referrals from this department, as well as the Department of Orthopedics at the University of Louisville.

(ii) amendments submitted to the IRB and USAMRMC HRPO for review
None

(iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.
None

Protocol 3 of 3 total:

Washington University School of Medicine HRPO# 201805155

Human Research Protection Office (HRPO) assigned A-number: A-18965.2e

Title: Harnessing neuroplasticity to enhance functional recovery in allogeneic hand transplant and heterotopic hand replant recipients

Submitted to and Approved by: Washington University School of Medicine Human Research Protections Office & USAMRMC HRPO.

WUSM HRPO

Submitted on: 05/22/18

Approval date: 06/20/18

6/5/2019	Mod to update contact information on recruitment flyer
5/28/2019	Continuing Review approved at Washington University in St. Louis
5/8/2019	Mod to add to study team
1/15/2019	Mod to submit updated MU protocols
11/5/2018	Mod to add to study team
8/2/2018	Mod to submit DOD approval and release ICFs
7/23/2018	Mod to add medical monitor

USAMRMC HRPO.

Approval date: 08/01/2018

(i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed:

Screened: 20; 8 excluded due to exclusion criteria or extreme distance from site

Contacted by phone for pre-screen: 12; 4 not interested, 2 excluded

Eligible: 6

Enrolled: 4

(ii) amendments submitted to the IRB and USAMRMC HRPO for review

Approval date	Description
8/1/2018	USAMRMC HRPO approval
6/11/2019	Continuing Review acknowledged

(iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation: None.

7. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.*

- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

8. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***