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

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CONTRACTING ORGANIZATION: **UNIVERSITY OF MISSOURI SYSTEM CURATORS OF THE UNIVERSITY OF MISSOURI 316 UNIVERSITY HALL COLUMBIA MO 65211-3020**

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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.

15. SUBJECT TERMS

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 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 within the reporting period 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:

- 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 continue for Aims 1 and 2. The COVID-19 pandemic has significantly slowed down progress on Aim 1 at all institutional sites (discussed in detail on Pages 21-22), however, we remain diligent in our recruitment efforts with modified safety protocols in place. Fortunately, Aim 2 data collection is conducted remotely and well-suited for safe administration during the pandemic with additional safety protocols. Our research team continues to employ our refined data analysis process on Aim 2 data, which includes novel data visualization techniques that will enable more effective dissemination of complex actigraphy data.

While progress continues on Aim 1 and Aim 2 data collection, we are preparing a manuscript reporting the results of three days of continuous limb activity monitoring from (4 below elbow unilateral transplants, 2 below elbow hand replants, 20 healthy controls, and 22 below elbow unilateral amputees). The main finding is that hand transplant and replant recipients exhibit a more symmetrical use of their limbs compared to the more one-handedness exhibited by unilateral

amputees with prostheses. However, levels of bilaterality in these limb reconstruction patients fall short of healthy matched controls.

Importantly, a no-cost time extension was approved in light of the effect of COVID-19 on our project's progress and to enable the completion of this clinical trial prior to the new award close date of 9/14/2021. This includes a revised Statement of Work (SOW; dated 5/20/2020). Updated SOW tasks are outlined in the Specific Objective section below.

Specific Objectives: Subtasks are defined in the Statement of Work to ensure timely completion of project goals. Below is a summary of subtask status for the project SOW dated 5/20/2020.

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 prior to this annual report.
2) ☑ Subtask 2: Preparation and submission of human use materials to local IRB and DoD HRPO.
    a. Status: Complete.
3) ☑ Subtask 3: Quarterly/Annual Report Preparation.
    a. Status: We have submitted all quarterly reports for this year.
4) ☑ Subtask 4: Coordinate with institutional sites for annual IRB report for continuing review.
    a. Status: Complete.
5) ☑ Subtask 5: Renewal of IRB protocols.
    a. Status: Please see Pages 25-28 for complete regulatory details. The University of Missouri maintains local IRB approval (1/24/18, CRR: 01/09/2019, 01/08/2020) and DoD HRPO approval (5/25/18, CRR 01/14/19, 2/23/20). Washington University maintains local IRB (6/21/18, CRR: 05/28/19, 5/5/20) and DoD HRPO approval (08/01/18, 06/21/19, 06/26/20). The University of Louisville (formally Christine M. Kleinert Institute; CMKI) maintains local approval (CMKI- 1/14/17, CRR: 1/11/18, 1/14/19, University of Louisville initial approval: 12/11/19) and DoD HRPO (CMKI - 10/09/18, 01/16/19, University of Louisville – 01/13/20).
6) ☑ Subtask 6: Submit amendments, adverse events and protocol deviations.
    a. Status: Complete. DoD HRPO acknowledged an unanticipated problem at University of Missouri regarding potential tDCS side effects and the withdrawal of a participant on 4/8/2020. University of Louisville and Washington University have nothing 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 support to Washington University and University of Louisville as needed via telephone, video conference, and email.
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: We have focused on data collection and analysis during this annual project period and have not presented any data at academic conferences.

4) ☑ Subtask 4: Organization, scheduling, and agenda coordination for biweekly teleconference meetings between all research teams.

Status: in progress as scheduled. We currently hold biweekly video conferences via Zoom. 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 as scheduled.

8) ☑Subtask 8: Hire/Identify therapists to administer protocol.

Status: Complete prior to beginning of the annual reporting period.

9) ☑ Subtask 9: Hire Research Associate at University of Missouri.

Status: Complete prior to the beginning of the annual reporting period.

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

Status: Complete prior to the beginning of the annual reporting period.

11) ☑Subtask 11: Prepare administrative pipeline.

Status: Complete prior to the beginning of the annual reporting period.

Major Task 3: Data Collection

Aim 1

1) ☑ Subtask 1: Gathering of data from medical records (relevant to Aim 1 only)

University of Louisville: In the last year multiple changes took place, including the transition of the VCA program and Dr. Kaufman's position in the CMKI and Jewish Hospital, to the University of Louisville and the Trager Transplant Center. The transition of the grant from CMKI to the University of Louisville was approved by both the University of Missouri and the DoD. Dr. Kaufman's full time faculty position was active as of 6/1/20. During this time Dr. Kaufman confirmed her 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, Dr. Kaufman has maintained contact with Dr. Tom Kaplan with the Indiana group, and Dr. Peter Stern and Dr. Keifhaber with the Cincinnati group.

Dr. Kaufman still has approved access to the list of previous replant recipients (from CMKI's electronic medical record). In addition, the Director of the Hand Service at the University of Louisville has transitioned to Dr. Lax Bhandari (this position was previously held by Dr. Galvis, who was associated with this study while Dr. Kaufman was located at CMKI). Dr. Bhandari worked with Dr. Kaufman when he was a fellow, and he and Dr. Kaufman have an established relationship. Dr. Bhandari is academically oriented, and has agreed to work with Dr. Kaufman to recruit subjects into the study.

In addition, as part of the transition, Dr. Bradon Wilhemi is now the lead transplant surgeon of the University of Louisville VCA program. Dr. Wilhelmi is a fellowship trained hand surgeon and also performs replant procedures. He has also agreed to help recruit subjects.

Washington University in St. Louis: No changes from the last annual report. The WU team gathered a list of potentially eligible participants from the medical record based on relevant medical codes (prior to the current annual reporting period), 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.

University of Missouri: Our team currently has a list of 152 potentially eligible Aim 1 participants that have received care at MU within the past ten years (obtained prior to the current annual reporting period) provided via a University of Missouri medical record recruitment service. Additionally, we remain open to the possibility that some individuals who receive care at Washington University may actually be located closer to the University of Missouri in Columbia, and encourage participants located between our two sites 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: Aim 1

University of Louisville: While the University has lifted restriction on the enrollment of subjects because of Covid-19 for non-essential clinical research, recruitment of subjects is still problematic. The majority of the hand transplant recipients in our program are from out of state, and many of the hand replant patients live a significant distance from Louisville and/or are from out of state. The University is still restricting non-essential travel to in state only. To further complicate matters, recent demonstrations in Louisville in response to the death of Breonna Taylor have been violent to the extent that parts of the downtown area, and for a time, parts of the UofL Health campus were boarded to reduce potential property damage. At least two subjects requested that any visits to Louisville be delayed until demonstrations were no longer a safety issue.

We do have a subject from out of state that was referred to our program by Dr. Frey (a hand replant recipient who recently completed Aim 2 at University of Missouri). As soon as the non-essential out of state travel ban is lifted, we will enroll this subject. Currently, the demonstrations are more peaceful, and the plywood is being removed from downtown and is gone from the Health Sciences Campus.

Washington University: A challenge for Aim 1 recruitment has been that many patients are referred/transferred from a distance to this Level 1 trauma center for their surgical treatment. Thus, these patients are not local and not able to fulfill the expectation of completing 7 visits within 2 weeks. There is not a work around for this challenge in light of the current pandemic (participants are hesitant to stay locally), but we continue to screen and attempt to enroll.

University of Missouri: The COVID-19 pandemic has also affected MU recruitment and enrollment. Similar to our institutional partners, many nerve injuries that require surgical repair are referred to the University of Missouri School of Medicine, which serves as a catchment hospital for surrounding rural areas. Travel is made more difficult by the pandemic, as potential participants are hesitant to commit to the protocol which involves 7 in-person visits. To ensure participant & staff health and safety, we have modified our

study protocols. These protocols are informed by both CDC and institutional-level guidance on how to safely conduct clinical research during the pandemic and include details on personal protective equipment, patient and staff monitoring of personal health and potential COVID-19 symptoms, and the use of EPA-approved disinfectants on all research materials and rooms. Additionally, we conduct the study at Rusk Rehabilitation Hospital, which requires a temperature and symptom screening for any individual who enters the building. These precautions are necessary and reduce risk, but recruitment has still slowed.

Aim 2:
University of Missouri: Aim 2 of this project (accelerometry) is conducted entirely through the mail, online, and over the phone: no in-person contact is necessary. With enhanced disinfection procedures, we have resumed Aim 2 safely.

Dr. Frey has a collaborative working relationship 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 with their staff on a renewed effort to distribute recruitment materials for Aim 2 through clinics across the United States. This recruitment avenue has been fruitful, and we continue to have a slow but steady stream of individuals with an amputation contact the lab with interest in the accelerometry study.

In addition to recruitment of amputee participants, our lab continues to work on the recruitment of age- and sex-matched controls. We regularly post IRB-approved recruitment advertisements in a campus-wide email listserv called MU Info, which is also posted online for the broader community to view. This has served as a very successful avenue for control recruitment.

3) ☐ Subtask 3: Patient Scheduling
Status: see Subtask 2 (above) for details.

4) ☐ Subtask 4: Protocol Administration
Status: Aim 1: In progress. Please see below for a summary of completed protocol administration at all sites. As discussed in previous reports and during our bimonthly updates with our DoD grant team, recruitment has been a challenge for Aim 1 of this project, particularly in light of the COVID-19 pandemic. However, we submitted a revised Statement of Work with updated recruitment goals with our no-cost time extension to account for delays. We are hopeful that as public health measures are better implemented by our local public health departments & local government leaders, outbreaks will stabilize and our communities will become accustomed to enhanced safety protocols in daily life. Study sites continue to forge ahead with recruitment.

We remain committed to the recruitment and protocol modifications we put forth in our no-cost extension request. For example, the multi-site design of this study allows for flexibility in and the shifting of recruitment goals if one or two sites are required to temporarily suspend recruitment due to high COVID-19 levels in their area. The locations of our sites are geographically separated enough to have very different-looking COVID-19 outbreaks. For instance, if Columbia's COVID-19 levels are significantly

lower than in St. Louis and/or Louisville, Dr. Frey's team can 'take on' some of our partner sites' enrollment load in order to account for differences in the ability to carry out in-person research. These recruitment goals will be adjusted continually during the project period in accordance with each institution's ability to conduct research and to ensure the success of our enrollment goals as outlined in our Statement of Work.

Current Enrollment for Aim 1:
Washington University: 4 complete (nerve repairs), 1 postponed
University of Louisville: 1 complete (replant; while team was located at CMKI)
University of Missouri: 1 complete (nerve repair), 1 withdraw, 2 postponed

Current Enrollment for Aim 2:
Protocol administration for Aim 2 at University of Missouri remains uninterrupted.
Individuals with amputations: 39
Healthy controls: 27
Transplant/replant recipients: 4
Enrolled, completing protocol at time of report: 5
Withdrawals – 13

7) ☑ Subtask 5: Coordinate pre/post actigraphy testing on all participants
Status: In progress as planned. MU has successfully coordinated pre/post actigraphy testing on all Aim 1 participants to date.

Major Task 4: Data Analysis/Manuscript Script

1) ☑ Subtask 1: Analyze data sets.
Status: in progress. Please see Pages 9-21 for highlighted results.
2) ☑ Subtask 2: Lead preparation of manuscripts from all projects.
Status: in progress.
3) ☑ Subtask 3: Assistance in preparing manuscripts from all projects.
Status: in progress.
4) ☑ Subtask 4: Final report preparation.
Status: Not yet applicable.

Major Task 5: Dissemination of Results

1) ☑ Subtask 1: Presentation of data at conferences.
Status: We did not present data at any academic conferences during the annual reporting period.
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?

This project has provided training to Dr. Frey's postdoctoral fellow, Dr. Binal Motawar, who developed novel data visualization and data analysis techniques with Dr. Frey's guidance. The study coordinator, Kelli Buchanan, continues to develop clinical trial management and administration skills. Additionally, the study research assistant, Mr. Sean Morrow, is a recent University of Missouri graduate who has developed study recruitment and administration skills with guidance and

supervision from Kelli Buchanan and Dr. Frey. Mr. Morrow plans to pursue a career in medical research. Overall, this grant has provided many training and professional development opportunities for Dr. Frey's team, all of whom have careers in research. All members of the study team maintain training in the application of transcranial direct current stimulation (tDCS), constraint induced movement therapy (CIMT), and sensory re-education training. Finally, 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?
We have not participated in dissemination activities during the current reporting period.

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 5/20/20) for this project. This includes finishing data collection on Aim 1 and Aim 2, completing data analysis, manuscript preparation, and dissemination of results into the scientific community and patient populations. Specifically, University of Louisville will work with Dr. Bhandari and Dr. Wilhelmi to seek local subjects that can be completed safely without the travel considerations of their currently interested subject. Similarly, Washington University and the University of Missouri will continue to search for suitable local candidates that can attend our Aim 1 study sessions safely. Finally, Dr. Frey's team will continue to coordinate closely with Washington University and the University of Louisville 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?
During the current reporting period, the project has not made an impact on the development of the principal disciplines of the study. However, we anticipate the following impact for Aim 1: 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.

Additionally, we anticipate the following impact for Aim 2: we expect that the results of accelerometry analysis in a large population of amputee and control participants will functionally define upper extremity movement in the real world, which may subsequently inform the development of novel rehabilitation techniques and prosthesis technology that are better tailored to amputees' real-world needs. Further, the use of accelerometry in hand transplant recipients will illuminate patterns of functional recovery that may inform clinical decisions related to transplant recipients' medical care.

What was the impact on other disciplines?
During the current reporting period, the project has not made an impact on the development of the principal disciplines of the study. However, we anticipate that 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.

Significant/Key Results during the Reporting Period:
In Aim 2, 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. To briefly recap, subjects wore a total of four accelerometers (GT9X Link, ActiGraph Corp, Pensacola, FL) for a period of 3 days (72 hours). The accelerometers were worn on each forearm and each bicep. The duration covered was two weekdays and one weekend day. If subjects had an irregular work schedule (i.e., not Mon-Fri), the study team ensured data collection took place over two work days and one non-work day.

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.

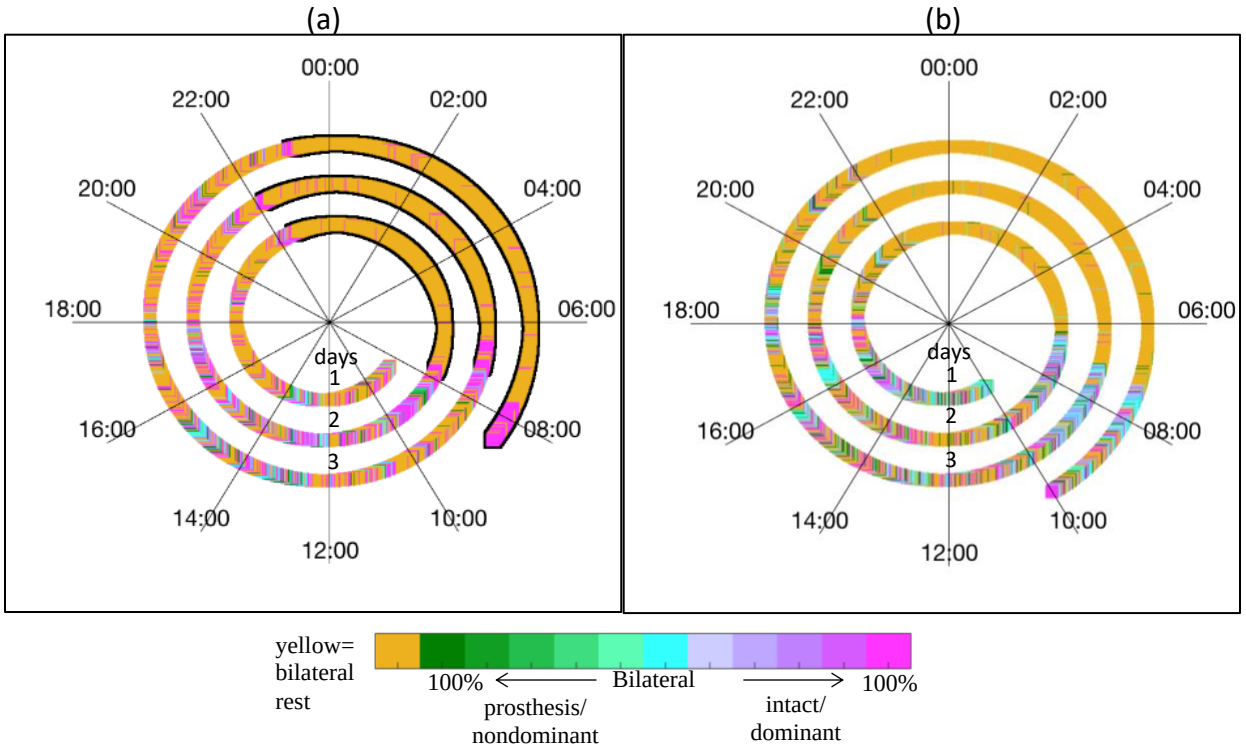


Figure 2 (above): spiral graph showing change in reliance on the intact forearm throughout the day in (a) prosthesis user and (b) control subject, over three days (72 hours). Black outline in prosthesis user represents prosthesis nonwear. The first day of the data collection is plotted in the inner most 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, below) (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 indicating greater reliance on the unaffected side throughout the day, as was previously shown (Chadwell et al., 2018).

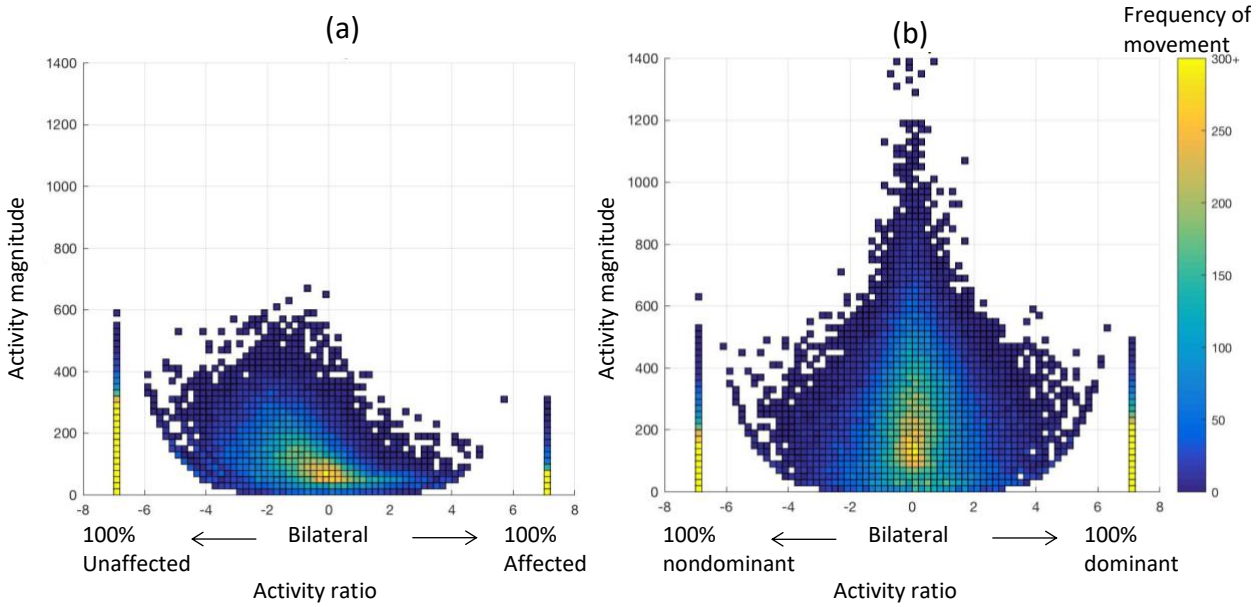


Figure 3: Density plots for forearm activity in (a) prosthesis user and (b) control subject.

Additional variables to characterize prosthesis use:

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.

To illustrate, Figure 4 Upper Panel (next page): Illustrates the case of EH, a right hand transplant recipient recorded 7 post-surgery. Figure 4 Lower Panel provides 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 (Upper Panel). After re-amputation (Lower Panel), he exhibits a pronounced left asymmetry reflecting considerably less use of right (dominant/prosthetic) vs. left (non-dominant/unaffected) limb.

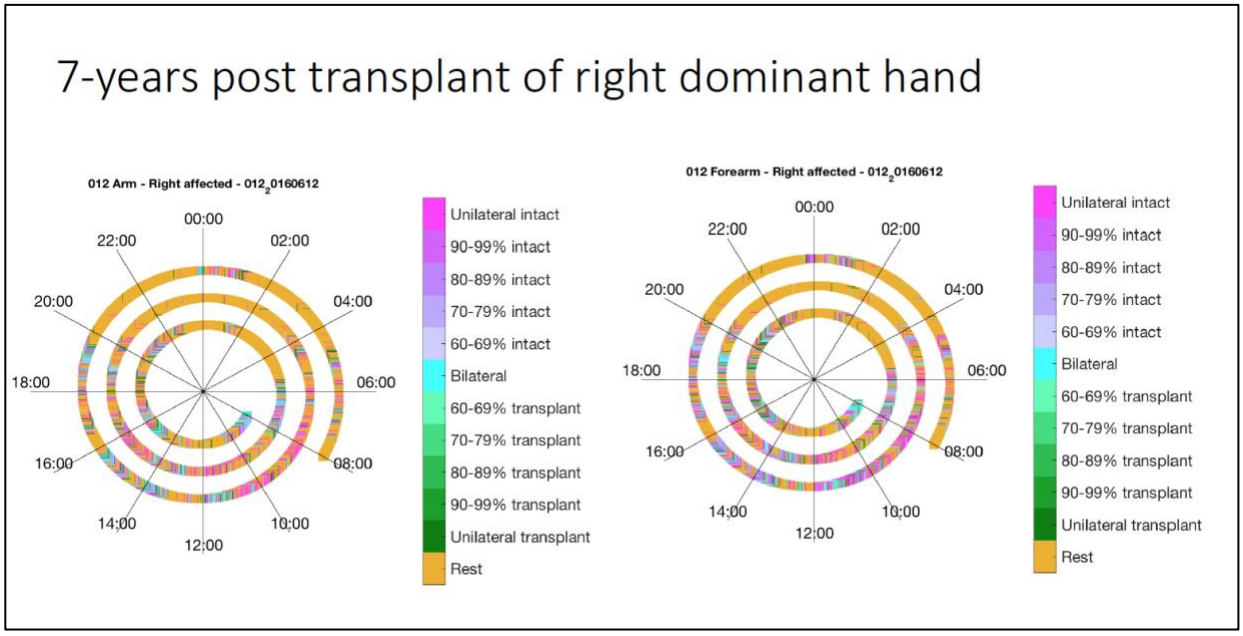
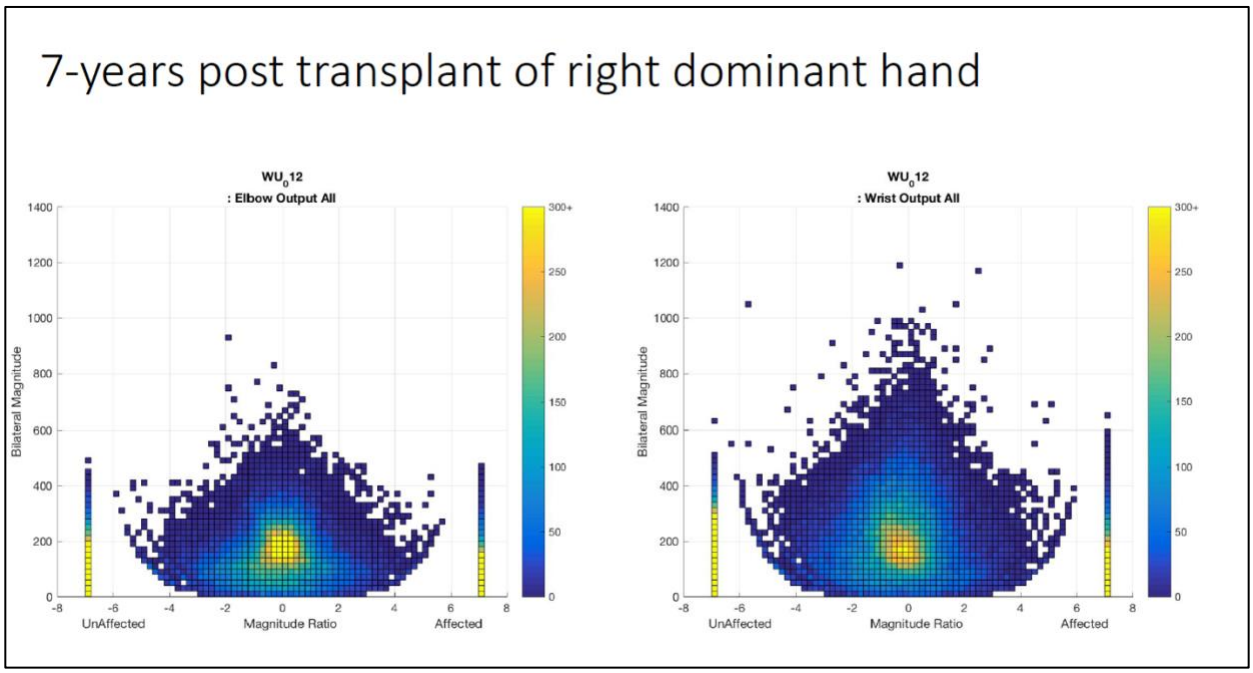


Figure 4 Upper Panels



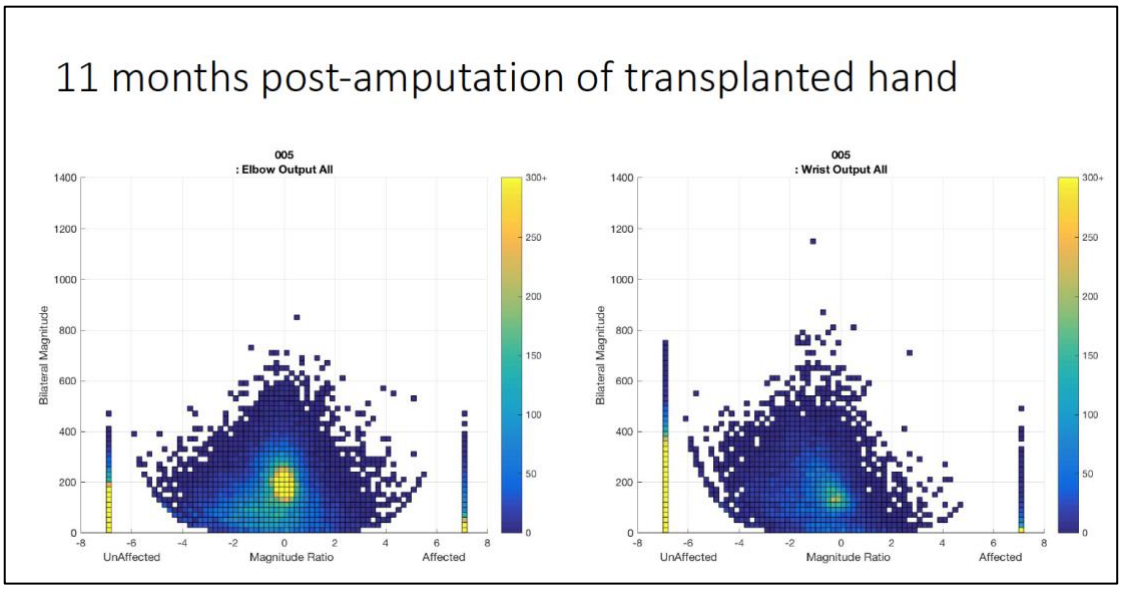
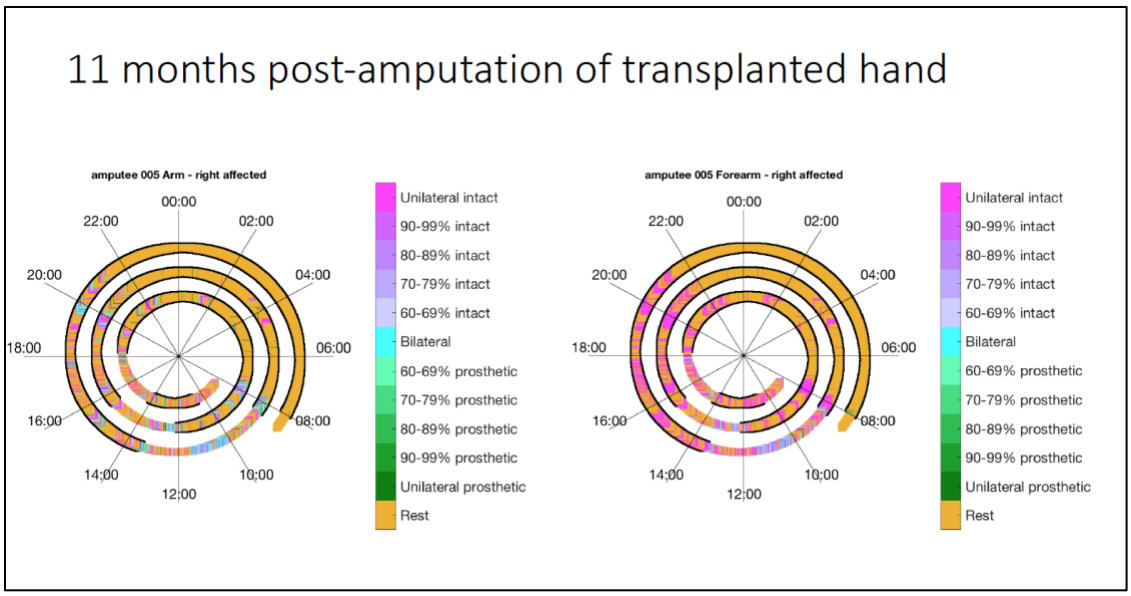


Figure 4 Lower panels



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).

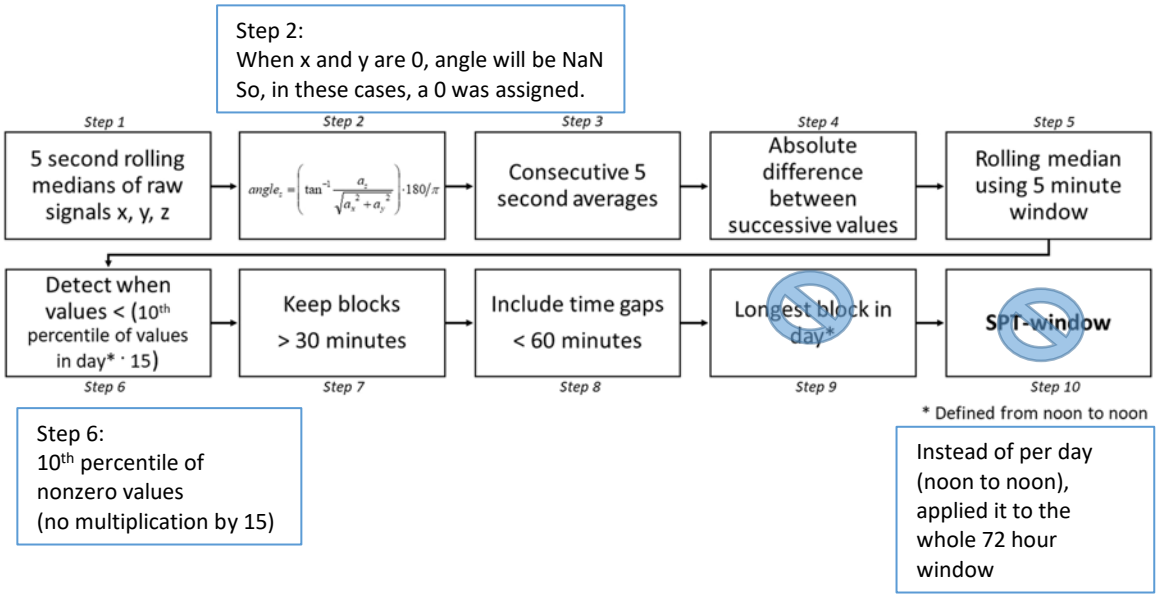


Figure 5: Adoption of the van Hees sleep/wake detection protocol for our study. The main differences were 1) the threshold was set as the 10th percentile of the rolling median using a 5 minute window, and 2) we applied this algorithm to our entire 72 hour period instead of doing a per-day analysis.

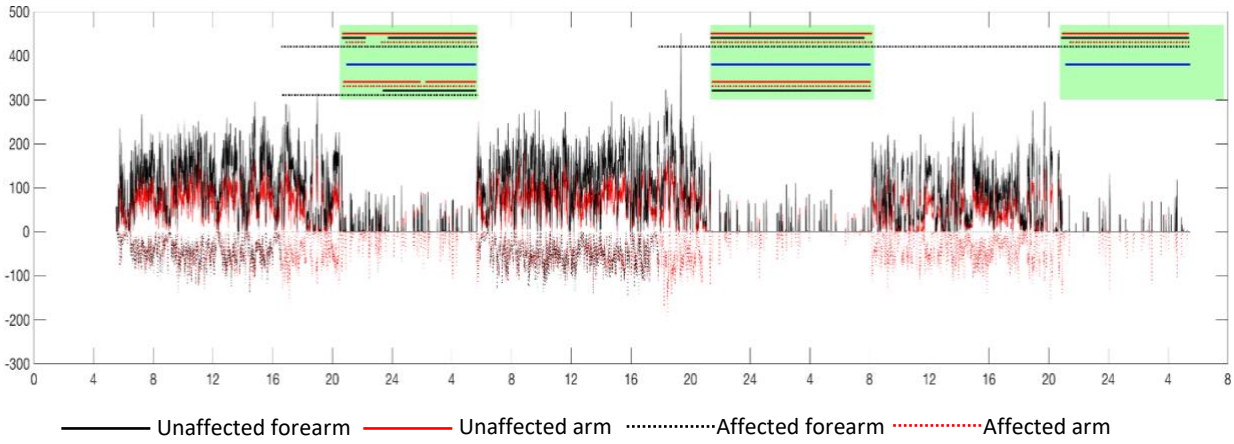


Figure 6: Vector magnitudes for each sensor plotted against time in the lower portion of the figure. In the upper portion, green patch shows sleep log sleep/wake times. Top four horizontal lines show van Hees protocol sleep/wake times, blue line shows eyeballing method sleep/wake times and bottom four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

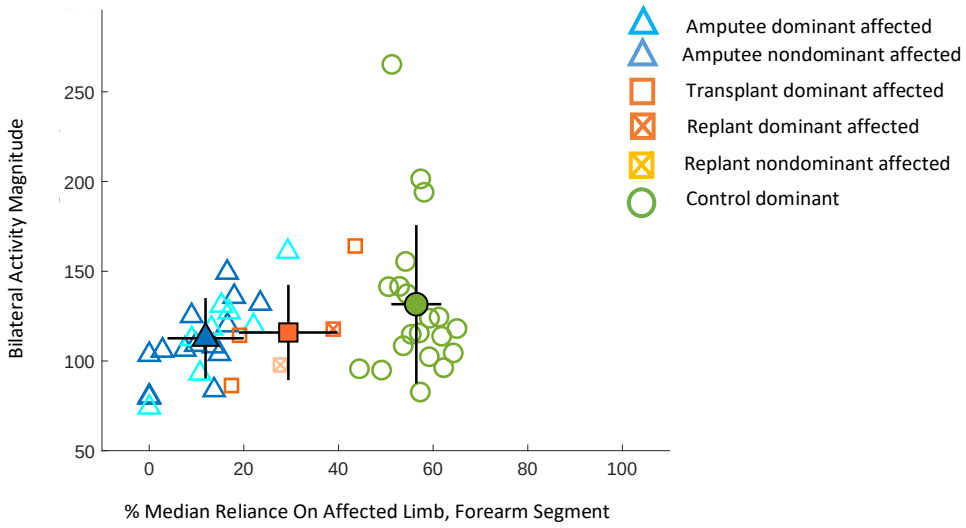


Figure 7 (above): Compared to unilateral prosthesis users, individuals with a transplanted hand used their affected and intact side more evenly. However, controls exhibited more even bilateral movement when compared to individuals with a transplanted hand. All three pairs (re/transplant vs. controls, re/transplant vs. amputees, amputees vs. controls) are significantly different. Error bars are SD.

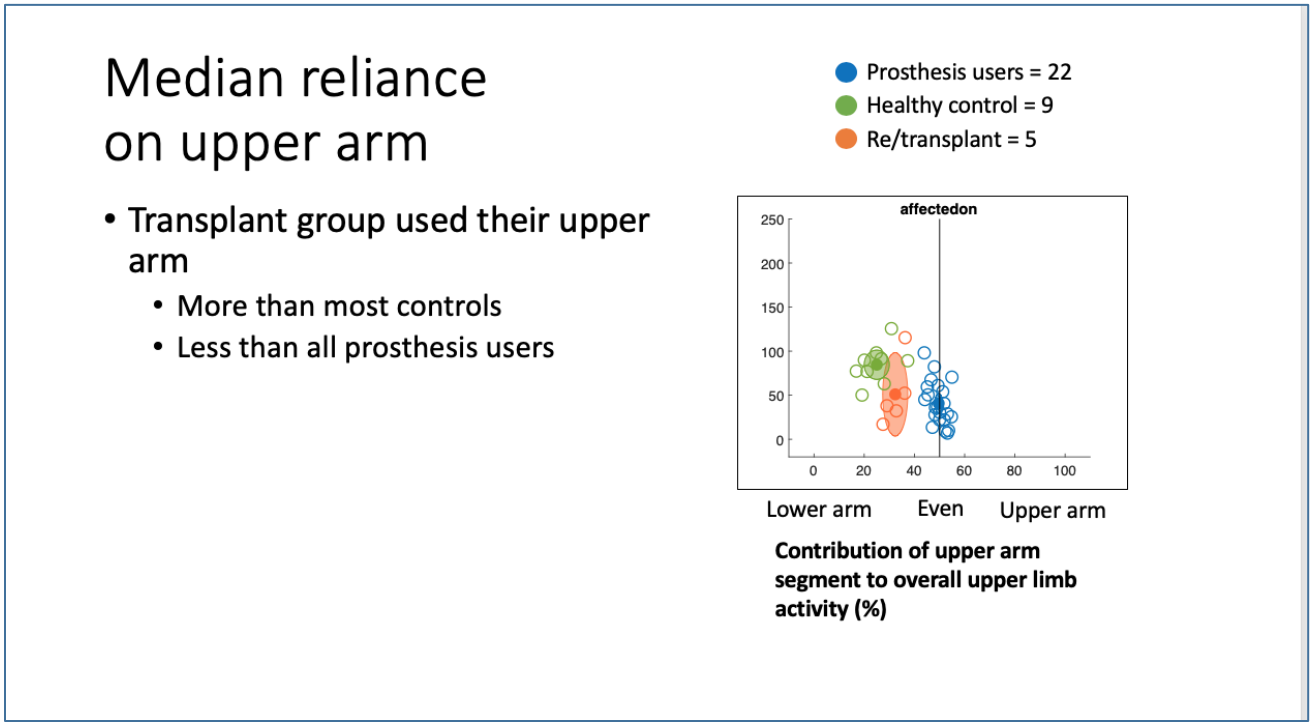


Figure 8 (above): Individuals with a transplanted hand used their upper arm more than more control subjects, but less than all prosthesis users.

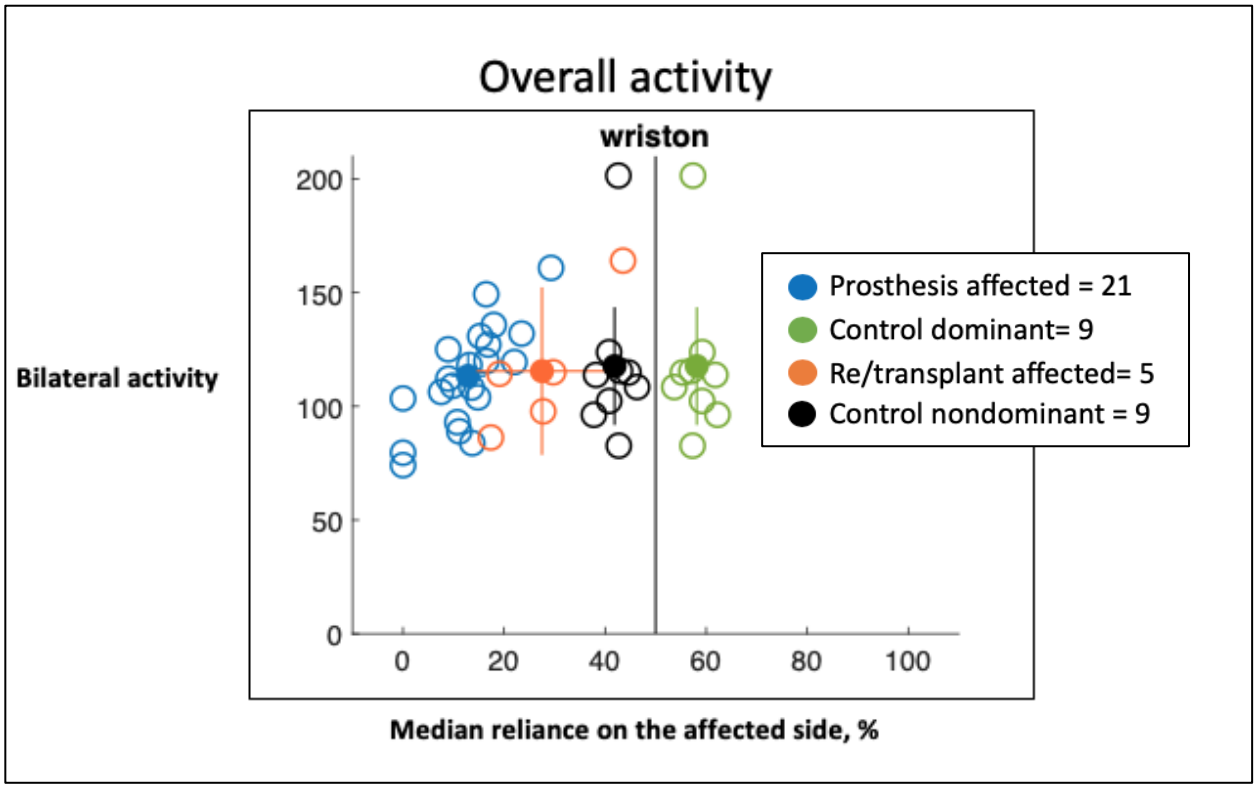


Figure 9. Prosthesis and hand transplant usage: overall activity. 0% reliance on the intact side indicates unilateral activity in the prosthesis forearm or the affected side arm. Similarly, 100% reliance on the intact side suggested unilateral activity in the unaffected forearm or arm. In our sample of hand transplant and replant recipients, we found that the affected hand was used more than amputees' use of their prosthesis ($p<0.05$). Additionally, the transplanted hand was used less than control subjects' dominant or nondominant hand ($p<0.05$). Finally, amputees used their prosthesis less than control subjects dominant or nondominant hand ($p<0.05$).

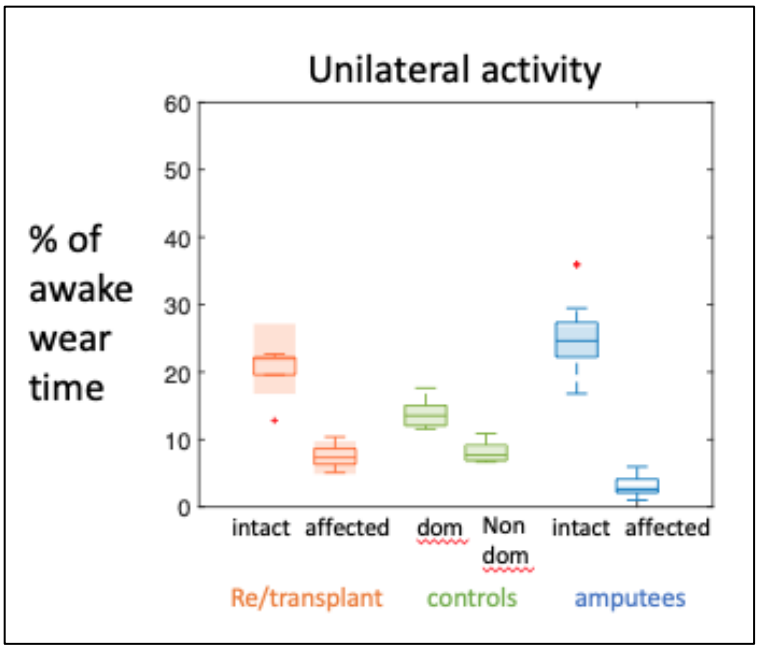


Figure 10. Unilateral activity involving the prosthesis or hand trans/replant during awake wear time.

Figure 10. Unilateral activity involving the prosthesis or hand trans/replant during awake wear time. Individuals with a replant or transplant spent less time engaged in unilateral activities when compared to the amount of unilateral activities that controls engaged in (involving their dominant or nondominant hand).

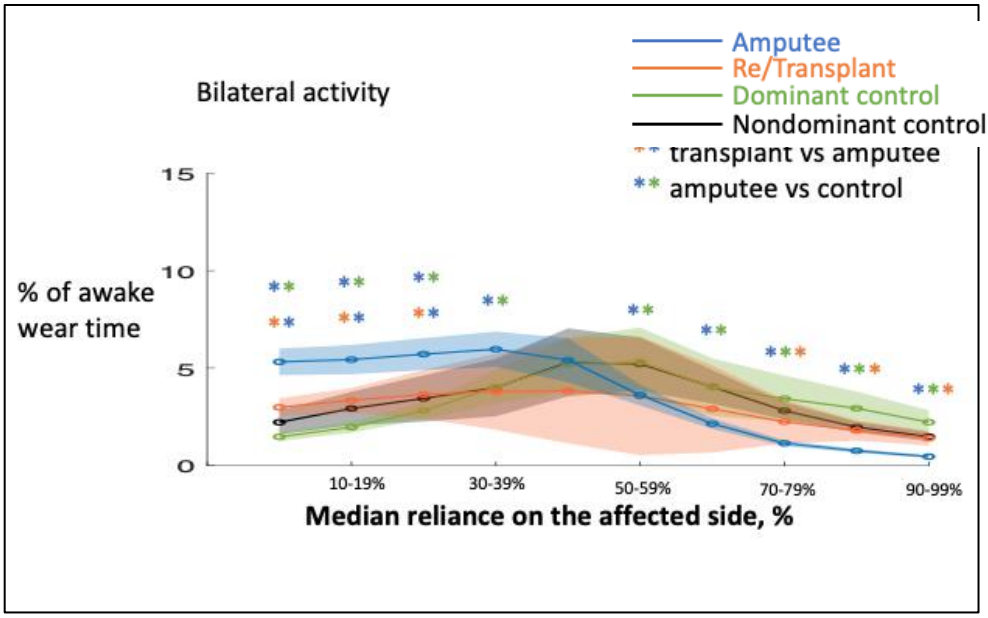


Figure 11: Bilateral activity involving the prosthesis or hand trans/replant during awake wear time.

Figure 11. Bilateral activity involving the prosthesis or hand trans/replant during awake wear time. Bilateral activity in individuals with a trans/replanted hand was comparable to the nondominant hand in our control sample ($p<0.05$). However, the trans/replanted hand was used less compared to the dominant hand of controls ($p<0.05$). Amputees showed a different bilateral hand activity compared to

controls and hand trans/replants: amputees showed a greater time spent at 1-39% median reliance on the affected side ($p<0.05$). This reflects their greater usage of the intact side.

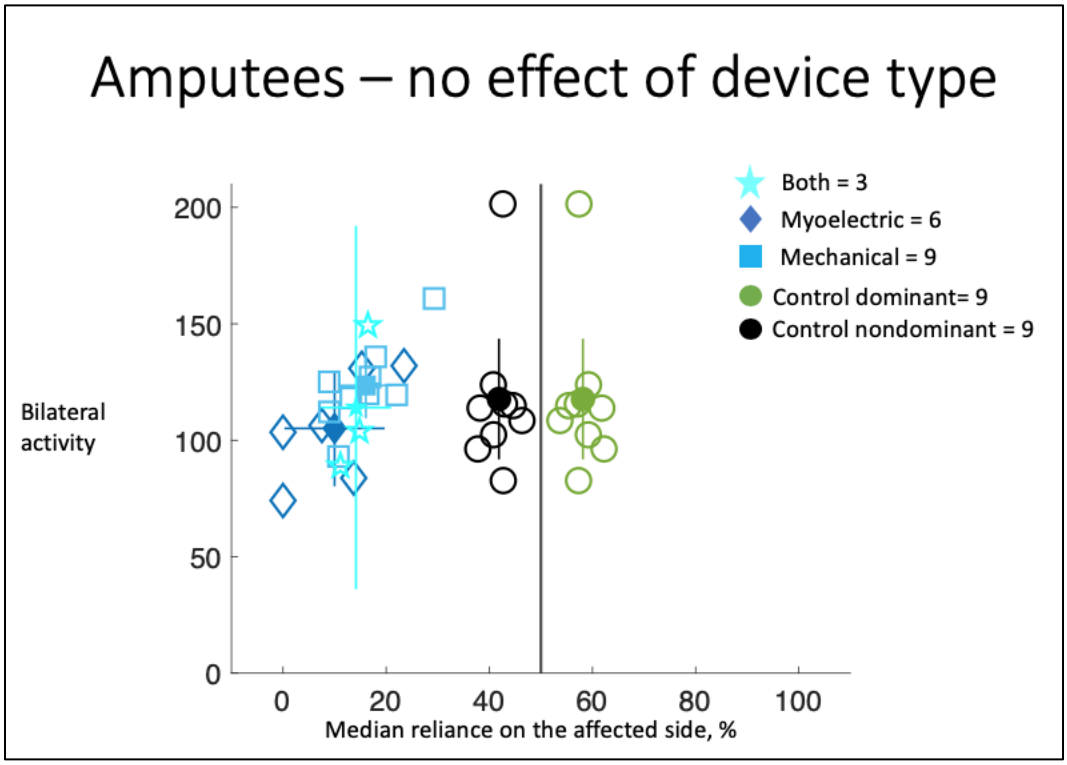


Figure 12: The type of prosthetic device had no impact on median reliance on the affected side in amputee users.

Figure 12: The type of prosthetic device had no impact on median reliance on the affected side in amputee users. The type of prosthetic device that amputees used (i.e., myoelectric, mechanical, or both) did not affect median reliance on their affected side.

Figure 13: Upper arm contribution to the affected upper limb activity with prosthesis on vs off:

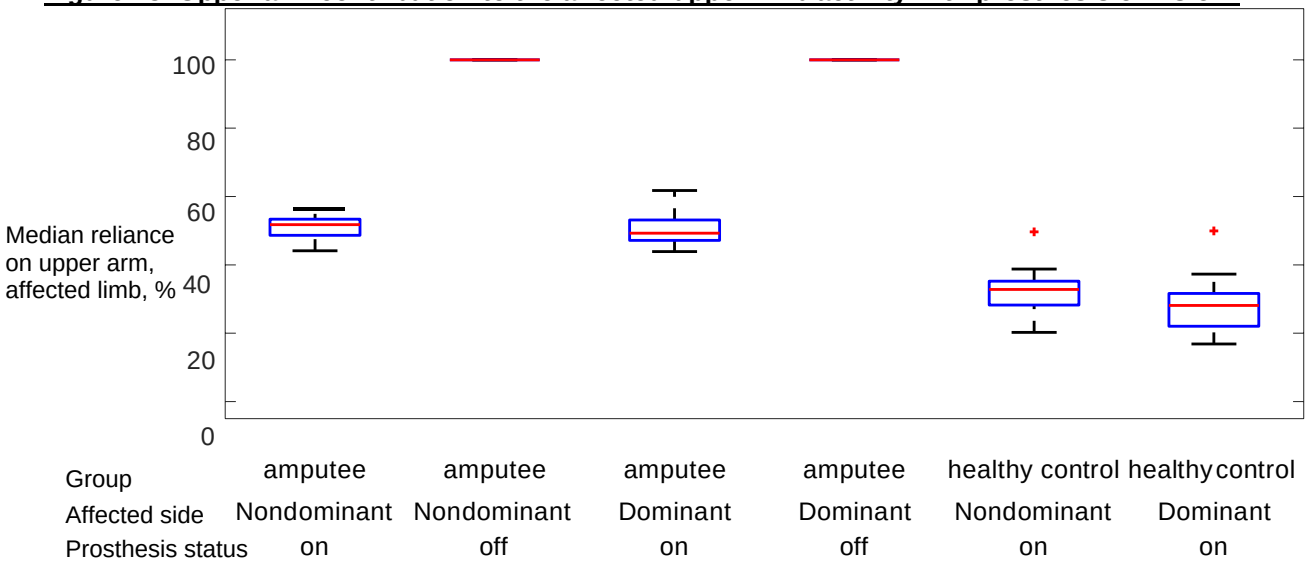


Figure 13: Amputees in general showed comparable use of their upper arms in relation to their lower arms in the affected upper limb, in contrast to higher lower arm use in healthy controls. This may contribute to over-use of the affected side upper arm in amputees, creating the possibility of overuse injuries.

Figure 14: Affected upper arm contribution to bilateral upper arm activity with prosthesis on vs off:

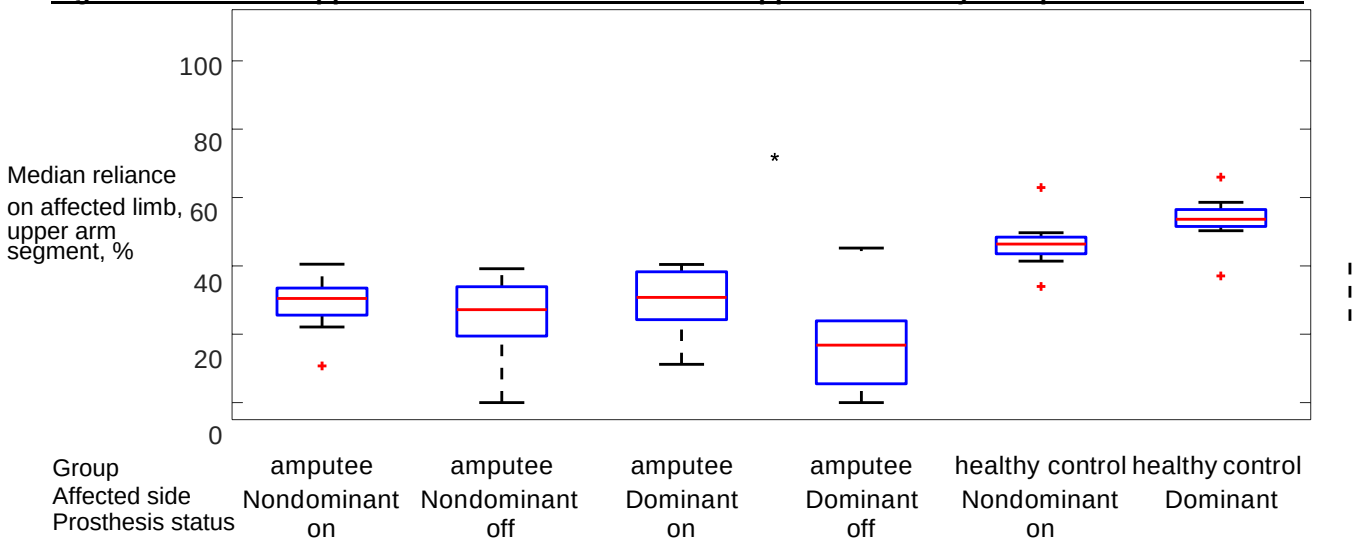


Figure 14: For the upper arm, amputees had different inter-limb reliance of upper arms reliance based on whether the dominant or nondominant hand was affected. In subjects with the dominant hand amputations, taking the prosthesis off resulted in lower use of the affected side upper arm compared to the intact side upper arm. The nondominant hand amputees showed no differences in the upper arm usage between prosthesis on and off conditions.

4. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change:
As discussed in our prior quarterly reports and our no-cost extension application, the impact of COVID-19 on our research progress has been substantial. In March 2020, all study institutions rapidly enacted research ramp-down plans that prohibited in-person research due to COVID-19 concerns. Our University of Missouri team was required to postpone several remaining Aim 1 sessions for an Aim 1 participant that was enrolled in the study on March 10, 2020. Additionally, MU's team was forced to postpone several pre-screened Aim 2 participants due to the uncertainty of the pandemic situation (these have since been completed). Similarly, Washington University postponed a then-current Aim 1 participant due to institutional guidance and in order to protect participant and research staff health and safety. During March and April 2020, our entire research staff was mandated to work-from-home to abide by state- and county-level quarantine orders. During this period, our team diligently worked on data analysis and manuscript preparation. Since Spring 2020, COVID-19 considerations have evolved significantly. Fortunately, all study sites are located within the School of Medicine of each institution, which provide a safe physical environment in which the entire population adheres to strict public health measures and social distancing guidelines. Additionally, our positions within medical institutions allow for access to personal protective equipment to ensure the safety of our participants and research staff.

Washington University: As the pandemic evolves, Washington University Medical Center continues to follow a carefully monitored, stepwise plan for re-starting in-person clinical research activities. We are currently at 'Clinical research Yellow Level 2' – A medium phase in which a broader range of clinical research can resume, if it does not pose undue risk to participants or other persons, and assuming excellent precautions are in place to ensure safety and avoid viral transmission. Our in-person study sessions have always been done outside of the ambulatory clinic area, where population density adjustments have been made to minimize contact among staff. However, we continue to search for local participants who are not at risk for COVID-19 complications and can safely travel to Washington University to complete the protocol.

University of Louisville: Currently, restrictions on interstate travel is still limiting enrollment as many patients travel to Louisville for microsurgical nerve repair. However, Dr. Kaufman will continue to work closely with her surgical colleagues, Dr. Brandon Wilhemi (lead transplant surgeon at University of Louisville), and Dr. Lax Bhandari (the Director of Hand Service at University of Louisville), to find suitable candidates whom are local and low-risk for COVID-19.

In addition to changes in recruitment strategy, we have modified our protocols for health and safety. These changes have been approved by our local regulatory bodies. Briefly, our protocols include the addition of: COVID-19 screening measures for participants and staff, including daily fever and symptom checks for in-person studies, modification of the physical environment to enable social distance, appropriate use of PPE, and a specified method of disinfecting research materials with EPA-approved cleaners to reduce the risk of transmission through research materials. Importantly, these protocols include instructions on how to quickly shift our Aim 2 research plan in accordance with current regional levels of COVID-19 community spread based on defined criteria set by our institution and the local public health departments. The research team has regulatory approval to continue to administer Aim 2 from home if the University of Missouri campus shuts down.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  As discussed above, COVID-19 has significantly slowed our Aim 1 recruitment. We have outlined several strategies to combat this in previous sections. In summary, we remain flexible as institutions to take on extra recruitment as our local environments allow – for example, if University of Missouri finds several eligible participants in excess of quarterly goals, we will complete them to relieve recruitment goals for other institutional sites. Washington University and University of Louisville are also flexible and ready to take on additional recruitment. In the interim, our University of Missouri team continues to focus heavily on Aim 2 recruitment, data analysis, and manuscript preparation.

- **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: nothing to report.
Journal publications: We are working on a manuscript that includes data from three days of continuous limb activity monitoring in 4 below elbow unilateral transplants, 2 below elbow unilateral replants, 20 healthy controls, and 22 below elbow unilateral 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: Nothing to report.
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: 11
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 leads administration of Aim 1.

Washington University School of Medicine
Name: Mitchell A. Pet, MD
Project Role: Principal Investigator
Nearest person month worked: 0.15
Contribution to project: Dr. Pet took over sub-site PI responsibilities at Washington University in St. Louis on 10/15/19, when the previous PI, Amy Moore took a new position at The Ohio State University. He has overseen all project activities at WU and contributed to ongoing protocol refinement.

Name: Carie Kennedy, BSN, RN
Project Role: Clinical Research Coordinator
Nearest Person Month Worked: 1
Contribution to Project: Ms. Kennedy has provided work to obtain local and HRPO approval at WU. She participates in subject recruitment, pre-screening, intervention administration, testing, and patient follow-up. Ms. Kennedy attends the biweekly coordinators' teleconference to maintain regular communication, updates and progress reports to Mizzou.

University of Louisville
Name: Christina Kaufman, PhD
Project Role: Principal Investigator
Nearest person month worked: 1

Contribution to the project: Dr. Kaufman has overseen all project activities at this site. She has contributed to protocol development and refinement, and submission of regulatory and financial documents. During the reporting period, she has moved to the University of Louisville and is a full-time faculty member in the Department of Cardiovascular and Thoracic Surgery, and has been named the Scientific Director of the University of Louisville Vascularized Composite Allograft (VCA) Program. She also retains her gratis faculty position in the Division of Hand Surgery in the Department of Surgery. Please note that as the hand therapists are currently only participating in the project by performing testing of subjects once they are enrolled, we have decided to pay the hand therapists on a consultant basis rather than a monthly percent effort.

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

University of Missouri: Nothing to report.

Washington University: Nothing to report.

University of Louisville: Yes, the following two grants were awarded. The first grant is active as of 9/30/2020. The second is still being negotiated. Dr. Kaufman's effort on W81XWH1520037 will not be affected.

RT190021 – FY19 Reconstructive Transplantation Research Program – Idea Discovery Award- "Effect of Peri-transplant C3d Blockade and Ischemia on Chronic Rejection and Vasculopathy in an Experimental OMC Flap Model of VCA."
September 15, 2020 – September 14, 2022 –
Kaufman – PI 5% effort

RT190094 – FY19 Reconstructive Transplantation Research Program – Quality Outcomes Research "Assessing the Comparative and Longitudinal Benefits of VCA of the Hand."
Scott Tintle, PI Walter Reed/Uniformed University of the Health Sciences
September 15, 2020 – September 14, 2022 –
Kaufman – subward PI 5% effort –

- **What other organizations were involved as partners?**

Washington University and the University of Louisville 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
  - Collaboration

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
  - Collaboration

- **Regulatory Information:** Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

Human Use Regulatory Protocols

TOTAL PROTOCOL(S): 3

PROTOCOL(S): List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.

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 IRB, USAMRMC HRPO.

1. MU IRB #2008784
   - submitted 12/19/2017
   - approved 1/24/18
   - CRR approved 01/09/2019
   - CRR submitted 12/10/2019, approved on 01/08/2020, submitted to USAMRMC HRPO on 1/24/20 (delay in submission was due to IRB delay in issuing our CRR approval letter until 1/23/20, though it was reviewed and approved on 1/8/20).

2. USAMRMC HRPO (A-18965.2b)
   - Submitted to USAMRMC HRPO on 3/26/18
   - Approved 5/25/18
   - Continuing Review Acceptance Memorandum issued 01/14/19
   - Submitted Continuing Review Report on 1/24/20, received Continuing Review Acceptance Memorandum on 2/23/20

(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.

Current Enrollment for Aim 1:
MU: 1 complete (nerve repair), 1 withdraw, 2 postponed
Current Enrollment for Aim 2:
Individuals with amputations: 39
Healthy controls: 27
Transplant/replant recipients: 4
Enrolled, completing protocol at time of report: 5
Withdrawals – 13

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

Approval date	Description
MU IRB- 9/28/20	Amendment 278650. Extending approval for HIPAA Authorization from Aim 1 to add Aim 2 as well in order to request portions of select participants' medical records (hand transplant and hand replant recipients).
MU IRB- 9/18/20	Amendment 277338. Updated age range in recruitment materials due to need for matched controls.
MU IRB- 8/11/20	Amendment Form 269928. Updated age range in recruitment materials due to need for matched controls.
MU IRB- 6/23/20	Amendment Form 266918. Changes to recruitment materials to reflect our COVID-19 mitigation plan, and request to reopen the study to enrollment under MU's research restart plan.
MU IRB- 5/15/20	Amendment Form 2648763. Changes to protocol to allow home administration for Aim 2.
MU IRB- 4/3/20	Amendment Form 263034. Temporary suspension of enrollment due to COVID-19 pandemic.
USAMRMC HRPO	COVID-19 Notification Acknowledgement Memorandum, received 3/30/20.
MU IRB- 3/11/20	Amendment Form 260042. Protocol changes to enact Corrective Action Plan from Event Report 259981.
MU IRB – 3/11/20	Event Report 259981. Reporting a potential adverse event experienced by a subject after receiving transcranial direct

	current stimulation. Also reported to HRPO and study medical monitor.
MU IRB – 2/12/20	Review ID 259265 (Expedited). Added an advertisement designed for use in a local University of Missouri advertising email called MU Info. Targeted to recruit matched adult controls.
MU IRB – 01/08/20	Review ID 256722 (Full Board). Annual Update.

Protocol 2 of 3 total:

University of Louisville Human Subjects Protection Program IRB #15.0950

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

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

Submitted to and Approved by: University of Louisville, USAMRMC HRPO.

1. University of Louisville IRB (#15.0950)
   - Initial Application Approval: approved 1/14/17
   - Continuing Review Report: approved 1/11/19
   - Continuing Review Report: Approved 2/5/20

2. USAMRMC HRPO (A-18965.2f)
   • Continuing Review Report submitted to USAMRMC HRPO on 2/12/20, received acknowledgement memorandum on 2/18/20.

(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:

Between relocation of the VCA program and Dr. Kaufman to the University of Louisville, and restrictions imposed by the COVID-19 pandemic, no subjects have been enrolled since the last study report.

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

- Local Amendment Approvals: Amendment to move project from CMKI to University of Louisville on submitted on 10/16/19, approved locally on 11/26/19.
- USAMRMC HRPO: Submitted amendment to move project from CMKI to University of Louisville on 12/12/19, Approval received from USAMRMC HRPO: 01/13/2020.

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

Protocol 3 of 3 total:

Washington University School of Medicine (WUSM) 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.

1. WUSM HRPO # 201805155
   Approval date: 06/20/18

   07/23/2018 Mod to add medical monitor
   08/02/2018 Mod to submit DOD approval and release ICFs
   11/05/2018 Mod to add to study team
   01/15/2019 Mod to submit updated MU protocols
   05/08/2019 Mod to add to study team
   05/28/2019 Continuing Review approved at Washington University in St. Louis
   06/05/2019 Mod to update contact information on recruitment flyer
   10/29/2019 Mod to change PI to Mitchell Pet, update to protocol 4.9 removing randomization, update information on ICF/phone screen script.
   12/13/2019 Mod for changes to study team
   05/05/2020 CR and Mod approved at WUSTL (updated to MU protocol V. 5.0 - the addition of headache and fatigue to the listing of possible study risks.) WU required a Reportable Event Form (REF) be submitted due to the delayed submission of this new risk information.
   05/06/2020 REF acknowledged - related to the delayed submission of new risk information. This event was found to represent noncompliance that is neither serious nor continuing.

USAMRMC HRPO (A-18965.2e)
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:
Enrolled: 5
Completed: 4

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

Approval Date	Description
08/01/2018	USAMRMC HRPO approval
06/11/2019	Continuing Review acknowledged
12/10/2019	Protocol Amendment Approval from USAMRDC 14
06/26/2020	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.***