

AWARD NUMBER: W81XWH-13-1-0496

TITLE: The Role of Cortical Plasticity in Recovery of Function Following Allogeneic Hand Transplantation

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REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2015		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2014 - 29 Sep 2015	
4. TITLE AND SUBTITLE The Role of Cortical Plasticity in Recovery of Function Following Allogeneic Hand Transplantation				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0496	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Scott H. Frey, Ed.M.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Missouri 205a Melvin H. Marx Building 1416 Carrie Francke Drive Columbia, MO 65211				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT In Year 2, we made significant progress on data collection in our multi-day protocol. Our preliminary findings continue to indicate that: 1) Hand transplant recipients utilize the former cortical sensorimotor hand territory when using the affected hand. 2) They continue to exhibit strong evidence for persistent, amputation-related, cortical reorganization. These persistent changes appear to diminish with recovery of hand function, suggesting that cortical reorganization is an important target for post-transplant rehabilitation. 3) Transplant and replant recipients show increased engagement of the parieto-frontal cortical networks involved in visually-guided grasping of objects, and these patterns appear related to recovery of functional hand use. 4) Transplant and replant recipients exhibit a remarkable ability to localize touch on their affected hand in the absence of vision. This level of recovery is difficult to understand given prevailing models in neuroscience that emphasize reinnervation errors. These results are summarized below.					
15. SUBJECT TERMS Hand transplantation, functional magnetic resonance imaging, hand replantation, cortical reorganization, functional recovery					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. Introduction

Hand transplant recipients provide a unique opportunity to investigate whether the central reorganizational changes that follow amputation are reversed when afferent and efferent signals between hand and brain are restored, and whether these changes are related to recovery of hand function. Our preliminary functional magnetic resonance imaging (fMRI) data suggest that areas of the sensory and motor cortex devoted to representing the hand prior to amputation come to represent the transplanted hand, even when received decades after amputation. We also find evidence that changes in cortical organization associated with unilateral hand amputation may not be fully reversed even a decade after transplantation; transplant recipients, like amputees, show increased activity in the former hand territory during movements of the adjacently represented face and of the intact hand. Importantly, the level of functional recovery appears to be associated with greater activity in the former hand territory when using the transplanted hand and with less evidence of persistent cortical reorganization. On the basis of our preliminary data, we hypothesize that experience-dependent central (brain) changes play a key role in the functional improvements known to continue throughout at least the first decade following hand transplantation, long after the expected completion of peripheral nerve regeneration. Developing a more complete understanding of the relationship between experience-dependent changes in brain organization and functional outcomes is critical to the long-term success of composite tissue transplantation. This project will yield new insights of fundamental relevance to improving the care and rehabilitation of transplant recipients, and broadly to other patients suffering from peripheral nerve or spinal cord injuries.

2. Keywords

Hand transplant, hand replant, amputation, brain reorganization, sensory, motor, hand function, recovery, functional magnetic resonance imaging

3. Accomplishments

Major Goals Achieved: Year Two

My lab is relocated to Washington University School of Medicine in St. Louis as of July 1, 2015. In an effort to complete data collection for the current project prior to the move, we scheduled and tested a total of 22 patients. These include recipients of hand transplants, N = 2, hand replants N = 3, and N = 17 amputees. This will allow us to now concentrate on completing very demanding data preprocessing, data analyses and the dissemination of our findings through conference presentations and manuscripts during the final period of the award. We also expect to gather one additional time point on our transplant recipients during this period.

Summary of Consents

Since January 2015, this project has consented two transplant patients, three replant patients and 17 healthy adult controls for a total of 22 consents. Since the project began, we have collected 6 transplant consents, 7 replant consents, and 48 control consents for an overall total of 73 consents over the total life of the project.

	2015 Summary	Total
Transplant	2	6
Replant	3	7
Amputee	17	48
Control	0	22
Total	22	73

Preliminary Data

A. Mapping sensorimotor reorganization in the brains of hand transplant and hand replant recipients.

fMRI sensorimotor mapping protocol. Participants were positioned supine in the scanner and moved different body parts according to auditory cues: “left hand”, “right hand”, “left foot”, “right foot”, or “lips”. Each cue was followed by a series of tones presented at a rate of 1 Hz, and participants were instructed to move the cued body part in pace with the onset of each tone. Movement blocks ended with the cue “stop”, and were 13s in duration, excluding movement instruction and stop cues. Each movement block was followed by 18s rest periods. Participants were asked to keep their eyes closed throughout.

Evidence for the reversal of amputation-related changes in cortical primary sensorimotor map organization. DR is a right-hand dominant male who suffered traumatic amputation of his left hand proximal to the wrist at the age 23 years. Thirteen years after amputation, patient DR underwent successful allogeneic hand transplantation. The data presented here were collected at 15 (Session 1) and 26 (Session 2) months post-transplant. During this interval of time, DR’s hand function improved substantially. Carroll Test from 67 to 86; Dash test from 24.14 to 8.6.

The fMRI results indicate marked reduction in the extent to which DR activates his ipsilateral sensorimotor cortex when moving his *non-transplanted hand* between testing session 1 & 2. This pattern reflects functional reorganization following the amputation that appear to persist even after the transplant (**Figure 1**). In patient DR, movements of his transplanted hand robustly activated contralateral sensorimotor cortex (SMC) and ipsilateral cerebellum, and the magnitude, location, and extent of this activity was remarkably consistent across Sessions 1 and 2. At 15 months post-transplant, movements of DR's non-transplanted hand also resulted in robust ipsilateral SMC activity, closely overlapping with preferential responses evoked by movements of his transplanted hand. *In other words, a considerable portion of DR's functionally-defined sensorimotor hand area contralateral to – and presumably devoted to the control of – his transplanted hand was also activated by movements of his non-transplanted hand.* Most exciting, the results from Session 2, at 26 months post-transplantation, reveal a striking reduction in the magnitude and extent of ipsilateral SMC activity during movements of DR's non-transplanted hand, and these changes are within the area of cortex preferentially responsive to movements of his transplanted hand. As this area of cortex continues to selectively respond to movements of DR's transplanted hand, paralleled by continuous improvements in sensory and motor function, response sensitivity to movements of the non-transplanted hand is reduced. As illustrated in **Figure 2**, these patterns differ from those exhibited by individual controls. Further, as is evident in **Figure 3**, we see very stable responses during hand movements in controls across two testing sessions, which suggests that the longitudinal changes in DR are valid and reliable markers of experience-dependent plasticity.

A second transplant – EH – also shows evidence of amputee-like bilateral SMC activity when moving his (left) non-transplanted hand. EH was tested 65 mths post transplant when his hand was functioning at a moderate level (Dash score = 53, Carroll score = 76). He was an amputee for 32 mths before receiving a transplant proximal to the wrist of his right dominant hand (**Figure 4**).

We find a different pattern in patient WH, who experienced an distal forearm amputation and immediate replantation. WH Following replantation of his amputated left hand, WH shows a different pattern than the transplant recipients. He exhibits very significant bilateral activation when moving either the affected or the unaffected hands (**Figure 5**).

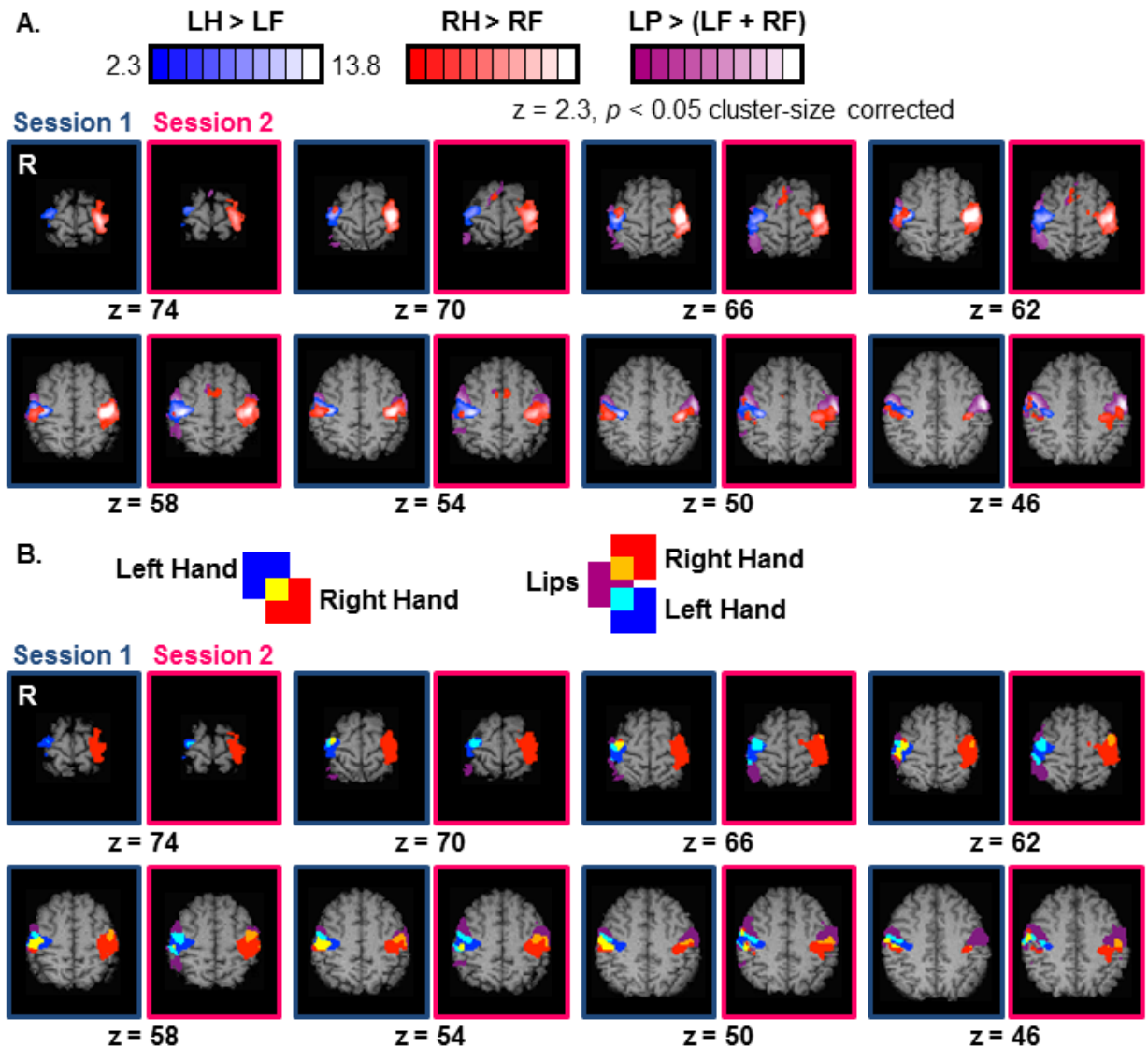


Figure 1. Patient DR: somatomotor map organization over time. (A) Activity maps showing preferential responses for movements of the left hand (blue-to-white), right hand (red-to-white), and lips (purple-to-white) in patient DR at 15 (Session 1) and 26 (Session 2) months post-transplant. Axial slice images from Session 1 (blue outline) and Session 2 (pink outline) are shown side-by-side to facilitate comparisons. Set to the same thresholds, the maps indicate the statistical strengths of the effects for each of the three independent contrasts, as well as the locations of peak

maxima. (B) The same activity maps shown in (A) masked as solid-colored-voxels to better illustrate the voxel-wise spatial extents and points of overlap between maps. Most notably, there is a striking reduction in the extent of activity preferentially responsive to movements of DR's right, non-transplanted hand within his right (ipsilateral) sensorimotor cortex from Sessions 1 to 2 (compare yellow-colored voxels, slices $z = 58$ through 46). LH = left hand; RH = right hand; LP = lips; LF = left foot; RF = right foot.

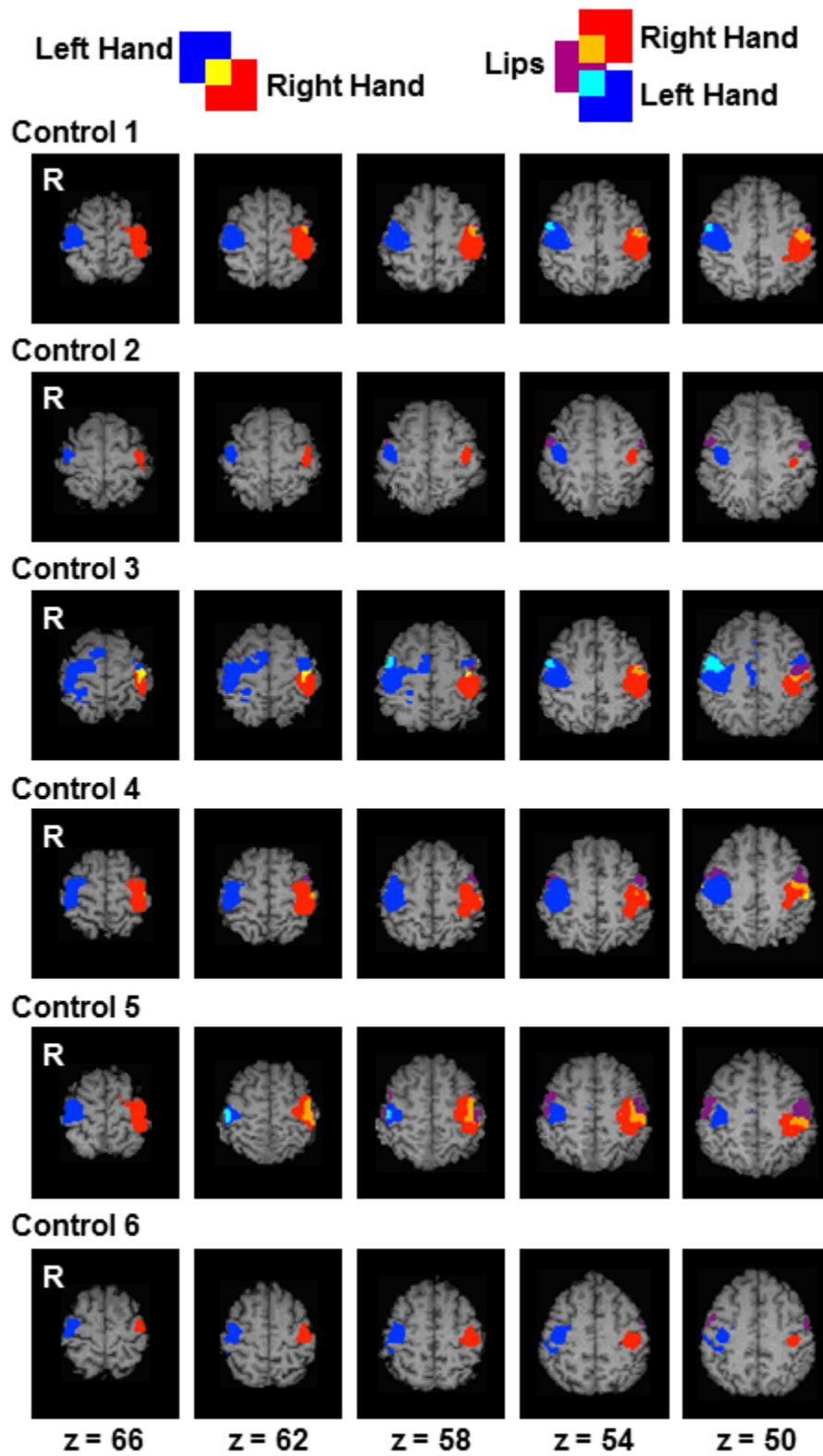


Figure 2. Data from 6 healthy, age- gender- and handedness-matched controls. Note the absence of areas exhibiting increased activity for movements of both hands.

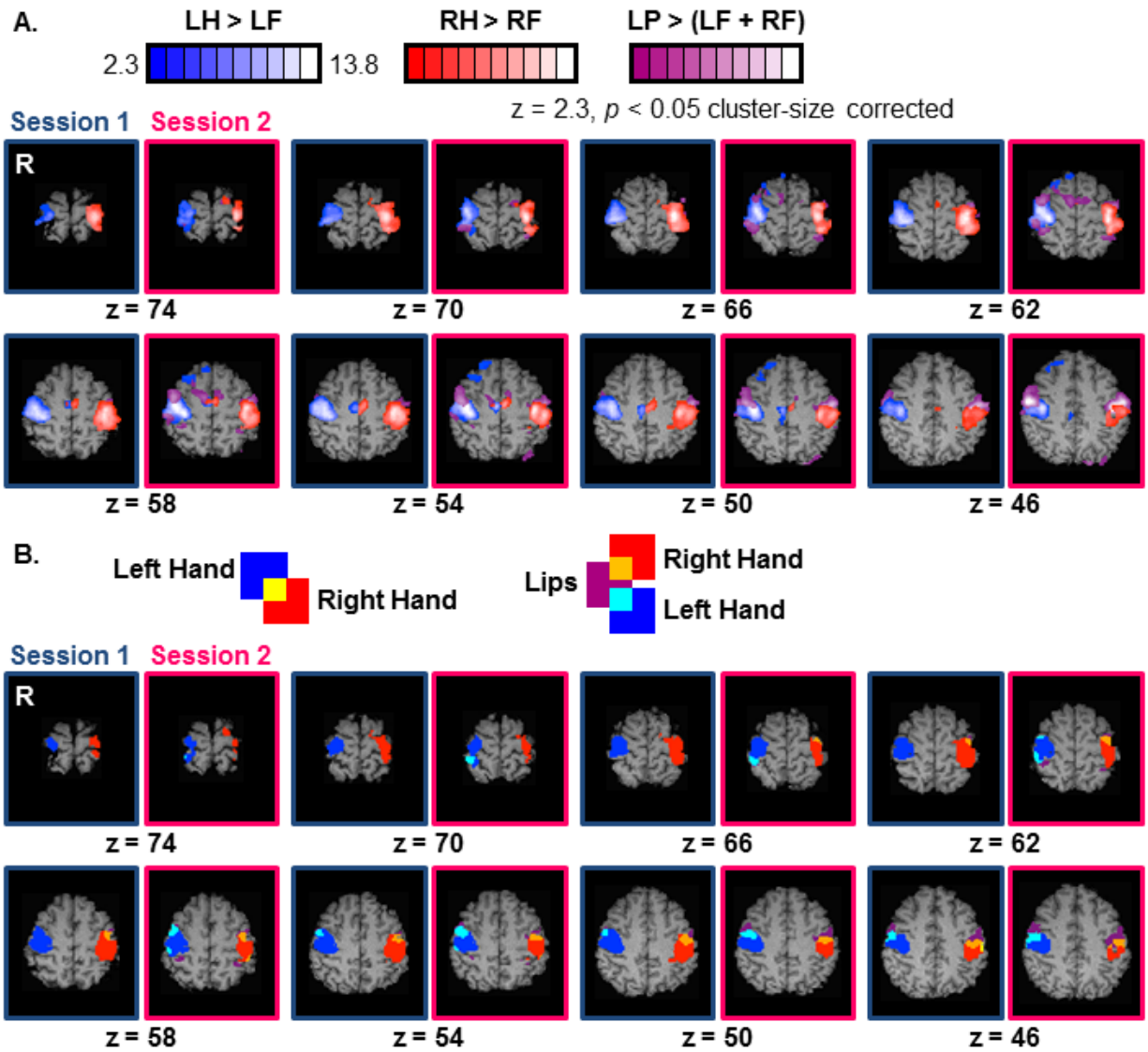


Figure 3. Example Control participant (C1): somatomotor map organization over time. Compare with Figure 1. A) Unlike patient DR, results from an age-, gender-, and handedness-matched healthy control participant show little evidence of ipsilateral sensorimotor cortex activity for movements of either hand, for either session. B) Specifically, in Session 1 only, a total of 58 voxels were activated within in left sensorimotor cortex for movements of the left (ipsilateral) hand, 37 of which overlapped with responses for movements of the right (contralateral) hand (evident as yellow-colored voxels in slice $z = 46$). LH = left hand; RH = right hand; LP = lips; LF = left foot; RF = right foot.

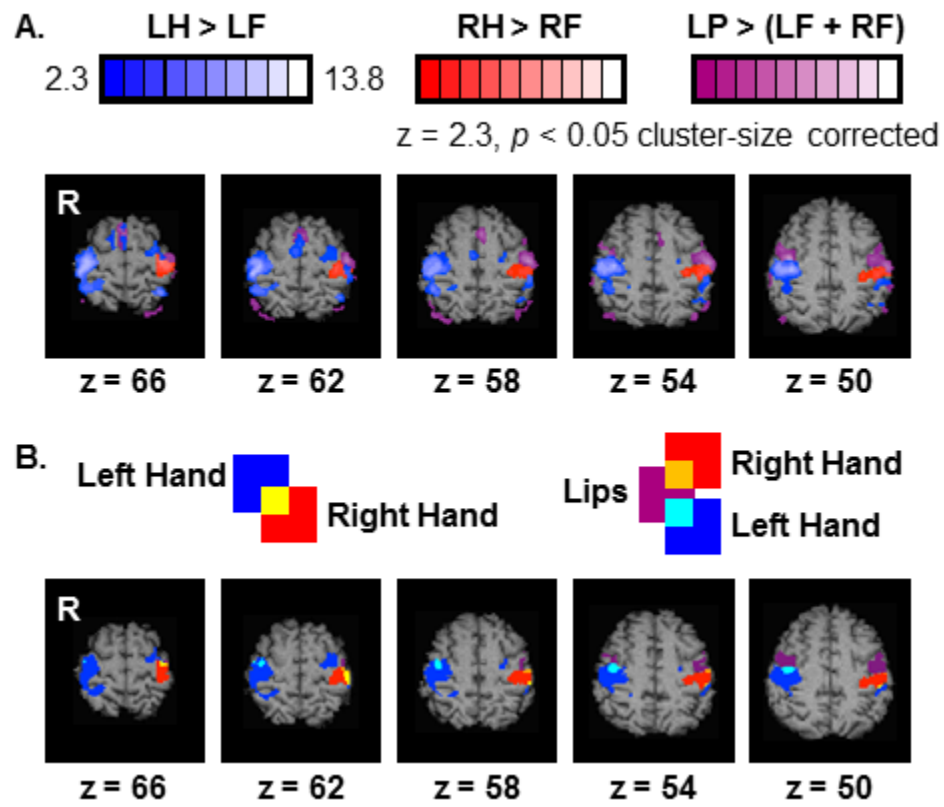


Figure 4. Data from right hand transplant patient EH. As for DR (Fig. 1), we see increased bilateral activity during movements of the intact hand.

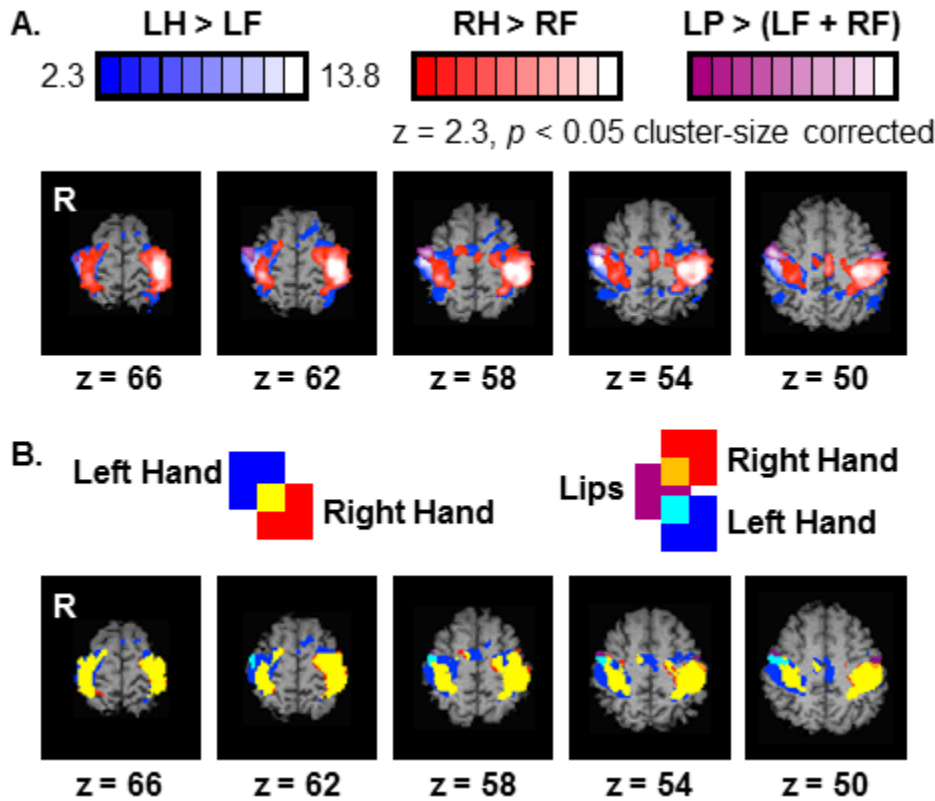
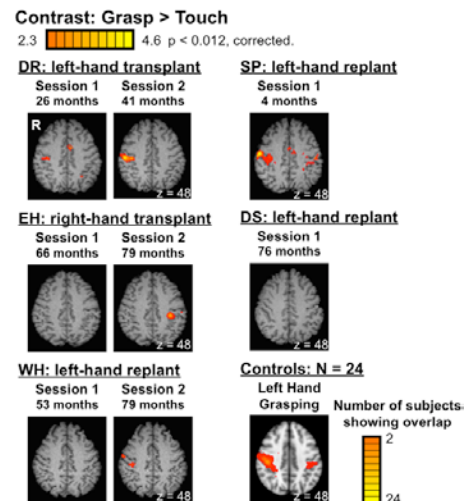


Figure 5. Data from hand replant recipient patient WH. Following replantation of his amputated left hand, WH shows a different pattern than the transplant recipients. He exhibits very significant bilateral activation when moving either the affected or the unaffected hands.

Figure 6: Grasp-selective activity in patients and controls. Activation maps shown per patient, per session are set to reliable statistical thresholds, and the data shown for controls reflects the number of subjects showing active voxels at these same single-subject level thresholds. In at least one testing session, nearly all (4/5) patients show significant activity for grasping within contralateral AIPC, similar to that seen in the majority (19/24) of controls. At reduced thresholds, (not shown here), this activity was also evident for patients EH and WH, Session 1, and DS, as well as for 3/5 of the controls that also failed to show significant effects at corrected levels. Patients DR, SP, and DS (at reduced thresholds) also showed evidence of ipsilateral AIPC activity, similar to many (7/24 corrected; 15/24 uncorrected) controls.



Neural Control of Reaching and Grasping with a Transplanted or Replanted Hand

We have collected data from five patients, two hand transplant and three replant cases, and 24 age-, gender-, and handedness-matched healthy control participants. One replant, and two transplant patients, and one control participant completed the paradigm twice, approximately one year apart. Although there is considerably variability across patients, grasping with the re/transplanted hand tends to activate contralateral anterior intraparietal cortex (AIPC) (**Figure 6**). This pattern of brain activity is qualitatively similar that that observed in healthy controls, and suggests that the functional organization of brain areas dedicated to the control of grasping can be reestablished after traumatic hand amputation and

surgical re/transplantation. In all three patients tested longitudinally, the statistical strength and extent of grasp-selective activity in contralateral AIPC increased over time (Figure 1). This suggests recovery associated with regaining stronger representation (more neural tissue) in the former cortical hand territory

Sensory Localization: Behavioral Consequences of Central Reorganization

We have been working on cleaning and analyzing locognosia data from both the amputee participants and the transplant/replant participants. Figure X is the latest data showing a clear improvement in the ability to localize touch on the palm of the surgically attached hand in transplant patients over time. We have been working on cleaning and analyzing locognosia data from both the amputee participants and the transplant/replant participants. Figure 1 is the latest data showing a clear improvement in the ability to localize touch on the palm of the surgically attached hand in transplant patients DR and EH over time. Transplant recipients GF and MS were only tested at a single time point, 8 and 10 years after their transplants respectively. Patients CH, JS, PP, and RW are hand replant recipients.

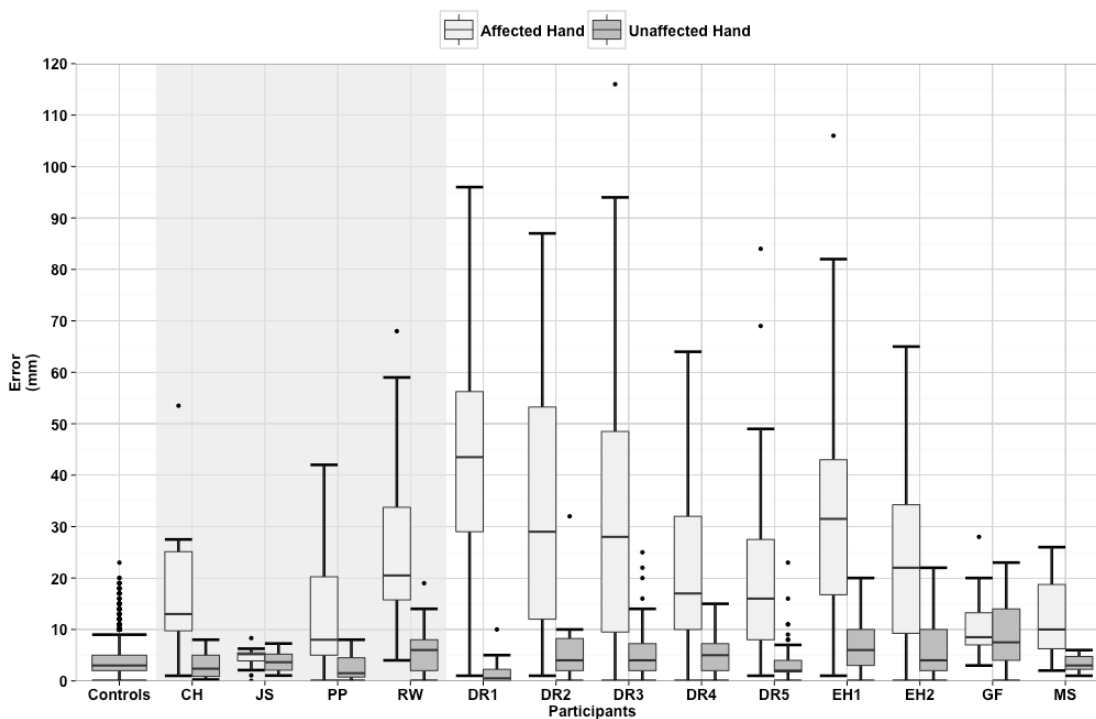


Figure 7 illustrates the accuracy with which our hand replant (gray shaded region) and hand transplant recipients are capable of localizing a light touch on the affected vs. unaffected hand. Note that DR has been evaluated on three different occasions. Overall, both groups exhibit lower mean error rates that are related to the time post-surgery. Transplant recipients on the far left show remarkably accurate recovery when tested 8 (GF) and 10 (MS) years post-transplant. This is consistent with evidence indicating continued gradual improvements in hand function long after the peripheral nervous system has completed reinnervation. We hypothesize that these changes are attributable to central (brain) adaptations.

4. Impact

It is too early to discern many of the broader impacts of this work. At this stage, we can say that the trainees involved in the project have benefited significantly in terms of their skills in this clinically-relevant area of research.

Training Opportunities

This project provided opportunities for postdoctoral and predoctoral trainees to gain additional experience in this area of research. They will be presenting aspects of this research at the annual meetings of the American Society for Neurorehabilitation and the Society for Neuroscience in November, 2015.

As noted below, several presentations about this on-going project have been given to professional groups during this initial year.

5. Challenges/Problems

- We experienced unanticipated artifacts in our electromyographic (EMG) recordings due to the delivery of transcranial magnetic stimuli (TMS, Aim 4). This required us to consult with other technicians and purchase a new set of amplifiers. We are working to get this system up and running with our protocol.
- We are experiencing some challenges scheduling several of the transplant recipients travels due to their work schedules.
 - We are investigating the possibility of conducting all portable aspects of the testing in Louisville during their required annual visits to the clinic there.
 - We have amended our IRB protocol to allow us to also recruit hand replant recipients through the Plastic Surgery Dept. at Southern Illinois University.

6. Products

Book Chapter

Frey, S.H. (2015). *Why Brain Science is Essential to the Success of Hand Allotransplantation*. In *The Science of Reconstructive Transplantation*. Gerard Brandacher (Ed.). Springer.

Presentations at Professional Conferences (Denotes invited address)*

Baune N.A., Philip B.A., Kaufman C., Kutz J. & **Frey S.H.** (November 2014). Improved sensation in a replanted or transplanted hand. Poster to be presented at the annual meeting of the American Society of Neurorehabilitation, Washington, D.C.

Baune N.A., Philip B.A., Kaufman C., Kutz J. & **Frey S.H.** (November 2014). Localization of touch on a replanted or transplanted hand: evidence for late improvements that may reflect central adaptations. Poster to be presented at the annual meeting of the Society for Neuroscience, Washington, D.C.

Cirstea M.C., Peng H., & **Frey S.H.** (November 2014). Spectroscopic evidence for lower neuronal metabolism and time-dependent increases in inhibition within the former sensorimotor hand territory of chronic unilateral amputees. Poster to be presented at the annual meetings of the American Society of Neurorehabilitation and Society for Neuroscience, Washington, D.C.

Peng H., Cirstea M.C., Valyear K.F., & **Frey S.H.** (November 2014). Diffusion tensor tractography study of sensorimotor pathways in unilateral hand amputees. Poster to be presented at the annual meetings of the American Society of Neurorehabilitation and Society for Neuroscience, Washington, D.C..

Philip, B.A., & **Frey S.H.** (November 2014). Stability of precision drawing skills acquired with the non-dominant hand and associated changes in functional connectivity between sensorimotor hand representations. Poster presented at the annual meeting of the Society for Neuroscience, Washington D.C.

Philip, B.A., & **Frey S.H.** (November 2014). Skill acquisition with the non-dominant hand and associated changes in functional connectivity between sensorimotor hand representations. Poster presented at the annual meeting of the Society for Neurorehabilitation, Washington D.C.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (November 2014). Amputation-related changes in inter-hemispheric interactions are reversible through transplantation of the human hand. Poster to be presented at the annual meeting of the Society for Neuroscience, Washington, DC.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (January, 2015). Cortical reorganization and recovery of limb function following allogeneic hand transplantation. Presented at Extremity War Injuries X: Advancing Health and Function. Washington, DC.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (May, 2015). Cortical reorganization and recovery of limb function following allogeneic hand transplantation. Presented at the Concept, Objects and Actions Meeting in Rovereto, Italy.

Invited Colloquia

Dr. Frey gave invited colloquia on this research at: Washington University, Vanderbilt University, and the University of Florida.

7. Participants and Other Collaborating Institutions

A. Participants

Name	Benjamin Philip
Project role	Postdoctoral fellow
Nearest person month worked	6.0
Contribution to project	Data collection, analysis, reporting
Funding support	W81XWH-13-1-0496

Name	Kenneth Valyear
Project role	Postdoctoral fellow
Nearest person month worked	6.0
Contribution to project	Data collection, analysis, reporting
Funding support	W81XWH-13-1-0496

Name	Noah Marchal
Project role	Senior Research Technologist
Nearest person month worked	6.0
Contribution to project	Hardware & Software Engineering
Funding support	W81XWH-13-1-0496

Name	Saadiya Aswad
Project role	Project Coordinator
Nearest person month worked	2.0
Contribution to project	Patient recruitment, scheduling, accounting
Funding support	W81XWH-13-1-0496

Name	Nathan Baune
Project role	Graduate Student
Nearest person month worked	2.0
Contribution to project	Patient testing, behavioral data analysis
Funding support	Departmental funding

B. Change in Other Support

Nothing to Report

C. Collaborating Organizations

Christine M. Kleinert Institute

Louisville, KY

Patient identification, recruitment, behavioral evaluation, referral