

## Translational Research Stimulation Award Progress Report for 1<sup>st</sup> year (January 2008 – December 2008)

**Title:** MRI of Human Cortex after Limb Loss

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### 1. Brief overview, specific aims, and long term objectives

#### Overview.

The University of Toledo (UT) Translational Research Stimulation Award (TRSA) program was designed to develop new translational research at UT, including generation of pilot data for seeking new extramural funding. Our TRSA funded studies involve a translational research project aimed at doing pilot tests of cortical structural changes in humans who have lost a limb. As stated in the timetable for our original proposal, our goal is to recruit, scan, and analyze 15 amputee and 15 age/gender matched control subjects within 1½ years of the January 2008 starting date. The final ½ year will be used to complete the planned analyses, explore unplanned analyses that may be prompted from the results, and prepare applications for external funding. Our studies to date follow this timetable.

#### Specific Aims.

Nearly two million living Americans have undergone limb amputation. Limb loss leads to sensory, motor, and cognitive-emotional symptoms that are individual-specific and can threaten quality of life. Amongst the most debilitating is pain that can emerge unpredictably at any time, and that may endure for life.

Post-amputation pain is difficult to manage, and treatments are often not effective. Treatment decisions are presently based on pain symptoms. Since pain generation involves major contributions of cortex, there is interest in developing brain-based approaches that can supplement assessments of pain symptoms with cortical measures that provide direct indications of pertinent cortical organization states.

It is likely that permanent loss of a part of the body has distinctive effects on cortex. Consistent with this view, limb loss causes cortical functional reorganization. At present, this functional reorganization is usually presumed to occur within a normal cortical structure. Our proposal seeks to address whether loss of a body part is also accompanied by cortical structural alterations and whether cortical structural organization is related to post-amputation pain.

#### Hypothesis and questions that are being tested.

We are interested in whether a partially bodiless cortex, i.e. a cortex that has lost normal contact with a major part of the body, becomes structurally changed. The research is testing the hypothesis that *loss of a limb and/or related post-amputation pain is associated with cortical structural reorganization that involves alterations in cortical thickness.*

Adult amputees and control subjects who have not lost limbs are studied using structural magnetic resonance imaging (sMRI) of the brain, computational morphometric techniques for measuring cortical thickness, and questionnaire assessments of pain and other symptoms/variables. Two questions are being addressed to test the hypothesis.

1) *Is limb loss associated with changes in the thickness of human cortex?* There is little understanding of how human cortical structure is affected by loss of a limb. This question

is being addressed with cross-sectional analyses that test if cortical thickness differs in subjects who have lost limbs and control subjects with normal limbs.

2) *Is pain due to limb loss related to the thickness of human cortex?* There is no understanding of whether post-amputation pain is related to measurable properties of cortical structure. To address this question, we are examining associations between post-amputation pain symptoms and measures of cortical thickness.

### Long-term objectives.

In addressing the above hypothesis and questions we are working toward long-term basic science and clinical objectives that are needed to understand how the human brain is affected by limb loss. With regard to basic science, an objective is to understand whether limb loss and related post-amputation pain are associated with structural changes in human cerebral cortex and, if so, basic principles that determine the location(s) and nature(s) of these changes. A clinical objective is to understand whether sMRI measures of human cortical thickness can contribute to an understanding of cortical organization that, in turn, can be translated into brain-based clinical approaches that use cortical thickness as a biomarker and therapeutic target for improving post-amputation pain treatment.

## **2. Progress to date on each of the questions being tested**

### **What has already been done?**

The proposed studies have been approved by the University of Toledo Institutional Review Board, and preliminary results have been derived from 9 amputee (9 male) and 10 control (7 male, 3 female) subjects (mean age: amputees = 53 yrs, controls = 45 yrs;  $t=2.15$ ;  $p=0.143$ ).

### ***Question 1: Is limb loss associated with changes in the thickness of human cortex?***

Question 1 is addressed with cross-sectional analyses of cortical thickness in amputee versus control subjects. Amputees have previously undergone lower limb amputations and have no history of chronic nonamputation pain, whereas controls have intact limbs and no history of limb abnormalities or chronic pain. T1-weighted images and FreeSurfer automated morphometric programs are used to measure cortical thicknesses at approximately 150,000 vertex locations spanning each hemisphere of each subject.

Current understanding does not permit prediction of whether cortical thickness alterations occur after limb loss and, if they do, whether they are extensive or restricted to delimited locations. To deal with these possibilities, we are using a search approach that examines thicknesses in amputation-related regions of interest (arROIs) that are empirically defined, cortical areas (CAs) that are structurally defined, and each hemisphere (global). Pilot results are providing indications that cortical thickness may change after limb loss.

### arROI analyses

arROI analyses entail tests of thickness differences at homologous vertex locations in amputees versus controls. Using FreeSurfer programs, data from each subject's cortex are registered to a spherical atlas that aligns analogous sulci and gyri of subjects to permit generation of a statistical difference map that indicates levels of statistical difference between mean cortical thicknesses at homologous vertex locations for the amputee versus control group. arROIs are defined empirically by contiguous vertices that have statistically different means. The mean thickness of an arROI, or thicknesses at selected vertices in an arROI,

can also be determined in each amputee and control subject and used to generate scatterplots that compare arROI thickness distributions for subjects in each group.

In pilot analyses, Freesurfer programs were set to identify vertices that differed with a  $p \leq 0.05$  (with false discovery rate (FDR) correction for repeated tests). These analyses suggest that amputees have decreased thicknesses relative to controls in multiple arROIs that occupy lateral and medial hemisphere locations. These include, for example, parietal and frontal arROIs involving one or more locations in (Fig. 1A, blue): postcentral (#1), superior parietal (#2), inferior parietal (#3), supramarginal (#4), precentral (#5), superior frontal (#6), middle frontal (#7), orbital frontal (#8), pars opercularis (#9), paracentral (#10), precuneus (#11), and isthmocingulate (#12) areas. arROIs involving these areas are seen in both hemispheres. Further thickness decreases in amputees are also seen in occipital and temporal locations (Fig. 1A). Adding to arROIs where thickness appears decreased in amputees are arROIs where thickness may be increased in amputees, including left postcentral (Fig. 1A, red, #13) and right orbital frontal and anterior cingulate areas.

Scatterplots comparing thicknesses of individual amputee and control subjects at homologous locations in arROIs provide indications of disparities in thickness distributions in arROIs of amputee versus control subjects. Locations in, for example, postcentral, inferior parietal, precentral, superior frontal, middle frontal, and paracentral arROIs have distributions that appear offset from each other, with thicknesses in amputees consistently at or below the lower end of control thickness distributions (Fig. 1B and see location number and asterisk indicating respective cortical location in Fig. 1A). The differences in the mean thicknesses in the amputee versus control groups at these locations ranged from 0.191 – 0.416 mm. These results are surprising in view of present assumptions that cortical structure remains normal after limb loss.

### CA analyses

To test for alterations across structurally pre-defined areas of cortex, we examine mean thicknesses in CAs that are defined by FreeSurfer parcellation programs on the basis of gyral and sulcal structure. Preliminary indications of decreases ( $p \leq 0.05$ ) or trends for decreases ( $0.05 > p \leq 0.08$ ) (t-tests; not corrected for repeated tests) in mean thicknesses of parietal and frontal CAs for corresponding left and right sides of amputees versus controls are found, for example, in postcentral, superior frontal, middle frontal, anterior cingulate, supramarginal, orbital frontal, and precentral areas on one or both sides (e.g., Fig. 1C, left). Further comparisons of CAs that are contra- or ipsi-lateral to limb loss (compared to respective right and left sides in controls) indicate decreases ( $p \leq 0.05$ ) or trends for decreases ( $0.05 > p \leq 0.08$ ) in mean thicknesses of amputees in, for example, contra-lateral precentral, middle frontal, orbital frontal, and posterior cingulate, and ipsi-lateral middle frontal, postcentral, anterior cingulate, and orbital frontal cortical areas (e.g., Fig. 1C, right). Preliminary analyses thus far have not revealed mean CA thickness increases in amputees relative to controls.

### Global analyses

The mean thickness of all vertices in each hemisphere is used to test for extensive global hemispheric alterations. Global mean thicknesses of amputees show trends for decreased thickness relative to controls. This is seen when corresponding sides are compared (t-tests) in amputees versus controls (left vs left:  $p=0.051$ ; right vs right:  $p=0.059$ ) and when contra- or ipsi-lateral sides (relative to limb loss) in amputees are compared with mean thicknesses in control hemispheres (e.g., contra-lateral vs right:  $p=0.054$ ; ipsi-lateral vs left:  $p=0.056$ ).

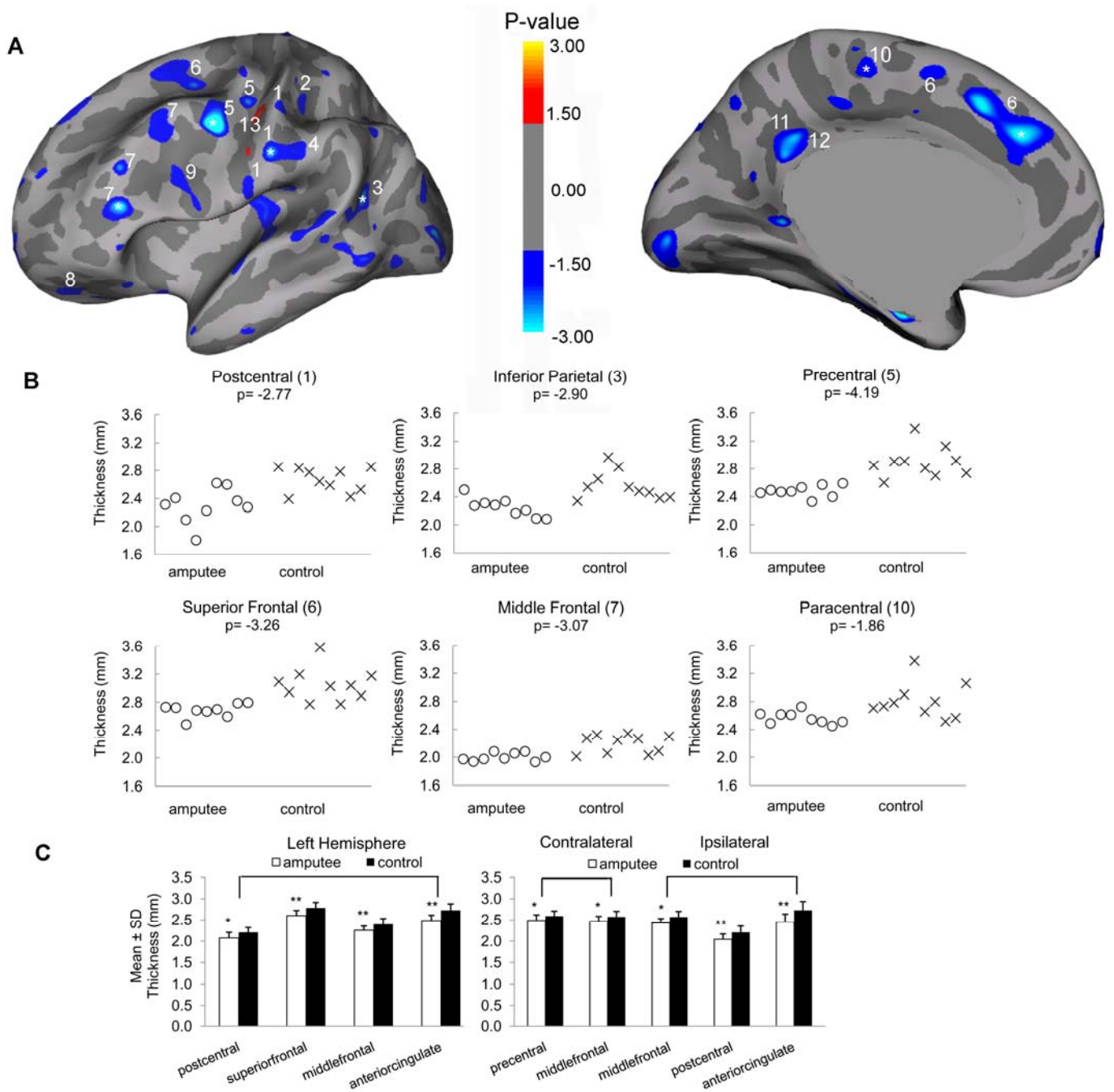


Figure 1. Preliminary results for question 1. **A**. Statistical difference map indicating left hemisphere arROIs where cortical thicknesses in amputees were significantly thinner (blue) or thicker (red) than in controls. Map is shown on lateral (left) and medial (right) surfaces of an inflated left hemisphere model (subcortical areas are shaded out on medial surface). arROIs where cortex was thinner in amputees include parts of postcentral (#1), superior parietal (#2), inferior parietal (#3), supramarginal (#4), precentral (#5), superior frontal (#6), middle frontal (#7), orbital frontal (#8), pars opercularis (#9), paracentral (#10), precuneus (#11), isthmocingulate (#12), and other areas (not numbered), whereas arROIs where cortex was thicker in amputees include parts of postcentral (#13) cortex. P-values on color bar of  $\leq -1.30$  (dark – light blue) or  $\geq +1.30$  (red - yellow) are significant at  $p \leq 0.05$ . **B**. Thickness distributions for amputee subjects were at or below distributions for control subjects at, e.g., locations of asterisks in areas #1, #3, #5, #6, #7, and #10 in A. **C**. CA analyses suggesting trends for decreases in mean cortical thicknesses in amputees in, e.g., left hemisphere CAs (left) and contra- and ipsi-lateral CAs (right). \*\* indicates  $p \leq 0.05$  and \* indicates  $0.05 > p \leq 0.08$ .

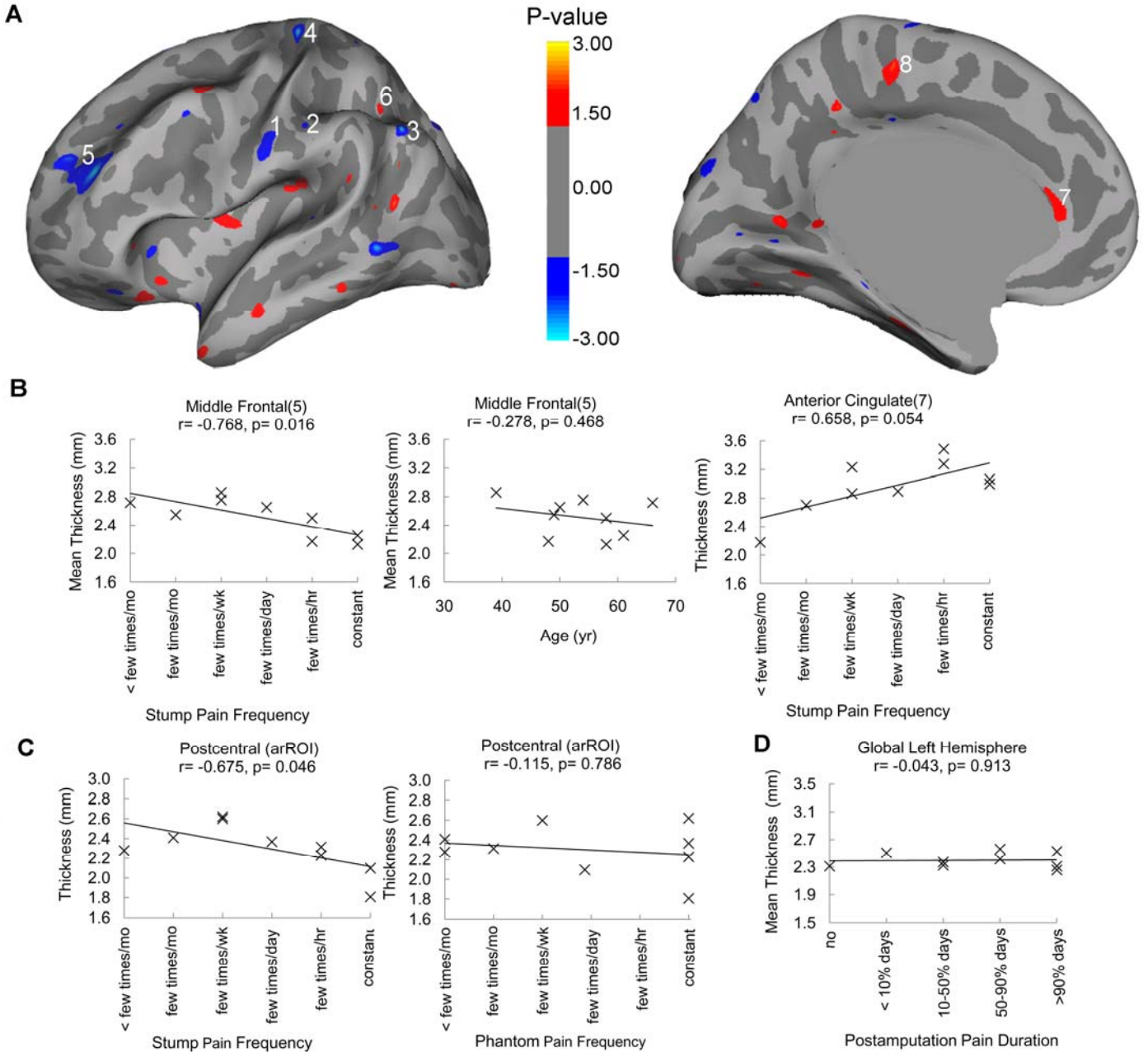


Figure 2. Preliminary results for question 2. **A**. Statistical correlation map indicating left hemisphere prROIs where cortical thickness in amputees was significantly negatively (blue) or positively (red) correlated with frequency of stump pain over the preceding 3 months. Map is shown on lateral (left) and medial (right) surfaces of an inflated left hemisphere model (subcortical areas are shaded out on medial surface). prROIs where stump pain frequency was negatively correlated with thickness (higher pain frequency related to thinner cortex) include parts of left postcentral (#1), supramarginal (#2), inferior parietal (#3), precentral (#4), middle frontal (#5), and other areas (not numbered), whereas prROIs where stump pain frequency was positively correlated with thickness (higher pain frequency related to thicker cortex) include parts of superior parietal (#6), anterior cingulate (#7), and paracentral (#8) cortex. P-values on color bar of  $\leq -1.30$  (dark – light blue) or  $\geq +1.30$  (red - yellow) are significant at  $p \leq 0.05$ . **B**. Mean thicknesses of the middle frontal prROI (#5 in A) were negatively correlated with stump pain frequency (left) but were not correlated with amputee age (middle). Thicknesses at a site within the anterior cingulate prROI (#7 in A) were positively correlated with stump pain frequency (right). **C**. Cortical thicknesses at a site in the postcentral arROI identified in question 1 analyses (see asterisk in #1 of Fig. 1A) were negatively correlated with stump pain frequency (left) but were not correlated with phantom pain frequency (middle). **D**. Left hemisphere global mean thicknesses in amputees did not appear correlated with one index of post-amputation pain duration.

## **Question 2: Is pain due to limb loss related to the thickness of human cortex?**

Given no precedents of pain indices that have proven useful for examining relationships between post-amputation pain and cortical thickness in amputees, we test for correlations between, on one hand, a range of post-amputation pain indices including, for example, frequency, duration, and intensity of phantom, stump, or combined phantom + stump pain and, on the other hand, a range of empirically and structurally defined cortical thickness measures, including pain-related ROIs (prROIs), arROIs, CAs, and global thicknesses. Our present sample is too small to identify effects with statistical confidence, but the following illustrates analysis approaches and examples of results from pilot tests to date.

### prROI analyses

Using FreeSurfer, MR data from each amputee's brain are registered to a spherical surface atlas that aligns cortices of amputees to permit correlation tests between a pain variable and thicknesses at homologous vertices. FreeSurfer generates a correlation map that identifies contiguous vertices where cortical thickness and a tested pain variable are significantly correlated. For convenience we identify these empirically defined regions as pain-related regions of interest (prROIs). In pilot analyses, we identified vertices where thickness correlated with pain at a  $p \leq 0.05$  (with FDR correction). A preliminary correlation map showing, for example, relationships between vertex thicknesses and frequency of stump pain over the preceding 3 months identifies parietal and frontal prROIs involving, e.g., left postcentral (#1), supramarginal (#2), inferior parietal (#3), precentral (#4), and middle frontal (#5) areas where higher stump pain frequency was related to thinner cortex (Fig. 2A, #1-5, blue). Potential left parietal and frontal prROIs where higher stump pain frequency was related to thicker cortex were also seen, for example, in superior parietal (#6), anterior cingulate (#7), and paracentral (#8) areas (Fig. 2A, #6-8, red). Further prROIs with potential negative or positive pain/thickness correlations were seen in other locations and the other hemisphere.

Using FreeSurfer, the mean thickness of all vertices in a prROI, or thicknesses for selected vertices in a prROI, can be mapped back to each amputee to provide scatterplots and regression analyses that can be used to further examine relationships between thickness and pain variables. For example, increased frequency of stump pain (over preceding 3 months) was related to decreased mean thickness across the middle frontal (#5) prROI seen in Fig. 2A (Fig. 2B, left,  $p=0.016$ ), whereas other variables such as amputee age did not appear related to mean thickness across this prROI (Fig. 2B, middle,  $p=0.468$ ). Other analyses that examine thickness at specific sites within prROIs, for example, a site within the anterior cingulate (#7) prROI seen in Fig. 2A, indicated a positive correlation between frequency of stump pain and thickness at that site (Fig. 2B, right,  $p=0.054$ ). We emphasize that the present sample size limits confidence in conclusions, but these preliminary results are consistent with the view that cortical thickness may be related to post-amputation pain in amputees.

### arROI analyses

The above prROIs are defined without regard to potential thickness differences between amputees and controls. To consider these differences, we also examine relationships between pain variables and thicknesses in amputees within arROIs that are identified in question 1 analyses. Mean thickness of an arROI, or thicknesses for selected sites in an arROI, are used as thickness correlates. For example, preliminary results suggest thicknesses in amputees at the postcentral arROI site identified in Figure 1A (see asterisk at

#1) were negatively correlated ( $p=0.046$ ) with stump pain frequency, suggesting increased frequency of stump pain may be related to thinner cortex (Fig. 2C, left). In contrast, thicknesses at this site did not appear to be correlated ( $p=0.786$ ) to frequency of phantom pain (Fig. 2C, right). These findings raise the interesting possibility that cortical locations where thickness is altered in amputees relative to controls may be further distinguished by specific, and potentially different, relationships to stump versus phantom pain.

### CA and global analyses

Pain and thickness relationships have also been tested for mean CA and global thicknesses on left/right and contra/ipsi-lateral (relative to limb loss) sides. Pilot findings pertaining, for example, to global mean thickness suggest there was no relationship between left global mean thickness and an index of post-amputation pain duration (i.e., percentage of days since amputation that post-amputation pain has been a problem) (e.g., Fig. 2D).

## **3. Summary of salient results**

### Summary of initial pilot findings for question 1

Assessments of arROI, CA, and global thicknesses are providing pilot evidence that limb loss may be associated with thickness alterations in multiple cortical locations and both hemispheres. Present conclusions are limited by the small sample. However, it appears that locations that contribute to sensori-motor and body image (e.g., postcentral, parietal, paracentral, precentral), emotional/affective (e.g., cingulate), cognitive (e.g., superior frontal, middle frontal, orbital frontal), and further (temporal, occipital) functions may undergo thickness decreases, whereas other locations that may also contribute to sensory (e.g., postcentral), emotional/affective (e.g., anterior cingulate), or other functions may undergo thickness increases. *Given current lack of evidence for cortical structural change in amputees, it is usually assumed that cortical structure remains normal after limb loss. The emerging pilot findings are provocative because they suggest this assumption may not be justified. Confirmation of these findings would significantly impact present theories and basic science and clinical understanding of how limb loss affects human cortex structure.*

### Summary of initial pilot findings for question 2

Our present sample is too small to identify correlations with statistical confidence. This acknowledgement aside, the emerging pilot results point to the possibility that post-amputation pain may be related to cortical thicknesses in multiple cortical locations of both hemispheres. Locations implicated thus far include areas that are usually considered as parts of a cortical pain matrix including, for example, postcentral, parietal, frontal, and cingulate areas. The results raise the further interesting possibility that different relationships may hold between stump versus phantom pain and thickness at specific cortical locations, including locations where thickness can also be shown to differ in amputees versus controls.

At present, there is no understanding of relationships between post-amputation pain and cortical structure. *The emerging pilot findings are provocative in suggesting that sMRI measures of cortical thickness may serve as useful structural correlates of post-amputation pain symptoms. A demonstration of relationships between cortical thickness and post-amputation pain could contribute significantly to development of brain-based approaches for post-amputation pain treatment.* For example, there is evidence that, under some conditions, structural changes in human cortex may be reversible. There are also indications that pharmacological, rehabilitation, transcranial magnetic stimulation, and other interventions can be used to affect particular cortical regions, sometimes by altering cortical structure. This

raises the possibility that pain- or amputation-related changes in cortical thickness may, to some degree, be reversible, and that a combination of sMRI imaging of cortical thickness and interventions that affect appropriate cortical regions may provide potential new avenues to treat cortical structural changes that may be associated with post-amputation pain.

#### **4. Problem areas**

Virtually all TRSA funding to date has been used for personnel costs that are absolutely required to getting the proposed work done, including costs for (a) data analysts who have expertise needed to run time-intensive procedures for prROI, arROI, CA, and global cortical thickness analyses and for organizing the data base that is used to assess cortical thickness and relationships between pain variables and cortical thickness, (b) MRI technologists used to run scans, and (c) subject recruitment and compensation.

The work is computer technology intensive. Our paid analysts work with UT IT personnel to maintain required programs, databases, inter-computer transmission, and data backup systems to make these studies possible. A main computer used in data analyses had problems last spring and again this last week. This computer was purchased with past NIH grant funding, is several years old, and needs to be replaced in the near future to continue progress in this project. UT currently does not have a program to replace computers. This upcoming year some TRSA funds will be used to replace this required tool.

#### **5. Extramural submissions**

This TRSA project represents new MRI translational work of our research group. Our group and UT are presently not recognized for MRI research. Attraction of extramural funding requires that grant reviewers see that proposed work can be done competently. To build credentials in MRI research, with the help of TRSA funding, this past year we published a paper and an abstract (presented at the Society for Neurosciences meeting in November) on pilot results that address the test-retest reliability of our cortical thickness measures. These publications can be used in grant applications to establish our competence in making thickness measures needed for our amputee study. Copies of this paper and abstract are attached.

Consistent with our original timetable (see above), we are using TRSA funding to generate pilot data that can be used to seek extramural support. We should be able to reach our original pilot sample and analyses of 15 amputee and control subjects over the next few months. During that time, and over the subsequent remaining part of the upcoming year, we will develop at least one NIH grant proposal (our first goal is an NIH R21 application), and 1 manuscript/abstract that can further establish our MRI credentials. Even though some grant application mechanisms (e.g. NIH R21) advertise that pilot data are not required, we have been advised that in the present funding climate applications with weak pilot data are not competitive with applications with strong pilot data. This is an important consideration because many grant applications are returned without undergoing review and, for those that are reviewed, funding rates of  $\leq 10\%$  of applications likely apply. In addition, as part of recent changes in NIH grant application procedures, grants will only be reviewed 2 times (not 3 times as previously possible). Since the very high majority of grant applications do not get funded after the first review, it is essential to have strong pilot data and minimal problems that can potentially be corrected with 1 revision. We are carefully developing a first grant application with these realities in view.

## 6. Attachments

### Paper

Wang, X., Bauer, W., Chiaia, N., Dennis, M., Gerken, M., Hummel, J., Kane, J., Kenmuir, C., Khuder, S., Lane, R., Mooney, R., Bazeley, P., Apkarian, V., and Wall, J. (2008) Longitudinal MRI evaluations of human global cortical thickness over minutes to weeks, *Neuroscience Letters*, 441, 145-148.

### Abstract

Wall, J.T., Bauer, W.R., Dennis, M.J., Kane, J.T., Mooney, R.D., Wang, X., Gerken, M., Khuder, S.A., Bazeley, P.S., Apkarian, A.V., Chiaia, N.L., and Lane, R.D. (2008) Longitudinal MRI evaluation of human global cortical thickness over minutes to weeks, *Society for Neuroscience Abstracts*.

(my docs: Dec 2008 TRSA progress report end of year 1)