

# The Penn State McNair Journal

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Summer 2015, Volume 20

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THE PENN STATE MCNAIR JOURNAL

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## WELCOME

Since 1991, the Penn State McNair Scholars Program has enriched the lives of students at Penn State. The McNair Program holds a very special place in our lives, as well as in the lives of the faculty and staff who work with our students. This publication celebrates their achievements and we offer it to our readers with pride and pleasure.

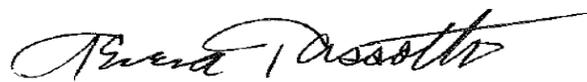
This is the twentieth issue of the Penn State McNair Journal. We congratulate the Penn State McNair Scholars and their faculty research advisors! This journal presents the research conducted in the summer of 2015 by undergraduate students from Penn State, who are still enrolled in the Penn State McNair Scholars Program.

The articles within this journal represent many long hours of mutual satisfying work by the Scholars and their professors. The results of their research are published here and have also been presented at various research conferences around the country. We are especially proud to see how these students have grown as researchers and scholars. The hard work, dedication, and persistence required in producing new knowledge through research is most evident in these articles.

We very much appreciate the guidance, expertise, caring and patience of our fine group of Penn State faculty research advisors. For their ongoing support and assistance, we thank Eric Barron, President of Penn State University; Nicholas Jones, Provost; Regina Vasilatos-Younken, Vice Provost and Dean of the Graduate School; Michael Verderame, Senior Associate Dean; Suzanne Adair, Assistant Dean, and Stephanie Danette Preston, Senior Director of the Office of Graduate Educational Equity Programs, the administrative home of the McNair Scholars Program.

We are also fortunate to have the support and encouragement of many faculty and staff members who have worked with our students as social mentors or who have presented workshops and seminars on the many aspects of graduate and faculty life. You give the most precious of gifts to our students – your time in volunteering to support, encourage and nurture our Scholars' hopes and dreams.

Lastly, we would like to acknowledge the work of five McNair scholars whose summer research is not included in this online publication. Their work has been submitted to peer-reviewed professional journals in their fields for first consideration to be published. We applaud these five scholars – Jordan Chapman, Houston Claire, Caroline Gooch, Francis Kwok, and Alyssa Palmer – for their professional journal submissions. Their faculty research advisors and social mentors are included in this journal's acknowledgements section.



**Project Director**

## **TRIO PROGRAMS ON THE NATIONAL LEVEL**

Since their establishment in the mid-sixties as part of Lyndon Johnson's War on Poverty Program, the federal TRIO Programs have attempted to provide educational opportunity and make dreams come true for those who have traditionally not been a part of the educational mainstream of American society. The TRIO programs are funded under Title IV of the Higher Education Act of 1965. While student financial aid programs help students overcome financial barriers to higher education, TRIO programs help students overcome class, social and cultural barriers to higher education. There are eight TRIO programs, which include the original three – Upward Bound, Talent Search and Student Support Services. The additional programs are Educational Opportunity Centers, Upward Bound Math & Science Centers, the Ronald E. McNair Post-Baccalaureate Achievement Program, Veterans Upward Bound, and a training program for TRIO staff. McNair programs are located at 156 institutions across the United States and Puerto Rico. The McNair Program is designed to prepare participants for doctoral studies through involvement in research and other scholarly activities.

## **TRIO PROGRAMS AT PENN STATE**

The ten TRIO Programs at Penn State comprise six of the eight TRIO programs. There is the Educational Opportunity Center serving Philadelphia, two Talent Search Programs serving select western Pennsylvania school districts and the York City school district, the Ronald E. McNair Scholars Program serving the University Park campus, two Student Support Services Programs serving the Greater Allegheny and Wilkes-Barre campuses, the Upward Bound and Upward Bound Migrant Programs serving central and southeastern Pennsylvania select school districts, and the Upward Bound Math and Science Program serving select central and southeastern Pennsylvania school districts. These programs annually serve more than 6,000 students, from 6th graders through adults, all with clear potential for academic success. Altogether, the programs operate across select Penn State campuses and in communities across the state, often linking with middle schools, high schools, and community agencies. The programs focus on helping students overcome economic, social, and class barriers so that they can pursue education beyond high school.

## MCNAIR SCHOLARS PROGRAM AT PENN STATE

Designed for low-income and first-generation college students, and students from groups underrepresented in graduate education, the McNair Scholars Program at Penn State encourages talented undergraduates to pursue the doctoral degree. The program works closely with these participants through their undergraduate career, encourages their entrance into graduate programs, and tracks their progress to successful completion of advanced degrees.

The goal of the McNair Program is to increase graduate degree attainment of students from the above-mentioned underrepresented segments of society. McNair Scholars are presented with opportunities to study and do research in the University's state-of-the-art facilities in order to hone those skills required for success in doctoral education. Through both academic year and summer program components, McNair Scholars are required to complete a series of steps that lead to their application and enrollment in a graduate program of their choice.

Since 1991, the McNair Scholars Program at Penn State has helped 279 students earn their baccalaureate degrees. Of these graduates, 226 or 85 percent have gone on to graduate school at institutions across the country and overseas. As of March 2016, 59 or 26 percent have earned their academic or professional doctorates. Of this group, 31 also earned their master's degrees prior to the doctorate. Another 131 or 53 percent have now earned at least their master's degrees. Currently, there are 65 or 23 percent of alumni, who are still enrolled in graduate programs. Among the institutions McNair alumni have attended or now attend are: Arizona State, Boston University, Columbia, Cornell, Harvard, Howard University, Indiana University, Iowa State, Johns Hopkins, New York University, Ohio State, Penn State, Purdue, Stanford, Temple, Texas A&M, UC-Berkeley, UC-Davis, UCLA, University of Chicago, University of Florida, University of Illinois-Urbana Champaign, University of Maryland-College Park, University of Michigan, University of North Carolina-Chapel Hill, University of Pennsylvania, University of Texas, University of Wisconsin and Yale, to name just a few.

### *Summer 2015 McNair Scholars and Program Staff*



**Summer 2015 Penn State McNair scholars and program staff gather together during the 2015 Penn State McNair-SROP Summer Research Symposium held July 27-28, 2015 at University Park.**

## ABOUT RONALD E. MCNAIR

Dr. Ronald Erwin McNair, the second African American to fly in space, was born on October 21, 1950, in Lake City, South Carolina. In 1971, he received a Bachelor of Science degree, magna cum laude, in physics from North Carolina A&T State University. He continued his education at the Massachusetts Institute of Technology (MIT) where, in 1976, he earned his Ph.D. in physics.



While at MIT, McNair performed some of the earliest development of chemical and high-pressure CO lasers. He went on to study laser physics at E'cole D'ete Theorique de Physique in Les Houches, France. He was well published and nationally known for his work in the field of laser physics through the Hughes Laboratory.

In 1978, McNair realized his dream of becoming an astronaut when he was selected from a pool of several thousand applicants to be included in the first class of thirty-five applicants for the space shuttle program. Ronald McNair and six other astronauts died on January 28, 1986 when the space shuttle *Challenger* exploded after launching from the Kennedy Space Center in Florida.



McNair was an accomplished saxophonist; held a sixth-degree, black belt in karate; and was the recipient of three honorary doctorates and a score of fellowships and commendations. He was married to the former Cheryl Moore and is the father of two children, Reginald Ervin and Joy Cheray. After his death, Congress approved funding to honor the memory of McNair by establishing the Ronald E. McNair Post-Baccalaureate Achievement Program, which became the sixth program funded under the TRIO Programs umbrella.

*“Historians, who will write about McNair, the man, will discover that there was much more to him than his scholastics achievements. Friends who knew him, say he walked humbly and never boasted about his achievements. They say his commitments were to God, his family and to the youths he encouraged to succeed.”* (Ebony, May 1986)

## **SPECIAL ACKNOWLEDGEMENTS**

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# *Lexical processing in child and adult beginning second language learners\**

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## Abstract

The objective of the present study was to investigate the neural processes underlying second language (L2) learning using child and adult English monolinguals in the early stages of L2 acquisition. The Revised Hierarchical Model (RHM) of L2 processing (Kroll & Stewart, 1994) predicts that both the child and adult beginning L2 learners will show no effect of semantic interference when presented with semantically related pairs because of their novice learner status. However, based on recent research with Dutch child learners of English, we predicted that the child participants would show semantic interference (Brenders, van Hell & Dijkstra, in revision; Poarch, van Hell, & Kroll, 2015). Stimuli consisted of three types of pairs: correct translations (*perro-dog*), incorrect semantically related translations (*perro-cat*) and incorrect unrelated translations (*perro-table*). Participants were asked to indicate correctness of the translation pairs of Spanish and English words while EEG was recorded. Event-related brain potentials (ERPs) were analyzed using mean amplitude, time-locked to the second word of the pair (English). We found a significant effect of semantic relatedness in both the child and adult participants. Preliminary patterns suggest conceptual activation is possible, even in beginning L2 learners. Further research should investigate how to best revise our current theories of second language processing to accommodate these findings.

\* This research reported in this paper was supported by NSF BCS-1349110 to Janet G. van Hell.

## **Introduction**

In the field of psycholinguistics, the mental lexicon is most easily defined as the mental “dictionary” containing all the words a person knows in a given language. This mental lexicon is typically considered to contain only one language; therefore, a bilingual is thought to have multiple lexicons. Additionally, it is assumed that these two lexicons share one conceptual system that stores the meaning of words. When processing language, words in the lexicon are mapped onto concepts to access meaning (Traxler, 2011). However, it is debated whether bilinguals with varying proficiency find meaning directly from the second language (L2) word or via mediation of the first language (L1) word (for a review, see Van Hell & Kroll, 2013). For

example, when a beginning learner of Spanish reads or hears the word *mesa* (table), does their word processing system map the word *mesa* directly to its meaning or does the system first translate the word into *table* and then access meaning from the word *table*?

Potter, So, Von Eckardt, and Feldman (1984) proposed two models to explain these two alternates. These models describe the mapping of L2 words to meaning and make the assumption that the L1 and L2 share one conceptual system but have separate lexicons. The first model of L2 processing—the concept mediation model—assumes that L2 words are mapped directly onto the meaning through the conceptual link between the second language word and the concept. The word association model claims that to find meaning from an L2 word, the system relies on the L1 lexicon. Therefore, this model states that meaning is found by first mapping the L2 word to its translation in the L1 and meaning is accessed using the person’s already well established L1 word to concept mappings.

The Revised Hierarchical Model (RHM) model of L2 processing states that mode of conceptual access depends on proficiency. This model predicts that in the early stages of learning a second language, learners process L2 words using the word association model such that meanings for L2 words are accessed by translating them to their L1 words (see Figure 1). As L2 proficiency increases, the RHM proposes that more proficient L2 learners follow the links proposed by the concept mediation model such that the conceptual system is activated directly when processing in both their L1 and their L2 (Kroll & Stewart, 1994).

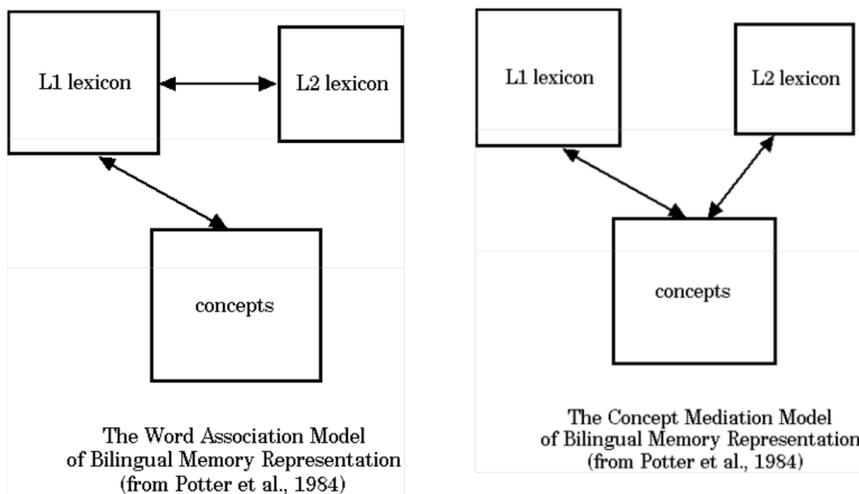


Figure 1: Word Association vs. Conceptual Mediation

De Groot (1992) developed the translation recognition task as a method to study the RHM consistently with people of differing L2 proficiency levels. The translation recognition task involves the presentation of a word in one language followed by the presentation of a word in a second language that is either a correct or an incorrect translation of the first. Participants are then asked to indicate whether the second word they viewed was a correct or incorrect translation of the first.

An adaption of this task to study proficiency and semantic interference was developed by Talamas, Kroll and Dufour (1999) in which half of the incorrect pairs were related in semantic category or form. For example, an incorrect pair that shares semantic category is *man-mujer* (woman) instead of *man-hombre* (man) because the words *man* and *woman* are semantically related. An example of an incorrect pair that shares word form was *man-hambre* (hunger) as

opposed to the correct translations *man-hombre* (man) due to the orthographic relatedness of the correct translation—*hombre*—and the incorrect word form related translation—*hambre*.

Using these stimuli, this study tested the RHM by comparing more fluent English-Spanish bilinguals with less fluent bilinguals. The RHM would predict that because conceptual activation will increase with increased proficiency, the more fluent English-Spanish bilinguals would show slower reaction times and make more errors for pairs that share a semantic category compared to unrelated controls. This is based upon the prediction that increased conceptual activation will lead to semantic interference. Additionally, the RHM predicts that in the less fluent bilinguals, due to low proficiency and reliance on lexical mediation, the word form pairs should produce slower reaction times and more errors in comparison to unrelated controls, and no semantic interference effects would be observed.

Talamas et al. (1999) found evidence for both predictions, providing support for the theorized transition from lexical to conceptual representation of the L2 as proficiency increased, as outlined in the RHM. These results thus confirm the RHM model as less proficient L2 learners showed mostly word form interference and the more proficient L2 learners showed a decrease in word form interference and an increase in semantic interference compared to less proficient L2 learners as they transition into more conceptual processing in the L2.

The adaptations of Talamas et al. (1999) became the basis of many studies looking at proficiency related effects in adult L2 learners to further investigate the link between semantic interference and proficiency (for a review, see Van Hell and Kroll, 2013). Fewer studies used this paradigm to investigate the degree of conceptual activation in and child beginning learners (Comesaña, Perea, Piñeiro & Fraga, 2009; Poarch et al., 2015). Guo, Tam and Kroll (2012) and Brenders et al. (in revision) additionally measured event-related brain potentials (ERPs) to further investigate the time course of these effects. These studies will be discussed in more detail below.

Comesaña et al. (2009) investigated semantic interference effects in fluent child Basque-Spanish bilinguals and Spanish child learners of L2 Basque. Participants in this study performed the translation recognition task. Key word pair sets contained Spanish and Basque words that were incorrect translations but semantically related and incorrect unrelated controls. Results demonstrated semantic interference in Spanish-Basque bilinguals when processing in their L2. The semantic interference effect was also found in early beginning learners of Basque. These findings contradict the Revised Hierarchical Model of second language acquisition because according to the model, learners in the early stages should rely on word form relations and should not develop conceptual connections until L2 proficiency increases. This study demonstrated that processing at a conceptual level can occur early in L2 acquisition in children.

Poarch et al. (2015) investigated whether children in the early stages of second language acquisition map a word in their second language directly to the concept or meaning. In this study, Dutch speaking children with eight months of classroom instruction in English (about 1 hour per week) performed a translation recognition task and a translation production task. In the translation recognition task, longer reaction times and decreased accuracy for semantically related pairs relative to incorrect controls suggest that semantic activation was present when processing in the L2. This evidence supports the idea that children can directly access the meaning of L2 words, even in the very early stages of L2 acquisition.

Guo et al. (2012) also used the translation recognition task to investigate the predictions of the RHM, but they tested fluent adult Chinese-English bilinguals. Replicating Talamas et al. (1999), semantic and orthographic relatedness of the two words were manipulated to investigate

the degree of conceptual and L1 lexicon activation when processing in the L2. Based on the behavioral and EEG/ERP data, Guo et al. (2012) found semantic and word form interference when processing in the L2. This evidence suggests that when fluent bilinguals process L2 words, both concepts and L1 words are active. The RHM predicts that in fluent bilinguals concept is directly activated when processing words in the L2. However, it does not predict that the L1 lexicon is active, as these results suggest.

Building on the findings of Guo et al. (2012), Brenders et al. (in revision) replicated the investigations of interference using fluent adult bilinguals and extended the study with the addition of child beginning second language learners. The children were fifth and sixth grade Dutch elementary school students learning English in a classroom setting. Fifth grade students had received English lessons for five months. The sixth grade students had received English lessons for 16 months.

The study consisted of three experiments: an off-line behavioral translation recognition study, an on-line behavioral translation recognition study and an Event-Related brain potentials translation recognition study. In Experiment 1, errors showed semantic and word form interference in the two groups of beginning child L2 learners and adult proficient bilinguals. Experiment 2 replicated these effects with the addition of reaction times providing converging evidence that both conceptual and lexical representation were active in all three age groups. The ERP data collected in Experiment 3 further supported this same conclusion. The ERP results showed a significant N400 effect present in all groups in reaction to both types of distracters. The N400 is a negative-going effect that reaches peak amplitude around 400ms after stimulus onset and is typically associated with lexico-semantic processing. The amplitude of the N400 was smaller in reaction to stimuli that are more closely semantically related. and this effect was found in both beginning L2 learners and adult bilinguals.

Brenders et al (in revision) presented evidence that child beginning learners process second language words similarly to fluent adult bilinguals. Additionally, the child beginning learners in this study showed an N400 effect similar to the adult bilinguals, providing neurocognitive evidence that child beginning learners process more like the proficient bilingual adult. This contradicts the predictions of the RHM which states that fluent bilinguals process L2 words using direct L2 word to concept mappings while early learners rely on mediation of the L1 lexicon to access meaning. However, this study is the first to provide physiological evidence that this may not be the case in child beginning learners.

A continued debate in the literature exists about the RHM. Furthermore, studies testing the RHM are highly varied in the population of use and type of manipulation. Guo et al. (2012) found that bilinguals can access their conceptual system directly, supporting the RHM. However, Brenders et al. (in revision) found neural and behavioral evidence that early child learners access their conceptual system during L2 processing. Further research collecting more EEG data during the translation recognition task would be beneficial as there are few papers out using EEG to investigate conceptual activation. Additionally, Brenders et al. (in revision) was the first to perform this task with children while recording EEG so further ERP data still needs to be collected on children to provide converging neural evidence for this effect in children.

Importantly, the early child learners in the Brenders et al. (in revision) study, living in the Netherlands, were also exposed to at least some English outside of the classroom setting through popular media (e.g., popular music, television) and the internet. Therefore, the children tested in this study not be true novice L2 learners, and may have had exposure to English in addition to their L2 English language classes. Therefore, the current study will explore early L2 learners

with little L2 exposure outside of classroom instruction, who can be qualified as true novice L2 learners.

The present study will extend Brenders et al. (in revision) by utilizing the translation recognition task with children and adults in the early stages of L2 learning. This study is the first to systematically compare two groups of L2 learners who differ in age but share comparable proficiency. This study will record continuous EEG to investigate L2 lexical processing as it relates to the RHM.

The RHM predicts that both child and adult beginning learners will show no effect of semantic interference due to their novice learner status. In the adult beginning learners, we expect to find patterns consistent with the predictions of the RHM. It is predicted that the adult learners will show no difference in N400 effects between the semantically related and unrelated pairs because the RHM predicts that in the early stages of L2 learning, L2 form to concept mappings are not formed yet and L2 processing relies on L2 form to L1 form mappings to access concept.

According to the RHM, children should show no effect of semantic interference; however recent evidence contradicts this prediction. Alternatively, if children have already built L2 form to concept mapping at the early stage of L2 learning, it is predicted that they will show an effect of the semantic interference. If so, the effect will be evident in the ERP data such that a smaller N400 to semantically related incorrect pairs will be detected in comparison to the unrelated incorrect translation controls. These alternative results would suggest L2 word form to concept mappings in novice L2 child learners.

## **Methods**

### **Design**

This study was a one factor within subjects design with the independent variable translation type such that there were three types of word pairs (correct, incorrect semantically related, incorrect unrelated). The materials consisted of correct translations (PERRO (*dog*) – DOG), incorrect translations with the second word semantically related to the correct translation (PERRO (*dog*) – CAT) and incorrect translations with the second word unrelated to the correct translation (PERRO (*dog*) – TABLE). Event-related potentials (ERPs) time locked to the second word in the pair were used as the dependent measure.

### **Participants**

The participants included adult L2 learners from two colleges in central Pennsylvania and kindergarten L2 learners from a local elementary school. Participants in both groups were right-handed and from English speaking homes. The adult participants did not acquire a second language until college and the children began learning Spanish in Kindergarten. We tested both groups of participants after approximately 20-25 hours of classroom instruction in Spanish.

### **Procedure**

Following the procedure of Brenders et al. (in revision), participants were presented with word pairs, the Spanish word followed by the English word, on a monitor with a black

background with letters in white Arial font. Each trial began with a fixation sign at the center of the computer screen for 500ms, followed by a 500ms pause. Next, the L2 word was presented for 350ms in the visual presentation and 500ms to 1200ms depending on the length of the word for auditory presentation. A blank screen was presented for 1000ms followed by the L1 word with timing the same as the L2 word timing. Following the pair presentation, there was a 3000ms pause until a screen appeared asking the participant if the word pair was a good or bad translation.

The participants responded using a button box to indicate whether they thought the pair was a correct or incorrect translation. This jittered time interval and the delayed decision procedure was used to prevent motor artifacts associated with pressing the buttons entering the EEG signal. After the participant responded, a smiley symbol appeared for 3 seconds, indicating to the participant that they were allowed to blink and swallow at this time if needed. All communication and directions during the experiment were in English.

### **EEG Recording and Data Analysis**

Participants were tested in a mobile van with modifications to control for interference with EEG recording. Data was collected on-site, at the participants' school where the van was parked for the duration of testing. The EEG system was a mobile ActiCHAMP system with 30 active scalp electrodes configured to the international 10-20 system and two eye-electrodes (vertical and horizontal) to track eye movement and blinks. EEG was continuously recorded across the entire scalp of the participants. We recorded data at a sampling rate of 500 Hz with a band pass filter of 0.1 to 40Hz. Impedances at each electrode site were maintained below 15kohms. Electrodes referenced online to the left mastoid electrode were later re-referenced offline to an average of the left and right mastoid electrodes. The EEG continuous data was filtered offline at 30Hz with an offline low-pass filter and at 0.5Hz with an offline high-pass filter. ERPs were averaged offline for each participant at each electrode site for each of the three experimental conditions, relative to a 200ms pre-stimulus baseline. Trials found to contain eye artifacts were identified and rejected from inclusion in analyses.

### **ERP Analysis**

In line with research on earlier L2 learners (e.g. Brenders et al., in revision; Tokowicz & MacWhinney, 2005), all trials were included in the analysis, regardless of whether the participant correctly determined whether the translations were correct or incorrect. EEG was time-locked to the onset of the second word (English word) in the pair. Analyses examined mean amplitudes with a pre-stimulus baseline of 200ms prior to the onset of the word. In accordance with prior research on the N400, the epoch of 300ms to 500ms was selected for analysis. Preprocessing and the measurement of the ERP data were done in ERPlab.

Two repeated measures analyses of variance (ANOVA) compared conditions by examining scalp distribution of the ERP effect. An ANOVA was performed for midline electrodes with a factor of electrode group (Fz, Cz, and Pz). Another ANOVA was conducted focusing on a factor of anteriority (anterior, posterior) and laterality (right, left hemisphere). Analyses examined if mean amplitude between 300ms and 500ms differed significantly by condition (correct, incorrect semantically related, incorrect unrelated).

## Results

### Adult Data

Preliminary results from the 10 adult participants tested at the time of analysis are reported. Additional data will be collected and analyzed in the future. Visual inspection of the waveforms indicated a visually detectable difference between the two types of incorrect conditions between 300ms and 500ms, such that the unrelated translation condition produced a more negative going waveform than the semantically related condition, and the two incorrect conditions produced a more negative going waveform than the correct condition in this time window (see Figure 2).

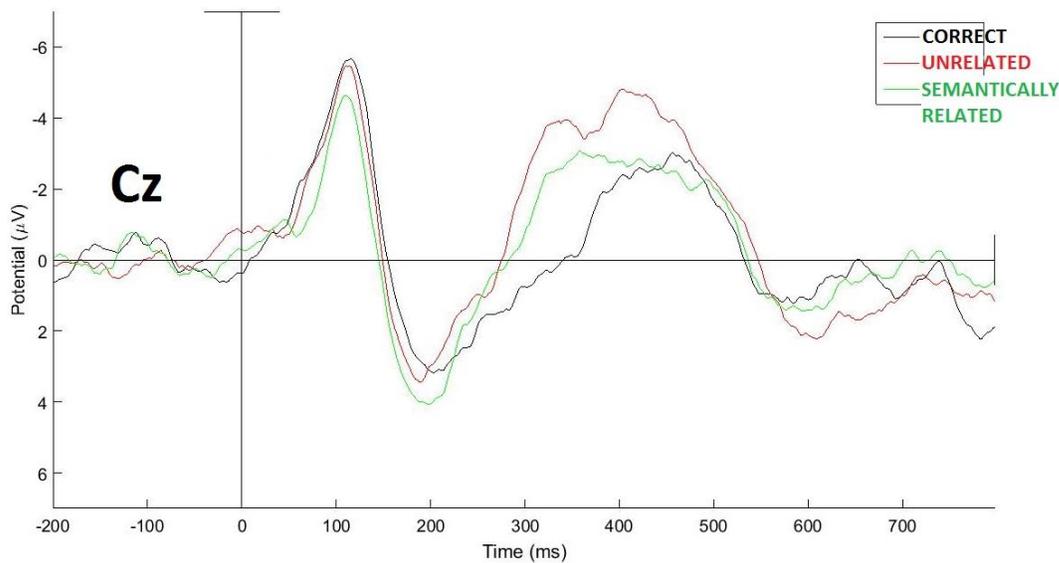


Figure 2: Adult data: Grand average event-related brain potentials at representative electrode Cz for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up. Significant difference in mean amplitude between correct translation, and both types of incorrect translations, 300ms - 500ms time window. Significant difference of mean amplitude between semantically related pairs and unrelated controls in 300ms - 500ms time window. (For additional electrode sites see Appendix A).

The analysis yielded a significant effect for translation type (correct, unrelated incorrect, semantically related incorrect) such that unrelated incorrect translation pairs produced a greater negativity than semantically related incorrect pairs which produced a greater negativity than correct pairs (midline:  $F(2,18) = 9.168, p = .005$ ; lateral:  $F(2,18) = 11.43, p = .002$ ).

Additionally, a significant interaction was found with topography (translation type x electrode:  $F(4,36) = 4.147, p = .032$ ) such that a significant difference was found between unrelated and semantically related conditions at front and central electrode sites but not posterior sites (Fz:  $F(1,9) = 6.102, p = .036$ ; Cz:  $F(1,9) = 8.216, p = 0.056$ ; Pz:  $F(1,9) = 2.218, p = .171$ ). A significant difference between correct and unrelated translations was found at central and parietal electrode sites Cz and Pz but no significant difference was found at frontal site Fz (Fz:  $F(1,9) = 2.835, p = .127$ ; Cz:  $F(1,9) = 11.7, p = .008$ ; Pz:  $F(1,9) = 15.83, p = .003$ ). A significant difference between correct and semantically related translations was found at central

and parietal electrode sites Cz and Pz but no significant difference was found at frontal site Fz (Fz:  $F(1,9) = 1.236, p = .295$ ; Cz:  $F(1,9) = 11.7, p = .008$ ; Pz:  $F(1,9) = 2.218, p = .171$ ).

Analysis of the lateral electrodes (CP6,CP2,P8,P4,F8,F4,FC2,FC6,CP5,CP1,P7,P3,F7, F3,FC1,FC5) was performed using four regions of interest (right anterior, left anterior, right posterior, left posterior). A significant interaction was found between translation type and anteriority (anterior, posterior)  $F(2,18) = 6.10, p = .014$ . Unrelated incorrect translation pairs produced a significantly greater negativity on average in the 300ms to 500ms time window than correct translation pairs at both anterior and posterior electrode sites (anterior:  $F(1,9) = 5.93, p = .083$ ; posterior:  $F(1,9) = 20.76, p = .001$ ). Semantically related incorrect translation pairs produced a significantly greater negativity than correct translations at posterior sites but not at anterior sites (anterior:  $F(1,9) = 0.196, p = .668$ ; posterior:  $F(1,9) = 21.87, p = .001$ ). Unrelated incorrect translation pairs produced a significantly more negative N400 effect compared to semantically related incorrect translations at anterior sites and a marginally more negative effect at posterior sites (anterior:  $F(1,9) = 8.433, p = .017$ ; posterior:  $F(1,9) = 4.449, p = .064$ ).

### Child Data

Preliminary results from the 10 adult participants tested at the time of analysis are reported. Additional data will be collected and analyzed in the future. Visual inspection of the waveforms indicated a visually detectable difference in the 300ms to 500ms time window between the two types of incorrect translations such that incorrect translations produced a more negative going waveform than correct translations. A marginally significant main effect for translation type was present at the midline such that semantically related translations produced the most negative going waveform, followed by unrelated translations which produced a more negative going waveform than correct translations ( $F(2,8) = 5.038, p = .054$ ) (see Figure 3). We found no significant effect of translation type at the lateral electrodes ( $F(2,8) = 3.37, p = .127$ ). Additionally, no significant interactions were found at the lateral electrode sites.

A significant effect of translation type was found at electrode site Fz such that semantically related translations produced on average a more negative effect than unrelated translations, followed by correct translations as least negative ( $F(2,8) = 6.03, p = .049$ ). At electrode Fz, unrelated translations were significantly more negative than correct translations ( $F(1,4) = 9.84, p = .035$ ) and semantically related translations were significantly more negative than correct translations ( $F(1,4) = 7.30, p = .054$ ). Unrelated and semantically related translations were not significantly different at electrode Fz ( $F(1,4) = 2.03, p = .228$ ).

A marginally significant effect of translations type was detected at electrode site Cz such that semantically related translation produced the most negative effect, followed by unrelated translations and then correct translations ( $F(2,8) = 3.92, p = .082$ ). Additionally, a significant difference was found between correct translations and semantically related translations such that semantically related incorrect translations produced on average a more negative effect than correct translations ( $F(1,4) = 8.86, p = .041$ ). No other significant differences existed at electrode site Cz (see Figure 3).

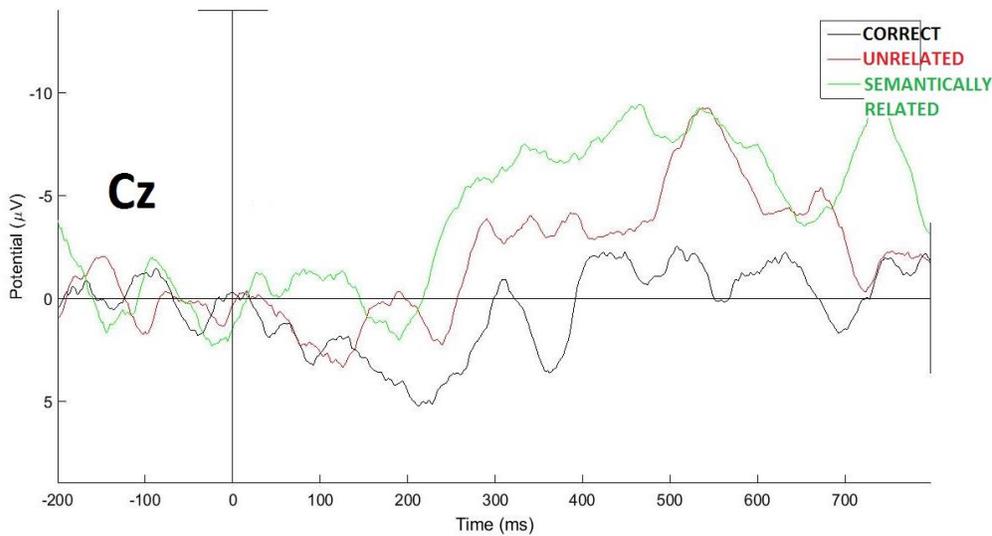


Figure 3: Child data: Grand average event-related brain potentials at representative electrode Cz for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up. Significant difference in mean amplitude between semantically related pairs and correct pairs, 300ms - 500ms time window. (For additional electrode sites see Appendix A).

No significant effect for translation type was found at posterior site Pz ( $F(2,8) = 3.40, p = .123$ ). However, further comparisons reveal emerging trends that could reach significance with added participants. A marginally significant difference between semantically related and correct translations is present such that semantically related translations produced a marginally more significant negativity on average than correct translations ( $F(1,4) = 6.982, p = .057$ ). Additionally, comparisons between unrelated and semantically related translations are significant such that semantically related translations produced on average more negative effect than unrelated translations ( $F(1,4) = 8.855, p = .041$ ).

## Discussion

The RHM predicts that beginning second language learners should not show sensitivity to the semantic manipulation during the translation recognition task. However, if beginning L2 learners do activate conceptual information when processing L2 words, then semantic interference should occur, even in these groups of beginning L2 learners. In this study, we found semantic effects in both the adult and child groups of L2 learners. Results found significant differences between the two types of incorrect translations in the 300ms to 500ms time window. This difference in N400 amplitude provides evidence for semantic interference in adults contradicting the predictions of the RHM. These results suggest that the conceptual system is active when beginning adult learners process words in their L2.

Brenders et al. (in revision) found evidence for semantic interference in beginning child L2 learners. However, it can be argued that those Dutch children were not true novice learners due to L2 English exposure outside of the classroom. The present study, testing child L2 learners in Central Pennsylvania with minimal exposure to their L2 Spanish outside the classroom, also found a semantic effect. It should be noted that the direction of the difference between semantically incorrect translations and incorrect controls (the semantically incorrect

translations more negative going) differed from the direction observed in Brenders et al. (in revision) who found that semantically incorrect translation were less negative going. Because the present study included only five children, it remains to be seen to what extent this effect holds with a larger group of children. Assuming it will, a possible explanation is that the direction of the effect is related to the age difference between the two groups (5<sup>th</sup> graders in Brenders et al. versus Kindergartners in the present study). Kindergartners more so than 5<sup>th</sup> graders are still developing conceptual links between L1 words and their meanings, in addition to the L2 word-to-concept mappings, and this developmental difference may be reflected in the children's neurocognitive patterns. Until more Kindergartners are tested the interpretation of these results remains speculative, but generally speaking developmental differences in L1 acquisition potentially affect L2 learning and the nature of the lexical-conceptual links child L2 learners develop.

Likewise, the adult beginning learners in the present study showed a reduced N400 on trials containing semantically related incorrect pairs relative to unrelated controls, whereas the children displayed an increase N400 in reaction to these same pairs in comparison to unrelated controls. The differing patterns of results found for children and adults in this study may be related to the fact that young children are still acquiring semantic categories and may not utilize them in the same way as adults do. Adults may rely on semantic categories more than the children because the adults have fully acquired categories and can use them to their advantage when processing. However, children who are still learning words and categories in their L1 may not use semantic categories yet as a means of facilitating processing. As also noted above, this issue remains speculative until more child data are collected.

A possible explanation for the conceptual activation found in both child and adults learners at this early stage of L2 learning is the context of processing. The present study utilizes a recognition task and past studies have found that the link between concepts and L2 words in early learners is more evident in recognition tasks than production tasks (for review see Van Hell & Kroll, 2013). Furthermore, in particular later versions of the RHM (e.g., Kroll et al., 2010) do not predict an inability of novice learners to access concept but merely *weaker* L2 word to concept links. Therefore, the mixed results regarding conceptual activation suggest that the strength of this link may vary depending on the task and learner circumstances (for review see Kroll et al., 2010; Van Hell & Kroll, 2013; Poarch et al.(2015).

Additionally, ERP measures may be a more sensitive measure of conceptual activation than behavioral measures. Future analyses will also examine the accuracy data of the presently tested child and adult L2 learners. Moreover, it should be noted that Brenders et al. (in revision) is one of the very few papers, if not the only one, that examined ERP evidence related to translation recognition in L2 learners. Therefore, a wide base of empirical evidence is not yet present. However, both Brenders et al. (in revision) and the present study provide evidence for activation of conceptual information early in L2 learning.

To conclude, this study provides preliminary physiological evidence regarding semantic processing during the translation recognition task. Evidence of semantic activation was present suggesting that even in the very early stages of L2 learning, L2 form to concept mappings are available to both child and adult L2 learners. Further data collection and analysis of behavioral data recorded will provide further insights into the nature of these semantic effects. However, preliminary patterns suggest conceptual activation is possible, even in beginning L2 learners. Further research should investigate how to best revise our current theories of second language processing to accommodate these findings.

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## Appendix A

### Adult and Child Data: Additional Waveforms

Figure 4: Adult data: Grand average event-related brain potentials at all scalp electrodes for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up. Significant difference in mean amplitude between semantically related pairs, unrelated pairs and correct translation pairs in 300ms - 500ms time window.

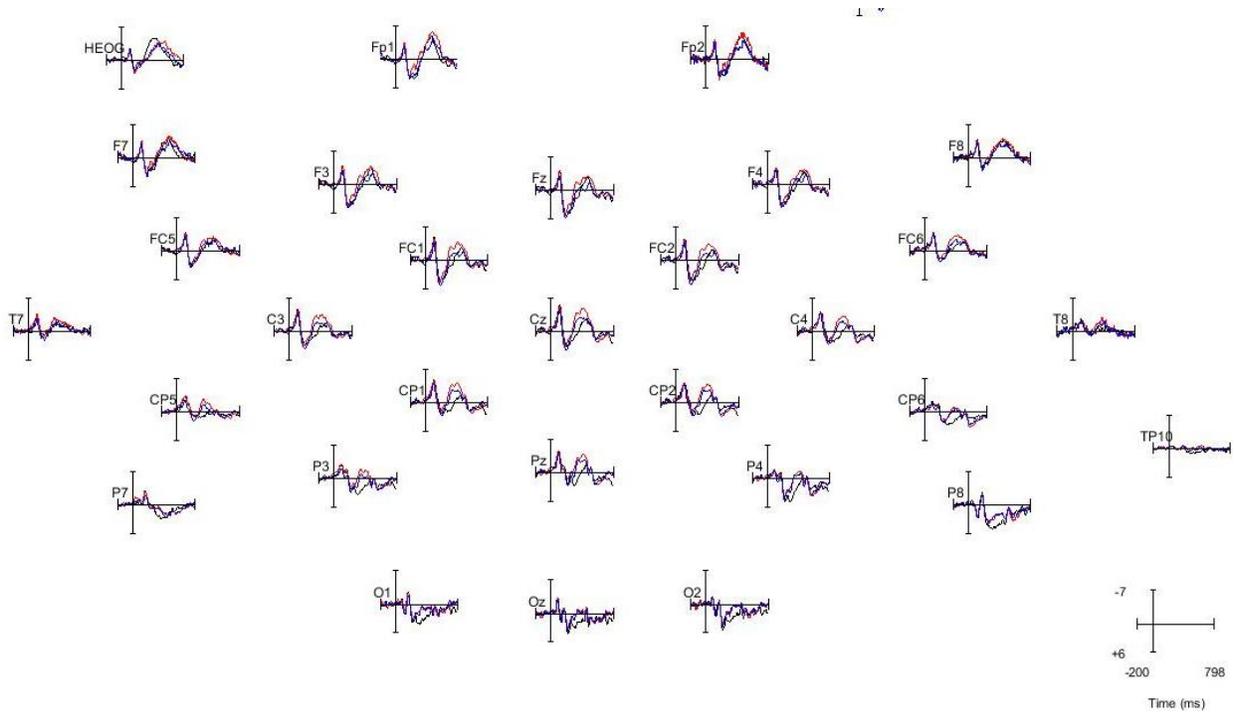


Figure 5: Adult data: Grand average event-related brain potentials at midline electrodes Fz, Cz and Pz for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up.

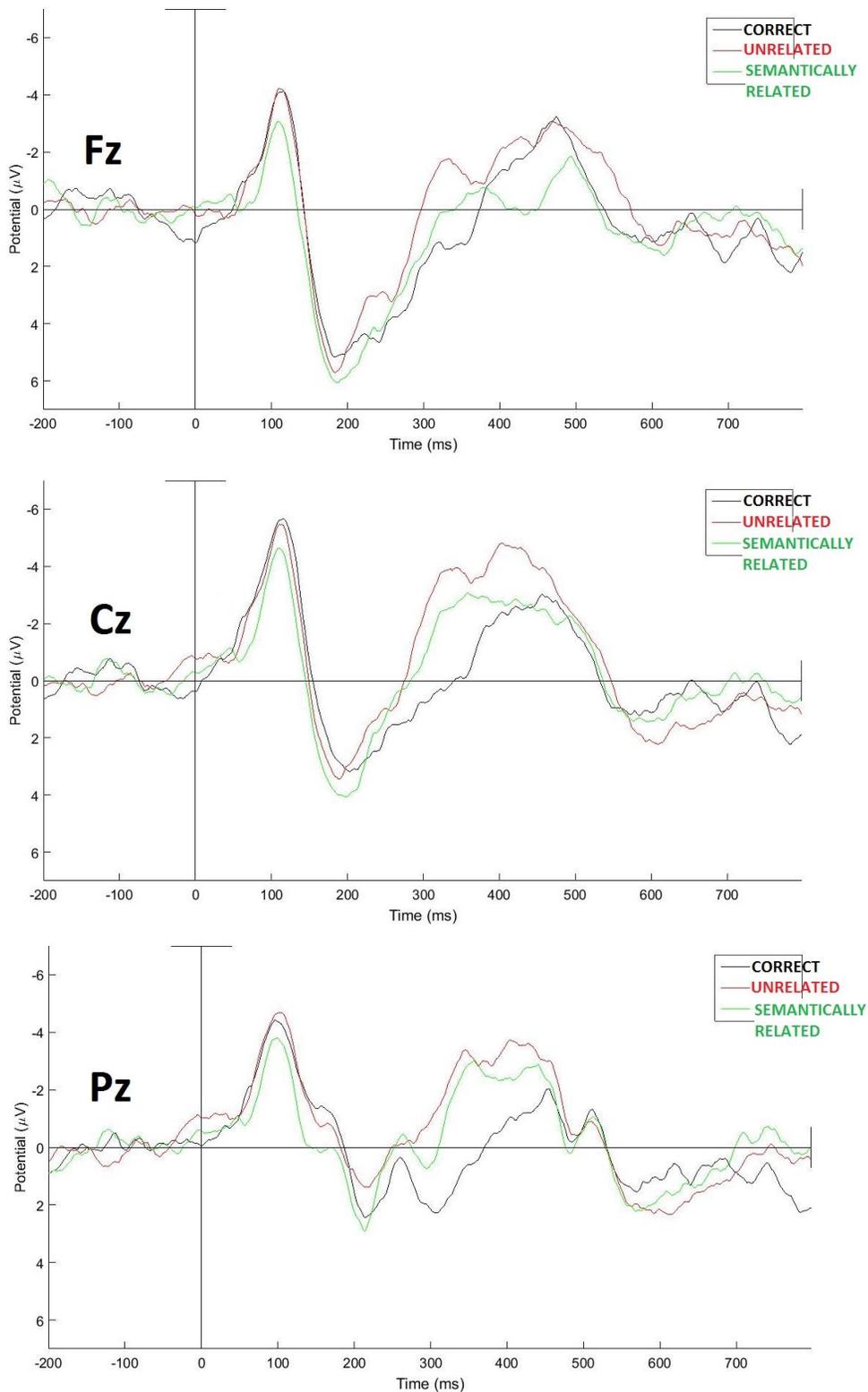


Figure 6: Adult data: Grand average event-related brain potentials for regions of interest (left anterior, left posterior, right anterior, right posterior) for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up.

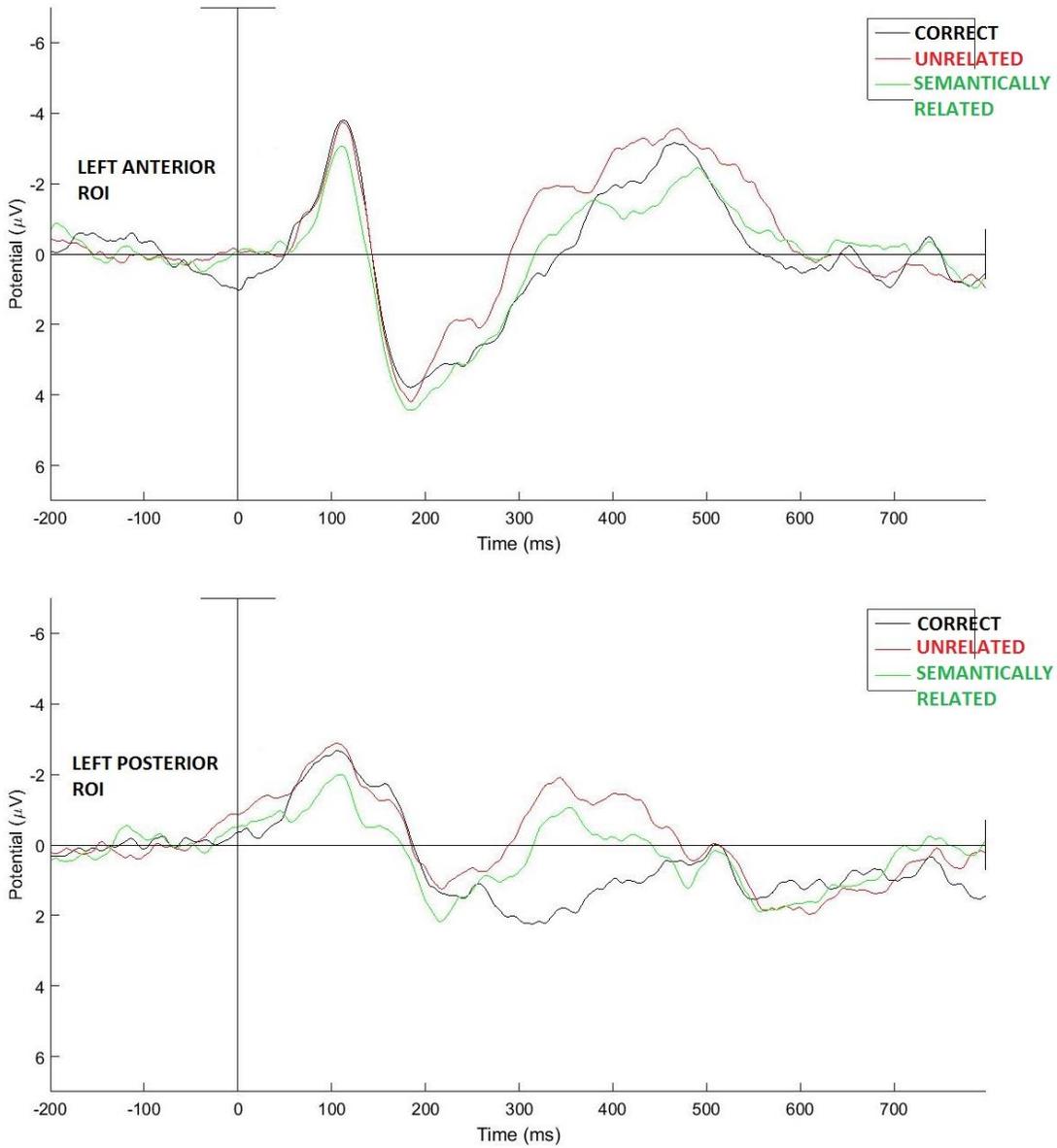


Figure 6 continued

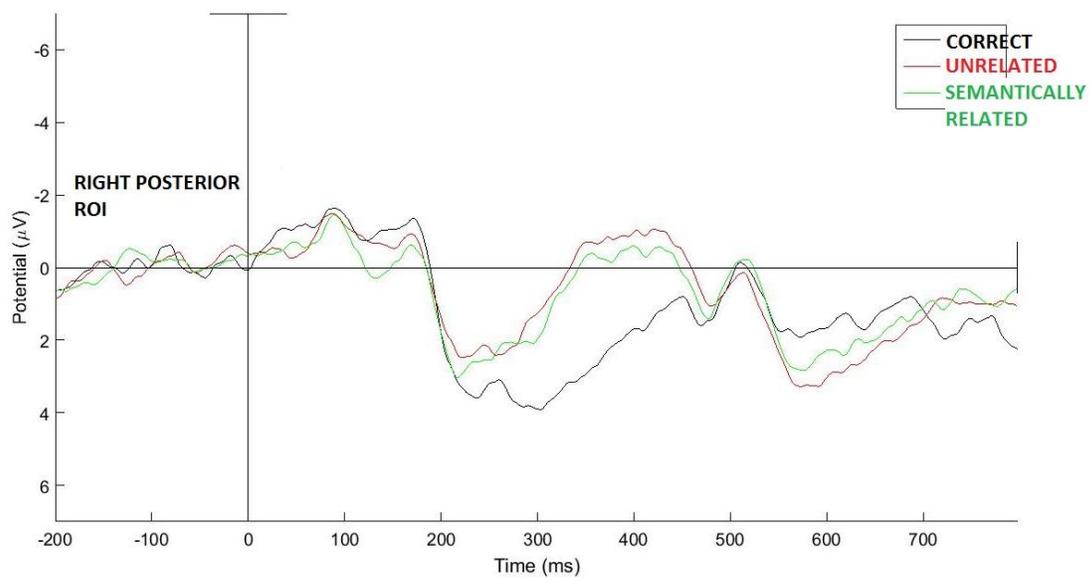
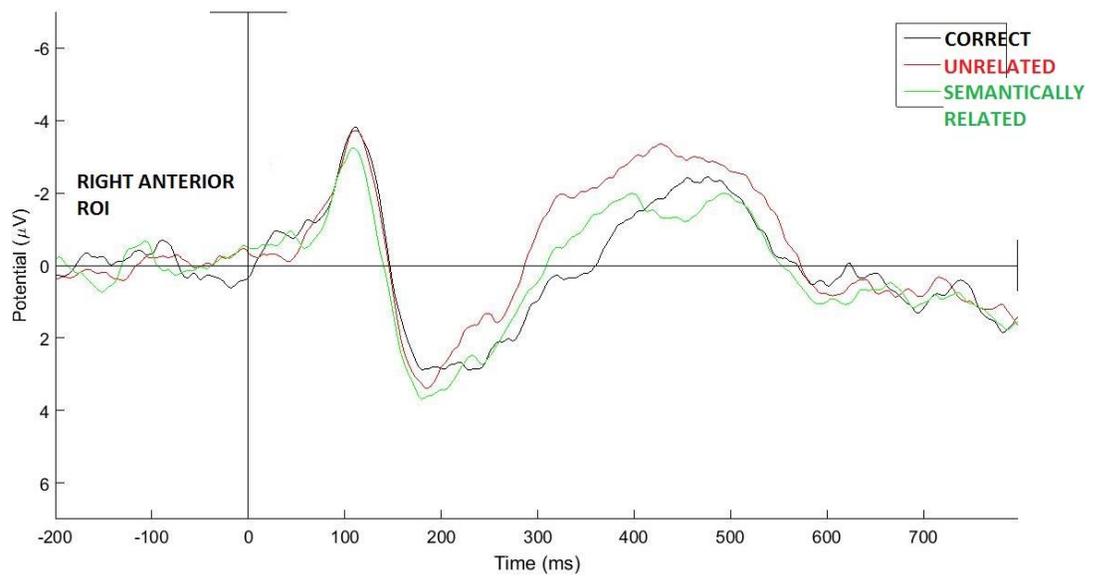


Figure 7: Child data: Grand average event-related brain potentials at all scalp electrodes for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up. Significant difference in mean amplitude between semantically related pairs, unrelated pairs and correct translation pairs, 300ms - 500ms time window.

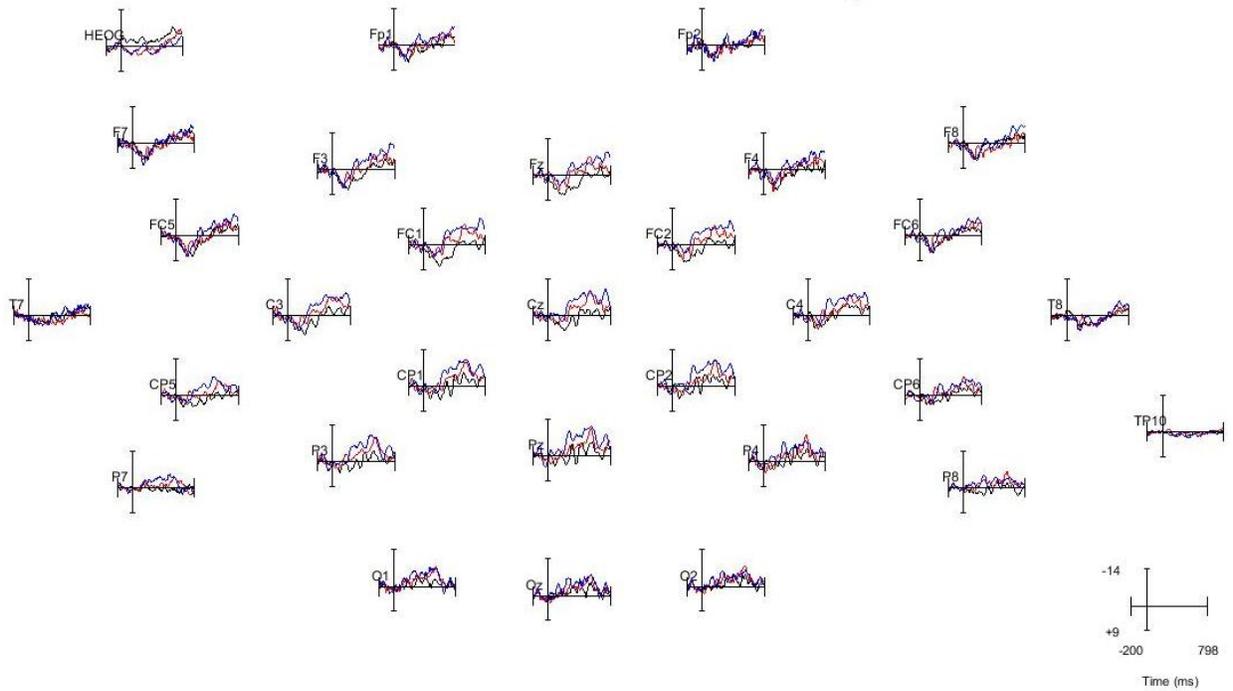


Figure 8: Child data: Grand average event-related brain potentials at midline electrodes Fz, Cz and Pz for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up.

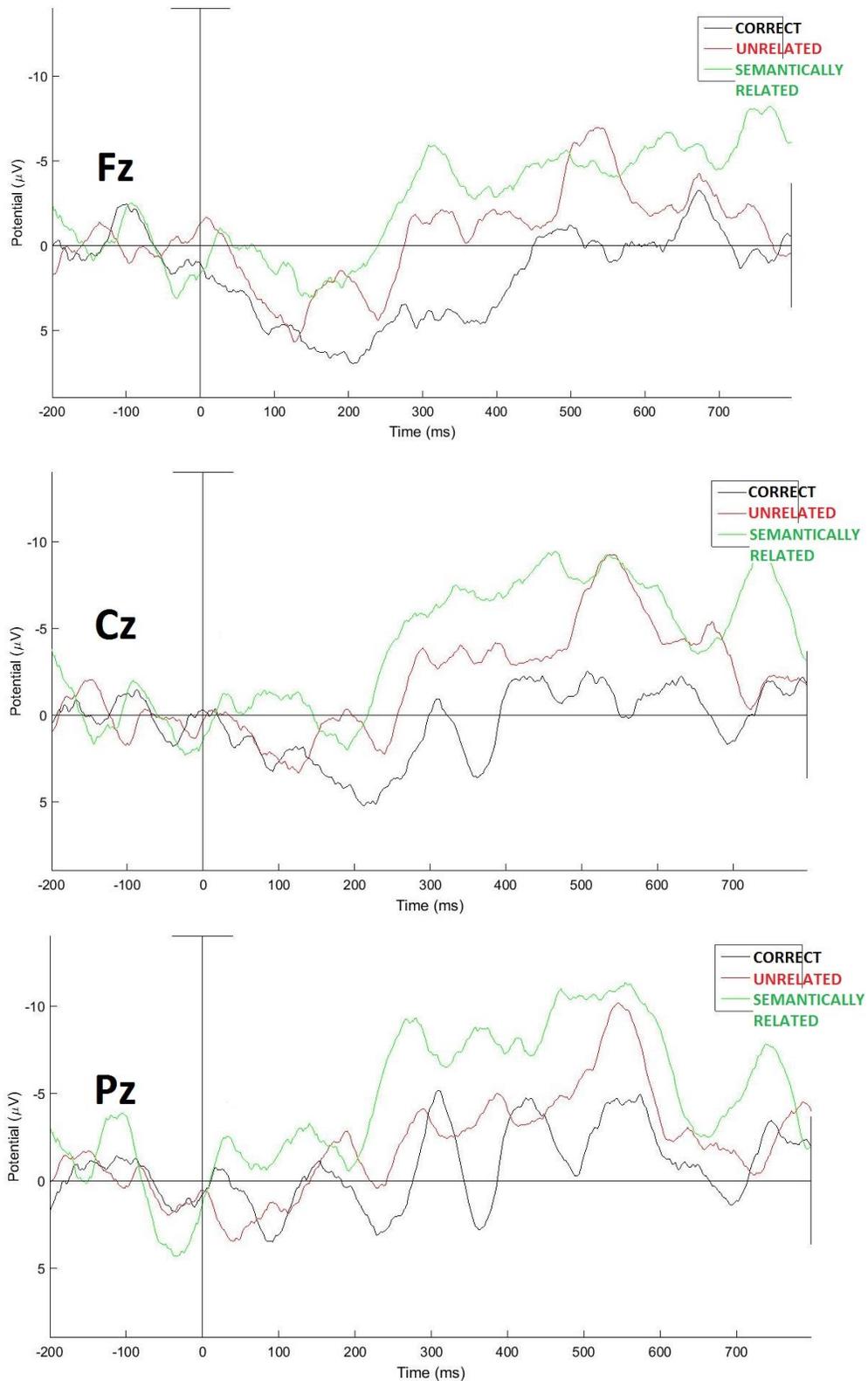


Figure 9: Child data: Grand average event-related brain potentials for regions of interest (left anterior, left posterior, right anterior, right posterior) for correct translations (black), unrelated incorrect controls (red) and semantically related pairs (green) plotted from 200ms prior to stimuli onset to 800ms post stimuli onset. Negative is plotted up.

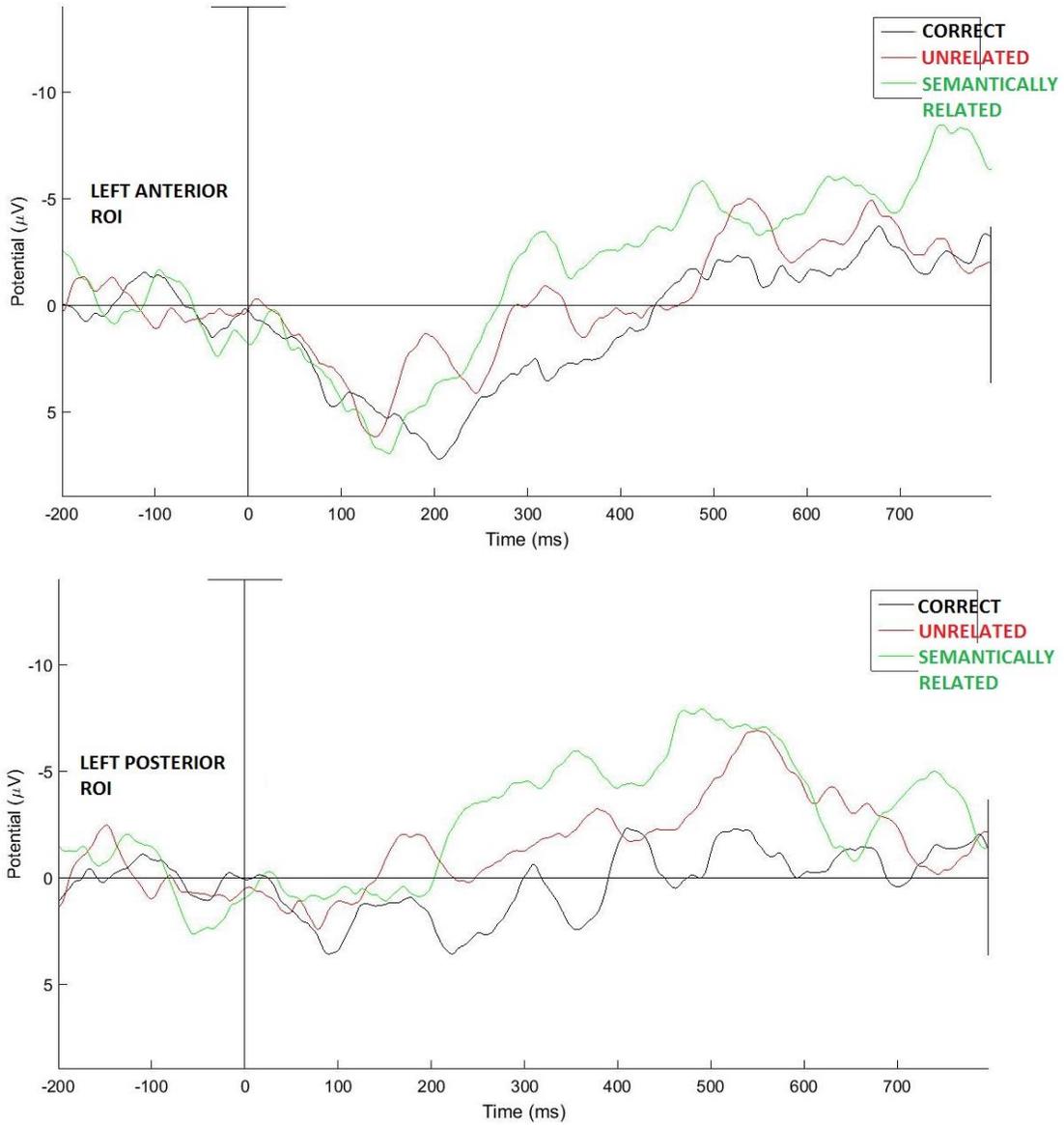
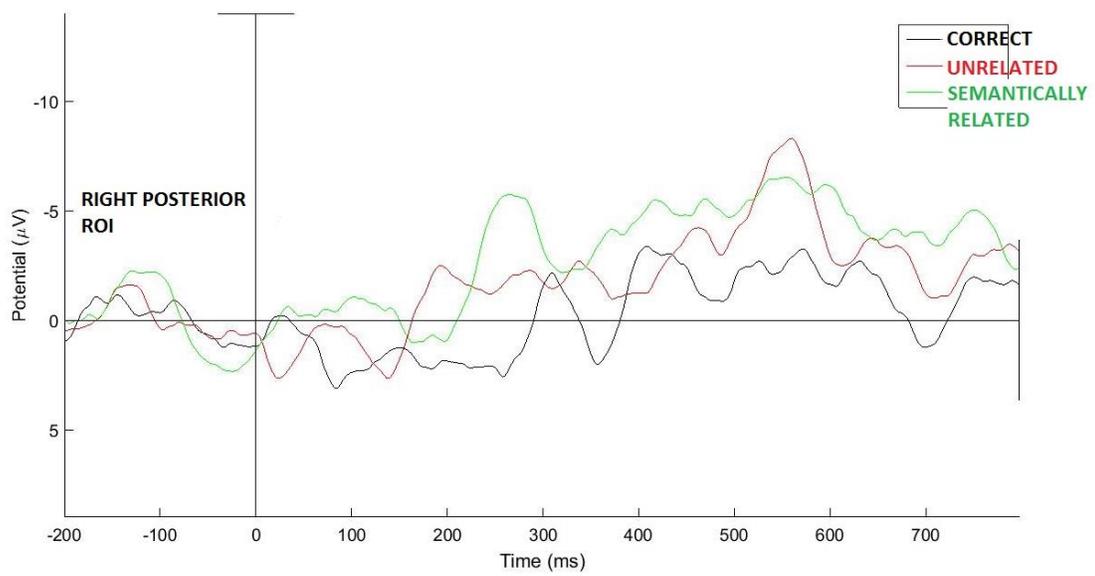
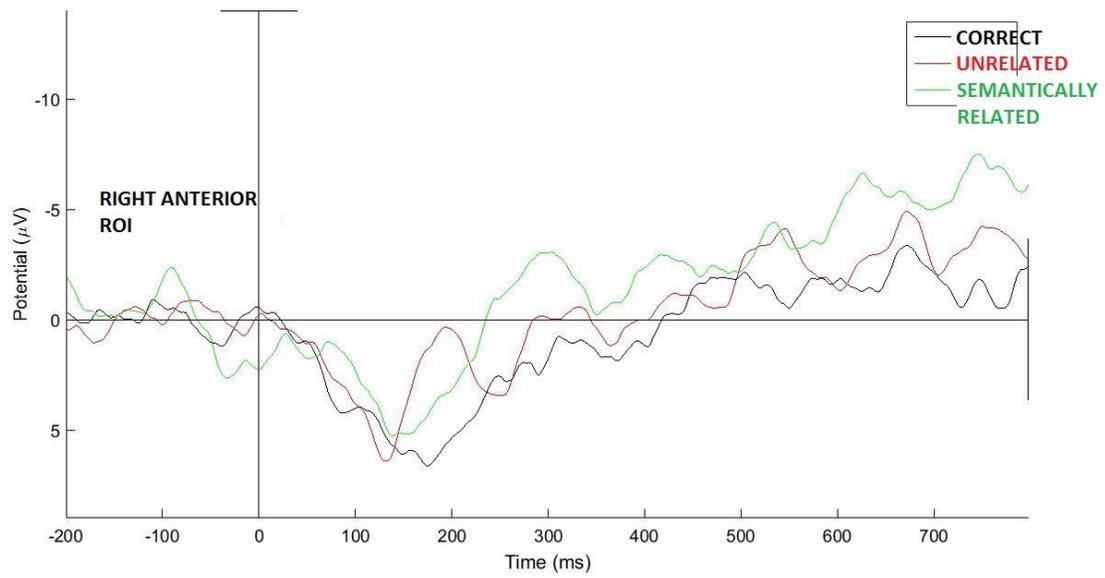


Figure 9 continued



# *Eritrean and Eritrean American Health Assessment*

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## **Abstract**

**Background:** Eritrean Immigrant health and health care access has not been explored, to the same extent as other African immigrants. Given the increasing population of Eritreans in America, understanding Eritreans health status will be important for developing public health policy for African communities. **Methods:** An anonymous 1-point cross sectional survey, including 28 questions, assessed information on chronic disease status, health care access, health status, and demographics. Surveys were distributed through social media forums such as Twitter and Facebook. Cross-tabulations were used to draw descriptive conclusions. **Results:** Of 101 completed surveys, 63.3% of participant's insured actually having had a routine checkup in the last year. 45.5% uninsured did not visit a doctor due to cost. 54.5% did not have one doctor thought of as their personal health care provider. **Discussion:** Future research with community-based participation may help to further understand the needs of Eritrean and Eritrean-American communities to create culturally appropriate health interventions.

## **Introduction**

### **African Immigrant Health in the U.S.**

According to the 2008-2012 Census of the 1.6 million foreign-born from Africa in the U.S., 36 percent were from West Africa, 29 percent from East Africa, 17 percent from the Northern region, 5 percent from South Africa, and 5 percent from Central Africa (U.S. Census Bureau, 2014). But even with the increasing number of East Africa populations, Ethiopia, most closely culturally related to Eritrea in the horn of Africa, consists of one of the largest African-born populations but has the second lowest educational attainment among these having a bachelor's degree or higher at 26.2 percent (U.S. Census Bureau, 2014). Nigeria, at 60.9 percent,

and Egypt, at 63.9 percent, both have the highest percentage of educational achievements beyond bachelor's degrees (U.S. Census Bureau, 2014).

A lack of education can result in poor basic communication of health needs in specific populations, ultimately leading to bad health outcomes. Researchers have failed to observe that health literacy can include the variables of culture and ethnicity as potential barriers to understanding and utilizing of U.S. health care system (Shaw, 2009). Furthermore, low health literacy is most prevalent in ethnic minorities, low-income, and elderly populations (Shaw, 2009; McLean, 2013; Gonzalez, 2008; Lucas, 2003). Additionally, ways in which African populations in general view chronic disease, health care, and health issues vary from region to region.

Although papers have examined culturally appropriate health care interventions for African immigrants, often these studies are combining findings from participants across the continent of Africa, which includes a variety of cultural differences. Furthermore, the use of the data found in African populations is often used to observe the "immigrant health effect," comparing the health of Africans to Black people in America. Although important data exists, including findings that immigrants appear to have lower rates of hypertension compared to American-born Blacks, such data should not dismiss the importance of further reducing these numbers in the African community (Hyman, 2000). Drawing most data from West Africans, which may be due to language barriers, cultural differences on views on health care, or the educational barrier mentioned, can limit healthcare providers' opportunities to reach out to other African communities to access their health care needs (Adeyinka, 2014).

Health disparities in the U.S. are still prevalent, and with the immense growth and diversity of cultural backgrounds present, the country is not equipped with culturally appropriate health care or education. Awareness of cultural norms and ideals faced in different populations is important to establish health interventions. More specifically, African immigrants represent one of the fastest growing groups of immigrants to the United States. Between 1990 and 2000, the total number of African immigrants increased by 166 percent (Venters, 2011). Eritrean populations originating from the Horn of Africa are increasing in the U.S. population, with many pockets of communities in places such as Washington, D.C, Washington State, Texas, and California. With this growth in mind, as future or current public health specialists, we must have an understanding of populations and how we can reach them to begin health care interventions.

## **Previous Research Methods and Culturally Appropriate Health Interventions**

### ***Surveys, focus groups, and one-on-one interviews***

Studies on the Eritrean community have used methods such as focus groups, surveys, and one-on-one interviews. A cross-generational survey found that among 40 participants, two-thirds knew someone with diabetes and 3 of the 40 participants had diabetes (McGuigan, 2010, p1). "Most middle-aged and older adult respondents named lifestyle factors such as poor diet, lack of exercise, and stress as contributing factors to diabetes;" they mentioned diet and exercise as primary treatments, with few acknowledging medications. (McGuigan, 2010 pp. 1-2). In addition, both Ethiopian and Eritrean participants had concerns about becoming addicted to medication and Western medication being too strong, as well as other concerns (McGuigan, 2010). These same interviewees expressed the need for intensive counseling and education to build knowledge within their communities, but such counseling and education may not always be cost effective (McGuigan, 2010). Few published studies reveal a thorough health assessment or

specific health interventions molded to fit the needs of Eritrean populations at risk for chronic disease.

A multidisciplinary intervention for diabetes in Eritrea, including the training of diabetes educators, enhanced physician education, patient-teaching materials, and glucose monitoring promotion, worked in improving sustainable diabetes care in developing countries (Windus, 2007). This study alone reveals that when immigrants are migrating, they might be arriving with chronic illnesses. Research abroad and interviews in Eritrean communities in Seattle indicate a need for education on how to prevent such health outcomes, as well as a need for disease management over a lifetime. Expanding the field of research on specific populations in Africa will directly impact health care outcomes in different areas across the U.S. for the benefit of the people and the health care system.

Focus groups have made great strides in understanding the beliefs Eritreans have about health concerns, such as psychosocial barriers to obtaining preventive dental care for children; barriers for HIV testing; and differences among acculturation experiences, food beliefs, and perceived health risks (Amin, 2012; Lindkvist, 2015; Wilson, 2014). Important findings from these studies include barriers stemming from cultural health beliefs, such as denial and fear of social isolation as a result of having HIV and roots in cultural beliefs from experiences in Eritrea, such as traditional eating habits (Amin, 2012; Lindkvist, 2015; Wilson, 2014; Ghezehay, 2010). Having used focus groups as a method to reach a community, we see that the community, when receiving health interventions, is receptive to speaking about its views and possibly its needs, which will allow health care providers to fulfill these needs.

### ***Social media as community outreach***

Community based participatory research is an approach to improving health status in communities based on recommendations and participation of the community to bring about the change they would like to see. Social media as an outlet to easily reach this community has been used to target specific immigrant populations, usually distributed in their native language. To encourage Vietnamese women to get pap tests, one health intervention took data from their previous study, which included suggestions from Vietnamese women on the best way of reaching them through social media (Lam, 2003). Working with Vietnamese language TV networks, newspapers, radio stations, as well as creating posters and information pamphlets, they found that, post-intervention, more woman knew about cervical cancer, also increasing the number who went to get pap tests (Lam, 2003). Social media in this case was used as the community thought best, specific to their language needs, in overcoming a cultural barrier to better health. Lam also employed the use of community forms to receive feedback on their intervention strategies of social media, helping to refine their intervention as suggested by the community.

Identifying community leaders as a way to reach the community, Keimer used them to distribute informational flyers and posters for Turkish immigrant communities (Keimer, 2011). Finding that their target audience was hard to recruit for the study, it revealed barriers of limited time to participate, location as an issue, and the content not being of interest to them. Using the community to help identify, and potentially solve, issues they face allows for health care providers to better understand the structural or cultural barriers faced by populations.

What is missing in the research for African immigrants is the assessment and utilization of social media forums such as Facebook and Twitter to assess health status and risk. Eritrean

immigrants living in the U.S. adopt a new way of living, and reaching the community through focus groups interventions may be limiting. This paper will begin to look into ways to further reach Eritrean populations. Furthermore, the research will first use the strategy of reaching these populations through survey form on Facebook and Twitter, as well as a health assessment based on responses to questions about health care access, exercise, chronic disease, and health status.

## **Methods**

### **Participants**

Eritrean American and Eritrean participants were recruited from different forums/pages on Facebook and Twitter. These residents were reached by posting the survey link and a short message explaining the purpose of the survey. Public Eritrean Facebook pages, personal messages, and the sharing of posts by participants themselves were the main forms of distribution. Eligibility to participate was determined by age (18+), being a U.S resident or citizen, and Eritrean or Eritrean-American status.

### **Procedure**

An anonymous 1-point cross sectional survey, including 28 questions from the Behavioral Risk Factor Surveillance System (BRFSS), was distributed through social media forums including Twitter and Facebook. Consent was given on the first page of the survey, only allowing access to the survey to those answering “yes”. Included in the survey were questions of U.S citizenship or residency and confirmation of being Eritrean; these questions were added after about 100 submissions. Survey stuffing was disabled (not allowing access once a survey was completed).

### **Measures**

#### ***Behavioral risk factor surveillance system (BRFSS)***

The Behavioral Risk Factor Surveillance System is a telephone survey collecting annual data on health risks, chronic disease, and use of preventative care services. Pulling from the 2014 survey, questions were taken from the chronic disease, health care access, health status, exercise, and demographics sections as measures.

Chronic disease information included five questions about whether the participant or a family member, has ever had, or been told they have had a heart attack, angina or coronary heart disease, stroke, cancer, or diabetes. Health care access information included whether they had any kind of health coverage (including Medicaid, HMO’s, etc.); having someone they considered their personal doctor or health care provider; whether money was a factor for not seeing a doctor in the past year; and the time since their last visit for a routine checkup (12 months, 1-2 years, 2-5 years, 5+ years, Don’t know/Not Sure, or Never).

Health status included one self-assessed question about general health including answer choices “excellent” (1), “very good” (2), “good” (3), “fair” (4), “poor” (5), or “don’t know/not sure” (6). Exercise also included one question asking “yes” or “no” to engaging in exercise outside of work in the last month (running, walking, gardening, etc). Demographics included

height, weight, age, sex, race identification, military services, marital status, the number of children in the home less than 18 years of age, highest academic achievements, employment, annual household income, renting or owing a home, and whether physical/mental/emotional problems may limit activities.

## **Participant Recruiting**

### ***Facebook and Twitter***

Facebook pages “Eritrea Meadi Deki Hade Libi” (Eritrea, children of one heart-563 members); “Eritrea Niana, Nhna N’Eritrea” (Eritrea for us, us for Eritrea-414 members); “I am Eritrean and I am proud” with 680 members; and “We are all Isaias Aferwki” with 9,009 members are pages used to share news about political issues and policies being passed in Eritrea. The main initiative is to keep those in the diaspora informed. They also host communities for people to share any other news locally they may have.

“YFPDJ and Young Eritreans” with 5,058 members; “Harrisburg YFPDJ” with 27 members; “YFPDJ: Hidri Dallas Chapter” with 472 members; and Young PFDJ with 4,724 members are groups specific to the organization of “Young People’s Front for Democracy and Justice (YFPDJ).” YFPDJ is a nationalist Eritrean Diaspora Youth Organization branch. This is where political news is shared, but also things pertaining to identity and community for those in the Diaspora. Many of the topics discussed are from the National conference held annually, last year’s topic being identity. Many videos are posted on what the different *hidri* (youth) across the U.S. are doing for their Eritrean communities locally.

Finally, there is the “21<sup>st</sup> anniversary of the Eritrean Independence” which is an older page but has 12,843 members. It is now the 24<sup>th</sup> anniversary of Eritrea’s liberation from Ethiopia. It is a page that updates the Eritrean communities across the U.S. about the festivals/celebrations happening in D.C., since there is a big population there.

Twitter was used as a way to directly contact individuals in the Eritrean community through the hashtag Eritrea. Through the Facebook page of YFPDJ, a Twitter page link was found allowing reaching contacts quicker through personal tweets. Individuals were asked to take the survey for the Eritrean and Eritrean American Health Assessment. In addition, participants on Facebook and Twitter were able to retweet or repost the survey to their personal pages. The number of friends each participant reposting or retweeting had was accounted for and added to the total number of contacts.

## **Variables and Analytic Strategies**

Overall, 276 submissions were recorded and descriptive conclusions were made using IBMs Statistical Package for the Social Science (SPSS). Small conclusions based on the data collected included a bivariate analyses exploring health risk by SES; chronic disease history and health risk; whether online surveys are an effective method to collect data from this community; how often Eritreans engage in exercise; and cross tabulations of how much health care access Eritreans and Eritrean Americans have and utilization of their access. For the purpose of a descriptive study, submissions were used to generate hypotheses for future health interventions or methods to reach Eritrean populations in the U.S. based on research of these varying methodologies.

## Results

### Response Rate

Taking into account the number of members on the various Facebook pages and the number of friends or twitter followers of those who reposted the link, about 64,936 points of contact were made. There is no data on how many of these contacts are repeats or whether they actually had the chance to view the link/survey. 276 participants did give consent to take the survey with 220 actually completing the survey. Of the 220 who completed the survey, 101 were recorded after the addition of the question confirming their being Eritrean or Eritrean-American.

### Descriptive Results

Basic demographics including age, BMI, self-reported health status, gender, educational level, marital status, employment status, number of children under 18 in household, rent or owning home, and household income are summarized in Table A1. The mean age for respondents was 28, with a mean BMI of 25, considered healthy. Mean self-reported health status of 1.9 indicates most respondents believe they are in either very good or excellent health, supporting the BMI mean outcome.

More than half of the respondents (55.6%) were female. Educational attainments show that more than 80% either completed 1-3 years of college or 4+ years. Only 28.6% of respondents chose “student” for employment status; “wages” was the largest with 58.2%, which may indicate that some students are also working while attending school. Marital status was second largest with 17.6, with most respondents, 72.5%, answering “never married”. About 53.9% of participants made less than 50,000, 19.1% making less than 75,000, and the remaining 27% made 75,000 or more.

54.7% of participants reported that either they, or a family member, had diabetes. Only 12.3% reported myocardial infarction, 10.4% for angina coronary heart disease, and 20.8% for stroke, which are all known for being co-morbid with diabetes. A number of participants also reported that either they or a family member had cancer (29.2%). Most of respondents reported having engaged in exercise outside of work (82.8%), with only 4.4% reporting limitations due to physical, mental, or emotional problems.

Most respondents had access to health care (Table A3), with 63.3% ( $P = 0.03$ ) of insured participants actually having had a routine checkup in the last year (Table A4). A little fewer than half of respondents (45.5%,  $P = 0.03$ ) who were uninsured did not visit a doctor due to cost (Table A4.) More than half of those uninsured (54.5%,  $P = 0.0015$ ) also did not have one doctor they thought of as their personal health care provider (Table A4).

## Discussion

Social media served as an effective way to reach the Eritrean community, with 220 completed responses in about 3-4 weeks. The mean age of 28 was telling of respondents being young and old and actively participating in social media such as Facebook and Twitter. Similar to Lam’s study targeting immigrant Vietnamese populations through social media, our survey was shared through the social networks of our participants. In Lam’s study, they observed the community for 16 years before conducting a successful health intervention. The researchers

spent the time understanding cultural patterns of seeking and sharing information within the Vietnamese community (Lam, 2003). Their findings showed the Vietnamese had a strong emphasis on community ties and found that community leaders were primary distributors of important information, helping to spread information on cervical cancer prevention. Similar to Lam's study findings, Eritrean community leaders worked to distribute the survey. These indications of strong ties between those in the community shows that future research on community outreach can be strategically planned based on the understanding of cultural norms of information sharing (Lam, 2003; Keimer, 2011).

BMI calculated by using self-reported weight and height found a mean of 25, which is considered healthy, although close to being overweight. A mean health status was reported at 1.9 (1 being excellent health and 2 being very good). Interestingly, 82.8% reported engaging in exercise outside of their workplace. The reasons for the mean being close to overweight should be explored, as well as the perception of body size. A study examining overweight prevalence across countries in Africa and other underdeveloped countries found that in the 1990s to 2000s, there was an increase in urban and rural areas, with the exception of Nigeria and Rwanda (Jaacks, 2015). From the 2000s to early 2010, there was an increase in the percentage overweight in both urban and rural areas (Jaacks, 2015). Additionally, a Senegal study looked into the perceptions of body size among Senegalese women, finding that about 27% in the study were overweight, and one-third viewed those overweight, not obese, as being most socially desirable body size (Holdsworth, 2004). Although culturally and geographically different, a South Africa study found the same positive association of overweight, but not obese, body sizes in women (Draper, 2015). Overweight and perceptions of overweight are of increasing concern in Sub-Saharan Africa. Weight perceptions in Eritrea may continue to affect Eritrean immigrants. Furthermore, perceptions about body sizes may interfere negatively with future health interventions to reduce rates of overweight and obese populations.

The prevalence of chronic diseases such as diabetes is highly associated with overweight and obese populations. Overall, 54.5% of participants reported that they or a family member had diabetes. Non-communicable diseases (NCDs) are expected to be reaching about half of the deaths in Sub-Saharan African by 2030 (The World Bank, 2011; Jaacks, 2015). The dilemma faced in these at-risk countries is the economic burden, developing NCDs while still economically developing, cutting resources to prevent bad health outcomes (The World Bank, 2011).

In relation to health care access in the Eritrea study, 36.7% of Eritreans responding "yes" to having access to health care indicated that they did not visit a doctor in the past year for a routine checkup. Previous research has found that refugee or asylum-seeking immigrant populations coming from countries with less developed health care systems expect hospital referrals to serve their needs in developed countries, which typically rely on primary care to address such needs (Burnett, 2001). Understanding how to utilize the health care system is a struggle for native-born Americans; so examining the understanding of specific African immigrants' beliefs about the system is needed (Bernstein, 2003). Furthermore, this finding raises the need to question and further explore potential barriers, whether cultural, individual, or structural, to utilizing health care even when insured.

Additionally, in 2013, 13.4% of the U.S. population was uninsured and 10.9% of participants in this study were uninsured or did not know if they were insured (Smith, U.S. Census, 2013). A little less than half of those uninsured did not visit the doctor due to cost; more than half also did not have anyone they considered their doctor or personal health care provider.

A lack of education may exist regarding being insured and benefits that are available for use. Furthermore, refugees moving to a new country will need health professionals and organizations to make contact with them to build their network of health care resources (Burnett, 2001). Future research needs to answer why Eritrean and Eritrean-American populations may be uninsured.

### **Limitations**

The age of those active on social media may be limiting of the audience that needs to be reached those who may not know how to use them. Having been a self-reported health assessment, all responses on chronic disease were open to being specific to himself or herself or a family member. Specificity on family history and the participant having the chronic diseases will need to be further researched. BMI was also calculated using self-reported height and weight, which may include estimations rather than confirmed results.

A language barrier may also limit the number of people able to access information online. Future research must be conscious of the possibility of a language barrier. Additionally, the number of responses was limited because the question of whether they were Eritrean was not added until about 100 responses were documented. Overall, 101 responses met all requirements to make conclusions. Furthermore, the method used in the study to keep track of the response rate limited knowing how many people were new and old points of contact. There was no way to know how many of the contacts were repeats, in order to effectively calculate the percentage of people reached compared to the number who completed the survey.

### **Future Research**

Further research must begin to explore views Eritreans may hold about the chronic diseases they have, as well as their methods of management. For example, the rate of diabetes reported was 54.7% with few reporting having diseases usually associated as co-morbid, raising the question of the rate of comorbidity for these populations. With the help of doctors/hospitals, confirmation of chronic disease prevalence and health status of individuals can be produced to begin targeting health issues. Furthermore, exploration and understanding of cultural norms within Eritrean communities will begin to reveal different views in relation to chronic disease management and effective methods of communicating with the community.

As previous research in one Eritrean community showed, fear of becoming addicted to medication, exists; therefore, researchers must understand other beliefs about chronic disease management (McGuigan, 2010). The best methods observed with immigrant populations seem to be community-based participatory research (CBPR) involving the community to identify problems related to health. The most basic barrier is language. To overcome language barriers, the youth may be utilized, as well as the use of photo voice in CBPR to provide a way of communicating concerns through photos. The methods used in Windus's study in Eritrea training diabetes educators, enhancing physician education, developing patient-teaching materials, and more involved the use of those within the Eritrean community who understood the effective ways of communicating with the people (Windus, 2007).

Giving the community a voice and central position in the health related research being conducted would raise questions that may have otherwise been overlooked. Furthermore, having an ethnographic mindset when entering into communities of a different culture will allow the community to show the issues it faces rather than health professionals making educated

assumptions. All these considerations will lay the foundation of research that must be explored before health interventions can take place.

### **Personal reflection**

I was already an active member of the Eritrean social media community, so leaders and members of the Eritrean community who recognized me reposted, asked questions about the study, and emailed their Eritrean friends to take the survey. These actions were also supported with encouragement and recognition for the work I was doing in the development as furthering Eritrean people as a whole through public health. The support received as a member of the community also shows that the community is very close-knit with the quick circulation of the survey, support, and outcome of responses. I found participants who said they were already contacted before I personally sent a message or tweet. Not being a member of the Eritrean community may be a limitation to reproducing this research because another will not be as connected to the community. However, my membership is a bigger advantage for reaching this population for future research.

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## Appendix A

Table A1

Demographics (n=101)

<u>Variable</u>	<u>M (Sd)</u>	<u>%</u>
Age	28 (9.5)	
BMI	25 (7.9)	
Health Status	1.9 (.37)	
	Excellent – 1	30.8
	Very Good – 2	43.9
	Good – 3	21.5
	Fair – 4	2.8
	Don't Know/ Not Sure – 5	.9
Gender		
	Male	44.4
	Female	55.6
Educational Level		
	Grades 9-11	1.1
	Grade 12 or GED	13
	College 1-3 years	40.2
	College 4 years or more	45.7
Marital Status		
	Married	17.6
	Divorced	6.6
	Separated	2.2
	Never Married	72.5
	Member of an unmarried couple	1.1
Employment Status		
	Wages	58.2
	Self-employed	9.9
	Out of work for less than a year	1.1
	A homemaker	1.1
	A student	28.6
	Unable to work	1.1
Number of children in home under 18 years of age		
	0	57.6
	1-2	33.7
	3-4	7.6
	5-6	1.1
Rent or Own Home		
	Own	38.9
	Rent	45.6
	Other arrangements	10
	Don't know/Not sure	1.1
	Do not want to answer	4.4
Annual household income		
	Less than 25,000	15.7
	Less than 35,000	20.2
	Less than 50,000	18
	Less than 75,000	19.1
	75,000 or more	27

Table A2

Health Conditions (n=101)

Variable	Answer Choices	%
Myocardial infarction	Yes	12.3
	No	84.9
	Don't know/ Not Sure	2.8
Angina or coronary heart disease	Yes	10.4
	No	84.9
	Don't know/ Not Sure	4.7
Stroke	Yes	20.8
	No	72.6
	Don't know/ Not Sure	6.6
Cancer	Yes	29.2
	No	68.9
	Don't know/ Not Sure	1.9
Diabetes	Yes	54.7
	No	40.6
	Don't know/ Not Sure	4.7
Engaged in exercise	Yes	82.8
	No	16.2
	Don't know/ Not Sure	1
Limited by physical, mental, or emotional problems	Yes	4.4
	No	95.6
	Don't know/ Not Sure	1.1

Table A3

Access to care (n=101)

Variable	Answer Choice	%
Health care coverage	Yes	89.1
	No	8.9
	Don't know/ Not Sure	2
Time in past 12 months when not seeing doctor due to cost	Yes	20.8
	No	78.2
	Don't know/ Not Sure	1
Having one person as personal doctor or health care provider	Yes, only one	54.5
	More than one	27.7
	No	14.9
	Don't know/ Not sure	3
Time since last doctor visit for physical/routine checkup	Past year (less than 12 months)	60.4
	Within past 2 years	23.8
	Within past 5 years	11.9
	5 or more years	4

Table A4

Cross Tabulations (n=92)

Variable	Insured % N=9	Uninsured % N=83*	P-value
Do you have one person you think of as your personal doctor or health care provider?			
-Yes	55.6	45.5	
-More than 1	31.1	0	0.0015
-No/Don't know/Not sure*	13.3	54.5	
About how long has it been since you visited a doctor for a routine checkup? (a routine checkup is a general physical exam for a specific injury, illness, or condition)			
-Within the past year (anytime less than 12 months ago)	63.3	36.36	
-Within the past 2 years (1 year but less than 2 years ago)	22.22	36.36	0.03
-Within the past 5 years	12.22	9.09	
-5 years or more	2.22	18.18	
BMI (SD)	24.5 (4.51)	31.2(21.3)	.3784
Was There a time in the past 12 months when you needed to see a doctor but could not because of cost?			
-Yes	15.8	45.5	0.03

***Analyzing Fire Hazard Risk:  
A Case Study in Table Mountain National Park,  
Cape Town, South Africa***

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**Abstract**

Fieldwork is the traditional basis for creating fuel hazard maps, but it is not always cost or time effective. This study utilizes remote sensing and GIS technologies to analyze Landsat 8 and other geographic layers, exploring the use of the Normalized Difference Vegetation Index (NDVI) and the Normalized Difference Moisture Index (NDMI) as surrogates for fieldwork data to provide an inexpensive alternative for deriving a wildfire hazard map. The study focuses on the fire that occurred March 1, 2015, on Table Mountain National Park in Cape Town, South Africa, where the primary vegetation is the fire-adapted Fynbos shrubland. Calculation of NDMI values was coupled with a traditional NDVI analysis to provide additional information about plant moisture. A pre-burn Landsat 8 image was analyzed for fire hazard using five variables: vegetation moisture, slope, aspect, elevation and distance from roads and then compared to post-burn imagery to test the method's accuracy. The results indicate that NDMI may be a stronger indicator of fire hazard than the more popular NDVI.

**Keywords:** Landsat, remote sensing, NDVI, NDMI, wildfire, South Africa, Fynbos

## Introduction

Wildfires are complex events that occur as a result of natural and human factors and are hazardous to people and the natural environment (Merlo and Rojas Briales 2000; Wenliang et al. 2010). Recent changes in both climate and anthropogenic factors related to fire hazard could transform traditional fire regimes and exacerbate the risk of fire and its negative impacts. Among these factors, the influence of climate warming on increased fire frequency and intensity has been documented in several ecosystems (Kasischke and Turetsky, 2006; Westerling et al., 2006). The encroachment of the built environment into areas of high fire risk is another important factor. This situation provides urgency for understanding the risk that wildfire hazards pose.

Evaluating the risk of fire hazard is a critical part of preventing fires and reducing fire's negative impacts. The fire hazard risk is defined as a combination of the hazard and potential damage. By determining areas of high fire risk it is possible to minimize threats to life, property, natural and economic resources (Adab et al., 2013). Pre-fire planning resources require objective tools to monitor when and where a fire is more prone to occur, or when it will have more negative effects (Martinez et al., 2009). To understand fire hazard, fieldwork is traditionally used to collect vegetation information via sample plots. This usually requires sending researchers to sample vegetation over large areas. This research is often time consuming and expensive because it requires multiple skilled workers. The use of satellite imagery with a GIS offers a cheaper alternative that could potentially save time and money.

The wildfires of Cape Town, South Africa were used as a case study for the potential application of remote sensing and GIS technologies for wildfire hazard analysis. This study sought to determine whether vegetation information derived from remote sensing data could serve as a sufficient surrogate for collecting field data when mapping fire hazards. It investigated the potential of using the Normalized Difference Vegetation Index (NDVI) and the Normalized Difference Moisture Index (NDMI). However, the fuels are difficult to discriminate between using only these indices, and therefore the Structural Fire Index (SFI) was used to incorporate other influential factors.

## Study Area

The study region is Table Mountain National Park. The park extends across the Cape Peninsula to include Table Mountain and the Silvermine Nature Reserve and is adjacent to the city of Cape Town (Figure 1). The wildfire studied here occurred within the Silvermine area of the park.

The area surrounding Cape Town, South Africa, is prone to burning because of the natural fire cycle in the Fynbos biome, a fire-adapted shrubland and heath environment located only on the Cape Peninsula. This study looks at the fire that began March 1, 2015, and burned intensely across the landscape. Such fire events are natural in Fynbos, which on average burns every 10-15 years (Cowling 1992), but the fire's close proximity to this highly urbanized area presents a danger to humans. There has been limited research conducted on Fynbos fires. Historically, fires were ignited at high elevations from lightning strikes (Cowling 1992) and then burned downslope into the

valleys. However, urbanization has fragmented the landscape and slowly encroached up the mountainside. This landscape change has altered the fire cycle by preventing the natural spread of the fires from mountains into the valleys.

The climate is generally categorized as Mediterranean, and although lightning strikes occur infrequently in Mediterranean climates, they are a higher risk than human-caused fires. Fires ignited by lightning tend to burn larger areas because they occur in more isolated and steeper areas and frequently have various simultaneous ignited spots. The resultant fires are more difficult to control (Wotton and Martell, 2005). Many developed countries, such as the United States and Canada, have meteorological stations available to monitor lightning strikes and relate them to wildfires (Wotton and Martell, 2005; Martinez et al., 2009). In contrast, despite its proximity to an urban area, South Africa does not have meteorological stations capable of monitoring lightning strikes close to the site of the fire studied in this paper.

### **Remote Sensing of Surface Fuel**

Remote sensing offers a wide range of sensors that can assist in fire fuel mapping. There are many limitations to this application, such as the complexity of fuel types and high spatial and temporal variability (Keane et al., 2001). The most important limitation of these sensors is their inability to penetrate forest canopies and detect surface fuels (Keane et al., 2001), especially where two or more canopies are present. The application of this technique to shrubland areas such as the Fynbos biome, which have no overstory, is potentially more viable.

The earliest applications of remote sensing used medium to low-resolution multispectral approaches to identify fire fuels, classifying an image into vegetation categories and then assigning fuel characteristics to each category. Kourtz (1977) introduced several digital techniques for Landsat fuel-type classification including supervised classification (maximum likelihood), unsupervised classification, and principal components analysis. These methods require an input of spectral signatures from specific fuel classes that are usually obtained from fieldwork.

In research that applied remote sensing without fieldwork, several researchers attempted to map fuel types using multispectral sensors such as Landsat Multispectral Scanner (Landsat MSS) or Thematic Mapper (TM) (Salas and Chuvieco, 1995; Castro and Chuvieco, 1998; van Wagtenonk and Root, 2003). Fuel types have also been mapped by applying maximum likelihood decision rules to Landsat MSS and SPOT data (Chuvieco and Congalton, 1989; Chuvieco and Salas, 1996; Castro and Chuvieco, 1998) with accuracies ranging from 65% to 80% (Chuvieco et al., 1999). Studies have explored the use of tasseled cap transformation of Landsat TM multispectral data. These studies include Van Wagtenonk and Root's (2003) use of an unsupervised classification of NDVI, combined with graphical, visual and statistical techniques to identify 30 fuel classes. The accuracy reported by these authors was 65% and with the combined use of ancillary data (NDVI, slope, texture, illumination) accuracy improved up to 85.9% (Riano et al., 2002; Francesetti et al., 2006). Other studies utilized National Oceanographic and Atmospheric Administration-Advanced Very High Resolution Radiometer (NOAA-AVHRR) images (McGinnis and Tarpley, 1985). AVHRR imagery

is popular for fire monitoring; however, its coarse resolution limits the use to regional and global scales (Dennison et al., 2005; Chuvieco and Congalton 1989; Chuvieco et al., 2004).

## Methods

This study employed a minor modification to the fire hazard mapping method introduced by Chuvieco and Congalton (1989). In contrast to the original method, this study uses NDVI and NDMI values calculated from Landsat 8 imagery collected before the fire events. The fire occurred on March 1 and cloud-free images collected closest to that date occurred on February 23, 2015 (pre-burn) and March 11, 2015 (post-burn). The two images were clipped to the area extent (Figure 1, below).

### *Vegetation Mapping*

The pre-burn imagery was analyzed using the Normalized Difference Vegetation Index (NDVI) and Normalized Difference Moisture Index (NDMI). For Landsat 8, the equations are as follows:

$$\text{Equation 1} \quad NDVI = \frac{(NIR-R)}{(NIR+R)}$$

where NIR and R are the Landsat 8 Near-Infrared (5) and Red (4) bands, respectively; and

$$\text{Equation 2} \quad NDMI = \frac{(NIR-SWIR)}{(NIR+SWIR)}$$

where NIR and IR are the Landsat 8 Near-Infrared (5) and Shortwave Infrared (6) bands, respectively.

NDVI values vary according to radiation absorption by the chlorophyll in the red spectral area and its reflectance in the near infrared spectrum (Dragomir and Petrosani 2012). The index values correspond to the consistency of the green vegetation and are useful for mapping vegetation health. This study assumed low NDVI values are usually associated with unhealthy and drier plants, while high values indicate good health.

NDMI values vary according to radiation absorption of the short-wave infrared band and reflectance of the near infrared. The index evaluates the different content of humidity from the landscape (soils, rocks and vegetation) and is an excellent indicator of dryness (Dragomir and Petrosani 2012). Vegetation moisture condition is important because it influence the flammability of the fuel.

### *Topographic Data*

DEM. Elevation influences vegetation structure, fuel moisture, and air humidity (Castro and Chuvieco 1998). Two digital elevation model (DEM) files were obtained from the Shuttle Radar Topography Mission (SRTM) and mosaicked to form a single DEM of the Cape Town area. The resulting DEM was reclassified into three classes and assigned a

hazard level of low, medium or high. Elevations between -17m and 100m were assigned a low hazard value. These areas are dominantly composed of highly urban flatland. The urban areas have limited Fynbos vegetation and therefore were considered a lower risk. Elevations between 100m - 200m were considered a medium hazard. The elevation of 200m is roughly the average elevation of the street that demarcates the bottom of the mountain in the Silvermine Nature Reserve and serves as a boundary between the natural area and the city of Cape Town below. This boundary serves as a firebreak between the reserve and the city below. The Fynbos vegetation primarily occurs at elevations above 200m and constituted the high hazard zone. Lightning is the natural ignition source of the fires and strikes at high elevations.

*Aspect.* Aspect is correlated with the amount of insolation an area receives. Aspects that experience higher insolation are assumed to have drier vegetation than aspects that are usually shaded. In the Southern hemisphere, northern facing aspects experience more insolation and are therefore more likely to have higher temperatures and drier vegetation. Western facing slopes also receive more insolation than eastern facing slopes because the sun's rays are strongest during the afternoon. Because of that relationship, fuels on north-facing and west-facing slopes present a higher fire hazard. An aspect map was derived from the DEM and then reclassified based on approximate insolation and then hazard risk. The classes included three sectors: west - north (high), northeast - southeast (medium) and south - southwest (low).

*Slope.* Slope is a variable that influences the spread rate of the fire: fire moves more quickly upslope and less quickly downslope. Steep slopes over 40% are reported as a crucial threshold for fire operations and increase the rate of fire spread (Brass et al., 1983). The classes for slope values were created based on natural breaks in the histogram to reflect the topography. The classes included 0 - 13% (low), 13 - 38% (medium) and 38 - 65% (high).

#### *Fire Hazard Modeling - Structural Fire Index (SFI)*

The Structural Fire Index (SFI) was used to delineate fire risk. The SFI is an empirical weighted index based on the combination of five variables influencing fire risk in Mediterranean and semi-arid climates: vegetation moisture, slope, aspect, elevation, and distance from roads (Chuvieco and Congalton 1989). The variables in the index are the basic factors that affect forest fires in Mediterranean areas (Chuvieco and Congalton 1989). Vegetation is the most influential factor, with a weight of 100, whereas elevation is the least influential with a weight of 2. This index has previously been employed in Spain (Chuvieco and Congalton 1989), Portugal (Pelizzari et al., 2008) and Iran (Adab et al., 2013) to map forest fire risk.

All topographic files were converted to raster and reclassified with integer values corresponding to fire hazard rank. NDMI and NDVI values were multiplied by 1000 to reflect the decimal values. Variables are ranked from highest to lowest influence on fire hazard, respectively: vegetation, slope, aspect, and elevation. The following formulas respectively represent the original SFI of Chuvieco and Congalton (1989) and the modified SFI used here:

$$\text{Equation 3} \quad SFI = 1 + 100v + 30s + 10a + 5r + 2e$$

Where  $v, s, a, r, e$  = vegetation, slope, aspect, distance from roads, and elevation, respectively.

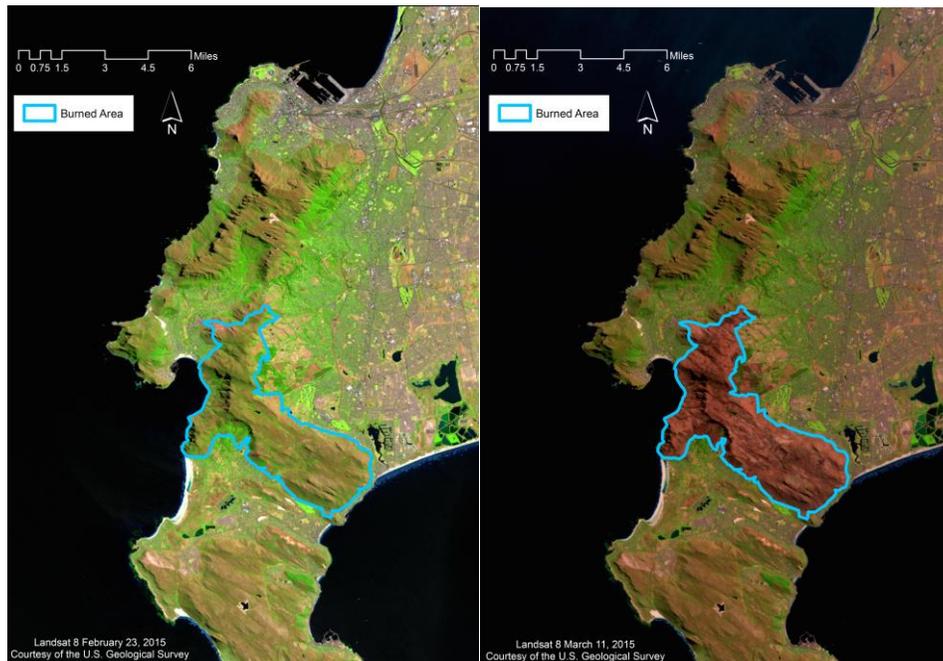
$$\text{Equation 4} \quad \text{Modified SFI} = 1000v + 30s + 10a + 2e$$

Where  $v, s, a, e$  = vegetation index (NDVI or NDMI), slope, aspect, and elevation, respectively. Distance to roads was dropped because there were no road data available in the study area.

The resulting index was used to provide a visual depiction of areas with predicted high fire hazard. The final values were displayed as a continuous raster rather than divided into hazard classes to allow better visual emphasis on the differences.

## Results

The following figures present the processed data layers that were combined to form the hazard model; i.e., the modified SFI. Figure 1 shows Landsat 8 images of Table Mountain National Park and adjacent Cape Town, South Africa.



*Figure 1:* Landsat 8 pre-burn imagery (February 23 2015) and post-burn imagery (March 11 2015). Courtesy of the U.S. Geological Survey.

The mountainous area outlined in blue is Silvermine Nature Reserve, an area within the larger Table Mountain National Park. The images highlight the difference

between vegetation in Silvermine before (left image) and after the fire (right image), with the brown area in the post-burn image showing the extent of the burnt area. The fire burned approximately 17,000 acres of the reserve.

Slope (Figure 2) was not found to be a strong indicator of fire hazard. The total burned area of high-risk slopes was minimal. Although there was correspondence between medium to high-risk slopes and burned areas, there was also a large portion of low-risk slope that burned. However, it should be considered that slope is an indicator of high risk for fire spread and not necessarily an indicator of highly burnable material.

The elevation layer (Figure 2) was divided to discriminate between the city of Cape Town and the mountains in Table Mountain National Park, with a major road (around 200m) serving as the break point. In the natural state, Fynbos would burn at lower elevations as well as higher elevations, but the city has been built over the natural vegetation. The higher elevations were all predicted to be high-risk areas as this is where lightning, a primary source of Fynbos fires, is most likely to strike.

The aspect layer introduced the most noise into the equation. Although there was correspondence between the predicted medium-risk to high-risk areas (Figure 2), there was also a large portion of the city predicted to burn due to the aspect layer being weighed so significantly in the SFI formula. While aspect does provide insight into which areas may have drier vegetation, it does not seem to be extremely important in this analysis.

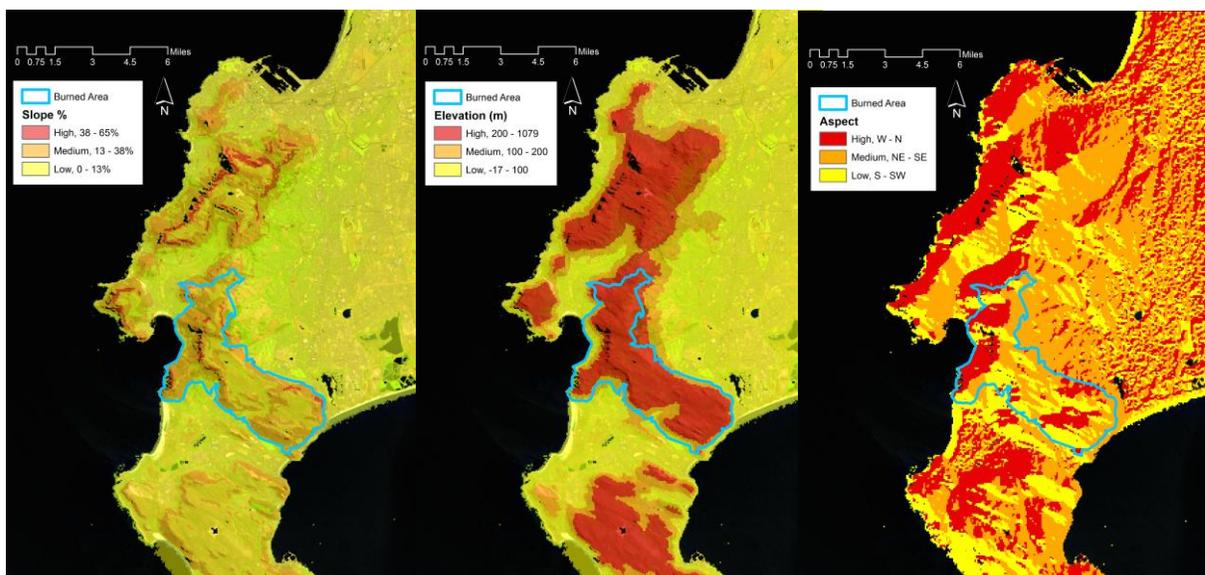


Figure 2: Slope elevation and aspect classes for fire hazard model, respectively

The first model was run with NDVI values substituting for discrete vegetation classes. By itself, the NDVI layer was a good visual indicator of greenness. However, when used in the formula, the results were too broad (Figure 3). By not presenting multiple vegetation layers, the low Fynbos shrubland allows NDVI to represent the entire range of vegetation. Nonetheless, the highly flammable shrubs do not produce high positive NDVI values and, when combined with other landscape attributes, do not appear to be clearly at risk. Ironically, many agricultural areas are identified as high risk.

The second model was run with NDMI values as a substitute for discrete vegetation classes. When compared to the NDVI model, this model displays better correspondence with the burned areas (Figure 4).

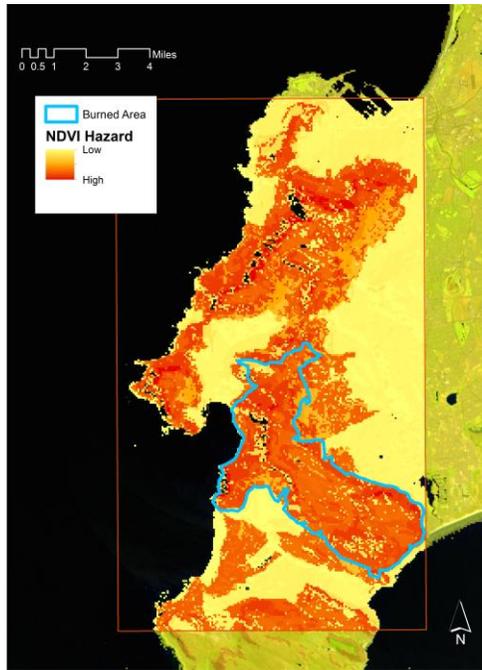


Figure 3: Fire hazard model with NDVI substitute for vegetation

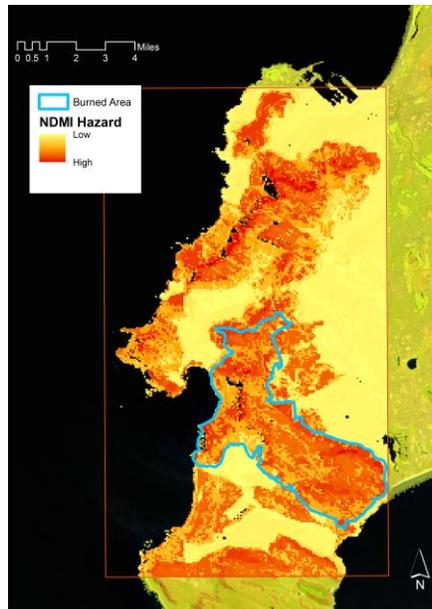


Figure 4: Fire hazard model with NDMI substitute for vegetation

## Conclusions

The knowledge of fuel characteristics is essential to fire management because it can be used to estimate fire hazard, risk, and impacts. Although fire fuel mapping has traditionally been performed through fieldwork, this is a time-consuming and expensive method. Remote sensing and GIS systems potentially offer a cost-effective alternative to fieldwork. This study investigates that possibility of combining topographic information with NDVI and NDMI to produce a structural fire index in order to identify areas most likely to burn.

The results suggest that the highest elevation areas are at greatest risk. This is most likely because higher elevations are where the Fynbos vegetation is present and is also most exposed to lightning strikes. Steep-sloped areas are also high-risk areas because of chimney effects. Low-slope areas can be at high risk, too, but only if they occur at high elevation where they are exposed to lightning strikes. Aspect does not appear to contribute to hazard risk, and NDVI does not by itself discriminate fuels adequately to be a valuable tool in this application. NDMI shows promise for future applications because it does a better job discriminating fuel loads; higher resolution imagery or better ancillary information could improve future results using NDMI. In the end, however, the results are too indefinite to be valuable for fire forecasting.

Although the findings are inconclusive, they do offer the potential for better results with improved analytical techniques and data. While the hazard index was incapable of adequately predicting risk, the coupled NDMI and NDVI did provide information on the range of Fynbos. The indices also showed where the vegetation was driest and therefore at a risk of burning. By combining that information with different topographic variables, the analysis demonstrated that areas of lesser and great risk could be mapped.

The analysis was limited by the short period analyzed, the subjective weighting system used in the formula, and the lack of a comprehensive fire database for the area. The study only compared pre- and post-burn imagery, but a long-term analysis would allow for more insight into average NDVI and NDMI values for the vegetation. Comparing long-term average NDVI and NDMI values coincident with fire events could lead to a better understanding between these indices and wildfire. The subjective weightings of the formula were also a limitation because they only reflected general values of how different factors influence fire hazard; a formula specifically developed for the Fynbos vegetation and the Cape Town context would allow a better analysis. For example, the findings reported here suggested that elevation should be weighed more heavily because those values determine occurrence of the vegetation. The final limitation was the lack of a comprehensive fire database. Detailed records including date, weather, and vegetation type and area burned would help establish a better understanding of the fire cycle in the area and relationships to the variables tested here.

It is important to note that the model only aims to identify risk of fire hazard affecting an area and not actual fire behavior, which could be affected by other real-time factors such as weather and human factors (e.g., arson or fire fighters). Ultimately, improvements of the model could allow forest fire managers and emergency responders to initiate preventative policies and actions that could limit future fire damages to humans and nature.

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# *Post 9/11 War Stories: Between Therapy and Critique*

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## **Abstract**

Veterans struggle with the significance and moral effects of war, namely reconciling fighting wars of occupation ostensibly for Iraqi freedom or 9/11 retaliation. Therapeutic approaches to war trauma often use narrative as a technique for recovery and social integration. However, the method also gives rise to moral tensions that are not necessarily psychological problems, but *moral injuries*. How is the remedy for war trauma dependent upon narrative reflection? What psychological damage occurs when action, for instance killing, is considered inadequate, wrong, or misguided? When does that remedy give rise to a philosophical discussion about moral injuries of war? Distinguishing these spheres grants access to paradoxes generated by the asymmetric demand for soldier narratives. How does the demand for stories in therapy intertwine with the American market for soldier narratives? How does re-envisioning soldier narratives through moral injury repurpose them as access points for national conversation? To approach these questions, I surveyed psychological literature on combat trauma and narrative theory that engages both morality and the challenge of self-integration including Judith Herman's *Trauma and Recovery*, Paul Eakin's *Living Autobiographically*, and Phil Klay's *Redeployment*.

## **Introduction**

The narratives US soldiers tell about their war zone experiences lend themselves to therapeutic and critical examination. These narratives shape part of the conversation surrounding investigation into the morality of the US led wars in Iraq and Afghanistan when analyzed simultaneously from therapeutic perspectives, narrative genre perspectives, and public discussions. Stemming from the therapeutic PTSD model of reliving war trauma in a safe, structured environment through narration, soldier narratives facilitate discussion about war trauma, moral complications, and dilemmas in revealing war crimes when viewed through distinct lenses on intimate and national levels.

Paradoxes abound when considering the war narratives born from US soldier participation in Iraq and Afghanistan post 9/11 through the separate lenses of post-traumatic stress disorder (PTSD), moral injury, and witnessing and/or committing war crimes. One such paradox is the displacement of occupation narratives when using the moral injury or PTSD models as the ethical framework for exploring soldier stories. The market demand for soldier

narratives in the United States remains constant, especially in the wake of its post 9/11 wars. Yet, the demand is asymmetric—prioritizing the narratives of soldiers over those of noncombatants affected by each war.

The aim of this research project was to investigate how recovery techniques involving narrative reflection posed by the PTSD model illuminated moral qualms within US soldiers. From there, I determined to resolve how the moral injury model attempts to encapsulate the moral trauma that the PTSD model misses. What are the strengths of the moral injury framework in examining soldier narratives, and what are the shortcomings in utilizing it as an ethical framework? If inadequate, what lens should be used to bolster moral injury, and/or replace it, as a means of interpreting all war narratives ethically?

### **Methodology**

To examine the relationships and questions posed by the moral injury framework I developed an interdisciplinary model. The aim of this model is to understand how soldier narratives are produced from a joint demand, therapeutic recovery and ethical examination, to assist soldiers through the PTSD healing process by telling heroic stories and how the emerging model for moral injury came to the fore. It also investigates how both models may operate as an ethical framework for interpreting war narratives. However, my research also aims to investigate any deficiencies in doing so.

My research model grounds itself in the model and recovery techniques for combat trauma posed by Judith Herman and the developing framework researchers, such as Shira Maguen and Brett Litz, have devised for moral injury. It also employs a humanistic approach proposed by Paul Ricoeur's *philosophical anthropology* and Immanuel Kant's *practical philosophy* as a means to explain differences in individual moralities and reflections upon morality. The premise is that *bios*, where a person's life is located in space and time, and *logos*, a person's ability to use reason to grasp universal concepts, create a disproportion in the human experience. This disproportion masks the *self* from the individual—making it impossible for someone's self to be fully apparent to them. This same gap distinguishes people from one another, accounting for individual personalities, and the capacity to perform good, evil, and moral reflection (Ricoeur, xvi).

How does the service member or veteran incorporate the morally injurious event into their identity without it dominating their personality? I turned to Antonio Damasio and Paul Eakin as a means to understand narration engaged in self-integration. Both were instrumental in cultivating an understanding of self-narration and the act of reconstructing memory to form a narrative identity. Both posit that this reconstruction is culturally linked, meaning that we draw on the resources of the cultures we inhabit to specify how we ought to behave in certain circumstances (Eakin 22). Gillian Whitlock likewise contributed to a holistic comprehension of how soldier narratives, particularly memoir, operate and appease the market for which they are published. She claims “when texts coincide with warfare, the uses to which they are put are called into question,” as well as “Popular fiction and nonfiction alike are caught up in the mechanisms of the marketplace, they are conscious of their readers and determined to please them” (Whitlock 94, 95). From Whitlock's perspective, soldier narratives are being used for a purpose and that readers in the American marketplace are equally responsible for the proliferation of war stories as are the authors who pen them.

With the model in place, I selected an array of soldier narratives ranging from first-hand accounts of combat (*Lone Survivor*), a third person non-fiction chronicling the struggles of soldiers attempting to reassimilate to non-combat life (*Thank You for Your Service*), a collection of short stories (*Redeployment*), well-known narratives that surfaced via the media (Abu Ghraib atrocities and Sgt. Joseph Darby), and a novel translated from Arabic to English as a counter example to the all-soldier perspective that comprises popular post 9/11 literature (*The Corpse Washer*).

### **PTSD Model for Recovery**

In her study of combat trauma and recovery techniques, *Trauma and Recovery*, Judith Herman tells us that the foundational component for recovery is reconstructing and reflecting upon the traumatic incident in full detail with the associated emotions intact. The aim of the technique is to integrate the trauma into the soldier or veteran's self—devaluing the importance of the traumatic event until the story becomes a memory like other memories (195). Paramount to this exercise is the establishment of safety. “The acutely traumatized person needs a safe refuge. Finding and securing that refuge is the immediate task of crisis intervention” (Herman 162). Without a supportive therapeutic relationship, the soldier's rendition of the trauma will prove to be a useless exercise.

Often times, in the context of US soldiers returning from wars in either Iraq or Afghanistan, or both, the memory of the traumatic incident is fragmented or distorted. The act of narrating the traumatic instance serves as a means of structuring the story linearly. In turn, the story the veteran or soldier conveys becomes the event itself. “This work of reconstruction actually transforms the traumatic memory, so that it can be integrated into the survivor's life story” (Herman 175). Reconstruction inevitably lends itself to reflection. It is precisely this continuous cycling of reconstructing memories into narrative and reflecting upon those narratives that is narrative identity. As Oliver Sacks states, “It might be said that each of us constructs and lives a ‘narrative,’ and that this narrative *is* us, our identities” (Sacks 110). This is evident in modern soldier narratives, too, as seen in Phil Klay's collection of short stories, *Redeployment*, when a narrator states, “...I'd told him that if he gave this girl his story, it wouldn't be his anymore...your story *is* you” (Klay 225).

The Diagnostic Statistical Manual V defines the trigger for post-traumatic stress disorder as “exposure to actual or threatened death, serious injury or sexual violation” (309.81). Furthermore, the manual states that the exposure must arise from witnessing the trauma, direct experience, repeated exposure, or learning that the traumatic incident happened to a close friend or family member. For soldiers, examples of such triggers may include witnessing explosions, hearing gunshots, seeing a close friend injured or killed, etc. The model encompasses soldiers who have experienced fear-induced trauma and the infringement the memory of it has on assimilating to non-combat environments. However, as the number of returning post 9/11 veterans with mental wounds increased, their stories illuminated a gap in the model. Take the following passage from David Finkel's *Thank You for Your Service* for instance:

‘And it really hit me when I saw my first baby come in burned’ is what the medic is saying. He is no longer reading, just talking, surely a step toward habituation. ‘Dipped in boiling water and skin sloughing off,’ he says. ‘And you know what? I got to the point where I started carrying extra fucking medical supplies. I got to the point where I started feeling kind of sorry for them. I started feeling sorry that we're sitting there fucking

beating these people and it's just like that fucking baby. We're just using them, like they're fucking nothing. Like they're not even human. And you get to a point...'

And now he is shaking and sobbing in an otherwise silent room until one of the other soldiers comes to his rescue. (62)

In the passage, it is arguable that the medic relating the story has a moral quandary once confronted with the suffering of a non-soldier referent. His narration takes place in a Veteran's psychiatric facility and employs Herman's model for trauma recovery. Notice, however, how the relationship between the medic and the baby is established. The medic, the soldier "I" protagonist is introduced to *his* first baby. The passage inserts the narrator into the context of war relative to the pain experienced by a non-combatant. It is the infant's suffering, not the medic's fear that is the impetus for alterations in the medic's behavior. The emotions that the medic feels, as he makes discernible through his narration, act as his reflective evaluation of the event itself. The moral tension the medic has in regard to his interaction with the baby is not encapsulated by the PTSD model because the trauma is not life threatening for the medic, but does contain moral and ethical implications. This is indicative that there are circumstances which give rise to symptoms mimicking those in PTSD, but are provoked by a non-fear based catalyst.

### **Moral Injury Framework Emerges from PTSD Model**

The technique of narrating war zone experiences has proven to be of therapeutic value for US veterans of Iraq and Afghanistan. However, the narration technique that the PTSD model employs demonstrates the ethical and moral challenges service members are confronted with in war that are not necessarily captured by the PTSD framework. As a result, research psychologists proposed that the symptoms that soldiers encounter are not always psychological conflicts, but *moral injuries*. Thus a new framework materialized from PTSD that attempts to act as the moral interjection within a soldier narrative.

The moral injury framework maintains that the service member's symptoms such as anguish, shame, etc., are indications of an intact conscience about expectations of moral conduct; the dissonance between what is experienced and a moral belief system is evidence that the moral belief system itself is still intact (Litz et al, 698). There is a gap between expectations of moral conduct and the disappointment soldiers have when those expectations are not met. A fundamental assumption within the framework is that issues of morality demand individual attention, distinct from PTSD and adjustment disorders. Researchers justify the need for the new model by noting that scant focus has been concentrated on the lasting moral conflict trauma in the clinical science community, in favor of attending to the impact of life-threatening trauma. Even so, the intervention strategies apply the same therapeutic approach as does PTSD in the realm of emotional narration of the morally injurious event. When comparing soldier narratives viewed through the PTSD framework and the moral injury framework, the most glaring difference is the necessity of a non-soldier referent present in moral injury.

For US veterans of Iraq and Afghanistan, moral injury has been defined as performing, allowing, witnessing, or learning about acts that deeply transgress moral beliefs and expectations (Litz et al, 698). What the preliminary definition discounts is a sphere of personal ethical responsibility; it engages soldier discontent but evades questions about moral injury when war atrocities are committed by soldiers *intentionally*. The definition instead opts to explore trauma soldiers endure that stems from morally ambiguous combat situations. Among the list of combat

environment events highly correlated to moral injury include killing, learning about amoral behaviors perpetrated by others, witnessing intense human suffering and cruelty, exposure to dead bodies, etc. Again, symptoms indicative of moral injury, shame, guilt, withdrawal, etc. parallel those of PTSD. The impact of soldier's actions upon non-soldier referents is the distinction.

Reconsider the passage from *Thank You for Your Service* and the relationship between the medic and the baby. Moral injury establishes its importance by representing a relationship between a combatant and a non-soldier subject, both in literature and narrative reflection. Within the text, the medic can evaluate his personal moral objections and speculation in relation to the non-combatant referent, the baby. Coincidentally, within the moral injury framework, the passage also operates as an inquiry into the morality of the wars in general for the reader. If US soldiers are proxies for American action, then it is arguable to state that American non-combatants who supported both wars may likewise feel their own moral injury. In that light, moral injury creates a space for conversation about the morality of the wars in Iraq and Afghanistan and delineates the dual purposes soldier narratives may serve.

### **Dual Purposes of Soldier Narratives**

Consider Marcus Luttrell's memoir *Lone Survivor* as an example. The book is a first-hand account, narrated by Luttrell, of a Navy SEAL combat operation in Afghanistan. The mission was a failure; all US combatants involved, save for Luttrell, were killed in action. Throughout the course of the text, Luttrell persistently works to venerate his fallen comrades while concurrently expressing his anguish over losing them. Arguably, Luttrell's relation of his story may function therapeutically, allowing him to overcome the trauma of his experience, and his trauma of survival or "survivor's guilt", via Herman's trauma recovery model.

Retrospectively, the wars in Iraq and Afghanistan may have been incited by the 9/11 atrocities, but have proven to be largely unrelated to them. The war stories centered on soldier experience may act as victories for the American public in two wars that have provided comparatively little. The soldier narratives *themselves* are the victory that would otherwise be absent, providing tangible evidence of US troops' heroism. Gillian Whitlock tells us that memoirs are conscious of their markets and determined to please their readers (Whitlock, 95). The moral injury framework allows us to interpret Luttrell's story as a tool for restoring American national patriotism which has been wounded by two wars that have arguably paid few dividends, but have come at an enormous price.

However, there is a complication in regarding soldier narratives as tales of victorious heroism and as texts by which we can analyze the moral injury framework. What moral belief system would consider stories comprised of killing and warfare as valiant? Moreover, how would that belief system impede an ethical evaluation, as opposed to a strictly moral one, of both war narratives and the wars themselves?

## Question of Religion in Moral Injury Framework and Just War Theory

While the framework proposed for moral injury does not exclusively apply religious principles, there is an undercurrent of religious commitment in military service. Analyze Article VI of the United States Military Code of Conduct:

I will never forget that I am an American, fighting for freedom, responsible for my actions, and dedicated to the principles which made my country free. I will trust in my God and in the United States of America. (*Federal Register* 53)

The Code of Conduct is not official law, but a US Department of Defense implemented ethical guide for service members should act while in combat, especially when faced with capture and ascribed Prisoner of War status. However, the article instructs the soldier to trust in their God—a suggestion that raises questions about the military, a secular institution, being guided by religious principles. Consider the following passage from Phil Klay’s collection of short stories, *Redeployment*:

‘If you killed somebody,’ he said, ‘that means you’re going to hell.’

Marines had asked me about that before, so I thought I had an answer. ‘Killing is a serious thing,’ I said, ‘no doubt about that. And...’

‘I mean’—Rodriguez looked away, down at the candy—‘somebody you’re not supposed to.’

That brought me up short. At first I didn’t understand what he was talking about, though I suppose it should have been obvious. ‘You’re not responsible for Fujita’s death...’

‘That’s not what I’m talking about,’ Rodriguez snapped, eyes back on me, angry. ‘I mean, not Marines. I mean, out in the city.’ He took a breath. ‘And, if other people did it too, when you’re out there, and you don’t stop them. Do you go to hell, too?’ (139)

Why invoke hell? Rodriguez acknowledges the non-soldier referent as ‘people you’re not supposed to kill.’ Yet, he does not appear fixated on the welfare of the implied deaths of the non-soldier subjects, but on the ramifications of not only perpetrating the killing, but facilitating it. Applying the moral injury framework to the passage demonstrates that the focal point of the model is the moral transgression felt by the protagonist without a necessity for accountability, even when confronted with alleged war crimes. What is the chaplain’s ethical responsibility after this passage?

As the short story continues, the chaplain does attempt to take action through the proper channels of the chain-of-command. He is met with stifled results from higher military authorities exemplified by the response the chaplain received from his superior, Major Eklund. “‘You think Lieutenant Colonel Fehr will ever become Colonel Fehr if he tells higher, ‘Hey, we think we did some war crimes?’” (Klay 144). If the military chain-of-command is ineffective or stymies investigations of accountability, then there is no viable infrastructure in immediate reality by which we can explore ethical accountability in this context. Perhaps, then, the invocation of hell and a religious line of thought in general serves as an ethical framework which the soldier believes is inescapable. However, the explanation may be more profound after considering the Christian ideologies deeply entrenched in rationales provided for wars in Iraq and Afghanistan.

At a Palestinian-Israeli peace summit in Egypt during 2003, former US President George W. Bush publicly stated that he was on a divine mission regarding the invasions of Iraq and Afghanistan (MacAskill, par. 1). Reportedly, Bush stated “I am driven with a mission from God. God would tell me, ‘George, go and fight these terrorists in Afghanistan.’ And I did. And then God would tell me, ‘George, go and end the tyranny in Iraq’. And I did” (MacAskill, par. 2). The irony of these statements is that they strongly resemble, if not mimic, jihadist rhetoric that likewise employs divine instruction as a means to rationalize violent intervention. The former President’s sentiments echo another long-standing tradition embedded in Christian thought: Just War Theory (JWT).

According to Robert Meagher, the foundation of JWT does not exist in religious texts i.e. the New Testament, but in the Roman Empire’s adoption of Christianity as the state’s official religion (Meagher xv). The theory allowed the Romans to reshape previously accepted theories about war and killing by differentiating ‘killing’ from murder so long as killer’s intentions are to do the will of God. It legitimizes state violence under the guise of a convenient differentiation between murder, a defiance of God’s will, and necessary killing ‘for God and country’ (Meagher xix).

Paramount to comprehension of JWT is the respect in which it reveres WWII. To Europeans and Americans, WWII is the definitive proof needed to vindicate war as a morally justified course of action. The irony is that WWII brought about an unprecedented level of military forces indiscriminately massacring civilians. “Every war is just from the perspective of those waging it, and every killer is a hero, to the side they are on” (Meagher xv). JWT constrains the space for critical examination of war, specifically here the US-led wars in Iraq and Afghanistan. If the secular governing body is commanding the US military from a religious standpoint, then there can be no moral injury done unto service members because the aims of the war are just. This is one of several observable complications in utilizing moral injury as an overarching ethical framework.

### **Difference between Morals and Ethics**

“A man is moral if he conforms to the established practices and customs of the group in which he is. He is *ethical* if he voluntarily obligates himself to live in the light of an ideal good” (Weiss 381).

Moral injury may function as the ethical impulse, albeit a structurally limited one, within soldier narratives. It functions well as a method for individual recovery in the wake of a moral trauma, but does minimal to highlight profound ethical dilemmas which percolate from the circumstances of both wars in Iraq and Afghanistan. Consider the definition the preliminary framework and intervention model for moral injury provides for morals: “the personal and shared familial, cultural, societal, and legal rules for social behavior, either tacit or explicitly stated” (Litz et al, 699). From the definition it is arguable that morality functions the individual’s personal disposition in regard to good and evil and/or that of the group which they inhabit. The dilemma is that its function is limited to the ends and well-being of the group; it is not applicable universally. If the Other, an individual, group, or institution, falls outside of that specific group’s moral definition, then they are not considered as an intrinsic part of the framework.

The atrocities exhibited within the Abu Ghraib prison are a primary example of an ethical impulse for an idealized good that does not coincide with the moral injury framework. The

soldiers commanded that the prison's detainees were stripped naked, forced to stand in isolation for hours upon end, coerced into homoerotic positions with other prisoners, etc. Amoral action was enabled within the prison by what Phillip Zimbardo calls situationalism. Using the landmark Stanford Prison Study as an example, Zimbardo asserts that it is not that the individual soldiers were inherently evil, but that the system in which they were placed facilitated abuse and amoral actions. The soldier's responsible for the prisoners' torture were not trained for prison assignment; moreover, those same soldiers were ordered by higher officials to 'soften up' the detainees prior to interrogation in order to make them more likely to produce actionable information.

The order is a collapse of the chain-of-command military structure. When the prisoners' treatment surfaced and disseminated through the media, military officials immediately decried the abuses as the handiwork of a few 'bad apples' (Zimbardo 325). However, the 'bad apples' received the order that facilitated these atrocities to be committed and that is the failure of the chain-of-command. It is a structure that is laterally intended to mitigate unlawful orders from being conscripted and/or followed, not inspire war crimes to be committed.

While the question of the chain-of-command structure and its effectiveness in preventing violations of the Geneva Conventions and/or Rules of Engagement (ROEs) is powerful, it raises a line of inquiry into a service member's personal sphere of responsibility. Consider Article IV and, again, Article VI of the US military Code of Conduct:

Article IV: If I am senior, I will take command. If not, I will obey the lawful orders of those appointed over me, and will back them up in every way.

Article VI: I will never forget that I am an American, fighting for freedom, responsible for my actions, and dedicated to the principles which made my country free. I will trust in my God and in the United States of America. (*Federal Register* 53)

Was the soldier's responsibility to follow orders or to object to war crimes? If articles IV and VI are interpreted literally, then a soldier has no obligation to an unlawful order—one that violates the Uniform Code of Military Justice, Geneva Conventions, or ROEs. Article VI also implies an obligation for personal accountability. Nonetheless, "groupthink"—a way of thinking that promotes the group's consensus with the leader's, in this case military official(s)'s position or order, disinclines individual soldiers from speaking out against the commonly accepted.

When Sergeant Joseph Darby exposed the Abu Ghraib crimes to the proper military authorities in his chain-of-command—doing so with the initial promise of anonymity—his family was threatened and shunned by their community, their property had been vandalized, and they eventually went into hiding under protective military custody (Bryan, sec. 4). Arguably, Darby was acting ethically, sacrificing his family's safety, as well as his own, to cease the unlawful torture of detainees. "To be ethical they [people] must voluntarily choose and pursue an ultimate and universal good" (Weiss 382). However, this raises questions about the moral injury framework when it confronts the predicament with witnessing war crimes. Morality, again, seems to only belong to the narrator who feels morally transgressed. Where does Darby's narrative then belong? What options are left for veterans of Iraq and Afghanistan who feel *ethically* as opposed to morally violated when their chain-of-command is impotent?

## Problems with Moral Injury framework

At a glance, the moral injury framework accounts for the moral damage done to US service members that is discounted in the fear-based model posed by PTSD. Researchers have shown that the preliminary model and intervention strategies have been well received by soldier and veteran participants (Litz, 705). The stories told by US servicemen and women have an enormous capacity to enhance our understanding of the moral ambiguities and horrors attributable to both wars in Iraq and Afghanistan. However, it is essential to realize that the moral injury framework and intervention strategies emerged from PTSD recovery methods and is therefore reliant upon narrative reconstruction solely from a soldier's perspective.

Recall the baby's suffering in *Thank You for Your Service*; think of the maltreatment of the prisoners in Abu Ghraib; remember the people Rodriguez and his compatriots were not supposed to kill in *Redeployment*. In each instance, the soldier protagonist is left to negotiate the moral trauma, but it is the non-soldier subject who endures the most acute pain and suffering. As Dr. Elliot Colla points out, in post 9/11 war narratives, as told by US soldier protagonists, "the Iraq invasion and occupation again appear as almost exclusively American events...Iraqis are largely absent from the frame...torment and pain—and humanity—belong to US soldiers rather than Iraqi civilians" (Colla, par. 3).

Consider the following passage from Sinan Antoon's novel, *The Corpse Washer*:

'I'm a taxi driver trying to make a living—I picked up this poor man—He seemed like a good and honest man. We started yapping about this miserable situation we are in and about the massacres and politics of it all—We argued a bit, but we were in agreement and were consoling each other. I had to take a leak and I asked to stop for a minute. I parked the car on the side next to the trees on al-Qanat Highway. There were choppers hovering overhead that day. Something had happened in al-Sadr City between the Americans and Mahdi Army—I'd just unzipped my pants when I heard a huge explosion—I looked back and saw that my car had become a ball of fire. I...saw an American Apache up in the air whirling—I didn't know what to do and was afraid it would fire at me too—I opened the door—I don't know how I managed to pull him out. He was in flames.' (145)

In this passage—with a non-soldier narrator protagonist—the regard in which the American soldiers are held is not that of heroic liberators, but agents of destruction. Here, torment and pain belongs to the civilians killed indiscriminately by soldierly action and not to the soldiers agonizing over their conduct.

Why are stories like Antoon's so scarce among American audiences? As Whitlock argues, "The management of testimony is almost always strategic and in the national interest" (Whitlock, 78). While rare in popular American post-9/11 literature, novels and poetry similar to Antoon's abound in Arabic. From the perspective of moral injury, the non-proliferation of occupation narratives by Iraqi and/or Afghan authors in the US market serves to prioritize the narratives of US service members over those who military occupation.

Again, soldier narratives have a large capacity to help achieve understanding of the moral ambiguities posed in the course of the two wars as well as the horrors they wrought. But by examining and circulating stories told only by U.S. soldiers, we are exploring tremendously complicated issues with a limited ethical framework that discounts occupied voices.

## Conclusion and Discussion

Moral injury is not the ethical framework by which we should conduct such investigations. While therapeutically valuable for service members and veterans struggling to reintegrate themselves into non-combat life and alleviating their symptoms, it is structured around morality intrinsic to the soldier and does not aim for an overall good. Its aim is for the veteran or service member to feel acceptable in society and in their notion of their self—to get them to reject the morally injurious act while accepting the imperfect self responsible for the act (Litz 703). It does not account for the suffering endured by hundreds of thousands of Iraqis and Afghans who have lost loved ones through the course of US occupation—either as a result from moral ambiguity or intentional depravity. It is arguable that moral injury can serve as an ethical framework. However, it is limited solely to soldier narratives and evades the problematic topic of personal accountability when either witnessing or perpetrating war crimes.

What is needed is a revamped concept, one that allows for interpretation of war crimes as such and not merely moral grievances against soldiers, of moral injury as an ethical framework, or a new ethical framework altogether. This is most clearly observable when considering Sergeant Joseph Darby and the treatment to which he was subjected after revealing war crimes. Darby arguably operated ethically, voiding the groupthink within Abu Ghraib that kept the atrocities shrouded in secrecy at the expense of his own safety and comfort. He revealed the injustices through the proper channels in the chain-of-command and was vilified regardless. What incentive is there for service members who witness war crimes to report it via chain-of-command if doing so socially castigates them anyway?

There seems to be a binary opposition between soldier responsibility and committing treason. Sgt. Darby performed his military duties to the point that they remained lawful and then continued to properly engage the appropriate military authorities when revealing the war crimes committed at Abu Ghraib. It is as though Darby had committed treason even when conforming completely to military standards. Where do the spheres for treason and soldier responsibility intersect or overlap? When does the ethical imperative become renouncing the chain-of-command in the face of egregious offenses? Finally, what responsibilities do we as readers have in incorporating all war narratives, including occupied subjugation and war atrocities, into the ethical fabric and discussion of the US led wars in Iraq and Afghanistan?

The ethical framework by which military personnel can take action when witnessing war crimes does not seem to exist. Instead, we are left with a compromised situation where illuminating war crimes, perpetrated by the United States, is treasonous for service members regardless of whether they employ the chain-of-command or not.

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# *Exploration of Hardware Acceleration for a Neuromorphic Visual Classification System*

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## Abstract

Neuromorphic visual perception algorithms have become increasingly popular as they enable a wide array of vision based applications. HMAX is an example of a neuromorphic visual feature extraction algorithm that has been shown to perform well for large scale object and scene recognition tasks. While the accuracy of HMAX is considerable, its high computational latency makes it prohibitive for many real time applications. Hardware acceleration is a widely accepted technique for mitigating the computational latency of complex algorithms and has been investigated for HMAX specifically. However, prior investigations of hardware accelerated HMAX have not produced latencies that are suitable for large-scale real-time classification. Using a holistic approach, this work proposes both algorithmic optimizations and hardware customization techniques to accelerate HMAX beyond current state-of-art implementations. Results show confirmation of a future version of HMAX with potentially improved execution time, while still performing at a reasonable accuracy.

## **Introduction**

Neuromorphic vision algorithms is a popular topic within computer vision. These algorithms mimic the way the mammalian visual cortex processes visual stimuli and have fostered a wide range of applications [1,2,3]. For example, utilizing the efficiency and robustness of neuromorphic algorithms, a visual prosthesis device can be engineered to augment the quality of life for visually impaired persons. A key task in such a system is object identification which relies on visual features to distinguish object classes. HMAX is one such neuromorphic algorithm that extracts visual features from an image for use in classification. HMAX is able to achieve considerable accuracy; however this accuracy comes at a high computational cost [4].

## HMAX Model

HMAX is a four stage feature extraction model derived as an extension of the algorithm developed by Mutch & Lowe [5]. The model uses a combination of template matching, and maximum response collection to extract the edge-based features that are present within an image.

### *S0 Stage*

This first stage acts as a preprocessing stage that creates a multiscale image pyramid from the original input image. This process is necessary to enable scale invariant feature extraction for objects that may appear at arbitrary sizes. In this work we extract features from twelve image scales.

### *S1 Stage*

The S1 stage then takes all twelve scales and convolves it with an 11x11 Gabor filter to detect edges present within the image. The Gabor filter is described by Eq (1) where  $X = x\cos(\theta) + y\sin(\theta)$  and  $Y = -x\cos(\theta) + y\sin(\theta)$ ,  $x$  and  $y$  varies between -5 and 5,  $\theta$  varies between 0 and  $\pi$ , and the wavelength ( $\lambda$ ), width ( $\sigma$ ), and aspect ratio ( $\gamma$ ) are 5.6, 4.6, and 0.3, respectively.

$$G(x, y) = -\exp\left(\frac{(X^2 + \gamma^2 Y^2)}{2\sigma^2}\right) x \cos\left(\frac{2\pi}{\lambda} X\right) \quad (1)$$

### *C1 stage*

The C1 stage utilizes a 10x10x2 3D max filter to find maximum responses from the Gabor convolution for each orientation across two different scales.

### *S2 stage*

This stage of the HMAX model then takes a dictionary of prototypes and correlates every applicable prototype to the output of the C1 stage. Eq (2) describes this stage. The numerator of Eq (2) details the correlation of each C1 output,  $X$ , against a prototype,  $P$ , and, the accumulation of those responses across orientations. The denominator denotes the calculation of the normalization patch, where  $x_i$  is a single C1 output ( $n=\{4,8,12,16\}$ ;  $m=12$ ). After the accumulation across orientations is finished pixel wise division is done with the normalization patch.

$$R(X, P) = \frac{X \cdot P}{\sqrt{\sum x_i^2 - \frac{(\sum x_i)^2}{n^2 * m}}} \quad (2)$$

### *C2 stage*

The C2 stage does a global pooling of all the S2 stage output data, by removing all spatial information and recording maximum responses across two sets of scales. Once these four stages have been completed the information is then passed to a classifier to detect the object within the image.

## The Computational Cost of HMAX

The main bottleneck of the HMAX algorithm is in the S2 stage where the output from the C1 stage is correlated with the template dictionary [4]. The dictionary contains over five thousand prototypes which must be spatially correlated with each C1 output. Spatial correlation can be a costly process for a large number of inputs, as spatial correlation requires numerous multiplications and accumulations. But correlation in the Fourier Domain costs less, as those multiplications and accumulations now become single point-wise multiplications [6]. This project demonstrates using frequency correlation in the S2 stage to reduce execution time while still maintaining the baseline accuracy. In addition, this project also explores adding the spatial information originally removed in C2 stage, as adding spatial information along with a feature vector has shown to improve classification accuracy in other areas [7].

## Methodology

The HMAX algorithm, written in C++ by Jim Mutch, served as the foundation of the experimentation. The project consisted of seven experiments total. The purpose of the first five experiments was to confirm our hypothesis of doing correlation in the Fourier domain, while still maintaining the baseline accuracy. The sixth and seventh experiment were used to evaluate the accuracy from adding spatial information to the final C2 vector.

### Experiment 1: Fourier-A

The objective of this experiment was to perform a Fourier transform of the C1 output to do frequency correlation in the S2 stage, but then return back to the spatial domain to normalize the data as in the original algorithm. This information was then sent to the C2 stage for the global pooling.

### Experiments 2-4: Fourier-B, Fourier-C, Fourier-D, & Fourier-E

These experiments again used frequency correlation in the S2 stage, but remained in the Fourier domain for the C2 stage. The data was normalized beforehand using an approximated normalization patch. This was done to test whether we could still achieve a reasonable accuracy without the calculated normalization patch. Four experiments were done to find the best value, which represents the maximum response from pooling in the Fourier domain. In *Fourier-B*, the final C2 vector was composed of the average power of each max Fourier coefficient [8]. *Fourier-C*'s final C2 vector was composed of the peak magnitude. *Fourier-D* and *Fourier-E*'s maximum response was composed of the max real and max imaginary value respectively.

### Experiments 6-7: Spatial A and Spatial B

These two experiments were conducted to evaluate adding spatial information to the C2 output to improve classification accuracy. This was done in two ways using time-domain correlation. In experiment *Spatial A*, a linear combination of the (x,y) coordinate and the scale size was given along with the max response. In experiment *Spatial B*, the (x,y) coordinate pair was given along with the max response to evaluate the accuracy. The linear combination technique was done to minimize the amount of information given to the classifier, while still adding spatial info for better accuracy.

Ten classes of images from a dataset of grocery images were used for evaluation, with approximately 1200 images used for training, and 300 images used for testing. For each experiment the accuracy was recorded for comparison against the baseline HMAX implementation.

Three different classifiers were used for evaluation of experiments six and seven. This was done to gather data on the type of classifier which would yield the highest accuracy. The RLS and the SVM-linear classifier was used to evaluate the accuracy from using a linear classifier. The SVM-RBF was used to evaluate the accuracy from using a non-linear classifier. Since the purpose of the first five experiments was to confirm our hypothesis of doing the template correlation in the Fourier domain, classification was only done with the RLS classifier. After finishing the experiments, the next phase of the project was to model a hardware implementation with the information gathered from Experiments 1-5. This was done to observe a reasonable decrease in execution time from using frequency correlation in the S2 stage.

### Results

Table 1 shows the results from the experiments *Fourier-A*, *Fourier-B*, *Fourier-C*, *Fourier-D*, and *Fourier-E*. The table shows the accuracies recorded from each individual experiment conducted using the RLS classifier. *Baseline* refers to the baseline HMAX accuracy for the ten classes of images.

Experiment	RLS Classifier
Baseline	96.67%
Fourier-A	96.06%
Fourier-B	69.58%
Fourier-C	72.68%
Fourier-D	75.49%
Fourier-E	79.44%

Table 1. From the results it shows that we can achieve comparable accuracy to the baseline Accuracy using the architecture of Fourier-A

Table 2 shows the results from the experiments *Spatial-A* and *Spatial-B*. The table shows the accuracies recorded from each individual experiment conducted using a specific classifier. *No Spatial Data* refers to the baseline HMAX accuracy for the ten classes of images.

### Discussion

With the results from experiments one through five, it can be seen that we can perform the S2 stage of HMAX in the Fourier domain, while still achieving considerable accuracy by doing the C2 stage in the time-domain. However, the low accuracies in experiments two through five may be attributed to the approximated normalization values used for these experiments. More favorable results may have been achieved if we had instead used the same normalization patch as in experiment *Fourier-A*. As for adding spatial info to the final C2 vector, in all cases, doing so decreased the accuracy considerably. This was most likely due to not enough variance between the data for any classifier to adequately make distinctions between different images.

Classifier	No Spatial	Spatial-A	Spatial-B
RLS	96.67%	88%	87.83%
SVM-Linear	81.16%	75.36%	73.62%
SVM-RBF	82.03%	74.49%	61.16%

Table 2. Adding spatial info to the final C2 vector in all cases hindered the classification accuracy.

### Theoretical Architecture

A high-level architecture of the modified HMAX model was developed using the data gathered from Experiments 1-5. Equation 3 and Table 3 describe a model for the baseline HMAX architecture, as well as the modified architecture to conduct correlation in the Fourier-Domain.  $S2(Baseline)$  denotes the S2 stage with the baseline HMAX architecture and  $S2(Fourier-A)$  denotes the S2 stage with the modified Fourier HMAX architecture. The purpose of this model was primarily to observe the speed in the S2 stage from doing correlation in the Fourier domain. Therefore, the cost of going into and out of the Fourier domain was not included.

$$StageLatency = \frac{(\#OutputPixels * LatencyPerPixel + FSizeLatency) * (ZSize)}{ClockFrequency} \quad (3)$$

Stage	#OutputPixels	LatencyPerPixel (cycles)	FSizeLatency (cycles)	ZSize
S1	60516	241	0	12
C1	2209	99	0	12
S2(Baseline)	1936	511	21296	5120
S2(Fourier-A)	2209	1	24299	5120
C2(Baseline)	5120	11615	0	5120
C2(Fourier-A)	5120	13253	0	5120

Table 3. Listing of latency and output values for both the baseline and the modified architecture

Figures 1 and 2 detail a block diagram of architecture from using the modifications from Experiment *Fourier-A*. Theoretical execution times were drawn from both models using Eq (3) and Table 3 at a 100 MHz clock frequency,

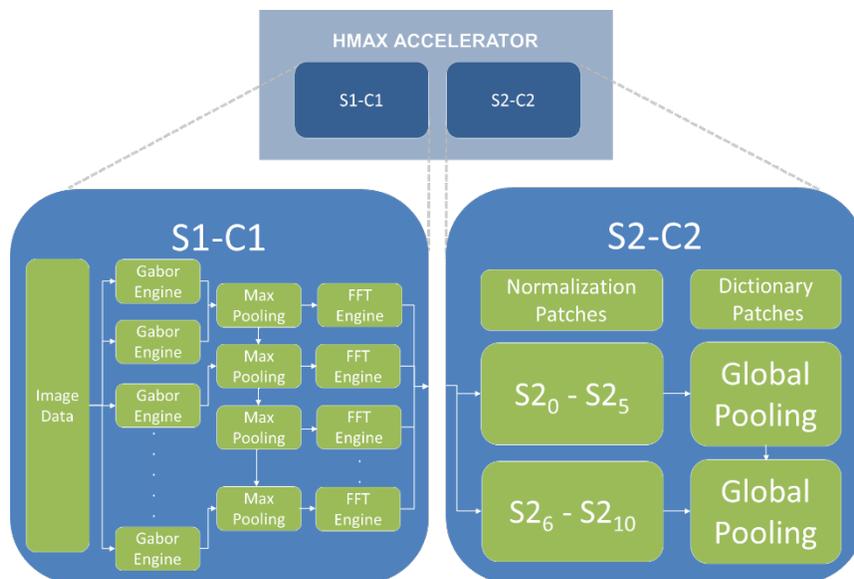


Figure 1. Depicts an overview of the S1-C1 stage and the S2-C2 stages. The S1-C1 stage consists of multiple Gabor engines each with their own max pooling unit

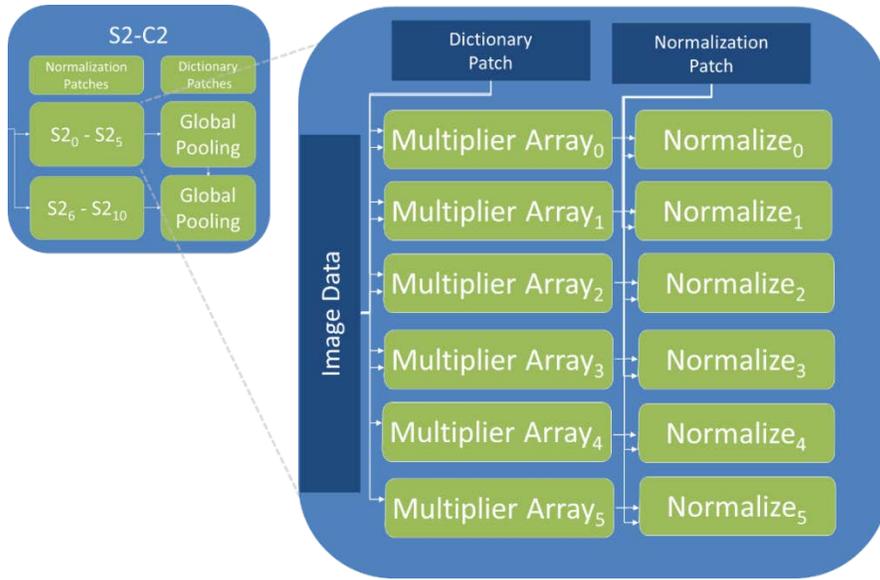


Figure 2. Depicts the S2 stage architecture, with several multiplier arrays

Memory resources were also recorded for both models. As can be noted from figures 3 and 4, Model *Fourier-A* has a 50x speedup versus the *Baseline* model, but on the other hand uses far more memory. This increase in memory consumption is due to extra padding needed for frequency correlation at various scales [6]. An alternative to this approach would be to convert the prototypes to the Fourier domain online, but this would require more DSP resources.

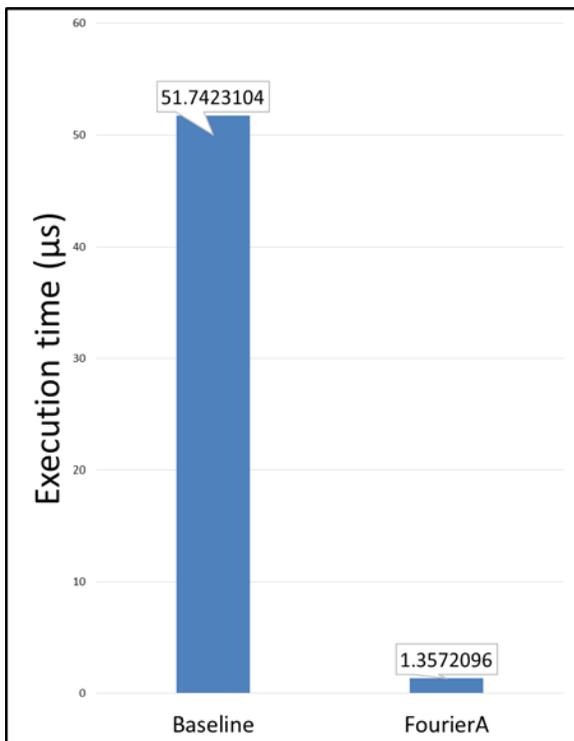


Figure 3. Graph shows a 50x Speedup from using frequency correlation

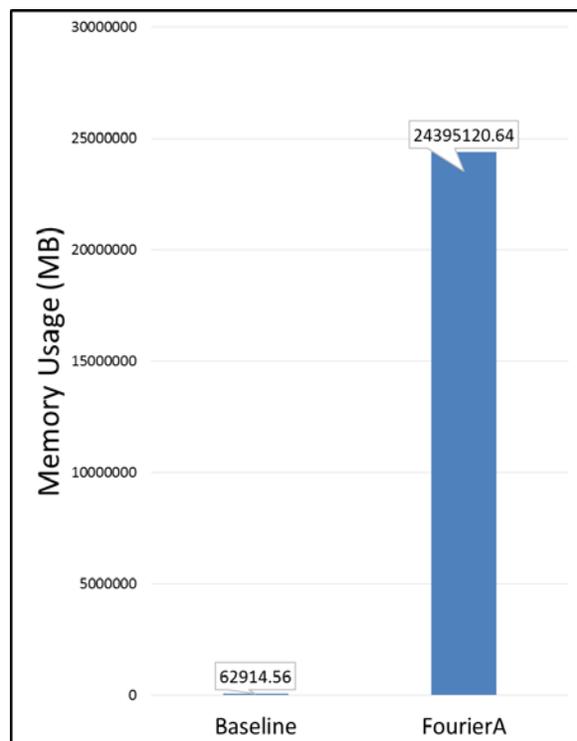


Figure 4. Graph shows a 387x increase in memory resources consumed

## Future Work

The S2 stage currently uses a general dictionary composed of templates collected using a variety of photographs from the natural world. Using this dictionary, HMAX can perform at a significant accuracy, but this accuracy was only for ten classes of images. As can be seen in previous work [9], HMAX has been known to top out in accuracy at around twenty classes of images. This is due to the edge features extracted from HMAX not containing enough variance for more than twenty classes of images. This lack of variance then hinders any classifier from being able to yield a decent accuracy. In order to compensate for this, future work will look at using a more customized dictionary in the S2 stage. Future work should encompass using a more specialized dictionary, whereby the patches are instead extracted from the dataset being tested on.

In addition to a customized dictionary, future work will also investigate the effects of zero padding the image before doing a Fourier transform. Because of the amount of zero padding that needs to be done at different scales, the size of pre-converted patch coefficients is rather large. This extra padding was done to avoid wrap around error when converting to the Fourier domain [10]. However, the error produced from neglecting to zero pad the image may be negligible and may still yield satisfactory results. If the findings are true, then the amount of storage needed for the template coefficients will be cut down to one-fourth the original size.

The theoretical architecture developed does not account for the cost of going into and out of the Fourier domain. At the front end, the cost to convert the C1 data to the Fourier domain is negligible. However at the backend, that 50x speed up would be lost from having to convert all the data from the S2 stage back to the spatial domain. This is motivation for looking at schemes to extract viable features from the Fourier domain, making the large number of inverse Fourier transforms unnecessary.

## Conclusion

In this paper, we describe various methods to increase both the accuracy and the execution time of the state-of-art HMAX feature extractor. We developed several experiments in software to test our theories of improving accuracy using spatial info, and conducting correlation in the Fourier domain. Results show that adding spatial information to the final C2 vector hinders the classification accuracy. However, HMAX can be accelerated using the Fourier domain for correlation while still maintaining the baseline accuracy.

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# *Quantitative Analysis of Cortical and Trabecular Bone in Three Human Populations*

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## **ABSTRACT**

Bone mass is known to vary as a result of age, sex, behavior, and diet; however, little is known about the differences between populations. This study's objective is to analyze cortical and trabecular bone structure in the proximal femur and to examine differences related to ethnicity and behavior. MicroCT scans were used to collect data from two Native American populations, the forager Black Earth and the agricultural Norris Farms, and an African agricultural population, the Kerma. Femoral neck cortical bone and 3D femoral head trabecular bone were examined quantitatively. Results show that the foragers have significantly more robust cortical and trabecular bone than the agriculturalists. These data support previous claims that a more sedentary lifestyle increases cortical and trabecular bone fragility, suggesting that susceptibility to osteoporosis is more prevalent now than it was then. Future research could be done to better understand the factors contributing to the variation across populations.

## INTRODUCTION

Osteoporosis causes brittle and fragile bone resulting from loss of tissue due to hormonal changes, or deficiency of calcium or vitamin D. According to the American Academy of Orthopaedic Surgeons, “Osteoporosis is a global public health problem currently affecting more than 200 million people worldwide. In the United States alone, 10 million people have osteoporosis, and 18 million more are at risk of developing the disease. Another 34 million Americans are at risk of osteopenia, or low bone mass, which can lead to fractures and other complications.” According to the National Osteoporosis Foundation, approximately one in two women and up to one in four men, age 50 and older, will break a bone due to osteoporosis. The most common sites of osteoporotic fracture are the distal forearm (wrist), vertebrae (spine), and proximal femur (hip). Hip fractures are strongly related to low bone mineral density (BMD), and therefore, have been used internationally to gauge osteoporosis (Cummings and Melton, 2002). The amount of bone mass a person has, along with bone structure, varies between individuals and populations due to ethnicity, sex, age, diet, or even behavior (Pollitzer and Anderson, 1989). It is important to understand how different factors affect bone composition so that we can more effectively treat osteoporosis and other bone related diseases.

Bone composition is determined by analyzing two types of bone tissue found in whole bones - cortical and trabecular bone. Cortical bone is dense and compact. It forms the outer layer of the bone. Trabecular bone, also known as spongy bone, is a complex network of rods and plates, and is present in joint regions, short bones, and flat bones. Variation in bone structure and bone mass determines bone strength. The amount of bone mass directly correlates with the strength of bone - more bone generally means stronger bones. Cortical thickness and the width of the femoral neck are two important factors of bone strength in the proximal femur and hip joint. Although there does not appear to be significant variation in cortical thickness between sexes, men tend to have wider femoral necks than women (Duan et al., 2003). Even though a wider bone may inhibit bending, the femoral neck is still at risk for fragility if trabecular and cortical bone is thin, especially at older ages. It has been shown that cortical bone in the femoral neck is thicker inferiorly (Ohman et al., 1997). Bone volume fraction (BV/TV) and degree of anisotropy (DA) are two variables of trabecular bone that are important to bone strength (Ryan and Walker, 2010; Hodgskinson and Currey, 1990a, b; Turner et al., 1990). Essentially, the amount of trabecular bone present and its distribution in space are great determinants of how strong the bone will be.

Past work has demonstrated that bone structure varies in populations with different behaviors. The shift from a hunter-gatherer lifestyle to an agricultural lifestyle has negatively impacted bone health because of a decrease in a nutritional diet and an increase in sedentism (Larsen, 1995). Becoming more sedentary causes a decrease in biomechanical loading, which causes more gracile bones. Studies have shown that people who are more active tend to have more robust cortical bone (Larsen, 1995; Ruff, 2005) and trabecular bone (Ryan and Shaw, 2014). With the shift to agriculture came a significant change in mobility patterns that had profound effects on bone mass and bone health (Bridges, 1989).

Clinical studies suggest bone also varies due to ethnicity or ancestry. It has been shown that people of African descent have greater bone mass than Caucasians (Pollitzer and Anderson, 1989; Wang et al., 1997; Ortiz et al., 1992). People of Hispanic origin are more similar to Caucasians with Asians having lower bone mass than Caucasians (Pollitzer and Anderson, 1989; Barrett-Connor et al., 2005; Cundy et al., 1995). While these groups are unnecessarily broad and

tend to follow traditional conceptions of racial categories, it is not clear how much variation in bone structure exists between groups or what is driving these differences. Few studies have examined the variation in cortical and trabecular bone in diverse human groups to determine the relative importance of behavioral versus genetic/ancestral differences.

The goal of this study is to bring an anthropological perspective to these questions of bone structural variation by analyzing cortical and trabecular bone structure in the proximal femur, and examining differences related to ethnicity and behavior across multiple recent human populations. Specifically, this study attempts to assess how increases in sedentary lifestyles affect bone health and the effects of ancestry on bone structure. This study uses three different populations: Native American hunter-gatherer, Native American agriculturalist and African agriculturalist. I predict that the hunter-gatherers will have more robust femoral neck cortical bone and femoral head trabecular bone than the agriculturalists and that the African agriculturalists will have more robust bone than the Native American agriculturalist group.

## MATERIALS AND METHODS

### Skeletal Sample

The skeletal sample used in this study consisted of three populations with different behavioral patterns and ancestry. The Kerma individuals came from the Kingdom of Kerma located in Egypt and Sudan. The individuals date to about 3600 B.P and come from the Middle Kingdom period (Buzon, 2006). These individuals were primarily agriculturalists. The Norris Farms #36 site is a cemetery site from the central Illinois River valley from about 700 yrs. B.P. This site is linked to more sedentary agriculturalists of the Oneota cultural tradition (Ryan and Shaw, 2014). The Black Earth site dates to about 5000 yrs. B.P. and the individuals were highly mobile foragers (Ryan and Shaw, 2014). Details of the sample are listed in Table 1. Only adult individuals with no signs of pathologies were used in this study

**Table 1. Skeletal sample used in this study**

Population	<u>Cortical Bone</u>			<u>Trabecular Bone</u>			Unidentified
	N=	Males	Females	N=	Males	Females	
Kerma	4	4	0	20	11	7	2
Black Earth	10	5	5	20	10	10	0
Norris Farms	10	5	5	19	10	9	0
Total	24	14	10	60	32	26	2

### MicroCT Scan Data Collections

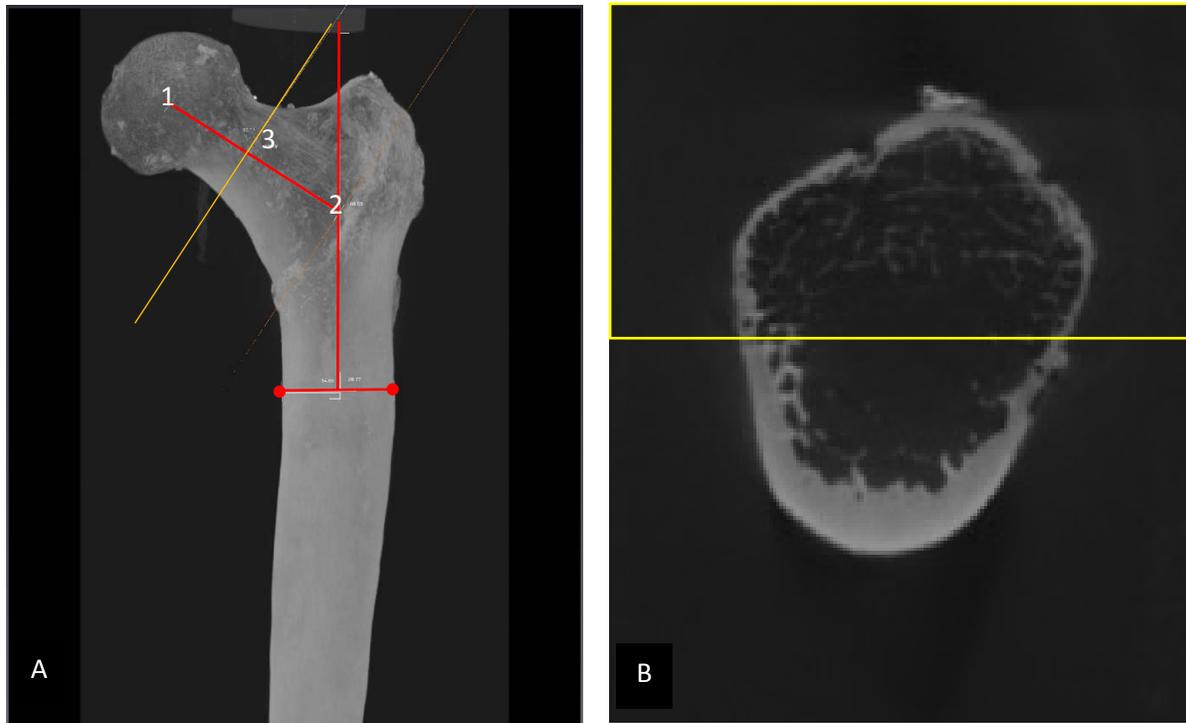
One femur from each individual was microCT scanned in order to quantify internal bone structure non-destructively. The Kerma individuals were scanned at the Cambridge Biotomography Centre at the University of Cambridge using a Nikon Metrology XT H 225 ST High Resolution CT Scanner. The Norris Farms and the Black Earth individuals were scanned at the Center for Quantitative Imaging (CQI) at Pennsylvania State University using the OMNI-X HD-600 industrial  $\mu$ CT system. High resolution scans of the proximal femur and low-resolution

scans of the entire femur were used in this study. The voxel sizes ranged from 0.0378 to 0.058 for trabecular bone and 0.110 to 0.125 for cortical bone.

## Quantitative Analysis of Bone Structure

### *Cortical Bone*

A multi-step process was used to define the femoral neck cross-section for this analysis. First, a maximum intensity projection of the proximal end of the femur, viewed anteriorly, was created. The femoral shaft axis was defined as a vertical line, viewed on the anterior portion of the bone, which extended from the midpoint of the shaft diameter to the superior portion of the bone (Fig. 1). Cortical bone thickness of the femoral neck was quantified on a single two dimensional slice positioned perpendicular to the long axis of the femoral neck. This slice was located at the midpoint of the femoral neck, which was defined as the distance between the center of the femoral head and the femoral shaft axis line. The center of the femoral head was defined as the intersection of the two lines at the midpoints of the mediolateral and superoinferior extents of the head. The midshaft slice produced contained trabecular bone and cortical bone (Fig. 1). A cortical bone separation algorithm used a three-step method to isolate the cortical bone from the trabecular bone. The Avizo 8.01 software was used to identify the slices needed for analysis of the cortical bone in the femoral neck.



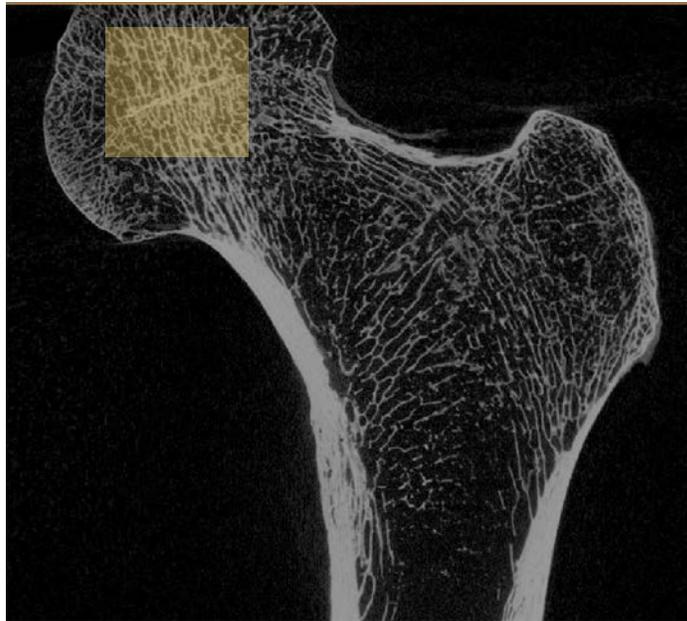
**Fig. 1.** A: Midshaft slice selection method. FNL was defined as the distance between the center of the femoral head (1) and a point on the femoral shaft axis line (2). The midpoint between (1) and (2) was calculated and labeled the midshaft slice (3). B: The midshaft slice was split into a superior half and an inferior half based on superior-most and inferior-most “y” dimensions.

Each femoral neck cross-section was split in half along the midline in the superoinferior axis, producing two new datasets representing the superior and inferior halves of the femoral neck (Fig. 1). Each image was segmented using the iterative algorithm in ImageJ that determined

what was bone and what wasn't bone. The mean and maximum cortical bone thickness was calculated for the superior and inferior half of each cross-section using a model-independent method implemented in BoneJ within ImageJ.

### ***Trabecular Bone***

Trabecular bone structure was quantified in three dimensions in cubic volume of interest (VOI) extracted from the center of each femoral head (Fig. 2). The femoral head was isolated for each specimen by using a bounding box; this defined the region of interest (ROI). The size of each VOI was equal to 50% of the ROI to ensure that variability in femoral head size was accounted for. Further explanation on the method used to define VOI can be found in Ryan and Shaw (2012), Ryan and Walker (2010), and Ketcham and Ryan (2004). Seven morphometric variables were quantified using the BoneJ plugin in ImageJ: BV/TV, Tb.Th, Tb.Sp, Conn.D, DA, BS/BV, and SMI. BV/TV is defined as the amount of bone in the VOI. Tb.Th is the thickness of the trabeculae and Tb.Sp is the amount of separation between trabeculae. Conn.D determines how well the trabeculae are interconnected. DA defines how similarly oriented the trabeculae are and SMI measures the distribution of rod – to plate – like trabeculae. Lastly, BS/BV is defined as the amount of surface area the bone occupies in the VOI (Table 2). A correction factor was used to correct the variables for different voxel sizes. These variables were chosen because they were proven to be the best determinants of trabecular bone mechanical behavior, functional behavior, elasticity of structure, and strength (Ryan and Walker, 2010; Hodgkinson and Currey, 1990a, b; Turner et al., 1990, Kabel et al., 1990b; Odgaard et al., 1997; Cowin, 1997; Mittra et al., 2005).



**Fig. 2.** The center of the femoral head was determined for each sample and a cubic volume of interest (VOI) was extracted.

### **Statistical Analyses**

Mean and maximum thickness for the superior and inferior portions of the femoral neck were compared between populations using ANOVA. A paired-t test was used to compare

superior and inferior thickness means within each population. An ANOVA test, along with a post-hoc test, was used for the statistical analyses of trabecular bone. In order to determine what comparison test to use, Levene’s test for equal variances was applied. Minitab 17.1 was used to run statistical analyses on all data.

**Table 2. Measurements of trabecular architecture**

Measurement	Abbreviation	Units	Description
bone volume fraction	BV/TV	(-)	ratio of bone volume to total volume of interest
degree of anisotropy	DA	(-)	extent to which trabeculae are similarly oriented
structure model index	SMI	(-)	measure of distribution of rod- to plate-like trabeculae
corrected trabecular thickness	Tb.Th	mm	measure of average strut thickness corrected for body size
trabecular separation	Tb.Sp	mm	measure of average distance between struts
connectivity density	Conn.D	mm <sup>-3</sup>	relative quantity describing how well are the struts interconnected
bone surface to bone volume	BS/BV	%	the ratio of trabecular bone surface area to total trabecular bone volume in the VOI

(Fajardo et al., 2007; Ryan and Shaw, 2012)

## RESULTS

### Femoral Neck Cortical Bone Thickness

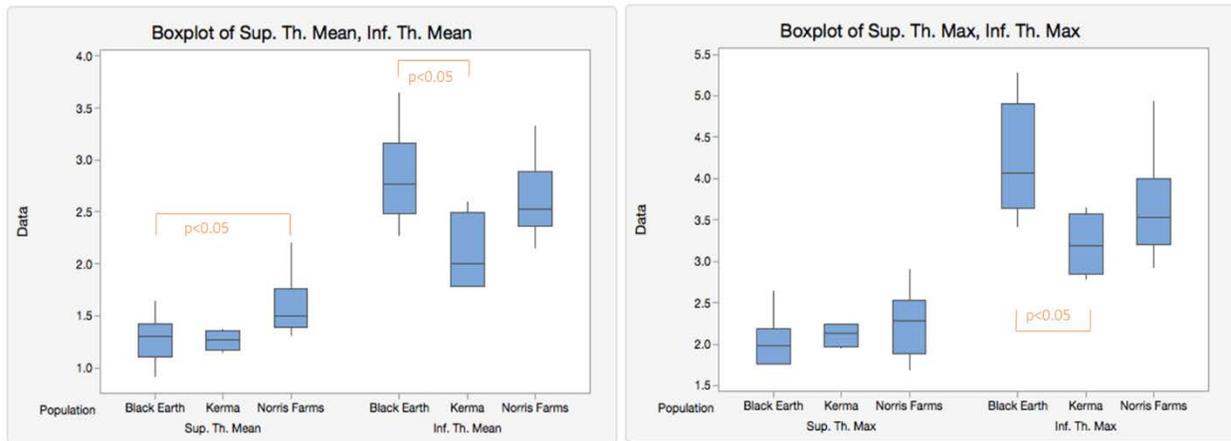
Population means and standard deviations for each measured cortical bone variable are listed in Table 4 and boxplots for each variable in each population are provided in Figure 3. A statistically significant difference was found between thickness means for the superior and inferior portions in each population. There are no statistically significant differences between populations for maximum thickness in the superior neck. The Norris Farms group has a significantly higher mean superior thickness than the Black Earth. However, the Kerma are not significantly different from Norris Farms or Black Earth in this variable. The Black Earth foragers have a significantly higher mean and maximum thickness in the inferior neck than the Kerma. The Norris Farms agriculturalists, however, are not significantly different than either the Black Earth or the Kerma in the inferior femoral neck thickness (Fig. 4).

**Table 4. Basic Statistics for Cortical Neck Thickness Analysis, Mean (St Dev)**

Population	<u>Superior</u>		<u>Inferior</u>	
	<i>Thickness Mean</i>	<i>Thickness Max</i>	<i>Thickness Mean</i>	<i>Thickness Max</i>
Black Earth	1.286 (0.234)	2.008 (0.278)	2.847 (0.468)	4.188 (0.665)
Kerma	1.266 (0.097)	2.110 (0.147)	2.092 (0.387)	3.202 (0.374)
Norris Farms	1.586 (0.268)	2.245 (0.377)	2.603 (0.352)	3.645 (0.598)

**Table 5. Basic Statistics for Trabecular Thickness Analysis, Mean (St Dev)**

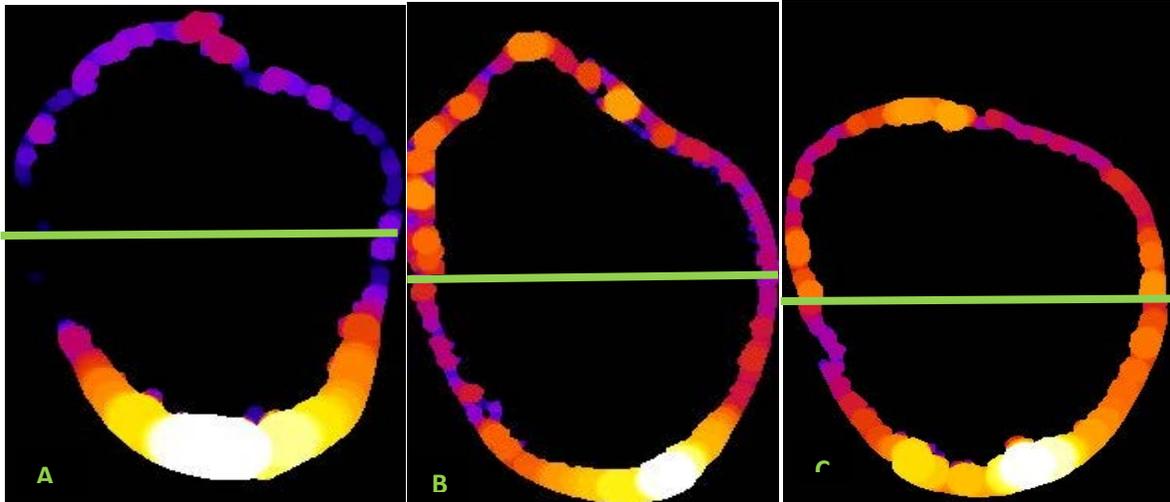
Population	BV/TV	Tb.Th	Tb.Sp	Conn.D	DA	SMI	BS/BV
Black Earth	0.465	0.438	0.696	1.959	0.627	1.130	4.670
Kerma	(0.060)	(0.074)	(0.101)	(0.505)	(0.059)	(0.761)	(0.630)
Norris Farms	0.302	0.258	0.731	5.684	0.597	2.575	7.942
	(0.045)	(0.033)	(0.091)	(2.289)	(0.067)	(0.488)	(0.670)
Norris Farms	0.387	0.337	0.712	2.619	0.702	2.350	5.465
	(0.034)	(0.057)	(0.087)	(0.696)	(0.056)	(0.415)	(0.392)



**Fig. 3.** Boxplots showing the results of morphometric analyses of cortical bone in the femoral neck for each population. Statistically significant population differences, based on the ANOVA, are marked with their respective p-values.

### Femoral Head Trabecular Bone Structure

Population mean and standard deviations for each measured trabecular bone variable are listed in Table 5 and boxplots for each variable can be found in Figure 5. There are no statistically significant differences between populations for average distance between struts (Tb.Sp) and body mass. Norris Farms has significantly higher DA than Black Earth and Kerma, while Black Earth and Kerma were not significantly different. Black Earth has significantly higher Tb.Th than both Norris Farms and Kerma. Norris Farms has significantly higher Tb.Th than the Kerma population. Kerma and Norris Farms has significantly higher SMI than Black Earth. Kerma has significantly higher Conn.D than Norris Farms and Black Earth. Norris Farms has significantly higher Conn.D than the Black Earth population. Kerma has a significantly higher BS/BV than Norris Farms and Black Earth. Norris Farms has a significantly higher BS/BV than Black Earth. Black Earth has a significantly higher BV/TV than Norris Farms and Kerma. Norris Farms has a significantly higher BV/TV than the Kerma population.

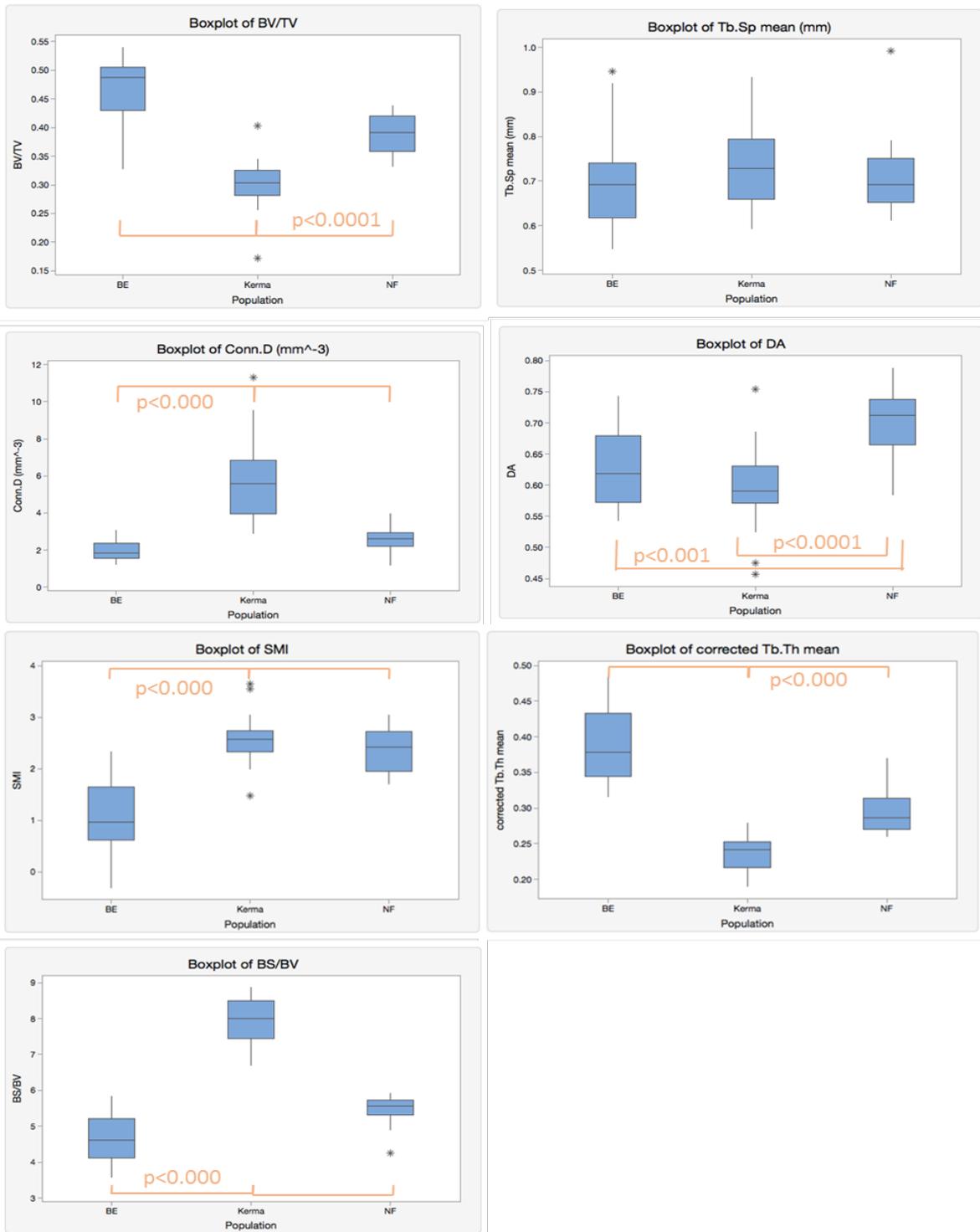


**Fig. 4.** Thickness Maps for cortical bone from the three populations: (A) Black Earth, (B) Kerma, and (C) Norris Farms. The line separates the superior half from the inferior half and represents where the cortical bone was split. The images have been adjusted to show the thickest point (shown in white) of the inferior region.

## DISCUSSION

The goal of this study was to better understand human skeletal variation and its relationship to age-related bone loss in contemporary human populations. Osteoporosis is a systemic bone disease with significant global health impacts that is characterized by low bone mass and microarchitectural deterioration, leading to increased bone fragility and fracture risk (Ryan and Shaw, 2014). Although trabecular architecture is usually more susceptible to bone loss, this study analyzed cortical and trabecular bone because they both contribute to bone strength. The thicker the trabeculae found in the femoral head of bones, along with thicker cortical bone in the femoral neck, corresponds with greater bone mass and ultimately stronger bones.

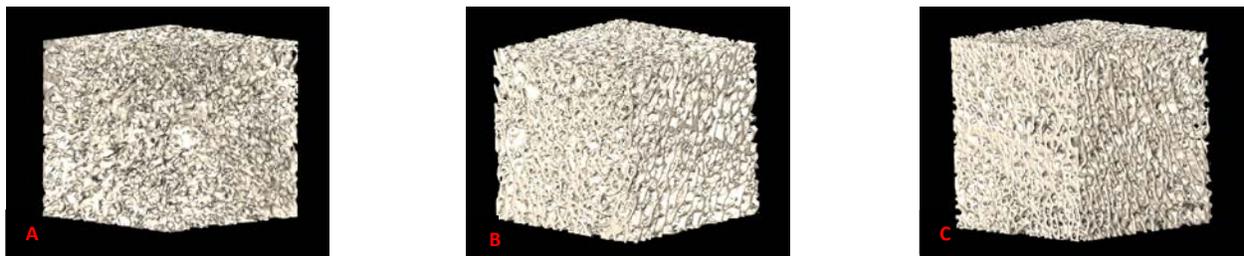
The goal of this analysis was to investigate cortical and trabecular bone structure in the proximal femur and to examine differences related to ethnicity and behavior. As expected, the results show that the Black Earth hunter-gatherers have the thickest inferior femoral neck cortical bone. They have the highest inferior thickness max and mean with the Norris Farms following close behind. This suggests that mobility and associated loading have effects on the cortical bone of the femoral neck. Surprisingly, there are no significant differences between the Norris Farms agriculturalists and the African agriculturalists. This suggests that behavior may be more important than ancestry when it comes to cortical bone. Overall, the results indicate that patterns of trabecular structural variation differ between the three populations (Fig. 6). As predicted, the Black Earth hunter-gatherers have more robust trabecular bone. It was shown that the Black Earth had the highest BV/TV, meaning they have more bone overall than the other populations. However, the Kerma were shown to have the highest Conn.D and BS/BV values but the lowest Tb.Th. This means that even though the Kerma have the greatest number of trabeculae, their trabeculae are significantly thinner than the other two populations. This suggests that loading and activity may be a significant factor in building robust bone.



**Fig. 5.** Boxplots showing the results of morphometric analyses of trabecular bone in the femoral head for each population. Statistically significant population differences, based on the ANOVA, are marked with their respective p-values. Outliers are marked by asterisks (\*).

Many factors could explain the variation in cortical and trabecular bone: ancestry, complexity of societies, behavior, age, sex, etc. Studies have suggested that people who are more sedentary, agriculturalists, have more gracile bone when compared to more active individuals, hunter-gatherers (Ryan and Shaw, 2014). The shift from a foraging lifestyle to an agricultural lifestyle led to more gracile human bones for a number of reasons. The main reason was the decrease in physical activity. Hunter-gatherers were naturally more mobile because of the work they had to perform to find food. As humans adopted agriculture, the amount of mobility decreased, therefore, less biomechanical loading on our bones, especially the femur. With the shift to agriculture came the change in the division of labor, as well as, eventually, more stratified and societies. Another potential force in gracilization is the significant change in diet. By moving to an agricultural lifestyle, much of the nutritional diversity and possibly quality was lost, for instance, the high levels of proteins and carbohydrates (Agarwal and Grynepas, 1996; Ryan and Shaw, 2014).

There were some limitations to this study. As mentioned before, diet potentially has a dramatic effect on bone strength and bone growth. Because we weren't aware of each population's specific diet, we were not able to determine if that was a factor that significantly affected their bone structure. Also, our sample size was limiting in two other ways. The sample consisted of only two Native American groups (one hunter-gatherer and one agriculturalist) and one African group (agriculturalist). If we were able to obtain a hunter-gatherer African group, and possibly even samples from Europe or Asia, then we would be able to determine the relative importance of behavior and ancestry on bone structure and composition. Also, including samples from different regions of Africa could help to determine more specifically how ancestry affects bone composition.



**Fig. 6.** Three-dimensional reconstructions of cubic trabecular bone specimens from the femoral head for each population: (A) Kerma, (B) Black Earth, and (C) Norris Farms. These cubes were used for analysis of trabecular bone.

Further analyses are needed in order to better assess whether or not behavior or ancestry is more important to bone composition. If the sample variation was increased to include more ethnic groups and more diverse behavioral styles, we could better address which factor, behavior or ancestry, affects bone composition more. Bone samples from older individuals within each population would be useful to analyze as well. This would tell which populations were affected by osteoporosis, or other related bone diseases, more frequently. From that information, along with the analysis of the populations' behavior, diet, economic status, etc., it could be determined why they experienced more osteoporosis. Lastly, the research could potentially help better treat osteoporosis and other related bone diseases. If it were determined that ancestry played a bigger role in bone composition, then medical treatment could become more personalized. Hopefully

with a more specific treatment, more people would not need artificial bone and/or joints or die from the fractures.

## CONCLUSION

Overall, the main conclusion made from this study is that mobility and associated loading (behavior) seems to play a bigger role in building more robust bone than ancestry. However, the variability in bone composition still exists and it affects the health of the bone and the person, specifically in the occurrence of osteoporosis. It increases the chance of fracture risk and is a common concern among people in the medical field. Ancestry is often excluded when discussing bone health in past and present human populations. Understanding bone loss and bone health, as it pertains to ethnicity, presents a new and exciting direction and could help conclude why people experience common bone disorders differently. Understanding this will help determine how, or if, people should be treated for these disorders and if they can be prevented.

Although first recognized more than 250 years ago, the clinical and epidemiological knowledge about osteoporosis is largely limited to the last 70 years. The study of osteoporosis in past populations increases knowledge about bone modifications related to age, menopausal status or lifestyle (Curate, 2014). For instance, studies have shown that the way osteoporosis is experienced today is different than how past populations experienced it. Past *Homo sapiens* populations had cases of osteoporosis in both sexes and across all age ranges. Today, osteoporosis is found more in women, and usually in older women. In more modern *Homo sapiens* populations, osteoporosis tends to cause fragility fractures in the skeleton. The fractures that occur due to osteoporosis tend to be more fatal and are the reason the medical field wants to learn more about the disease; however, past *Homo sapiens* populations did not have a large occurrence of fragility fractures (Agarwal, 2008). Although the risk factors for osteoporosis between blacks and whites are similar, osteoporosis and related fractures occur half as much in African American women than Caucasian women (Bohannon, 1999; Barrett-Connor et al., 2005).

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# *Characterization of $\beta$ -heavy Spectrin Self-interaction*

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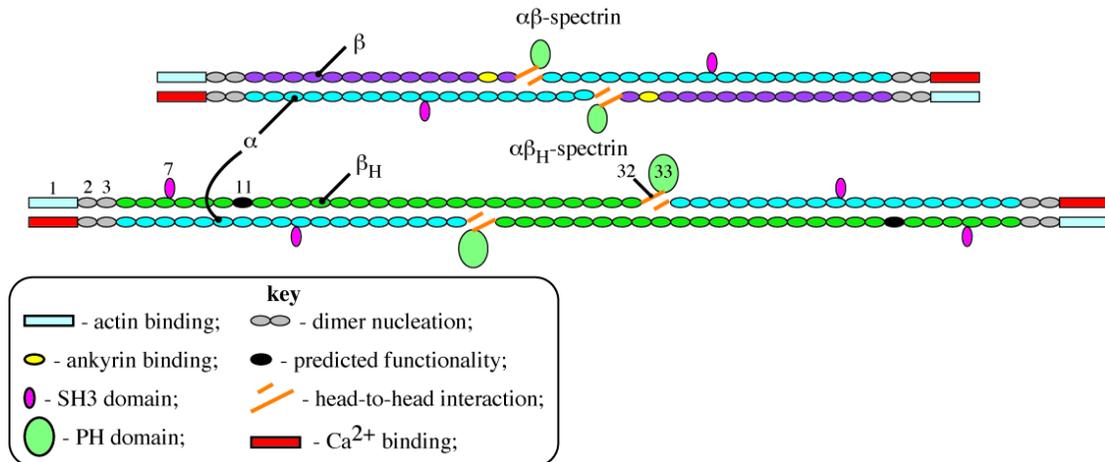
## **Abstract**

Spectrin is a protein which serves to form the cytoskeletal network in cells for structural support. The protein forms tetramers consisting of two  $\alpha$  and  $\beta$  subunits. This study's objective is to investigate the possibility that  $\beta$ -heavy spectrin functions independently of  $\alpha$ -spectrin to form higher order structures contrary to the classical tetramer-based structure found in erythrocytes. An *in vitro* western blot overlay method is utilized to observe a self-interaction of  $\beta$ -heavy spectrin. A self-interaction was observed, offering new insight into how spectrin may function in biological processes and generate networks.

## **Introduction**

First discovered in 1968, spectrin was found to be a defining part of the membrane skeleton in red blood cells, where it maintains a structural role to form and shape erythrocytes (Marchesi and Steers, 1968). The structural role of spectrin was thought to be its only function, but further research has revealed that non-erythroid spectrin plays a role in many more biological processes. Some of these processes include cell polarity, the organization and formation of cell adhesion complexes, and protein trafficking (Zarnescu and Thomas, 1999; Xiuli An *et al.*, 2008; De Matteis and Morrow, 2000).

In *Drosophila*, there are three isoforms of spectrin:  $\alpha$ ,  $\beta$ , and the larger  $\beta_H$ -spectrin. Vertebrates possess multiple spectrin isoforms in the forms of  $\alpha I$ ,  $\alpha II$ , and  $\beta I$  through  $\beta V$ . Spectrin functions and forms networks through its several binding domains and interactions with other proteins.  $\alpha$ -spectrins contain a Src homology 3 (SH3) domain as well as a C-terminal calmodulin related  $Ca^{2+}$  binding site.  $\beta$ -spectrins possess an N-terminal actin binding domain as well as ankyrin binding sites. In addition to the extended length on  $\beta_H$ -spectrin, these spectrins also differ from  $\beta$  in that they possess an SH3 domain and lack ankyrin binding sites (Bennet and Baines, 2001). These subunits organize into a cytoskeletal network by forming heterodimers in which  $\alpha$  spectrin subunits connect to  $\beta$  spectrin in an antiparallel, side-to-side fashion (Fig. 1). These  $\alpha/\beta$  heterodimers then connect to form tetramers in which the N-terminus of each  $\alpha$  subunit connects to the C-terminus of each  $\beta$  subunit in the head-to-head interaction (Fig. 1). This tetramerization allows the formation of long spectrin protein chains, which make up the membrane skeleton network.



**Figure 1. Spectrin Tetramerization and Binding Domains (Schematic provide by G. Thomas and used with permission)**

Spectrin mutations cause improper erythrocyte network formation, and the compromised networks result in weak and fragile membranes. Humans with these mutations exhibit hereditary hemolytic anemias and spinocerebellar ataxia (Bennet and Healy, 2008). Spectrin is recognized as an essential non-erythroid protein. However, recent studies on the *aspec*<sup>R22S</sup> mutation, in which the tetramerization of  $\alpha$  and  $\beta$  subunits is disrupted, have revealed that the tetramerization of spectrin in non-erythrocyte cells is not crucial. In fact, *Drosophila aspec*<sup>R22S</sup> mutants complete normal development with only minor abnormalities (Khanna *et al.*, 2014). Some of these observed abnormalities included compromised fusome integrity during oogenesis, fewer individuated synaptic boutons in the neuromuscular junction, and irregular shaping and position of cuprophillic cells in the midgut. Despite these abnormalities, viable adult flies were produced. The evidence suggests that the tetramer network is not essential for the major function of spectrin. This would suggest that a new model needs to be developed for the function and structure of the spectrin membrane skeleton in non-erythroid cells and that the importance of tetramerization in erythrocytes is a special case. One possibility is that spectrins may form a network that differs from the erythrocyte network to function in non-erythroid cells.

A recent discovery related to spectrin binding was made by Samantha Papal *et al.*, (2013) during an investigation of  $\beta_V$ -spectrin, the  $\beta_H$  ortholog in humans. In this paper, *in vitro* and *in vivo* evidence demonstrated a homodimeric interaction between repeats 26-30 and 29-30 of  $\beta_V$  (Papal *et al.*, 2013). The proposed model arising from this observation is that  $\beta_V$  has the ability to form homodimers through its C-terminal region. This model is supported by *in vitro* western blot evidence, which demonstrated a self-interaction between  $\beta_V$  protein fragments. Homodimer formation is also supported by *in vivo* evidence of  $\alpha$  independent function of  $\beta_V$  along opsin trafficking routes in photoreceptor cells. The discovery of this interaction suggests that there are  $\alpha$  independent functions for  $\beta_V$  and that new molecular pathways could result in network formation in a system where  $\beta_V$  spectrin is the key component. Since  $\beta_V$  is the human ortholog for  $\beta_H$ , I set out to investigate whether this self-interaction is also present in the *Drosophila* protein using the *in vitro* method of a western blot overlay.

## Methods

### Polymerase chain reaction (PCR) and Cloning

PCR was performed using the primers 3'-AGCGGATCCTCTCACCAATTGCAGGAGTTCCTGCGCAGTCTCTA-5' (top primer) and 3'-ATGCGAATTCTTACTGGTGACGGAATAGCTGCTCAA-5' (bottom primer) to amplify the region S25-32 of  $\beta$ -heavy using the preexisting cDNA p3F1 as a template. The 2.47kb PCR product (Fig. 3) was then purified by running it on an agarose gel and the band was extracted using a Qiagen gel extraction kit (Germantown, Maryland). This was followed by digestion with the restriction enzymes BamHI and EcoRI and ligation into similarly digested pGEX-4T-1 plasmid to create a fused open reading frame for GST-S25-32. Next, this plasmid was transformed into *Escherichia coli* (*E. coli*) strain XL10. The transformed bacteria containing the positive clone were grown overnight to stationary phase for plasmid extraction using a Qiagen maxi prep kit. This DNA was sequence verified. Following verification, the plasmid was transformed into *E. coli* BL21-CodonPlus (DE3)-RIPL (Agilent Technologies, Santa Clara, CA) for expression using standard methods. These *E. coli* were optimized for induction for the process of protein expression and purification.

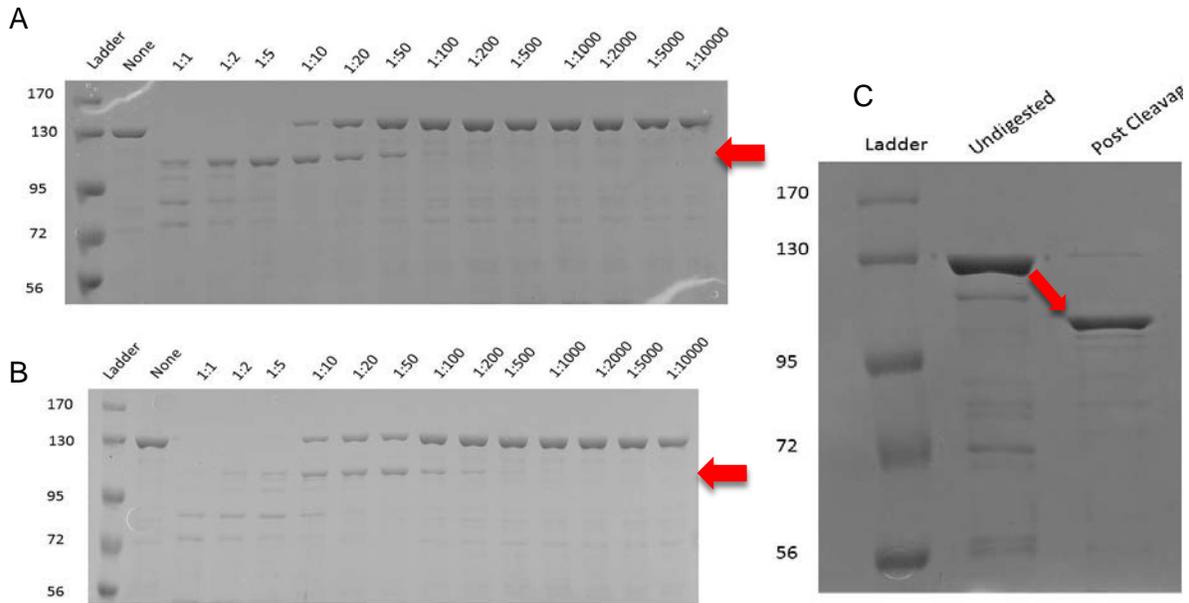
### Expression and Purification

6 X 600 mL cultures of BL21 cells containing the GST-S25-32 fusion were grown to log phase and induced for 5 hours at room temperature using 1mM IPTG. After centrifugation, the pellets were combined and re-suspended together in lysis buffer (50 mL, 50mM Tris-Cl, pH 8.0, 50mM NaCl, 1mM  $\beta$ -mercaptoethanol, 5mM EDTA, 150 $\mu$ M PMSF, 1 $\mu$ g/ml of leupeptin, 1  $\mu$ g/ml of pepstatin, 1  $\mu$ g/ml of diisopropyl fluorophosphate). The bacteria were then sonicated (6 times for two minutes each) on ice to extract the fusion protein. The sonicated sample was centrifuged and the supernatant was mixed with glutathione agarose beads and tumbled for 1 hour at 4°C. Next, the beads were packed into a column and washed with 1X PBS + EDTA/PMSF (PBEP, 10 mM NaPO<sub>4</sub>, 130 mM NaCl, pH 7.3, 1 mM  $\beta$ -mercaptoethanol, 5 mM EDTA, and 150  $\mu$ M PMSF), followed by PBEP without PMSF. Finally, the fusion protein was eluted with G buffer (50 mL, 50 mM Tris-Cl, pH 8.0, 10 mM reduced glutathione, 1 mM  $\beta$ -mercaptoethanol, 5mM EDTA, 1 $\mu$ g/ml of pepstatin).

### Thrombin Titration, Cleavage, and GST Subtraction

Thrombin titration was performed on 50  $\mu$ L aliquots of GST fusion protein that were digested with 50  $\mu$ L of decreasing concentrations of thrombin (Sigma) mixed with PBEP with no PMSF. The thrombin ratios ranged from 1:1 to 1:10,000 and the titration was repeated in conditions of 0.15 M NaCl (Fig. 2, A and B). By running the titration samples on a gel, it was observed that the thrombin ratio of 1:10 in 0.15 M NaCl yielded maximum cleavage with little fragmentation of protein. Thrombin (400  $\mu$ L) was added to a 20 mL sample of protein at a ratio of 1:10 with a 0.15 M NaCl concentration and incubated for 3 hours at 37°C to cleave off the GST and eventually isolate the S25-32  $\beta_H$  fragment (Fig. 2C). The digest was stopped by the addition of PMSF (80  $\mu$ L) and chilled until dialysis with PBEP. Once dialyzed, glutathione agarose beads were added to the sample and tumbled for 1 hour at 4°C. The sample was then packed into the column and the isolated S25-32 flow-through was collected. The GST was eluted

out of the column with G buffer and the column was washed with PBEP. This process was repeated to maximize the removal of GST from the isolated S25-32 protein sample.



**Figure 2. Thrombin Titration and Cleavage Results**

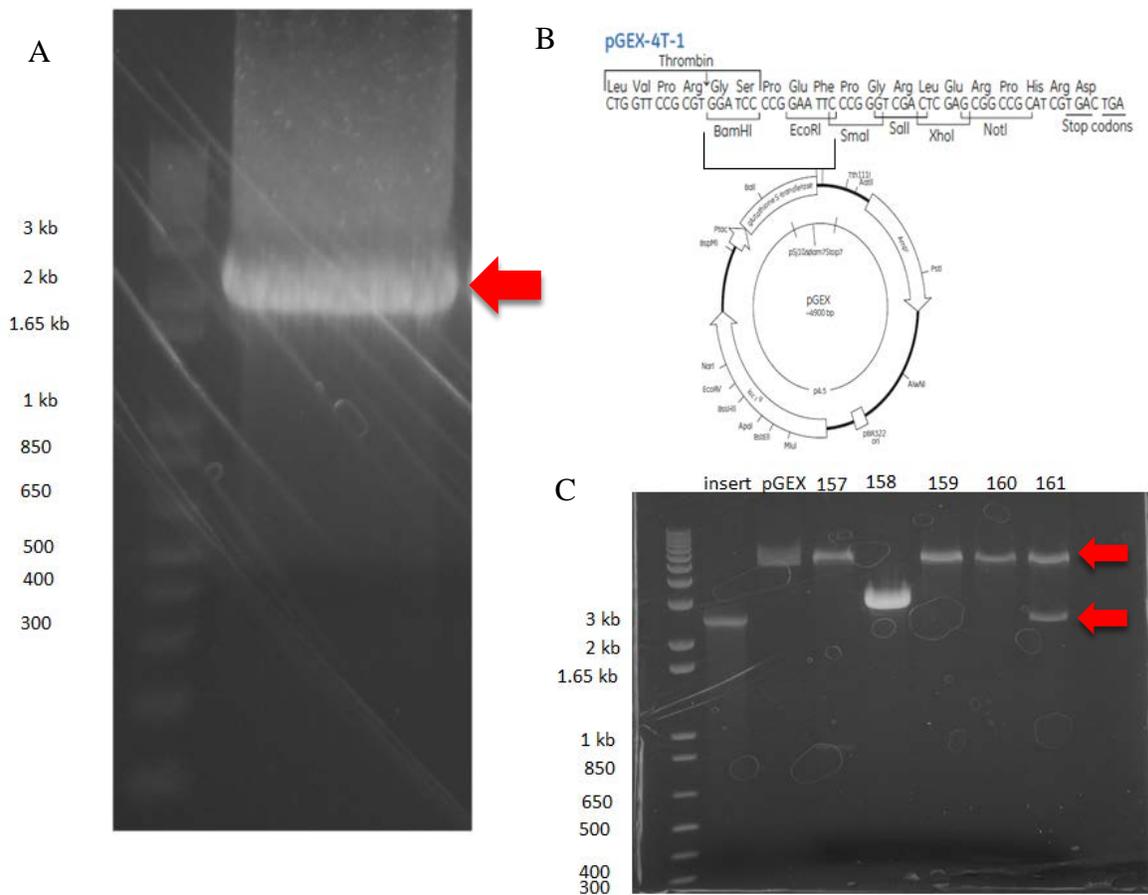
(A) Thrombin titration performed with 0.15 M NaCl (B) Thrombin titration performed in absence of NaCl (C) Large-scale thrombin cleavage of fusion protein divulges successful cleavage with expected band at ~95kDa.

### In Vitro Western Blot Protein Interaction Assay

100  $\mu$ L of GST subtracted  $\beta_H$  S25-32 was run on a wide-lane 7% SDS page gel, transferred onto nitrocellulose paper, and sliced into strips. Individual strips were blocked (20 mM Tris pH 7.5, 150 mM NaCl, 5% nonfat milk), and rocked overnight at 4°C in various dilutions of uncleaved GST-S25-32 fusion protein. Following the overlay, the membrane was washed in incubation solution (4 x 5 minutes, 20 mM Tris pH 7.5, 150 mM NaCl, 5% nonfat milk, 0.1% Tween) and incubated in anti-GST primary antibody (GE Health Care) at a 1:500 dilution for 1 hour at room temperature. Following primary antibody incubation, the blot was washed in incubation solution (4 x 5 minutes) and incubated in secondary antibody (HRP donkey anti-goat) for 1 hour at room temperature. The blot was then washed in Tris Buffered Saline with Tween (4X 5 minutes, 20 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween) and an ECL chemiluminescence system was used for detection.

## Results

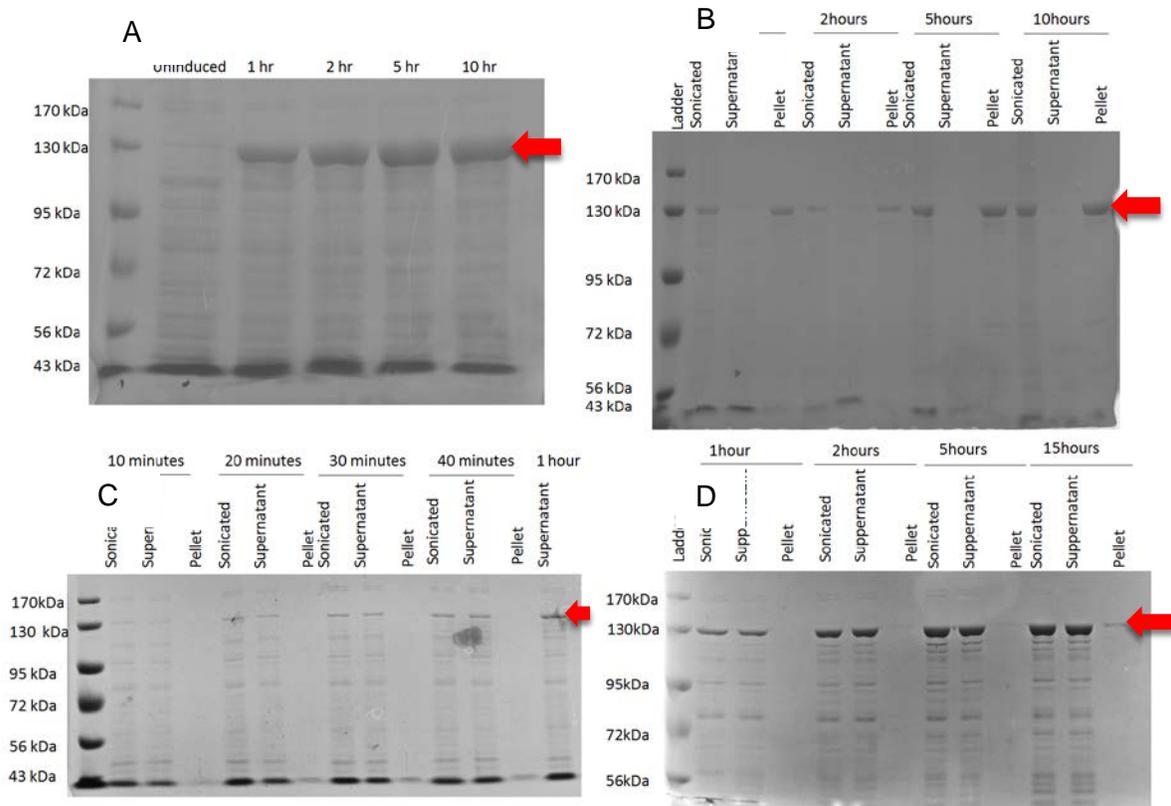
Through an *in vitro* western blot overlay method, Papal *et al.*, (2013) was able to identify self-interaction between repeats 26-30 of  $\beta_V$ . I used PCR to amplify segments of the  $\beta_H$  sequence which are equivalent to this region. Amplification, using the primers described in the methods section and plasmid p3F1 as a template, resulted in the expected band of 2.467 kb (Fig. 3A). The primers used to amplify the fragment also added the restriction sites for BamHI and EcoRI for the purpose of cutting with these enzymes and cloning into pGEX-4TI, which was similarly digested (3B). After I performed the digest and ligated together the vector and PCR fragment, the plasmid was transformed into XL10 *E.coli*. The resulting colonies were then tested for successful plasmid intake. Analysis of several clones revealed that the insert was successfully transformed into colony #161 of the XL10 cell line, as indicated by the 2.4 kb band (Fig. 3C).



**Figure 3.** PCR and Cloning Results. (A) PCR product ran on 1% gel with expected band size of 2.46 kb. (B) Model of pGEX-4TI cloning vector with restriction enzyme sites (C) Insert with expected size of 2.46 kb was revealed by diagnostic digest to have successfully transformed into colony 161 when compared with S25-32 and pGEX-4TI positive loading control.

I retransformed the plasmid containing  $\beta_H$  S25-32 into the BL21 cell line for the purpose of expression.  $\beta_H$  GST::S25-32 is expected to have a band weight of 120 kDa. Test inductions with the BL21 cell line confirm this band weight and show that strong induction takes place

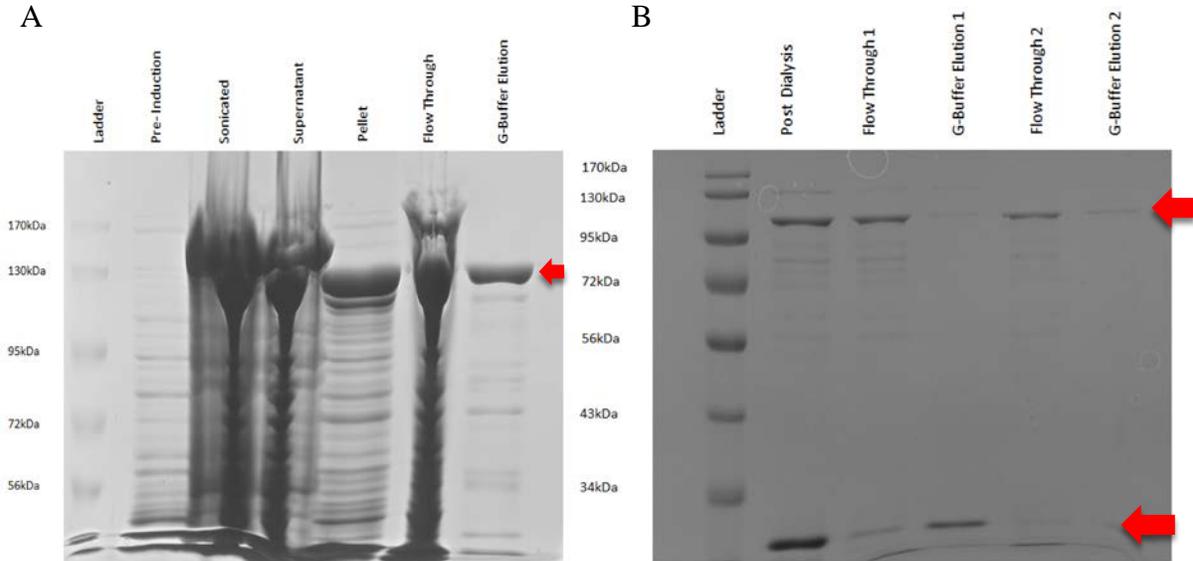
within a relatively short time (Fig. 4A). Induction time was shown to have little impact on the yield of fusion protein after 5 hours as the band intensities at five and ten hours are similar (Fig. 4A). I then conducted solubility tests to optimize the solubility of the protein in lysis buffer. Tests demonstrated that induction at 37°C results in an insoluble protein which remains in the pellet (Fig. 4B). Since flies live at ambient temperature, I decided to test the induction at room temperature which might improve the proper folding and conformation. Solubility tests at room temperature supported this prediction and yielded a highly soluble protein (Fig. 4C and D). From this result, it can be inferred that proper folding conformation of the GST::S25-32 fusion protein requires an optimal temperature range based on the environment in which the protein would normally function. Additionally, induction time was shown to have little effect on the solubility and yield of the protein (Fig. 4C and D).



**Figure 4.** Transformed BL21 cells are shown to induce successfully and GST::S25-32 fusion protein is soluble when induced at room temperature. (A) Induction is shown to be successful with a band weight of 120 kDa and high concentrations of protein after only 1 hour. (B) Induction at 37°C reveals that the fusion protein is insoluble and remained in the pellet. (C) Induction at short time and room temperature reveals that the protein is soluble and remained in the supernatant. (D) Induction at room temperature for long periods of time reveals that the protein is nearly completely soluble and will remain in the supernatant as long as induction takes place at room temperature.

After a large-scale induction was performed, I extracted the fusion protein from the induced bacteria sample and isolated it using glutathione agarose beads, as described in the methods section. Some loss of protein was shown in the pellet and flow-through samples, but

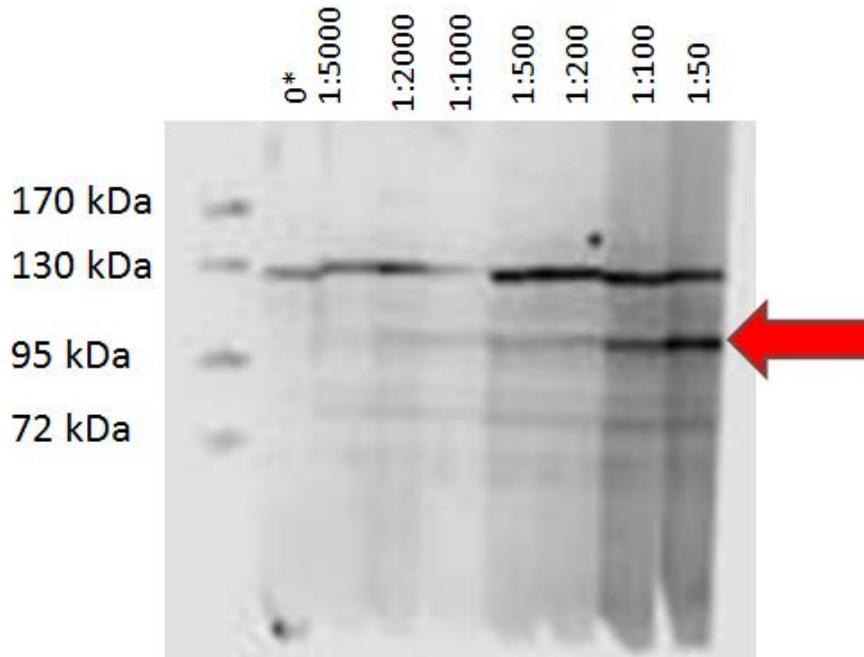
despite these losses a significant amount of purified protein was eluted from the column (Fig. 5A). Following the successful purification, I went on to the cleavage and subtraction of GST from the S25-32. The subtraction was shown to be effective as the gel exposes no GST band at 25 kDa in the second flow-through sample.



**Figure 5.** Protein purification and thrombin cleavage was shown to be successful. (A) Purification of GST S25-32 fusion protein was shown to be successful with some loss of protein in the flow-through and pellet. (B) Gel reports that thrombin cleavage and isolation of S25-32 from GST was successful after two flow-through collections.

In order to look for self-interaction, I applied the western blot overlay technique (Papal *et al.*, 2013) in which GST subtracted S25-32 was embedded in a nitrocellulose membrane and overlaid with GST::S25-32. The membranes were then incubated with primary and secondary antibodies to target the overlaid GST protein followed by imaging of the blot using HRP chemiluminescent detection of secondary antibody. Final imaging of a spaghetti western revealed that interaction did occur as the appearance of bands indicates that GST::S25-32 fusion protein bound to the GST subtracted S25-32 on the blot (Fig. 6A). It should be noted that the top band which appears in the spaghetti western is the result of GST::S25-32 contaminant in the GST subtracted sample of S25-32. The control strip with 0 fusion protein overlay reveals antibody targeting despite the expected absence of GST. The upper band also appears at the expected 120 kDa weight of GST::S25-32.

A



**Figure 6.** Spaghetti Western Overlay of GST Subtracted  $\beta_H$  S25-32 with  $\beta_H$  GST::S25-32. (A) Blot reveals that GST::S25-32 fusion protein binds with GST subtracted S25-32 near predicted band weight of 95 kDa. Band intensity is shown to increase with increasing concentration of overlay.

## Discussion

The *in vitro* western results indicate that self-interaction does occur within the region of terminal repeats of *Drosophila*  $\beta_H$  spectrin (Fig. 6A). The appearance of a band in the blot indicates that the antibodies had targeted GST::S25-32 which was localized at 95 kDa due to a specific binding interaction with GST subtracted S25-32. This is in line with the results shown by Samantha Papal *et al.* (2013) in human  $\beta_V$ . This finding could give a possible explanation as to how spectrin may have been able to form a network in R22S mutants and led to normal developmental pathways (Khanna *et al.*, 2014). It is plausible that if network formation is still occurring in the absence of  $\alpha/\beta$  dimerization, spectrin could function normally and still be able to cross link F-actin since the F-actin binding domain is located on the  $\beta$  subunits (Brenner and Korn, 1979; Karinch *et al.*, 1990). If further supported, this new finding would significantly emphasize the importance of the  $\beta$  subunit for proper functionality of spectrin. The characteristic  $\beta_H$  and  $\beta_V$  independent network formation would support findings in vertebrates in which  $\beta$ -spectrin is reported to function in neurons and epithelial cells independent of  $\alpha$ -spectrin (Papal *et al.*, 2013; Dubreuil *et al.*, 2000). If  $\beta_H$  self-interaction is found to be conserved in both vertebrates and invertebrate species, further studies should attempt to identify examples of  $\beta_H$  independent function in *Drosophila*.

Further studies are required in order to properly confirm and characterize the interaction occurring between  $\beta_H$  subunits. If the interaction involves specific binding domains, an additional spaghetti western protocol should use higher concentration overlays of fusion protein in order to identify a point of saturation in which band intensity plateaus. Showing a point of saturation will

reveal the specificity of the self-interaction and whether it occurs through binding domains. In addition to *in vitro* western blotting evidence, additional protocols could also confirm self-interaction. One protocol which could potentially identify interaction is the use of chemical cross linking in which specific amino acid side chains, in close proximity of one another due to protein interaction, are covalently linked together (Life Technologies). Immunoprecipitation is another potential method in which anti-GST antibodies would be used to precipitate GST::S25-32 and anything binding it out of solution. In order to propose a specific model of where and how the binding occurs between two  $\beta_H$  chains, additional variants of  $\beta_H$  fusion proteins will need to be cloned, such as GST::S28-32 or GST::S30-32. In understanding the occurrence of self-interaction, the scientific community can develop better models as to how spectrin may form networks as well as explanations for observed  $\alpha$  independent functions of  $\beta$ -spectrin.

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