INDIVIDUALIZED COGNITIVE DECLINE AND THE IMPACT OF GUT MICROBIOME COMPOSITION.

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A dissertation presented to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Psychology & Neuroscience (Developmental Psychology).

Chapel Hill
2020

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ABSTRACT

L. Grant Canipe: Individualized Cognitive Decline and the Impact of Gut Microbiome Composition
(Under the direction of Carol L. Cheatham)

The U.S. population is aging at its greatest rate in history. An older average population will increase the number of age-related cognitive issues. Elucidation of factors that contribute to decline with age and methods to prevent or decrease the incidence of cognitive dysfunction in the aging population is vital to offset the impact of the age shift. Validation of tests to identify and predict decline is the first step, but must be paired with an increased understanding of the inter- and intra-individual differences that influence cognitive decline. One difference, gut microbiome diversity, changes within the person across their lifespan and varies among individuals. An individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior.

The study was focused on identification and prediction of cognitive decline using CANTAB and visual ERP as well as exploring the relation between gut-microbiome diversity and cognitive performance. Participants underwent tests to evaluate cognitive decline over time: the MoCA, a CANTAB battery for behavioral cognitive assessment, and an electrophysiological evaluation via a passive oddball paradigm and an active detection task. The role of microbiome diversity in cognitive decline was investigated, ERP measures were validated against CANTAB measures, the predictive relation between MoCA and future cognitive outcomes were characterized, and the utility of ERP PCA factors and CANTAB outcomes to predict future ERP and CANTAB performance were shown.

Three CANTAB measures (RTI, SWM, and RVP) were independently confirmed to significantly relate to selected ERP measures in both the active detection and the passive oddball tasks. Baseline MoCA score and change in MoCA score significantly predicted outcomes in the CANTAB battery and

ERP tasks at follow-up. The study also included the design and implementation of novel methodology with two-step temporospatial PCA to successfully predict future performance on ERP with baseline performance on the same task, which, to this author's knowledge, is the first known use of this method for this purpose. Finally, significant relations between gut-microbiome diversity and healthy cognitive function were revealed, where lower microbial diversity significantly relates to poorer cognitive performance on both behavioral (CANTAB) and electrophysiological (ERP) measures.

ACKNOWLEDGEMENTS

I would like to acknowledge my advisor, Dr. Carol L. Cheatham. Thank you for your advice and support and providing the motivation to reach each milestone and, “get out of here.” Thank you to my dissertation committee: Dr. Jean-Loius Gariepy, Dr. Kelly S. Giovanello, Dr. Peter A. Ornstein, and Dr. Eva H. Telzer. Thank you to the members of the Cheatham Lab over the years and, in particular to, Andrea Armer, Lerin Eason, and Thomas Jackson. Thank you, most of all, for the many laughs and sharing of tears/frustration through all the successes and setbacks. I certainly would not have survived this without you. Thank you to the faculty, students, and staff at the Nutrition Research Institute and the Department of Psychology & Neuroscience. Thank you to all of our amazing participants and families – You are the reason we do our research.

Possibly the largest thanks go to my parents and grandparents, who provided all the scaffolding throughout my development to reach this goal. To Earl & JoAnn Lester, thank you for the many summer nights of story-telling to me and Mr. Bear, 4H camps, and the lessons of what “living” really means. To Richard & Julie Davidson, thank you for the beach trips where you supported and inspired my inquisitive mind. To Bobbie & David Hadley, thank you for teaching me physical and social/emotional wellness at tennis camps and sharing your lives with me in Georgia – Memaw, even if you couldn’t be here at the end, I know you would have helped me incorporate some cheetah print into the doctoral hood somehow. To Larry and Deanna Canipe, the biggest thank you of all for your ceaseless support and belief that I could accomplish my goals and survive “it” through to the end. To Hope Canipe, my sister, thank you for always providing the love only a sibling could share. To my dog, Blu, you were always a ray of sunshine on the cloudiest of research and writing days.

To everyone, but especially my family and friends, thank you for always listening, for always offering great advice (even when I wouldn’t take it), and when all else failed, for always just being there.

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LIST OF ABBREVIATIONS

MCD - Mild Cognitive Decline

MRI – Magnetic Resonance Image

DTI – Diffuse Tensor Imaging

WM – Working Memory

MoCA – Montreal Cognitive Assessment

ERP – Event Related Potential

CRUNCH – Cognitive ReUtilization of Neural Circuits Hypothesis

STAC – Scaffolding Theory of Aging and Cognition

STAC-r – Scaffolding Theory of Aging and Cognition - revised

PFC – Prefrontal Cortex

MTL – Medial Temporal Lobe

BMI – Body Mass Index

MCI - Mild Cognitive Impairment

AD – Alzheimer's Disease

GIT – Gastrointestinal Tract

CANTAB – Cambridge Neuropsychological Test Automated Battery

MMSE – Mini Mental State Examination

WAIS-IV – Weschler Adult Intelligence Scale

EEG – electroencephalogram

RTI – Reaction Time

SWM – Spatial Working Memory

RVP – Rapid Visual information Processing

NDSR – Nutrition Data System for Research

PCA – Principle Components Analysis

PCoA – Principle Coordinates Analysis

VIF – Variable Inflation Factor

CI – Condition Index

PAL – Paired Associates Learning

CHAPTER 1: INTRODUCTION

The proportion of the U.S. population aged 65 or older will peak at approximately one in five adults by the year 2035. Rising healthcare needs and costs are the paramount issues caused by a shifting-age landscape; decline in cognitive function paired with an increase in age-related cognitive disorders will impact American lifestyle. Elucidation of methods to prevent or decrease the incidence of cognitive dysfunction in the aging population could serve as a tool to offset the impact of the age shift. Understanding, identifying, and predicting cognitive decline are the first steps in determining the factors that contribute to declining brain health and in developing methods to prevent and ameliorate its effects.

Executive control measures, such as speed of information processing, working memory, and inhibitory control, decline linearly with age. The neurobiological changes and psychological functions associated with aging and mild cognitive decline (MCD) have been studied, but there are inconsistencies in methodology and study paradigms. Auditory event-related potentials (ERP), an electrophysiological technique, and the Cambridge Neuropsychological Test Automated Battery (CANTAB) have shown promise in the identification of MCD. However, due to the potential for loss of hearing with age, I suggest that visual ERP paradigms should be utilized and, moreover, validated with standardized behavioral assessments. Researchers have identified markers in ERP waveforms for early detection of age-related decline and prediction of future decline, but they require further elucidation. With this research study, my goal was to identify early indicators of cognitive decline and MCD using CANTAB and visual ERP. Participants underwent a set of tests to evaluate cognitive decline over time: the Montreal Cognitive Assessment (MoCA), a CANTAB battery for behavioral cognitive assessment, and an electrophysiological evaluation via the passive oddball paradigm and an active detection task.

Inter- and intra-individual differences are essential points of consideration when studying cognition. Differences like genetics, nutrient intake, education, and many other factors within and between individuals complicate studies of cognitive decline. The identification and control of relevant covariates are imperative to identifying and understanding factors for cognitive decline. One difference between individuals - gut microbiome diversity and composition - changes within the person across his or her lifespan as well as varying among individuals. Recent research on the gut microbiome has shown that an individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior by indirectly causing changes in internal homeostasis. Effects occur directly via immune activation or indirectly, through the production of neuro-metabolites. In this study, I investigate the role of microbiome diversity in cognitive function for older adults.

Defining Cognitive Aging

Aging can be defined in a myriad of ways, but most definitions describe some form of change of abilities and/or functions across time such as changes in cognition. “Older age” can be defined as age in years - 65-80 with >80 being “elderly.” For most, aging often means a decline in many facets of life, such as cognitive function and development of dementia. Whereas many theories have aimed to explain why we age, and some have stood the test of time by indicating ways to slow or fight aging, many outcomes remain inevitable such as declining short-term and working memory function, delayed reaction time, and slowed recall of semantic and episodic memory (Beckman & Ames, 1998; Brown & Park, 2003; Harman, 1956; Hayflick, 1979, 2007; Medawar, 1952; Salthouse, 2000; Salthouse, Atkinson, & Berish, 2003; Vina, Borras, Abdelaziz, Garcia-Valles, & Gomez-Cabrera, 2013; G. C. Williams, 1957).

For the present study, mild cognitive decline (MCD) is defined as greater than expected change in cognitive function after considering age and education. If confirmed by clinical diagnosis, this change may then be defined as mild cognitive impairment (MCI). No MCI diagnoses were obtained in this study. I argue that by providing the proper characterization, methods that provide MCD classification could allow for the study of differences between typical and atypical cognitive aging without relying on a more costly, time-consuming, and difficult to obtain clinical diagnosis. Individuals who are experiencing

MCI/D may show an increased risk for Alzheimer's disease (AD) and other dementias (Etgen, 2015; Levy, 1994). However, no definitive evidence exists that MCI/D individuals progress to a demented state (Etgen, 2015; T. Smith, Gildeh, & Holmes, 2007). Some cognitive domains are impacted heavily in typical (non-diseased) aging (e.g., working memory, processing speed, inhibitory control), and changes in these domains are to be expected, while other domains should remain relatively unaffected (e.g., verbal acquisition and semantic memory; Baltes, 1993; Brown & Park, 2003; P. A. Reuter-Lorenz & Park, 2010). Non-demented MCI/D could represent an accelerated form of typical aging, as indicated by a decline in processing speed and in specific aspects of executive control such as working memory and inhibitory control (Karbach & Verhaeghen, 2014; Salthouse et al., 2003; Verhaeghen, 2011; Y. Zhang, Han, Verhaeghen, & Nilsson, 2007).

The early identification and accurate assessment of cognitive decline is imperative to creating successful public health interventions and preventing future decline. The first step would be to identify factors that improve cognitive function across the lifespan to maximize positive outcomes (Salthouse, 2009). Therefore, we must identify factors that contribute to and predict decline before it advances.

Is aging developmental?

Although growth and change in the structure and function of the human brain do not end after adolescence, it does not occur as rapidly as in the first two decades of life. Studies of cognition in adulthood span a much broader age range compared to childhood. Measurable decline in performance in key domains accompanies typical aging. The development of cognitive dysfunction, or age-related decline, varies significantly between individuals, and often these differences can be attributed to noticeable alterations in structure and function of critical brain regions in individuals experiencing global (not domain specific) cognitive decline when compared to healthy individuals experiencing no decline (Braver & Barch, 2002; Braver et al., 2001; Etgen, 2015; Levy, 1994; Persson & Reuter-Lorenz, 2008; Pudas et al., 2013).

The human brain undergoes significant changes in both structure and function across our lifespan (Casey, Tottenham, Liston, & Durston, 2005), and it is essential to consider what is meant by

development, or change, with age. Advances in neuroimaging techniques allow the tracking of changes in healthy adults across time. Findings indicate a fine-tuning of cortical function as individuals develop from infancy to adolescence (Casey et al., 2005). Essential functions – such as sensory and motor processing – and their supporting brain regions, mature first. Following the development of these regions, associated areas involved in top-down control of behavior develop (Gogtay et al., 2004; Sowell et al., 2003; Sowell et al., 2004). Studies of young to elderly adults suggest that behaviors, and their associated brain regions, decline in the reverse pattern (Albright, Kandel, & Posner, 2000; Persson et al., 2006; Persson & Reuter-Lorenz, 2008; Pfefferbaum et al., 1994; Pudas et al., 2013; Sowell et al., 2003). Essentially, basic functions are the least susceptible to decline, and higher-order functions are the first to be affected. During both development and decline, there are numerous contributing factors to the timing and outcome.

Neuroscientific and psychological investigations in the last two decades have resulted in publications on the healthy aging brain and the articulation of neural-based theories of cognitive aging (P. A. Reuter-Lorenz & Park, 2010). Still, a great deal of disagreement exists as to the exact mechanisms behind cognitive decline with age. Advances in tracking of cognitive changes in healthy adults across time indicates that decline mimics the patterns of brain development in early life in a backwards fashion where higher order functions decline first and basic functions go largely unaffected (Harada, Natelson Love, & Triebel, 2013). In development, there is a fine-tuning of cortical function as individuals develop from infancy to adolescence (Casey et al., 2005) and essential functions, such as sensory and motor processing (and their corresponding brain regions), mature first. Then, associated areas involved in top-down control of behavior develop (Gogtay et al., 2004; Sowell et al., 2003; Sowell et al., 2004). As such, basic functions are the least susceptible to decline, and higher-order functions are the first to be affected (Baltes, 1993; Harada et al., 2013). During both development and decline there are numerous contributing factors to the timing and outcome.

Several neurobiological variables have emerged as potential characteristics for decline. Some of the variables that have been found to exhibit nearly continuous age-related declines from age 30 onward are measures of regional brain volume (Arfanakis et al., 2013; Bartzokis et al., 2001; Bryant et al., 2013;

Colcombe et al., 2006), myelin integrity (Hsu et al., 2008), cortical thickness (Salat et al., 2004), serotonin receptor activity (Sheline, Mintun, Moerlein, & Snyder, 2002), hippocampal and striatal dopamine binding (Backman, Lindenberger, Li, & Nyberg, 2010; Backman, Nyberg, Lindenberger, Li, & Farde, 2006; Verney et al., 1985), accumulation of neurofibrillary tangles (Del Tredici & Braak, 2008), and concentrations of various brain metabolites (Kadota, Horinouchi, & Kuroda, 2001). Along with these biological factors, cross-sectional comparisons of cognitive abilities across adulthood indicate declines in average cognitive performance (e.g. processing speed and executive control) in individuals tracked from age 30 to 75 (Salthouse, 1996; Salthouse et al., 2003; Schroeder, Lipton, Ritter, Giesser, & Vaughan, 1995).

Empirically documenting aging

Since the early 1990s, psychology has experienced the emergence of cognitive neuroscience and a surge of novel information about the brain. Whereas molecular biology approaches have combined many scientific fields, cognitive neuroscience owes much of its rise to the joining of psychology and systems approaches of neuroscience (Albright et al., 2000). The field of developmental psychology has provided a roadmap to use in surveying the structure and function of the brain. Performing any higher-order cognitive task is linked to the activation of many parts of the brain. In cognitive tasks of higher complexity, such as memory formation and delayed recall, several brain areas are involved. Cognitive neuroscience approaches afford cognitive scientists the ability to draw parallels between human brain anatomy and behavior, allowing for identification of relevant brain regions and actions (Albright et al., 2000).

Brain aging is heterogeneous in the pattern of structural decline (Lockhart & DeCarli, 2014). Atrophy is present across the whole brain, but it is evident that the degree of change varies by area and type of tissue. The effects of aging are most evident in the cerebral cortex (e.g., superior frontal, middle frontal, and superior parietal cortex), and the rates of atrophy fluctuate, where some areas show more significant atrophy early and other areas later (Lockhart & DeCarli, 2014). Regions of the prefrontal cortex (e.g., dorsolateral & inferior frontal gyrus) are heavily recruited in working memory tasks.

Evidence from comparing performance on simple tasks, such as digit span, with more complex tasks requiring executive function, such as the N-back task, between younger and older adults shows that in the simpler tasks there is little difference in success or time to completion between the age groups. However, functional imaging indicates that whereas recognition (behavioral measure) is equivalent, there are pronounced activation (structural measure) differences (see Reuter-Lorenz & Park, 2010 for review). For example, it has been shown that frontally-mediated executive processes are recruited to support working memory performance but are more cognitive resource-dependent as well as cognitive resource-limited, resulting in diminished activation and performance on high-load tasks in older adults. If significantly more of the prefrontal cortex (PFC) is recruited for simple tasks, and it fails to activate on complex tasks, measures of activation in working memory tasks could be an excellent marker to track typical aging (relative to younger adults).

The study of memory serves as a useful example for illustrating changes in cognition with typical aging and helps differentiate between typical and atypical decline. Cognitive neuroscientists have elucidated some of the mechanisms of memory. There are two independent forms of memory: explicit and implicit memory (Albright et al., 2000). The study of these types of memory is itself subdivided into two parts: first, the systems problem of memory, concerned with the where, and second, the molecular problem of memory, concerned with the how (Albright et al., 2000). Explicit memory has been shown to involve, although briefly, the medial temporal lobe (MTL) - initially shown by work with patients like the famous H.M. in the studies of Penfield and Milner (1958), and confirmed in animal models (Milner, 1998). Studies support the possibility that the MTL, which commonly includes the hippocampus, may direct reorganization by binding together separate cortical regions that store the whole event memory. Researchers suggest that after a sufficient amount of time has passed, the hippocampal formation may no longer be needed for storage or retrieval, and long-term memory may become wholly dependent on the neocortex (Squire & Alvarez, 1995).

Studies of amnesic patients and healthy adults have confirmed that multiple memory systems exist and that those systems are in themselves mediated by distinct neural systems (Giovanello &

Schacter, 2012; Giovanello & Verfaellie, 2001). For example, working memory is unaffected in those experiencing amnesia, even when those patients show little to no long-term memory for new events (Baddeley & Warrington, 1970; Giovanello & Verfaellie, 2001). However, the reverse pattern is also observed. Patients with damage to the left inferior parietal lobe show decreased repetition and reduced auditory verbal span (Giovanello & Verfaellie, 2001; Murray, Ramage, & Hopper, 2001). Patients with damage to the left frontal regions are characterized by diminished phrase length and poor articulation (Murray et al., 2001; Shallice & Warrington, 1977). Additionally, imaging studies of healthy adults have shown that executive control processes are mediated by the cingulate and dorsolateral prefrontal cortices (Giovanello & Schacter, 2012; Giovanello & Verfaellie, 2001; E. E. Smith & Jonides, 1999). Thus, advances in testing and theory offer the opportunity to improve our assessment strategies, leading to the development of novel techniques for identifying, rehabilitating, and monitoring cognitive function across time.

The hippocampus is crucial for long-term episodic memory, but the exact role this structure plays is still controversial (Bird & Burgess, 2008). Electrophysiological studies in rodents have elucidated the neural bases of episodic memory (Aggleton & Brown, 1999; Bird & Burgess, 2008). Knowledge about the hippocampus has been acquired from a variety of sources. Some of this knowledge has been gleaned from studying patients with bilateral MTL damage or, with milder effects, selective damage to the hippocampus itself (Bird & Burgess, 2008; Milner, 1998; Scoville & Milner, 1957; Zola-Morgan, Squire, & Amaral, 1986). In aging, these patients are impaired in acquiring new explicit memories, while short-term memory, procedural memory, and long-term episodic memories are preserved. Given the evidence discussed above, it is unlikely that the hippocampus itself that is responsible for the decline in function for healthy aging adults as once thought, but decline in this area may be a useful indicator of atypical decline and future diseased states.

Relevant to the current study is the localization of higher order cognitive functions. Using cognitive neuroscience methods, one can discern both spatial and temporal resolution of brain function during a given task (Albright et al., 2000; P. A. Reuter-Lorenz & Park, 2010; Zamroziewicz & Barbey,

2016). In other words, these methods offer the ability to say when and for how long an anatomical area (where) is active and when information is being exchanged between areas. The use of joint or paired event-related potential (ERP), magnetic resonance imaging (MRI), and functional MRI (fMRI) methods allow the study of the brain based on electrophysiological, electromagnetic, and hemodynamic measurement, respectively. From this, one can determine which brain areas are activated by a task, how long the task is being processed, and the transfer of information and communication with other brain areas. I explain in the theoretical section below how the transfer of information and the bidirectional communication between brain areas may be vital to understanding cognitive aging. Recent work illustrates that it is possible to follow the flow of processing during cognitive tasks across brain areas and to determine whether areas have circular or bidirectional interactions (Anwar et al., 2016). For example, in cognitive aging, advances in cognitive science allow tracking of pathology across time. Using cognitive neuroscience approaches, scientists can now search functional changes such as the speed of processing, total activation of brain regions, and measures of executive control and memory function.

As noted in this section, age-related differences in cognitive function are well defined in the spatial domain. As such, I have primarily used spatial descriptions of cognitive aging. In the temporal domain, brain activation measures such as ERPs have been used to help understand atypical cognitive decline. However, in typically aging older adults, temporal activation is not clearly defined, and a greater understanding of temporal brain activation in healthy older adults would be beneficial. In this study, I attempt to show these similarities between task performance and temporal measures of brain activation using behavioral and electrophysiological assessments by evaluating the relation between behavioral outcomes in the MoCA and CANTAB with the temporal activation measures of ERPs.

Processing Speed

Processing speed underlies the ability to identify, discriminate, integrate, interpret, and respond to visual and verbal information. Responses for speeded tests are typically motoric (e.g., written response, check a response, etc.) or oral (e.g., saying an object's name, reading numbers or letters aloud). Behavioral measures of processing speed provide an estimation of how efficiently a subject can perform

basic tasks or processing of novel information. Whereas these tasks usually do not assess higher-order processes, they frequently require some degree of simple decision making and can indicate the automaticity of the process being evaluated, the efficiency of discrimination, and the speed of decision-making. From a neuroscience perspective, processing speed may be measured temporally as time to peak processing (ERP), or spatially as peak change in blood flow (fMRI, fNIRS). In cognitive aging, slowing in processing speed may be an indicator of decline.

The evidence for the role of age-related cognitive slowing as the cause of cognitive decline is pervasive in the literature (Salthouse, 1996), but is not the only contributing factor (Finkel, Reynolds, McArdle, & Pedersen, 2007; Sliwinski & Buschke, 1999). Methods such as measuring decision speed or perceptual speed can be used to evaluate the processing speed of older adults (Salthouse, 2000). Decision speed is based on the time to respond to complex content and confounded by an individual's relevant cognitive abilities. Perceptual speed, in contrast, is measured by the speed of responding to simple content such as simple comparisons. Additionally, psychomotor speed (e.g., repetitive finger tapping) or reaction time can be used. Mental chronometry, the study of reaction time, can be assessed in a choice reaction time paradigm with visual stimuli and manual keypress responses. Finally, cognitive neuroscientists observe variables that are postulated to reflect the time course of an innate response, such as the latency of a particular component of a brain's response to a stimulus (e.g., event-related potentials; Salthouse, 2000). Latency to peak processing in the ERP and reaction time in the CANTAB were used in the present study to investigate cognitive decline.

Working memory

Speed of processing is highly correlated with short-term and working memory (Salthouse, 1991; Schneider-Garces et al., 2009; Verhaeghen, 2011). Working memory (WM) is typically defined as a system for temporarily holding information and using it for complex cognitive tasks (Baddeley, 1992) and has acted prominently in cognitive theories of aging (Salthouse, 1996). Additionally, WM has become the central focus of neuroscience research on aging (Rajah & D'Esposito, 2005; Rypma & D'Esposito, 2000). WM can be tested using span tasks, where stimuli such as shapes, letters, or sounds are presented in a

specific order, and the participant must repeat the stimuli in the correct order immediately following a delay. WM declines with age and is linked to changes in activation in the dorsolateral prefrontal cortex (Rypma & D'Esposito, 2000). Recruitment of additional prefrontal circuitry at the less challenging levels of a WM task results in a higher level of peak activation in older adults at lower task demands when compared to younger adults (Cappell, Gmeindl, & Reuter-Lorenz, 2010; Salthouse, 1991; Schneider-Garces et al., 2009). In this study, behavioral and electrophysiological measures were used to investigate the age-related decline in WM.

Executive Control

Executive control is comprised of mechanisms that modulate various operations of cognitive sub -processes and regulate the dynamics of cognition (Carlson & Moses, 2001; Miyake et al., 2000; Pennington & Ozonoff, 1996). Cognitive control is the brain's ability to configure itself for the performance of tasks through adjustments in perception, response bias, and the active maintenance of contextual information (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Botvinick, Cohen, & Carter, 2004). In aging, deficits in cognitive function can be widespread across the cognitive system. Theories have emerged (e.g., CRUNCH and STAC-r;Goh & Park, 2009; P.A. Reuter-Lorenz & Cappell, 2008; P. A. Reuter-Lorenz & Park, 2014) in which scientists suggest that increased activation in the cortical areas of the brains of older adults indicates decline in neural efficiency and compensation by activation of additional brain areas to perform cognitive tasks (Cappell et al., 2010; Mattay et al., 2006; Persson et al., 2006; Pudas et al., 2013; P.A. Reuter-Lorenz & Cappell, 2008; Schneider-Garces et al., 2009). Thus, changes in overall cortical function (e.g., slower processing) are likely, and a diverse set of executive control functions may show a decline.

The excitatory or activational aspects of attention are preserved in older adults, while inhibitory processes become deficient (Hasher & Zacks, 1988; R. West & Alain, 2000). However, more recently it has been suggested that aspects of attentional selection may also be compromised with age (Amer, Campbell, & Hasher, 2016; Johnson, Mitchell, Raye, & Greene, 2004; P. A. Reuter-Lorenz & Park, 2010). With the emergence of brain imaging, specific inhibitory impairment has been documented in

older adults. In contrast to Hasher et al. (1988), more recent work suggests that activational aspects are indeed compromised with age (Johnson et al., 2004) showing a marked difference in dorsolateral prefrontal cortex. There are several correlates of inhibitory dysfunction with age that indicate altered engagement of prefrontal processes and impaired top-down control (P. A. Reuter-Lorenz & Park, 2010). Quantifying the ability to inhibit prepotent responses or the ability to detect differences between rapidly occurring stimuli has been proven an effective method to differentiate cognitive function between individuals (Carlson, Moses, & Breton, 2002). The use of inhibitory control and attention selection to define cognitive aging have been challenged, but brain imaging techniques support their utility by documenting impairments in associated brain areas. Changes in attentional selection and the ability to successfully perform detection tasks are evidenced in brain-based measures that involve an altered engagement of prefrontal processes and impaired top-down control at earlier stages of input processing (Amer et al., 2016; Braver et al., 2001; Hasher, Stoltzfus, Zacks, & Rypma, 1991; P.A. Reuter-Lorenz & Cappell, 2008; P. A. Reuter-Lorenz & Park, 2010; Weisz & Czigler, 2006; R. West & Alain, 2000; R. L. West, 1996; Wolk, Manning, Kliot, & Arnold, 2013). By identifying changes in attentional selection and performance on detection tasks we may be able to more accurately identify age-related cognitive decline and do so earlier.

Detection tasks could prove to be a reliable way to differentiate atypical from typical cognitive aging (Friedman, 2003; Kirino, Belger, Goldman-Rakic, & McCarthy, 2000; Pato & Czigler, 2011; Setti et al., 2011; Sugamata, Zeng, Hozumi, Tanaka, & Hirata, 2002). In this study, the ability to perform detection tasks will be tested in the RVP and active detection task and will be used to investigate age-related decline. In order to appropriately investigate age-related decline, however, it is crucial also to understand other ways cognitive aging is explained and some of the factors that contribute to (and possibly prevent) decline.

How do we explain cognitive aging?

As discussed earlier, in the past learning and memory deficits in older adults were attributed to hippocampal damage, but advances in the specificity of testing and better imaging techniques indicate

that this may not be true in typical aging (Allegri, Glaser, Taragano, & Buschke, 2008; Bizon, Lee, & Gallagher, 2004; Claesson, Cusack, O'Sullivan, & Greene-Diniz; Driscoll, 2003; Driscoll et al., 2006). In typical aging, a decline in the frontal cortical activity is suggested as a better identifier of cognitive decline (Gutchess et al., 2005; Lister & Barnes, 2009; MacPherson, Phillips, & Della Sala, 2002; Persson et al., 2006; Persson & Reuter-Lorenz, 2008; Pudas et al., 2013). I suggest that there is a distinct difference between cortical activation in individuals with decline that is, or will become, atypical compared to those experiencing typical decline. In the present study, I have identified these differences by comparing cortical activity in a well-characterized cohort of typically and atypically declining older adults. This study provided the unique opportunity to follow-up with an original cohort to track the progression of, and advancement to, mild cognitive decline (MCD) while also tracking changes in characterizing information and cognitive and behavioral assessments.

Even though aging research has a rich history that resulted in theories on which we can base interventions, cognitive issues in the aging population are still pervasive (Beckman & Ames, 1998; Brown & Park, 2003; Harman, 1956; Hayflick, 1979, 2007; Medawar, 1952; Salthouse, 2000; Salthouse et al., 2003; Vina et al., 2013; G. C. Williams, 1957). Historically, researchers relied on psychological constructs of cognitive aging using behavioral assessments to infer problematic states (Botwinick, West, & Storandt, 1978; Neugarten, 1979). However, as discussed, researchers utilizing cognitive neuroscience approaches to cognitive aging have provided structural and functional information to define age-related cognitive decline (Goh & Park, 2009; Kugler, Taghavy, & Platt, 1993).

Theoretical Approaches

In an attempt to explore the “black box” of the brain, cognitive scientists have been attempting to incorporate new models of cognition into theories of cognitive aging (Clark, 2013). When observed on a basic level, the brain is just a bundle of cells that create what we call perception. This bundle of cells accomplishes “perception” by matching incoming inputs with top-down expectations and predictions using hierarchical generative models (Clark, 2013) that aim to minimize prediction error within a cascade of cortical processes. When utilized in developmental cognitive neuroscience approaches, the hypothesis

of the brain as a hierarchical prediction machine provides a framework to understand cognition and behavior. If the brain is applying error-correction to a cascade of mental processes, then it is likely to generate unintended errors in later stages by replacing pertinent information. This natural occurrence of errors has led to an evolution of a system for brain processing that contains many checks and balances in underlying processes. Many of these processes are a one-way cascade with checkpoints throughout, but some of these checkpoints may send information backward.

In recent studies, it has been shown that some cortical processes may be bidirectional or circular and not just unidirectional (see Anwar et al. (2016); Zainuddin and Thuret (2012) for logic). If true, in these cascades of processing, high-level systems (e.g. MTL-PFC) show they may be predicting inputs and influencing, or "communicating," these inputs to lower-level systems (ex. sensory system). This influence is likely based on the high-level system's emerging model of casual structure - the source of the signal. An error in predicting the lower-level input results in the higher-level model adapting to reduce discrepancy (Casey et al., 2005; Clark, 2013).

We have all experienced being fooled by illusions of depth or size as our higher-order executive functions attempt to correct, or make sense of, what our lower-level systems are observing. These processes, when operating over multiple high-level models that are, allow for a brain that encodes a rich body of information that identifies the source of the signals that frequently flood it (Clark, 2013). The variety and consistency with which stimuli bombard these systems mean that the brain is continuously computing multiple probability distributions at any given moment. This probability calculation is constant, where the predictive probability of a given outcome is continually changing as the different sets of outcomes are distinguished only by their relative probability of actually occurring.

Not all systems have been proposed as bidirectional, but many have (such as memory). Bidirectionality is important because the bidirectional hierarchical structure allows the system to infer the prior stimulus and calculate new probabilities. By doing this, the system can use its best current model by employing "iterative estimation" (Clark, 2013; Dempster, Laird, & Rubin, 1977; Neal & Hinton, 1998). By slight extension of the idea of hierarchical generative models, one can assume that the brain can also

adapt, generating new routines to reduce the discrepancy between high- and low-level systems, diminishing the impact of a poorly functioning cog in the machine that is the brain. Via this extension, I am suggesting an hypothesis of compensation to explain (at least to some extent) cognitive dysfunction. I suggest an application of this theory, that some form of compensation or reutilization is occurring, where over-activation (compensation) would be measurably higher in MCD individuals compared to non-MCD individuals, or, as suggested below, at high levels of difficulty re-utilization cannot account for the differences and a failure in performance is observed.

In older adults, in order to reduce error and maintain minimal discrepancy between cognitive systems, there is activation of additional brain areas resulting in over-activation. This over-activation would indicate that scaffolding of resources among systems could diminish resource deficits due to age. The Scaffolding Theory of Aging and Cognition (STAC; P.A. Reuter-Lorenz & Cappell, 2008) model integrates these ideas with ideas from cognitive neuroscience. STAC is based on the idea that the aging brain is subject to a range of challenges, which can include but are not limited to, amyloid deposits, neuronal atrophy, and deterioration of white matter. The result is functional alterations, such as dedifferentiation and decreased medial-temporal lobe activation (Persson & Reuter-Lorenz, 2008; P. A. Reuter-Lorenz & Park, 2010). According to STAC, the brain responds to these challenges by forging alternative neural circuitry. The result is a network that may function less efficiently than the networks of younger individuals, but still produce the same outcomes and behaviors. This scaffolding process permits individuals to maintain a high level of cognitive function even in advanced age. Its occurrence is apparent in the pattern of over-activation, primarily in the frontal cortex. It is important to note, however, that whereas the capacity for the aging brain to perform processes such as neurogenesis decline with age, the STAC model assumes that all mechanisms remain functional enough to provide the means for creating new neural circuitry. This assumption is not particularly viable as a wholistic application to cognitive aging, however, because in some circumstances new neural circuitry is not created.

Neuroimaging has indicated that older adults sometimes show greater brain activation than younger adults when observed on identical tasks (Goh & Park, 2009; P.A. Reuter-Lorenz & Cappell,

2008; P. A. Reuter-Lorenz & Park, 2014). One interpretation of this finding is that the brain of older adults recruits greater resources on lower-level cognitive tasks than young adults. Declines in neural efficiency with age lead to engaging more neural circuits (P.A. Reuter-Lorenz & Cappell, 2008; P. A. Reuter-Lorenz & Park, 2010; Schneider-Garces et al., 2009; Souza, 2016). I suggest that this fits the conclusion above: correction made by the predictive machine. This over-activation in older adults results from utilization of frontal and bilateral recruitment, compared to more focal activation in younger adults. As load increases younger adults will shift to an overactive or bilateral pattern to address the increased demands (Goh & Park, 2009; P.A. Reuter-Lorenz & Cappell, 2008; P. A. Reuter-Lorenz & Park, 2014). Older adults have likely tapped out their neural resources at the lower load. As a result, in the more demanding task, older adults will show under-activation and a decline in task performance compared to their younger counterparts - known as the compensation-related utilization of neural circuits hypothesis, or CRUNCH. CRUNCH is upheld in studies of executive control and working memory (Cappell et al., 2010; Mattay et al., 2006; Schneider-Garces et al., 2009), and a longitudinal model indicates that overactivation predicts future cognitive decline (Persson et al., 2006).

A crucial component to STAC and CRUNCH is that this scaffolding is not a process that begins in old age, but a process that is occurring throughout the lifespan. Neuroscientific findings indicate that neurogenesis, synaptogenesis, neuronal apoptosis, and synaptic pruning are occurring at a rapid rate in early life as the child's existing circuity is utilized to scaffold the creation of new connections, thereby allowing acquisition of new cognitive skills (Petersen, van Mier, Fiez, & Raichle, 1998). The STAC model was updated in 2014 (Reuter-Lorenz & Park, 2014) to reflect this utilization of the model at all ages. The revised STAC (STAC-r) is redefined in light of more recent longitudinal data that incorporate measures across the lifespan. STAC-r improves upon the previous model, allowing it to better predict and lend understanding to cognitive status and the rate of cognitive change over time.

Factors that influence cognitive decline

Just as describing age-related cognitive decline is important, understanding the factors that contribute to it is of equal value in attempting to slow or stop this change. There is a large list of factors

that may contribute to cognitive decline, but the first factor should come as no surprise: age. Aging is the most prevalent risk factor for experiencing cognitive decline and developing cognitive impairments like dementia (Sahathevan, 2015). Other non-modifiable factors, aside from age, may include someone's sex or genetic background. A person's genotype and resulting phenotype can have profound impacts on the decline observed with aging. An analysis in twin studies has indicated that some cognitive domains associated with aging are highly heritable, such as processing speed and general cognitive ability (Lee et al., 2011). Furthermore, a decline in the expression of genes related to mitochondrial metabolism is predictive of selective neuronal vulnerability, a characteristic of neurons in brain areas most affected by typical aging (X. Wang, Michaelis, & Michaelis, 2010). Nutrigenomics indicate an individual's genotype impacts his/her ability to obtain and process certain vital nutrients for healthy brain function (Kussmann, Krause, & Siffert, 2010). Moreover, with age comes years of exposure to other factors that may influence cognitive changes such as environmental exposure, diet and nutrition differences, physical activity, job type, social wellbeing, and educational attainment, just to name a few. Whereas there have been significant advancements in describing cognitive aging, the significant intraindividual differences of studying someone with 65+ years of "factors" makes studying and applying theories of cognitive aging difficult.

Individual Characteristics and Disease States Contributing to Cognitive Decline

Body composition is heavily linked to development of diseases as well as cognitive function and the risk for development of cognitive disturbances in adults. Obesity in middle age has been identified as a risk factor for developing cognitive decline in old age (Wirth & Smoliner, 2015). This relation is also true in the opposite direction, where lower body mass index showed lower rates of decline in old age (Yaffe et al., 2009; Zhou, Flaherty, Huang, Lu, & Dong, 2010). Other studies conclude that higher BMI is related to lower cognitive performance throughout all age groups (Gunstad et al., 2007; Yaffe et al., 2009; Yaffe et al., 2004). Importantly, intentional weight loss in obese participants is shown to enhance cognitive performance in adults without dementia (Siervo et al., 2012).

Body mass itself is likely not the cause of decline in mental capacities and likely serves as a proxy for underlying issues, especially in the elderly, because increased BMI is significantly correlated with increased incidents of diseases that increase inflammation, reduce blood flow, and limit nutrient uptake. Moreover, the distinction between intentional weight loss and weight loss that is unexpected is important in older adults. In elderly individuals, a decline in BMI that was unintentional could include an unhealthy loss of muscle and bone in addition to fat (Shatenstein, Kergoat, & Reid, 2007). The likelihood for a loss of muscle and bone may be so high that, for elderly who are obese, staying that way could be a sign of stable cognitive function (Inelmen, Sergi, Coin, Girardi, & Manzato, 2010). Because obesity and fat mass throughout the lifespan are modifiable risk factors for cognitive decline with age, I collected data on the BMI, waist circumference, exercise level, and diet of my participants.

Aging is associated with an increase in inflammation due to typical environmental exposure, cellular aging, certain diseases (e.g. diabetes), or even dysbiosis in our body systems such as the gut that can cause numerous health effects. Neuroinflammation in particular involves microglia that contribute to deficits in neural plasticity. Inflammation, which is evidence of an immune response, is one of several factors known to regulate adult neurogenesis (Kohman, DeYoung, Bhattacharya, Peterson, & Rhodes, 2012). This impact on neurogenesis is indicated by microglia shown to express an inflammatory phenotype which reduces cell proliferation, survival, and function of new neurons. In contrast, microglia displaying the alternate phenotype have been shown to support adult hippocampal neurogenesis (Kohman et al., 2012). Therefore, the level of inflammation in otherwise healthy older adults should correspond with cognitive function. Arfanakis and colleagues (2013) showed these hypothesized differences in microglia and inflammation to be accurate by performing MRI DTI on non-demented, otherwise healthy, elderly subjects with varying levels of inflammation. Their findings indicated that higher levels of inflammation might be associated with lower integrity of microstructures of the corpus callosum of elderly individuals, which was also paired with reduced higher-order visual cognition with increased inflammation. But again, the causes of inflammation can be widespread. With this study, I attempted to address a few believed causes of inflammation by controlling for age and some body composition factors,

evaluating measures of gut dysbiosis and, on a limited scale, determining the relations among dietary factors, cognitive function, and the gut-microbiome.

Protective Factors

Several factors, such as higher education attainment and higher socioeconomic status, are protective against age-related cognitive decline (Alwin, McCammon, Wray, & Rodgers, 2008; Sheffield & Peek, 2011). For this review, however, the focus will be on three closely related and easily modifiable lifestyle factors that have been shown to have a positive impact on brain function across the lifespan: physical activity, diet, and nutrition. I then argue that these last two may be crucially linked to the gut microbiome for processing and utilization by the body.

In humans, epidemiological evidence shows that physical activity is associated with increased cerebral blood flow and neuronal connectivity (Burdette et al., 2010) and improved brain volume (Colcombe et al., 2006). However, results of randomized trials of physical activity and tests of cognitive function have been mixed, at best. Some promising evidence exists that better physical fitness is associated with improved attention and processing (Pontifex, Hillman, & Polich, 2009), and that exercise has mediating effects on cognition by positively influencing depression scales, stress levels, and sleep and diet quality (Sibley, 2008). A key example with a large cohort of older adults, Sink and colleagues (2015), was a clinical trial designed to test if a 24-month physical activity program resulted in improved cognitive function when compared to a health education program in a large cohort (n=1,635) of elderly individuals. Whereas the physical activity group experienced no decline in cognitive function, there were no significant differences between those older adults who participated in the physical activity compared to the health education only group. This study would benefit from a control group to identify the potential cognitive effect of the health education. Despite epidemiological evidence showing support for the positive impact of exercise on cognitive function in elderly adults, clinical trials have failed to reinforce this finding. Therefore, there is the potential that physical activity may keep the aging brain stable for some time, but it may not improve function when used alone. It is possible that there is a synergy between

the different aspects of a healthy lifestyle (e.g., between diet and exercise) that must be present for the benefits to emerge.

In today's modern world, food has become increasingly plentiful, palatable, and affordable, while physical activity has become increasingly unnecessary and obstructed. In America, for example, the chronic excess of calories eaten compared to calories burned is an important factor contributing to decline in cognitive function (Cheatham, 2014; Mirowsky, 2015). The ever-growing substitution of mechanical power for human physical activity and increasingly sedentary work-life activities challenge the body's need for cardiovascular, respiratory, and metabolic fitness (Mirowsky, 2015). Aside from the increased incidence of obesity and its impact (see previous discussion above), the increase in caloric intake does not coincide with an increase in vital nutrients (Davis, 2009; Davis, Epp, & Riordan, 2004). Diet and resulting nutrient intake of the individual could have a substantial impact on cognition and be an important protective factor against age-related cognitive decline (Donini, Poggiogalle, Pinto, Giusti, & del Balzo, 2015; Guesry, 1998; Mirowsky, 2015).

In addition, many foods contain fewer vital nutrients than their earlier forms from decades past (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011). The United Nations Standing Committee Report in 2006 stated that even in industrialized nations diet alone was not sufficient to attain needed nutrient for healthy body function (*UN Report of the Standing Committee on Nutrition at its thirty-third session*, 2006). More recently, however, the National Institute on Aging have begun to recommend against many supplements that were once recommended for older adults as they are ineffective or, in some cases, harmful (DHS, 2015; W.H.O., 2017). Regardless of over- or under-nutrition, older adults can still be malnourished due to a plethora of nutritional, social, functional, or psychological factors (Donini et al., 2015). Therefore, it is not only important that an individual monitor energy expenditure and activity level but also that he or she consume nutrient-dense diets that support cognitive function.

In this study, I investigate, on a limited scale, the impact of some diet and nutrition measures especially as they relate the to gut microbiome. Recent studies have shown that consumption of diets rich in antioxidants and anti-inflammatory components may lower the risk of cognitive decline and alter our

gut-microbiome (discussed in the next section) (Dauncey, 2014; Joseph, Cole, Head, & Ingram, 2009; Mena, Calani, Bruni, & Del Rio, 2015; J. Spencer, 2010).

A review of nutritional compounds thought to be memory enhancers (e.g., phosphatidylcholine (PC), citicoline, antioxidants) suggested mild effects (McDaniel, Maier, & Einstein, 2003). Antioxidants are suggested to help neutralize free-radicals and oxidative stress, which damage tissues and increase with age. Results with vitamin E and C showed no effect in MCI or AD patients on memory measures (McDaniel et al., 2003). However, antioxidants like polyphenols have shown an impact on reduction of oxidative stress and may improve cognitive functions in older adults (Cabezas et al., 2015; Casadesus et al., 2004; Krikorian et al., 2010). Polyphenol rich foods have also been shown to attenuate microglial activation and reduce inflammatory markers that are both attributed to improved gut microbiome diversity (Mena et al., 2015; Shukitt-Hale et al., 2008; C. M. Williams et al., 2008; Willis et al., 2010).

The Microbiome

Emerging hypotheses about the gut microbiome and its relation to the brain are revealing an all-encompassing system within the body that can explain, in part, the effects of diet on cognitive function (Caracciolo, Xu, Collins, & Fratiglioni, 2014; Leung & Thuret, 2015b; Noble, Hsu, & Kanoski, 2017). Inter- and intra-individual differences are constant points of consideration in studies of age-related cognitive decline. Non-modifiable factors of the individuals can explain some of the differences. Others, such as an individual's ability to take in and process nutrients, can change across the course of the lifespan or as the result of lifestyle choices of the individual. An individual's gastrointestinal flora can have an enormous impact on the ability of the gut microbiome to deliver the ingredients needed for a healthy brain.

The human gastrointestinal tract (GIT) is a natural habitat for a large and active community of microbiota known as microflora. These bacteria number 10^4 cells and can be classified into over 1,000 diverse types (Lim et al., 2015). Recent exploration of the gut microbiome has shown that an individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior (Claesson et al., 2011; Cryan & Dinan, 2012; Dinan & Cryan, 2017; Dinan, Stilling, Stanton, & Cryan,

2015). This influence is bidirectional and essential for maintaining homeostasis. Top-down communication of the brain to the GIT can change blood flow and secretions of digestive factors (Grenham, Clarke, Cryan, & Dinan, 2011).

With the exception of the study of infection, the fields of neuroscience and microbiology are rarely studied together. Progress in the study of gut microbiota, however, and its influence on human health and disease has triggered an interest in the manner in which this community affects normal physiology. The skin and mucosal surfaces of most vertebrates contain a vast array of microbiota containing bacteria, fungi, parasites, and viruses. Specifically, more than 100 trillion bacteria reside in the human GIT. This number is remarkably 10-100 times more than the quantity of eukaryotic cells in our bodies (Morgan & Huttenhower, 2012). Colonization of gut microbiota begins at birth and is established by the first three years of life resulting in a mutualistic symbiosis between host and microorganism. Gut microbiota contribute to a variety of important developmental and homeostatic processes in adult life. For example, gut microbes play a key role in metabolic function by breaking down complex polysaccharides in the diet, regulation of gut motility, GI barrier homeostasis, and fat distribution. Although the variety of individual microorganisms varies widely between individuals, it has been suggested they all fall into three separate enterotypes, each described by a single genus; *Bacteroides, Prevotella*, and *Ruminococcus*.

The influence on gut-brain communication, brain function, and behavior is bidirectional and essential for maintaining homeostasis (Grenham et al., 2011). Moreover, the gut microbiome can influence brain function and behavior directly via production of metabolites essential for cognitive function (i.e., memory and attention; Manderino et al., 2017; Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014), immune activation (e.g., inflammation; Bajaj et al., 2012; Biagi et al., 2010; Grenham et al., 2011; McHardy et al., 2013; Noble et al., 2017), and microbial neuro-metabolites (Cryan & Dinan, 2012; Dinan et al., 2015; Moloney, Desbonnet, Clarke, Dinan, & Cryan, 2014; Vernocchi, Del Chierico, & Putignani, 2016). There are multiple ways the gut microbiome can influence CNS function. One aspect that has amassed a convincing number of confirmatory results is the role of the vagus nerve. Vagus-dependent pathways have been shown to be involved in microbiota-brain communication, with vagotomy

preventing microbiota-modulated changes in behavior (Leung & Thuret, 2015b). With both efferent and afferent divisions, the vagus nerve plays a fundamental role in enabling signals in both directions. Activiation of the vagus nerve is also known to have marked anti-inflammatory capacity, which is protective against microbial-induced sepsis (Dinan et al., 2015). Potentially one of the most important examples of neurotransmitter control by the microbiome is the relation between levels of the probiotic bacterium *Bifidobacterium infantis* and altered levels of metabolized tryptophan into serotonin (Grenham et al., 2011; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Furthermore, indirect effects of the gut microbiota on the innate immune system can result in alterations in behavior due to changes in circulating levels of pro- and anti-inflammatory cytokines that directly affect brain function in areas such as the hypothalamus – where corticotrophin releasing hormone, the dominant regulator of the HPA axis, is released. Brain derived neurotrophic factor (BDNF) plays a pivotal role in supporting the survival of existing neurons, and encourages the growth of new neurons and synapse formation. Studies in germ free animals have produced evidence that the lack of a few key microbes in the gut limits or completely stops the generation of BDNF, resulting in significant reductions in activity observed in the dentate gyrus of the hippocampus and related behavioral disfunction.

The emerging hypotheses about the gut microbiome and its relation to the brain are crucial to understanding the effects of diet on cognitive function (Caracciolo et al., 2014; Leung & Thuret, 2015b; Noble et al., 2017). Microbial diversity decreases with age, but there is stability in the total number of microbes (Biagi et al., 2010; Claesson et al., 2011). Scientists are beginning to build a body of research on the so-called "gut-microbiome-brain" axis. Through this axis, the gut microbiome affects behavior and modulates brain plasticity via mechanisms such as inflammation and altered blood flow (Faraco et al., 2018; Santoro et al., 2014) and change in neurotransmitter availability (Bravo et al., 2011; Janik et al., 2016). So-called “inflammaging,” or chronic low-grade inflammation, as well as specific inflammatory diseases like the metabolic syndrome, arthritis, and fibrosis, has been shown to have a strong relation to all forms of aging - particularly cognitive decline (Arfanakis et al., 2013; Bennett et al., 2015; Howcroft et al., 2013; Kohman & Rhodes, 2013; Misiak, Leszek, & Kiejna, 2012; Mu, Ogawa, & Kawada, 2010;

Noble et al., 2017; Santoro et al., 2014; X. Wang et al., 2010; Yaffe et al., 2004). I predict that this inflammation may be partially mediated by microbes in our gut and therefore, dysbiosis of the gut microbiome.

Researchers exploring the gut-microbiome-brain axis provide evidence for the gut microbiome's modulation of brain function, which could contribute to changes in cognition during aging, but more investigation is required (Anderson et al., 2017; Caracciolo et al., 2014; Leung & Thuret, 2015a; Lim et al., 2015; Manderino et al., 2017). Pyrosequencing-based characterization of the human intestinal microbiome using 16s rRNA-based methods has provided evidence of altered bacterial composition in individuals experiencing cognitive decline compared to individuals with typical cognitive function (Bajaj et al., 2016; Bajaj et al., 2012; Rampelli et al., 2013), but those studies have been limited to institutionalized populations. Studies investigating cognitive function in healthy older adults are incredibly limited (Anderson et al., 2017; Manderino et al., 2017), and fail to use any of the cognitive neuroscience or developmental methods used in the current study. By adding to the growing evidence for the association with cognitive function and differences in microbiome diversity and composition, a study such as this one could provide evidence for the role of the gut microbiome in age-related cognitive decline. I hypothesized that individuals with poorer microbial diversity as measured by a calculated Shannon alpha diversity score would show poorer cognitive function as measured by behavioral (CANTAB) and electrophysiological (ERP) measures.

Several metrics were considered for Specific Aim 4a of the present study to best classify the microbiome samples of the participants. The raw metrics of Richness (total number of identified genera) and their Relative Abundance was too variable across the sample (6,000 reads to 22,000 reads for 90 to 240 genera), and unidentified genera could not be included in counts. One common solution for poorly understood and classified biomes is the use of diversity indices. A diversity index is a mathematical measure of species diversity in a community, and these metrics typically provide more information about community composition than simple Richness because they take the relative abundance of different species into account. The use of relative abundance provides important information about rarity and

commonness of a species in a community, which is essential when trying to understand a poorly understood community such as the microbiome of the healthy older adult gut. There are two types of diversity indices relevant to this study, alpha and beta diversity. Alpha and beta diversity indicate the number of species found in a particular community (e.g., an individual's gut) and the variation of the species composition between two communities (e.g., comparing two microbiome samples), respectively. Beta diversity accomplishes its comparison by taking into account the alpha diversity of the communities being compared and the number of unique species in each community.

Shannon alpha diversity is one of the most common alpha diversity metrics in gut-microbiome research, and was selected as the alpha metric for Specific Aim 4. Shannon diversity accounts for both the abundance and evenness of the species present – the proportion of species relative to the total number of species is calculated, and then multiplied by the natural logarithm of this proportion before being summed across all species and multiplied by -1.

Beta diversity calculation is more complicated and can be seen as the sum of two components: turnover and nestedness. Turnover is the difference between communities solely in relation to which species exist in each. Nestedness is how much the species composition of a site with a lower species richness is a subset of a site with higher species richness. Therefore, one could calculate and compare beta diversity values based on species presence/absence and/or relative abundance, which means using a measure of alpha diversity to calculate beta diversity. Some commonly used beta metrics used in microbiome analyses include Bray Curtis, Euclidean, and Unifrac (weighted (quantitative) or unweighted (qualitative)). Unifrac is unique in that it uses comparison to the phylogenetic tree, allowing it to factor in evolutionary distances to the results (Hamady & Knight, 2009). A weighted unifrac highlights diversity differences due to changes in relative taxon abundance (e.g., a set of taxa flourish because a limiting nutrient source becomes abundant), whereas an unweighted unifrac is only informative when communities differ primarily by what can live in them (e.g., high temperatures), in part because abundance information can obscure significant patterns of variation (Lozupone et al., 2007).

Screening & Behavioral Testing

Now that some of the factors known to impact cognitive aging have been discussed, it is vital to understand in more detail how age-related cognitive decline is tested. Fluid cognitive abilities (i.e., memory, attention, and cognitive speed) can be tested alongside crystalized abilities (i.e., language, general knowledge, reading) in cognitive tests to evaluate the cognitive fitness of an individual. In cognitive aging, particularly in cases of decline with dementia, crystallized as well as fluid abilities can be influenced. Targeted assessments can serve as quick screening tools to determine the cognitive state of older adults and serve as an inexpensive way to determine if additional evaluation is necessary. However, certain considerations should be taken in to account when selecting a test, such as which test is most appropriate to use and the procedure for testing. A balance must be struck between a test that is brief enough to be done quickly and efficiently to minimize burden on participants and a test that is detailed enough to evaluate relevant domains of cognitive function effectively to provide an in-depth assessment of an individual's strengths and weaknesses. Ease of administration, scoring, and test-taking time are important aspects in the choice of cognitive tests, as is the sensitivity and specificity of the test. An additional consideration when serial assessments are required is whether practice effects bias the performance.

The following descriptions and proposed testing practices have been adapted from the APA Working Group on Older Adults and its evaluation by Mahendran and colleagues (Mahendran, Chua, Feng, Kua, & Preedy, 2015; "What practitioners should know about working with older adults," 1998). It is important to note, though, that there is a great deal of inter-individual difference in age-related cognitive decline as well as intra-individual variability in the extent of decline across domains. Physical health, disease, current life events, diet, nutrition, and the individual's premorbid cognitive function all play vital roles in the individuals' testing outcomes (Mahendran et al., 2015). These factors can vary by day, and even hour or minute, depending on the factor. These points are particularly important for clinicians whose goal is to efficiently and effectively diagnose patients who are experiencing decline. The development of clinical cognitive assessments that combine tests of multiple domains, such as MMSE,

MoCA, WAIS-IV, and CANTAB might be the answer for clinicians as long as they maintain a grounding in the primary literature.

However, this is only true if the clinician or researcher controls/tests for the additional, non-domain-specific factors that may be at play (Mahendran et al., 2015). To diminish these confounds and improve validity and reliability of these tests, the clinician or researcher should limit stress on the individual, use only tests designed for older adults, be certain the individual is in no pain or distress, and make sure testing time and duration is suitable for optimal cognitive functioning in older adults (de Jager et al., 2014; Mahendran et al., 2015). The researcher can use questionnaires to identify outside stress, anxiety, and depression in the individual's life, as well as get information about when and what they eat and the amount of exercise they get daily. In the testing environment, the researcher should be sure to familiarize the individual with the environment, ensure adequate lighting and comfortable temperature, and make certain the participant understands the instructions (Mahendran et al., 2015).

MMSE, MoCA, and WAIS

The two most common assessments used with older adults are the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). The MMSE consists of 30 items that cover verbal function, memory abilities, and construction (Mahendran et al., 2015). Because the MMSE screens crystallized abilities, its utility in detecting early or mild cognitive changes in healthy adults is limited and when compared to other screening methods scores the lowest at sensitivity to MCD with a ceiling effect observed in healthy populations. The MoCA is a well-validated and reliable screening tool for detecting age-related changes in cognition as well as detecting other cognitive dysfunctions (Mahendran et al., 2015; Nasreddine et al., 2005; T. Smith et al., 2007). The cognitive functions tested are attention and working memory, short-term recall, visuospatial abilities, language, and executive control, such as divided attention, and semantic fluency and abstraction. The MoCA is superior to other screening tests of cognitive decline in detecting MCI and early AD (Nasreddine et al., 2005). When compared to the other most common screening tool for cognitive decline - MMSE - the sensitivity and specificity of MoCA to detect MCI were 90% and 87% compared to 18% and 100% using the MMSE

(Nasreddine et al., 2005), respectively. The sensitivity of the MoCA is vital to the study for characterization of the study population.

The Weschler Adult Intelligence Scale (WAIS-IV) is one of the most commonly used tests in clinical practice (Archer, Buffington-Vollum, Stredny, & Handel, 2010). The WAIS-IV is an excellent predictor of several measures due to its sensitivity to the neuropsychological deficit (Lezak, Howieson, & Loring, 2004). Additionally, the test provides the individual with strengths and weaknesses that could be used for treatment planning or intervention. WAIS-IV assesses multiple areas of intellectual ability, which provides the researcher or clinician with an overall IQ score as well as the specific index scores. WAIS-IV is comprised of 10 core subtests and five supplemental subtests that span four indices. Those indices are verbal comprehension, perceptual reasoning, working memory, and processing speed (Wechsler, 2014).

CANTAB

As the study of age-related cognitive decline evolves and matures, it is crucial that there are reliable, valid, and sensitive measures developed to identify and track the development of decline. Behavioral tests that can act as proxies for the underlying processes identified by expensive and time-consuming brain imaging techniques will be an essential step in improving health outcomes in an aging population. These tests should assess how the individual responds to everyday challenges and should discriminate between different states with the sensitivity to parse intra- and inter-individual differences (de Jager et al., 2014).

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a computer interface for assessing cognitive function. The use of semi-automated computer interfaces for assessment of cognitive function has gained traction in both clinical and research settings in the last decade. Because they can be run individually or within a customizable battery, they have great appeal for researchers and clinicians in several areas of cognitive science and the study of various populations. High levels of adoption are likely due to the numerous logistical advantages when compared to the traditional testing practices. Scoring is automated; raw scores can be compared to normative data; and you can assess

multiple cognitive domains within one automated program. Also, due to the computerized nature of these batteries, score reports and data can be efficiently and accurately produced and entered into study databases (Schatz & Browndyke, 2002). A recent review of computerized cognitive testing for older adults concluded that large numbers of available batteries and an ease of use could be beneficial to researchers and clinicians. However, selection of the correct battery for each application is imperative (Zygouris & Tsolaki, 2015)

The CANTAB has also been shown to discriminate between typical adults and various other clinical populations, including those with MCI and AD (Egerhazi, Berecz, Bartok, & Degrell, 2007). Studies in these populations are still limited. Lack of measurement validity in healthy adults makes it challenging to make predictions and track the potential cognitive decline in healthy aging. The good news is that many researchers are beginning to show CANTAB's validity in the last few years, with undoubtedly more to come (Egerhazi et al., 2007; Lenehan, Summers, Saunders, Summers, & Vickers, 2015; P. J. Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013). Currently, CANTAB has a set array of tests and specific battery for cognitive decline, and an abbreviated version of this battery was utilized in the present study.

The CANTAB has been successfully validated against traditional neuropsychological tests (P. J. Smith et al., 2013). Of the computerized testing batteries, the CANTAB is probably the most widely utilized, with a mention in over 1,300 peer-reviewed article or book chapters as of 2015 (Lenehan et al., 2015). As such, it has been shown to have adequate discrimination abilities, successfully showing differentiation within healthy, typically-aging, adult samples; normative samples (De Luca et al., 2003); and clinical populations of mild cognitive impairment and AD (Egerhazi et al., 2007). A lack of studies to measure healthy young and older adults have limited the ability for researchers to use the CANTAB to make predictions and track the potential cognitive decline in healthy aging. However, researchers have taken up the task of improving the validity of the CANTAB to predict future decline and differentiate between the different domains of cognitive function (Egerhazi et al., 2007; Lenehan et al., 2015; P. J. Smith et al., 2013). Furthermore, researchers have used the CANTAB in conjunction with brain imaging

techniques, to begin to understand the structure-to-function relation of the CANTAB outcomes (Chamberlain et al., 2011; Egerhazi et al., 2007), and have successfully shown relations to spatial cognitive function (e.g. WM and DLPFC), but the understanding of the relation to temporal measures (ERP) remains limited.

Imaging

Electroencephalography (EEG) is a recording of electrical signals given off by cortical neurons in the brain, resulting in the output of brain "waves" or "waveforms" used to analyze brain function. When an EEG is time-locked to a stimulus and averaged across multiple trials, an event-related potential (ERP) is produced. The ERP is made up of several components or waves (e.g., N200, P300, and slow wave (SW); Bressler & Ding, 2006; Sur & Sinha, 2009). The study of ERPs has shown promise for use in diagnosis of age-related cognitive decline (Bamidis et al., 2014; Bennys, Rondouin, Benattar, Gabelle, & Touchon, 2011; Dustman, Shearer, & Emmerson, 1993; Jackson & Snyder, 2008; Papaliagkas, Kimiskidis, Tsolaki, & Anogianakis, 2008; Vallesi, 2011). Most researchers center their work on identification or diagnosis of cognitive decline, MCI, and AD, but do not track or attempt to predict age-related decline.

Researchers are exploring the potential for ERP measures to predict and track decline using the N200 and P300 components (Duncan et al., 2009; Papaliagkas, Kimiskidis, Tsolaki, & Anogianakis, 2011; Polich, 2007), but these studies may be confounded in that they rely on auditory paradigms. I suggest that loss of hearing with age could confound the evidence reported in these studies. Less recent ERP studies have utilized visual paradigms, but were not designed to differentiate types of decline; lack the benefit of current definitions and theories in the field; and rely on equipment that produce less specificity (Friedman, 2000; Kugler et al., 1993; Polich, 1996; Schroeder et al., 1995). Only three out of thirteen studies in a meta-analysis of recent studies of P300 and cognitive decline by Jiang et al. (2015) used any form of a visual stimulus (Parra, Ascencio, Urquina, Manes, & Ibanez, 2012; P. Wang et al., 2013).

The P300 waveform refers to the positive peak of the waveform occurring 250-400 msec after an auditory stimulus but as late as 700 msec after a visual stimulus (Sur & Sinha, 2009). The P300 waveform is clinically useful as an index of cognitive function because it involves functions originating in widespread brain regions, including temporal and parietal lobes (Papaliagkas et al., 2011). When elicited in the oddball paradigm, the P300 latency (time to peak processing) has been shown to increase with normal aging while amplitude (the difference between the pre-stimulus baseline voltage and the largest peak) decreases (Dustman et al., 1993; Golob, Irimajiri, & Starr, 2007). Along with increased latency, the P300 amplitude is reduced in MCI and AD patients compared to healthy controls (Bennys et al., 2011; Brannon, Libertus, Meck, & Woldorff, 2008; Lai, Lin, Liou, & Liu, 2010; Papaliagkas et al., 2008; Papaliagkas et al., 2011).

The most common task used to elicit the P300 is the oddball task. In this task, a participant is instructed to attend to a string of stimuli, either auditory or visual, occurring one at a time. In the study, I used visual oddball tasks, in which familiar and novel pictures are presented. Depending on the age group and goals of the study, the length, number, and ratio of novel/familiar varies (Polich, 2007). The participant is instructed to either merely observe (passive) or instructed to press a button when the novel stimulus appears on the screen (active). The utility of the passive oddball task is the removal of the button pressing confound, meaning the only brain activity observed should be the activity of processing novel vs. familiar pictures. Conversely, an active oddball paradigm allows for examining the ability to perform a detection task, an activity shown to be affected by age and related to prefrontal activation (Kirino et al., 2000; Kutas, Iragui, & Hillyard, 1994; O'Connell et al., 2012). In the study, I compared an ERP detection task with cognitive behavioral detection tasks from CANTAB.

To my knowledge, no researchers have used a passive visual oddball ERP paradigm to study cognitive decline. In the study, I investigated if visual oddball paradigms will show similar identification and predictive qualities as auditory paradigms. I have generated evidence of the utility of the visual oddball paradigm in studying age-related cognitive decline. The use of passive and active tasks will facilitate the identification of changes in processing speed and cortical activation.

Conclusions

It is important to the prediction and treatment of age-related cognitive decline that knowledge of the factors that contribute to decline are paired with and assessed by behavioral and biological predictors of cognitive aging. The study was designed to elucidate evidential and predictive neuropsychological and behavioral indicators of cognitive decline while examining potential biological influences, such as the gut microbiome. The goal was accomplished by utilizing electrophysiological, behavioral, and biological data collected from an existing cohort of older adults. I extended the findings of a 6-month intervention study by reevaluating the cohort cross-sectionally at 6, 18, and 30 months after the final appointment.

Specific Aims

The specific aims of the present study were:

Specific Aim 1: To validate electrophysiological measures against behavioral cognitive measures of cognitive decline. I will collect data from 66- to 82-year olds at 6, 18, or 30 months after the final assessment from the parent study. I will then determine the relation between behavioral performance on the CANTAB and electrophysiological measures on two ERP tasks. I will enter the data into a regression analysis. I hypothesize that a significant relation will exist between ERP measures of latency and amplitude during active and passive oddball paradigms and working memory, reaction time, and inhibitory control tests in the CANTAB.

Specific Aim 2: **To determine the relation between baseline characteristics (MoCA and known aging resiliency factors) and cognitive outcomes (ERP and CANTAB) across time.**

Specific Aim 2a: To determine the relation between baseline MoCA and task-based ERP outcomes. I will determine if performance on the electrophysiological paradigms are predicted by baseline MoCA score. To accomplish this, electrophysiological measures will be regressed on to MoCA score. Time since baseline and changes in mental health and lifestyle will be included as covariates. I hypothesize that lower scores on the MoCA will relate to longer latencies to respond and to peak amplitude on correct trials on the active oddball task, and less differentiation between a novel and familiar stimuli in the passive oddball task.

Specific Aim 2b: To determine the relation between baseline MoCA score and behavioral task performance on CANTAB. I will determine if outcomes of the RTI, SWM, and RVP tasks of CANTAB are predicted by MoCA score at baseline. Again, I will determine these differences using regression analysis where CANTAB measures will be regressed on to MoCA score. Time since baseline and changes in mental health and lifestyle will be included as covariates. I hypothesize that lower MoCA score will relate to poorer performance on CANTAB tasks of SWM, RTI, and RVP.

Specific Aim 3: To identify behavioral and brain activity measures that predict cognitive decline. I will examine the relation between performance on behavioral and electrophysiological tasks at baseline and after 6, 18, or 30 months from the final appointment to determine if performance at baseline predicts performance at follow-up. I will regress follow-up outcomes on baseline outcomes, controlling for baseline MoCA score. Time since baseline and changes in mental health and lifestyle will be included as covariates. I hypothesize that ERP and CANTAB outcomes at baseline will successfully predict performance on those same tasks, where individuals initially showing poorer performance (classified as MCD at baseline) will continue showing poorer performance at follow-up.

Specific Aim 4: To explore microbiome factors that may influence brain activity and may be related to the progression of cognitive decline. I will collect microbiome specimens from participants returning at follow-up. I will then examine the relation between microbiome diversity and measures of brain activity, working memory, and reaction time. I expect that microbiome diversity will predict brain activity outcomes.

Specific Aim 4a: To determine measures of microbiome diversity and composition. I will use the outcomes of 16s rRNA sequencing of the microbiome from the participants of the follow-up to determine the appropriate outcome variable(s) of the microbiome to use in answering specific aim 4b. After sequencing, the genera constituting the microbiome will be identified. The selection of the appropriate variable(s) for specific aim 4b will depend on the overall composition of the study sample. Based on the number and types of genera present in my study population, potential

variables will be identified, such as total number of species, number of known symbiotic species, or number of identified unique species. Additionally, individual genera that may impact cognitive function will be identified based on the compositions of the study sample and investigation of the literature at the time of analysis.

Specific Aim 4b: To determine whether microbiome diversity relates to brain activity. I will use multiple regression to determine if microbiome composition relates to latency and amplitude in the cortex during the two oddball ERP paradigms and identify additional covariates of microbiome composition, such as diet and pharmaceutical use, to include in analyses. I hypothesize that individuals with a lower score on the microbiome diversity measure identified in specific aim 4a will have increased latency on both tasks and show less differentiation in amplitude between the novel and familiar pictures on the oddball task. Furthermore, I hypothesize that there will be a linear relation between the decline in the microbiome measure and decreased brain activity. Additionally, regression analysis will be used to determine if microbiome composition relates to decline from baseline to follow-up appointments. I hypothesize that lower diversity measures will relate most strongly with individuals with more significant decline. Finally, I hypothesize that several genera will be identified within the study population that relate to brain activity measures and group classification and that some, if not all, of these relations will be supported by recently published literature or can be supported by the rich dataset of this study population.

Specific Aim 4c: To determine if microbiome composition relates to decline across time. I will utilize multiple regression to determine if microbiome composition relates to decline from baseline to follow-up appointments. The relation will be assessed for MoCA score, CANTAB outcomes, and ERP outcomes. Chosen microbiome composition variable(s), change in MoCA score, from time 1 to time 2, age, education, IQ, and Time will be entered in to the model to predict cognitive outcomes while controlling for baseline MoCA score.

CHAPTER 2: METHODS

The Study Sample

Baseline

In the parent study (baseline), participants were enrolled in a 6-month randomized controlled trial of the effects of blueberry consumption on MCD in older adults 65 to 79 years old. Potential participants were screened for MCD and were invited to enroll if they were healthy and were beginning to experience MCD (but not dementia) or experiencing no cognitive decline as measured by the MoCA. Inclusion criteria were consumption of less than 5 daily servings of fruits and vegetables; no diagnosis of dementia, AD, central nervous system disorders, psychiatric disorders, gastrointestinal or digestive problems, or diabetes; body mass index (BMI) less than 35; not taking medications known to enhance cognition, to produce cognitive side effects, or to restrict cerebral blood flow); and right-handed. Informed consent was obtained from all subjects, and the study remains approved by the IRB for follow-up. A total of 133 participants were enrolled, with eighty-eight (n=88) individuals being classified as experiencing cognitive decline (MCD) and forty-five (n = 45) classified as no decline. The baseline data from the parent study were used as the baseline in the proposed study. In the parent study, a number of cognitive and behavioral tests were performed as well as diet recalls and self-report questionnaires for potential covariates. All participants also underwent vision screening to rule out visual impairments.

Follow-Up

For the current study, I contacted all participants (N = 133) via phone call and invited them back to the Cheatham Nutrition & Cognition Lab at the UNC-CH Nutrition Research Institute (NRI) to participate in a follow-up study that took place 6, 18, or 30 months after the final appointment of the parent study (baseline measurement), over two-thirds (n = 92) returned for the follow-up. See **Table 1** for characteristics of the cohort. Participants were compensated for their time and travel expenses at the same

rate as the initial study. I screened for all initial inclusion criteria and repeated all tests (described below) from the baseline appointment including diet recall and covariate questionnaires. **Table 2** outlines all assessments performed at each session and the number of individuals performing each assessment at each session. A power analysis using G*Power 3.1 (Faul, Erdfelder, Buchner, & Lang, 2009) based on similar studies indicated that in the analyses planned to answer SAs 1, 2, 3, and 4 to achieve a power of at least 90% (Cohen, 1977), using a moderate effect size of $f^2 = 0.3$, I needed to bring back at least 80 individuals. Since the proposal of this study, and the resulting data collection and analysis, I chose to add additional statistical tests to the original design in order to better address my specific aims. Each statistical approach is described below by specific aim.

Screening/group assignment

The participants were originally grouped using the MoCA. The MoCA consists of eight sections to test recall memory, executive function, attention, orientation, abstraction, visuospatial skills, and naming. Each section is weighted and scored according to the guidelines established by Nasreddine et al. (2005). Cognitive decline group status was determined as a score less than 26 out of 30 on the MoCA (Nasreddine et al., 2005; T. Smith et al., 2007). The same researcher scored all MoCAs, and 25% were reassessed by a second researcher. Reliability in this sample reached 92.6%. Participants were screened and divided into groups at baseline in the parent study based on their MoCA score. MoCA was used again at the follow-up to assess participants, and I have used parent study group membership as a covariate in analyses where appropriate to control for the potential confound in trajectories caused by the berries.

Methods

Diet Recalls

The NDSR 24-hour dietary record procedure was used to obtain three days of dietary intake. Participants were instructed to choose three days in the same week leading up to their appointment to record their diet for 24 hours in a diet journal. The diet journal was then brought to the session and reviewed with a researcher using the 4-pass methodology. Twenty-four hour diet recalls are considered the most valid mechanism for determining a person's diet (Baranowski, 2013; Thompson et al., 2002).

Diet recalls were conducted using the 4-pass methodology described below. Data collection was guided by and entered into the Nutrition Data System for Research (NDSR).

First pass - Quick list: The interviewer entered the data from the participants diary while the participant completed several of the paper-based assessments. Then, the interviewer did an initial walk-through of the information. “We’ll be talking about what you ate or drank on XX. After you got up on XX morning, what was the first time you had something to eat or drink? What did you eat or drink at that time? Did you eat or drink anything else at that time? What was the next time on XX that you had something to eat or drink? What did you eat or drink. . .?” The interviewer repeated this process to cover XX’s intake in chronological order. Then, the participant was asked “Can you remember any other times on XX that you had something to eat or drink?”

Second pass - Review: The interviewer repeated back everything the participant reported at each time, and asked “Can you think of anything else you ate at that time?” and “Can you think of anything else you drank at that time?” (The interviewer repeated this process for XX’s intake in chronological order.) Due to the access to the diary, this step was often unnecessary, but if many things had been added to the recall list that had not been recorded, the interviewer completed these steps before moving to the third pass.

Third pass - Details: The interviewer asked the interviewer to name each eating occasion (response options: work breakfast, breakfast, work lunch, lunch, dinner, supper, snack), identify the location of each meal (response options: home, school, somewhere else), provide details about each item, indicate additions to items, and indicate amounts consumed for each item. The interviewer began with the earliest time on XX morning and continued in chronological order to cover XX’s intake.

Fourth pass - Final review: Each eating occasion was reviewed with the participant for correctness. The interviewer began with the earliest time the first morning and repeated this process in chronological order to cover intake. Then, the participant was asked one final time “Can you remember any other times that you had something to eat or drink on XX?”

ERP acquisition

In a protocol consisting of three tests, EEG was recorded from each participant and from recordings, ERPs were extracted. For recording, the participant was fitted with a 128-sensor geodesic net (GSN; Philips Neuro, Inc., Eugene, OR, USA). Measurements of the participant's head were obtained to ensure proper net size, and correct placement of the landmark sensors. Application of the net required approximately 10 minutes and is well tolerated by the participants. Impedances were checked and corrected, if necessary, to below 50 kΩ. The participant was then seated 45 cm from a monitor in the testing room (separate from the acquisition room) while stimuli were presented by E-Prime 2.0 software (Psychology Software Tools, Inc, Sharpsburg, PA, USA). Data were digitized, referenced to a single point at the vertex, and stored by NetStation software (Philips Neuro, Inc., Eugene, OR, USA). The NetStation system utilizes a digital clock to time-lock the E-prime presentation of the stimuli to the NetStation EEG recording.

For all tests, stimuli were displayed on a computer in the testing room, and pictures are displayed on a black background. In the first test (reaction time), the participant was instructed to respond with a button press: 1 for a red car and 2 for a blue car. Reaction times were displayed on the screen between stimulus presentations. For the second test (active detection), participants were instructed to respond accordingly to a series of Xs and Os that appeared on the screen by pressing a key for an X, and not pressing a key for an O. The active response (X) occurred in 20% of trials, whereas the inhibited response (O) occurred in the other 80% of trials. I utilized this task as a detection task. Finally, on the third test (passive visual oddball), the participant was instructed to observe pictures that occurred on the screen. After an habituation period of 10 presentations of the familiar picture, the participant viewed 120 pictures randomly ordered by E-Prime consisting of the familiar (40), a frequent-novel (40), or a trial-unique (40) image. In the study, I utilized the active detection and oddball tasks for examination of SAs 1, 2, 3, and 4. The reaction time test was tested for its appropriateness as a covariate and was found to not be necessary.

ERP waveforms were visually assessed for anomalies, after being subjected to lowpass (30 Hz) and highpass (0.3 Hz) filtering and segmented into individual segments corresponding to stimulus

presentation and trial length. Bad channels were detected by the software using moving averages and a threshold of 250 μV. Segments that included more than 12 (>10%) bad channels were rejected. After completion of the visual inspection, individual channels were replaced as necessary using spherical spline interpolation. Data were then baseline corrected using the mean voltage during the 100 ms that precede the stimulus presentation. Finally, trials were averaged within the condition (i.e., novel, novel unique, and familiar; Target and Standard). The resulting file was visually examined, and negative and positive deflection windows of interest were chosen. Windows for active detection were 50-190 ms and 100-800 ms, respectively. Windows for passive oddball were 210-350 ms and 350-900 ms, respectively. After visual inspection of the continuous data (and review of the relevant literature), sensor clusters of interest were chosen, and data were averaged across those clusters. See **Appendix A** for sensor clusters on the EGI 128-sensor net. Then, clusters were individually assessed for mean amplitude, peak amplitude, and latency to peak amplitude within the determined segment window for each deflection (positive or negative) for both tasks. In the current study, windows and sensor clusters were consistent with the parent study. Visual representations of these windows in the grand averaged waveform data for the passive oddball and detection tasks are displayed in **Figures 1 & 2**, respectively. Finally, in order to compare ERP variables across time, as well as to explore additional ways to evaluate the study sample, the averaged-referenced and baseline corrected files were entered into MatLab, and using EEGLab, Fieldtrip, and ERPToolkit base program code was written to perform temporospatial principle components analysis (PCA) on each test at each timepoint to produce unique factors for analysis (Barry, 2014; Dien, 2012). The process of the two-step PCA is outlined in greater detail in the results section.

CANTAB

The CANTAB system consists of standardized measures of cognitive function that have been computerized on a touch-screen instrument that automatically stores data as the participants complete the test battery. The CANTAB offers several benefits, as many tests can be done on one platform. Language ability does not skew results and requires minimal reading and direction. The tests chosen for this study

were determined by Cambridge Cognition as the recommended battery (Egerhazi et al., 2007; Lenehan et al., 2015). All participants completed the tasks below in the same order.

Motor Task (MOT): Motor Task is a baseline test of motor function to ensure the participant can correctly press the screen and participate in the study. Thus, outcome measures from this task are not used in analyses. The participant will see a series of flashing Xs on the screen that they must touch.

Reaction Time (RTI): The Reaction Time task (RTI) is a measure of reaction time, movement time, and response accuracy for a condition in which the stimulus is predictable (simple reaction time) and for a condition in which the stimulus is unpredictable (5-choice reaction time). It familiarizes the participant with the press-pad and provides simple and choice reaction and movement times. The participant will first hold down a button on the press pad. A yellow dot appears inside a circle. The participant releases the button as quickly as possible and touches the spot where the yellow dot appeared. The second section will show five circles on the screen, and the yellow dot could appear in any of them. The participant will again release the button as quickly as possible and press the spot where the yellow dot appears.

Spatial Working Memory (SWM): Spatial Working Memory is a working memory and planning task that incorporates heuristic strategy. The participant will see boxes on the screen – each of which contains a blue token. The objective is to find the blue tokens in the correct order. The participant must remember which boxes have already been searched, and identify those containing a blue token. The test starts with four boxes and increases to eight boxes.

Paired Associates Learning (PAL): Paired Associates Learning is a memory task requiring participants to identify where they have seen patterns on the screen. The participant was first shown a screen with 6 boxes. The boxes revealed patterns in random order. Participants had to remember the location of each pattern, and the number of patterns to remember increased from 2 to 8 with each successful trial.

Rapid Visual Information Processing: Rapid Visual Information Processing (RVP) measures the ability to sustain attention over a period of time, which requires both working memory and selective attention, and is a sensitive measure of frontal-parietal function. A white box appears in the center of the screen, and single digits appear inside the box in pseudo-random order. Participants are instructed to watch the digits change and press the button when a 3-digit target sequence appears. The task is presented in two parts. The practice involves one 3-digit target sequence. The test stage involves three 3-digit sequences. Increases in latency to respond to the target sequences and the participant's ability to successfully identify the target have been negatively correlated with cognitive function (Chamberlain et al., 2011).

Microbiome samples

After they consented to provide fecal samples following cognitive testing, participants were provided a fecal specimen collection kit and instructions for collection at home. The kit included all necessary items for home collection of fecal samples and provided four individual samples from the same stool for analysis. The kit included a shipping container and a prepaid shipping label to be mailed back to

The Cheatham Nutrition & Cognition Lab where the sample was processed for storage and later DNA extraction. A survey (**Appendix B**), to be filled out at the time of collection, was also included with the kit. The survey asks participants about any recent antibiotic use as well as other health and lifestyle factors that could influence their sample. Alternatively, the participant could also drop off the samples in person during operating hours at the NRI. Once all samples were collected, DNA was isolated and sent to the Human Microbiome Core at UNC-Chapel Hill for library preparation.

Processing of 16S rRNA sequence data was completed with BiolockJ, a bioinformatics pipeline frame work for metagenomics analysis written in the department of bioinformatics at UNC Charlotte by Michael Sioda (Sioda, 2018; https://github.com/msioda/BioLockJ). Paired-end sequences were merged with Paired-End read merger (PEAR, v 0.9.10) (J. Zhang, Kobert, Flouri, & Stamatakis, 2014) using default arguments, excluding sequences for which primers do not match or for which ten base pairs do not overlap.

In the initial analysis performed prior to the pipeline output, the taxonomic assignment was performed with the Ribosomal Database Project (RDP) Classifier v2.12 (confidence threshold=80%) (Q. Wang, Garrity, Tiedje, & Cole, 2007). In secondary analysis, sequences were processed through QIIME v1.9.1 (Quantitative Insights Into Microbial Ecology;Caporaso et al., 2010), where BioLockJ multiplexed the pEAR merged reads as QIIME input, UCLUST for deriving Operational Taxonomic Units (OTUs) by clustering sequences at 97% similarity (Edgar, 2010), and open-reference assignment of OTUs using the Silva (132 release) reference database (Quast et al., 2013). In the pipeline, BioLockJ was directed to run the QIIME alpha_diversity script to calculate the Shannon Alpha diversity metric (Peet, 1974). Beta-diversity was assessed with Principal Coordinates Analysis (PCoA), using Bray-Curtis dissimilarity matrixes of microbial-abundance-based distances (Faith, Minchin, & Belbin, 1987). For taxonomy-specific analysis, we excluded operational taxonomic units (OTUs) that are present in <25% of participants, and transform raw taxonomic counts as $\log_{10}[(RC/n)(x/N)+1]$, where RC is the total raw taxon count for a participant and n is the total count across all taxa for a participant, x is the total across all OTUs and participants and N is the total number of participants (McCafferty et al., 2013). We

conducted multivariable-adjusted regression models for the diversity measure of microbial community composition concerning measures of cognitive function. Regression analysis of individual genera controlled for multiple comparisons using the Benjamini-Hochberg method for false discovery rate (FDR) (Benjamini & Hochberg, 1995).

CHAPTER 3: RESULTS

Data Reduction and Analyses

All data were inspected for assumptions of the general linear model, including normal distribution, linearity, and homoscedasticity of residuals. All regressions included collinearity diagnostics – tolerance, Variance Inflation Factor (VIF), and Condition Index (Belsley, Kuh, & Welsch, 1980; Menard, 1995; Snee & Marquardt, 1984). A VIF >10, and sometimes more conservatively >5, has been considered to be an unacceptably high level of collinearity, which would place the interpretation of results in question. Belsley et al. (1980) suggest, however, a condition index (>30 tolerance) is a more appropriate measure because even low correlations among each independent variable can add up to high collinearity in the full model. Moreover, these rules should be interpreted in the context of other factors that influence the stability of the regression coefficient (O'Brien, 2007). Due to the large number of ERP variables entered into the regressions in all four specific aims, and the assumed cross-correlations (and likely collinearity) between the sensor clusters in the ERP outcomes, I report Condition Index (CI) measures for all models. I had neither a VIF above 6 nor CI above 30 in my analyses, indicating the results meet moderate to conservative levels of control. The Greenhouse-Geisser adjustment was used where relevant for ERP data. Age, Gender, Education, Current Occupation, Marital Status, Patient Health Questionnaire Score, Body Mass Index, Healthy Eating Index, MoCA, GAD, Stressful Life Events, PASE, and Time Since Baseline were tested as potential covariates before each set of analyses by regressing the outcome variables onto each potential covariate in turn. Relevant covariates are described for each model for each specific aim below. Statistical analyses were conducted with SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 26.0) except for the principal components analyses (PCA), which was conducted with Matlab (version 9.6.0 (R2019a). Natick, Massachusetts: The

MathWorks Inc.) and the ERP PCA Toolkit (Dien, 2012). A detailed description of PCA analyses is included in the results for Specific Aim 3.

Before beginning the study, several variables of *a priori* interest were selected for CANTAB and ERP. CANTAB variables of interest were selected by, first, choosing those variables that quantify speed of processing, planning time, and working memory function. After data collection, the variables were evaluated with bivariate correlations to determine a single variable that best represented all measures for a given test based on which variable within each test was most related to all others. The three chosen variables were, RTI five-choice reaction time, SWM strategy score (high scores indicate a poor use of the best strategy), PAL total errors (adjusted for the number of trials), and RVP mean latency to (correct) response (adjusted for the number of responses). In ERP, the Frontal, Frontal Right, Frontal Left, Midline, Temporal Right, and Temporal Left were selected from the calculated clusters as relevant based on the literature.

Specific Aim 1

Three CANTAB measures (RTI, SWM, RVP) were independently assessed via multivariate linear regression to determine the extent to which they predicted selected ERP measures. To avoid issues of multiple comparisons, data compiled in *a priori* ERP clusters of interest (Frontal Z, Frontal Right, Frontal Left, Temporal Left, Temporal Right, and Midline) were regressed onto the CANTAB variable of interest, resulting in six models for each of the two ERP tasks - minimum amplitude, maximum amplitude, mean amplitude of the negative deflection, latency to the negative deflection, mean amplitude of the positive peak, and latency to the positive peak. A stepwise method was also employed to determine if the determined covariates, Baseline Group and Education, were appropriate in the model. Significant multivariate results were followed up in reduced models when appropriate.

Reaction Time

Detection task. Reaction time significantly predicted activity in the Temporal Left cluster for two measures during the active response. First, it significantly predicted the mean amplitude of the negative deflection, $F(1,62) = 5.353$ $p = .024$ adj. $R^2 = .066$ $b = -.284$ VIF = 1 CI = 10.933. Second, reaction time

significantly predicted the maximum amplitude when education was included as a covariate, $F(1,62) = 7.057$ $p = .002$ adj. $R^2 = .163$ $b = -.369$ CI = 4.395 (**Figure 3**). No other ERP outcomes were significant at any cluster locations. The models indicate that as reaction time increases the mean amplitude of the negative deflection and maximum amplitude of the positive peak decrease.

Passive oddball. Reaction time significantly predicted the latency to the negative deflection in the Frontal Right cluster and the latency to the positive peak in the Temporal Left cluster during the Novel stimulus, $F(1,75) = 4.026$ $p = .048$ adj. $R^2 = .038$ $b = -.226$ VIF = 1 CI = 11.383, and $F(1,75) = 5.225$ $p = .025$ adj. $R^2 = .044$ $b = .255$ VIF = 1 CI = 11.383, respectively. The mean amplitude of the negative deflection in the Temporal Left cluster during the Familiar stimulus was also significantly predicted by reaction time, $F(1,75) = 4.513$ $p = .037$ adj. $R^2 = .044$ $b = -.238$ VIF = 1 CI = 11.383 (**Figure 4**). No other ERP outcomes were significant at any cluster locations. The models indicate that, during the Novel stimulus, as reaction time increases the latency to the negative deflection decreases and the latency to the positive peak increases.

Spatial Working Memory

Detection task. Spatial working memory (strategy score) significantly predicted the latency to the positive peak during the inhibited response in the Midline and Frontal Clusters, $F(1,64) = 10.444$ $p = .002$ adj. $R^2 = .127$ $b = .375$ VIF = 1 CI = 12.645, and $F(1,64) = 5.414$ $p = .023$ adj. $R^2 = .064$ $b = .279$ VIF = 1 CI = 12.645 (**Figure 5**). No other ERP outcomes were significant at any cluster locations. The models indicate that as strategy score on SWM increases (poorer strategy use), the latency to the positive peak increases as well.

Passive oddball. Spatial working memory significantly predicted the latency to the negative deflection in the Frontal Right cluster during the Familiar stimulus, $F(1,78) = 7.316$ $p = .008$ adj. $R^2 = .074$ $b = -.293$ VIF = 1 CI = 12.353. Next, it predicts the mean amplitude of the negative deflection in the Temporal Right cluster during the Novel stimulus and the Frontal Left cluster during the Familiar stimulus, $F(1,78) = 4.163$ $p = .045$ adj. $R^2 = .038$ $b = -.225$ VIF = 1 CI = 12.353, and $F(1,76) = 3.456$ $p = .021$ adj. $R^2 = .085$ $b = .346$ VIF = 1 CI = 19.337 (**Figure 6**). No other ERP outcomes were significant at

any cluster locations. The models indicate that as SWM strategy increases (poorer strategy use) latency to the negative deflection decreases and mean amplitude increases during the Familiar stimulus, while mean amplitude during the Novel stimulus increases.

Rapid Visual Information Processing

Detection task. Mean latency to response in the RVP task significantly predicted minimum amplitude in the Temporal Right cluster during the active response when Education was included, $F(1,63) = 6.635$ $p = .002$ adj. $R^2 = .148$ $b = -.294$ VIF = 1.026 CI = 12.349. RVP also significantly predicts the maximum amplitude in the Frontal Right and Temporal Right clusters during the active response, $F(1,64) = 4.995$ $p = .029$ adj. $R^2 = .058$ $b = .269$ VIF = 1 CI = 9.621, and $F(1,64) = 14.983$ $p < .000$ adj. $R^2 = .177$ $b = .269$ VIF = 1 CI = 9.621 (**Figure 7**). No other ERP outcomes were significant at any cluster locations. The models indicate that as the mean latency to a response increases in the RVP task the minimum amplitude decreases and maximum amplitude increases.

Passive oddball. RVP (mean latency to response) significantly predicted two attributes of the positive peak in the Frontal Left cluster during the Familiar stimulus; first, the mean amplitude of the positive peak, $F(1,79) = 6.648$ $p = .012$ adj. $R^2 = .066$ $b = .279$ VIF = 1 CI = 10.245; then, the maximum amplitude, $F(1,79) = 7.829$ $p = .006$ adj. $R^2 = .079$ $b = .3$ VIF = 1 CI = 10.245 (**Figure 8**). No other ERP outcomes were significant at any cluster locations. The models indicate that as the latency to response in RVP increases the mean amplitude of the positive peak and the maximum amplitude during a familiar stimulus increases.

Specific Aim 2

Specific Aim 2 was investigated in two different ways to fully capture the relation between baseline characteristics of my participants and their cognitive outcome at follow-up. First, ERP and CANTAB variables were regressed onto the baseline MoCA score as initially designed, and those results are discussed first. Second, I was interested in whether the change in MoCA score may be a better predictor of follow-up cognitive assessment performance, so a MoCA change score was created where the baseline score was subtracted from follow-up score. A positive change score indicates a decline in

performance on the task. In all analyses, multiple linear regression was used, and Age, Time Since Baseline, and Education were included as covariates.

Baseline MoCA

ERP. Baseline MoCA score significantly predicted the mean amplitude of the positive peak in the Frontal Right cluster, $F(1,64) = 3.233$ $p = .046$ adj. $R^2 = .063$ $b = .294$ VIF = 1 CI = 23.843. No other ERP outcomes were significant at any cluster locations. The model indicates that as baseline MoCA score increases, in the detection task, the mean amplitude of the positive peak increases.

CANTAB. Baseline MoCA score significantly predicted SWM and PAL outcomes when Time Since Baseline and Age were included in the model, $F(3,86) = 8.444$ $p < .000$ adj. $R^2 = .201$ $b = -.437$ VIF = 1.088 CI = 8.545, $F(3,86) = 8.683$ $p < .000$ adj. $R^2 = .204$ $b = -.265$ VIF = 1.074 CI = 8.533. No other CANTAB outcomes were significant. The models indicate that as MoCA score increases (improves) SWM strategy score decreases (improves) and total errors on PAL also decreases.

Change in MoCA Score

ERP. Change in MoCA score significantly predicts the latency to the negative deflection in the Frontal Right cluster during the active response of the detection task, $F(1,65) = 4.807$ $p = .032$ adj. $R^2 = .055$ $b = -.262$ VIF = 1 CI = 1.626. The mean amplitude and latency to the positive peak in the Midline cluster and the latency to the positive peak in the Frontal cluster during the Familiar stimulus in the passive oddball task is significantly predicted by change in MoCA score, $F(1,80) = 6.874$ $p = .034$ adj. $R^2 = .043$ $b = -.234$ VIF = 1 CI = 1.555, and $F(1,80) = 7.282$ $p = .008$ adj. $R^2 = .072$ $b = -.289$ VIF = 1 CI = 1.555, $F(1,80) = 6.136$ $p = .015$ adj. $R^2 = .060$ $b = -.267$ VIF = 1 CI = 1.555, respectively. Also, Change in MoCA predicts latency to positive peak in the Temporal Left and Frontal clusters during the Novel stimulus, $F(1,80) = 7.272$ $p = .009$ adj. $R^2 = .072$ $b = -.289$ VIF = 1 CI = 1.555. and $F(1,80) = 4.100$ $p = .046$ adj. $R^2 = .037$ $b = -.221$ VIF = 1 CI = 1.555, respectively. No other ERP outcomes were significant at any cluster locations. The models indicate that as change in MoCA score increases (better performance) latency to the negative deflection in the detection task decreases. As change in MoCA score increases the

mean amplitude of and latency to the positive peak in the passive oddball task decrease during the familiar stimulus, and latency to the positive peak during the novel stimulus decreases.

CANTAB. Change in MoCA score significantly predicts mean latency to response in the RVP task when Time Since Baseline and Age are included in the model, $F(3,87) = 5.391$ $p = .002$ adj. $R^2 = .128$ $b = -.227$ VIF = 1 CI = 2.093. No other CANTAB outcomes were significant. The model indicates that as the change in MoCA score increases (better performance) latency to response decreases in the RVP task.

Specific Aim 3

Specific aim 3 presented a unique challenge when deciding how to properly compare the ERP outcomes at both time points because the ERPs from the baseline appointment were collected on an older version of NetStation. Such that baseline ERPs were sampled at 250 samples/sec whereas the follow-up ERPs were sampled at 1000 samples/sec. Moreover, the attributes of the ERPs used in Specific Aims 1-3 (e.g., latency to peak at six different clusters) are too numerous to test individually for fear of multiple comparisons and producing Type I errors. Therefore, even though the windows and clusters were consistent, the variables of interest could not be compared in the initially proposed regression model. A solution that allowed for the normalizing of all ERP outcomes irrespective of timepoint and would produce better, less correlated components for comparison was to perform a temporal-spatial principal components analysis (PCA; Dien, 2012).

Generating the PCA Factors

A temporal-spatial PCA was performed on the ERPs from baseline appointment for the active detection and the passive oddball tasks to determine unique spatiotemporal factors that could help determine the relation among the individual ERP components across both time points (Curran & Dien, 2003; K. M. Spencer, Dien, & Donchin, 1999). Methods are described in detail in Dien (2012) and briefly here. A spatial PCA followed a temporal PCA. A Promax rotation was used, which involved first applying a Varimax rotation and then relaxing it to allow for correlated factors. At each step of the PCA the number of factors retained was determined by evaluating a Scree plot (Cattell, 1966; Cattell &

Jaspers, 1967) and the parallel test (Horn, 1965), which compares the Scree of the dataset to that obtained from an entirely random dataset, and the intersection of these two lines is determined as the number of factors. At the temporal step, 22 factors were retained for the Oddball task and 23 for the active detection task.

Separate spatial PCAs were performed on each of the 22 or 23 temporal factors. Seven spatial factors were produced for each of the temporal factors for the oddball task and 12 for the detection task. Seven temporospatial factors were retained for the oddball and five for the detection task based on statistical modeling and windowing performed on the jack-knife PCAs using the EP Toolkit (Barry, 2014; Curran & Dien, 2003; Dien, 2012). The spatial PCA procedure forced identical scalp topographies and sample rate for a given factor across all conditions, but factors (and individuals) are free to differ in amplitude. The application of identical scalp topographies and sampling rate at the second step was essential for the comparisons of the two time points

. Finally, the determined factor loadings for the baseline study were applied to the ERPs from the follow-up study, which produced temporospatial factors for each test that are identical in time course and sensor cluster but differ individually by timepoint and participant on amplitude (microvolts).

Predicting follow-up ERP with Baseline ERP

The newly generated PCA factors for each time point were then entered into a hierarchical multiple linear regression model. Baseline MoCA score was forced into the model to control for differences in MCD characterization, leaving only the change in brain function. The inclusion of baseline MoCA score did increase measures of collinearity (CI, specifically), but tolerance and VIF remained within conservative levels. Time Since Baseline, Baseline Group, Age, and Education were tested in subsequent hierarchical models but were not necessary. Each baseline factor was regressed onto its paired follow-up factor, and due to the exploratory nature and initial selection of factors not being *a priori*, a Bonferroni correction was made setting the significant p-value at .003.

Oddball. Factors 2, 3, and 4 (TF2SF1, TF2SF2, TF2SF3; **Figure 9 A, B, & C**) for the Familiar condition significantly predicted their counterparts, $F(2,52) = 6.405$ $p = .003$ adj. $R^2 = .167$ $b = .449$ VIF

= 1 CI = 21.942, $F(2,52) = 7.899$ $p = .001$ adj. $R^2 = .204$ $b = .460$ VIF = 1 CI = 20.010, and $F(2,52) = 6.417$ $p = .003$ adj. $R^2 = .167$ $b = .408$ VIF = 1 CI = 20.197, respectively. Factors 2 and 3 (TF2SF1, TF2SF2; **Figure 9 D & E**) also significantly predicted their counterpart for the Novel condition, $F(2,52) = 11.993$ $p < .000$ adj. $R^2 = .289$ $b = .562$ VIF = 1 CI = 21.870, and $F(2,52) = 6.754$ $p = .002$ adj. $R^2 = .176$ $b = .448$ VIF = 1 CI = 22.138, respectively. Thus, higher amplitude at baseline is predictive of continued higher amplitude at follow-up. Regressions were followed up with robust ANOVAs with Bonferroni correction (alpha = .008) to test the difference between Familiar and Novel amplitude at each selected factor. The model for Factor 5 (TF3SF3) was significant $T_{WJt}/c(3, 40) = 10.13$ $p = .00014$ MSe = 0.792262 and contrasts indicated that Familiar was significantly different than Novel, $T_{WJt}/c(3, 48) = 14.41$ $p = .00054$ Mse = 0.792262.

Detection task. Factors 2 and 3 (TF2SF3, TF3SF3; **Figure 10 A&B**) for the inhibited response condition significantly predicted their counterparts, $F(2,58) = 13.277$ $p < .000$ adj. $R^2 = .290$ $b = .554$ VIF = 1 CI = 20.912, and $F(2,58) = 11.989$ $p < .000$ adj. $R^2 = .268$ $b = .551$ VIF = 1 CI = 21.656, respectively. Factors 1 and 4 (TF1SF1, TF5SF1; **Figure 10 C&D**) also significantly predicted their counterparts for the active response condition, $F(2,58) = 10.291$ $p < .000$ adj. $R^2 = .236$ $b = .512$ VIF = 1 CI = 20.703, and $F(2,58) = 13.729$ $p < .000$ adj. $R^2 = .298$ $b = .552$ VIF = 1 CI = 24.359, respectively. The model indicates that, again, higher amplitude at baseline predicts higher amplitude at follow-up. Again, I evaluated the chosen factors for differences between the inhibited response and active response conditions using robust ANOVAs with Bonferroni correction (alpha = .008). The model for Factor 4 (TF5SF1) was significant, $T_{WJt}/c(1, 60) = 121.86$ $p < .00001$ Mse = 1.531.

Predicting follow-up CANTAB performance with Baseline performance

To assess whether baseline CANTAB performance predicted CANTAB performance at follow-up, measures were regressed onto the counterpart baseline measure controlling for baseline MoCA score. Three of the four models did not include additional covariates, whereas the RVP model included Time Since Baseline. A hierarchical model indicated that Education could increase the explained variance of the SWM model significantly ($p = .04$), but inclusion in the model caused a significant increase in the

Condition Index (CI = 34.153). Education and Baseline MoCA score significantly predict each other (r = -.402, $p < .001$). Therefore, I chose to exclude Education from the SWM model. Similarly, the inclusion of Time Since Baseline was suggested in the RVP model ($p = .04$).

RTI, PAL, SWM, and RVP all significantly predicted their counterpart outcome at follow-up, $F(2,84) = 16.517$ $p < .000$ adj. $R^2 = .265$ $b = .528$ VIF = 1 CI = 25.913, $F(2,88) = 33.272$ $p < .000$ adj. $R^2 = .418$ $b = .573$ VIF = 1 CI = 24.670, $F(2,87) = 36.611$ $p < .000$ adj. $R^2 = .445$ $b = .531$ VIF = 1 CI = 29.268, and $F(3,87) = 16.718$ $p < .000$ adj. $R^2 = .344$ $b = .577$ VIF = 1 CI = 29.051, respectively. Interpretation of the model suggests that performance at baseline on the CANTAB is significantly predictive of follow-up performance, where poorer performance on the task at baseline predicts continued poorer performance at follow-up.

Specific Aim 4

Determining the Measure of Microbiome Composition

Shannon alpha diversity, one of the most common alpha diversity metrics in gut-microbiome research, was selected as the alpha metric for Specific Aim 4. Shannon diversity accounts for both the abundance and evenness of the species present – the proportion of species relative to the total number of species is calculated, and then multiplied by the natural logarithm of this proportion before being summed across all species and multiplied by -1.

As a reminder, Beta Diversity is a much more complicated measure of diversity, and can be seen as the sum of two components: turnover and nestedness. Turnover is the difference between communities solely in relation to which species exist in each. Nestedness is how much the species composition of a site with a lower species richness is a subset of a site with higher species richness. Therefore, one could calculate and compare beta diversity values based on species presence/absence and/or relative abundance, which means using a measure of alpha diversity to calculate beta diversity. Several variables of interest were chosen on which to calculate beta diversity, Healthy Eating Index, MoCA score, Education, Berry Consumption (discussed later), and the four selected CANTAB variables. Unfortunately, after significant time and effort, the calculation of either weighted or unweighted unifrac on the present microbiome

dataset was unsuccessful. The principal coordinates analysis (PcoA) run to quantify the turnover and nestedness of the alpha metric failed. With the PcoA I attempted to determine the individual components (the change in relative taxa relative to a metadata field) that uniquely explain the variance between communities (participants microbiomes). In all instances, the PcoA could explain approximately 95% of the variance with a single metadata field, which changed with each calculation (not the metadata attribute itself causing the significance). The significantly high predictive quality of each PcoA rendered the results useless as a predictive indicator of diversity. Therefore, for testing specific aims 4b and 4c, Shannon diversity was used as the characterizing metric of the microbiome data.

Statistical Analyses

Data met assumptions of normality and were subjected to stepwise multiple regression analysis, with controls for repeated measures and relevant covariates, to assess the relation between microbiome diversity measures of interest and the CANTAB and ERP variables using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 26.0). Before beginning the analysis of the data, several variables of interest were selected for CANTAB and ERP variables and are discussed below for their respective assessment. Significant results were followed up in reduced models when appropriate. Age, Gender, Education, Current Occupation, Marital Status, Patient Health Questionnaire Score, Body Mass Index, Healthy Eating Index, MoCA, GAD, Stressful Life Events, PASE, and Time Since Baseline were tested as potential covariates. It was determined that Education was necessary (p = .020).

Stepwise multiple regressions were performed for each assessment where Shannon diversity (the unknown) was regressed onto the predictors of interest. Whereas the design of this model is atypical in the fields of psychology and neuroscience, it is standard practice in the microbiome literature to predict Shannon alpha. In the specific aims, it has been indicated that ERP and CANTAB are known measures of cognitive function and are sensitive to changes due to aging. The gut-microbiome, is therefore, the unknown in the equation. Dynamic interactions exist among environment, microbiome, and host. To study the complicated interactions among these factors, unique models must be built. One such model to explore the association between microbiome and host is to evaluate whether the composition of the

microbiome (a “dysbiotic” microbiome) is linked to the health or disease of host. Based on the research hypotheses, the null statistical hypothesis is “there is no difference of microbiome composition in age-related cognitive decline.” Therefore, in Specific Aim 4, placing microbiome diversity as the dependent variable in all models is warranted (Xia & Sun, 2017).

Relation Among CANTAB Measures and Microbiome Diversity

Two of the CANTAB outcome variables used in Specific Aim 4 differed from those in Specific Aims 1-3 due to the exploratory nature of this aim. The selected variables were as follows: Simple Reaction Time (RTI) as a measure of reaction time, Total Errors Adjusted (PAL) as a measure of visual memory, Mean Time to First Success (six-step; SWM) as a measure of working memory and planning, and Mean Latency to Response (RVP) as a measure of sustained attention (working memory and selective attention).

Data were entered into a stepwise regression using all the predictors (RTI, PAL, SWM, RVP, and Education). Shannon alpha was predicted by PAL and SWM ($p = .007$). The model, including only PAL ($p = .025$) and SWM ($p = .024$), accounted for 11.7% of the variance and statistically significantly predicted Shannon alpha, $F(2,56) = 4.846$ adj. $R^2 = .117$, $b = -.293$ and $b = -.248$, respectively. The model indicates that as the total number of errors (PAL; **Figure 11**) and mean trials to success (SWM; **Figure 12**) increases alpha diversity decreases. See **Table 3** for results of the model.

Relation Among ERP Measures and Microbiome Diversity

The same *a priori* clusters of interest from specific aims 1-3 (Frontal Z, Frontal Right, Frontal Left, Temporal Left, Temporal Right, and Midline) were entered into a stepwise regression to predict microbiome diversity measures.

Detection task. For the detection task, six stepwise regressions were performed to determine if ERP measures predicted microbiome diversity. Each regression tested a model for an individual ERP attribute (minimum amplitude, mean amplitude of the negative deflection, or latency to the negative peak) for Target or Standard conditions and included all clusters of interest. Education was not selected to remain in the model for any of the regressions.

The minimum amplitude as well as the mean amplitude for the Frontal Z cluster for the Target condition significantly predicted Shannon alpha, $F(1,46) = 4.202$ $p = .022$ adj. $R^2 = .089$ $b = .330$ VIF = 1 CI = 3.347 (**Figure 13**), and $F(1,46) = 4.361$ $p = .042$ adj. $R^2 = .067$ $b = .294$ CI = 1.771 (**Figure 14**), respectively. The model indicates that as the amplitude the negative deflection increases alpha diversity also increases and as the mean amplitude across the window (50-190ms) increases diversity also increases. See **Table 4** for results of these two models. When these significant variables are placed into the same model, the model emerges as insignificant, $F(2,45) = 2.887$ $p = .066$ $R^2 = .114$.

When clusters were tested in a stepwise regression together (e.g. Frontal for each ERP attribute), there is an indication that frontal and midline clusters show a significant relation to microbiome diversity. In the Target condition the minimum amplitude significantly predicts Shannon alpha in the Frontal. Frontal Right, and Midline sensors, $F(1,46) = 5.611$ $p = .022$ adj. $R^2 = .089$ $b = .164$ VIF = 1 CI = 3.347, $F(1,46) = 4.742$ $p = .035$ adj. $R^2 = .074$ $b = .194$ VIF = 1 CI = 3.251, and $F(1,46) = 4.206$ $p = .046$ adj. $R^2 = .064$ $b = .289$ CI = 3.630, respectively.

Passive Oddball. For the passive oddball task, stepwise multiple regressions were performed in an identical fashion to the detection task to evaluate if the minimum or maximum amplitude, mean amplitude of the positive or negative deflection, or latency to the positive or negative peak for Familiar and Novel Unique stimuli predicted microbiome diversity as measured by Shannon alpha. Education was not selected to remain in the model for any of the regressions.

The latency to the negative deflection for Familiar in the Frontal Right cluster significantly predicted Shannon alpha, $p = .024$ $F(1,55) = 5.373$ adj. $R^2 = .072$ $b = -.298$ CI = 20.726, suggesting that as latency increases to the negative deflection alpha diversity decreases (**Figure 15**). See **Table 5** for results of this model.

The max amplitude as well as the mean amplitude from 350-1500ms at the Temporal Left cluster predicted Shannon alpha during the Familiar condition, $F(1,55) = 5.456$ $p = .023$ adj. $R^2 = .074$ $b = -.300$ CI = 4.735 (**Figure 16**); $F(1,55) = 5.225$ $p = .026$ adj $R^2 = .070$ $b = -.295$ CI = 1.888 (**Figure 17**). The model for the positive peak when observing a familiar stimulus suggests that as the amplitude of the peak

increases alpha diversity decreases and as the mean amplitude increases across the window diversity decreases. See **Table 5** for results of this model. When these significant variables are placed into the same model, the model again emerges as significant, $F(2,54) = 3.397$ $p = .041$ $R^2 = .112$.

When clusters were tested in a stepwise regression together (e.g. Frontal for each ERP attribute), there is an indication that frontal and temporal clusters show a significant relation to microbiome diversity. In the Familiar condition negative latency and maximum amplitude significantly predict Shannon alpha. The negative latency in the Frontal Right cluster significantly predicts Shannon alpha, $F(1,55) = 5.373$ $p = .024$ adj. $R^2 = .072$ $b = -.012$ VIF = 1 CI = 20.726. The maximum amplitude in the Temporal Left cluster significantly predicts Shannon alpha, $F(1,55) = 5.164$ $p = .024$ adj. $R^2 = .074$ $b = -.374$ VIF = 1 CI = 4.735.

Added Hypotheses for Conference Presentation

Other Individual Factors Influencing Cognition and Microbiome Diversity

To address Specific Aim 4c, several additional variables collected about the participants were evaluated for their relation to microbiome diversity. In particular, several dietary variables were evaluated, the Healthy Eating Index, three fiber content measures of the diet (total, soluble, and insoluble), total fat content of the diet, two protein content measures (animal and plant), and berry consumption. Interestingly, plant protein content, total dietary fiber, and insoluble dietary fiber significantly predict a change in MoCA score, but do not predict Shannon diversity, $F(1,90)=5.503$, $p=.021$, $R^2=.047$, $F(1,90)=5.133$ $p=.026$ $R^2=.043$, and $F(1,90)= 5.104$, $p=.026$, $R^2=.043$, respectively. Only berry consumption was significantly related to Shannon diversity.

Berry consumption. Evidence is emerging regarding the modulatory activity of dietary polyphenols on the gut microbiome, but the influence is still poorly understood. In turn, the gut microbiome is known to contribute to the production of polyphenol metabolites. Moreover, recent evidence suggests that gut microbiome diversity relates to many health outcomes, such as cognitive function. Still, there are no published studies investigating a free-living human sample of older adults to determine the relation between berry consumption and gut microbiome composition. I quantified

associations between berry consumption and gut microbial diversity and composition. I hypothesized that a positive linear relation exists between berry consumption and Shannon diversity. Based on existing literature (Bamberger et al., 2018; Mena et al., 2015; Wu et al., 2011), I further hypothesized that berry consumption would be associated with *Enterobacteriaceae* and *Clostridium*, specifically.

Using the individual 3-day diet recalls collected at the follow-up appointment, I queried the NDSR database of calculated food totals for each participant and extracted daily individual food totals. I then isolated those individuals who consumed any polyphenol-rich berry. Once berry consumers were identified, the total number of servings per day was calculated and summed across the three days. The resulting variable, Total Berry Servings, was then used to evaluate the relation between berry intake and Shannon diversity.

Multivariable-adjusted regression models were used to assess whether Total Berry Servings predict Shannon diversity. As before, Education was included in the model. Among participants who consumed berries (n=22; servings=0.13-4.87), consumption was significantly positively related to the Shannon Diversity Index, $F(2,18)=4.310$, $p=.03$, $r^2=.324$. Further, berry consumption was associated with a higher relative abundance of *Enterobacteriaceae* and *Clostridium spp.*, $F(1,19)=4.310$, $p=.046$, $r^2=.152$, and $F(1,19)=5.822$, $p=.026$, $r^2=.194$, respectively. The model indicates that berry consumption was positively associated with gut microbial diversity and two genera known to be influenced by polyphenols, confirming the association found in controlled studies using a free-living sample. See **Appendix D** for poster from conference presentation.

CHAPTER 4: DISCUSSION

In the present study, I identified early indicators of cognitive decline using CANTAB and visual ERP as well as explored the relation of gut-microbiome diversity to cognitive performance. Participants underwent a set of tests to evaluate cognitive decline over time: the Montreal Cognitive Assessment (MoCA), a CANTAB battery for behavioral cognitive assessment, and electrophysiological evaluation via the passive oddball paradigm and an active detection task. I identified a collection of unique relations among the variables tested. One difference between individuals, gut microbiome diversity and composition, changes within the person across his or her lifespan as well as varying among individuals. Recent research on the gut microbiome has shown that an individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior by indirectly changing internal homeostasis. In this study, I investigated the role of microbiome diversity in cognitive decline, validated ERP against CANTAB measures, characterized predictive relation between the MoCA and future cognitive outcomes, and showed the utility of ERP PCA factors and CANTAB outcomes to predict future ERP and CANTAB performance.

In older adults, executive functions are in decline most likely due to changes in activation and utilization of specific brain areas. Healthy aging is associated with increased brain activity in the PFC (during simple tasks)(Persson et al., 2006), alterations in the MTL (Pudas et al., 2013), altered hippocampal function during working memory tasks (Pudas et al., 2013), and improved performance and transfer of working memory skills (Borella et al., 2014). This pattern is part of the CRUNCH (Schneider-Garces et al., 2009), which posits that older adults use more cortical resources than younger adults in order to perform at the same level, and with the most difficult tasks, reduced cortical activation is exhibited (See STAC-R; Goh & Park, 2009). These changes in and attributes of cognitive function can be

observed in behavioral and electrophysiological tasks such as the ones in this study, and some aspects of these changes have been confirmed by my results.

Relation of CANTAB and ERP

Three CANTAB measures (RTI, SWM, and RVP) were independently confirmed to significantly relate to selected ERP measures in both the active detection task and the passive oddball task.

The N1 Component of the ERP

The peak of the negative deflection in a visual active oddball or detection task, commonly referred to as the "visual N1," typically occurs around 75 to 180ms post-stimulus, is most commonly evaluated at central and frontal electrode sites, and is part of the visual evoked potential – a series of voltage deflections observed in response to presentation of visual stimuli (Luck & Hillyard, 2009; Mangun, 1995; Mangun & Hillyard, 1991; Naatanen, 1982; Thorpe, Fize, & Marlot, 1996). This time course and spatial specificity match the negative window (50-190ms) and clusters selected in the active detection task. In my study, the amplitude of the visual N1 is influenced by selective attention and is therefore used to study attentional processes (Kok, 1999; Mangun, 1995; Martinez et al., 2006; McDowd & Filion, 1992). The visual N1 is a sensory component evoked by any visual stimulus, reflecting a critical mechanism of attention that indicates whether or not attention was appropriately allocated, and is a manifestation of a critical sensory gating mechanism of attention (Luck & Hillyard, 2009; Vogel & Luck, 2000). One would expect the N1 component to be enhanced under conditions that require a decision, such as the detection task of the present study. As a result, individuals with better cognitive function (improved attention allocation) would be expected to have a higher peak amplitude of the N1 when identifying a target stimulus. Below I relate two CANTAB tasks – RTI and RVP – to the N1 component.

RTI. Reaction time was assessed as the response time during the five-choice task in RTI. The five-choice task was selected due to the more complex nature compared to the simple (one-choice) task as it is expected that more significant differentiation would exist at higher cognitive demand. Reaction Time (RTI) was a measure of simple and choice reaction time, movement time, and vigilance during several reaction time trials. Both the simple (one-step) and the 5-choice serial reaction time variable used for the

analyses in this study were chosen because they are analogues to a well-characterized animal behavior paradigm (5-Choice Serial Reaction Time; 5-CSRT). In the rat, the 5-CSRT shows sensitivity to discrete lesion sites in the PFC (Fizet, Cassel, Kelche, & Meunier, 2016). It would be expected that participants with MCD would show declined accuracy and slower latency on the RTI task.

During the non-response of the detection task, RTI predicted a reduction in the total deflection (negative amplitude) of the N1 component (Figure 3B). The reduction in the amplitude of the N1 as reaction time increases indicates poorer attentional allocation, resulting in poorer decision making about the stimulus (Luck & Hillyard, 1994; Luck & Hillyard, 2009). It was hypothesized that as reaction time increases, ERP measures of cognitive function would decline. Results in the active detection task support to this hypothesis at the N1. With cognitive decline, the N1 component should show a reduction in amplitude and does, thereby supporting the conclusion of poor activation and attentional selection with poorer cognitive function as measured by RTI. The use of 5-choice time when comparing to the detection task in ERP may introduce a confound of test dissimilarity, because when compared to the simple reaction time task, the 5-choice task requires encoding of spatial location and the ERP detection task does not. As I discuss below, this confound could explain some of the results that do not support the hypothesis.

RVP. In order to evaluate what even higher task demands would do to processing ability, the mean latency to a response during the RVP task was selected. Mean latency differs from the reaction time task in that memory of a sequence has to be maintained, and evaluation of a string of numbers must be done before a response is given. Rapid Visual Information Processing (RVP) was intentionally placed last in the battery of tests. By placing the most difficult task last, I intended to take advantage of some cognitive fatigue late in the appointment to assess the underlying mechanisms of CRUNCH and STAC-r – declines in more difficult cognitive tasks. Performance on the RVP task has been shown to be associated with activation in the frontal and parietal lobes and the networks that connect them (Coull et al., 1995). Therefore, RVP was an accurate measure of the ability to utilize multiple connected systems

and assess higher order cognitive processes while still using a variable that could easily be compared to the temporal activation measures in the ERP.

During the detection task, RVP predicted that as latency to response increases the minimum amplitude decreases (Figure 7B). A reduction in N1 amplitude during the active response indicates cognitive dysfunction as attentional allocation should be increased (eliciting an increase in N1 amplitude). A decrease in N1 amplitude supports the hypothesis that RVP performance predicts cognitive decline measures in ERP attributes.

The N2 Component of the ERP

Another essential ERP component is the N2, the negative waveform that peaks between 200-350ms, which has been found to reflect executive cognitive control functions in adults – particularly a measure of attentional processing (Luck & Hillyard, 1994; Luck & Hillyard, 2009). In a passive oddball task, such as the one in the present study, the latency to peak amplitude should differ between familiar and novel pictures. When an individual has been exposed repeatedly to an image (the familiar), the need to process the information about that image diminishes over time and attention to the image will fall linearly to that need and latency should decrease. Therefore, better cognitive function as measured in the passive oddball paradigm during the selected window of the negative deflection (210-350 ms) would be indicated by shorter latency in the Familiar condition.

RTI. During the novel stimulus of the passive oddball task, latency to the N2 decreases as reaction time increases (Figure 4A). Shorter latency to the N2 indicates shorter attentional processing and is not what one would expect during the Novel stimulus for typical cognition. Instead, latency is expected to be longer during the novel stimulus, indicating this reduced latency may indicate poorer attentional activation (Luck & Hillyard, 1994; Luck & Hillyard, 2009). Reaction time also significantly predicts the reduced amplitude of the N2 during the familiar stimulus (Figure 4C), indicating what would be considered better cognitive function, which does not support my hypothesis that increased reaction time would indicate poorer performance. So, the results provide mixed information about the relation between RTI reaction time and the N2 ERP component. The most likely explanation for this relation is the

dissimilarities between the two tasks, namely the lack of a response in passive oddball and significant differences in the type of visual stimulus. I propose here that visual dissimilarity of the two tasks may confound the results and a simple reaction time test may not adequately characterize the activational ERP aspects of a low-stakes visual oddball paradigm.

SWM. The Spatial Working Memory (SWM) task measured the ability to retain spatial information and to manipulate remembered items in working memory. The test is a sensitive measure of frontal lobe and executive function dysfunction. SWM performance is impaired by damage to the PFC, especially the dorsolateral PFC (Owen et al., 1990; Manes et al., 2002). Moreover, in neuroimaging studies in healthy participants, SWM performance is associated with activations in the dorsolateral and mid ventrolateral PFC (Owen et al., 1996). Therefore, SWM performance would be significantly impaired in mild to moderate AD and should show significant differences between MCD and non-MCD participants. The spatial working memory strategy score was selected as it is the most inclusive variable of the different factors required to perform well in the SWM task. A higher strategy score on the SWM task indicates an increase in the number of attempts and/or time to discovery of all objects corrected to the comparison of a "perfect" strategy versus the strategy utilized. The results indicate several significant findings between the SWM task and ERP outcomes.

Spatial Working Memory significantly predicted two aspects of the ERP during the passive oddball task (during the familiar stimulus; Figure 6). Poorer strategy score predicts reduced latency to and increased amplitude of the negative deflection. Reduction in latency to the N2 is historically indicative of better cognitive function, so this finding is perplexing, but the relation may be indicative of poor attentional control in general (Luck & Hillyard, 1994; Luck & Hillyard, 2009). In support of the literature, however, one would predict a decrease in amplitude of the N2 for the familiar stimulus in healthy cognitive function. So, in contradiction to the latency, the increased amplitude of the N2 confirms the relation between SWM and the N2 component. This contradictory evidence could be explained by the simplicity of the passive oddball paradigm. As with the RTI task, the lack of a response in the passive oddball task may be a confound for which we should control. In future studies, an active oddball task will

be added to better elucidate the relation between the two tasks. It is important to add, though, that during the novel stimulus, the finding that amplitude is higher when SWM performance is poorer may indicate that SWM performance is a valid measure of the additional cognitive utilization during tasks in cognitive decline as posited by STAC-r and the CRUNCH.

The P3 Component of the ERP

A third visual ERP component, the P3, is a positive peak occurring after 300ms and typically before 700ms following a visual stimulus presentation and alterations in it have been strongly linked to typical and atypical cognitive aging (Jiang et al., 2015; Knott et al., 2004; Papaliagkas et al., 2011; Parra et al., 2012; Polich, 2007; Pontifex et al., 2009). The P3 differs from the N1 and N2 components as it does not link to a physical attribute of a stimulus but rather to a person's cognitive response to the stimulus. For this reason, it is commonly considered an "endogenous potential" and is most commonly attributed to the process of decision making. More accurately, the P3 reflects processes involved in stimulus evaluation or categorization; commonly measured in an oddball paradigm – low-probability (Novel) items mixed with high-probability (Familiar) items (Jiang et al., 2015; Kirino et al., 2000; Pato & Czigler, 2011; Polich, 1996). Much like the N2 component, the overall existence or amplitude of a P3 should be higher for less-probable, novel, events and small in amplitude or non-existent for more probable familiar events. This observed outcome, however, is reliant upon healthy cognitive function – the ability to successfully discriminate between stimuli via appropriate attentional orientation and effective visual memory storage and retrieval. Therefore, individuals experiencing cognitive decline might be expected to show increased latency to and amplitude of the P3 component, especially in the Familiar condition. If this is true, during the passive oddball paradigm, older adults experiencing cognitive decline would have a higher maximum amplitude during the positive inflection window.

RTI. During the non-response of the detection task RTI predicted a reduction in the positive peak (positive amplitude) of the P3 (Figure 3A). The reduction in the maximum amplitude during what would likely be a P3 component could indicate that participants with poorer reaction time do not reach full activation during the decision-making of whether to press the button. However, the literature would

suggest that the amplitude of the P3 would show no change or an increase with decline in cognitive performance when there is no decision or action to be made (Jiang et al., 2015; Kirino et al., 2000; Pato & Czigler, 2011; Polich, 1996). The opposite relation is observed when performance is measured by the RTI task. Because the RTI task is visually different than the detection task, the relation between the two tasks may not be reflective of the hypothesized cognitive processes and may represent an unknown attribute. In a future study, I will design tasks that are more visually similar. It is also possible that I have incorrectly characterized the functional aspects of the reaction time variable for RTI, where the task cannot appropriately characterize the activational aspects of the ERP during the detection phase of processing. Additionally, the two tasks are both relatively simple in that they do not require a great deal of resources for success. The relation should be tested again with tasks of increased complexity and greater similarity in visual stimuli.

During the novel stimulus of the passive oddball task, latency to the P3 increased as reaction time increased (Figure 4B). The potential for reduced attentional activation noted in the N2, above, is also supported by the increased latency to the P3 component as this indicates time to decision making and response to the stimulus has been increased. Evaluation of the results indicates support for the conclusion that increased reaction time predicts poorer attentional activation, resulting in a delay in processing and subsequent decision making indicated by the P3 component.

RVP. During the detection task, RVP predicted that as latency to response increases the maximum amplitude (P3) increases (Figure 7A). The increase in maximum amplitude may indicate overactivation in cognitively declined individuals compared to those with better RVP performance, but these data cannot provide support for this conclusion at this time.

Evaluation of the relation between RVP and passive oddball ERP attributes indicates a significant relation between RVP performance and two aspects of the P3 component during the familiar stimulus (Figure 8). As mean latency to response increase in the RVP task, the mean and maximum amplitude of the P3 increases. In healthy cognitive function, the P3 should be reduced during the familiar stimulus,

indicating that poorer RVP performance predicts overactivation, and therefore cognitive decline, during the ERP task.

SWM. SWM strategy score significantly predicted longer latency to the positive peak during the non-response of the detection task in two ERP clusters (Figure 5). For the non-response and more common stimulus, analysis of the literature would suggest that the P3 would be shorter in time to activation and reduced in amplitude (Jiang et al., 2015; Kirino et al., 2000; Pato & Czigler, 2011; Polich, 1996). An increase in latency to the P3 during the common stimulus indicates slower decision making about the stimulus and therefore poorer cognitive function, supporting the hypothesis that poorer performance on the SWM task would relate to poorer cognitive function as measured by the ERP task.

Conclusion

In consideration of the relation between ERP and CANTAB measures, I suggest that there are inviting relations for future exploration, but designing ERP tasks that are more visually similar to the CANTAB tasks is highly desirable. The goal of Specific Aim 1 was to explore the relation between CANTAB and ERP measures, and in that effort, RTI and ERP tasks are related, but additional exploration needs to be performed to determine the underlying mechanisms. SWM and RVP tasks show more straightforward and clear relations to the two ERP tasks than RTI, but all three would benefit from a study designed to reduce the noted confounds. Even without these improved investigations, significant conclusions can be drawn. I suggest that these relations are highly valuable in both a research and clinical settings. These results indicate that a relation does exist between the behavior measured by the CANTAB and the processes occurring in the brain of older adults. This knowledge of form to function could reduce the necessary burden of identifying decline in older adults by reducing the tests needed during physician visits and screening for new studies of cognitive decline.

How does baseline global measure predict follow-up performance?

I chose to explore this question in two ways to best capture the relation between baseline characteristics of my participants and their cognitive outcomes at follow-up. First, I regressed follow-up ERP and CANTAB variables onto baseline MoCA scores including covariates. Then, I repeated the

regressions with a MoCA change score instead of the baseline MoCA score including covariates. The difference score was calculated by subtracting baseline score from the score at follow-up. Both approaches indicated significant relations, and I discuss the merits of each below.

An additional CANTAB task to test paired associate learning was utilized in Specific Aim 2. Paired Associates Learning (PAL) assesses visual memory and learning, and has been shown to be particularly useful for assessing patients with non-demented types of cognitive dysfunction. Satisfactory performance on PAL is dependent on functional integrity of the temporal lobe, particularly the entorhinal cortex (Owen et al., 1995), and older adults with AD show a marked deficit on this task that predates the gross cognitive decline associated with the diagnosis (Blackwell et al., 2004). Moreover, PAL performance is sensitive to subtle cognitive impairments that are present in individuals who will likely progress to MCI and AD (Juncos-Rabadan, Pereiro, Facal, Reboredo, & Lojo-Seoane, 2014).

Baseline MoCA

When assessing the active response in the detection task, baseline MoCA significantly predicted the mean amplitude of the positive peak (P3). Higher MoCA scores at baseline predicted the increased mean amplitude of the P3. During the active response, increased activation at the P3 is suggested by the literature in healthy cognitive function (Bourisly, 2016; Chapman et al., 2011; Cid-Fernandez, Lindin, & Diaz, 2014; Fjell & Walhovd, 2001), so the relation with better MoCA score is supported.

Baseline MoCA score significantly predicted outcomes in the SWM and PAL tests for CANTAB at follow-up. The hypothesis that better MoCA score at baseline predicts better performance on CANTAB at follow-up is supported by the data as an increase in MoCA score predicts lower (better) strategy score on SWM and fewer errors in the PAL task. The identification of this relation between CANTAB and MoCA improves our ability for early diagnosis. True to the history of the MoCA, a brief and inexpensive screening with the MoCA could be used clinically to determine if further testing with the CANTAB is warranted.

Change in MoCA Score

Change in MoCA score significantly predicts several ERP outcome measures at follow-up. First, as the change in MoCA score increases (better performance) latency to the N1 decreases. Reduction in latency to the N1 component indicates better attention. Thus, these results support the hypothesis that relative stability between baseline and follow-up on the MoCA predicts better brain function. MoCA change also significantly predicts mean amplitude of and latency to the positive peak in multiple ERP clusters during the familiar stimulus in the passive oddball task: Better MoCA change score predicts the reduced amplitude of and latency to the P3 when viewing the familiar stimulus. In the familiar condition, a healthy brain should have reduced activation and earlier peak processing as there is no processing to be performed on the stimulus; therefore, these findings support the relation between better change score and better cognitive function at follow-up. Finally, change in MoCA also predicted the latency to the P3 in the novel stimulus, where a better change score predicts decreased latency to the P3 increases. Again, better cognitive function as measured by the passive oddball task should be indicated by faster processing and shorter latency to peak processing; therefore, the results continue to support the hypothesis.

Change in MoCA score also significantly predicts mean latency to response in the RVP task. Better MoCA change score predicts a shorter latency to correct response. This relation with latency supports the improved processing speed results above and further supports the hypothesis that reduced change in MoCA score from baseline to follow-up predicts better cognitive function at follow-up. These associations of CANTAB to MoCA change score are key findings for developing tools for early identification of decline and prediction of future decline. As with the relation to better baseline MoCA score, the knowledge that stability in MoCA score is also predictive of better cognitive function allows clinicians to better understand the needs of aging adults and provides the potential for earlier intervention.

Do behavioral and electrophysiological tasks predict follow-up performance on those tasks?

Both ERP and CANTAB measures from baseline were successfully used to predict future performance on those tasks at follow-up. The use of two-step PCA transformation on the ERPs proved to be a successful method to allow for the comparison of ERP factors at two time points and to determine if

baseline ERP measures can predict later ERP outcomes. To my knowledge, this is the first execution of a temporospatial PCA on ERP tasks like those in this study, and furthermore, this is the first use of this method to predict ERP outcomes across time.

Predicting Follow-Up ERP with Baseline ERP

In the passive oddball paradigm, three temporospatial (440-445ms) factors that represented similar electrode locations to the Frontal Left (Factor 2, TF2SF1, Factor 4, TF2SF3) and the Frontal Right (Factor 3, TF2SF1) clusters showed significant predictive value to determine performance in these locations from baseline to follow-up. For the Familiar condition, factors 2, 3, and 4 from baseline significantly predicted their counterpart at follow-up where greater amplitude at baseline predicted greater amplitude at follow-up. Additionally, Factors 2 and 3 show the same positive relation in the novel condition. The temporal component of these factors is likely indicative of P3 amplitude at three unique spatial locations. In the future, with the data in hand, I will be able to select for temporospatial factors such as these *a priori* at each step of the PCA, attending to both sensor location and temporal relativity to better predict the attributes measured in the task for older adults.

An additional factor, Factor 5 (TF3SF3), did not significantly predict future ERP outcomes, but did show promise for identification of other factors. Even though Factor 5 did not significantly predict future outcomes, it does closely resemble the positive slow wave that was observed at the end of the epoch in my older adults. Its temporal location was 769-773ms, and it incorporated electrodes most closely related to the Frontal Right and CentralZ clusters. An overall ANOVA model was significant, indicating significant differences in the conditions among individuals, and a contrast indicated a significant amplitude difference between the Familiar and Novel conditions. By predicting a difference between Novel and Familiar conditions, Factor 5 proves itself as an avenue for future exploration to differentiate between individuals experiencing decline and those who are not.

In the detection task, factors that represent a wider range of temporal and spatial variety showed significant predictive value to determine performance in those factors from baseline to follow-up. Factor 1 (TF1SF1) represents a similar spatial location to the Frontal and Midline clusters and amplitude from

819-823ms. Factor 1 was included because the positive slow wave in the detection task appeared to extend to the end of the 1500ms window for many older adults, and this factor is a good candidate for future exploration. Factor 2 (TF2SF3) spatially represents electrodes in the Frontal and Frontal Left clusters while representing the temporal window of 480-487ms, which is likely a P3b component. Factor 3 (TF3SF3) is very close to Factor 2 in temporal space at 319-323ms and likely represents the P3a, but is best represented by electrodes between the Temporal Right and Frontal Right clusters. Lastly, Factor 4 (TF5SF1) represents temporally (191-195ms) what would likely be the N1 and is represented by electrodes located on the base of the skull along the backline outside of any of the clusters, but is most closely related to the Temporal Right cluster. Factor 4 was also significant in the robust ANOVA evaluating amplitude differences between inhibited and active conditions indicating that it may be a valid measure of the N1 component. Like the factors produced for the oddball task, I will use these results to support the *a priori* selection of factors at each step in future studies to help better elucidate what these factors can tell us about change in cognitive function in older adults.

These PCA results provide a robust new avenue for exploration of electrophysiological attributes of healthy older adults and could be used to develop new methods for early identification of cognitive decline. The identification of individual factors that predict future activation one, two, or three years later could be instrumental in developing new methodology for clinicians to identify and track cognitive decline.

Predicting Follow-up CANTAB Performance with Baseline Performance

The evaluation of CANTAB measures to predict themselves over time was highly significant, but if repeated in the future, reduction in collinearity should be explored. Even with high Condition Index levels, the VIF and tolerance factors remained low, and models were highly significant easily passing the conservatively adjusted p-value of .0003. RTI, PAL, SWM, and RVP at baseline all significantly predicted their counterpart at follow-up where poorer performance on the task at baseline predicts poorer performance at follow-up.

Conclusions

Specific Aim 3 became one of the most novel and learning-intensive explorations of the four aims due to the utilization of PCA methods that have not been used in this way previously. As a first step into exploring the predictive qualities of initial testing on future outcomes, the aim was a success. As noted, there are some methodological changes that I will make in future studies such as using these findings to develop an *a priori* approach to factor selection and the addition of additional ERP tasks that would add diversity in the types of waveforms present in the ERPs. The significant findings that indicate predictive quality of both ERP and CANTAB measures could prove promising for early intervention and treatment of MCD and the prevention of dementia.

How does microbiome diversity relate to cognitive function in older adults?

Behavioral and electrophysiological measures of cognitive function in older adults were investigated in relation to alpha diversity, a measure of the varieties of species in the gut-microbiome, to test the hypothesis that a relation exists between gut microbial diversity and cognitive performance. The results indicate that there is an association between behavioral measures (paired-associate learning and spatial working memory) and calculated alpha diversity of the gut microbiome, where poorer performance (indicative of cognitive dysfunction) predicted lower gut-microbiome diversity (**Appendix C** – Canipe, Sioda, & Cheatham, Submitted). The findings support the hypothesis that cognitive performance, as measured by a standardized behavioral assessment, is related, negatively, to poor microbial diversity in the gut. Furthermore, results indicate that there is a significant predictive relation between several electrophysiological cognitive measures and gut-microbiome diversity.

The results indicate several significant findings. First, the minimum and mean amplitude of the negative deflection in the Frontal region of the ERPs of participants during the Target condition of a detection task significantly predict alpha diversity. The same relation is observed in the passive oddball task where the latency to the negative deflection in the Frontal Right cluster was shown to predict Shannon alpha significantly. Adding to the significant relation between Shannon alpha and brain activity

during the passive oddball task, the max and mean amplitude of the positive deflection for the Familiar condition in the Temporal Left cluster was also predictive of Shannon alpha.

One would expect the N1 component to be enhanced under conditions that require a decision, such as the detection task of the present study. As a result, individuals with better cognitive function (improved attention allocation) would be expected to have a higher peak amplitude of the N1 when identifying a target stimulus. The relation between alpha diversity of the microbiome and amplitude of the N1 for Target in the ERPs of participants suggests that individuals with greater amplitude (more negative) have greater microbiome diversity, supporting the hypothesis that increased microbial diversity in the gut relates to better cognitive function.

Better cognitive function as measured in the passive oddball paradigm during the selected window of the negative deflection (210-350 ms) is assumed to be an N2 and as such, would be indicated by shorter latency in the Familiar condition. In the present study, it was shown that an increase in latency to peak amplitude in the Familiar condition predicts a decrease in alpha diversity of the gut microbiome. This finding supports the hypothesis that microbial diversity in the gut is related to sustained attention.

Much like the N2 component, the overall existence or amplitude of a P3 should be higher for less-probable novel events and small in amplitude or non-existent for the more probable familiar events. This observed outcome, however, is reliant on healthy cognitive function – the ability to successfully discriminate between stimuli via appropriate attentional orientation and effective visual memory storage and retrieval. Therefore, individuals experiencing cognitive decline might be expected to show increased latency to and amplitude of the P3 component, especially in the familiar condition. If this is true, during the passive oddball paradigm, older adults experiencing cognitive decline would have a higher maximum amplitude during the positive inflection window. Indeed, measures for mean amplitude from 350-1500ms during the Familiar condition predicted Shannon alpha, where an increase in both measures of amplitude predicted a decrease in gut-microbiome diversity. Again, this supports the hypothesis that gut-microbiome diversity relates to healthy cognitive function.

Finally, as part of presentation given at a nutrition conference (**Appendix D**), I showed that berry consumption significantly predicts microbiome diversity. In a free-living human sample, total servings of berries were positively associated with gut microbial diversity. Further, I confirmed associations between specific genera and polyphenols in controlled studies previously reported by others. The inclusion of berries in the daily diet may contribute to increases in the diversity of gut microflora, which in turn may lead to improvements in related health issues such as inflammatory disease and cognitive dysfunction. Future studies and analyses should aim at refining our understanding of the interplay among berries, influential gut microflora, and health outcomes.

Significance

The interpretation of the findings suggests significant implications about the relation that exists between gut-microbiome diversity and healthy cognitive function. Conclusions of previous studies in humans suggested a relation between poor microbiome health and observed behavioral measures in individuals diagnosed with illnesses or taking medications known to impact the gut microbiome negatively, but specific measures of microbial disposition and measured cognitive or clinically quantified behavioral outcomes have been limited (Anderson et al., 2017; Bajaj et al., 2016; Bajaj et al., 2012; Caracciolo et al., 2014; Dinan et al., 2015). Animal models of microbiome diversity and behavior have revealed a clear link between specific gut bacteria and unique cognitive and behavioral outcomes (Bravo et al., 2011; Janik et al., 2016; Marchesi et al., 2016; O'Mahony et al., 2015; Sampson & Mazmanian, 2015), but high levels of specificity in the individual microbes discovered to impact the gut-brain axis have made it challenging to translate useful predictions for humans (especially older adults).

With this study, I have attempted to bridge this gap between loosely attributed relations among behavior and gut-brain interactions in humans and the understanding of the microbial impact on brain function observed in highly-controlled animal studies. The bridging of these gaps and previously disparate fields of science (psychology, neuroscience, microbiology, and bioinformatics) has been accomplished by combining clinically validated behavioral measures and electrophysiological measures of brain activity with robust quantification and characterization of gut-microbiome data collected from a

large cohort of older adults. The initial analysis of the data from this large cohort will contribute to the growing knowledge about the gut-brain axis. The present study is limited in that it has only evaluated a diversity metric, and further analyses of the dataset should be performed as improved knowledge of individual microbes becomes available. Without a doubt, however, the findings presented have revealed new hypotheses and future directions for impactful follow-up work. Most importantly, the work reported here is the first in a program of research that will add to our knowledge and ability to reduce the effects of age-related cognitive decline in our population by identifying individual biomarkers of cognition in healthy older adults.

General Discussion and Conclusions

The present study adds to our understanding of age-related cognitive decline by elucidating some of the measures of cognitive function sensitive to change and proving the validity of these tests to predict future outcomes. The study supports many ideas in the literature on cognitive decline and fills gaps in knowledge I previously identified by validating CANTAB and ERP and showing, for the first time, the use of a passive visual oddball paradigm with older adults. The utility of ERP in cognitive decline assessment has been strengthened as it has been validated with common clinical assessments of CANTAB and the MoCA, and a new technique for the longitudinal study of ERPs has been introduced with PCA analysis.

The improved validation of visual ERP to identify and predict cognitive (dys)function in healthy older adults adds strength to previous results that indicated that auditory ERPs were effective predictors of MCD and different dementias. In the first and second specific aims, attributes of the visual ERP are effectively shown to relate to valid behavioral measures in the CANTAB, and will allow clinicians to better understand the activational aspects of patient's brains during reaction time and memory tasks without costly and time-consuming brain imaging techniques like EEG. In Specific Aim 2 I further strengthen the validity of visual ERP and CANTAB to identify and predict cognitive decline by presenting relations between baseline and change scores of the global assessment the MoCA. As mentioned, validation to predict both baseline and change score of the MoCA could greatly improve our

ability for early identification of cognitive decline in presently healthy adults before atypical decline begins as well as reduce the burden and cost of unneeded tests by eliminating onerous testing on patients who do not need them.

Also of critical importance in my study was the development of two new methodologies (PCA and gut-microbiome) not previously used in the study of age-related cognitive decline, especially in healthy older adults. First, the utilization of two-step PCA methods to compare to ERP timepoints had never been performed. This new method shows promise in the development of several new lines of investigation to use ERP attributes to identify and predict cognitive decline, possibly before it even begins on a measurable level. I plan to use the data collected from this initial method building step to develop new *a priori* hypotheses in future studies with older adults and publish these methods, and predictions to help grow the field.

Furthermore, with this study, I have described and investigated some of the contributing factors to cognitive decline, the most important of which is the novel understanding provided by the newly defined relations between cognition and gut-brain interactions in healthy older adults. I add several new ideas to the body of literature that have not been directly investigated before, such as gut-microbiome diversity in free-living older adults. Cognitive decline is highly individualistic in its development and involves the influence of the many factors that can contribute to its progression. However, with proper early identification, and continued growth in knowledge of its influencers, we may succeed in slowing or stopping it in the population before it becomes a burden too significant for humanity to bear.

TABLES

Table 1. Cohort Characteristics at Baseline

N	92
Age at baseline (years)	71.98 ± 4.21
Sex (%)	
M	44.57
F	55.43
Race (%)	
White	96.77
Black or African American	2.15
American Indian/Alaskan Native	1.08
Native Hawaiian or other Pacific Islander	0.0
Marital Status (%)	
Single/Never Married	3.23
Married	75.27
Separated/Divorced	6.45
Widowed	15.05
Education (%)	
Less than high school degree	3.33
High school degree	14.44
Some college	30.00
2 year college degree	11.11
4 year college degree	23.33
Master's Degree	16.67
Doctoral Degree	0.00
Professional Degree	1.11
Avg BMI	
Baseline	27.41 ± 3.85
Avg MoCA Score	
Baseline	24.54 ± 2.49
Avg Full IQ Score	
Baseline	106.18.2 ± 10.87
Avg PASE Score	
Baseline	146.45 ± 62.27

Table 2. Chart of Assessments at Each Session

Assessment	Baseline	Follow-up	Time to collect	Both Sessions		
				6m	*18m*	*30m*
Medical History Questionnaire	113	92	10m	9	22	53
Eye Test	113	-	3m	-	-	-
Demographic Questionnaire	113	-	3m	-	-	-
Montreal Cognitive Assessment	113	92	15m	9	22	53
Multifactorial Memory Questionnaire	113	92	10m	9	22	53
Self-report of Memory	113	92	5m	9	22	53
Informant Report	113	92	-	9	22	53
Generalized Anxiety Disorder-7	113	92	3m	9	22	53
Patient Health Questionnaire	113	92	3m	9	22	53
Anthropometric (Ht, Wt, Waist of Circumference)	113	92	3m	9	22	53
Blood pressure	113	92	5m	9	22	53
Physical Activity Questionnaire	113	92	5m	9	22	53
Stressful Life Event	113	92	5m	9	22	53
3-day Diet Recall	113	92	30m	9	22	53
Weschler Adult Intelligence Scale-IV	113	-	75-90m	-	-	-
Electrophysiological Paradigm/ ERP	113	92	45m	9	22	53
CANTAB	113	92	45m	9	22	53
iDXA	113	-	15m	-	-	-
Blood Draw	113	-	5m	-	-	-
Urine Collection	113	-	5m	-	-	-
Fecal stool sample collected	-	68	~1 wk*	8	12	43

Number indicates number of individuals completing the assessment at each time point. * Fecal samples arrived on average 1 week from the follow-up appointment.

Table 3. Model-building results from stepwise regressions

	Variables Entered	β	SD	p-Value	Model R-Square
Shannon Alpha	Education	.098	1.519	.139	.148
	Reaction Time (RTI)	.070	64.284	.210	
	Mean Time Success (SWM)	-.248	1391.810	.029*	
	Total Errors (PAL)	-.293	22.635	.012*	
	Mean Latency (RVP)	-.025	71.672	.373	

* Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

Table 4. Model Building results from stepwise regressions

	Variables Entered	β	SD	p-Value	Model R-Square
Shannon Alpha	Education	.110	1.519	.279	.087
	Target mean amplitude FL	.002	1.110	.131	
	Target mean amplitude FR	-.075	1.198	.127	
	Target mean amplitude M	.006	1.051	.038	
	Target mean amplitude TL	-.053	.870	.103	
	Target mean amplitude TR	-.021	.814	.093	
	Target mean amplitude F	.294	1.275	.021*	
Shannon Alpha	Education	.136	1.152	.339	.109
	Target minimum amplitude FL	-.144	1.430	.469	
	Target minimum amplitude FR	.169	1.427	.335	
	Target minimum amplitude M	-.131	1.386	.734	
	Target minimum amplitude TL	-.091	1.417	.519	
	Target minimum amplitude TR	-.006	1.459	.968	
	Target minimum amplitude F	.330	1.819	.022*	

* Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

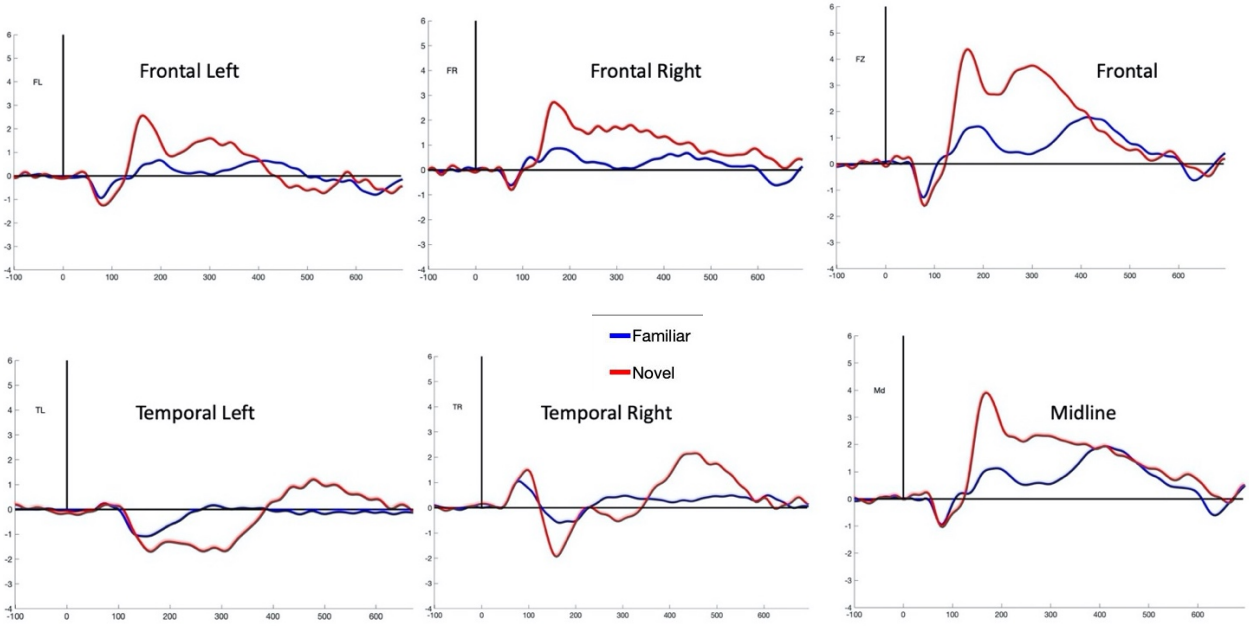
Table 5. Model Building results from stepwise regressions

	Variables Entered	β	SD	p-Value	Model R-Square
Shannon Alpha	Education	.118	1.532	.368	.089
	Familiar Negative Latency FL	-.298	32.447	.871	
	Familiar Negative Latency FR	-.023	26.117	.024*	
	Familiar Negative Latency M	.062	28.162	.703	
	Familiar Negative Latency TL	-.057	39.937	.669	
	Familiar Negative Latency TR	.023	40.353	.862	
	Familiar Negative Latency F	.023	34.248	.894	
Shannon Alpha	Education	.136	1.532	.280	-.295
	Familiar Positive mean Amplitude FL	-.144	1.067	.214	
	Familiar Positive mean Amplitude FR	.169	.996	.729	
	Familiar Positive mean Amplitude M	-.131	1.363	.437	
	Familiar Positive mean Amplitude TL	-.091	.641	.026*	
	Familiar Positive mean Amplitude TR	-.006	.876	.472	
	Familiar Positive mean Amplitude F	.330	1.896	.898	
Shannon Alpha	Education	.228	1.532	.084	.090
	Familiar Max Amplitude FL	-.051	1.464	.695	
	Familiar Max Amplitude FR	.061	1.559	.640	
	Familiar Max Amplitude M	.126	1.661	.350	
	Familiar Max Amplitude TL	-.300	.8116	.023*	
	Familiar Max Amplitude TR	.182	1.253	.218	
	Familiar Max Amplitude F	.113	2.280	.428	

* Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

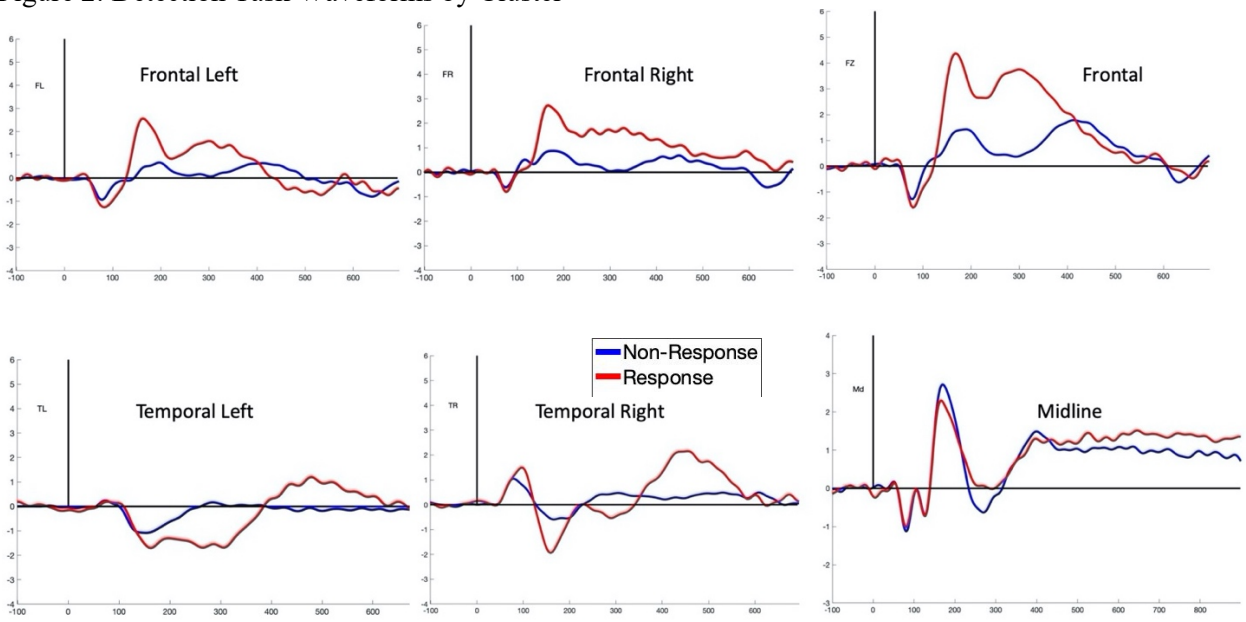
FIGURES

Figure 1: Passive Oddball Waveforms by Cluster



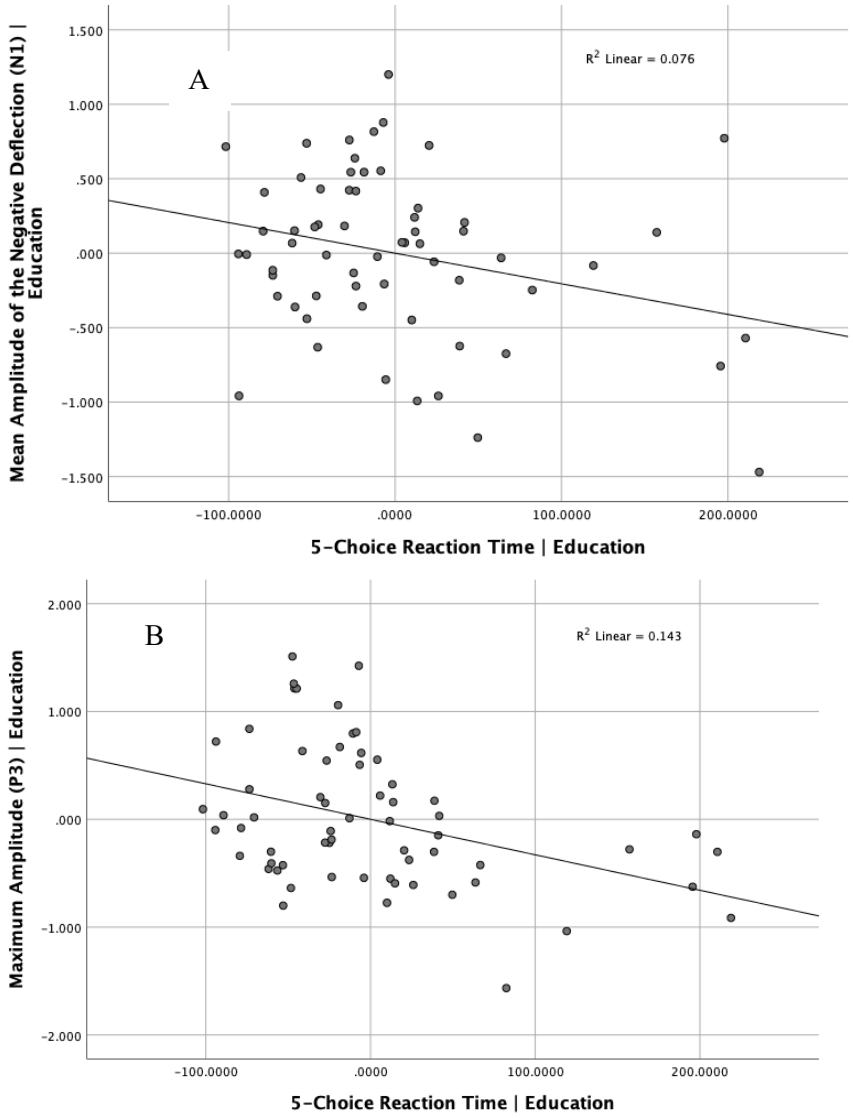
Visual representation of the windows selected in Specific Aims 1,2, and 4 and referenced to factor locations in Specific Aim 3. Windows for passive oddball were 210-350 ms (N2) and 350-900 ms (P3), respectively. Representations show the grand averaged (all participants) waveform data for the passive oddball task in each of the clusters represented in Appendix A; Frontal Left, Frontal Right, Frontal, Temporal Left, Temporal Right, and Midline.

Figure 2: Detection Task Waveforms by Cluster



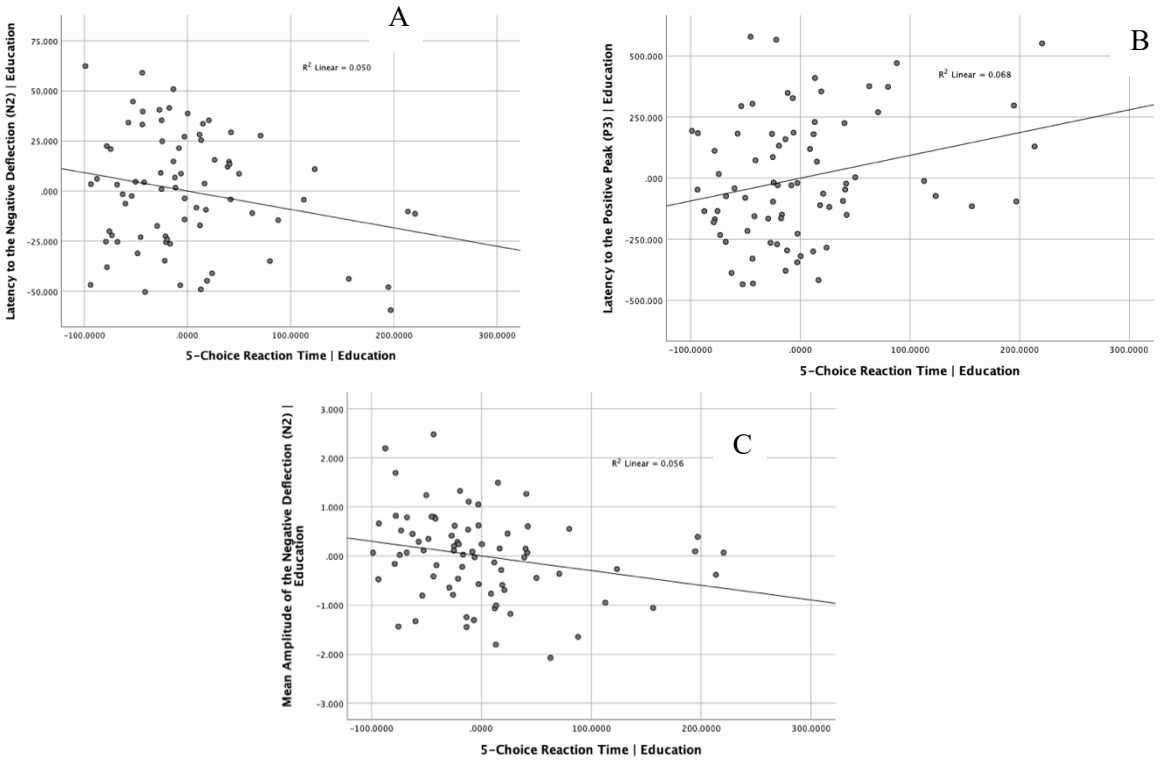
Visual representation of the windows selected in Specific Aims 1,2, and 4 and referenced to factor locations in Specific Aim 3. Windows for active detection task were 50-190 ms (N1) and 100-800 ms (P3), respectively. Representations show the grand averaged (all participants) waveform data for the detection task in each of the clusters represented in Appendix A; Frontal Left, Frontal Right, Frontal, Temporal Left, Temporal Right, and Midline.

Figure 3: Partial Regression Plot of Reaction Time and ERP Detection Task



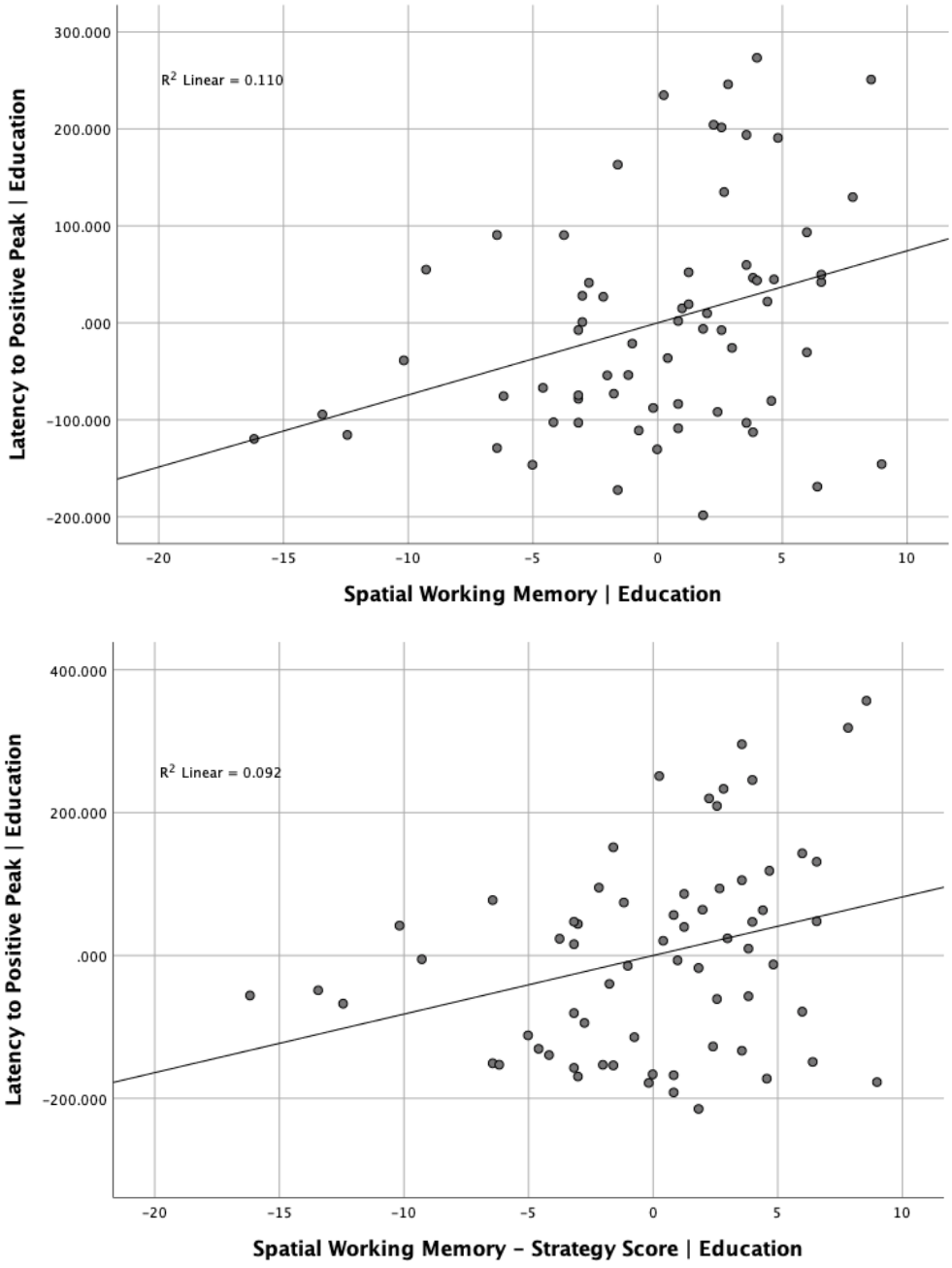
Partial regression plots visually represent the relations between the 5-choice reaction time task during the CANTAB and ERP attributes P3 (3A) and N1 (3B) during the detection task when controlling for education. As reaction time in the RTI task increases the mean amplitude of the negative deflection (N1) and maximum amplitude (P3) of the positive peak decrease; $F(1,62) = 5.353$ $p = .024$ adj. $R^2 = .066$ $b = -.284$, $F(1,62) = 7.057$ $p = .002$ adj. $R^2 = .163$ $b = -.369$, respectively.

Figure 4: Partial Regression Plot of Reaction Time and ERP Oddball



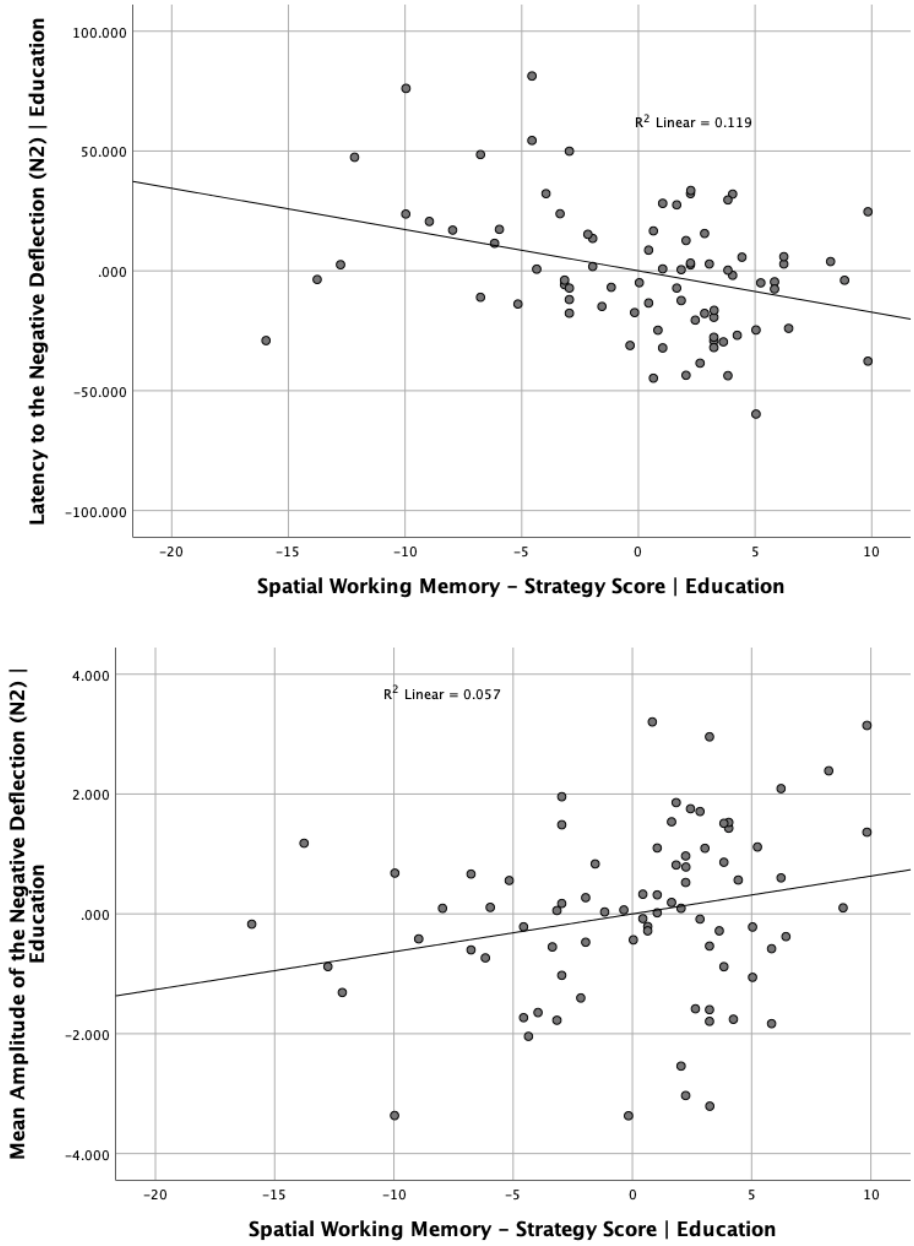
Partial regression plots visually represent the statistically significant relations between performance on the 5-choice reaction time task in the CANTAB and ERP attributes, N2 and P3, during the passive oddball task when controlling for education. During the Novel stimulus, as reaction time increases the latency to the negative deflection (N2) decreases (4A) and the latency to the positive peak (P3) increases (4B). Furthermore, as reaction time increases the mean amplitude of the negative deflection (N2) decreases (4C). $F(1,75) = 4.026$ $p = .048$ adj. $R^2 = .038$ $b = -.226$, $F(1,75) = 5.225$ $p = .025$ adj. $R^2 = .044$ $b = .255$, $F(1,75) = 4.513$ $p = .037$ adj. $R^2 = .044$ $b = -.238$, respectively.

Figure 5: Partial Regression Plot of Spatial Working Memory and ERP Detection Task



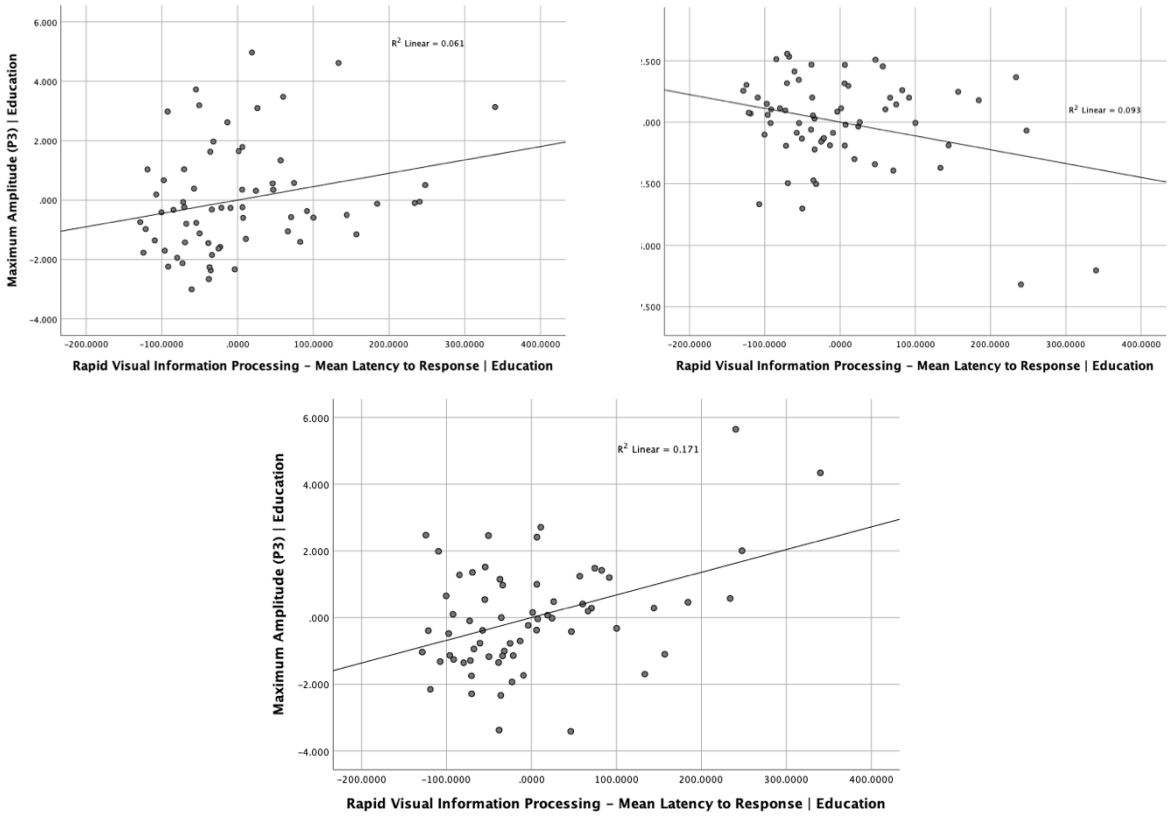
Partial regression plots visually represent the statistically significant relation between spatial working memory strategy score on the CANTAB and the latency to the P3 in two clusters during the ERP detection task when controlling for education. As strategy score on SWM increases, the latency to the positive peak (P3) increases in frontal and midline clusters. $F(1,64) = 10.444$ $p = .002$ adj. $R^2 = .127$ $b = .375$, and $F(1,64) = 5.414$ $p = .023$ adj. $R^2 = .064$ $b = .279$, respectively.

Figure 6: Partial Regression Plot of Spatial Working Memory and ERP Oddball Task



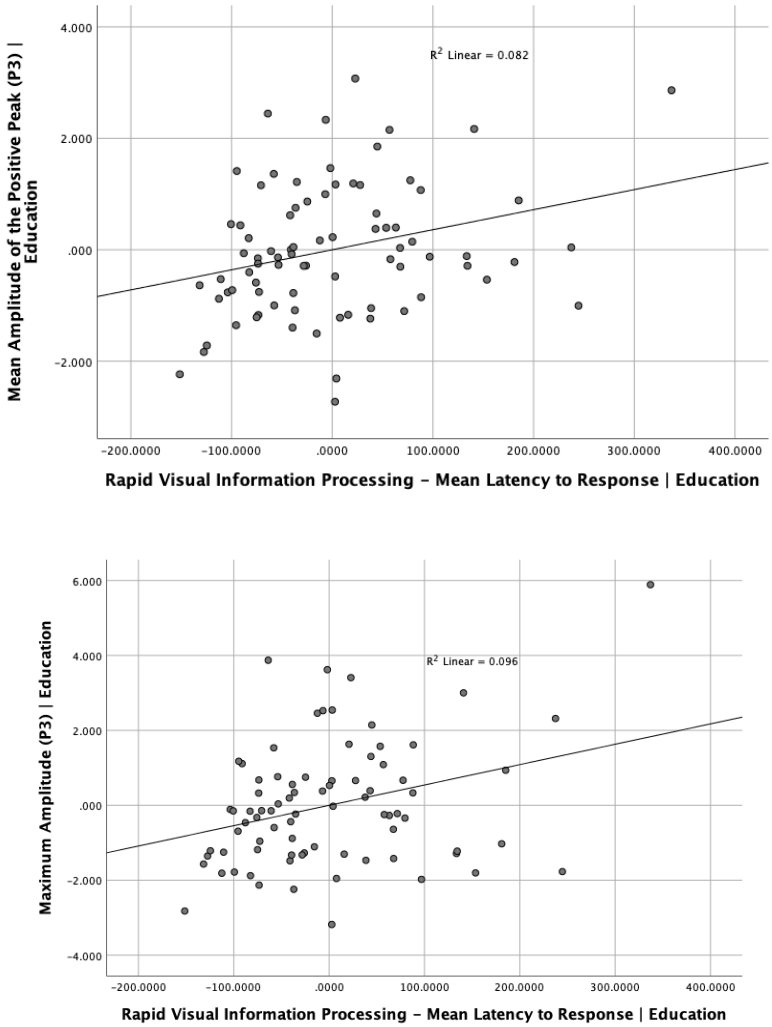
Partial regression plots visually represent the statistically significant relation between spatial working memory strategy score on the CANTAB and the latency to and mean amplitude of the N2 during the ERP oddball task when controlling for education. As SWM strategy increases (poorer strategy use) latency to the negative deflection decreases and mean amplitude increases during the Familiar stimulus. $F(1,78) = 7.316$ $p = .008$ adj. $R^2 = .074$ $b = -.293$ & $F(1,78) = 4.163$ $p = .045$ adj. $R^2 = .038$ $b = -.225$.

Figure 7: Partial Regression Plot of Rapid Visual Information Processing and ERP Detection Task



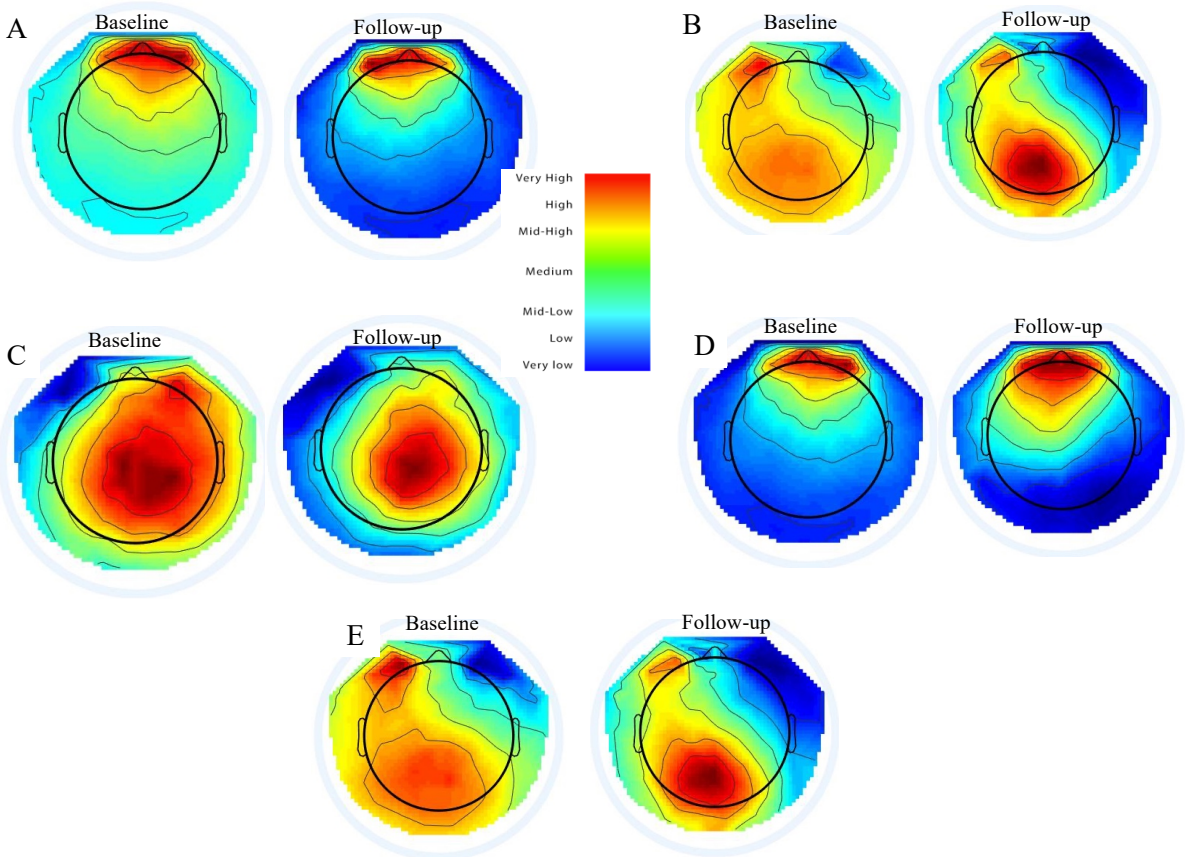
Partial regression plots visually represent the statistically significant relations between the latency to correct response in the RVP task in the CANTAB and N1 and P3 attributes of the ERP during the Target response of the detection task when controlling for education. As the mean latency to a response increases in the RVP task the minimum amplitude decreases (N1) and maximum amplitude (P3) increases (at frontal right and temporal right clusters). $F(1,63) = 6.635$ $p = .002$ adj. $R^2 = .148$ $b = -.294$, $F(1,64) = 4.995$ $p = .029$ adj. $R^2 = .058$ $b = .269$, and $F(1,64) = 14.983$ $p < .000$ adj. $R^2 = .177$ $b = .269$, respectively.

Figure 8: Partial Regression Plot of Rapid Visual Information Processing and ERP Oddball Task



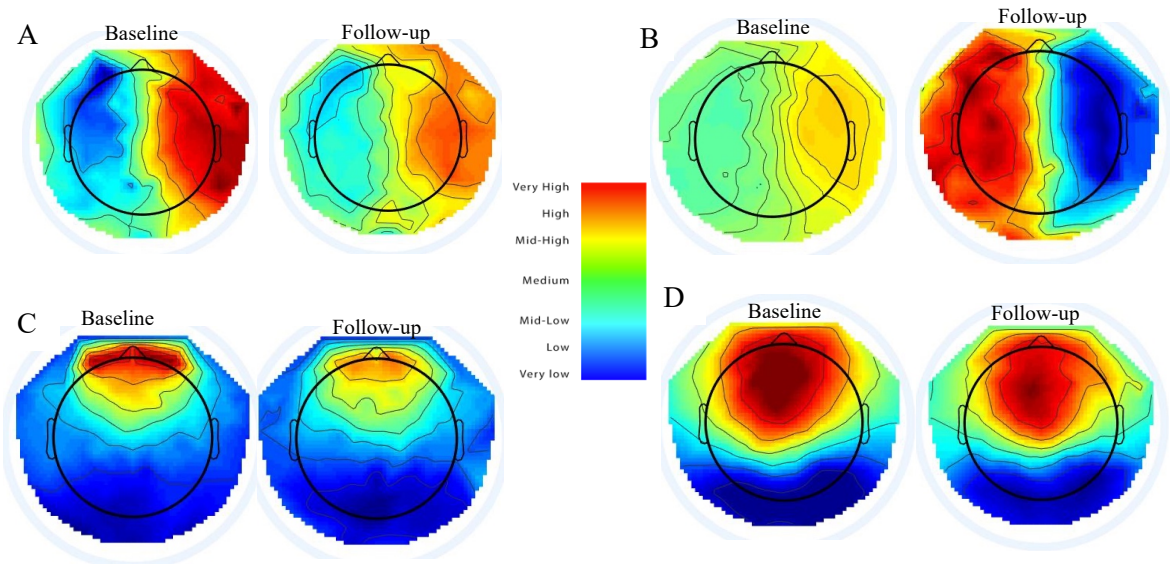
Partial regression plots visually represent the statistically significant relations between the mean latency to correct response during the RVP task of the CANTAB and the P3 attribute of the ERP during the passive oddball task when controlling for education. As the latency to response in RVP increases the mean amplitude of the positive peak (P3) and the maximum amplitude during a familiar stimulus increases. $F(1,79) = 6.648$ $p = .012$ adj. $R^2 = .066$ $b = .279$ VIF = 1 CI = 10.245, $F(1,79) = 7.829$ $p = .006$ adj. $R^2 = .079$ $b = .3$.

Figure 9: Relative Amplitude Intensity of PCA Factors During ERP Oddball Task



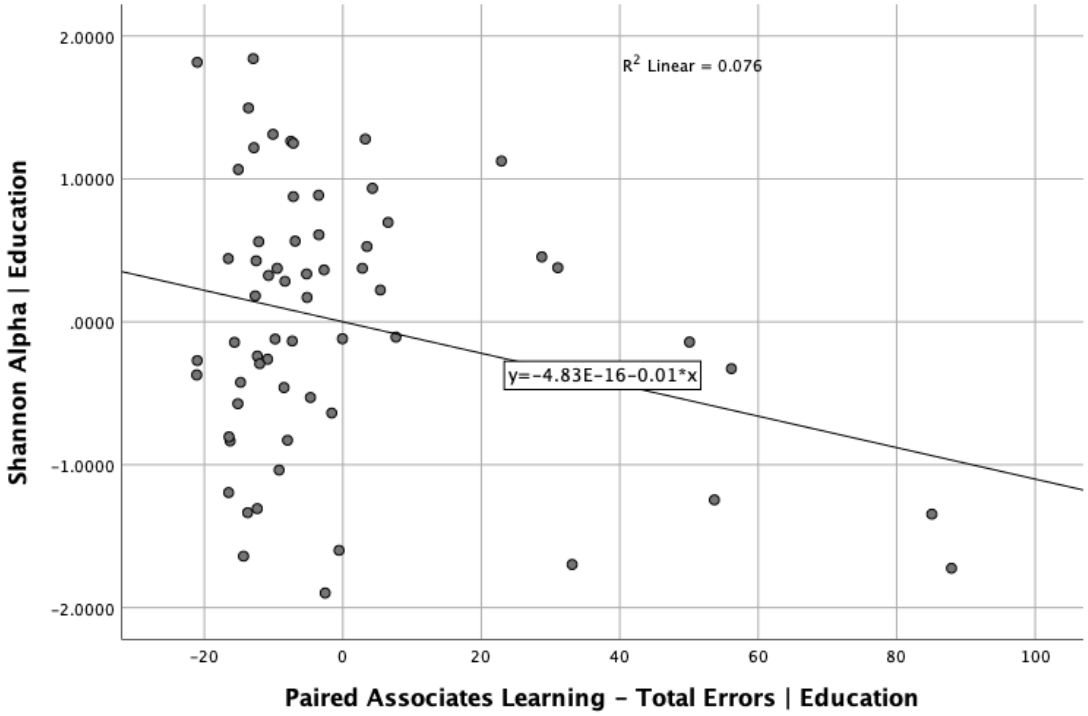
Visual representation of relative amplitude intensity of the statistically significantly related PCA factors (p<0.001) from baseline to follow-up during the passive oddball task. Heatmaps are rescaled and rereferenced to the relative amplitude of the scalp at the individual factor's temporal and spatial location. A) Familiar TF2SF1 B) Familiar TF2SF2 C) Familiar TF2SF3 D) Novel TF2SF1 E) Novel TF2SF2

Figure 10: Relative Amplitude Intensity of PCA Factors During ERP Detection Task



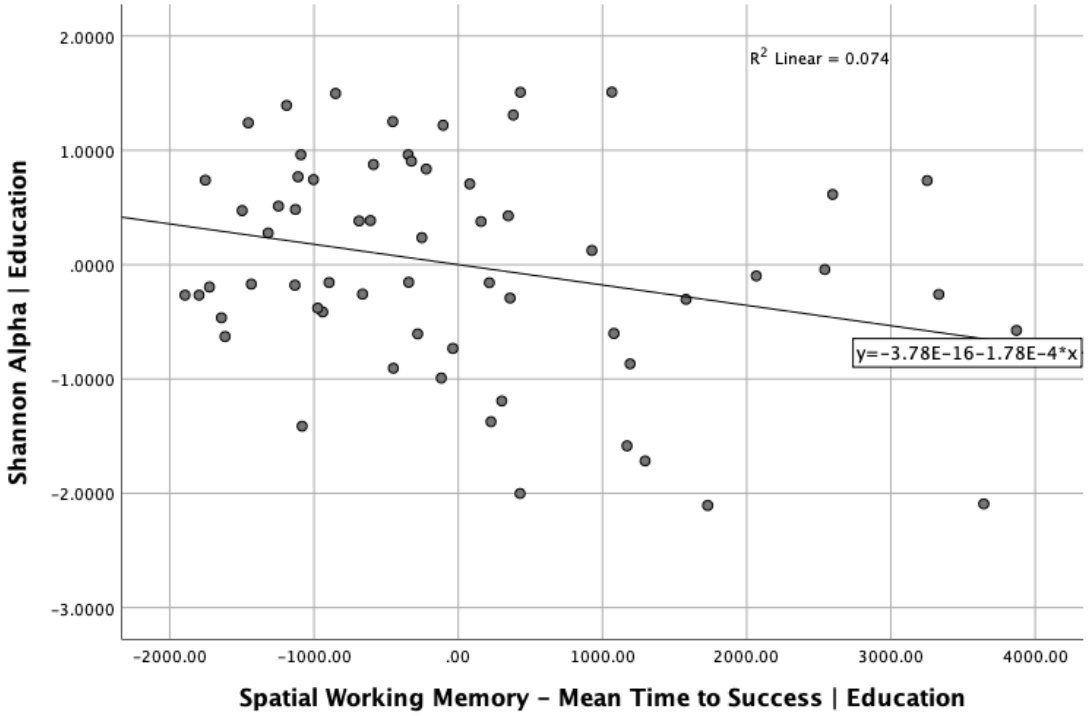
Visual representation of relative amplitude intensity of the statistically significantly related PCA factors (p<0.001) from baseline to follow-up during the detection task. Heatmaps are rescaled and rereferenced to the relative amplitude of the scalp at the individual factor's temporal and spatial location. A) Standard TF2SF3 B) Standard TF3SF3 C) Target TF1SF1 D) Target TF5SF1

Figure 11: Partial Regression Plot of Alpha Diversity and Paired Associates Learning



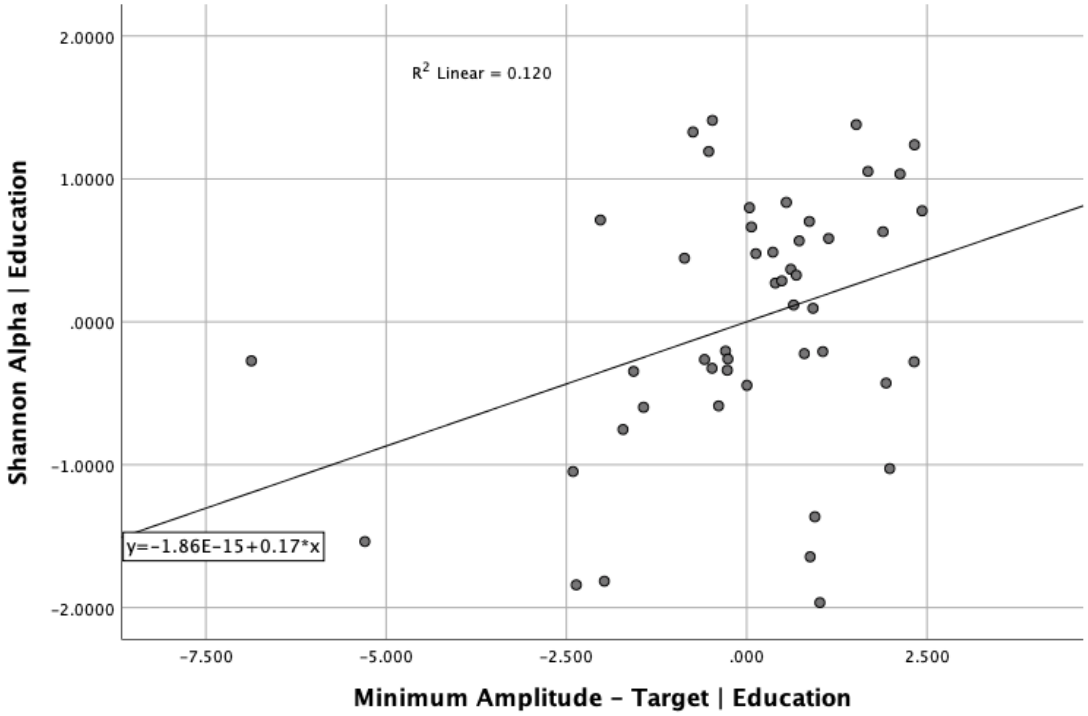
Partial regression plot visually represents the statistically significant relation between Shannon alpha diversity and performance on the paired associates learning (PAL) task (total errors made) when controlling for education. As the total number of errors on PAL increases alpha diversity decreases, $p<0.05$.

Figure 12: Partial Regression Plot of Alpha Diversity and Spatial Working Memory



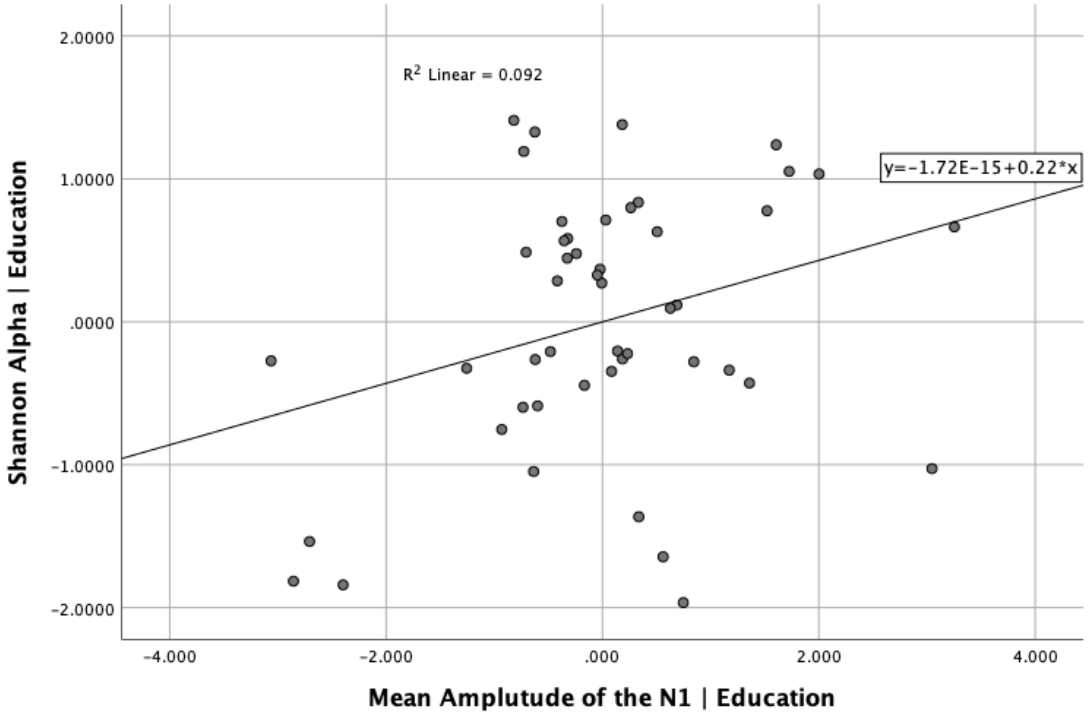
Partial regression plot visually represents the statistically significant relation between Shannon alpha diversity and performance on the spatial working memory task (mean time to success) when controlling for education. As mean time to success on SWM increases alpha diversity decreases, $p<0.05$.

Figure 13: Partial Regression Plot of Alpha Diversity and Minimum Amplitude During the Target



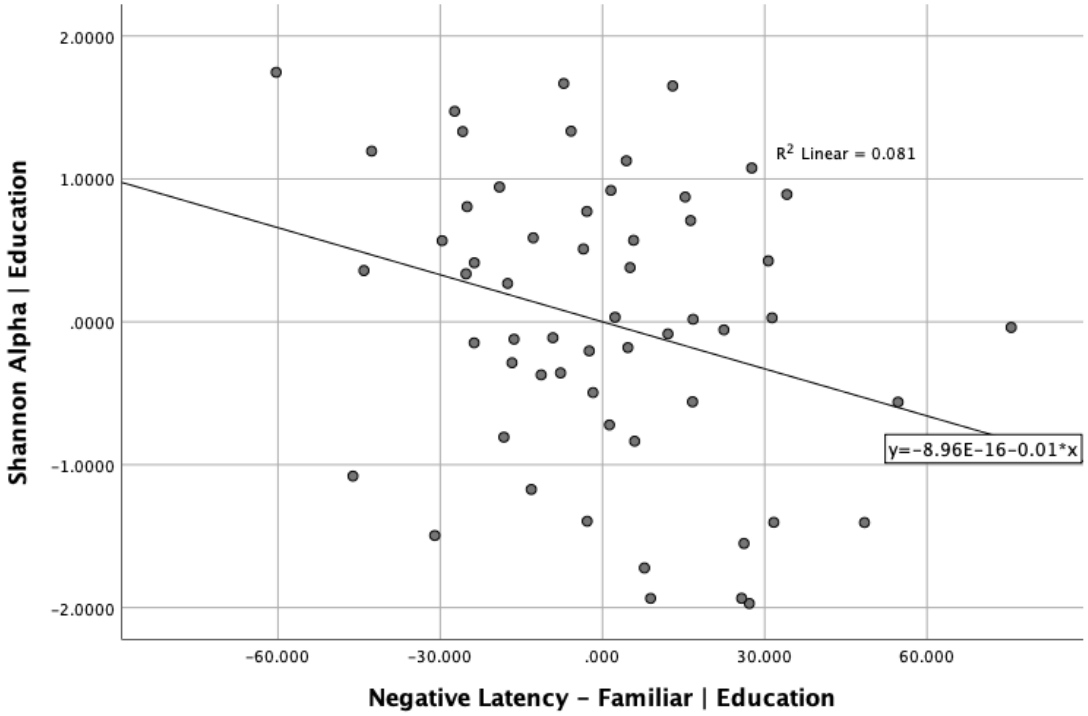
Partial regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the N1 attribute of the ERP during the detection task when controlling for education. The minimum amplitude for the Frontal cluster for the Target condition significantly predicted Shannon Alpha $p<0.05$. As the amplitude of the negative deflection increases alpha diversity also increases.

Figure 14: Partial Regression Plot of Alpha Diversity and Mean Amplitude During the Target



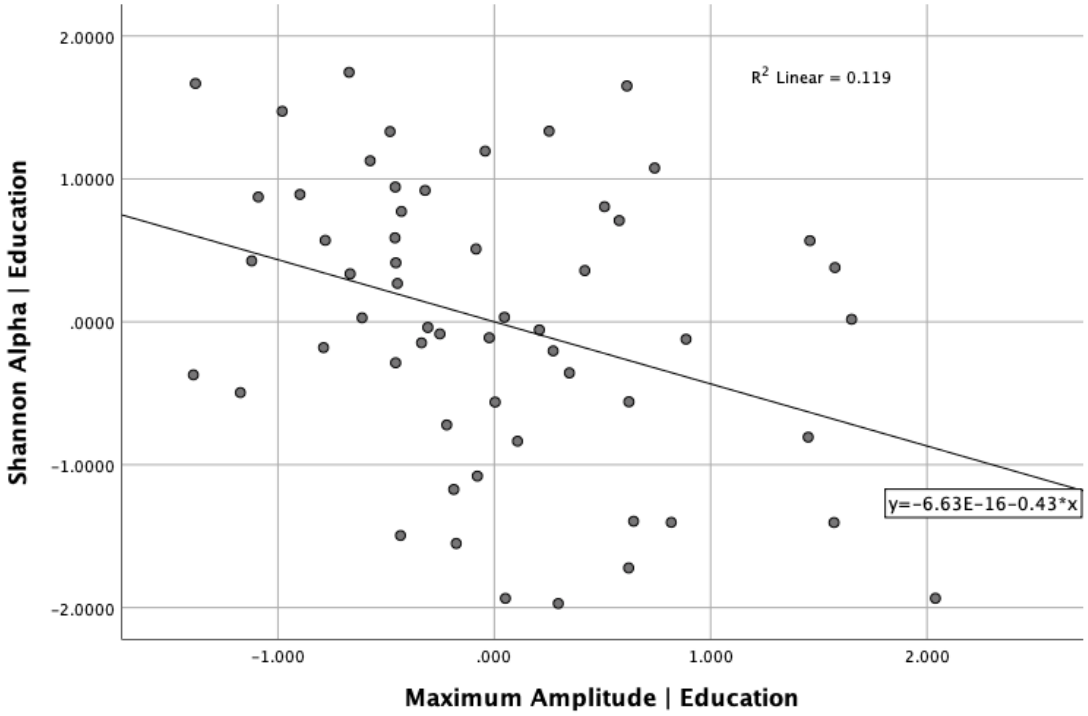
Partial regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the N1 attribute of the ERP during the detection task when controlling for education. The mean amplitude for the Frontal cluster for the Target condition significantly predicted Shannon alpha, $p<0.05$. As the mean amplitude across the window increased diversity also increased.

Figure 15: Partial Regression Plot of Alpha and Latency to the Negative Deflection During Familiar



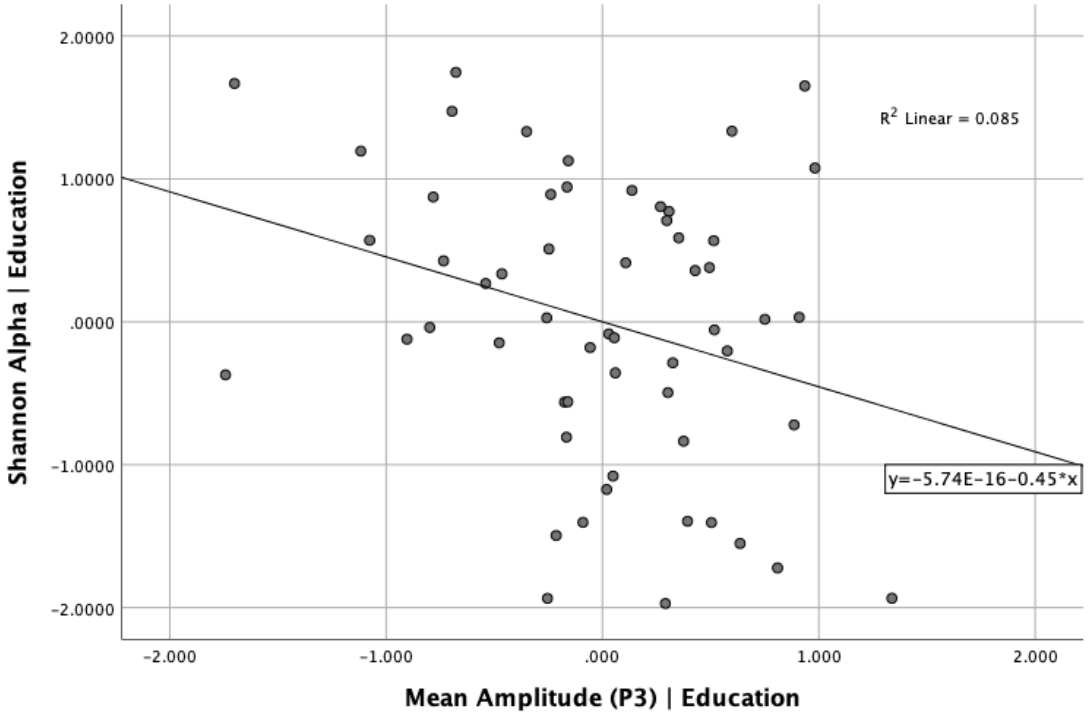
Linear regression plot visually represents the statistically significant relation between Shannon alpha and the latency to the N2 attribute of the ERP during the oddball task when controlling for education. The latency to the negative deflection for Familiar in the Frontal Right cluster significantly predicted Shannon alpha, $p<0.05$. As latency to the N2 increases alpha diversity decreases.

Figure 16: Partial Regression plot of Alpha and Max Amplitude During the Familiar Condition



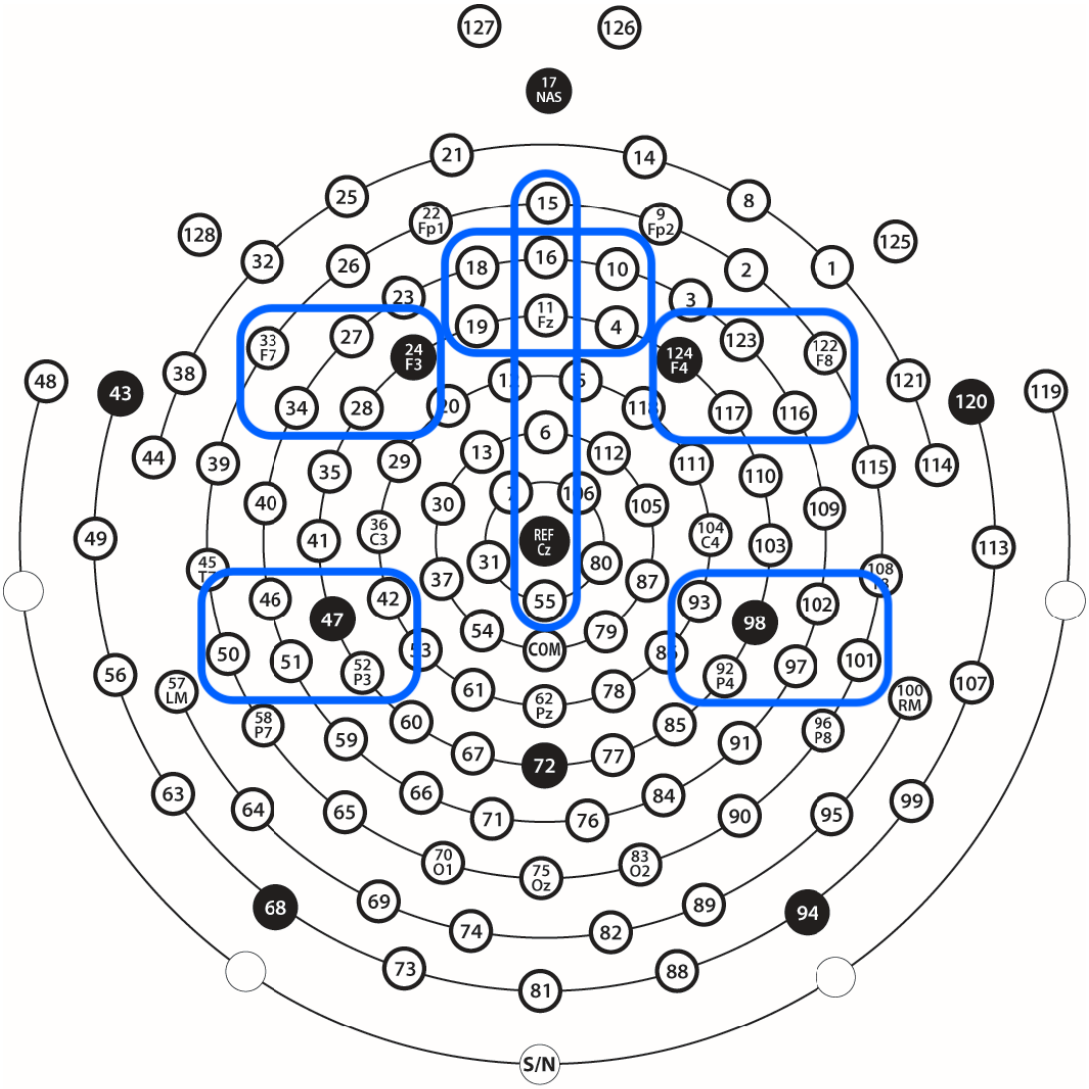
Partial regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the P3 attribute of the ERP during the Oddball task when controlling for education. The maximum amplitude at the Temporal Left cluster predicted Shannon alpha during the Familiar condition, $p<0.05$. As the amplitude of the P3 increases alpha diversity decreases.

Figure 17: Partial Regression Plot of Alpha and Mean Amplitude During the Familiar Condition



Partial regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the P3 attribute of the ERP during the oddball task when controlling for education. The mean amplitude at the Temporal Left cluster predicted Shannon alpha during the Familiar condition, $p<0.05$. As the mean amplitude of the P3 increases alpha diversity decreases.

APPENDIX A: SENSOR MAP INDICATING CLUSTERS



Sensor map showing the clusters used in analyses: FrontalZ (4,10, 11, 16, 18, 19), FrontalL (24,27,28,34,33), FrontalR (116, 117, 122, 123, 124), TemporalL (50, 46, 51, 47, 42, 52), TemporalR (93, 92, 98, 97,102,101), and Midline (15, 16, 11, 6, VREF, 55).

Gut Microbiome Survey

INSTRUCTIONS: Please complete this survey and place it in the zip-lock bag and send it in the same box as the completed stool samples.

Subject ID: _____

Date stool sample collected: _____/_____/_____

Time stool sample collected: _____:_____ (am/pm)

1) In the past **2 weeks**, have you received either of the following types of medications as pills or through the vein (DO NOT INCLUDE INHALERS):

   ☐ chemotherapy
   ☐ immunosuppressants (e.g., oral corticosteroids)

2) In the past **2 weeks**, have you undergone a colonoscopy or other procedure requiring bowel preparation?

   ☐ Yes ☐ No

3) In the past **2 weeks**, have you used an oral contrast agent for a CT scan or x-ray?

   ☐ Yes ☐ No

4) In the past **2 weeks**, have you had diarrhea?

   ☐ Yes ☐ No

5) In the past **2 weeks**, have you been hospitalized for any reason?

   ☐ Yes ☐ No

6) Have you ever had bowel surgery?

   ☐ Yes ☐ No

7) In the past **6 months** have you used antibiotics?

   ☐ Yes ☐ No

8) In the past **month** have you used antibiotics?

☐ Yes ☐ No

9) In the past **2 weeks** have you used probiotic supplements? Probiotic supplements are live bacteria that may be taken with the goal of improving digestive health. There are many probiotic supplements on the market. Many have "probiotic," "biotic," or "flora" in their name; some include: Culturelle, Renew Life Ultimate Flora Critical Care, NOW Foods Probiotic-10, Healthy Origins Probiotics, All-Flora, and Florastor.

☐ Yes ☐ No

10) Yogurt is a natural probiotic. Thinking over the past month, please report the frequency of your yogurt consumption.

☐ Daily ☐ Every week, but not every day ☐ A couple of times ☐ None

APPENDIX C: CANIPE, SIODA, CHEATHAM

Diversity of the gut-microbiome relates to cognitive behavioral outcomes in healthy older adults

Gut-microbiome diversity in older adults

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#words abstract; # words Introduction; # words discussion

Conflict of interest statement

Acknowledgements

Abstract

The human population is aging at its greatest rate in history, resulting in an increase in age-related cognitive disorders, and is likely to negatively impact society. Differences in factors within and among individuals that influence cognitive decline complicate studies on the topic. One difference among individuals, gut microbiome diversity and composition, changes within the person across their lifespan as well as varying among individuals. An individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior. Little research has been done to evaluate the gut-brain relation in non-clinical populations, with no previous studies to our knowledge in healthy older adults. In the present study we investigated the role of microbiome diversity and cognitive decline. Sixty-eight healthy older adults between 67-83 years of age were invited to provide a fecal microbiome sample and underwent Event-Related Potential (ERP) and the Cambridge Neuropsychological Test Automated Battery (CANTAB) testing. Electrophysiological and behavioral data were related to alpha diversity, a measure of the variety of species in the gut-microbiome, supporting the hypothesis that a relation exists between gut microbial diversity and cognitive performance in healthy older adults as measured by both CANTAB and ERP. Our results begin to bridge the gap between loosely attributed relations among behavior and gut-brain interaction in humans and the understanding of the microbial impact on brain function observed in highly controlled animal studies and clinical human populations, and has shown, for the first time, the relation between ERP outcomes and gut-microbiome diversity.

Significance Statement

The impact of a rapidly aging population in developed countries on the success and wellbeing of our society is likely to be tremendous. Methods to predict onset of age-related cognitive decline and provide early intervention or reversal of damage will be one vital approach to curbing the impact of aging. The rich, symbiotic, microbial environment present in the human gut has emerged in the past decade as an important factor in controlling brain function and behavior through the gut-brain axis. The present study indicates that poor gut microbial diversity in otherwise healthy older adults relates to poorer cognitive function as measured by behavioral and electrophysiological methods.

INTRODUCTION

The proportion of the U.S. population classified as "old" will climb from 13% in 2014 to approximately 20% by 2035 (Colby and Ortman, 2015). A decline in cognitive function and an increase in the diagnosis of age-related cognitive disorders is likely to have a substantial impact on the American lifestyle. Methods to prevent or decrease the incidence of cognitive dysfunction in the aging population could serve as a tool to offset the impact of the age shift. Differences in factors like genetics, nutrient intake, and education, within and among individuals complicate studies of cognitive decline. One difference among individuals, gut microbiome diversity and composition, changes within the person across their lifespan as well as varying among individuals, and has been shown to relate to cognitive function. In the current study, we investigate the role of microbiome diversity and cognitive decline using behavioral and cognitive developmental neuroscience techniques (e.g., The Cambridge Neuropsychological Test Automated Battery (CANTAB) and Event-Related Potentials (ERP)).

Scientists report that an individual's gut microflora can significantly influence gut-brain communication, brain function, and behavior (Claesson et al., 2011; Cryan and Dinan, 2012; Dinan et al., 2015; Dinan and Cryan, 2017). This influence is bidirectional and essential for maintaining homeostasis (Grenham et al., 2011). Moreover, the gut microbiome can influence brain function and behavior directly via production of metabolites essential for cognitive functions (i.e. memory and attention; Mayer et al., 2014; Manderino et al., 2017), immune activation (e.g., inflammation; Biagi et al., 2010; Grenham et al., 2011; Bajaj et al., 2012; McHardy et al., 2013; Noble et al., 2017), and microbial neuro-metabolites (Cryan and Dinan, 2012; Moloney et al., 2014; Dinan et al., 2015; Vernocchi et al., 2016).

Emerging hypotheses about the gut microbiome and its relation to the brain are showing the effects of diet on cognitive function (Caracciolo et al., 2014; Leung and Thuret, 2015b; Noble et al., 2017). Microbial diversity decreases with age, but there is stability in the total number of microbes (Biagi et al., 2010; Claesson et al., 2011). A large body of research is beginning to build on the so-called "gut microbiome-brain" axis, where the gut microbiome affects behavior and modulates brain plasticity via mechanisms such as inflammation and altered blood flow (Santoro et al., 2014; Faraco et al., 2018) and

change in neurotransmitter availability (Bravo et al., 2011; Janik et al., 2016). So-called "inflammaging," or chronic low-grade inflammation, as well as specific inflammatory diseases like the metabolic syndrome, arthritis, and fibrosis, have been shown to have a strong relation to all forms of aging - particularly cognitive decline (Yaffe et al., 2004; Mu et al., 2010; Wang et al., 2010; Misiak et al., 2012; Arfanakis et al., 2013; Howcroft et al., 2013; Kohman and Rhodes, 2013; Santoro et al., 2014; Bennett et al., 2015; Noble et al., 2017). We predict that this inflammation may be partially mediated by microbes in our gut and therefore dysbiosis of the gut-microbiome.

Researchers exploring the gut-microbiome-brain axis provide evidence for the gut-microbiome's modulation of brain function, which could contribute to changes in cognition during aging, but more investigation is required (Caracciolo et al., 2014; Leung and Thuret, 2015a; Lim et al., 2015; Anderson et al., 2017; Manderino et al., 2017). Pyrosequencing-based characterization of the human intestinal microbiome using 16s rRNA-based methods has provided evidence of altered bacterial composition in individuals experiencing cognitive decline compared to individuals with typical cognitive function (Bajaj et al., 2012; Rampelli et al., 2013; Bajaj et al., 2016), but those studies have been limited to institutionalized populations. Studies investigating cognitive function in healthy older adults are incredibly limited (Anderson et al., 2017; Manderino et al., 2017), and fail to use any of the cognitive neuroscience or developmental methods used in the current study. We hypothesized that individuals with poorer microbial diversity as measured by a calculated Shannon Alpha score would show poorer cognitive function as measured by behavioral (CANTAB) and cognitive neuroscience (ERP) measures.

MATERIALS & METHODS

Participants

Participants (n=68), 67-83 years of age (M = 75, SD = 4.27), were recruited from an existing cohort of older adults in the context of a larger longitudinal study conducted in the southeastern United States between 2012 and 2016. The analyses include participants from the larger study who had clean ERP data (defined as a minimum of 8 artifact-free segments for each condition) and provided a fecal stool sample; n=47 for task one and n=56 for task two.

Exclusion criteria included: (1) history or recent diagnosis of neurological, developmental, or severe psychiatric disorder (i.e. Alzheimers or dementia); (2) Antibiotic use in the last 30 days or history of significant gastrointestinal surgery; (3) Taking certain medications known to produce cognitive side effects; and (4) left-handedness. Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board. Several cognitive and behavioral assessments were performed as well as diet recalls and self-report questionnaires for potential covariates such as demographics, physical activity (Physical Activity Scale for the Elderly –PASE), general anxiety disorder (GAD), stressful life events, and memory (Multi-factorial Memory Questionnaire – MMQ).

The Montreal Cognitive Assessment (MoCA)

The MoCA consists of eight sections to test recall memory, executive function, attention, orientation, abstraction, visuospatial skills, and naming. Each section is weighted and scored according to the guidelines established by Nasreddine et al. (2005). MoCA's were scored by the same researcher, and 25% were scored for reliability by a second researcher. Reliability in this sample reached 93%. MoCA was used in analyses as a predictor of cognitive decline in some assessments.

ERP acquisition

In a protocol consisting of two tasks, we recorded event-related potentials (ERP; electroencephalogram (EEG) time-locked to stimuli presentation) were recorded from each participant. For recording, the participant was fitted with a 128-sensor Geodesic Net (GSN: Electrical Geodesics, Inc., (EGI) Eugene, OR, USA). Measurements of the participant's head were obtained to ensure proper net size, and correct placement of the vertex, mastoid, and other landmark sensors. Application of the net required approximately 10 minutes and was well tolerated by the participants. Impedances were checked and corrected, if necessary, to below 50 kΩ. The participant was then seated 45 cm from a monitor in the testing room (separate from the acquisition room) while stimuli were presented by E-Prime 2.0 software (Psychology Software Tools, Inc, Sharpsburg, PA, USA). Data were digitized at 1000Hz, referenced to a

single point at the vertex, and recorded by NetStation software (Version 5.1; Electrical Geodesic, Inc., Eugene, OR, USA). The NetStation system utilizes a digital clock to time-lock the E-Prime presentation of the stimuli to the NetStation EEG recording. The tasks included a discrimination task and a passive visual oddball task.

For all tasks, stimuli were presented on a black background on a computer screen in the testing room. In the first task (active discrimination) participants were instructed to respond to a series of Xs and Os that appeared on the screen by pressing a key for an X, and not pressing a key for an O. The active response (X) occurred in 20% of trials, whereas the non-response (O) occurred in the other 80% of trials. In the second task (passive oddball), the participant was instructed to observe pictures that occurred on the screen. After a habituation period of 10 presentations of the familiar picture, the participant viewed 120 pictures randomly ordered by E-Prime consisting of the familiar (40), a frequent-novel (40), or a trial-unique (40) image.

ERP waveforms were visually assessed for anomalies, after being subjected to lowpass (30 Hz) and highpass (0.3 Hz) filtering and segmented into individual segments corresponding to stimulus presentation and trial length. For task one, two categories (Standard (O) or Target (X)) were created with 1000ms segments. Task two was divided into three categories (Familiar, Frequent-Novel, and Novel-Unique) with 1500ms segments. Bad channels were detected by the software using moving averages and a threshold of 250 μV. Segments that included more than 12 (>10%) bad channels were rejected. After completion of the visual inspection, individual channels were replaced as necessary using replacement state interpolation. Data were then baseline corrected using the mean voltage during the 100 ms that precede the stimulus presentation. Finally, trials were averaged within the condition (i.e., novel, novel unique, and familiar; Target and Standard). The resulting file was visually examined, and negative and positive deflection windows of interest were chosen for each task. Windows for active oddball were 50-190 ms and 100-800 ms. Windows for passive oddball were 210-350 ms and 350-1500 ms. After visual inspection of the continuous data, sensor clusters of interest were chosen, and data were averaged across those clusters. See **Figure 1** for sensor clusters on the EGI 128-sensor net. Then, clusters were

individually assessed for mean amplitude, peak amplitude, and latency to peak amplitude within the determined segment window for each deflection (positive or negative) for both tasks.

Cambridge Neuropsychological Test Automated Battery (CANTAB)

The CANTAB system consists of standardized measures of cognitive function that have been computerized on a touch-screen computer that automatically stores data as the participants complete the test battery. The CANTAB offers several benefits, as many tests can be done in one place with minimal equipment. Language ability does not skew results and requires minimal reading and direction. The tests chosen for this battery were recommended by CANTAB because they are sensitive enough to distinguish typical cognitive aging from atypical decline and AD (Egerhazi et al., 2007; Lenehan et al., 2015). All participants completed the tasks below in the same order.

Motor Task (MOT): Motor Task is a baseline test of motor function to ensure the participant can correctly press the screen and participate in the study. Thus, outcome measures from this task are not used in analyses. The participant will see a series of flashing Xs on the screen that they must touch.

Reaction Time (RTI): The Reaction Time task (RTI) is a measure of reaction time, movement time, and response accuracy for a condition in which the stimulus is predictable (simple reaction time) and for a condition in which the stimulus is unpredictable (5-choice reaction time). It familiarizes the participant with the press-pad and provides simple and choice reaction and movement times. The participant will first hold down a button on the press pad. A yellow dot appears inside a circle. The participant releases the button as quickly as possible and touches the spot where the yellow dot appeared. The second section will show five circles on the screen, and the yellow dot could appear in any of them. The participant will again release the button as quickly as possible and press the spot where the yellow dot appears.

Spatial Working Memory (SWM): Spatial Working Memory is a working memory and planning task that incorporates heuristic strategy. The participant will see boxes on the screen – each of which contains a blue token. The objective is to find the blue tokens in the correct order. The participant must remember which boxes have already been searched, and identify those containing a blue token. The test starts with four boxes and increases to eight boxes.

Rapid Visual Information Processing: Rapid Visual Information Processing (RVP) measures the ability to sustain attention over a period of time, which requires both working memory and selective attention, and is a sensitive measure of frontal-parietal function. A white box appears in the center of the screen, and single digits appear inside

the box in a pseudo-random order. Participants are instructed to watch the digits change and press the button when a 3-digit target sequence appears. The task is presented in two parts. The practice involves one 3-digit target sequence. The test stage involves three 3-digit sequences. Increases in latency to respond to the target sequences and the participant's ability to successfully identify the target have been negatively correlated with cognitive function (Chamberlain et al., 2011).

Microbiome Samples

After they consented to provide fecal samples following cognitive testing, participants were provided a fecal specimen collection kit and instructions for collection at home. The kit included all necessary items for home collection of fecal samples and provided four individual samples from the same stool for analysis. The kit included a shipping container and a prepaid shipping label to be mailed back to The Cheatham Nutrition & Cognition Lab where the sample was processed for storage and later DNA extraction. A survey (**Appendix A**), to be filled out at the time of collection, was also included with the kit. The survey asks participants about any recent antibiotic use as well as other health and lifestyle factors that could influence their sample. Alternatively, the participant could also drop off the samples in person during operating hours at the NRI. Once all samples were collected, DNA was isolated and sent to the Human Microbiome Core at UNC-Chapel Hill for library preparation.

Processing of 16S rRNA sequence data was completed with BiolockJ, a bioinformatics pipeline framwork for metagenomics analysis written in the department of bioinformatics at UNC Charlotte by Michael Sioda (2018; https://github.com/msioda/BioLockJ). Paired-end sequences were merged with Paired-End read merger (PEAR, v 0.9.10) (Zhang et al., 2014) using default arguments, excluding sequences for which primers do not match or for which ten base pairs do not overlap.

In the initial analysis performed prior to the pipeline output, the taxonomic assignment was performed with the Ribosomal Database Project (RDP) Classifier v2.12 (confidence threshold=80%) (Wang et al., 2007). In secondary analysis, sequences were processed through

QIIME v1.9.1 (Quantitative Insights Into Microbial Ecology;Caporaso et al., 2010), where BioLockJ multiplexed the pEAR merged reads as QIIME input, UCLUST for deriving Operational Taxonomic Units (OTUs) by clustering sequences at 97% similarity (Edgar, 2010), and open-reference assignment of OTUs using the Silva (132 release) reference database (Quast et al., 2013). In the pipeline, BioLockJ was directed to run the QIIME alpha_diversity script to calculate the Shannon Alpha diversity metric (Peet, 1974). Beta-diversity was assessed with Principal Coordinates Analysis (PCoA), using Bray-Curtis dissimilarity matrixes of microbial-abundance-based distances (Faith et al., 1987). For taxonomy-specific analysis, we excluded operational taxonomic units (OTUs) that are present in <25% of participants, and transform raw taxonomic counts as $\log_{10}[(RC/n)(x/N)+1]$, where RC is the total raw taxon count for a participant and n is the total count across all taxa for a participant, x is the total across all OTUs and participants and N is the total number of participants (McCafferty et al., 2013). We conducted multivariable-adjusted regression models for the diversity measure of microbial community composition concerning measures of cognitive function. Regression analysis of individual genera controlled for multiple comparisons using the Benjamini-Hochberg method for false discovery rate (FDR) (Benjamini and Hochberg, 1995).

Determining the Measure of Microbiome Composition

Shannon alpha diversity, one of the most common alpha diversity metrics in gut-microbiome research, was selected as the key measure of microbiome diversity in the sample. Shannon diversity accounts for both the abundance and evenness of the species present – the proportion of species relative to the total number of species is calculated, and then multiplied by the natural logarithm of this proportion before being summed across all species and multiplied by -1.

Statistical Approach

Data met assumptions of normality and were subjected to stepwise multiple regression analysis, with controls for repeated measures and relevant covariates, to assess the relation

between microbiome diversity measures of interest and the CANTAB and ERP variables using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 26.0). Before beginning the analysis of the data, several variables of interest were selected for CANTAB and ERP variables and are discussed below for their respective assessment. Significant results were followed up in reduced models when appropriate. Age, Gender, Education, Current Occupation, Marital Status, Patient Health Questionnaire Score, Body Mass Index, Healthy Eating Index, MoCA, GAD, Stressful Life Events, PASE, and Time Since Baseline were tested as potential covariates. It was determined that Education was necessary (p = .020).

Stepwise multiple regressions were performed for each assessment where Shannon diversity (the unknown) was regressed onto the predictors of interest. Whereas the design of this model is atypical in the fields of psychology and neuroscience, it is standard practice in the microbiome literature to predict Shannon alpha. In previous work with a larger dataset of these data (Canipe & Cheatham, In prep) and previous literature (Dustman et al., 1993; De Luca et al., 2003; Chapman et al., 2011; Lenehan et al., 2015), it has been indicated that ERP and CANTAB are known measures of cognitive function and are sensitive to changes due to aging. The gut-microbiome, is therefore, the unknown in the equation. Dynamic interactions exist among environment, microbiome, and host. To study the complicated interactions among these factors, unique models must be built. One such model to explore the association between microbiome and host is to evaluate whether the composition of the microbiome (a "dysbiotic" microbiome) is linked to the health or disease of host. Based on the research hypotheses, the null statistical hypothesis is "there is no difference of microbiome composition in age-related cognitive decline." Therefore, in the present study, placing microbiome diversity as the dependent variable in all models is warranted (Xia and Sun, 2017).

RESULTS

Data met assumptions of normality and were subjected to stepwise multiple regressions to explore potential models of import using the cognitive or behavioral measure and relevant covariates to

predict the microbiome diversity measure of interest using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0). Before beginning analysis of data, several variables of *a priori* interest were selected for CANTAB and ERP variables and are discussed below for their respective assessment. Significant results were followed up in reduced models when appropriate.

Data were examined to ensure that assumptions were met for regression analyses. Age, Gender, Education, Current Occupation, Marital Status, Patient Health Questionnaire Score, Body Mass Index, Healthy Eating Index, MoCA, GAD, Stressful Life Events, and PASE were tested as potential covariates by regressing the outcome variables onto each one in turn. It was determined that Education was necessary ($p = .020$; all others $p > .05$).

Relation Among CANTAB Measures and Microbiome Diversity

In order to avoid issues of reduced power and multiple comparisons, the number of tested variables was limited to four, or one for each test in the CANTAB battery. CANTAB outcome variables were identified *a priori* based on the cognitive ability they represented, and no multicollinearity issues existed. The selected variables were as follows: Simple reaction time (RTI) as a measure of reaction time, Total errors adjusted (PAL) as a measure of visual memory, Mean time to first success (six-step; SWM) as a measure of working memory and planning, and Mean latency to response (RVP) as a measure of sustained attention (working memory and selective attention).

Data were entered into a stepwise regression using all the predictors (RTI, PAL, SWM, RVP, and education). Shannon Alpha was predicted by PAL and SWM ($p = .007$). The model, including only PAL ($p = .025$) and SWM ($p = .024$), accounted for 11.7% of the variance and statistically significantly predicted Shannon Alpha, $F(2,56) = 4.846$ adj. $R_2 = .117$, $b = -.293$ and $b = -.248$, respectively. The model indicates that as the total number of errors (PAL; **Figure 2**) and mean time to success (SWM; **Figure 3**) increases alpha diversity decreases. See **Table 1** for results of the model.

Relation Among ERP Measures and Microbiome Diversity

The same *a priori* clusters of interest from specific aims 1-3 (Frontal Z, Frontal Right, Frontal Left, Temporal Left, Temporal Right, and Midline) were entered into a stepwise regression to predict microbiome diversity measures.

Detection task. For the detection task, six stepwise regressions were performed to determine if ERP measures predicted microbiome diversity. Each regression tested a model for an individual ERP attribute (minimum amplitude, mean amplitude of the negative deflection, or latency to the negative peak) for Target or Standard conditions and included all clusters of interest. Education was not selected to remain in the model for any of the regressions.

The minimum amplitude as well as the mean amplitude for the Frontal Z cluster for the Target condition significantly predicted Shannon Alpha, $F(1,46) = 4.202$ $p = .022$ adj. $R_2 = .089$ $b = .330$ VIF = 1 CI = 3.347 (**Figure 4**), and $F(1,46) = 4.361$ $p = .042$ adj. $R_2 = .067$ $b = .294$ CI = 1.771 (**Figure 5**), respectively. Interpretation of the model suggests that as the amplitude the negative deflection increases alpha diversity also increases and as the mean amplitude across the window (50-190ms) increases diversity also increases. See **Table 2** for results of these two models. When these significant variables are placed into the same model, the model emerges as insignificant, $F(2,45) = 2.887$ $p = .066$ $R_2 = .114$.

When clusters were tested in a stepwise regression together (e.g. Frontal for each ERP attribute), there is an indication that frontal and midline clusters show a significant relation to microbiome diversity. In the Target condition the minimum amplitude significantly predicts Shannon alpha in the Frontal. Frontal Right, and Midline sensors, $F(1,46) = 5.611$ $p = .022$ adj. $R_2 = .089$ $b = .164$ VIF = 1 CI = 3.347, $F(1,46) = 4.742$ $p = .035$ adj. $R_2 = .074$ $b = .194$ VIF = 1 CI = 3.251, and $F(1,46) = 4.206$ $p = .046$ adj. $R_2 = .064$ $b = .289$ CI = 3.630, respectively.

Passive Oddball. For the passive oddball task, stepwise multiple regressions were performed identical fashion to the detection task to evaluate if the minimum or maximum amplitude, mean amplitude of the positive or negative deflection, or latency to the positive or negative peak for Familiar and Novel Unique stimuli predicted microbiome diversity as measured by Shannon Alpha. Education was not selected to remain in the model for any of the regressions.

The latency to the negative deflection for Familiar in the Frontal Right cluster significantly predicted Shannon Alpha, p = .024 $F(1,55)$ = 5.373 adj. R_2 = .072 b = -.298 CI = 20.726, suggesting that as latency increases to the negative deflection alpha diversity decreases (**Figure 6**). See **Table 3** for results of this model.

The max amplitude as well as the mean amplitude from 350-1500ms at the Temporal Left cluster predicted Shannon Alpha during the Familiar condition, $F(1,55)$ = 5.456 p = .023 adj. R_2 = .074 b = -.300 CI = 4.735 (**Figure 7**); $F(1,55)$ = 5.225 p = .026 adj R_2 = .070 b = -.295 CI = 1.888 (**Figure 8**). The model for the positive peak when observing a familiar stimulus suggests that as the amplitude of the peak increases alpha diversity decreases and as the mean amplitude increases across the window diversity decreases. See **Table 3** for results of this model. When these significant variables are placed into the same model, the model again emerges as significant, $F(2,54)$ = 3.397 p = .041 R_2 = .112.

When clusters were tested in a stepwise regression together (e.g. Frontal for each ERP attribute), there is an indication that frontal and temporal clusters show a significant relation to microbiome diversity. In the Familiar condition negative latency and maximum amplitude significantly predict Shannon alpha. The negative latency in the Frontal Right cluster significantly predicts Shannon alpha, $F(1,55)$ = 5.373 p = .024 adj. R_2 = .072 b = -.012 VIF = 1 CI = 20.726. The maximum amplitude in the Temporal Left cluster significantly predicts Shannon alpha, $F(1,55)$ = 5.164 p = .024 adj. R_2 = .074 b = -.374 VIF = 1 CI = 4.735.

DISCUSSION

Behavioral and electrophysiological measures of cognitive function in older adults were investigated in relation to alpha diversity, a measure of the varieties of species in the gut-microbiome, to test the hypothesis that a relation exists between gut microbial diversity and cognitive performance. The results indicate that there is an association between behavioral measures (paired-associate learning and spatial working memory) and calculated alpha diversity of the gut microbiome, where poorer performance (indicative of cognitive dysfunction) predicted lower gut-microbiome. The findings support the hypothesis that cognitive performance, as measured by a standardized behavioral assessment, is related,

negatively, to poor microbial diversity in the gut. Furthermore, results indicate that there is a significant predictive relation between several electrophysiological cognitive measures and gut-microbiome diversity.

The results indicate several significant findings. First, the minimum and mean amplitude of the negative deflection in the Frontal region of the ERPs of participants during the Target condition of a detection task significantly predict alpha diversity. The same relation is observed in the passive oddball task where the latency to the negative deflection in the Frontal Right cluster was shown to predict Shannon alpha significantly. Adding to the significant relation between Shannon alpha and brain activity during the passive oddball task, the max and mean amplitude of the positive deflection for the Familiar condition in the Temporal Left cluster was also predictive of Shannon alpha.

The peak of the negative deflection in a visual active oddball or discrimination task, commonly referred to as the "visual N1," typically occurs around 75 to 180ms post-stimulus and is most commonly evaluated at central and frontal electrode sites and is part of the visual evoked potential – a series of voltage deflections observed in response to presentation of visual stimuli (Naatanen, 1982; Mangun and Hillyard, 1991; Mangun, 1995; Thorpe et al., 1996; Luck and Hillyard, 2009). The amplitude of the visual N1 is influenced by selective attention and is therefore used to study attentional processes (McDowd and Filion, 1992; Mangun, 1995; Kok, 1999; Martinez et al., 2006). The visual N1 is a sensory component evoked by any visual stimulus, reflecting a critical mechanism of attention that indicates whether or not attention was appropriately allocated, and is a manifestation of a critical sensory gating mechanism of attention (Vogel and Luck, 2000; Luck and Hillyard, 2009). One would expect the N1 component to be enhanced under conditions that require a decision, such as the discrimination task of the present study. As a result, individuals with better cognitive function (improved attention allocation) would be expected to have a higher peak amplitude of the N1 when identifying a target stimulus. The relation between alpha diversity of the microbiome and amplitude of the N1 for Target in the ERPs of participants suggests that individuals with greater amplitude (more negative) have greater microbiome diversity, supporting the hypothesis that increased microbial diversity in the gut relates to better cognitive function.

Another essential ERP component is the N2, the negative waveform that peaks between 200-350ms, which has been found to reflect executive cognitive control functions in adults – particularly a measure of attentional processing (Luck and Hillyard, 1994; Luck and Hillyard, 2009). In a passive oddball task, such as the one in the present study, the latency to peak amplitude should differ between familiar and novel pictures. When an individual has been exposed repeatedly to an image (the Familiar), the need to process the information about that image diminishes over time and attention to the image will fall linearly to that need and latency should decrease. Therefore, better cognitive function as measured in the passive oddball paradigm during the selected window of the negative deflection (210-350 ms) would be indicated by shorter latency in the Familiar condition. In the present study, it was shown that an increase in latency to peak amplitude in the Familiar condition predicts a decrease in alpha diversity of the gut-microbiome. This finding supports the hypothesis that microbial diversity in the gut is related to the cognitive function of sustained attention.

A third visual ERP component, the P3, is a positive peak occurring after 300ms and typically before 700ms following a visual stimulus presentation and alterations in it have been strongly linked to typical and atypical cognitive aging (Knott et al., 2004; Polich, 2007; Pontifex et al., 2009; Papaliagkas et al., 2011; Parra et al., 2012; Jiang et al., 2015). The P3 differs from the N1 and N2 components as it does not link to a physical attribute of a stimulus but rather to a person's reaction to the stimulus. For this reason, it is commonly considered an "endogenous potential" and is most commonly attributed to the process of decision making. More accurately, the P3 reflects processes involved in stimulus evaluation or categorization; commonly measured in an oddball paradigm – low-probability (Novel) items mixed with high-probability (Familiar) items (Polich, 1996; Kirino et al., 2000; Pato and Czigler, 2011; Jiang et al., 2015). Much like the N2 component, the overall existence or amplitude of a P3 should be higher for less-probable, Novel, events and small in amplitude or non-existent for more probable events, Familiar. This observed outcome, however, is reliant upon healthy cognitive function – the ability to successfully discriminate between stimuli via appropriate attentional orientation and effective visual memory storage and retrieval. Therefore, individuals experiencing cognitive decline might be expected to show increased

latency to and amplitude of the P3 component, especially in the Familiar condition. If this is true, during the passive oddball paradigm, older adults experiencing cognitive decline would have a higher maximum amplitude during the positive inflection window. Indeed, measures for mean amplitude from 350-1500ms during the Familiar condition predicted Shannon Alpha, where an increase in both measures of amplitude predicted a decrease in gut-microbiome diversity. Again, this supports the hypothesis that gut-microbiome diversity relates to healthy cognitive function.

Significance

The interpretation of the findings suggests significant implications about the relation that exists between gut-microbiome diversity and healthy cognitive function. Conclusions of previous studies in humans suggest a relation between poor microbiome health and observed behavioral measures in individuals diagnosed with illnesses or taking medications known to impact the gut microbiome negatively. However, specific measures of microbial disposition and measured cognitive or clinically quantified behavioral outcomes have been limited (Bajaj et al., 2012; Caracciolo et al., 2014; Dinan et al., 2015; Bajaj et al., 2016; Anderson et al., 2017). Animal models of microbiome diversity and behavior have revealed a clear link between specific gut bacteria and unique cognitive and behavioral outcomes (Bravo et al., 2011; O'Mahony et al., 2015; Sampson and Mazmanian, 2015; Janik et al., 2016; Marchesi et al., 2016), but high levels of specificity in the individual microbes discovered to impact the gut-brain axis have made it challenging to translate useful predictions for humans (especially older adults).

With this study, we have attempted to bridge the gap between loosely attributed relations among behavior and gut-brain interactions in humans and the understanding of the microbial impact on brain function observed in highly-controlled animal studies. The bridging of this gap and previously disparate fields of science (psychology, neuroscience, microbiology, and bioinformatics) has been accomplished by combining clinically validated behavioral measures and electrophysiological measures of brain activity with robust quantification and characterization of gut-microbiome data collected from a large cohort of older adults. The initial analysis of the data from this large cohort will contribute to the growing knowledge about the gut-brain axis. The present study is limited in that it has only evaluated a diversity

metric, and further analyses of the dataset should be performed as improved knowledge of individual microbes become available. Without a doubt, the findings presented have revealed new hypotheses and future directions for impactful follow-up work. Most importantly, the work reported here is the first in a program of research that will add to our knowledge and ability to reduce the effects of age-related cognitive decline in our population by identifying individual biomarkers of cognition in healthy older adults.

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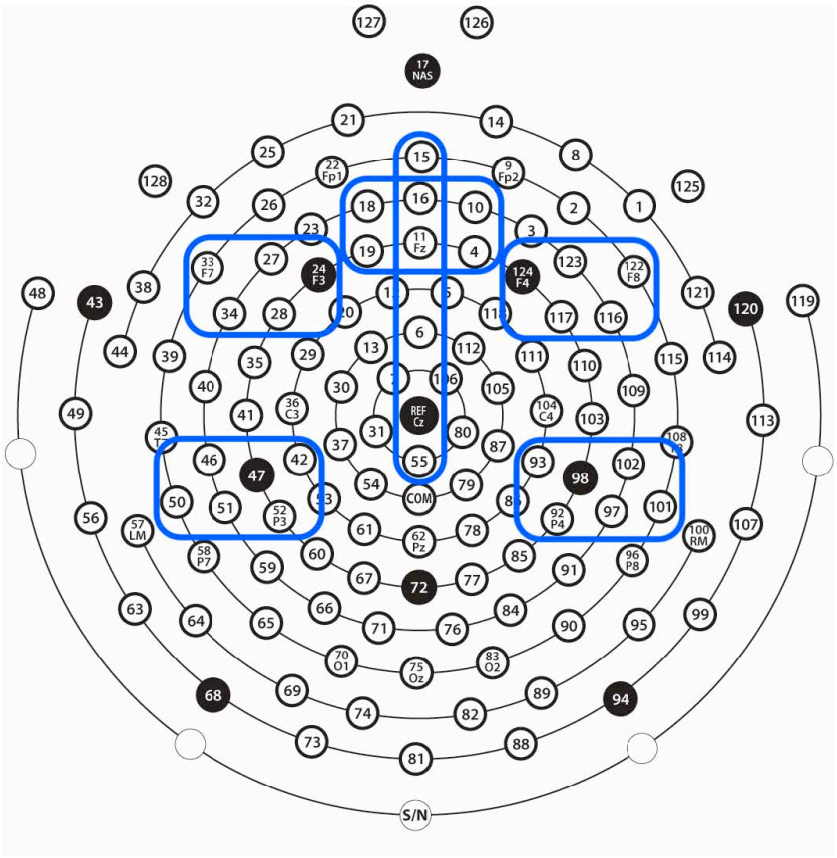
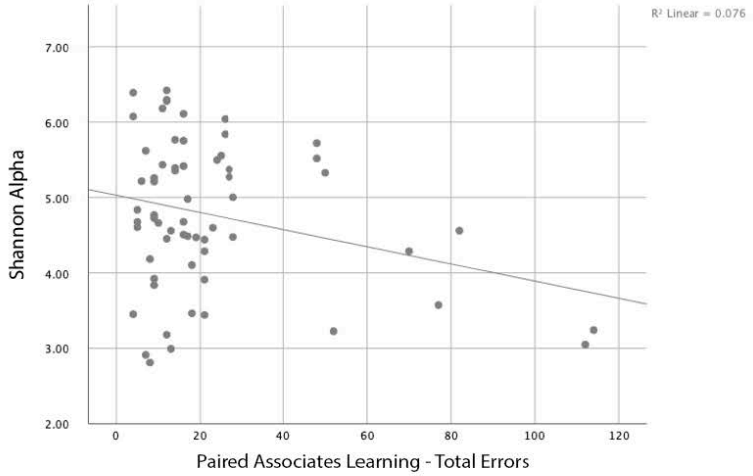
Figure 1. Sensor Clusters



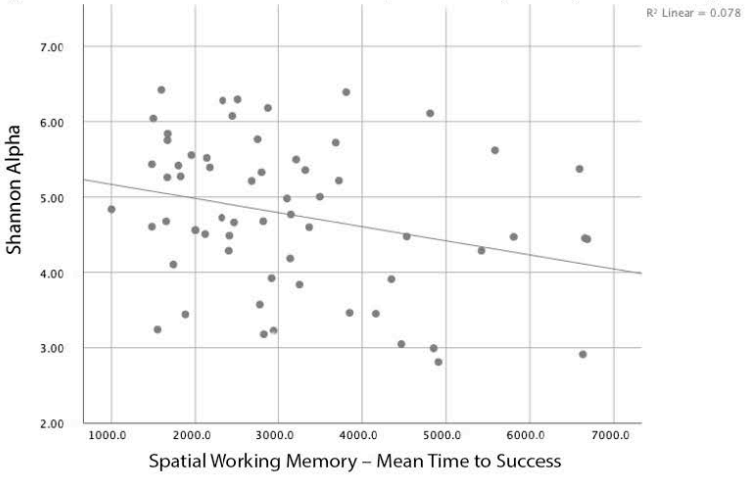
Sensor map showing the clusters used in analyses: FrontalZ (4,10, 11, 16, 18, 19), FrontalL (24,27,28,34,33), FrontalR (116, 117, 122, 123, 124), CentralZ (7, 31, 55, 80, 106, REF), ParietalZ (61, 62, 67, 72, 77, 78), TemporalL (50, 46, 51, 47, 42, 52), TemporalR (93, 92, 98, 97,102,101), and Midline (15, 16, 11, 6, VREF, 55).

Figure 2. Relation Between Shannon Alpha Diversity and Paired Associates Learning Performance



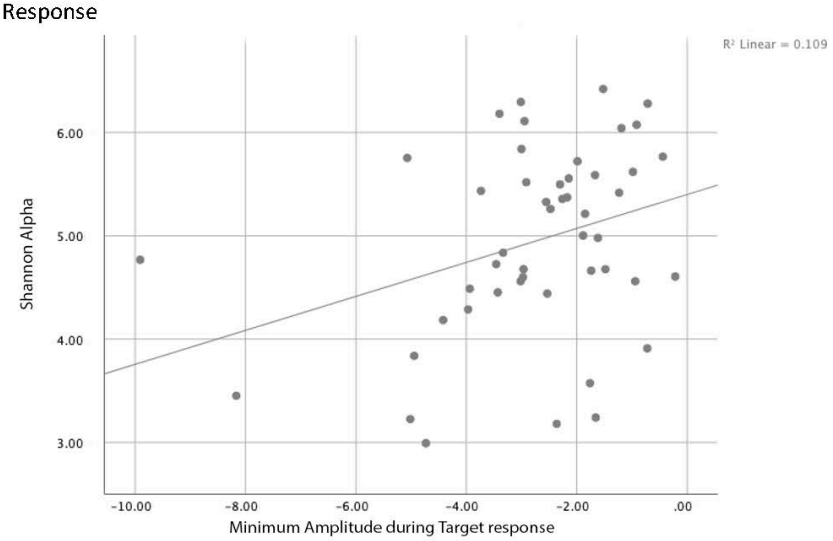
Linear regression plot visually represents the statistically significant relation between Shannon alpha diversity and performance on the paired associates learning (PAL) task (total errors made). As the total number of errors on PAL increases alpha diversity decreases, $p<0.05$.

Figure 3. Relation Between Shannon Alpha Diversity and Spatial Working Memory Performance



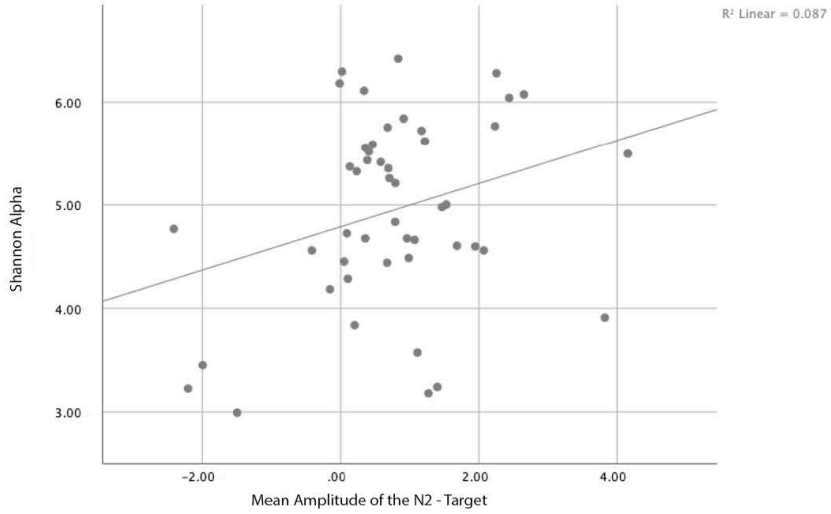
Linear regression plot visually represents the statistically significant relation between Shannon alpha diversity and performance on the spatial working memory task (mean time to success, ms). As mean time to success on SWM increases alpha diversity decreases, $p<0.05$.

664 **Figure 4.** Relation Between Shannon Alpha and Minimum Amplitude During the Target
665 Response



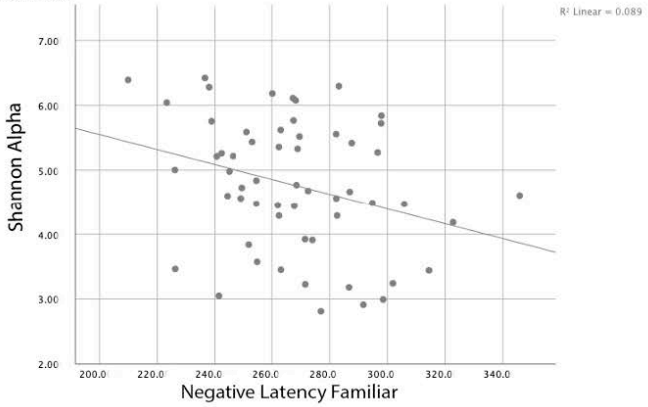
666
667 Linear regression plot visually represents the statistically significant relation between Shannon
668 alpha and the amplitude of the N1 attribute of the ERP during the detection task. The minimum
669 amplitude for the Frontal cluster for the Target condition significantly predicted Shannon Alpha
670 $p<0.05$. As the amplitude of the negative deflection increases alpha diversity also increases.
671
672

673 **Figure 5.** Relation Between Shannon Alpha and Mean Amplitude During the Target Response



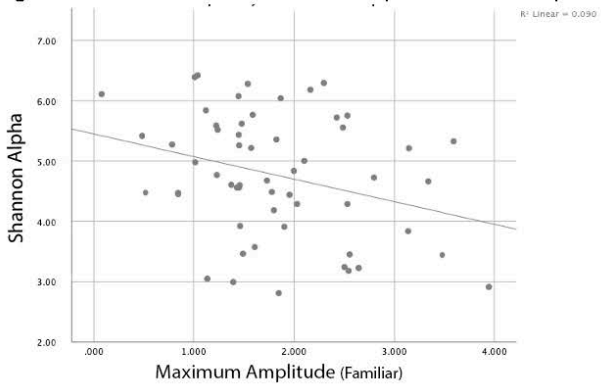
674
675 Linear regression plot visually represents the statistically significant relation between Shannon
676 alpha and the amplitude of the N2 attribute of the ERP during the detection task. The mean
677 amplitude for the Frontal cluster for the Target condition significantly predicted Shannon alpha,
678 $p<0.05$. As the mean amplitude across the window increased diversity also increased.
679
680

Figure 6. Relation Between Shannon Alpha and Latency to the Negative Deflection During Familiar



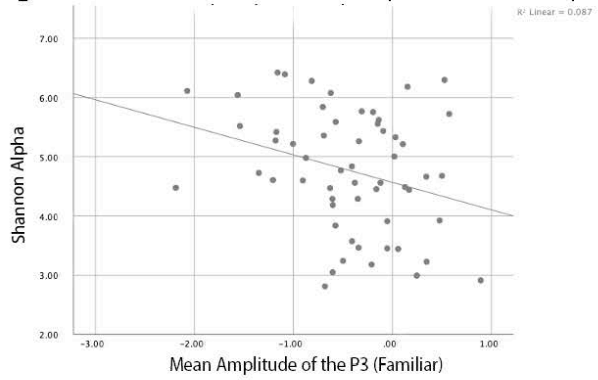
Linear regression plot visually represents the statistically significant relation between Shannon alpha and the latency to the N2 attribute of the ERP during the oddball task. The latency to the negative deflection for Familiar in the Frontal Right cluster significantly predicted Shannon alpha, $p<0.05$. As latency to the N2 increases alpha diversity decreases.

Figure 7. Relation Between Shannon Alpha and Max Amplitude During the Familiar Condition



Linear regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the P3 attribute of the ERP during the Oddball task. The maximum amplitude at the Temporal Left cluster predicted Shannon alpha during the Familiar condition, $p<0.05$. As the amplitude of the P3 increases alpha diversity decreases.

Figure 8. Relation Between Shannon Alpha and Mean Amplitude During the Familiar Condition



Linear regression plot visually represents the statistically significant relation between Shannon alpha and the amplitude of the P3 attribute of the ERP during the oddball task. The mean amplitude at the Temporal Left cluster predicted Shannon alpha during the Familiar condition, $p<0.05$. As the mean amplitude of the P3 increases alpha diversity decreases.

705 **Table 1.** Model-building results from stepwise regressions predicting microbiome diversity from *a priori*

706 CANTAB tests

	Variables Entered	β	SD	p-Value	Model R-Square
	Education	.098	1.519	.139	.148
	Reaction Time (RTI)	.070	64.284	.210	
Shannon Alpha	Mean Time Success (SWM)	-.248	1391.810	.029*	
	Total Errors (PAL)	-.293	22.635	.012*	
	Mean Latency (RVP)	-.025	71.672	.373	

707 * Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

708

709

Table 2. Model Building results from stepwise regressions predicting microbiome diversity from *a priori* ERP clusters in the detection task

	Variables Entered	β	SD	p-Value	Model R-Square
Shannon Alpha	Education	.110	1.519	.279	.087
	Target mean amplitude FL	.002	1.110	.131	
	Target mean amplitude FR	-.075	1.198	.127	
	Target mean amplitude M	.006	1.051	.038	
	Target mean amplitude TL	-.053	.870	.103	
	Target mean amplitude TR	-.021	.814	.093	
	Target mean amplitude F	.294	1.275	.021*	
Shannon Alpha	Education	.136	1.152	.339	.109
	Target minimum amplitude FL	-.144	1.430	.469	
	Target minimum amplitude FR	.169	1.427	.335	
	Target minimum amplitude M	-.131	1.386	.734	
	Target minimum amplitude TL	-.091	1.417	.519	
	Target minimum amplitude TR	-.006	1.459	.968	
	Target minimum amplitude F	.330	1.819	.022*	

* Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

714 **Table 3.** Model Building results from stepwise regressions predicting microbiome diversity from *a priori*

715 ERP clusters in the passive oddball task

	Variables Entered	β	SD	p-Value	Model R-Square
Shannon Alpha	Education	.118	1.532	.368	.089
	Familiar Negative Latency FL	-.298	32.447	.871	
	Familiar Negative Latency FR	-.023	26.117	.024*	
	Familiar Negative Latency M	.062	28.162	.703	
	Familiar Negative Latency TL	-.057	39.937	.669	
	Familiar Negative Latency TR	.023	40.353	.862	
	Familiar Negative Latency F	.023	34.248	.894	
Shannon Alpha	Education	.136	1.532	.280	-.295
	Familiar Positive mean Amplitude FL	-.144	1.067	.214	
	Familiar Positive mean Amplitude FR	.169	.996	.729	
	Familiar Positive mean Amplitude M	-.131	1.363	.437	
	Familiar Positive mean Amplitude TL	-.091	.641	.026*	
	Familiar Positive mean Amplitude TR	-.006	.876	.472	
	Familiar Positive mean Amplitude F	.330	1.896	.898	
Shannon Alpha	Education	.228	1.532	.084	.090
	Familiar Max Amplitude FL	-.051	1.464	.695	

Familiar Max Amplitude FR	.061	1.559	.640
Familiar Max Amplitude M	.126	1.661	.350
Familiar Max Amplitude TL	-.300	.8116	.023*
Familiar Max Amplitude TR	.182	1.253	.218
Familiar Max Amplitude F	.113	2.280	.428

716 * Significant at $p < 0.05$; R-squared: coefficient of determination; SD: standard deviation

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Berry Consumption in Older Adults: Gut-Microbiome Composition and Diversity

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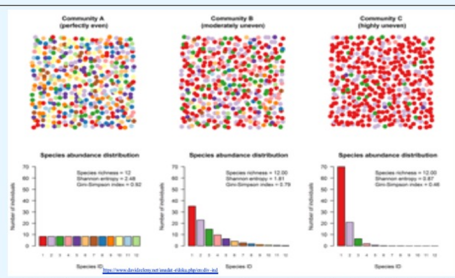
Cheatham Nutrition & Cognition Lab

Background

- Dietary polyphenols modulate the gut microbiome, but the influence is still poorly understood.
- Gut microbiota are known to contribute to the production of polyphenol metabolites.
- High gut microbiome diversity relates to several positive health outcomes, including better cognitive function.
- There are no published studies investigating a free-living human sample of older adults to determine the relation between berry consumption and gut-microbiome composition.
- Associations were quantified between berry consumption and gut microbial diversity and composition.
- It was hypothesized that a positive linear relation exists between berry consumption and gut microbial diversity. Also, that berry consumption would be associated with *Enterobacteriaceae* and *Clostridium spp*.

Participants and Methods

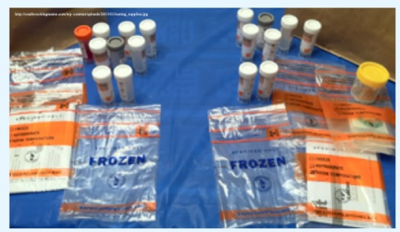
- Three-day diet recalls were conducted with 83 healthy adults aged 66-82y and fecal samples were requested.
- Total servings of berries were calculated using the Nutrition Data System for Research (Minneapolis, MN). 22 participants reported berry consumption.
- DNA was isolated from fecal samples, and 16S rRNA sequences were processed to assign taxonomy and to calculate the Shannon Diversity Index, a measure of within-person microbial diversity.



The A, B and C labels within the figures note three example communities, each with an equal number of biota. Shannon Diversity (Shannon Entropy above) is a measure of the abundance of unique species present in the population and is a commonly used metric to quantify the diversity of the microbial community.

Processing of Gut-Microbiome Samples

- Fecal DNA was isolated and sent to the Human Microbiome Core at UNC-CH for genomic library preparation of 16S rRNA.
- Sequence data was then processed using BiolockJ, a bioinformatics pipeline framework for metagenomics analysis.
  - Paired-end sequences were merged using default PEAR arguments, excluding sequences for which primers did not match or for which ten base pairs did not overlap.
- Sequences were processed through QIIME v1.9.1, where BiolockJ multiplexed the PEAR merged reads as QIIME input, utilizing UCLUST for deriving OTUs by clustering sequences at 97% similarity.
- In the pipeline, BiolockJ was directed to run the QIIME alpha-diversity script to calculate the Shannon Alpha diversity metric.



Analyses

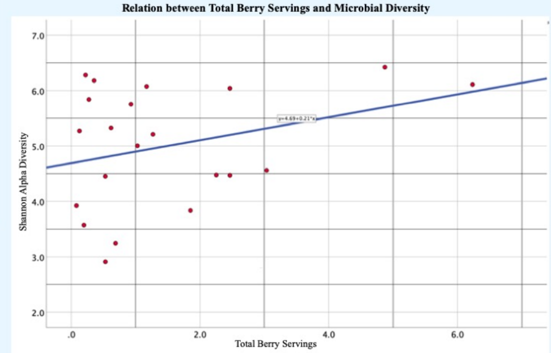
- Multivariable-adjusted regression models were used as follows:
  - Shannon Diversity of microbial community composition concerning Total Berry Servings was evaluated.
  - Further, relation of Total Berry Servings to *Enterobacteriaceae* and *Clostridium spp* were assessed.

Results

- Number of berry servings across three days ranged from 0.13 to 6.24
- Consumption was significantly positively related to the Shannon Diversity Index, [F(2,18)=4.310, p=.03, r^2=.324].
- Further, berry consumption was associated with higher relative abundance of *Enterobacteriaceae* and *Clostridium spp.* (p=.026 & p=.046, respectively)

Results

Relation between Total Berry Servings and Microbial Diversity



Discussion

- In a free-living human sample, berry consumption was positively associated with gut microbial diversity.
- Associations between specific genera and polyphenols found in controlled studies were confirmed.

The inclusion of berries in the daily diet may contribute to increases in the diversity of gut microflora, which in turn may lead to improvements in related health issues. Future studies and analyses will further refine our understanding of the interplay among berries, influential gut microflora, and health outcomes.

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Acknowledgements: The authors thank the participants. This research was funded by funds from the UNC-CH Nutrition Research Institute, and by a smaller grant from the NC Translational & Clinical Sciences Institute.

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