

**INHIBITORY CONTROL CHANGES IN HEALTHY AGING,
ALZHEIMER'S DISEASE AND AMNESTIC MILD COGNITIVE
IMPAIRMENT: AN FMRI STUDY OF THE STROOP TASK**

by

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by impairments in cognition coupled with a predictive pattern of neural atrophy beyond changes associated with healthy aging. Divergence from normative aging occurs years before clinical diagnosis of AD, a prodromal period classified as amnesic mild cognitive impairment (aMCI). Inhibitory control has been investigated across healthy aging, AD and aMCI through utilization of the Stroop task. Inhibition is required to suppress the automatic reading response in order to identify the ink color of word stimuli with matching (congruent) or non-matching (incongruent) ink color. Whether differences exist behaviorally, or if compensatory brain mechanisms to maintain task performance occur across these groups remains unclear. To address this knowledge gap, we utilized functional magnetic resonance imaging (fMRI) to evaluate the relationship between behavioral measures, neural correlates, and functional connectivity during performance of the Stroop task in young, middle-aged, and older adults, as well as patients with AD and aMCI. We found evidence of over-recruitment in middle-aged adults, which became more discernable after age 65 with both over-recruitment and loss of specificity to stimuli. Despite changes detected using fMRI, behavioral performance was similar across groups, with generalized slowing in older adults. Patients with aMCI performed similarly to controls, however showed reduced BOLD response within the parietal ROIs and over-recruitment of frontal ROIs when faced with incongruent trials. Patients with AD did not elicit different activity from controls in any ROI but did produce significantly more incongruent errors. Patients with AD had reduced functional connectivity in frontal brain areas, which correlated with default mode network (DMN) seeds. Functional connectivity was similar between aMCI and controls, suggesting that functional connectivity of Stroop and DMN ROIs remain spared early in the disease. Our results suggest

that reaction times alone may not be sensitive enough to show changes in aging or aMCI, but incongruent errors may be particularly useful at discriminating AD. Our studies support the literature indicating compensation, as we found evidence of neural plasticity in both healthy and pathological aging. Methodological factors including Stroop design, fMRI analysis, and participant variability may contribute to inconsistencies within the literature.

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List of Abbreviations

Aβ	Cerebral amyloid- β
ACC	Anterior Cingulate Cortex
AD	Alzheimer's Disease
ADL	Activities of Daily Living
aMCI	Amnesic Mild Cognitive Impairment
ANOVA	Analysis of Variance
B₀	Main Static Magnetic Field
B₁	Rotating Radio Frequency Field
BOLD	Blood Oxygenation-Level Dependent
CEN	Central Executive Network
CSF	Cerebral Spinal Fluid
CVLT-II	California Verbal Learning Test (version 2)
dACC	Dorsal Anterior Cingulate Cortex
DMN	Default Mode Network
DLPFC	Dorsolateral Prefrontal Cortex
DRS-2	Mattis Dementia Rating Scale (version 2)
FDR	False Discovery Rate
FDG-PET	Fluorodeoxyglucose Positron Emission Tomography
fMRI	Functional Magnetic Resonance Imaging
FoV	Field of View
HRF	Hemodynamic Response Function
IADL	Instrumental Activities of Daily Living

IFG	Inferior Frontal Gyrus
IFJ	Inferior Frontal Junction
IPS	Intraparietal Sulcus
ISI	Interstimulus interval
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MPRAGE	3D magnetization-prepared rapid gradient echo
MRI	Magnetic Resonance Imaging
NINCDS-ADRDA	National Institute of Neurological Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association
OFC	Orbitofrontal Cortex
PG	Precentral Gyrus
PET	Positron Emission Tomography
P-tau	Phosphorylated Tau
RF	Radio Frequency Pulse
ROI	Region of Interest
rs-fMRI	Resting state functional magnetic resonance imaging
SG	Supramarginal Gyrus
T1	Longitudinal relaxation time
T2	Transverse relaxation time
T2*	Effective transverse relaxation time
TE	Echo Time
TR	Repetition Time

TMT	Trail Making Test
T-tau	Total Tau (CSF)
VAA	Visual Association Area
WCST	Wisconsin Card Sorting Test
WMS	Wechsler Memory Scale

Chapter 1

Introduction

This thesis will describe an investigation of inhibitory control in both healthy and pathological aging, specifically mild Alzheimer's disease (AD) and the prodromal phase amnesic mild cognitive impairment (aMCI), through use of the Stroop task. Using a computerized trial-by-trial version of the Stroop task developed for functional magnetic resonance imaging (fMRI), we probed measures of behavioral performance, average magnitude of the blood oxygenation-level dependent (BOLD) response in regions of interest (ROIs) underlying the Stroop task, as well as functional connectivity of these ROIs across healthy aging (young, middle-aged and older adults), as well as comparing patients diagnosed with AD and aMCI to healthy age-matched controls.

1.1 Inhibitory Control and the Stroop Task

1.1.1 Theoretical Framework of Inhibitory Control as an Executive Function

Inhibition, as it relates to psychology, is defined as the ability to assert cognitive control over certain behaviors, and voluntarily suppress dominant or automatic actions or responses (Amieva, Phillips, Della Sala, & Henry, 2004; Miyake, et al., 2000; Stroop, 1935; Luna & Sweeney, 2004). As such, inhibition is closely related to self-control and attention, and has been linked to working memory (Miyake, et al., 2000). It has been hypothesized that in the context of working memory, the ability to exert inhibitory control over incoming information and filter out irrelevant details prevents the overload of working memory capacity, allowing for successful performance on cognitive tasks (Borella, Carretti, & De Beni, 2008).

1.1.2 Development of Inhibition

Simple inhibition can be described as a response or goal-directed behavior that requires little to no working memory (Garon, Bryson, & Smith, 2008; Best & Miller, 2010). According to a review by Garon et al. (2008), simple inhibition begins to emerge in infancy, with rapid increases into preschool years. Not surprisingly, the ability of a child to complete simple or reflexive inhibition tasks precedes the ability to successfully complete complex inhibitory tasks (Garon et al., 2008).

In contrast, complex inhibition tasks require working memory, often requiring the participant to keep instructions or a rule in mind while completing the task (Garon et al., 2008). The Day/Night task is one example that is designed to measure complex inhibition (Gerstadt, Hong, & Diamond, 1994). In this task participants are shown cards with pictures of either a sun on a white background, or a moon and stars on a black background. Participants are instructed to say “day” for the black moon card and “night” for the white sun card. This not only creates conflict but also requires children to remember arbitrary rules throughout the duration of the task. In a study of 160 children ranging in age from 3.5 to 7 years that employed the Day/Night task, the authors found performance increased with age both in terms of accuracy and decreased response latency (Gerstadt et al., 1994). A similar task was conducted with different stimuli: big and small circles (Ikeda, Okuzumi, & Kokubun, 2014). Participants ranged in age from 3 – 12 years old and were instructed to say “big” when viewing a small circle and “little” when viewing a big circle. Ikeda et al. (2014) found that 3 – 4-year-olds made more errors than all other age groups, with small improvements in 5 – 6-year-olds and children aged 7 and up were not significantly different from young adults. As for reaction times, there were

steady improvements up to age 9 – 10, at which the children were performing at the adult level.

In attempt to ascertain the developmental trajectory of inhibitory control measures across slightly older age groups, Huizinga, Dolan, & van der Molen (2006) explored a variety executive functioning tasks, including three measures of inhibition and two complex executive functioning tasks, in 7, 11, 15 and 21-year-olds. The three inhibition tasks were Stop-signal, Eriksen Flankers, and Stroop. For the Stop-signal task participants were shown either left or right pointing arrows and had to press a corresponding button (left or right) as quickly as possible. The arrows were color coded in that when they were green, they were to press the button for each arrow, but when they were red, they had to inhibit the button response. In the Eriksen Flanker task, a target arrow in the middle was surrounded by two arrows on each side, some of which were all going in the same direction (congruent), and some that were going the opposite direction of the target (incongruent). Participants had to press a left or right button indicating the direction of the target arrow. The Stroop-like task involved participants having to pay attention to color and/or orientation of a smiley face. For example, if the smiley face was outlined in blue, they had to make a response with their dominant hand and withhold a response when it was red, or vice versa. In addition, another block involved participants having to combine stimuli, for instance, making a response but only when the face is red and in the right-side-up orientation. The results show that across all three inhibition tasks, performance increased with age. More specifically, performance on the Stop-signal and Eriksen Flankers task increased until age 15, when their performance became like the young adults. However, the Stroop-like task showed that even the 15-year-olds had

longer reaction times than the adults, suggesting continued development on this task into young adulthood. Furthermore, on the complex executive functioning tasks that required the addition of working memory, set shifting, and inhibition, some performance measures reached adult levels at age 15, while others show evidence of continued improvements into young adulthood. Similarly, performance on the anti-saccade task, which requires inhibition of an automatic saccade toward a salient visual stimulus and generation of a voluntary saccade away from the stimulus, also improves dramatically throughout childhood and only reaches adult levels of control around age 20 (Yep, et al., 2022).

Overall studies of complex inhibition point to development of this ability around preschool age, with performance increases across middle childhood and even into adolescence and young adulthood, although it varies depending on task difficulty (Garon et al., 2008; Gerstadt et al., 1994; Ikeda et al., 2014; Huizinga et al., 2006).

The developmental differences in the performance of simple versus complex inhibition tasks may be linked with the development of language and abstract thinking (Garon et al. 2008). Some research has found that giving children strategies can help improve performance. For example, in the delay of gratification tasks that are driven by motivational and rewarding factors, giving children strategies such as focusing on neutral aspects of the reward (e.g., shape of the marshmallow rather than the taste) lengthens delay and therefore improves inhibitory control (Mischel, Shoda, & Rodriguez, 1989). While many studies have been conducted to understand how inhibition changes across development, there are some limitations. Attaining a direct comparison of task performance across ages is difficult because cognitive abilities vary greatly with age, for example some complex tasks might be too difficult for young children, but easier

inhibition tasks would be too easy for older children/adolescents, possibly resulting in ceiling effects. Variations of tasks and methodologies also exist in the literature, making comparison across studies challenging (Huizinga et al., 2006). For instance, the day/night task and big/small circle task are similar, but children as young as 3 were able to complete the big/small task and not the day/night task. The authors suggest that this may be in part because the day/night task is more abstract thus making it more difficult (Ikeda et al., 2014).

1.1.3 The Stroop Task as a Measure of Inhibitory Control

The Stroop task is a well-studied tool used to study inhibition (Stroop, 1935). Seminal work done by Stroop (1935) extended earlier explorations of color and word naming and corresponding interference effects (MacLeod, 1991). In the late 19th century, it was discovered that it took longer to identify the color of blocks of colors than to read the matching color word, for example naming the color “blue” versus reading the word “blue” (Cattell, 1886). It was postulated that since reading is so well-practiced it becomes automatic in adults with the ability to read, while color naming is not a skill used on a regular basis and therefore requires additional attentional demand (Cattell, 1886; MacLeod, 1991). Building on this observation, Stroop (1935) focused on understanding attentional control and interference between color and word naming by combining colors and color words. The study included several experimental manipulations. The first experiment involved participants reading aloud a list of words written in both black and colored ink (blue, red, green, purple and brown) as quickly as possible while ignoring the print color. For the second experiment, participants were instructed to ignore the printed

stimulus and say the color of ink each word or symbol, a solid color-filled square, was written in. Importantly, in some stimuli the color of ink was incongruent with the written word, for example the word “blue” written in red. The main findings were that when instructions were to read the word aloud the color of the words (black ink versus colored ink) did not impact response times, suggesting that there was no interference or conflict created by the addition of colored ink. However, for the second experiment, color naming incongruent word stimuli was slower than color naming symbols. Despite instructions to say the color participants may unintentionally read the word, and thus inhibition is required to suppress the automatic response to read the word in order to identify the ink color (Stroop, 1935; MacLeod & MacDonald, 2000). This bolsters the idea that reading is a more practiced skill in every-day life and therefore requires less effort than color naming (MacLeod, 1991; MacLeod & MacDonald, 2000).

Neutral stimuli are frequently included as experimental conditions, for instance neutral words, shapes or symbols, and non-word letter combinations. Neutral words for instance contain two possible responses, which are naming the ink color (task relevant) or reading the word (task irrelevant) (MacLeod & MacDonald, 2000). This is like the incongruent condition, however, in the latter the word meaning represents a color, adding to the increased demand on inhibitory control mechanisms. Incorporation of neutral stimuli into the Stroop test provides important control data and allows for the Stroop effect to be calculated. The Stroop effect is the average incongruent reaction times minus the average neutral reaction times and is essentially a measure of interference that reflects the conflict arising from color naming the incongruent stimuli versus the neutral stimuli. While Stroop (1935) did not investigate congruent stimuli specifically, it is also

frequently included in current versions of the Stroop task (MacLeod & MacDonald, 2000).

Congruency in the Stroop task is when the color word matches the color of ink it is written in, for example “green” written in green. Responses to congruent stimuli are often faster and more accurate relative to control or neutral stimuli, a concept known as facilitation (MacLeod & MacDonald, 2000).

The Stroop task has been utilized extensively in neuropsychological test batteries in both healthy and clinical populations. Benefits of the Stroop lie in its simplicity to administer and understand, as well as its test-retest reliability (MacLeod, 1991). However, literature on the Stroop task highlights how susceptible the task is to manipulations in experimental design. Studies have shown that varying different aspects of the task can impact Stroop performance. For instance, Stroop interference and facilitation effects can be impacted by interstimulus interval (ISI) timing (Parris, 2014). Furthermore, altering the proportion of incongruent and congruent stimuli can force the adaptation of different strategies, for instance participants might adapt a reading strategy if blocks of trials are mostly congruent, which can be negated by a mixed stimuli trial-by-trial design (MacLeod & MacDonald, 2000; MacLeod, 1991). Frequency of stimuli presentation can also impact reaction times. Responses were found to be faster to compatible stimuli mixed with infrequent incompatible stimuli and were also faster to incompatible stimuli that were presented more frequently (Logan & Zbrodoff, 1979). Task modality can also affect accuracy and Stroop effect, with disparities noted between a card reading version of the task and a computerized trial-by-trial version (Perlstein, Carter, Barch, & Baird, 1998). In terms of response modality, vocal responses have been

shown to produce larger Stroop effects compared to manual button responses (Augustinova, Parris, & Ferrand, 2019; MacLeod, 1991). Recent versions of the Stroop task are also often computerized allowing for more accurate calculations of reaction times for individual stimuli (MacLeod & MacDonald, 2000). Aside from task manipulations, the selection of a control condition also requires careful consideration. To this point, congruent stimuli have been shown to produce some interference effects when contrasted against neutral stimuli containing symbols instead of words (e.g., string of X's), which may be due to a combination of inadvertent reading during congruent trials and the absence of words interfering in neutral trials (Sichel & Chandler, 1969; MacLeod & MacDonald, 2000).

1.1.4 Neural Correlates of the Stroop Task

From a developmental perspective, inhibitory control follows a prolonged trajectory of change, and continues to develop later in life (Luna & Sweeney, 2004). This is largely due to the fact that inhibition is linked to the prefrontal cortex, which unlike other brain areas that develop early on, continues to develop into adolescence and even early adulthood (Best & Miller, 2010; Luna & Sweeney, 2004; Gogtay, et al., 2004).

Given the popularity of the Stroop task many fMRI studies have investigated the neural correlates underlying its performance. Considering the link between inhibitory control and prefrontal cortex, it is not surprising that activation of the frontoparietal network is commonly reported in studies of the Stroop task (Parris, et al., 2019; Laird, et al., 2005; Nee, Wager, & Jonides, 2007; van Veen & Carter, 2005). As mentioned earlier the Stroop task often contains incongruent, congruent and neutral or control conditions, allowing for several possible contrasts depending on the research question. A meta-

analysis of a selection of tasks involving the resolution of interference, including the Stroop task, carried out by Nee, Wager & Jonides (2007) in healthy young adults found that in studies contrasting incongruent versus neutral or incongruent versus congruent conditions there was involvement of the left dorsolateral prefrontal cortex (DLPFC), left insula, medial frontal cortex including the anterior cingulate cortex (ACC), left posterior parietal cortex (PPC), as well as smaller peaks in both the right DLPFC, PPC, and thalamus. Separating the studies by the control condition, either neutral or congruent, showed some differences in terms of activations. Contrasting incongruent versus neutral conditions resulted in greater activity in the left DLPFC and PPC, whereas incongruent versus congruent involved greater activity in the ACC. The authors suggest that since congruent stimuli contain color words as a possible competing source of information involvement of the left DLPFC is still required to assess relevant and irrelevant details, however there is no conflict at the response level since the ink color and color word match, therefore requiring less ACC activity (Nee, Wager, & Jonides, 2007). A recent meta-analysis employing activation likelihood estimation by Huang, Su, & Ma (2020) in healthy young adults revealed activations consistent with Nee, Wager, & Jonides (2007). Incongruent relative to neutral condition resulted in peak areas including the right IFG, left DLPFC, bilateral medial frontal cortex, bilateral inferior parietal lobule, precuneus, and left insular cortex. Contrasting the incongruent with congruent conditions yielded similar results, with the addition of the right ACC, left IFG, right superior frontal gyrus, and the left fusiform gyrus.

The neural correlates underlying Stroop performance have been shown to overlap with other tasks involving inhibition and interference resolution, including the right

DLPFC, ACC, and presupplementary motor area with the Flanker task, go/no-go task, and the stimulus-response compatibility task (Nee, Wagner, & Jonides, 2007). However, what appears to be unique to the Stroop task is the strong left lateralization. Imaging studies involving the Stroop task often report activations in the left hemisphere, which may reflect the verbal nature of the task, as well as increased task difficulty (Mead, et al., 2002; MacLeod, 1991; Nee, Wager, & Jonides, 2007). According to Mead et al. (2002), contrasting incongruent with neutral trials showed significant activation of the left precentral sulcus, close to the left IFG as well as Broca's area, indicating its possible involvement in language processing (Zhu, et al., 2013). Peak activity of the IFG bilaterally was found in another study, preceding activation in the ACC, which the authors suggest may reflect the role of the IFG in response selection (Carter, et al., 2000). The role of the left hemisphere in Stroop performance is furthered by early lesion studies. Perret (1974) noted that patients with left frontal lobe lesions performed worse on the Stroop task compared to patients with right hemisphere lesions and healthy controls.

Whether ACC activity is due to conflict arising from incongruent stimuli or just attentional control in general has been debated in the literature (Parris, et al., 2019). It has been argued that the role of the ACC during the Stroop task is to monitor for conflict and prepare a motor response as opposed to processing interference (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Zysset, Müller, Lohmann, & von Cramon, 2001; Milham, et al., 2002). In this view, the DLPFC becomes engaged along with the parietal cortices to resolve conflict (Bunge, Hazeltine, Scanlon, Rosen, & Gabrieli, 2002). Floden, Vallesi & Stuss (2011) found that increased ACC activity within the incongruent condition was greater for an unblocked, random trial-by-trial design compared to pure blocks of one

stimulus type, suggesting that ACC activity during the Stroop task may be due to arousal instead of conflict monitoring. In order to discern where along the information processing pipeline conflict occurs, van Veen & Carter (2005) implemented a button response fMRI Stroop paradigm where the left finger was to respond if the color was red or yellow and the right finger to blue or green. Stimuli were either congruent (color and word matched), semantically incongruent (color and word were different but required the same finger, e.g., 'green; written in blue), and response incongruent (color and word required opposing finger response, e.g., 'yellow' written in blue). Contrasting semantically incongruent with congruent revealed activations in the ACC, middle frontal gyrus (MFG), and bilateral inferior parietal lobule. Response incongruent relative to semantically incongruent trials showed distinctly different activations, including the inferior frontal gyrus (IFG), ACC (more anterior location), precentral gyrus, middle temporal gyrus, insula and thalamus. This finding of separate brain areas involved in different forms of conflict may reflect specific allocation of attentional resources (van Veen & Carter, 2005).

The importance of study design is also evident in the literature. For instance, block design fMRI studies of the Stroop task where each run consists of a single stimulus condition, such as congruent, are susceptible to habituation leading to practice effects (Bush et al., 1988). Participants may adapt different strategies, for example reading, during the congruent condition instead of color naming. The Stroop task is often considered a verbal task; however, many fMRI studies have adopted a manual button response in attempts to reduce head movement during fMRI that may cause motion artifacts (Nee, Wager, & Jonides, 2007). Laird et al. (2005) compared activations

between verbal and manual responses and found overlap in the ACC, left IFG and left inferior parietal lobe (IPL). Furthermore, papers that implemented manual responses showed left lateralization, while verbal responses showed activations in both hemispheres (Laird, et al., 2005). While button-responses have been shown to produce Stroop effects, this method may rely more on response selection to the mapped-out response set by the experimenter (MacLeod, 1991; Nee, Wager, & Jonides, 2007). Task manipulations can also impact the involvement of brain areas. For instance, varying the degree of task difficulty can impact the magnitude of ACC involvement between stimulus types (Carter, et al., 2000).

Nonetheless, it is apparent that activation of the frontoparietal network is involved in performance of the Stroop task (Huang, Su, & Ma, 2020; Nee, Wager, & Jonides, 2007). Knowledge of the brain areas involved in healthy young adults provides a foundation for further exploration of how the relationship between these brain areas and task performance may change with healthy aging and pathological aging, such as Alzheimer's Disease (AD) and amnesic mild cognitive impairment (aMCI).

1.2 Healthy Aging

1.2.1 Cognitive Changes Associated with Healthy Aging

Aging is a heterogeneous process, with considerable variability in terms of rate of decline and changes to cognitive functioning with increasing age at the individual level (Harada, Natelson, & Triebel, 2013; Craik & Bialystok, 2006; Fabiani, 2012). Factors such as genetics, overall health, fitness and environment all contribute to cognition (Craik & Bialystok, 2006). Susceptibility to age related declines also varies across cognitive

domains, with some areas less affected by age than others. Crystallized intelligence, for example, remains relatively stable over time, even showing improvements with increasing age (Harada, Natelson, & Triebel, 2013; Salthouse, 2012). Crystallized intelligence refers to skills and knowledge that is acquired over time, such as vocabulary, whereas fluid intelligence represents the ability to adapt and learn, for instance problem solving (Cattell R. B., 1936; Salthouse, 2012; Harada, Natelson, & Triebel, 2013). In contrast, some aspects of cognition, including markers of fluid intelligence, show evidence of decline in older age. Processing speed, attention, and memory are examples of domains that are vulnerable to normal age-related declines (Oschwald, et al., 2020; Harada, Natelson, & Triebel, 2013). Performance in these areas, for example novel problem solving, typically peaks in young adulthood, followed by drop-offs in performance around age 30 that continues to decline with increasing age (Salthouse, 2012). In fact, generalized slowing is often reported as underlying much of the cognitive changes noted in older age (Salthouse T. A., 1996). It is generally understood that executive functioning and cognitive control coincides with the development of the frontal lobes, peaking in the early twenties, and becomes susceptible to age-related declines first (Craik & Bialystok, 2006). This may be due to the complex relationship between aspects of cognition requiring more cognitive demand, for example working memory and inhibitory control (Craik & Bialystok, 2006; Hasher & Zacks, 1988). Included in the domains negatively affected by age and central to this thesis is attention and inhibitory control.

The concept that inhibitory control diminishes with advancing age was discussed by Hasher & Zacks (1988), where it was proposed that reductions in inhibitory control

mechanisms with older age result in irrelevant information being entered into working memory causing disruptions to cognitive functioning and in turn worse task performance (i.e., longer reaction times, more errors). Since then, however, there have been a multitude of studies that contradict inhibitory deficits with aging (Rey-Mermet & Gade, 2018). Several possibilities for this discrepancy are outlined by Rey-Mermet & Gade (2018), which include differences in experimental design and tasks used, participant characteristics (i.e., could happen to include high functioning group of older adults with preserved inhibition), and possible effects of generalized slowing as opposed to a true deficit in inhibitory control. A meta-analysis investigating age effects and inhibition found a task specific deficit as opposed to a general decline in inhibition with age (Rey-Mermet & Gade, 2018). More specifically, age effects were noted for studies employing the go/no-go and stop-signal tasks, but not for the Stroop, Flanker, global-local, or N-2 repetition cost tasks. Studies investigating older versus younger adults showed that older adults produced longer reaction times for the stop-signal task and more errors on the go/no-go task compared to young adults. According to Rey-Mermet & Gade (2018), more studies are needed in order to gain a clearer picture on whether inhibition deficits are found in healthy older adults, especially in certain behavioral tasks.

1.2.2 Brain Changes Associated with Healthy Aging

During typical development the brain undergoes tremendous changes, including increases in size, myelination and synaptogenesis, as well as decreases through apoptosis and synaptic pruning (Gogtay, et al., 2004; Giedd, et al., 1999). This coinciding loss of gray matter and increase in white matter is an important part of brain maturation and can be viewed as a developmental period where more efficient connections are being made

while pruning away redundancies, and in part being shaped through experiences in life (Gogtay et al., 2004).

As far as healthy aging is concerned, numerous longitudinal and cross-sectional studies have attempted to capture age-related changes in the brain (for a review see Oschwald et al., 2020). As far as gray matter goes, studies generally point to a gradual loss of volume with advancing age (Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012). An annual whole brain volume loss at a rate of 0.2% was found after age 35, increasing to approximately 0.5% or more after the age 60 (Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012). However, certain brain areas are more susceptible to initial atrophy over others. Areas of the frontal cortex, as well as medial temporal regions (e.g., hippocampus), tend to show selective deterioration first compared to posterior regions (Tamnes, et al., 2013) . Evidence from the Seattle Longitudinal Study suggest that cortical thickness measurements collected over a period of 8 years in middle-aged to older adults follows a similar pattern (Rast, et al., 2017). Another cross-sectional study that implemented voxel-based morphometry of white matter in healthy adults observed a non-linear trajectory, with total white matter volume increasing in the 40's, peaking in the 50's, and declining into the 60's (Liu, et al., 2016). As for specific regions, the frontal and temporal lobes were the first regions identified to show age-related reductions, with the occipital lobe remaining stable until older age (Liu, et al., 2016). Another marker of older age is the presence of white matter hyperintensities, which are lesions indicative of small vessel disease, and tend to increase in prevalence with older age (Merino, 2019). The structural organization of white matter tracts also declines with age (Bennett & Madden, 2014). Both the presence of white matter hyperintensities and

reduced organization of tracts has been linked with cognitive decline (Bennett & Madden, 2014; Merino, 2019). A review of the relationship between brain structure and cognition in healthy aging focusing on longitudinal studies presented mixed findings, with some positive associations between brain volume and cognition (e.g., hippocampal volume and episodic memory) as well as some negative associations (Oswald, et al., 2020). The authors highlight the fact that relatively few studies exist and vary widely in their methodologies and outline the need for future large-scale longitudinal studies to be conducted to advance our knowledge of the brain-behavior relationship in healthy aging.

In order to counteract some of these negative brain changes associated with aging, several theories have been put forth that describe compensatory mechanisms (Oswald, et al., 2020). More specifically, studies have shown that older adults may recruit additional (Reuter-Lorenz & Cappell, 2008) or bilateral areas when completing a cognitive task compared to younger adults (Cabeza, 2002), and that compensation often results in the maintenance of behavioral performance. A more recent model, the Scaffolding Theory of Aging – revised (STAC-r) by Reuter-Lorenz & Park (2014) incorporates the idea that experiences acquired and shaped throughout the lifetime can impact both brain structure and function and should be considered in the view of compensatory mechanisms and aging. This addition in turn reflects neural plasticity, with the idea that there can be positive or protective effects (e.g., engagement in intellectually stimulating activities, educational achievements etc.) as well as detrimental effects (e.g., lifestyle factors including smoking and obesity, stress, and genetics, for example the presence of the APOE-4 gene). Taken together the STAC-r model attempts to gain a comprehensive view on aging by accounting for individual differences in lived

experiences and the complex relationship between compensation, brain structure and function and cognitive outcomes (Reuter-Lorenz & Park, 2014).

Another term often discussed in aging literature is cognitive reserve. The concept of cognitive reserve in healthy aging can be broken down further into either physical e.g., brain reserve: brain size, number of neurons, and synapses, and neural reserve: capabilities of neural networks, or mental e.g., cognitive reserve: adoption of strategies or processing mechanisms, that provide an individual with the ability to fend off age-related decrements to cognition (Stern, 2009). Varying levels of cognitive reserve or compensation may reflect the heterogeneous nature of healthy aging and contribute to difficulties in comparing aging across studies (Oschwald, et al., 2020).

1.2.3 The Stroop Task and Healthy Aging

1.2.3.1 Behavioral Measures of the Stroop Task and Healthy Aging

The Stroop effect emerges with the capability to read, as studies in young children just learning to read report faster color naming (MacLeod, 1991). Although, as children age it is generally accepted that they become more susceptible to interference compared to adults. A study including children in the second grade aged 7-8 showed a greater Stroop effect compared to adults (Tipper, Bourque, Anderson, & Brehaut, 1989). This may be due to limited development of inhibitory control coinciding with the strong development of automaticity involved in reading.

It has been suggested that the Stroop effect becomes greater again in older age, with healthy older adults more effected by interference compared to younger or middle-aged adults (MacLeod, 1991; Spieler, Balota, & Faust, 1996; Stroop, 1935). That is, older

adults beginning around the age 60 have disproportionately longer reaction times when faced with incongruent stimuli compared to neutral stimuli compared to young adults in their 20's (Spieler, Balota, & Faust, 1996). Other studies report no difference in reaction times or Stroop effect between older and younger adults (Langenecker, Nielson, & Rao, 2004; Milham, et al., 2002). In corroboration with the latter, a recent meta-analysis found that older adults showed no impairment in response inhibition or ability to ignore irrelevant information during the Stroop task compared to younger adults (Rey-Mermet & Gade, 2018). In fact, potential age-related differences in Stroop performance may be a result of generalized slowing as opposed to a true breakdown of inhibitory control (Verhaeghen & De Meerssement, 1998). Zysset et al. (2007) found that older adults were generally slower overall at responding to stimuli but did not produce a greater Stroop effect. In contrast to previous meta-analyses conducted by Verhaeghen & De Meerssement (1998) and Rey-Mermet & Gade (2018) that focused on group-level mean reaction times using plot-based methods of analysis, a recent meta-analysis by Nicosia, Cohen-Shikora & Balota (2021) took a different approach by taking into account individual variability in trial-by-trial reaction times as well as measures to control for generalized slowing (e.g., using z-scores, proportion scores: $\text{incongruent RT} - \text{congruent RT} / \text{incongruent RT}$). Using this alternate technique, Nicosia, Cohen-Shikora & Balota (2021) found evidence for disproportionate age-effects in the Stroop task. Applying the same plot-based analysis, Brinley and state-trace, the authors were able to replicate the generalized slowing findings of previous studies. Inconsistencies across studies and methodologies highlight the complexities involved in quantifying cognitive tasks and aging effects.

Therefore, there remains no clear consensus as to whether healthy aging negative impacts inhibitory control as measured by the Stroop task.

1.2.3.2 Neural Correlates of the Stroop Task in Healthy Aging

Several studies have probed inhibitory control in healthy older adults using fMRI and neuroimaging techniques in combination with the Stroop task, yielding mixed results. Some investigations of the neural correlates underlying healthy aging and the Stroop task have revealed evidence of compensatory brain activations. For example, older adults have been found to recruit similar brain areas to young adults, but to a greater extent (Langenecker, Nielson, & Rao, 2004; Turner & Spreng, 2012). Older adults may also rely on more wide-spread activation of brain areas during interference (Laguë-Beauvais, Brunet, Gagnon, Lesage, & Bherer, 2013; Mathis, Schunck, Erb, Namer, & Luthringer, 2009). Another study found that comparable Stroop-related areas were activated across age groups, but that middle-aged adults recruited additional areas, including the IFG, in addition to increased BOLD response in similar areas to younger adults (Zysset, Schroeter, Neumann, & von Cramon, 2007). Conversely, young adults have been found to have greater activity in frontal areas important for attentional control, including the DLPFC, ACC, middle frontal gyrus and superior parietal lobe relative to older adults (Milham, et al., 2002). In the same study by Milham et al. (2002) the authors found that older adults had more extensive activation of the anterior versus posterior temporal lobe seen in the younger adults, which they suggest indicates greater visual processing of the word stimuli. Schulte et al. (2011) described different activation patterns between young and older adults with sustained behavioral performance on a Stroop match-to-sample task

between groups, which the authors interpret as a form of reorganization akin to compensation when faced with increasing task demands in older age.

Understanding the temporal progression of compensation with healthy aging remains unclear in the literature as relatively few studies exist that encompass a wide age range. However, initial changes in inhibitory control as measured by the Stroop task may emerge around age 50 (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). Considering behavioral and fMRI data across a large age range would help give insight into how and if inhibition changes as a function of healthy aging and the relationship between neural correlates and cognitive performance.

1.3 Pathological Aging: Alzheimer's Disease (AD) and Amnesic Mild Cognitive Impairment (aMCI)

1.3.1 Cognitive Changes Associated with AD and aMCI

Aging is associated with cognitive changes, some of which are considered normal age-related declines and others that fall outside of the normal range (Knopman et al., 2015). One common reason underlying cognitive impairments in aging populations is due to dementia. Dementia is an umbrella term that encompasses a wide range of symptoms common across several diseases, including Alzheimer's disease (AD), vascular dementia, dementia with Lewy bodies, and frontotemporal dementia (The Alzheimer Society of Canada, 2022). There is significant overlap in symptoms across dementias, for example memory loss, declines in problem solving, verbal skills, attention and behavioral disturbances (The Alzheimer Society of Canada, 2022). Close to 600 000 people were estimated to be living with some form of dementia in 2020 in Canada alone, with that number projected to rise to close to 1 million by the year 2030 (The Alzheimer Society of

Canada, 2022). The prevalence of AD, the most common form of dementia, in Canada is on the rise. With the growing number of older Canadians, and age being a significant risk factor for development of AD, estimates of AD are expected to increase from over 300 000 in 2020 to well over 1 million cases by 2050 (The Alzheimer Society of Canada, 2022). Furthermore, the associated costs with AD are staggering, impacting both the health care system as well as families. As a result, current research efforts are underway to better understand these diseases with emphasis on prevention or the delay of disease onset (The Alzheimer Society of Canada, 2022). One way in which the field is moving is to better identify the earliest stages of AD, even pre-clinically, which may be a more effective target for early interventions partly because pathology may have not accumulated enough to cause irreversible, widespread cognitive and neural damage (Dubois et al., 2016; Peterson et al., 2014).

It is well accepted that the course of AD pathology is a long process with an asymptomatic period occurring over 20 – 30 years before clinical symptoms appear (Morris, 2005; Sperling et al., 2011). Mild cognitive impairment (MCI) is a term used to describe symptoms of slight cognitive changes that are considered outside of the norm for someone of similar age and years of education (Albert et al., 2011). These cognitive changes are pronounced enough to be detected on formal neuropsychological tests, however, not enough to disrupt daily functioning. Long-term epidemiological studies of older adults propose that of people aged 65 and older, approximately 10 – 15% have MCI (Knopman et al., 2015; Petersen, 2004). While MCI is often viewed as the stage between healthy aging and dementia related to AD, MCI as a definition is heterogeneous, both in terms of causes, subtype, and prognosis (Knopman et al., 2015; Peterson et al., 2014).

Commonly, MCI is divided into the amnesic subtype (aMCI) and non-amnesic (single or multidomain) subtype based on cognitive symptoms. Amnesic MCI is characterized by deficits in memory, for example forgetting recent conversations or events (Albert et al., 2011). In contrast, non-amnesic MCI involves deficits in one (single domain) or more (multidomain) cognitive domains, such as problem solving and judgement, language, or perception (Albert et al., 2011). Amnesic MCI and non-amnesic MCI have distinct differences in terms of underlying pathology and disease trajectory. According to Petersen et al. (2004), the risk of future conversion from aMCI to AD is higher than non-amnesic MCI. Approximately 80% of individuals with aMCI go on to develop AD after 6 years (Petersen et al., 1999). Thus, MCI of the amnesic subtype is viewed as a continuum of AD.

1.3.1.1 Cognitive Decline in Alzheimer's disease

According to Jack et al. (2013), cognitive decline is the last symptom to appear along the disease continuum in AD. In AD there is significant loss of cognitive abilities that progress over time (McKhann et al., 2011). Usually memory, along with other cognitive domains are affected, like executive functioning (e.g., planning, problem solving, reasoning), visuospatial skills (e.g., face or object recognition), and language (e.g., word finding difficulties), that are corroborated by a reliable informant, and impairment is measurable on cognitive tests (McKhann et al., 2011). In AD disruptions to cognitive functioning also interfere with instrumental activities of daily living (IADLs), such as complex household tasks like handling finances, preparing food etc. (Lawton & Brody, 1969). Eventually activities of daily living (ADLs) are affected (e.g., dressing and

feeding oneself) and as the disease progresses, ultimately the ability to function independently (McKhann et al., 2011).

1.3.1.2 Cognitive Decline in Amnesic Mild Cognitive Impairment

Neuropsychological measures can help differentiate aMCI from AD. In terms of cognitive functioning, aMCI is defined as having mild, yet noticeable, impairment in memory (Albert et al., 2011). While early memory complaints are often common to both AD and aMCI, in AD there are at least two cognitive domains affected that show significant deficits compared to normal scores from healthy adults (McKhann et al., 2011). In comparison, aMCI is associated impairments only in memory, and these mild changes do not affect ADLs or IADLs, although they may slightly interfere with the latter (e.g., make more errors or perform complex tasks less efficiently) (Albert et al., 2011). On cognitive assessments people with aMCI typically perform 1 to 1.5 standard deviations below the mean, controlling for sex and years of education (Albert et al., 2011). This contrasts with the significantly reduced cognitive performance seen in patients with AD (McKhann et al., 2011).

The California Verbal Learning Test (CVLT) is one example of a neuropsychological test that interrogates verbal memory skills (Delis, Kramer, Kaplan, & Ober, 1987). The CVLT consists of a 16-word list with 4 words related to a specific category (e.g., clothing, animals, and food items). The list of words is read aloud by the experimenter and repeated immediately after by the participant (immediate recall). Next, a distractor list is read, after which participants must recall the previous list, and semantic clues are given. After fifteen-minute delay participants are again asked to recall the initial list, again semantic clues are given at the end of their free recall (delayed recall). Finally,

a list of words, some of which are from the first list, second list and not from the list at all, are read and participants must identify if the word was from the first list or not, which measures word recognition. Thus, the CVLT provides detailed information about acquisition of memory (i.e., learning the word list through semantic clustering), as well as consolidation. One study investigated differences on CVLT performance between aMCI, AD and healthy controls and found that participants with aMCI performed better than the patients with AD, but worse than healthy controls on several measures (Ribeiro, Guerreiro, & De Mendonça, 2007). More specifically, the aMCI group made more recall errors, had reduced learning performance on immediate recall trials, had more forgetting during the long delay free recall, and used semantic clustering less than the controls, but better than the AD group. However, when given semantic cues, aMCI participants generated a similar number of correct words as controls. Reduced semantic clustering in aMCI compared to healthy controls was corroborated by another study, which in addition found that controls that later progressed to aMCI also had impairments in semantic clustering (McLaughlin et al., 2014). Ribeiro, Guerreiro, & De Mendonça, (2007) suggest that this lack of spontaneous strategy use may reflect the beginning of impaired executive functioning. In fact, studies have been able to predict conversion from aMCI to AD using delayed verbal recall scores with sensitivity ranging from 73-89% and specificity from 70-97% (Gainotti, Quaranta, Vita, & Marra, 2014). While measures of memory are the hallmark cognitive symptom of aMCI, a post-mortem study compared longitudinal cognitive measures of verbal fluency (animal naming), constructional praxis (visuospatial abilities), and word list delay, after classifying individuals as preclinical AD or pathologically normal after autopsy (Riley et al., 2011). Results from this study found

that despite having within normal range on cognitive tests, preclinical AD as determined through autopsy was associated with faster declines on those cognitive measures (Riley et al., 2011).

1.3.2 Brain Changes Associated with AD and aMCI

Characteristic neuropathology of AD has been identified through post-mortem studies and includes a build-up of senile plaques through accumulation of beta amyloid ($A\beta$), and neurofibrillary tangles that contain hyperphosphorylated tau protein (Blennow, de Leon, & Zetterberg, 2006). In combination with the shift towards early detection, biomarkers that aid in classification of disease stages, as well as aiding in differential diagnoses, is under heavy investigation within the field. The current literature on biomarkers for AD and aMCI has shed much needed light onto AD progression. Biomarkers can provide significant links with known AD neuropathology and characteristic cognitive decline, and importantly, can be measured in living individuals thus providing insight into disease stage or classification as well as measure efficacy of new therapeutic treatments (Blennow, Hampel, Weiner, & Zetterberg, 2010). Work is being done to understanding the temporal development of biomarker abnormalities in AD in several core areas, including fluid, neuroimaging, and neuropsychological (Jack et al., 2013).

1.3.3 Fluid Biomarkers

Several fluid biomarkers have been identified that relate to AD pathophysiology. Biomarkers in cerebral spinal fluid (CSF) are of particular interest in AD since CSF surrounds the brain and can therefore provide insight into changes occurring in the brain (Blennow et al., 2010). These core biomarkers reflect the development of $A\beta$ plaques and

neurofibrillary tangles, which are well established hallmarks of AD pathology (Blennow et al., 2010). Currently, the fluid biomarkers commonly used in studies of AD measure amyloid beta ($A\beta$) deposition through cerebral spinal fluid (CSF) $A\beta_{42}$, one of the least soluble forms of $A\beta$ peptides (Jack et al., 2013; Shaw et al., 2009), and neuronal injury through CSF tau levels (Jack et al., 2013; Sämgård et al., 2010).

1.3.3.1 CSF $A\beta_{42}$ and Tau in Alzheimer's disease

It is thought that with AD $A\beta$ begins to accumulate in the brain due to lack of clearance of $A\beta_{42}$ forming insoluble plaques, and as a result can be detected in CSF as a reduction in $A\beta_{42}$ (Hardy & Selkoe, 2002; Blennow et al., 2010; Shaw et al., 2009).

Evidence of this comes from measures of CSF $A\beta_{42}$ in relation to post-mortem studies of brain $A\beta$. For example, one post-mortem study of confirmed AD cases found a negative correlation between CSF levels of $A\beta_{42}$ and $A\beta$ in the brain (Tapolia et al., 2009).

According to some models (Jack et al., 2013), CSF $A\beta_{42}$ is the first biomarker to show abnormalities in the pathological process of AD. CSF $A\beta_{42}$ levels have been shown to discriminate cognitively normal adults from patients with AD with fairly high accuracy, about 80 – 90 % (Shaw et al., 2009; Blennow et al., 2010; Shaw et al., 2011; Blennow & Hampel, 2003, Tapolia et al., 2009), with individuals diagnosed with AD having about 50% of the levels of $A\beta_{42}$ compared to age-matched healthy controls (Blennow & Hampel, 2003; Hampel et al., 2008).

As for CSF tau, studies have looked both at CSF total tau (t-tau) and phosphorylated tau (p-tau) as biomarkers in AD (Blennow et al., 2010). Tau protein is located in the axons of neurons and plays a role in microtubule stability (Hampel et al., 2008). Its release into extracellular space and consequently in CSF is viewed as a marker

of neurodegeneration (t-tau) (Hampel et al., 2008). When tau becomes abnormally hyperphosphorylated (p-tau), the result is disruption of the microtubules resulting in the formation of neurofibrillary tangles (Wang, Xia, Grundke-Iqbal, & Iqbal, 2013). Abnormally elevated tau levels appear after A β reduction (Jack et al., 2013), and elevated levels of p-tau have been consistently found in patients with AD compared to controls (Hampel et al., 2008). Furthermore, elevated p-tau levels have been found to correlate with neurofibrillary tangle burden in AD post-mortem (Tapiola et al., 2009), as well as positively correlate with whole brain atrophy and expansion of the ventricles (Josephs et al., 2008). It has been reported that in terms of distinguishing AD from healthy older adults, patients with AD have an increase of approximately 300% in CSF t-tau levels, independent of normal age-related increases in t-tau (Hampel et al., 2008). Within a population of people with AD, higher levels of t-tau (greater than 800 ng/L), and not A β 42 levels, were associated with a more rapid decline in cognitive scores, especially those reflecting memory and orientation performance, over a three-year period (S  mg  rd et al., 2010). Although, increased t-tau is not only AD specific, and elevated CSF levels have been found in cases with head trauma and stroke, as well as Creutzfeldt-Jakob disease (Ros  n et al., 2013; Hampel et al., 2008). On the other hand, elevated p-tau levels have been shown to discriminate AD from control groups as well as other dementias, like frontotemporal (sensitivity of 88% and specificity of 92%), dementia with Lewy bodies (sensitivity of 94% and specificity of 64%), and dementia related to Parkinson disease (Hampel, et al. 2004; Hall et al., 2012). Like the relationship between t-tau and cognitive decline within AD, elevated levels of p-tau have also been associated with worse cognitive performance over time (S  mg  rd et al., 2010). Studies have also looked at the

ratios of low CSF A β 42 with high levels of t-tau and p-tau and found that the combined analysis of both CSF biomarkers gives the best sensitivity (91.6%) and specificity (85.7%) for differentiating AD from healthy adults (Tapiola et al., 2009; Albert et al., 2011).

1.3.3.2 CSF A β 42 and Tau in Amnesic Mild Cognitive Impairment

CSF biomarker profiles of A β 42 and tau in aMCI show similarities to that of AD. The pattern of low A β 42 and high t-tau has been found in people with aMCI; however, the degree of abnormality falls between healthy adults and people with AD (Hempel et al., 2004). Furthermore, one study found that out of their participants, this AD-like CSF profile was found to be most common in individuals with aMCI (79%) compared to controls (31%), was associated with worse performance on the Mini Mental State Examination and measures of daily functioning and was associated with later conversion to AD (Visser et al., 2009). Even people with subjective memory complaints (52%), but not yet fitting diagnostic criteria for aMCI, were more likely to have the AD-like CSF profile of decreased A β 42 and increased p-tau (Visser et al., 2009). One large study that established CSF biomarker abnormality cut-offs for A β 42, t-tau, and p-tau from an independent group of control and AD participants, found that when these cut-offs were applied to a group of MCI participants that later developed AD, the combination of A β 42/p-tau ratio and t-tau levels could accurately identify future AD conversion with a sensitivity of 83% and specificity of 72% (Mattsson et al., 2009). Another study investigated the predictive value of CSF biomarker profiles in conversion from aMCI to AD between 4.1-11.8 years after initial lumbar puncture (Buchhave et al., 2010). Of those that converted to AD during follow-up (median 9.2 years later), Buchhave et al. (2010)

found reduced baseline levels of A β 42, and increased t-tau and p-tau compared to those that did not convert to AD. Additionally, participants that converted to AD earlier (0-5 years after initial visit) had increased levels of t-tau and p-tau compared to those that converted to AD later (5-10 years after initial visit), while A β 42 did not differ. Different from some other studies, Buchhave et al. (2010) found a sensitivity of 88% and a specificity of 90% for predicting future AD conversion based on baseline A β 42/p-tau. These results provide support for early A β 42 changes in AD, and that changes occur early on during the aMCI stage.

In terms of CSF biomarkers A β 42, p-tau and t-tau, a combination is the most accurate at distinguishing AD from healthy adults, as well as other dementias (Tapiola et al., 2009; Buchhave et al., 2010). Even the early disease stage of aMCI revealed AD like patterns of decreased A β 42 and increased p-tau and t-tau, however to a lesser degree, showing lower levels of A β 42 and higher levels p-tau and t-tau than controls, but higher levels of A β 42 and lower levels of p-tau and t-tau than AD groups.

1.3.4 Neuroimaging Biomarkers

Several neuroimaging biomarkers pertaining to classifying stages of AD have been explored using tools including magnetic resonance imaging (MRI) and positron emission tomography (PET). More specifically, both MRI and PET imaging have been viewed as ways to measure neurodegeneration and correspond with increased t-tau and p-tau that result in neurofibrillary tangles and neuron loss (Jack et al., 2013). One model of AD progression suggests that abnormalities on MRI and fluorodeoxyglucose positron

emission tomography (FDG-PET) show up after CSF A β 42 and tau biomarkers, and precede cognitive impairment (Jack et al., 2013).

1.3.4.1 MRI and PET Studies in Alzheimer's Disease

Structural MRI has allowed for non-invasive measures of brain volume in both AD and healthy adults. Measures of whole brain atrophy over time have found that yearly atrophy rates are faster for AD compared to normal aging alone, with 1% and 0.5% global reductions, respectively (Fotenos, Snyder, Girton, Morris, & Buckner, 2005). AD is associated with cortical atrophy beginning with early degeneration in the medial temporal lobes, including the hippocampus and entorhinal cortex, which was confirmed by post-mortem studies (Braak & Braak, 1991). Since the hippocampus is an anatomically well-defined structure, a site associated with early neurofibrillary tangle formation and neuron loss, and atrophy of the hippocampus has been linked with memory deficits associated with AD, hippocampal volume comparisons between AD and healthy adults have been widely investigated (Jack et al., 2011). Indeed, MRI measures of hippocampal volume have been able to distinguish healthy controls from patients with AD, with significantly decreased volumes bilaterally in AD, as well as atrophy in the precuneus (Ryu et al., 2010). Patients with AD also have significantly increased atrophy rates ranging from 3-7% per year, compared to the 0.9% found in healthy adults (Hempel et al., 2008).

The development of PET imaging with 2-[18F] fluoro-2-deoxy-d-glucose (FDG) has also been investigated in AD, which measures fluctuations in the cerebral metabolic rates for glucose (Mosconi et al., 2009). Indeed, studies of FDG PET in AD have shown hypometabolism in parieto-temporal and posterior cingulate cortices that progress to

frontal brain areas as disease severity increases (Mosconi, 2005; Hampel et al., 2008). This reduction in glucose metabolism is suspected to reflect synaptic dysfunction as a result of the pathological process occurring in AD, like the loss of synapses through neurofibrillary tangles (Mosconi, 2005).

1.3.4.2 MRI and PET Studies in aMCI

Structural MRI techniques have also been useful in distinguishing aMCI from healthy elderly, as well as AD. (Desikan et al., 2009; McEvoy et al., 2009). Like other biomarkers, volume measurements of certain brain areas in aMCI fall between average values for healthy adults and AD and can be classified using automated techniques (Desikan et al., 2009; Leung et al., 2010). Leung et al. (2010) investigated change in hippocampal volume over 12 months in groups of controls, AD, and MCI participants. The MCI group was further delineated into those that converted to AD, those that remained stable, and those that reverted to normal one-year post baseline visit. Consistent with the literature, the AD group showed the most baseline hippocampal atrophy, followed by the MCI group, and then the controls with the largest baseline hippocampal volumes. Similarly, the AD group had larger atrophy rates over the year compared to the MCI group and controls. Within the MCI group, those that converted to AD had significantly reduced hippocampal volumes and faster atrophy rates compared to the MCI individuals that remained stable or reverted to normal. As for the predictive value of hippocampal volume in future conversion from MCI to AD, baseline hippocampal and entorhinal cortex volumes, along with other regions, were able to predict with 64.7% sensitivity and 96.4% specificity (Nesteruk et al., 2015). Another study found improved classification when hippocampal and entorhinal cortex volumes were combined with age

and cognitive test scores (Devanand et al., 2007). Therefore, while many studies find differences in volumes between aMCI and AD, the predictive value of volumes alone may not be sufficient in determining conversion from aMCI to AD.

FDG-PET studies have also identified early changes associated with aMCI (Hampel et al., 2008). One study found that a group of aMCI participants that had a baseline AD-like profile consisting of elevated amyloid (identified using PET imaging) and evidence of neurodegeneration (hippocampal atrophy), over time showed reduced FDG uptake in the medial and lateral temporal lobe and lateral parietal lobe (Knopman et al., 2016). This reduction was more than the controls that had similar amyloid and hippocampal profiles, but less than those diagnosed with AD. In addition, a combination PET and MRI study using carbon 11-labeled Pittsburgh Compound B (PiB) with PET, which allows for in vivo analysis of amyloid burden in the brain, revealed higher global amyloid burden and reduced hippocampal volumes in AD compared to controls, with aMCI again falling in between the two groups (Jack et al., 2008). A recent review of FDG-PET findings in terms of predicting conversion from aMCI to AD found a wide range of sensitivities and specificities (Smailagic et al., 2015). Consequently, FDG-PET studies of aMCI seem to reveal early changes associated with the AD disease process; however, whether FDG-PET can reliably predict future conversion to AD remains unclear (Smailagic et al., 2015).

Therefore, evidence from both structural MRI and PET studies also find aMCI results in brain atrophy and decreased glucose metabolism that resembles AD-like pathology, however to a lesser extent. Again, this neuroimaging data bolsters the idea that aMCI can be considered an early stage of AD.

1.3.5 MCI: Amnestic versus Non-amnestic Subtypes

Amnestic and non-amnestic MCI are associated with different underlying pathologies and the development of different diseases later on (Albert et al., 2011). While aMCI is considered the early stages of AD, non-amnestic MCI has been linked to future development of other dementias, including Lewy Body Dementia (Albert et al., 2011). In some cases, people with MCI will revert to normal (Koepsell & Monsell, 2012), and causes of MCI may be due to other underlying conditions, including depression, medication side effects, or vascular events (Albert et al., 2011).

Several experiments have shown differences between aMCI and non-amnestic MCI. For example, structural differences on MRI were found between aMCI and non-amnestic MCI (Csukly et al., 2016). Csukly et al. (2016) found that those diagnosed with aMCI had significantly smaller volumes in the hippocampus and entorhinal cortex compared to controls and non-amnestic MCI groups, as well as reduced cortical thickness of the entorhinal cortex, fusiform gyrus, and isthmus of the cingulate gyrus, and precuneus only compared to controls. Neuropsychological measures also differed between groups, with aMCI participants having decreased performance on memory and verbal fluency compared to non-amnestic MCI and controls. Coutinho et al. (2015) also reported differences on FDG-PET and CSF A β 42 measures between aMCI and non-amnestic MCI. As expected, an AD-like profile was seen in aMCI, and both MCI subtypes had hypometabolism in the precuneus, however non-amnestic MCI was associated with higher A β 42, suggesting less amyloid deposition in the brain, and more prefrontal hypometabolism.

1.3.6 Summary of Biomarkers

The current research supports the idea that aMCI is the period before a diagnosis of AD dementia can be made (Albert et al., 2011). Collective biomarker evidence from CSF, neuroimaging and neuropsychology all point to a similar underlying pathological process, which results in slow, progressive changes over time (Jack et al, 2013; Albert et al., 2011). Biomarker abnormalities in aMCI are not atypical to the same extent as seen in AD, but rather exists somewhere in the middle between healthy aging and AD pathophysiology (Jack et al., 2013). The current standards for diagnosis of either aMCI or AD rely largely on clinical presentation (Albert et al., 2011). However, a wealth of literature supports the ability of biomarkers to distinguish between groups of healthy adults, aMCI and AD, and can aid in ruling out of other potential underlying causes for cognitive decline (e.g., other dementias, vascular pathologies, depression) (Albert et al., 2011). However, each biomarker on their own may be associated with other aetiologies, such as hippocampal atrophy in frontotemporal lobar degeneration (Jack et al., 2002), and increased CSF tau with stroke (Rosén et al., 2013). One study attempted to characterize CSF A β 42, t-tau and p-tau levels within a large cohort of people with various forms of dementia (Schoonenboom et al., 2012). While they were able to classify 92% of AD participants, the AD-like CSF profile was also seen in several patients with Lewy Body Dementia, corticobasal degeneration, frontotemporal lobar degeneration, and vascular dementia. This may be a result of misdiagnosis or mixed dementias, but nonetheless highlights the fact that a multifaceted diagnosis is currently the most reliable (Schoonenboom et al., 2012; Albert et al., 2011; McKhann et al., 2011). Thus, more longitudinal studies including large pools of participants, with multimodal biomarker exploration will help in discerning biomarker abnormality with disease state in AD.

The investigation for novel biomarkers is ongoing. For example, BACE1 (cleaving enzyme involved in the production of A β), toxic A β oligomers, and even blood biomarkers (e.g., plasma A β 42) may help reliably identify stages of AD (Rosén et al., 2013). In terms of neuroimaging, new techniques allowing for in vivo tau imaging, functional connectivity, as well as functional MRI tasks are also being developed and tested to identify early disease states (Albert et al., 2011). However, the reliability and validity of these measures remains unclear.

1.3.7 The Stroop Task and AD and aMCI

1.3.5.1 Behavioral Measures of the Stroop Task in AD and aMCI

The Stroop task has been utilized to evaluate inhibitory control in patients diagnosed with AD and aMCI. Several studies have reported that people diagnosed with AD show increased reaction times and error rates compared to healthy adults (Bondi et al., 2002; Duong, Whitehead, Hanratty, & Chertkow, 2006; Fisher, Freed, & Corkin, 1990; Koss, Ober, Delis, & Frieland, 1984; Spieler et al., 1996). Deficits in the Stroop task have also been discovered in the earlier disease stage, aMCI (Kramer, et al., 2006; Sung, Kim, Jeong, & Kang, 2012). However, several studies have failed to find differences in Stroop performance between patients with AD and healthy controls (Coubard, et al., 2011; Stawarczy, Grandjean, Salmon, & Collette, 2012). An exaggerated decreased speed of processing beyond normal age-related changes and degraded color-vision have been offered as potential mitigating factors in AD as opposed to a true break down of inhibitory control as measured by the Stroop task (Ben-David, Tewari, Shakuf, & Van Lieshout, 2014). Therefore, it remains unclear as to whether the

pathology underlying AD alters patients' ability to inhibit the automatic reading response required by the Stroop task. One factor to consider that may contribute to the lack of consistency in findings pertains to methodology. The variability in presentation style of the Stroop task, for instance computerized (Balota, et al., 2010), physical cards (Sarazin, et al., 2007), verbal and button-response, may contribute to variability in results across studies and make a definitive characterization of inhibitory control changes in AD difficult.

1.3.7.1 Neural Correlates of the Stroop Task in AD and aMCI

The neural underpinnings of the Stroop task have also been investigated in AD and aMCI, yielding conflicting results. One fMRI study including participants with aMCI reported no underlying activation differences in a direct comparison to healthy controls during the Stroop task (Puente, Faraco, Terry, Brown, & Miller, 2014), while others report evidence of compensation in aMCI; an increase in activity to compensate for detrimental disease-related changes to sustain cognitive functioning (Li, Zheng, Wang, Gui, & Li, 2009; Rosano, et al., 2005). Many studies suggest that AD is accompanied by a decrease in frontal lobe activity during the Stroop task (Li, Zheng, Wang, Gui, & Li, 2009; Yun, et al., 2011). This falls in line with studies that suggest frontal lobe impairment earlier on in the disease process (Braak & Braak, 1991; van der Flier, et al., 2002). However, this contrasts with another study that found no difference in activations between patients with AD and controls (Bowes, Stroman, & Garcia, 2012). Another possibility is that additional brain areas may become engaged, as another study employing a memory task discovered (Grady, et al., 2003). Accordingly, changes associated with the development of AD may not be captured by all methods, for example

ROI versus whole-brain contrasts. Considering the overlap between the neural correlates of the Stroop task with AD pathology it is not surprising to see a decrease in activity in patients diagnosed with AD in the Stroop task. However, the relatively small number of studies, especially including patients with aMCI, highlights the need for further investigation.

1.4 Functional Magnetic Resonance Imaging

1.4.1 The Magnetic Resonance Imaging (MRI) Signal

Magnetic resonance imaging (MRI) and functional MRI (fMRI) are widely used imaging tools that can investigate both brain structure and inferred neural function, including in aging and AD research. MRI allows for reliable discrimination of tissue types, including fat, water, muscle and soft tissue, in the absence of radiation and contrast materials such as positron emissions tomography or computerized tomography, making it a popular choice for both scientific and health-related studies.

The MRI signal is derived from the nuclei of hydrogen atoms found in tissues containing water and lipids (see Stroman, 2011 for a thorough review). Hydrogen atoms have a single proton, are positively charged, and are always spinning, which gives rise to a net magnetic moment. While hydrogen atoms spin at the same frequency, they are randomly oriented until placed in a strong magnetic field, such as an MRI machine, which causes the nuclei to align with the field (B_0) and results in a net magnetization proportional to strength of the field (i.e., the stronger the magnetic field the greater the net magnetization (Plein, Greenwood, & Ridgeway, 2010; Stroman, 2011). The net

magnetization of the hydrogen atoms within a volume of space is the available signal that can be measured and detected by the MRI system and has two components: parallel or antiparallel to the magnetic field (Stroman, 2011). Alignment of the nuclei to the magnetic field is a precession around B_0 with an angular frequency (Larmor frequency). The radio frequency (RF) pulse is a second magnetic field (B_1) applied to B_0 to disrupt the equilibrium, causing the nuclei to tip over and align in phase. This process results in a decrease in the longitudinal magnetization and establishes a new transverse magnetization plane. Removal of the RF pulse results in the loss of transverse magnetization (T_2) while the system seeks to return to equilibrium, restoration of magnetization along the longitudinal plane (T_1). A receiver coil measures the signal created by this process. Additionally, magnetic field gradients are applied to the uniform field B_0 that cause the strength of the field to vary linearly in space, which provides spatial information of the body part being imaged. As mentioned earlier the signal begins to decay as the system returns to equilibrium, so spin and gradient echos aid in refocusing the signal to allow for adequate sampling of the data.

Different tissue types contain different longitudinal relaxation times with white matter being the shortest, followed by gray matter, and cerebral spinal fluid (CSF) having the longest. Altering the repetition time (TR) (the time between consecutive RF pulses to the same slice) and the echo time (TE) (the time between excitation via the RF pulse and data collection/reception of the echo signal) dictates if the measured signal depends more on T_1 or T_2 relaxation times. Discrete data measurements are collected across k-space that are transformed using the inverse Fourier transform (Stroman, 2011). A matrix of numbers is created that code for various intensity levels that form pixels corresponding to

a spatial location and ultimately a gray scale image is formed. A shorter TR and TE means less time for nuclei to return to equilibrium resulting in T1 signal being measured. Conversely, a longer TR and TE allows for the signal from the decay of the transverse magnetization to be measured (T2). T2* also describes the decay of transverse magnetization (T2) but is additionally sensitive to inhomogeneities in the field from different tissues and fluids (e.g., blood, air, adipose, bone). Since T2* weighted sequences are sensitive to blood flow and oxygenation, they are at the center of fMRI using the blood oxygenation level dependent (BOLD) contrast technique.

1.4.2 Functional Magnetic Resonance Imaging (fMRI) and the BOLD Contrast

Brain function can be inferred using fMRI and the BOLD contrast. During an experiment a participant performs a task while the area of interest is imaged, for example the brain or spinal cord. Comparing signal changes over time in a three-dimensional space allows for interpretations to be made regarding task related activations or deactivations.

Neuronal signaling through excitatory or inhibitory presynaptic inputs causes the release of neurotransmitters and propagation of action potentials that ultimately effects the diffusion of blood flow to local tissues to meet cellular energy demands. The BOLD contrast is based on the different properties of hemoglobin and their effect on the magnetic field. Delivery of oxygen to tissues is through hemoglobin molecules traveling through blood. Hemoglobin contains four subunits with iron atoms that bind and unbind oxygen. Oxyhemoglobin is diamagnetic, containing no unpaired electrons, and thus does little to distort the magnetic field. Deoxyhemoglobin is formed when oxygen is released, resulting in four unpaired electrons and is paramagnetic. Therefore, the presence of

deoxyhemoglobin in capillaries and veins distorts the magnetic field. Performing a task increases the metabolic demand, oxygen consumption, of active neurons. The release of neurotransmitters results in an increase in blood flow to supply local brain areas with oxygen that surpasses the needs of the tissue leading to an increase in the ratio of oxy/deoxy-hemoglobin (Amaro & Barker, 2006; Matthews & Jezzard, 2004). The increased oxyhemoglobin results in a longer $T2^*$ and higher signal detected compared to baseline. The hemodynamic response function (HRF) models the temporal component regarding changes in blood flow and oxygen consumption that occur with the presentation of a brief stimulus during a task (Stroman, 2011). An initial dip is often seen as a result of an increase in deoxyhemoglobin as oxygen is released from the blood causing disruptions to the magnetic field and thus a decrease in $T2^*$ and faster signal decay. There is a brief lag between the rise in oxyhemoglobin prompted by increased neuronal activity and MR signal change, which peaks approximately 4-6 seconds after stimulus presentation and returns to baseline after 20 seconds (Stroman, 2011).

1.4.3 fMRI Study Design

Experiments need to be designed to adapt to the conditions and limitations of the MRI system while answering the desired hypotheses. Common fMRI experimental designs can be task based and include block, event and rapid-event related or resting-state (Stroman, 2011). Task based fMRI designs aim to localize active brain areas by detecting true signal while balancing noise. In block designs stimuli are presented for longer periods of time, typically 10 – 30 seconds followed by periods of the absence of stimuli. Event-related designs involve the serial presentation of several stimuli for shorter periods of time sandwiched by an inter-stimulus interval (ISI). In a similar vein, rapid event-

related designs are like event-related, however the ISIs are shorter in length and can be a constant duration or randomly jittered (varied durations). Another popular method of fMRI design is the absence of a task. A participant is scanned while at rest, which allows for examination of how different brain areas are functionally connected through measuring correlations between spatially separate brain regions.

Each experimental design has pros and cons including statistical power, participant anticipation and engagement, length of experiment, and HRF modelling. In addition, fMRI experiments are always a balance between spatial and temporal resolution and signal to noise. Spatial and temporal resolution trade-offs include time constraints, for example the longer you can take to acquire more data points the more detailed the image, but there could be increased susceptibility to motion artifacts and physical limitations of the participant to remain still. Signal-to-noise ratio refers to the balance between MR signal and background noise (e.g., physiological motion, electrical noise generated from the MRI system) and is dependent on the number of data points being sampled (more data means more noise but also larger voxel volume means more signal) and the time to acquire the image (Stroman, 2011).

1.5 Thesis Objectives and Hypotheses

The goal of this research is to gain a more comprehensive understanding of the changes in neural correlates of inhibitory control across healthy aging, as well as in AD and aMCI, using the Stroop task, which is a well characterized tool to measure inhibitory control (Stroop, 1935). The Stroop task has been extensively studied in healthy populations as well as various patient groups, hence there is continual interest to better understand the neural correlates underlying performance of the task. Given that

instructions for the color-word Stroop task are both easy to administer and are generally well understood by participants, including patient groups experiencing cognitive decline, it makes the Stroop task a useful means to investigate inhibitory control.

I set out to do this by using fMRI to investigate healthy aging in young, middle-aged and older adults as well as patients diagnosed with AD and aMCI. Given the challenges associated with the healthy aging brain and AD, I chose to examine independently defined regions of interest identified across studies of the Stroop task. The same regions of interest were analyzed to maintain consistency across the studies outlined in this thesis.

Chapter 2 investigated a computerized trial-by-trial version of the Stroop task adapted for fMRI in healthy young, middle-aged, and older adults to help establish brain changes associated with healthy aging in an inhibitory control task. Based on previous literature we expected to see evidence of compensation (Reuter-Lorenz & Cappell, 2008; Cabeza, Anderson, Locantore, & McIntosh, 2002), increased brain activity, in the middle-aged or older adults compared to young adults, especially in frontal brain areas.

Chapter 3 examined differences in neural correlates using the same fMRI Stroop paradigm in patients diagnosed with AD and aMCI compared to age-matched healthy controls. Considering previous studies of the Stroop and AD, we expected that patients with AD would show reduced activity compared to age-matched controls along with reduced behavioral performance on the Stroop task. Previous experiments in patients with aMCI are relatively sparse, however we predicted that patients with aMCI may have increased activation of some brain areas indicative of compensation with the maintenance of behavioral performance at a similar level to age-matched controls.

To extend this research further, in chapter 4 we utilized the same brain regions of interest identified in chapters 2 and 3 found to underly the Stroop task to investigate the strength of functional connectivity within these areas, also known hubs of the salience network (Seeley, et al., 2007), and their relation (anticorrelation) to the default mode network (Fox & Greicius, 2010) in AD, aMCI and age-matched controls (Dosenbach, et al., 2006; Menon & Uddin, 2011). Previous works have identified a temporary increase in functional network activity in patients early on in aMCI or AD (Brier, et al., 2012), although not unanimously, so accordingly we hypothesized that the group of aMCI participants may show increased functional connectivity with a decrease in patients with AD that are in a more advanced stage of the disease.

Overall, this work attempts to fill key gaps in the literature related to when initial changes inhibitory control as measured by the Stroop task may begin during healthy aging and when abnormal changes in inhibitory control can be detected in AD, and the prodromal condition aMCI and what brain areas may be involved in driving these behavioral changes.

Chapter 2

Inhibitory Control Across Healthy Aging Using the Stroop Task: An fMRI Study

2.1 Abstract

This study implemented a version of the Stroop task adapted for fMRI to investigate age effects in key brain areas across young, middle-age and older adults. The goal was to test whether inhibitory control, as measured by the Stroop task, changed as a function of healthy aging. Our study included three adult age groups: Young (N = 15, mean age 23.5, range 21-26 years), middle-aged (N = 31, mean age 54.5, range 43-64 years) and older adults (N = 28, mean age = 75.0, range 66-89 years). ROIs were selected independently using a meta-analytic search for the term “Stroop” in NeuroSynth. Brain activity in 11 ROIs was compared across the three different age groups during peak response to incongruent (interference condition, color word and ink color don’t match), congruent (facilitation condition, ink color and color word match) and neutral (non-color word) conditions. In terms of behavioral results from the Stroop task, all participants performed similarly regardless of age group. The older adults were slower overall in all conditions, but reaction times for incongruent (the interference condition) were not disproportionately longer compared to neutral trials, indicating no significant changes in inhibitory control with age. As for peak neural response during the Stroop task, older adults over-recruited the bilateral ACC, dACC, left DLPFC, bilateral parietal, and left IPS during neutral trials versus young adults, while the middle-aged adults only showed

over-recruitment in the right dACC during neutral and congruent trials and left parietal ROI during neutral trials compared to younger adults. The incongruent condition did not elicit a difference in response across any of the ROIs between the age groups. Within-group analysis showed that older adults produced a similar neural response to both incongruent and neutral conditions, while both the middle-aged and younger adults had greater responses to the incongruent versus neutral condition. Correlations between the magnitude of BOLD response, age, and behavioral measures revealed evidence for both the compensation and dedifferentiation hypothesis of aging. Taken together, the behavioral results support the idea that inhibitory control as measured by the Stroop task may not change with increasing age but reflects generalized slowing. The middle-age group showed some evidence of early brain changes in the right dACC and left parietal area but did not show the same dedifferentiation between conditions or correlations with behavior as the older adult group. This suggests that despite similar behavioral performance across the three age groups, neural changes associated with inhibitory control may begin to occur in middle-age and become more discernable after age 65.

2.2 Introduction

Healthy aging is a heterogeneous process that encompasses normal, widespread cognitive changes (Harada, Natelson, & Triebel, 2013; Buckner, 2004). Beginning in young adulthood some aspects of cognition begin to diverge from optimal performance, including attention and inhibitory control (Spieler, Balota & Faust, 1996; Madden, 2007). Considering the importance of inhibition in attentional processes and everyday life, it is important to understand how these cognitive mechanisms change as a function of age. It

is posited that declines in inhibitory control and working memory may contribute significantly to age-related cognitive decline (Borella, Carretti & De Beni, 2008, 2017). More specifically, reduced inhibitory control with increasing age may affect working memory capacity by decreasing the ability to filter out irrelevant details, making it increasingly difficult to focus on pertinent information necessary to successfully perform a task (Persad, Abeles, Zacks, & Denburg, 2002; Borella, Carretti, & De Beni, 2008).

A common task used to probe inhibitory control is the Stroop task (Stroop, 1935). Increased inhibition is required to suppress the automatic reading response in order to identify the ink color of a given stimulus (Stroop, 1935, MacLeod, 1991; MacLeod & Macdonald, 2000). There is greater attentional demand required to suppress the color word when the ink color and the word are incongruent, meaning they do not match, compared to neutral stimuli, such as non-color words, symbols or letters (MacLeod & Macdonald, 2000). Thus, the Stroop effect (average incongruent minus average neutral reaction times [RT]) reflects the increased inhibitory control required for the incongruent versus neutral condition (Stroop, 1935). Conversely, when the color word and ink color match, reaction times are typically faster, a process which is referred to as facilitation (MacLeod & MacDonald, 2000). It has been reported that compared to younger adults, older adults aged 60 + tend to have longer reaction times and produce more errors, especially for incongruent stimuli (e.g., the word blue written in yellow) (Stroop, 1935, MacLeod, 1991; Spieler, Balota & Faust, 1996). Early Stroop studies across the human lifespan put forward the idea that interference is greatest in younger school-aged children, coinciding with the development of the ability to read, and again with advancing age (see MacLeod, 1991 for a review). However, an increased Stroop effect in older adults is not

universally reported, and some studies have failed to find a difference in behavioral performance during the Stroop task across age groups (Langenecker, Nielson & Rao, 2004; Milham, Erickson, Banich & Kramer, 2002). A meta-analysis of behavioral Stroop measures in healthy adults concluded that interference arising from the Stroop task is not disproportionately larger in older adults (60 + years old) compared to young adults (30 + years old), and longer reaction times reported in older adults can be attributed to generalized slowing as opposed to a breakdown in inhibitory control (Verhaeghen & De Meerssement, 1998). Langenecker, Nielson & Rao (2004) implemented a calculation for interference that controls for generalized slowing by dividing the Stroop effect by the average neutral reaction time and found that both Stroop effect and percentage of interference was not significantly different between older and young adults, however older adults tended towards producing greater interference effects. In line with these results, Zysset, Schroeter, Neumann, & von Cramon (2007) reported that older adults were slower on neutral and incongruent conditions compared to younger adults, but there were no disproportionate age effects found between the two conditions, supporting the idea of generalized slowing as opposed to a breakdown of inhibitory control.

The neural network that functionally supports performance on the Stroop task has been well studied in the literature. The frontoparietal network has been implicated as playing a key role in performance of the Stroop task (Parris, et al., 2019; Nee, Wager, & Jonides, 2007; van Veen & Carter, 2005). A meta-analysis of Stroop fMRI studies carried out by Nee, Wager & Jonides (2007) observed consistent involvement of the left dorsolateral prefrontal cortex (DLPFC), left insula, medial frontal cortex including the anterior cingulate cortex (ACC), left posterior parietal cortex (PPC), and smaller peaks in

both the right DLPFC and PPC as well as the thalamus. Brain areas consistently reported from other fMRI studies relating to differences in incongruent versus control conditions include the ACC (Bush G. , et al., 1998; Carter, et al., 2000; Peterson, et al., 2002; van Veen & Carter, 2005; Huang, Su, & Ma, 2020), DLPFC (Huang, Su, & Ma, 2020; Parris, et al., 2019), inferior frontal gyrus (IFG) (Bush et al., 1998; Peterson et al., 2002; van Veen & Carter, 2005; Huang, Su, & Ma, 2020), superior parietal lobule , including the supramarginal gyrus (SG) (Bush et al., 1998), precentral gyrus (PG) (van Veen & Carter, 2005), visual association area (VAA) (Peterson et al., 2002), insula (Huang, Su, & Ma, 2020), and the frontal polar cortex (FPC) (Bench et al., 1993; Carter et al., 1995). There is also evidence in the literature that the Stroop task is often left lateralized, which may reflect the verbal nature of the task as well as interference effects arising from increased difficulty during the incongruent condition (MacLeod, 1991; Nee, Wager, & Jonides, 2007).

There have been several functional magnetic resonance imaging (fMRI) studies examining the neural correlates of healthy aging and the Stroop task. It has been suggested that older adults may recruit additional brain areas or show increased activity within similar areas activated in younger adults in order to compensate for normal age-related cognitive declines and support behavioral performance during various cognitive tasks (Reuter-Lorenz & Cappell, 2008; Nielson, Langenecker, & Garavan, 2002; Cabeza, Anderson, Locantore, & McIntosh, 2002; Cabeza, 2002). For instance, some studies have shown that older adults have increased activity in several frontal regions, including the left inferior frontal gyrus (IFG) compared to younger adults in the incongruent versus neutral condition (Langenecker, Nielson & Rao, 2004). In accordance with these

findings, another study found increased activity in the bilateral IFG, anterior inferior prefrontal cortex, as well as anterior portions of the temporal cortex, including the inferior temporal gyrus, middle temporal gyrus and superior temporal gyrus in older adults faced with incongruent stimuli (Milham, et al., 2002). In the same study, young adults overall showed increased activity during incongruent trials in areas including the DLPFC, ACC, left middle frontal gyrus, and superior parietal lobe compared to older adults. Furthermore, older adults had increased activity in the ACC to both incongruent and congruent stimuli compared to neutral trials, whereas young adults only showed increased ACC activity when faced with incongruent trials. This suggests that with increasing age the ACC may become dysfunctional when faced with competing information, such as two-color dimensions (incongruent or congruent) (Milham, et al., 2002). Age-related increases relating to Stroop interference have been found in the IPS, presupplementary motor area (superior frontal gyrus), and the left inferior frontal junction (IFJ) (Zysset, Schroeter, Neumann, & von Cramon, 2007). An alternative explanation for over-recruitment or recruitment of additional brain areas in older adults suggests that there is an increase in neural noise and loss of specificity with age (Li, Lindenberger, & Sikström, 2001). In other words, some areas may become active that wouldn't normally during a particular cognitive task and may not offer any performance benefit (Li, Lindenberger, & Sikström, 2001; Mathis, Schunck, Erb, Namer, & Luthringer, 2009).

Understanding the trajectory of inhibitory control as measured by the Stroop task across healthy aging remains unclear. fMRI studies of the Stroop task with the inclusion of middle-aged adults remains sparse (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). However, one study including young (range: 22-30 years), middle-age (range: 46-

55 years) and older (range: 60-68 years) adults found that similarly to previous studies, older adults demonstrated an over-recruitment of bilateral DLPFC, bilateral ventrolateral prefrontal cortex and bilateral parietal lobes during the incongruent and congruent condition compared to young adults, while the middle-aged adults showed only left ventrolateral prefrontal cortex and left parietal cortex over-recruitment compared to young adults during the incongruent condition (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). Furthermore, the middle-aged adults, like the older adults, were slower and less accurate in comparison to their younger counterparts, leading the authors to suggest that early changes to inhibitory control may begin around age 50. Another study including middle-aged adults by Zysset, Schroeter, Neumann, & von Cramon (2007) compared young (range: 22-36 years) and middle-aged (range: 45-75 years) adults using a button response version of the Stroop task adapted for fMRI. The contrast between incongruent versus neutral trials using a whole brain approach revealed a greater BOLD signal in middle-aged adults was most pronounced in the left inferior frontal junction (IFJ), bilateral IPS and presupplementary motor area (superior frontal gyrus) compared to younger adults. Additional areas showing similar increases in middle-aged adults included the insula, precuneus, fusiform gyrus, caudate nucleus, putamen and the cerebellum. In contrast to Mathis, Schunck, Erb, Namer & Luthringer (2009), no behavioral differences were identified in the middle-aged group aside from general slowing for all condition types.

Variability in how the Stroop effect is defined, for example incongruent versus neutral or incongruent versus congruent conditions, as well as experimental design (block versus event-related) and relative task difficulty may contribute to differences and

inconsistencies across studies of healthy aging and the Stroop task (Langenecker, Nielson, & Rao, 2004). In addition, whole brain contrasts between younger and older adults can pose issues relating to normal age-related neural changes, including gray matter atrophy, expansion of the ventricles and sulci, as well as neurovascular changes (Samanez-Larkin & D'Esposito, 2008; Milham, et al., 2002). An alternative technique to control for age-related brain changes is by conducting a region of interest (ROI) analysis. This restricts analysis to specific voxels thereby reducing issues relating to multiple comparisons as well as decreasing the impact of age-related brain morphometry changes by allowing for specific placement, for example ensuring selected areas aren't within the ventricles. The development of large-scale databases such as NeuroSynth allow for meta-analyses of brain imaging and cognitive studies and selection of independent ROIs from a plethora of studies (Yarkoni, Poldrak, Nichols, Van Essen, & Wager, 2011). The benefit of ROI selection using a meta-analytic approach from an external source is that regions are defined independently from the data being analyzed. In addition, greater confidence can be attained that selected ROIs are involved in a specific task or desired search term as well as control for areas that are often activated during a variety of cognitive tasks (Yarkoni, Poldrak, Nichols, Van Essen, & Wager, 2011).

The aim of the present study was to investigate inhibitory control using the Stroop task across healthy aging. We examined behavioral measures, reaction times, accuracy and interference, as well as independently defined ROIs relating to the Stroop task across healthy young, middle-aged and older adults using an fMRI version of the Stroop task. Given the evidence of age-related changes in the DLPFC and parietal cortex from the literature, we predicted that those ROIs may show alterations in older compared to

younger adults. More specifically, we expected an over-recruitment in ROIs from the frontoparietal network in older adults. Inclusion of a wide age range, especially middle-aged adults (40 – 65 years), who are an underrepresented group in the literature, will help to resolve the inconsistencies in the field pertaining to aging effects and the Stroop task (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). This will give further insight into when subtle age effects in inhibitory control may begin across the human lifespan. Implications can be furthered to potentially identifying when early changes to inhibitory control emerge in diseases such as mild cognitive impairment and Alzheimer's disease.

2.3 Methods

2.3.1 Participants

This study was approved by the research ethics boards of Queen's University and Hotel Dieu Hospital (#6004781). Healthy community dwelling adults from the greater Kingston area aged 20 and over were recruited to participate in this study. All participants were given a letter of information detailing the study and provided written informed consent prior to commencing the study. A total of 84 healthy adults were recruited into one of three age groups: young (range 21-26 years), middle-aged (range 40-65 years) and older adults (range 66-89 years). Ten participants were subsequently excluded from the analyses (4 from the middle-aged group and 6 from the older adult group) due to issues with the verbal recording of their responses or excessive movement within the MRI (greater than 3.3 mm, see section on head movement analysis). The final number of participants included in the study consisted of 15 young adults (10 females, mean age

23.5 ± 1.6 years, age range 21 – 26 years), 31 middle-aged adults (19 females, mean age 54.5 ± 6.9 years, range 43 – 64 years), and 28 older adults (18 females, mean age 75.0 ± 7.2 years, age range 66 – 89 years) (Table 2.1). All participants had normal or corrected vision, normal color vision, and complied with MRI safety standards. Of the included participants, the average number of years of education were 17.3 years for the young adults, 16.7 years for the middle-age group, and 16.5 years for the older adults. There were no statistical differences between groups based upon years of education.

	Young Adults n = 15	Middle-aged Adults n = 31	Older Adults n = 28
Demographics			
Age (years)	23.5 (0.4)	54.5 (1.2)	75.0 (1.4)
Sex (F/M)	10/5	19/12	18/10
Years of Education	17.3 (0.2)	16.7 (0.7)	16.5 (0.8)
MoCA (/30)	29.1 (0.3)	28.4 (0.2)	29.9 (2.0)
MMSE (/30)	29.5 (0.2)	29.5 (0.2)	29.0 (0.2)
fMRI Stroop Performance			
Incongruent RT (ms)	684.9 (37.2)	753.8 (15.6)	849.8 (33.9)
Neutral RT (ms)	606.0 (31.0)	654.0 (13.5)	733.0 (30.2)
Congruent RT (ms)	560.0 (29.2)	619.7 (15.5)	675.1 (26.9)
Stroop Effect (ms)	78.9 (12.1)	99.8 (8.1)	117.0 (10.5)
Interference (%)	13.0 (2.0)	15.5 (1.3)	16.3 (1.6)
Incongruent Errors (%)	5.3 (1.8)	2.9 (0.5)	4.8 (1.0)
Neutral Errors (%)	1.7 (0.6)	1.6 (0.5)	1.5 (0.4)
Congruent Errors (%)	1.7 (0.5)	1.0 (0.3)	0.6 (0.2)

Table 2.1 Demographic and mean Stroop performance data. Standard error is listed in brackets.

2.3.2 Study Design

Participants attended one study session comprised of a neuropsychological test battery and MRI component. The MRI component included a structural scan as well as an event-related version of the Stroop task adapted for fMRI. The neuropsychological

testing lasted approximately 1.5 hours, as did the MRI component, for a total duration of 3 hours.

2.3.3 fMRI Stroop Task

A rapid event related version of the Stroop task (Tam, Luedke, Walsh, Fernandez-Ruiz, & Garcia, 2015) was generated using Optseq (<http://surfer.nmr.mgh.harvard.edu/optseq/>). The experiment was run on a PC computer (Intel Core i7 3770K 3.5 GHz) and projected (NEC LT265 DLP projector) onto a rear projection screen located at the bore of the magnet near the participant's head. A mirror fixed to the head coil allowed participants to view the stimulus display screen. Each run began with the instructions "say the color of the ink", followed by the trial-by-trial presentation of each condition (Figure 2.1). The conditions were congruent (i.e., the color word matched the color of ink it is written in), incongruent (i.e., the color word did not match the color of ink it is written in) or neutral words (i.e., common neutral words matched for letter frequency written in the four possible colors of ink: red, blue, yellow and green). Each word was randomly presented at the center of the screen on a black background for a duration of 1000 ms. Inter-stimulus intervals (ISI) (white crosses) were centered on the screen and randomly jittered in between each trial for a period ranging from 1000 - 19000 ms. Random presentation of the incongruent, congruent and neutral stimuli as well as ISI time was used in attempt to reduce reading strategies and to maintain task engagement. Participants were instructed to say the color of the ink that each word was written in, and verbal responses were recorded. There were 15 trials per condition in each run. All participants underwent a short training period to familiarize them with the task, as well as to ensure correct color discrimination.

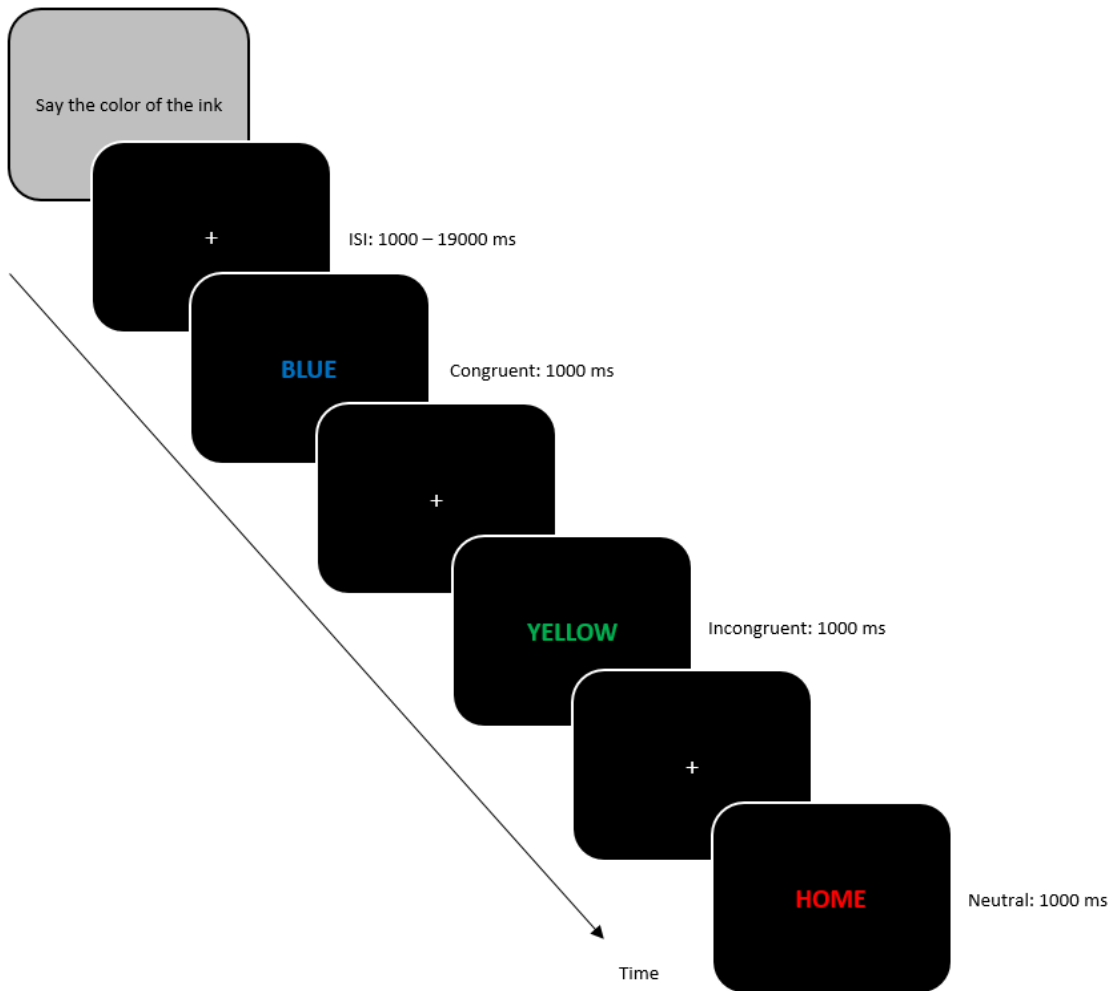


Figure 2.1. Example of the fMRI Stroop paradigm. The instructions were to say aloud the color of the ink for each condition: Congruent, neutral and incongruent. Each condition was presented individually at the center of the screen for a duration of 1000 ms, followed by randomly jittered interstimulus intervals (white crosses) that appeared for varying lengths of time ranging from 1000 – 19000 ms.

2.3.4 Neuropsychological Testing

A comprehensive neuropsychological test battery was carried out on all participants to ensure cognitive functioning was within the normal range. Tests of global cognition included the Montreal Cognitive Assessment (MoCA) (Nasreddine, et al.,

2005), Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), and the Mattis Dementia Rating Scale version 2 (Mattis, 1988). Verbal memory was assessed using the California Verbal Learning Test (CVLT) (Delis, Kramer, Kaplan, & Ober, 2000). The Wechsler Memory Scale (WMS) Third Edition was used to test working memory (forward/backward digit span and letter number sequencing) (Wechsler, 1997). Executive function was measured using the Trail Making Test (part B) (Reitan, 1958) and the Wisconsin Card Sorting Test (Heaton, Chelune, Talley, Kay, & Curtiss, 1993). Participants also completed a paper version of the Stroop task (Stroop, 1935). Color words were arranged in columns on a sheet of paper and instructions required participants to say aloud the color of ink (blue, red, tan and green) each word was written in. The score was the total number of correct responses out of a possible 112 in two minutes. As part of the inclusion criteria for study participation, scores on all cognitive tests had to fall within the normal range while accounting for age, sex, and years of education.

2.3.5 Behavioral Data from the fMRI Stroop Task

Verbal responses were recorded using an optical microphone (<http://www.magmedix.com>) during the Stroop fMRI task and analyzed outside of the MRI using Audacity (<http://audacity.sourceforge.net/>). Analysis of behavioral measures and correlations from the Stroop task was carried out using SPSS version 28.0 (SPSS, Chicago, IL, USA). All responses that were incorrect or inaudible were excluded from reaction time calculations. Reaction times for individual trials of the congruent, incongruent and neutral conditions were calculated by subtracting the time at which the participant made their response with the time of stimulus presentation and averaged

across conditions. The Stroop effect was calculated by subtracting the average incongruent reaction time from the average neutral reaction time for each group. Calculations were also computed for percentage of interference in order to control for generalized slowing $[(\text{average incongruent RT} - \text{average neutral RT})/\text{average neutral RT}]$ (Langenecker, Nielson, & Rao, 2004; Spieler, Balota, & Faust, 1996). The percentage of errors was calculated for each participant by dividing the number of errors for each condition by the total number of trials completed of each condition and averaged for each group. The behavioral data were assessed for normality and violations of parametric assumptions. The reaction time data failed to meet homogeneity of variance and were therefore log transformed. The error data showed a non-normal distribution and as a result underwent Aligned Rank Transformation (Wobbrock, Findlater, Gergle, & Higgins, 2011). Mixed ANOVAs were calculated on the transformed data in order to assess possible interactions with reaction times for condition type (incongruent, neutral, congruent) and each age group, as well as errors by condition type by age group. Group differences in Stroop effect (ms), interference (%) were analyzed using one-way ANOVAs with Tukey post-hoc test. We also interrogated sex differences in both behavioral measures and the magnitude of BOLD responses across all three conditions using Quade's non-parametric ANCOVA while controlling for age.

2.3.6 MRI Protocol

Each participant was scanned in the MRI Imaging Centre at Queen's University using the Siemens 3T Magnetom Trio system (Siemens Medical Systems, Erlangen, Germany) with a 12-channel head coil. The MRI protocol included a T1-weighted, 3D magnetization-prepared rapid gradient echo (MPRAGE) anatomical scan (single shot,

ascending sequence in the sagittal plane with 176 slices, TR = 1760 ms, TE = 2.2 ms, flip angle = 9° , FoV = 256 mm, 1 mm isotropic voxels, spanning the entire brain). There were eight functional runs of the Stroop task each taking 4 minutes and 12 seconds that acquired 125 functional images using T2*-weighted gradient echo-planar imaging parallel to the anterior commissure and posterior commissure (AC-PC) line (32 slices in the axial plane, TR = 2000 ms, TE = 30 ms, flip angle = 78° , FoV = 211 mm, 3.3 mm isotropic voxels).

2.3.7 fMRI Head Movement Analysis

Preprocessing of functional data in BrainVoyager QX (Brain Innovation, Maastricht, the Netherlands) allowed for head motion estimates to be evaluated in each participant for each functional run, excluding runs in which movement was greater than 3.3 mm. Strict motion cut-offs were used because our task involved verbal responses, and thus movement equal to or greater than 3.3 mm could have spilled over into neighboring voxels creating false activations. There were six motion estimated parameters that included both translation (in mm) in the X, Y and Z plane, as well as rotation (in degrees) in three dimensions: pitch, yaw, and roll. An overall average motion estimate was computed for the young, middle-aged and older adult groups across all viable runs in order to see if movement changed as a factor of age. Of all functional runs within each group, 1.7% of runs were excluded due to excessive movement in the young group, 0.8% in the middle-age group, and 4.0% in the older adult group. There were no significant differences between the groups on average head motion across all runs in all six motion estimate parameters (translation in X, Y and Z direction = dx, dy, dz, and rotation around X, Y and Z axis = rx, ry, rz).

2.3.8 fMRI Data Analysis

fMRI data from the Stroop task were analyzed using BrainVoyager QX (Brain Innovation, Maastricht, the Netherlands). The first two scans of each functional run were excluded for the MRI to reach steady state. Each functional run was preprocessed including slice scan time correction, head motion detection and correction and removal of linear and non-linear trends. The functional runs were then co-registered to anatomical scans and then transformed into Talairach space (Talairach & Tournoux, 1988). Spatial smoothing was applied with a full width at half maximum Gaussian kernel of 8 mm.

Individual time course protocols were created for each run for each participant, which consisted of only correct responses to incongruent, neutral and congruent trials across a run. A deconvolution analysis, with an additional correction for serially autocorrelated observations, was used to temporally divide the hemodynamic response into 10 points, each representing 2 seconds of the BOLD time course. Regressors based on the three Stroop conditions (incongruent, neutral, congruent) were included, resulting in a single study general linear model (GLM) with a total of 30 regressors.

Using a random effects analysis and correcting for serial autocorrelations with percent transform time course normalization, separate multi-study multi-subject GLMs were created for each age group: Young, middle-aged, and older adults. Within each group GLM planned contrasts were completed to investigate predetermined ROIs from NeuroSynth (See section 2.3.9, Table 2.2) associated with the Stroop effect by contrasting incongruent > neutral, incongruent > congruent, and congruent > neutral conditions during the peak of the hemodynamic response function, which was approximately 6 seconds following stimulus onset. This allowed for interrogation of the

beta weight estimates of activity, the magnitude of the blood oxygenation-level dependent (BOLD) response, both during incongruent, congruent and neutral trials while performing the Stroop task.

2.3.9 Region of Interest Analysis

Coordinates for ROIs were defined using the NeuroSynth database (www.neurosynth.org) (Yarkoni, Poldrak, Nichols, Van Essen, & Wager, 2011). A meta-analysis using the search term ‘Stroop’ from the NeuroSynth database identified 225 studies. The resulting association map was corrected for multiple comparisons using a false discovery rate (FDR) of $p < .01$ and was thresholded at $Z > 5.1$ to generate the peak of each cluster, which resulted in the selection of 11 ROIs for subsequent analysis (Table 2.2). The ROIs included three distinct clusters in the anterior cingulate cortex (ACC) (left, right and dorsal), bilateral inferior frontal gyrus (IFG), bilateral parietal lobes, left dorsolateral prefrontal cortex (DLPFC), left visual cortex, insula, and left intraparietal sulcus (IPS). The coordinates were transformed from MNI to Talairach space using the Lancaster transform (*icbm2tal*) in GingerALE (version 3.0.2). A spherical ROI consisting of 257 voxels was drawn around the foci of each cluster (Figure 2.4).

Hemisphere	ROI	Talairach Coordinates (X, Y, Z)
Right	ACC	4, 16, 39
Left	ACC	-5, 27, 34
Right	dACC	14, 3, 42
Left	DLPFC	-40, 16, 30
Left	IFG	-39, 2, 32
Right	IFG	40, 9, 37
Left	Parietal	-31, -53, 33
Right	Parietal	30, -53, 29
Left	IPS	-22, -70, 36
Left	Insula	-33, 7, 18
Left	Visual	-22, -77, 5

Table 2.2. List of 11 ROIs from the Neurosynth meta-analysis using the search term “Stroop”. Talairach coordinates represent the peak of the activation. ACC = Anterior cingulate cortex, dACC = Dorsal anterior cingulate cortex, IFG = Inferior frontal gyrus, IPS = Intraparietal sulcus, DLPFC = Dorsolateral prefrontal cortex.

Next, a random effects general linear model was performed within each spherical ROI. BOLD time courses were extracted and averaged for all three conditions, incongruent, congruent and neutral. Between group analysis of the peak BOLD signal (6 second time-lag) was also conducted using a one-way ANOVA with Tukey post-hoc test. In addition, repeated measures ANOVAs were calculated within each age group to assess how the neural response for each condition (incongruent, congruent and neutral) may change depending on the age group. A Greenhouse-Geisser correction was applied to those ROIs in which the assumption of sphericity was violated.

2.3.10 Correlations Between fMRI, Behavior and Age

Pearson correlations were conducted in order to assess the relationship between age, inhibitory control as measured by the Stroop task and neural activity from the ROIs. Correlations were completed between age and magnitude of BOLD response in each ROI. Additionally, correlations were completed between the transformed behavioral measures from the Stroop task including incongruent, congruent and neutral reaction times, Stroop effect, percentage of interference and percentage of incongruent errors with the magnitude of BOLD response from each ROI during peak response from incongruent, congruent and neutral conditions with all groups combined. In order to account for multiple comparisons a Bonferroni correction was applied to the p-value resulting in a significance of $p < .006$ ($p = .05/8$ behavioral measures).

2.4 Results

2.4.1 Behavioral Results from the fMRI Stroop Task

For all participants, behavioral measures were calculated from the fMRI version of the Stroop task, including average reaction times (ms) and error rates (%) for each condition type, Stroop effect (ms) and interference (%). Figure 2.2 shows the cumulative frequencies for each behavioral measure and condition type in each age group.

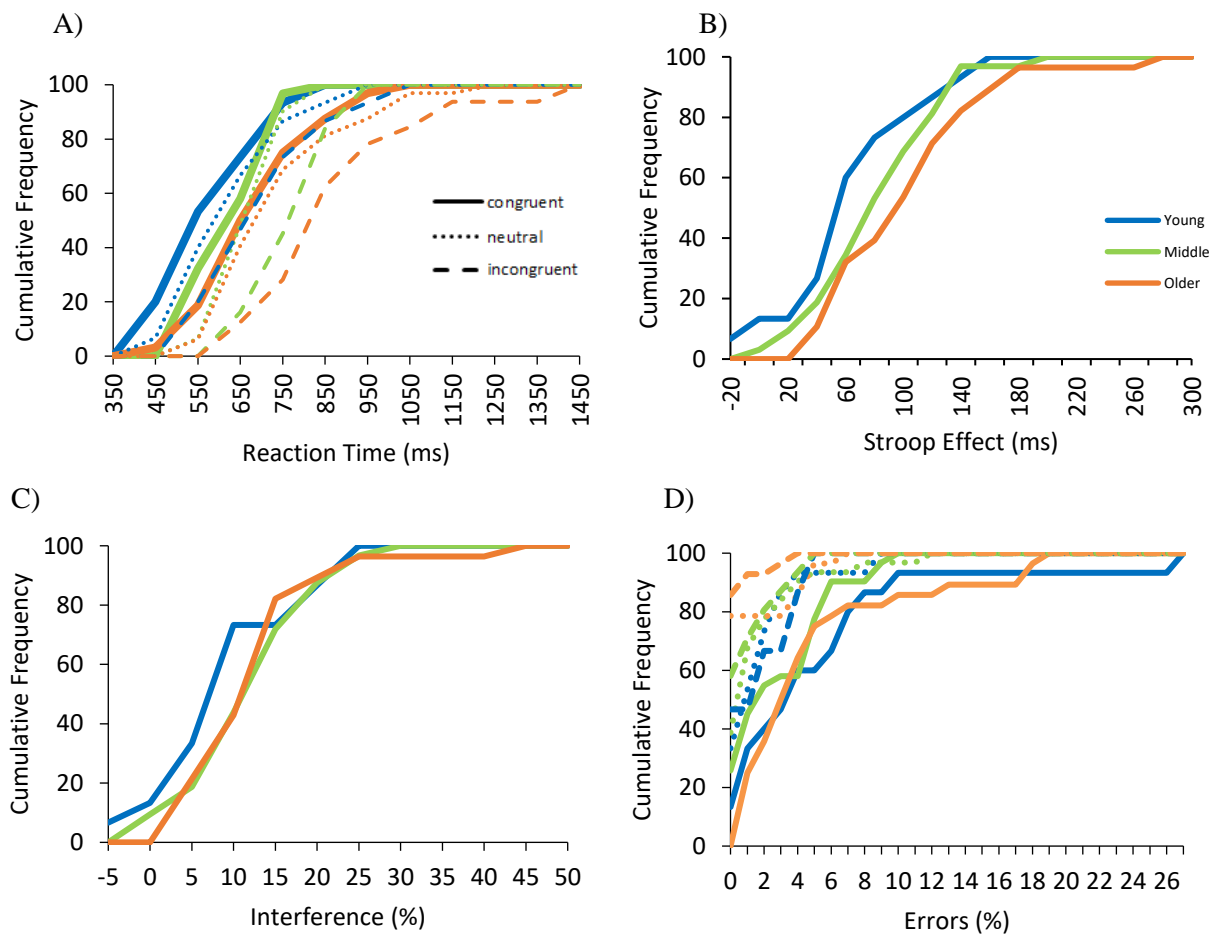


Figure 2.2. Cumulative frequency distributions for behavioral response data from the fMRI Stroop task. A) Reaction times (ms) for incongruent, congruent and neutral trials across each of the three age groups (blue = young, green = middle age, orange = older). B) Stroop effect (ms) (average incongruent reaction time – average neutral reaction time). C) Interference (%) (Stroop effect/neutral RT). D) Percentage of errors for incongruent, neutral and congruent conditions for each age group.

We set out to assess if there was an interaction between condition type (incongruent, neutral and congruent) and age group (young, middle-age and older) on reaction time (Figure 2.2 A). The dependent variable was reaction time, and the independent variables were condition (incongruent, congruent and neutral) as the within-subjects factor and age group (young, middle-aged and older) as the between-subjects factor. Since Levene's test for homogeneity of variance between groups was significant for incongruent $F(2, 71) = 3.13, p = .05$ and neutral reaction time $F(2, 71) = 4.08, p < .05$, the reaction time data was log transformed prior to performing the mixed ANOVA. There was a significant main effect of condition type on reaction time $F(1.66, 117.82) = 228.70, p < .001, partial \eta^2 = .76$, indicating that if we disregard age group there was a significant relationship between reaction times and condition of the stimulus, either incongruent, congruent or neutral. Pairwise comparisons revealed that reaction times for incongruent stimuli were significantly slower compared to both neutral $p < .001$ and congruent stimuli $p < .001$, and neutral stimuli were significantly slower compared to congruent stimuli $p < .001$. There was also a significant main effect of age group on reaction time, signifying that reaction times were different for young, middle-age and older adults $F(2, 71) = 7.01, p < .01, partial \eta^2 = .90$. Pairwise comparisons revealed that overall reaction times were significantly slower for older adults compared to young adults $p = .001$. We found no difference in reaction times between the older and middle-aged $p = .10$, or young and middle-age groups $p = .16$. The interaction between age group and condition type was not significant $F(3.32, 117.82) = .93, p > .05$. This suggests that reaction times for incongruent, neutral and congruent stimuli were not disproportionate across the different age groups, i.e., incongruent reaction times weren't

disproportionately longer than neutral reaction times for older adults versus young or middle-aged adults.

The percentage of errors for each condition was analyzed in a similar way (Figure 2.2 D). A Shapiro-Wilk test revealed that percentage of incongruent errors (young: $W(15) = .69$, $p < .001$, middle-age: $W(31) = .86$, $p < .01$, older: $W(28) = .76$, $p < .001$), congruent errors (young: $W(15) = .80$, $p < .01$, middle-age: $W(31) = .71$, $p < .001$, older: $W(28) = .59$, $p < .001$) and neutral errors (young: $W(15) = .70$, $p < .01$, middle-age: $W(31) = .65$, $p < .001$, older: $W(28) = .69$, $p < .001$) were significantly non-normal. In addition, Levene's test was significant for congruent errors $F(2,71) = 5.79$, $p < .01$. Due to violations in normality as well as the presence of outliers, the data was transformed using the Adjusted Rank Transform procedure followed by a mixed ANOVA and post-hoc pairwise comparisons using ART-C, resulting in a non-parametric analysis (Wobbrock, Findlater, Gergle, & Higgins, 2011; Elkin & Wobbrock, 2021). There was a significant main effect of condition type on error rates $F(2,71) = 33.27$, $p < .001$, *partial* $\eta^2 = .32$. Pairwise comparisons revealed that more incongruent errors were committed compared to both neutral $p < .001$ and congruent $p < .001$ conditions, irrespective of age group. No differences were found between percentage of neutral and congruent $p = .23$ errors committed. There was no significant main effect of age group, indicating that percentage of errors committed by young, middle-age and older adults were similar, $F(2,71) = 1.76$, $p > .05$, *partial* $\eta^2 = .05$. The interaction between percentage of errors in each condition and age group was not significant $F(3.69, 130.80) = 2.29$, $p > .05$. Like reaction times for each condition, this indicates that percentage of errors committed for different conditions did not vary disproportionately according to age group.

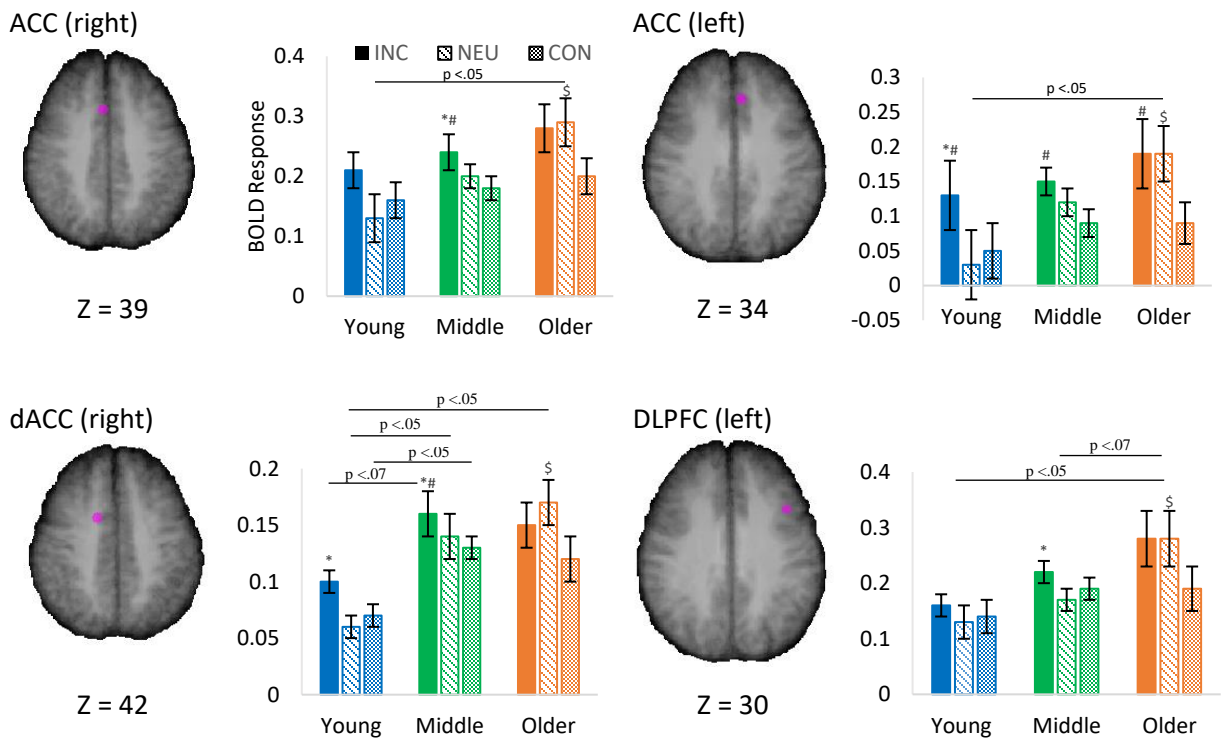
A one-way ANOVA was conducted to investigate differences in interference relating to the Stroop task and included the Stroop effect (average incongruent RT – average neutral RT) as well as percentage of interference [(average incongruent RT – average neutral RT)/average neutral RT] to control for generalized slowing across age groups (Figure 2.2 B, C). We found that differences between age groups for the Stroop effect was trending towards significance $F(2,71) = 2.94, p = .06$. A Tukey post-hoc test revealed a marginally larger Stroop effect in the older adult group (mean = 117.0 ms, SE = 10.5) compared to the young adult group (mean = 78.9 ms, SE = 12.1) $p = .049$. There was no significant difference in Stroop effect between the middle-age (mean = 99.8 ms, SE = 8.1) and older group ($p = .39$) or the middle-age and young group ($p = .38$). A one-way ANOVA for the percentage of interference yielded a non-significant result $F(2,71) = .92, p = .40$, indicating that percentage of interference was similar across young (mean = 13.0%, SE = 2.0), middle-age (mean = 15.5%, SE = 1.3) and older (mean = 16.3%, SE = 1.6) adults.

We investigated sex differences in both behavioral measures and magnitude of the BOLD response across all three conditions using Quade's non-parametric ANCOVA while controlling for age. Females tended to make more incongruent errors $F(1, 72) = 4.04, p < .05$ and congruent errors $F(1, 72) = 8.21, p < .01$. There were no significant differences in the magnitude of BOLD response in ROIs during the incongruent, congruent or neutral conditions.

2.4.2 ROI Imaging Results from the fMRI Stroop Task

2.4.2.1 Between Group ROI Results

A one-way ANOVA was conducted to compare the peak magnitude of BOLD response within each ROI identified using NeuroSynth (Table 2.2, Figure 2.3) for incongruent, congruent and neutral conditions across the three age groups. Repeated measures ANOVAs were calculated to assess how each ROI responded to different conditions of the Stroop task (incongruent, congruent and neutral) within each age group (Figure 2.3).



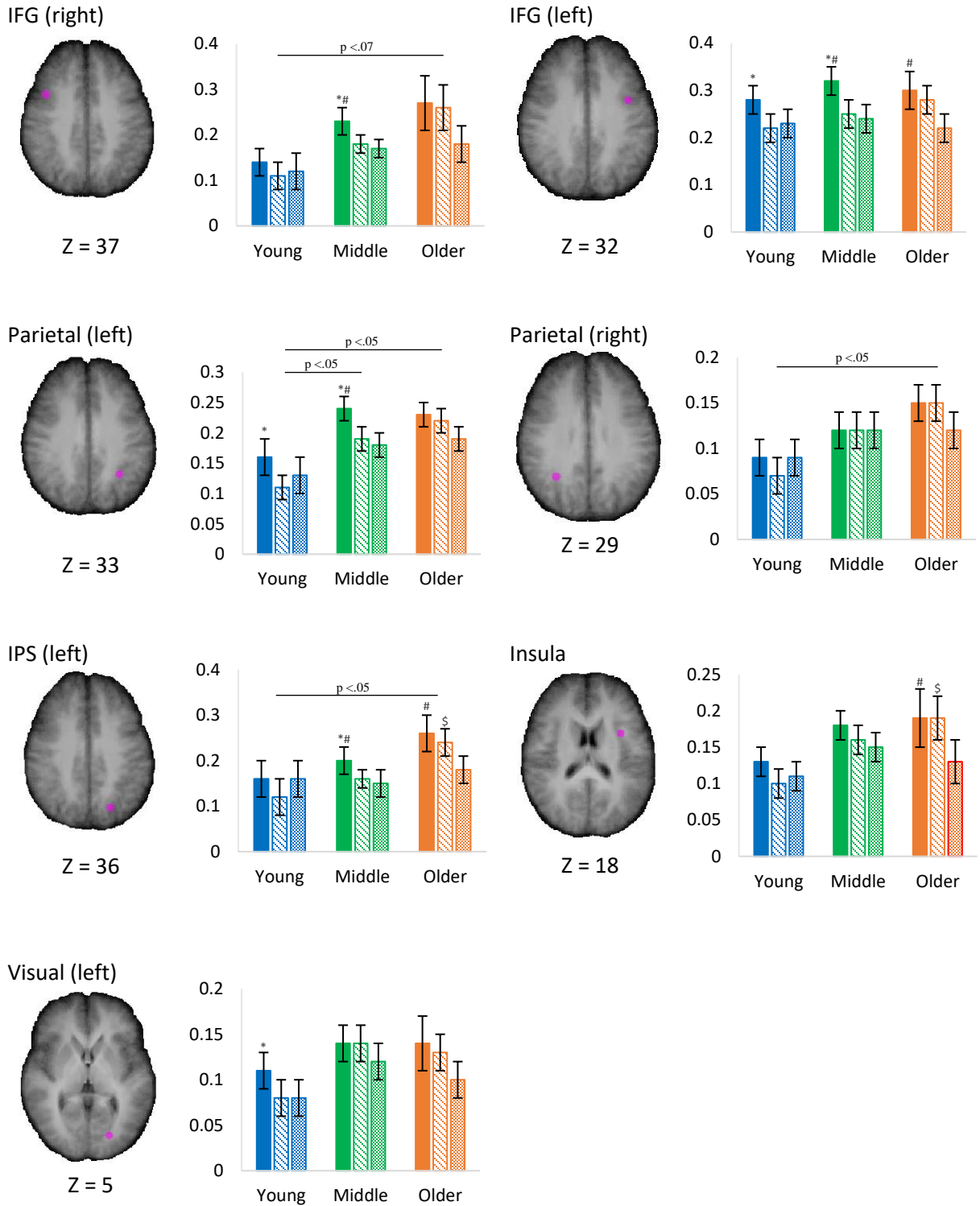


Figure 2.3 Depiction of spherical ROIs (purple) and corresponding mean magnitude of BOLD response during incongruent, congruent, and neutral trials during the Stroop task in healthy young, middle-age and older adults. Between group significance is denoted by

$p < .05$ or $p < .07$ and within group significance for incongruent > neutral is denoted by *, incongruent > congruent by #, and neutral > congruent by \$, $p < .05$.

There were significant group differences in the magnitude of BOLD response during the neutral condition in 7 ROIs, including the right ACC $F(2,73) = 5.22$, $p < .01$, left ACC $F(2,73) = 3.56$, $p < .05$, right dACC $F(2,73) = 7.75$, left parietal $F(2,73) = 5.85$, $p < .01$, right parietal $F(2,73) = 4.12$, $p < .05$, $p < .01$, left IPS $F(2,73) = 3.56$, $p < .05$, and DLPFC $F(2,73) = 4.57$, $p < .05$ (Figure 2.3). Tukey post-hoc tests revealed that older adults had greater activation compared to younger adults in the right ACC ($p = .009$), left ACC ($p = .027$), right dACC ($p = .001$), left parietal ($p = .003$), right parietal ($p = .015$), left IPS ($p = .044$), and DLPFC ($p = .023$). Middle-aged adults had greater activity in the right dACC ($p = .017$) and left parietal ($p = .037$) versus young adults. Older adults had slightly increased DLPFC activity in comparison to middle-aged adults ($p = .052$). The right IFG was also trending towards significance during the neutral condition $F(2,73) = 2.90$, $p = .062$, with older adults having marginally greater activity compared to younger adults ($p = .055$). A group difference was found in the congruent condition for the right dACC $F(2,73) = 3.17$, $p < .05$. Tukey post-hoc tests revealed that in the right dACC the middle-aged adults had greater activity compared to young adults during the congruent condition ($p = .049$), while estimates of activity were similar for older and middle-aged adults. The magnitude of the BOLD response during the incongruent condition did not differ across groups, although the right dACC was trending towards significance $F(2,73) = 2.82$, $p = .066$, with moderately greater activity in the middle-age versus younger adults ($p = .07$).

There was a significant age effect on the magnitude of the BOLD response in several ROIs during the fMRI Stroop task, with the most notable being that older adults elicited a greater response compared to young adults during the neutral condition. Middle-aged adults showed some increased recruitment compared to young as well, but only in the dACC and left parietal ROI.

2.4.2.2 Within-Group ROI Results

YOUNG GROUP: In the young adult group, the means for magnitude of the BOLD response were different across the three conditions in several ROIs. The magnitude of the BOLD response was found to be different depending on the stimulus condition in the left ACC $F(2,28) = 7.39, p < .01$. Post-hoc analysis with bonferroni correction revealed that the incongruent condition elicited a greater response compared to the neutral condition ($p = .005$) as well as compared to the congruent condition ($p = .045$). A similar result was found in the in the left IFG $F(2,28) = 5.47, p < .05$ (incongruent > neutral, $p = .038$), left parietal $F(2,28) = 4.01, p < .05$ (incongruent > neutral, $p = .041$), and left VAA $F(2,28) = 5.01, p < .05$ (incongruent > neutral, $p = .044$). A difference in mean magnitude of the BOLD response based on condition was also noted in the right ACC $F(2,28) = 5.02, p < .05$, with the incongruent condition approaching significance for eliciting a greater response compared to neutral ($p = .07$). A similar result was found for the right dACC $F(2,28) = 4.70, p < .05$ (incongruent > neutral, $p = .067$, incongruent > congruent, $p = .054$). There were no significant differences in the means for magnitude of BOLD response noted in the right IFG, right parietal, left IPS or insula across the three conditions.

MIDDLE GOUP: As for the middle-aged group, we found differences in the means for the magnitude of the BOLD response for the three conditions in the right ACC $F(1.38, 41.50) = 7.04, p < .01$ (incongruent > neutral, $p = .012$; incongruent > congruent, $p = .026$). Comparable results were identified in several of the other ROIs, including the left ACC $F(2,60) = 6.05, p < .01$ (incongruent > congruent, $p = .013$), right dACC $F(1.52, 45.56) = 5.67, p < .05$ (incongruent > neutral, $p = .036$; incongruent > congruent, $p = .042$), left IFG $F(2,60) = 15.14, p < .0001$ (incongruent > neutral, $p < .0001$; incongruent > congruent, $p < .005$), right IFG $F(1.52, 45.48) = 6.74, p < .01$ (incongruent > neutral, $p = .007$; incongruent > congruent, $p = .029$), left parietal $F(1.52, 45.58) = 8.02, p < .01$ (incongruent > neutral, $p = .001$; incongruent > congruent, $p = .014$), left IPS $F(1.65, 49.41) = 5.44, p < .05$ (incongruent > neutral, $p = .012$, incongruent > congruent, $p = .031$), and the DLPFC $F(2, 60) = 6.72, p < .01$ (incongruent > neutral, $p = .003$). Like the young adults, there were no significant differences in the means for the magnitude of the BOLD response in the right parietal or insula across the three conditions, with the addition of the left VAA also showing to difference in response to the three conditions.

OLDER GOUP: Differences in magnitude of BOLD response across the three conditions were also present in the older adult group. This included the right ACC $F(2,54) = 5.34, p < .01$ (neutral > congruent, $p = .004$; incongruent > congruent, $p = .065$), left ACC $F(2,54) = 6.39, p < .01$ (incongruent > congruent, $p = .029$; neutral > congruent, $p = .007$), right dACC $F(2,52) = 3.31, p < .05$ (neutral > congruent, $p = .037$), left IFG $F(2,54) = 5.37, p < .01$ (incongruent > congruent, $p = .026$; neutral > congruent, $p = .057$), right IFG $F(2,54) = 4.23, p < .05$ (incongruent > congruent, $p = .069$; neutral > congruent, $p = .060$), insula $F(2,54) = 8.23, p < .01$ (incongruent > congruent, $p = .011$; neutral >

congruent, $p = .002$), left IPS $F(1.65, 44.53) = 7.00$, $p < .01$ (incongruent $>$ congruent, $p = .013$; neutral $>$ congruent, $p = .008$), and the DLPFC $F(2,54) = 5.67$, $p < .01$ (incongruent $>$ congruent, $p = .058$; neutral $>$ congruent, $p = .012$). However, in comparison to both the young and middle-aged groups, there were no differences in mean magnitude of BOLD response between the incongruent and neutral condition in any of the ROIs. Furthermore, there were no differences in activity in the right IFG, bilateral parietal ROIs, and left VAA across the three conditions.

SUMMARY: In both the young and middle-aged adult groups, the incongruent condition elicited a greater neural response compared to neutral and congruent conditions in several ROIs. In contrast, the older adults showed no difference in terms of amount of activation between incongruent and neutral conditions, but in numerous ROIs activation in both incongruent and neutral conditions was greater than the response during the congruent condition. There were no instances of congruent activity being greater than incongruent or neutral.

2.4.3 Correlations Between ROIs and Age

There were several positive correlations between age and magnitude of the BOLD response during the neutral condition. As age increased so did activity in the right ACC ($r = .39$, $p = .001$), left ACC ($r = .32$, $p = .005$), right dACC ($r = .46$, $p < .0001$), DLPFC ($r = .35$, $p = .002$), right IFG ($r = .32$, $p = .005$) and left parietal ($r = .34$, $p = .003$) (Figure 2.4). Weak, positive correlations were also found in right parietal ($r = .30$, $p = .01$) and left IPS ($r = .30$, $p = .01$), although they did not reach significance. There were also several weak positive correlations with age and magnitude of the BOLD response during the incongruent condition. As age increased so did activity in the right IFG ($r = .26$, $p = .026$), left IPS ($r =$

.23, $p = .046$) and DLPFC ($r = .255$, $p = .028$). One significant positive correlation was identified during the congruent condition with age and the right dACC ($r = .25$, $p = .003$) (Figure 2.4).

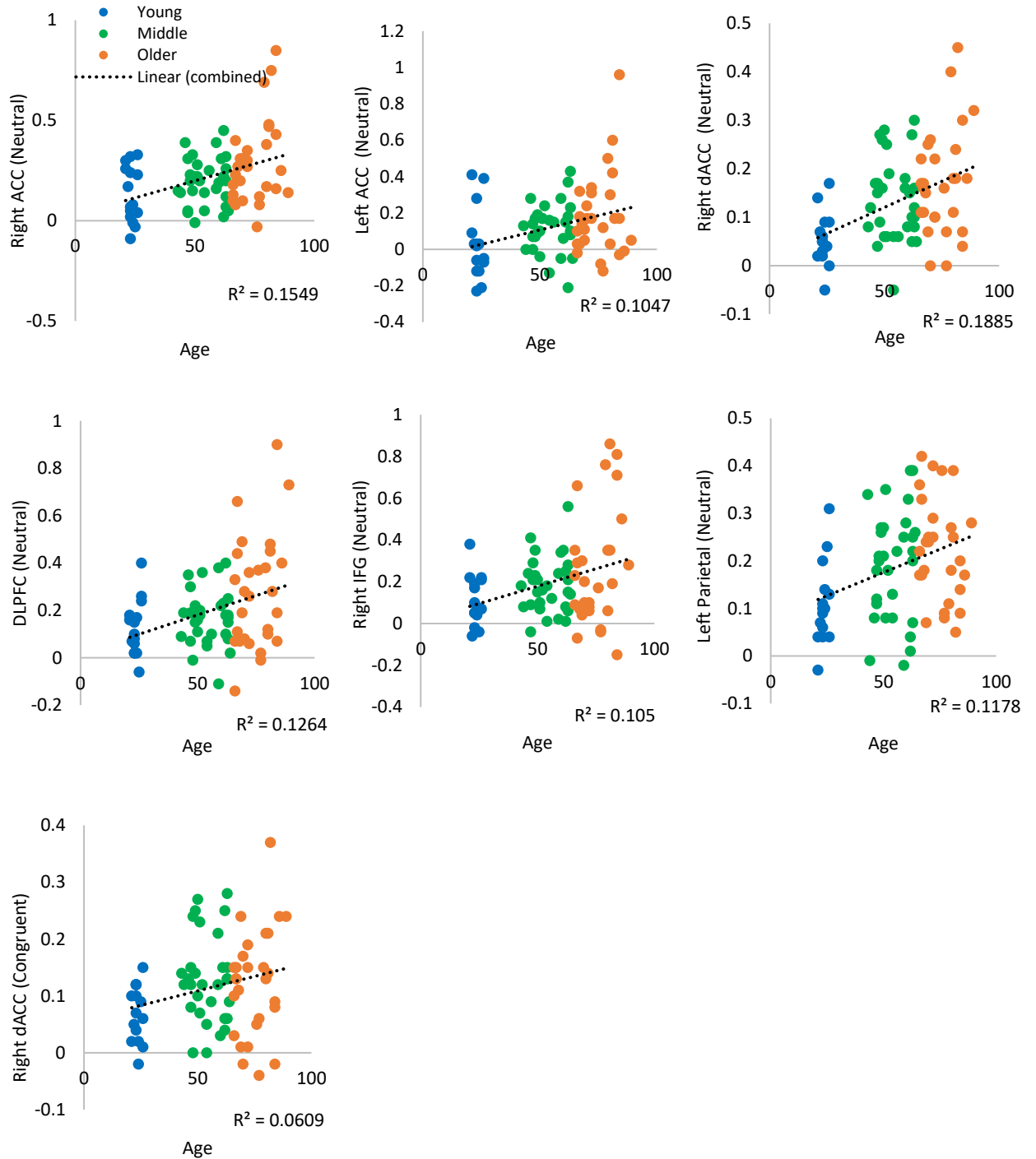


Figure 2.4. Significant Pearson correlations between age and the magnitude of BOLD response during neutral and congruent conditions in ROIs relating to the Stroop task.

2.4.4 Correlations Between ROIs and Stroop Behavior

To explore the relationship between ROIs associated with the Stroop task and behavioral measures from the Stroop task with aging, we conducted correlations analyses with all groups combined. Pearson correlations were calculated between the magnitude of the BOLD response from incongruent and neutral conditions within each ROI and transformed data for task performance: Average incongruent, neutral and congruent reaction times, Stroop effect (ms), interference (%) and percentage of incongruent, congruent and neutral errors for each group (Table 2.3).

Some ROIs did correlate with behavioral measures from the Stroop task (Table 2.3 A). More specifically, longer incongruent reaction times were significantly associated with greater activity during the neutral condition in the DLPFC ($r = .34, p = .003$) and right IFG ($r = .35, p = .002$), and weakly correlated with the left ACC ($r = .27, p = .02$). Similarly, longer neutral reaction times were associated with greater activity in the DLPFC ($r = .33, p = .003$) and weakly correlated with the right IFG ($r = .28, p = .016$) and the left ACC ($r = .23, p = .047$). Congruent reaction times were positively correlated with activity in the DLPFC ($r = .33, p = .004$) and to a lesser extent in the right IFG ($r = .25, p = .031$). In terms of accuracy, none of the correlations were significant after correcting for multiple comparisons, although incongruent errors ($r = .25, p = .031$) and neutral errors ($r = .24, p = .037$) were weakly correlated with activity in the left IFG. Measures of interference were also positively correlated with ROIs during the neutral

condition. The larger the Stroop effect, the higher the magnitude of BOLD response was in the right IFG ($r = .38, p = .001$). Weak correlations were also found between Stroop effect and the right ACC ($r = .25, p = .031$), left ACC ($r = .23, p = .047$) and left IFG ($r = .24, p = .044$). Greater interference was also weakly correlated with right IFG activity ($r = .26, p = .026$).

Correlations between ROIs during the incongruent condition and behavioral measures shared a similar result (Table 2.3 B). Incongruent reaction time was positively correlated with the DLPFC ($r = .32, p = .005$) and right IFG ($r = .36, p = .002$) ROI. Longer neutral reaction times were also associated with greater activity in the DLPFC ($r = .34, p = .003$) and to a lesser extent the right IFG ($r = .31, p = .007$). Longer congruent reaction times were also associated with greater activity in the DLPFC ($r = .38, p = .001$) and right IFG ($r = .33, p = .005$). In addition, a larger Stroop effect was linked with greater activity in the right IFG ($r = .31, p = .008$) and to a lesser degree the right ACC ($r = .24, p = .04$). There were no significant correlations between interference or accuracy and any of the ROIs during the incongruent condition. There were no significant correlations between any of the behavioral measures from the Stroop task and magnitude of the BOLD response during the congruent condition any of the ROIs (Table 2.3 C).

In summary, we found evidence to suggest that the relationship between behavioral measures and neural activity pertaining to the Stroop task changes with age. More specifically, older age was associated with greater magnitude of the BOLD response during the neutral condition of the Stroop task, and weakly linked in a few ROIs during incongruent and congruent conditions. In addition, greater activity was associated with worse performance (e.g., longer reaction times).

A)	INC RT (ms)	CON RT (ms)	NEU RT (ms)	INC Errors (%)	CON Errors (%)	NEU Errors (%)	Stroop Effect (ms)	Interference (%)
ACC (R)	.21	.09	.16	.15	.06	.08	.25*	.18
ACC (L)	.27*	.17	.23*	.18	.02	.10	.23*	.14
dACC (R)	.14	.05	.10	.12	-.09	.08	.17	.12
DLPFC	.34**	.33**	.34**	.13	.16	.19	.18	.02
IFG (L)	.23	.17	.19	.25*	.17	.24*	.24*	.11
IFG (R)	.35**	.25*	.28*	.17	.02	.04	.38**	.26*
Parietal (L)	.12	.12	.05	-.12	.10	.06	.18	.18
Parietal (R)	.18	.14	.13	.03	.17	-.03	.21	.17
IPS (L)	.11	.04	.06	-.12	-.10	.02	.16	.15
Insula	.14	.16	.13	.02	.18	.19	.09	.05
VAA (L)	.04	-.001	.01	.03	.03	-.08	.12	.11
B)	INC RT (ms)	CON RT (ms)	NEU RT (ms)	INC Errors (%)	CON Errors (%)	NEU Errors (%)	Stroop Effect (ms)	Interference (%)
ACC (R)	.21	.17	.16	.06	.03	-.05	.24*	.16
ACC (L)	.22	.17	.20	.12	.06	.03	.17	.07
dACC (R)	.09	.08	.06	.13	-.08	.03	.11	.07
DLPFC	.32**	.34**	.38**	.10	.15	.13	.13	-.03
IFG (L)	.15	.14	.12	.22	.19	.14	.18	.08
IFG (R)	.36**	.33**	.31*	.12	-.03	-.08	.31*	.17
Parietal (L)	.12	.11	.06	-.17	.09	-.04	.17	.16

Parietal (R)	.07	.02	.01	.01	.22	-.10	.19	.19
IPS (L)	.15	.05	.10	-.10	-.06	-.06	.17	.16
Insula	.10	.13	.08	-.04	.19	.11	.10	.07
VAA (L)	-.12	-.15	-.20	-.04	.10	-.11	.16	.22
<hr/>								
C)	INC RT	CON RT	NEU RT	INC Errors	CON Errors	NEU Errors	Stroop Effect	Interference
	(ms)	(ms)	(ms)	(%)	(%)	(%)	(ms)	(%)
ACC (R)	-.01	-.03	-.04	.15	.09	.05	.05	.08
ACC (L)	-.02	-.05	-.04	.16	.01	-.01	.03	.06
dACC (R)	-.03	-.002	-.01	.14	-.14	.10	-.07	-.06
DLPFC	.11	.17	.12	.09	.15	.15	.07	-.01
IFG (L)	-.02	.02	-.002	.22	.13	.19	-.01	-.07
IFG (R)	.08	.10	.04	.10	-.08	-.11	.15	.12
Parietal (L)	.04	.04	-.02	-.14	.14	.12	.17	.16
Parietal (R)	.02	.01	-.02	.01	.18	-.002	.11	.09
IPS (L)	-.02	-.05	-.05	-.15	-.09	.05	.06	.08
Insula	-.03	.01	-.02	.05	.21	.22	-.02	-.03
VAA (L)	-.16	-.13	-.17	-.03	.08	-.02	-.03	.002

Table 2.3. Pearson correlation coefficients between behavioral measures and the magnitude of the BOLD response in ROIs from the Stroop task. INC = incongruent, CON = congruent, NEU = neutral, RT = reaction time, R = right, L = left. A) Magnitude of BOLD response during the neutral condition. B) Magnitude of BOLD response during the incongruent condition. C) Magnitude of BOLD response during the congruent condition. Significant correlations are denoted by ** $p < .006$, and * $p < .05$.

2.5 Discussion

The goal of this study was to investigate how healthy aging affects inhibitory control and the neural underpinnings of the Stroop task. We examined whether there was an effect of age group (young, middle-age and older) on reaction times and errors by condition (incongruent, neutral and congruent), as well as any potential age group differences on measures of interference. Our analysis of behavioral measures from the Stroop task supports the idea of generalized slowing in older age, as we did not find that older adults produced disproportionately longer reaction times to incongruent versus neutral trials, maintained accuracy at levels comparable to younger and middle-aged adults, and did not have a greater percentage of interference.

We also explored how independently selected ROIs related to the Stroop task function within and across each age group. We probed possible relationships between brain and behavior by conducting correlations between the magnitude of the BOLD response in ROIs with age and behavioral measures from the Stroop task. Consistent with our hypothesis, older adults over-recruited frontal and parietal areas during neutral trials compared to young adults. The middle-aged group showed a similar trend, but to a lesser extent, suggesting that subtle changes to brain areas involved in inhibitory control may begin before the age 65. Within-group analysis revealed that older adult's lost specificity to stimuli, while both the middle-aged and younger adults produced a greater magnitude of the BOLD response during trials with more interference compared to the neutral condition. Correlations between the magnitude of the BOLD response, age, and behavioral measures provide evidence for both the compensation and dedifferentiation hypothesis of aging. The results from our study give insight into how inhibitory control

as measured by the Stroop task changes across healthy aging and gives insight into pinpointing when initial changes may occur across the human lifespan.

2.5.1 Age-related Changes in the Stroop Task: Behavioral Measures

In terms of behavioral performance on the Stroop task, we found that the young, middle-aged and older adult groups had similar reaction times to all three conditions, incongruent, congruent and neutral. Rather, all participants, regardless of age group, were slower at responding to incongruent versus neutral trials, incongruent versus congruent trials and neutral versus congruent trials, which is consistent with other studies investigating Stroop and aging (Langenecker, Nielson, & Rao, 2004; Mathis, Schunck, Erb, Namer, & Luthringer, 2009; Zysset, Schroeter, Neumann, & von Cramon, 2007). In contrast to Langenecker, Nielson & Rao (2004) that did not report differences in reaction times with age, we found that similarly to Zysset, Schroeter, Neumann & von Cramon (2007) and Mathis, Schunck, Erb, Namer & Luthringer (2009), older adults were slower overall compared to participants in the young and middle-age group for all conditions, with no differences found between middle-age and young adults. A similar study including middle-aged adults found additional slowing in the middle-age group (46-55 years) compared to young adults (22-30 years) (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). However, the interaction between age group and condition was not significant, which suggests that reaction times for incongruent, neutral and congruent trials were not disproportionately affected by age group. This finding is corroborated by other studies, which also failed to identify a group by condition interaction (Langenecker, Nielson, & Rao, 2004; Zysset, Schroeter, Neumann, & von Cramon, 2007; Mathis, Schunck, Erb, Namer, & Luthringer, 2009).

In terms of task accuracy, we found no differences in the percentage of errors committed by the age groups, which replicated other studies (Milham, et al., 2002; Zysset, Schroeter, Neumann, & von Cramon, 2007). Instead, all participants collectively made more incorrect responses to incongruent trials compared to both neutral and congruent trials. This contrasts with the study by Mathis et al. (2009), which reported significantly more incongruent errors committed by older and middle-aged participants compared with younger adults. Langenecker, Neilson & Rao (2004) also reported increased errors in the older adult group, regardless of condition. In terms of speed-accuracy trade off, it is often suggested that older adults will sacrifice speed in order to maintain a high level of accuracy (Starns & Ratcliff, 2010). This may relate to our findings that older adults were slower overall at responding yet maintained similar accuracy to younger and middle-aged participants.

Calculations for group differences in interference as measured by the Stroop effect and percentage of interference showed that older adults tended to produce a larger Stroop effect compared to young adults, but that when percentage of interference was calculated to control for generalized slowing there were no significant group differences. This finding is comparable to that of Langenecker, Nielson & Rao (2004), which in addition reported a trend towards a larger Stroop interference in older adults. While Milham et al. (2002) did not calculate percentage of interference, they also described a non-significant trend towards larger Stroop effect in older versus younger adults.

Collectively, the results from the behavioral analyses of Stroop task performance indicate that age did not disproportionately affect reaction time to any of the stimuli, i.e., reaction time for incongruent stimuli was not slower relative to neutral or congruent

stimuli in the older adults. Thus, all age groups appeared to show similar interference relating to the incongruent condition. This result is in line with previous findings and supports the idea that the longer reaction times found with increasing age may be a result of generalized slowing with increasing age as opposed to a breakdown of inhibitory control (Langenecker, Nielson, & Rao, 2004). This idea is again furthered by the finding that percentage of interference was not different between age groups. The conclusions from our behavioral results differ from those of Mathis, Schunck, Erb, Namer & Luthringer (2009). While Mathis, Schunck, Erb, Namer & Luthringer (2009) suggest based on their findings that changes to inhibition may occur after the age 50, we did not find differences in reaction time or accuracy between the middle-age and young adults. Early impairments in inhibitory control have been noted by other studies as well, for example one large study measuring attentional control using the Hayling test identified an initial dip in inhibitory control beginning at age 50, with accelerated drop-offs in the 60's and 70's (Borella, Carretti, & De Beni, 2008). There are limited studies including middle-aged adults. However, one possible reason for this difference could be due to our sample of participants. Studies of "successful" cognitive aging support the idea that several factors may contribute to the maintenance of cognitive abilities with increasing age, including genetics, as well as lifestyle factors such as exercise and mental and social engagement (Harada, Natelson, & Triebel, 2013). While we did not factor in those extra variables per se, our participants all had similar years of education, which in part may contribute to maintaining cognitive abilities with age (Harada, Natelson, & Triebel, 2013).

A recent study by Sjoberg, Wilner, D'Souza & Cole (2022) indicated that females tend to have improved Stroop task performance compared to males, which may be a result of better color naming or verbal abilities as opposed to heightened inhibitory control processes. We did not find evidence of any sex differences in our participants, and in fact found that females tended to make slightly more errors compared to males. However, our samples were uneven (females $n = 47$, males $n = 27$), which may have contributed to the overinflated error rates in female participants.

Thus, behavioral results from our study support the hypothesis that inhibitory control, as measured by the Stroop task, remains stable across healthy aging, and that interference arising from the incongruent stimuli does not change with increasing age in healthy adults. While behaviorally our age groups performed similarly, aside from some generalized slowing, we also probed ROIs involved in the Stroop task to investigate possible neural changes with age.

2.5.2 Age Related Changes and the fMRI Stroop Task

Applying the search term 'Stroop' in NeuroSynth yielded 11 ROIs, including the ACC, DLPFC, IFG, parietal, IPS, insula and left VAA. The ROIs identified through NeuroSynth coincide with brain areas identified in the literature to be associated with the Stroop task, including the frontoparietal network (Nee, Wager, & Jonides, 2007). We investigated the ROIs across three different age groups of cognitively normal adults, young, middle-aged and older, to see how the Stroop task, a measure of inhibitory control, and these brain areas function across healthy aging.

Our ROI analysis revealed several changes in the magnitude of BOLD response across three different age groups of healthy adults during the Stroop task. While we didn't find evidence of age group differences in neural activity during the incongruent condition, we found increased activity during the neutral condition in several ROIs in older and middle-aged participants compared to younger adults. Comparison of peak activity across age groups revealed that when faced with the Stroop task, older adults had an increased neural response during neutral trials compared to younger adults in the bilateral ACC, right dACC, DLPFC, bilateral parietal ROIs, and left IPS. Similarly, yet to a lesser extent, middle-aged adults showed a larger neural response to neutral trials compared to young adults in the right dACC and left parietal ROI. Older adults produced a slightly larger neural response compared to middle-aged adults only in the left DLPFC during neutral trials. Likewise, Langenecker, Nielson & Rao (2004) found older adults had greater activity in the left ACC during the neutral condition compared to young adults. However, in contrast to our results, older adults were found to have greater activity during the incongruent condition in several regions, including the IFG, middle frontal gyrus, precentral gyrus, medial frontal gyrus, inferior parietal lobule, precuneus, and the claustrum, while the neutral condition elicited greater activity in the younger adults in several areas. Experimental design may contribute to differences in activation patterns, including adopting strategies during block designs (e.g., reading during congruent trials versus color naming) (MacLeod & MacDonald, 2000), and button press where the participant needs to keep the motor response in mind versus vocal response, which is more intuitive for a verbal task such as the Stroop (Nee, Wager, & Jonides, 2007). In our study the young, middle-aged and older adults did not differ on activity

during the interference (incongruent) condition, which suggests that perhaps the control (neutral) condition, was more difficult due to the less predictable rapid-event related design (Langenecker, Nielson, & Rao, 2004). Comparing the Stroop effect across studies indeed shows that our participants had a greater Stroop effect, like that reported by Milham et al. (2002). It is possible that the increased activity when faced with the neutral condition in the middle-age and older adults reflects the increased demand regardless of condition to correctly respond to the task because of the random presentation of the stimuli and study design (Langenecker, Nielson, & Rao, 2004). A meta-analysis investigated the differences in contrasts separately and found that incongruent versus neutral conditions resulted in greater activity in the left DLPFC and PPC compared to incongruent versus congruent. Conversely, incongruent versus congruent resulted in greater involvement of the ACC, which they suggest indicates that the congruent condition evokes greater involvement in the DLPFC and PPC and less involvement of the ACC compared to neutral stimuli (Nee, Wager, & Jonides, 2007).

Other studies of Stroop and aging support our findings of increased activity during the Stroop task with older adults. Studies have found increased activity during the incongruent condition in frontal areas, including the left IFG/IFJ, left DLPFC, left parietal, IPS and ACC (Zysset, Schroeter, Neumann, & von Cramon, 2007; Mathis, Schunck, Erb, Namer, & Luthringer, 2009; Langenecker, Nielson, & Rao, 2004; Milham, et al., 2002). In our study activity in the right IFG was trending towards being significantly greater in older versus young adults. The IFG ROIs generated in Neurosynth (right: 40, 9, 37; left: -39, 2, 32) showed some overlap with the IFJ coordinates in Zysset et al. (2007) (right: 40, 8, 34; left: -44, 17, 28), and ours resembled IFG coordinates in

Langenecker et al. 2004 (right: 30, 30, -12; left: -40, 0, 32). Due to methodological differences in our study, namely a whole brain contrast versus ROI approach, a direct comparison cannot be made. However, the result that the IFJ shows age-related changes in activity is in line with our findings. The IFJ is part of DLPFC, and the coordinates used in Zysset, Schroeter, Neumann, & von Cramon (2007) (-44, 17, 28) are comparable to the one identified by Neurosynth in our study (-40, 16, 30). The role of the IFG in inhibitory control, especially in older adults, has been consistently reported (Nielson, Langenecker, & Garavan, 2002; Langenecker, Nielson, & Rao, 2004; Milham, et al., 2002). Studies of inhibition often cite activity predominantly in the left IFG, while we observed a moderate increase in older adults in the right IFG. This could be due to compensatory mechanisms such as increasing activity in bilateral or additional areas not specific to the task compared to younger adults (Cabeza, 2002).

The ACC was a key area we found to show differences across the age groups. We found that older adults had higher activity in the bilateral ACC, and dACC ROIs compared to young adults, as did the middle-aged adults, during the neutral condition. Other studies do report involvement of the ACC, including a meta-analysis that identified five main activation areas during Stroop interference, including the IFJ, inferior frontal sulcus (IFS), pre-SMA, and bilateral ACC (Neumann, Lohmann, Derrfuss, & von Cameron, 2005). The idea that the ACC is not solely involved in interference is bolstered by the finding that an increase in ACC activity was noted in older adults in both the incongruent and congruent condition (Milham, et al., 2002). This can extend to our findings of increased ACC activity in both the older and middle-aged adults in the neutral and congruent condition. This finding is somewhat relatable to the meta-analysis by Nee,

Wager & Jonides (2007) that ACC involvement is greater for neutral trials compared to congruent. Overall, our findings are in accordance with other Stroop aging studies that older adults tend to have an increased neural response when faced with certain cognitive tasks.

We also found that the neural response for older adults was similar in both incongruent and neutral conditions. In other words, the neutral condition with no interference between color and word elicited the same response as the condition with interference, yet they did not show decreased accuracy compared to middle-age or younger adults. This finding was related to Mathis et al (2009), except they reported increased activation during the congruent condition in older adults. Hence, an increase in neural response to stimuli from the Stroop task is not just specific to the interference condition in older adults (Mathis, Schunck, Erb, Namer, & Luthringer, 2009). The increased activity found within the neutral condition could again be related to increased task difficulty and is exacerbated in older age. In accordance with Mathis et al. (2009), within-group analyses showed both the younger and middle-aged adults had greater activity during the incongruent condition compared to neutral condition in several of the ROIs. In comparing peak estimates of activity across groups the middle-aged adults fell somewhat in the middle, with over-recruitment in the dACC and left parietal during non-interference conditions. Over-recruitment of the left parietal in middle-aged adults was also identified in the study by Mathis et al. (2009), however during the incongruent condition.

The finding that additional brain areas become active in older adults during a cognitive task is often described as compensation (Nielson, Langenecker, & Garavan,

2002; Cabeza, Anderson, Locantore, & McIntosh, 2002). It is posited that age-related neuronal loss may lead to other neural circuits being recruited to aid in performing a cognitive task. Several studies reported additional brain areas becoming active in older adults during the Stroop task (Zysset, Schroeter, Neumann, & von Cramon, 2007; Parris, 2014). Our study did not use a whole brain voxel wise approach, and therefore cannot make inferences regarding if additional brain areas became involved in the Stroop task across different age groups. Instead, since we relied on independent defined ROIs, we were able to interrogate the differences in activation levels within and between each age group, as well as relate activity with behavioral performance through correlations.

2.5.3 Correlations Between Age, ROIs and Stroop Task Performance

Correlations were conducted between the magnitude of the BOLD response during incongruent, congruent and neutral trials in ROIs with age and with behavioral measures from the Stroop task. Some ROIs that correlated with age, but not behavioral measures include the bilateral ACC and right dACC ROIs as well as the left parietal ROI. As age increased so did activity in the ROIs during the neutral condition, but this increase in activity was not correlated with reaction time, accuracy or measures of interference. This finding provides support for the dedifferentiation hypothesis and suggests that over-recruitment of the parietal area during neutral trials of the Stroop task in older adults provides no benefit to performance (Mathis, Schunck, Erb, Namer, & Luthringer, 2009; Li, Lindenberger, & Sikström, 2001). Similarly, increasing age was associated with higher activity in the ACC, but is not task related since correlations with ACC and behavior did not reach significance after correcting for multiple comparisons. Therefore, in our study the ACC showed susceptibility to age related increases during the Stroop

task, but again with no benefit to performance. Another study identified a similar finding with middle-aged adults within the ventrolateral prefrontal cortex during the incongruent condition (Mathis, Schunck, Erb, Namer, & Luthringer, 2009).

There was some overlap in ROIs that correlated with age and those that correlated with behavioral measures. For instance, activity in the right IFG during incongruent and neutral conditions increased with age and with reaction time and Stroop effect and interference. Similarly, activity in the DLPFC during incongruent and neutral trials also increased with age and had positive correlations with reaction times. The study by Mathis et al. (2009) also identified a correlation between age and reaction time, which may indicate that over-recruitment of these areas in older adults is maladaptive and lends support for the age-related compensation hypothesis. It is posited that age-related neuronal loss may lead to over-recruitment or recruitment of additional brain areas to aid in performing a cognitive task, known as compensation (Cabeza, Anderson, Locantore, & McIntosh, 2002). Compensation with aging has also been described in terms of the loss of lateralization, or the recruitment of bilateral areas, during a cognitive task (the HAROLD model) (Cabeza, 2002). Our study did not use a whole brain voxel-wise approach, and therefore we cannot make inferences regarding if additional or bilateral brain areas became involved in the Stroop task across different age groups, we did however identify several areas of over-recruitment in older adults compared to younger adults. Studies of inhibitory control and aging have also found evidence of compensation (Nielson, Langenecker, & Garavan, 2002). Only the right dACC showed a significant correlation with age during the congruent condition. Since there were no correlations with the congruent condition and behavior, this suggests that facilitation effects remain

relatively intact with increasing age. Indeed, this finding is comparable to Mathis et al. (2009) in which an over-recruitment of the network associated with the congruent condition (right DLPFC and ventrolateral prefrontal cortex and bilateral parietal cortex) was found in older adults with no detriment to reaction times or accuracy, indicating successful compensation. Therefore, the results from our study provide support for both the compensation and dedifferentiation theories of aging (Mathis, Schunck, Erb, Namer, & Luthringer, 2009).

2.5.4 Summary and Future Directions

Overall, the older adult group performed similarly on the Stroop task to the young and middle-age group and did not show any evidence of increased interference relating to a breakdown of inhibitory control. However, we identified several brain areas that showed changes related to aging. We found increased activity in older adults compared to young adults, and a loss of distinction between trial types as noted by overall increased activation during both incongruent and neutral trials. We also identified some early brain changes in the middle-age group, with increased activity in the right dACC compared to young adults. This suggests that despite having similar behavioral performance to the young adults, neural changes may begin to emerge in middle-age and become more marked after age 65. This is in line with another study that found cortical changes at age 60 (Mathis, Schunck, Erb, Namer, & Luthringer, 2009).

We found support for both the compensatory and dedifferentiation hypothesis of aging. Age was associated with higher activity in the DLPFC and right IFG and with worse Stroop performance, and activity in the right dACC and left parietal increased with

age but not with behavioral measures. Neurovascular changes with aging may also pose an issue with fMRI studies and interpretations of BOLD responses. Indeed, a study of aging and the Stroop task using a technique called calibrated fMRI with arterial spin labelling to account for age-related vascular changes concluded that increases in BOLD activity with age may be due to reduced oxygen metabolism (Mohtasib, et al., 2012). Future studies may benefit from using similar approaches to control for neurovascular changes. In addition, a larger sample size and longitudinal study design would allow for more insight into age related cognitive decline on the Stroop task. Understanding how inhibitory control and the Stroop task changes as a function of healthy aging provides valuable insight in the context of pathological aging too, for example in early detection of adults at risk of developing cognitive decline, or in Alzheimer's disease and amnesic mild cognitive impairment.

Chapter 3

Minimal changes to inhibitory control in Alzheimer's Disease and Amnesic Mild Cognitive Impairment: An fMRI Study of the Stroop Task

3.1 Abstract

Alzheimer's disease (AD) and the prodromal stage amnesic mild cognitive impairment (aMCI) are associated with widespread cognitive and neurological changes. However, whether deviations from healthy aging in measures of inhibitory control occur in people with AD and aMCI remains unclear. Using the Stroop task, a commonly used tool to measure inhibition, we investigated behavioral performance as well as neural correlates underlying the Stroop task in people diagnosed with AD, aMCI and healthy elderly. Participants performed the Stroop task while we measured blood oxygen level dependent (BOLD) signal. We found similar behavioral performance between both patient groups and respective control groups. However, participants with AD made significantly more errors during the incongruent condition compared to controls. We found no differences between AD and control groups in regions of interest (ROIs) related to Stroop performance. Participants with aMCI had decreased activity compared to controls in the bilateral parietal ROIs and marginally in the left ACC. We also investigated the relationship between Stroop performance measures and magnitude of BOLD response during the Stroop task. We found significant correlations between performance and the left parietal ROI in patients with AD, and correlations with all

groups combined suggest a possible dysfunction in the insula and right parietal relating to worse Stroop performance. Despite having similar behavioral performance to controls, it appears that brain areas involved in the Stroop task are already altered in aMCI, and errors made during stimuli containing the most interference seem to be a particularly sensitive marker to AD and aMCI.

3.2 Introduction

The neurodegenerative nature of Alzheimer's disease (AD) results in slow, progressive changes within various cognitive domains (Blennow, de Leon, & Zetterberg, 2006). In some instances, inhibitory control, the ability to maintain mental focus on relevant stimuli while ignoring irrelevant stimuli, has been shown to decline across normal aging; a change that may be exaggerated in AD (Spieler, Balota, & Faust, 1996). In fact, it has been suggested that deficits in inhibition often occur early on in AD and may even precede changes in visuospatial and verbal memory (Perry, Watson, & Hodges, 2000). Amnesic mild cognitive impairment (aMCI) is associated with an increased risk of future conversion to AD and is considered the prodromal stage of disease progression (Petersen, et al., 2001). Compared to AD, aMCI is characterized predominantly by deficits in memory, while activities of daily living remain intact; although, several studies have identified widespread deviations from the norm in several cognitive domains beyond isolated memory impairments, including inhibition (Kramer, et al., 2006). Providing further support for impaired inhibition in aMCI and AD, a study involving an antisaccade task found that participants with aMCI made more errors and were slower at initiating correct antisaccades (looking away from a target, requiring inhibition), similar to that of participants with AD (Peltsch, Hemraj, Garcia, & Munoz, 2014). Furthermore,

there was a negative correlation between Stroop scores and short-latency direction errors in the aMCI group, meaning that participants who had a higher Stroop score (number of correct responses – errors) made less short-latency antisaccade errors.

The Stroop task is a classic measure of selective attention and response inhibition (Stroop, 1935). When faced with color words written in either the matching color (congruent, e.g., red written in red) or non-matching (incongruent, e.g., green written in blue), inhibition is required to suppress the automatic response to read the word that is written in order to identify the color of ink in which the word is written. As a result of incompatible stimuli, and reading is faster than color naming, participants are generally slower to name the ink color on incongruent conditions compared to congruent or neutral conditions (Stroop, 1935; MacLeod, 1991; MacLeod & MacDonald, 2000).

In AD, a deficit in inhibitory control using the Stroop task is reflected by a decrease in the total number of responses when faced with interference resulting from the incongruent condition, essentially a longer reaction time, and increased number of errors compared to healthy controls (Bondi, et al., 2002; Koss, Delis, & Friedland, 1984; Fisher, Freed, & Corkin, 1990; Spieler, Balota, & Faust, 1996; Duong, Whitehead, Hanratty, & Chertkow, 2006). A recent review article also supports the idea that the Stroop task provides accurate discrimination between AD and healthy aging (Guarino, et al., 2019). Greater Stroop interference has also been found in people diagnosed with aMCI compared to controls (Kramer, et al., 2006; Sung, Kim, Jeong, & Kang, 2012). This difference in Stroop performance between people with AD and aMCI and healthy elderly suggests that the disease process alters patients' ability to quickly and successfully inhibit the automatic reading response (Fisher, Freed, & Corkin, 1990; Spieler, Balota, & Faust,

1996). However, not all studies report a reduction in Stroop performance in patients with AD (Stawarczy, Grandjean, Salmon, & Collette, 2012). One study that utilized several attention tasks to probe various aspects of attentional control, including the Stroop, found that people with AD differed from healthy aging on all tests except for the Stroop, and a decreased Stroop performance in people with AD was only found when compared to younger adults (Coubard, et al., 2011). Furthermore, the format that the Stroop test is delivered in also seems to affect performance. Studies focusing on predicting conversion from aMCI to AD using the Stroop task have found conflicting results. One study found no predictive value of a physical Stroop-card reading task between people that converted from MCI to AD and those that did not convert (Sarazin, et al., 2007). In contrast, another study reported significant differences between converters and non-converters using scores from a computerized trial-by-trial version of the Stroop task (Balota, et al., 2010). Incongruent errors, Stroop effect (incongruent – congruent reaction time), and reaction time distributions (more skewed in converters), were the most effective variables at predicting version to AD. Functional magnetic resonance imaging (fMRI) studies of the Stroop task have also identified differences between AD, aMCI and healthy elderly.

Numerous imaging studies have investigated the neural correlates underlying the attentional control required for the Stroop task. The frontoparietal network, including the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC) and posterior parietal cortex (PPC) have all been implicated in the ability to overcome interference in the incongruent conditions of the Stroop task (Nee, Wager, & Jonides, 2007; van Veen & Carter, 2005). As for studies investigating the Stroop task in patients with AD and aMCI, one study found that participants with aMCI exhibited an increased activation compared

to age-matched controls in several frontal lobe areas, with a decrease noted in patients with AD, and suggest that similar to healthy elderly showing greater activation than young adults, aMCI might result in an over activation as a mechanism of compensation to overcome early changes associated with underlying AD-related brain pathology (Li, Zheng, Wang, Gui, & Li, 2009). The idea of compensatory brain activity has been described as an over-activation of similar areas to younger or healthy counterparts, or recruitment of additional or bilateral areas during a cognitive task to circumvent age-related brain changes (Cabeza, 2002; Cabeza, Anderson, Locantore, & McIntosh, 2002). Increased activity when faced with incongruent stimuli has also been reported in the DLPFC and posterior parietal cortex (PCC) (Rosano, et al., 2005), as well as the ACC, inferior frontal gyrus (IFG), and insula (Li, Zheng, Wang, Gui, & Li, 2009) in persons diagnosed with aMCI. However, compensation, or an increase in activity in patients with aMCI is not universally reported. A different study did not identify any differences in activation between patients with aMCI and controls in inhibition related areas (Puente, Faraco, Terry, Brown, & Miller, 2014). Studies involving inhibition using the Stroop task and fMRI point to a general decrease in activity in patients with AD. One study reported that AD results in less ACC, bilateral middle and IFG, and inferior parietal lobule activation compared to controls, supporting the idea that attentional control is altered in AD (Li, Zheng, Wang, Gui, & Li, 2009). Another study found similar results using fluorodeoxyglucose positron emission tomography (FDG-PET) and reported hypometabolism in frontal regions in patients with AD compared to cognitively normal adults, and within the AD group higher metabolism of the DLPFC and ACC was associated with better Stroop performance (Yun, et al., 2011). In contrast, an fMRI study

of the Stroop task using a block design revealed that despite worse behavioral performance in the AD group, patients with mild AD recruited similar brain areas to healthy adults (Bowes, Stroman, & Garcia, 2012). Other fMRI studies employing different tasks, for example focusing on semantic and episodic memory, found evidence of recruitment of additional brain areas in participants with AD, which may indicate a compensatory mechanism similar to that seen in aMCI and healthy aging (Grady, et al., 2003).

While the location of early neuropathology in AD is often defined as beginning in the medial temporal lobes, the frontal lobes have also been shown to be susceptible to pathology (Braak & Braak, 1991; van der Flier, et al., 2002), which overlaps with areas underlying performance on the Stroop task (Nee, Wager, & Jonides, 2007). Thus, it remains unclear if compensation occurs in AD or if pathology accumulates enough at which point it can be detected by neuropsychological tests and compensation can no longer occur. The number of neuroimaging studies comparing AD and healthy elderly using the Stroop task are sparse, and relatively few have investigated aMCI.

The aim of this paper was to investigate independently defined regions of interest (ROIs) shown to be related to the Stroop task in patients diagnosed with mild AD and aMCI compared to healthy controls, and to see if any brain activity differences correlate with behavioral performance measures from the Stroop task. Based on previous research we expected behavioral differences on the Stroop task across controls, aMCI and AD groups. More specifically, we expected to find more incongruent errors committed by patients with AD and aMCI. We also expected to find increased activity in areas related to Stroop interference, a measure of inhibition, in aMCI compared to age-matched

controls, and a decrease in activity, especially prefrontal regions, in the AD group compared to controls. Hence, the goal of this study was to examine the differences in neural correlates of inhibitory control beginning in the earliest stages of the disease process in participants with aMCI, as well as mild AD.

3.3 Methods

3.3.1 Participants

This study was approved by the research ethics boards of Queen's University (#6004781) and Hotel Dieu Hospital. All participants were given a letter of information detailing the study and provided written informed consent prior to participation in the study. Healthy adults were recruited from within the community of the greater Kingston area. There was a total of 30 controls that were split into two groups: 13 age-matched to the aMCI group (7 females, mean age 63.5 ± 11.7 years), and 17 age matched to the AD group (9 female, mean age 74.4 ± 7.2 years) (Table 3.1). Participants received a diagnosis of aMCI based on the Petersen's criteria (Petersen, et al., 2001) and probable mild AD according to the NINCDS-ADRDA guidelines (McKhann, et al., 1984; Jack, et al., 2011) from a memory disorders clinic, and were subsequently invited to participate in the study. There were 14 participants diagnosed with aMCI (7 females, mean age 65 ± 11.8 years), and 19 participants diagnosed with mild AD (10 females, mean age 74.4 ± 7.9 years) that completed the study. Only patients with mild AD, as determined by a Montreal Cognitive Assessment (MoCA) score greater or equal to 18/30, were included in the study to ensure adequate comprehension of the task. Typically, the average MoCA score for AD is considered 16/30 (range: 11-21) and 22/30 (range:19-25) for patients with aMCI,

however there can be some overlap in scores between patients with AD and aMCI (Nasreddine, et al., 2005). All participants had normal or corrected vision, normal color vision, and complied with MRI safety standards. Two participants with AD and one with aMCI were excluded from the analysis due to excessive movement during the fMRI task.

	aMCI- Controls n = 13	aMCI n = 13	AD-Controls n = 17	AD n = 17
Demographics				
Age (years)	63.5 (3.2)	63.8 (3.2)	73.9 (1.9)	73.5 (1.7)
Sex (F/M)	7/6	7/6	10/7	10/7
Years of Education	18.1 (0.8)	15.4 (0.9)*	17.0 (0.9)	13.9 (0.7)*
Neuropsychological Test Battery				
MoCA	28.4 (0.3)	25.7 (0.6)*	27.7 (0.6)	21.9 (0.5)*
MMSE	29.3 (0.2)	28.1 (0.3)*	28.9 (0.3)	24.2 (0.3)*
Paper Stroop (/112)	96.3 (3.8)	85.8 (5.9)	95.4 (3.4)	54.9 (4.5)*
TMT (seconds)	83.2 (9.5)	103.8 (11.4)	96.6 (10.7)	229.7 (32.3)*
WMS: LNS	12.1 (0.4)	8.2 (0.9)*	11.7 (0.9)	6.8 (0.8)*
WMS: Fwd SS	9.5 (0.2)	7.8 (0.6)*	8.1 (0.3)	6.1 (0.3)*
WMS: Bck SS	9.0 (0.4)	7.4 (0.5)*	7.4 (0.4)	5.7 (0.3)*
CVLT-II: SDFR	1.4 (0.3)	-1.3 (0.2)*	1.0 (0.2)	n/a
CVLT-II: SDCR	1.1 (0.2)	-1.1 (0.2)*	0.7 (0.2)	n/a
CVLT-II: LDFR	1.3 (0.3)	-1.5 (0.2)*	1.0 (0.2)	n/a
CVLT-II: LDCR	1.0 (0.3)	-1.3 (0.2)*	0.8 (0.2)	n/a
DRS (total /144)	142.4 (0.5)	138.0 (0.9)	141.6 (0.6)	n/a
WCST: CC	> 16%	> 16%	> 16%	n/a
WCST: TCC	> 16%	> 16%	> 16%	n/a
WCST: FMS	> 16%	> 16%	> 16%	n/a
WCST: LL	> 16%	> 16%	> 16%	n/a
fMRI Stroop Performance				
Incongruent RT (ms)	804.4 (39.5)	799.7 (26.2)	848.8 (45.7)	901.8 (37.8)
Neutral RT (ms)	696.1 (36.1)	684.7 (22.3)	725.1 (40.6)	735.0 (27.7)
Congruent RT (ms)	652.8 (31.5)	626.2 (32.7)	669.1 (37.2)	613.1 (25.4)
Stroop Effect (ms)	108.3 (15.8)	115.0 (12.5)	123.7 (14.6)	166.8 (21.1)
Interference (%)	15.9 (2.5)	17.0 (2.0)	17.5 (2.2)	22.8 (2.7)
Incongruent Errors (%)	2.5 (0.7)	6.5 (1.6)	6.5 (1.5)	14.2 (1.8)*
Neutral Errors (%)	1.6 (0.6)*	0.3 (0.2)	2.1 (0.5)	1.7 (0.5)
Congruent Errors (%)	0.4 (0.2)	0.2 (0.1)	0.9 (0.3)	0.3 (0.1)

Table 3.1 Demographic, neuropsychological test battery, and Stroop fMRI task performance information. Behavioral measures are represented as the mean for each group and standard error is listed in brackets. MoCA = Montreal Cognitive Assessment, MMSE = Mini Mental State Examination, TMT = Trail Making Test, WMS = Wechsler Memory Scale, LNS = Letter Number Sequencing, Fwd SS = Forward Spatial Span, Bck SS = Backward Spatial Span, CVLT-II = California Verbal Learning Test (standard score), SDFR = Short delay free recall, SDCR = Short delay cued recall, LDFR = Long delay free recall, LDCR = Long delay cued recall, DRS = Mattis Dementia Rating Scale, WCST = Wisconsin Card Sorting Test (mode of percentiles, U.S. census age-matched), CC = Categories completed, TCC = Trials to complete 1st category, FMS = Failure to maintain set, LL = Learning to learn. Significant group differences * $p < .05$.

3.3.2 Neuropsychological Testing

All participants completed a cognitive tests battery that lasted approximately 1.5 hours (Table 3.1). Global cognition was assessed using the Montreal Cognitive Assessment (MoCA) (Nasreddine, et al., 2005), Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), and the Mattis Dementia Rating Scale version 2 (Mattis, 1988). Verbal memory was evaluated using the California Verbal Learning Test (CVLT) (Delis, Kramer, Kaplan, & Ober, 2000). Components of The Wechsler Memory Scale (WMS) Third Edition (forward/backward digit span and letter number sequencing) were used to test working memory (Wechsler, 1997). The Trail Making Test (part B) (Reitan, 1958) and the Wisconsin Card Sorting Test (Heaton, Chelune, Talley, Kay, & Curtiss, 1993) measured executive functioning. Participants also completed a paper version of the Stroop task (Stroop, 1935), which required participants to make a verbal response indicating the ink color from a possible 112 items on a stimulus sheet within two minutes. In order to be included in the study as a control participant, all cognitive tests had to be within the normal range considering age, sex, and years of education. Participants with aMCI underwent the same test battery and were diagnosed based on

clinical guidelines as well as performance on the CVLT with sub-scores below 1.5 standard deviations below the mean normalized for age, sex, and years of education, which is indicative of significant memory impairment.

The participants diagnosed with AD had significantly less years of education compared to their control group $t(28) = 2.17, p < .05$, as did the aMCI group $t(26) = 2.16, p < .05$ (Table 3.1). Participants in the aMCI group had significantly reduced scores on the MoCA $t(17.58) = 3.90, p < .05$, MMSE $t(21) = 2.66, p < .05$, SDFR $t(22) = 7.10, p < .001$, SDCR $t(22) = 5.75, p < .001$, LDFR $t(23) = 8.49, p < .001$, LDCR $t(23) = 6.79, p < .001$, LNS $t(21) = 3.47, p < .05$, forward spatial span $t(21) = 2.22, p < .05$ and backward spatial span $t(21) = 2.52, p < .05$. The Participants with AD completed a reduced version of the test battery. Compared to controls, participants in the AD group had reduced scores on all tests that were conducted, including the MoCA $t(32) = 7.42, p < .001$, MMSE $t(29) = 10.31, p < .001$, Stroop $t(26) = 6.69, p < .001$, LNS $t(26) = 4.00, p < .001$, forward spatial span $t(27) = 3.96, p < .001$, backward spatial span $t(27) = 3.02, p < .001$, and TMT $t(11.60) = -3.59, p < .01$ (Table 3.1).

3.3.3 MRI Protocol

Each participant underwent an MRI session lasting approximately 1.5 hours. Participants were scanned using the Siemens 3T Magnetom Trio system (Siemens Medical Systems, Erlangen, Germany) with a 12-channel head coil at Queen's University. The protocol included a T1-weighted, 3D magnetization-prepared rapid gradient echo (MPRAGE) anatomical scan (single shot, ascending sequence in the sagittal plane with 176 slices, TR = 1760 ms, TE = 2.2 ms, flip angle = 9°, FoV = 256

mm, 1 mm isotropic voxels, spanning the entire brain), and eight functional runs of the Stroop task, each taking 4 minutes and 12 seconds. The functional runs acquired 125 images using T2*-weighted gradient echo-planar imaging parallel to the anterior commissure and posterior commissure (AC-PC) line (32 slices in the axial plane, TR = 2000 ms, TE = 30 ms, flip angle = 78°, FoV = 211 mm, 3.3 mm isotropic voxels).

3.3.4 fMRI Stroop Task

We used a rapid event-related version of the Stroop task (Tam, Luedke, Walsh, Fernandez-Ruiz, & Garcia, 2015). Optseq (<http://surfer.nmr.mgh.harvard.edu/optseq/>) was used to randomly generate the timing of events for the rapid event-related design. A PC computer (Intel Core i7 3770K 3.5 GHz) was used to run the experiment, which was projected (NEC LT265 DLP projector) onto a rear projection screen located at the bore of the magnet near the participant's head where a mirror fixed to the head coil allowed participants to view the stimulus display screen. At the beginning of each run an instruction screen appeared reminding the participant to “say the color of the ink”, at which time the vocal recordings began, followed by the trial-by-trial presentation of each of the three conditions. The conditions were congruent (i.e., the color word matches the color of ink it is written in), incongruent (i.e., the color word does not match the color of ink it is written in) and neutral words (i.e., common neutral words matched for letter frequency to the color words used). The color words were green, yellow, red and blue, and the neutral words were home, day, finger and chair, and were all written in four possible colors of ink: red, blue, yellow and green. Each word was presented individually at the center of the screen on a black background for a duration of 1000 ms. Trials were

separated by interstimulus intervals (white crosses), which were centered on the screen and randomly jittered for a period ranging from 1000 - 19000 ms. Each run consisted of 15 trials per condition. Prior to entering the MRI, all participants underwent a short training period to familiarize them with the task, as well as to ensure correct color discrimination.

3.3.5 Behavioral Analysis from the fMRI Stroop Task

Vocal responses during the fMRI Stroop task were recorded using an optical microphone (<http://www.magmedix.com>) and analyzed outside of the MRI using Audacity (<http://audacity.sourceforge.net/>). Statistical analyses were carried out using SPSS version 28.0 (SPSS, Chicago, IL, USA). Incorrect or inaudible responses were excluded from reaction time calculations. For each participant, reaction times for individual trials of the congruent, incongruent and neutral conditions were calculated by subtracting the time at which the participant made their response with the time of stimulus presentation and averaged across conditions. The Stroop effect was calculated by subtracting the average incongruent reaction time by the average congruent reaction time for each participant, and then each participant's value was averaged to get the Stroop effect for each group. The percentage of errors was calculated for each participant by dividing the number of errors for each condition by the total number of trials completed of each condition and averaged for each group. Percentage of interference was calculated in order to control for generalized slowing $[(\text{average incongruent RT} - \text{average neutral RT}) / \text{average neutral RT}]$ (Langenecker, Nielson, & Rao, 2004; Spieler, Balota, & Faust, 1996).

Because we had a relatively small sample size in each group, determining the distributions of the behavioral data was important for choosing the appropriate statistical measures. A Shapiro-Wilk test revealed that incongruent reaction time $W(17) = .86$, $p < .05$, neutral reaction time $W(17) = .79$, $p < .01$, percentage of interference $W(17) = .83$, $p < .01$, percentage of incongruent errors $W(17) = .83$, $p < .001$, neutral errors $W(17) = .79$, $p < .01$, and congruent errors $W(17) = .74$, $p < .01$ were significantly non-normal in the AD-controls. In the AD group the Stroop effect $W(17) = .87$, $p < .05$, neutral errors $W(17) = .83$, $p < .01$, congruent errors $W(17) = .63$, $p < .001$ were significantly non-normal. Levene's test for homogeneity of variance revealed that the variances were equal for all behavioral measures except for congruent errors $F(1, 32) = 4.40$, $p < .05$ in the AD and AD-controls. The aMCI-control group revealed significantly non-normal data in neutral errors $W(13) = .73$, $p < .01$ and congruent errors $W(13) = .56$, $p < .001$, and in the aMCI group the Shapiro-Wilk test was significant for neutral reaction time $W(13) = .83$, $p = .017$, neutral errors $W(13) = .43$, $p < .001$ and congruent errors $W(13) = .45$, $p < .001$. Levene's test revealed that variances were equal for all behavioral measures except for incongruent errors $F(1, 24) = 11.40$, $p = .002$ and neutral errors $F(1, 24) = 7.87$, $p = .01$ in the aMCI and aMCI-control group. As a result of some of the reaction time and error scores being significantly different from a normal distribution, as well as the variances being significantly different between the groups, the data were transformed using the Aligned Rank Transform procedure followed by a mixed ANOVA and post-hoc pairwise comparisons using ART-C, resulting in a non-parametric factorial analysis (Wobbrock, Findlater, Gergle, & Higgins, 2011; Elkin & Wobbrock, 2021). We set out to assess if there was an interaction between condition type (incongruent, neutral and congruent) and

group (patient versus control) on reaction time. The dependent variable was reaction time, and the independent variables were condition (incongruent, congruent and neutral) as the within-subjects factor and group (AD, aMCI and healthy control) as the between-subjects factor. In the instance that sphericity was violated, a Greenhouse-Geisser correction was applied.

Since Stroop effect and percentage of interference did not violate assumptions of normality, Independent-Samples t-tests were conducted in order to assess if there were differences on average Stroop effect and percentage of interference between participants with AD or aMCI compared to controls.

3.3.6 fMRI Preprocessing and Head Movement Analysis

fMRI data from the Stroop task were analyzed using BrainVoyager QX (Brain Innovation, Maastricht, the Netherlands). The first two scans of each functional run were dropped for the MRI to reach steady state. Each functional run was preprocessed including slice scan time correction, head motion detection and correction and removal of linear and non-linear trends. Since participants were required to speak in the MRI potentially resulting in movement artifacts, any runs in which head movement was greater than 3.3 mm in translation (in mm) in the X, Y and Z plane, as well as rotation (in degrees) in three dimensions: pitch, yaw, and roll were discarded. Of all the viable runs, an overall head motion estimate was calculated for each participant and averaged across groups to see if there were any differences in movement between aMCI, AD and their respective control groups. Using a one-way ANOVA with Tukey's post-hoc test, there were no significant differences between the groups on average head motion across all

viable runs in all six directions (dx, dy, dz, rx, ry, rz). Of all functional runs for each group, 2.9% of runs were excluded in the aMCI matched controls, 1.0% in the aMCI group, 2.3% in the AD matched controls, and 6.3% in the AD group due to motion exceeding 3.3 mm in any direction. After ensuring the functional runs were under our cut-off for motion, each participant's functional run was then co-registered to their respective MPAGE anatomical scan and then transformed into Talairach space (Talairach & Tournoux, 1988). Spatial smoothing was applied with a full width at half maximum Gaussian kernel of 8 mm.

3.3.7 fMRI Data Analysis

Individual time course protocols were created for each participant for each run to include the following predictors: correct congruent, neutral and incongruent trials. A deconvolution analysis was performed on the predictors to estimate the hemodynamic response function by temporally sectioning it into 10 points, with each point representing 2 seconds of the BOLD time course. Regressors based on the three Stroop conditions (incongruent, neutral, congruent) were included that resulted in a single study general linear model (GLM) with a total of 30 regressors. The data from the 17 participants with AD, 13 participants with aMCI, 17 AD-matched and 13-MCI matched controls were input into separate multi-subject random effects general linear models and corrected for serial autocorrelations using percent transform time course normalization. Several planned contrasts were conducted to investigate neural activity related to the Stroop effect for each group. This included incongruent > neutral, incongruent > congruent, and

congruent > neutral conditions during the peak of the hemodynamic response function, which was approximately 6 seconds following stimulus onset.

3.3.8 Region of Interest Analysis

Coordinates for ROIs were the exact same as Chapter 2 and were defined using the NeuroSynth database (www.neurosynth.org) (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011). Using the search term ‘Stroop’ from the NeuroSynth database led to the generation of 11 ROIs from 225 studies. The resulting association map was corrected for multiple comparisons using a false discovery rate (FDR) of $p < .01$ and was thresholded at $Z > 5.1$ to generate the peak of each cluster (Table 3.2). The ROIs included three distinct clusters in the anterior cingulate cortex (ACC) (left, right and dorsal), bilateral inferior frontal gyrus (IFG), bilateral parietal lobes, left dorsolateral prefrontal cortex (DLPFC), left visual cortex, insula, and left intraparietal sulcus (IPS). The coordinates were transformed from MNI to Talairach space using the Lancaster transform (`icbm2tal`) in GingerALE (version 3.0.2). A spherical ROI consisting of 257 voxels was drawn around the foci of each cluster.

Hemisphere	ROI	Talairach Coordinates (X, Y, Z)
Right	ACC	4, 16, 39
Left	ACC	-5, 27, 34
Right	dACC	14, 3, 42
Left	DLPFC	-40, 16, 30
Left	IFG	-39, 2, 32
Right	IFG	40, 9, 37
Left	Parietal	-31, -53, 33
Right	Parietal	30, -53, 29
Left	IPS	-22, -70, 36
Left	Insula	-33, 7, 18
Left	Visual	-22, -77, 5

Table 3.2 List of 11 ROIs from the Neurosynth meta-analysis using the search term “Stroop”. Talairach coordinates represent the peak of the activation. ACC = Anterior cingulate cortex, dACC = Dorsal anterior cingulate cortex, IFG = Inferior frontal gyrus, IPS = Intraparietal sulcus, DLPFC = Dorsolateral prefrontal cortex.

Next, a random effects general linear model was performed within each spherical ROI. BOLD time courses were extracted and averaged for all three conditions, incongruent, congruent and neutral. Independent sample t-tests were conducted to compare the magnitude of the BOLD responses at the peak (6 second time lag) for the incongruent, congruent and neutral condition in each of the 11 ROIs identified using NeuroSynth in participants with AD and aMCI with their respective control groups. Repeated measures ANOVAs were conducted within each group to assess if activity during incongruent, congruent and neutral trials differed in the 11 ROIs. A Greenhouse-Geisser correction was applied to those ROIs in which the assumption of sphericity was violated.

3.3.9 Correlations Between fMRI and Behavior

Kendall’s partial correlations were conducted in order to assess the relationship between behavioral measures collected from computerized trial-by-trial version of the Stroop task and magnitude of the BOLD response from the 11 ROIs (IBM.com). We controlled for years of education since participants with AD and aMCI both had significantly less years of education compared to their respective control groups. We also controlled for age, since in our previous study (see Chapter 2) we found several Stroop related ROIs changed as a function of increasing age. More specifically, correlations were completed between reaction times and percentage of errors for incongruent,

congruent and neutral conditions, Stroop effect, and percentage of interference with the magnitude of the BOLD responses from each ROI during peak response from incongruent, congruent and neutral conditions in AD, aMCI, control groups, and all groups combined, while controlling for years of education and age. However, the sampling distributions are unknown for Kendall's partial rank correlations ($\tau_{xy,z}$) (Galla, 1987). In order to account for multiple comparisons a Bonferroni correction was applied to the p-value resulting in a significance of $p < .006$ ($p = .05/8$ behavioral measures).

3.4 Results

3.4.1 Behavioral Results from the fMRI Stroop Task

We calculated each participants average reaction times for each condition (incongruent, congruent, and neutral), Stroop effect (average incongruent minus average congruent reaction times), percentage of interference (Stroop effect/neutral reaction time), and percentage of incongruent, neutral and congruent errors across all viable runs. Individual measures were then averaged across groups to get an average Stroop performance measure (e.g., incongruent reaction time) for the aMCI-matched controls, aMCI, AD-matched controls and AD groups (Table 3.1, Figure 3.1).

Comparing reaction time data from participants with AD with their control group revealed that there was no significant effect of group, indicating that reaction times for each condition were generally the same for AD and AD-controls. However, regardless of group, reaction times to each condition (incongruent, congruent and neutral) were

significantly different $F(1.53, 48.79) = 115.67, p < .001, \text{partial } \eta^2 = .78$. Pairwise comparisons showed that incongruent reaction times were longer compared to both congruent ($p < .001$) and neutral trials ($p < .001$), and neutral trials took longer to respond to than congruent trials ($p < .001$). There was a significant interaction between group (AD or AD-control) and reaction time for each condition (incongruent, congruent and neutral) $F(1.41, 44.99) = 8.73, p < .01$. To break down this interaction contrasts were performed comparing reaction times for each condition across participant groups. These revealed a significant interaction when comparing AD and controls to incongruent trials compared to congruent trials $F(1, 32) = 9.88, p < .01, r = .49$. Reaction times were similar for neutral trials in both participants with AD and controls, however participants with AD produced longer reaction times to incongruent trials and healthy controls had longer reaction times for congruent trials. Therefore, participants with AD were more prone to having longer response times when there is a higher conflict between the stimulus dimensions (color and word do not match) versus trials with low conflict (congruent) than healthy elderly, and the opposite was found for healthy elderly.

An investigation of error rates revealed a significant effect of group $F(1, 32) = 11.78, p < .01, \text{partial } \eta^2 = .27$. Pairwise comparisons showed that participants with AD made significantly more errors compared to controls ($p < .01$). There was also a significant effect of condition on error rates $F(2, 64) = 72.81, p < .001, \text{partial } \eta^2 = .70$. Pairwise comparisons revealed that more errors were committed for incongruent trials versus congruent trials ($p > .001$) and neutral trials ($p < .001$), and more neutral errors compared to congruent ($p < .05$). The interaction between group and error rates for each condition was also significant $F(1.40, 44.68) = 20.46, p < .001$. Contrasts showed a

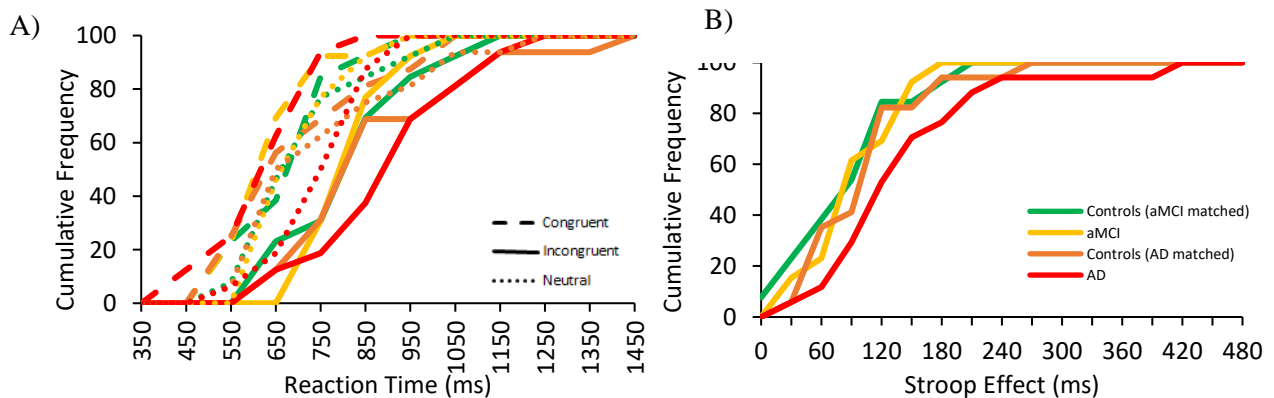
significant interaction between incongruent versus neutral errors committed $F(1, 32) = 22.25, p < .001$, comparing AD and controls, as well as incongruent and congruent errors committed $F(1, 32) = 23.69, p < .001$. Therefore, there were more incongruent errors produced when the trial was incongruent compared to neutral in participants with AD compared to healthy elderly. Conversely, there were more congruent errors committed by healthy elderly in comparison to incongruent errors. Therefore, a similar conclusion can be drawn to the reaction time data; patients with AD produced more errors when the stimulus was incongruent compared to healthy controls, and healthy elderly made more errors in low conflict trials.

Behavioral results from the aMCI comparison followed a similar pattern. There was a significant effect of condition on reaction times, irrespective of group $F(1.59, 37.94) = 62.24, p < .001, partial \eta^2 = .72$. Pairwise comparisons revealed that incongruent reaction times were longer compared to both congruent ($p < .001$) and neutral trials ($p < .001$), and responses to neutral trials were longer compared to congruent trials ($p < .001$). There was no significant effect of group on reaction time data comparing the aMCI with controls, nor was there a significant interaction between group (aMCI or control) and reaction time for each condition. This indicates that reaction times were not disproportionately different across conditions between participants with aMCI and aMCI-controls.

Analysis of percentage of errors for the aMCI and control groups revealed no significant effect of group, demonstrating that error rates were similar for both aMCI and control participants. There was a significant effect of condition on errors $F(1.62, 38.97) = 27.70, p < .001, partial \eta^2 = .54$. Pairwise comparisons revealed more errors were

produced across the groups in the incongruent condition compared to the neutral condition ($p < .001$) as well as the congruent condition ($p < .001$). More errors were also committed during neutral trials compared to congruent trials ($p < .05$). There was a significant interaction between group and errors committed across the three conditions $F(1.36, 32.59) = 8.89, p < .01$. Contrasts showed that there was a significant interaction between incongruent errors and neutral errors, comparing aMCI and controls $F(1, 24) = 16.09, p < .001$, as well as incongruent versus congruent errors $F(1, 24) = 5.23, p < .05$. Participants with aMCI and aMCI-controls differed on error rates for incongruent compared to neutral trials, with participants in the aMCI group producing more incongruent errors and less neutral errors. Healthy controls produced more congruent errors compared to incongruent errors in comparison to participants with aMCI.

On average, participants with AD produced a larger Stroop effect ($M = 166.8$ ms, $SE = 21.1$) and percentage of interference ($M = 22.8, SE = 2.7$) compared to controls ($M = 123.7, SE = 14.6, M = 17.5, SE = 2.2$), however results from the independent-samples t-test revealed that this difference was not significant (Table 3.1, Figure 3.1). Similarly, participants with aMCI also had a larger Stroop effect ($M = 115.0, SE = 12.5$) and percentage of interference ($M = 17.0, SE = 2.0$) compared to controls ($M = 108.3, SE = 15.8, M = 15.9, SE = 2.5$), yet again these differences were non-significant.



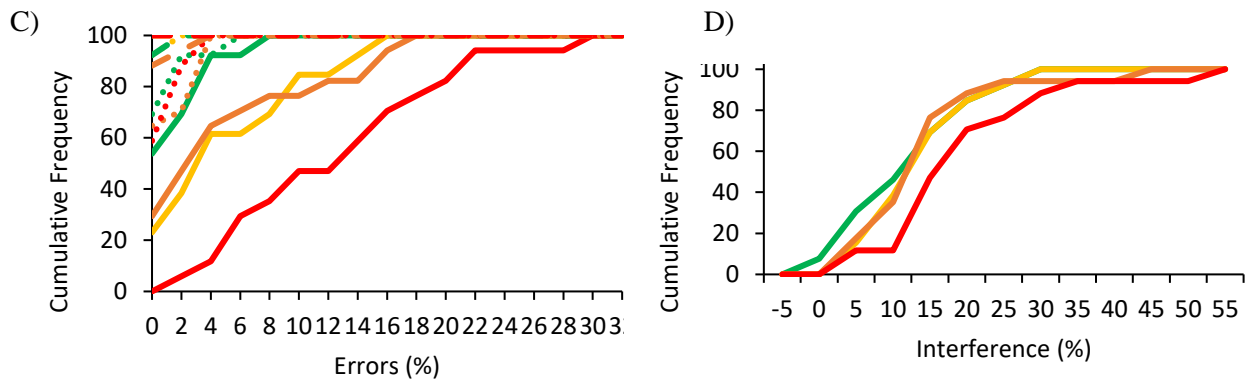


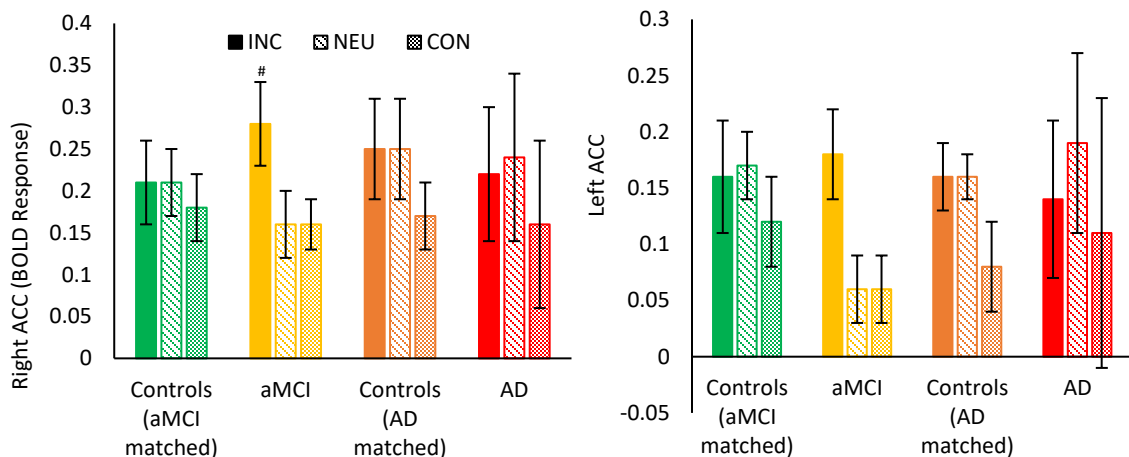
Figure 3.1 Cumulative frequency distributions for behavioral response data for the fMRI Stroop task for each of the four groups (green = MCI-matched controls, yellow = MCI, orange = AD-matched controls, red = AD). A) Reaction times (ms) for each condition: Incongruent, neutral and congruent. B) Stroop effect (average incongruent reaction time – average congruent reaction time) for each group. C) Interference (%) (Stroop effect/neutral RT). D) Percentage of errors for incongruent, neutral and congruent conditions for each group.

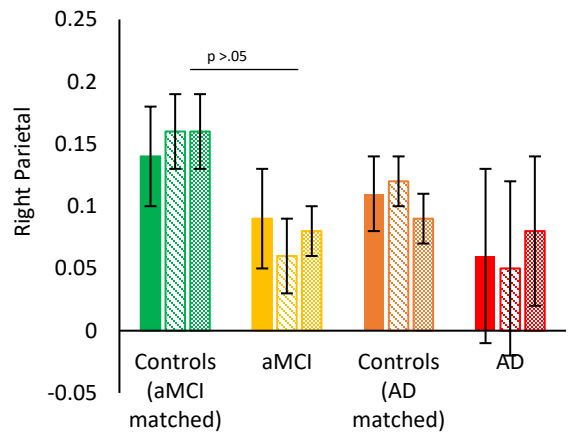
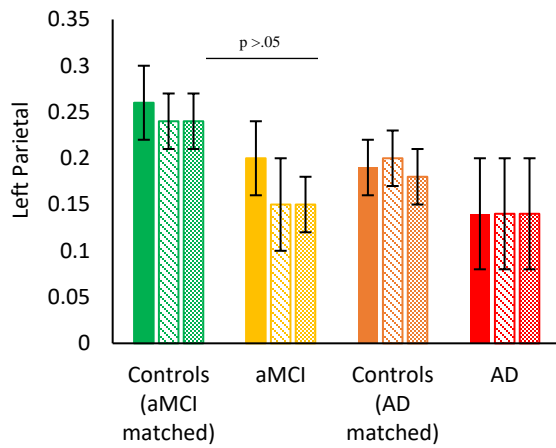
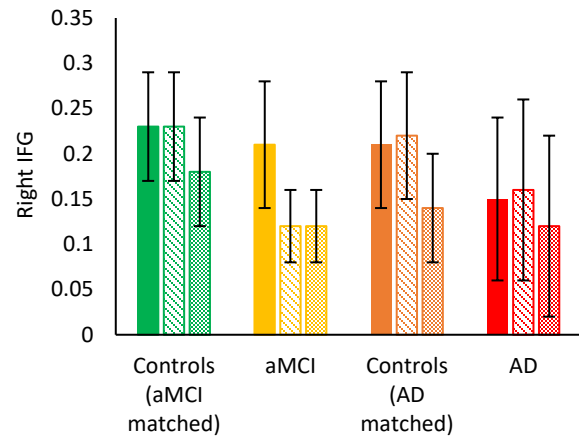
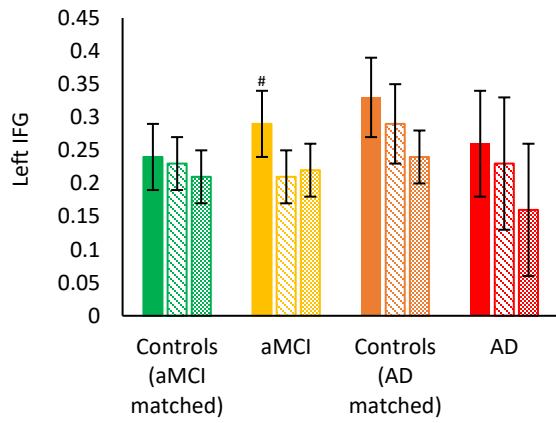
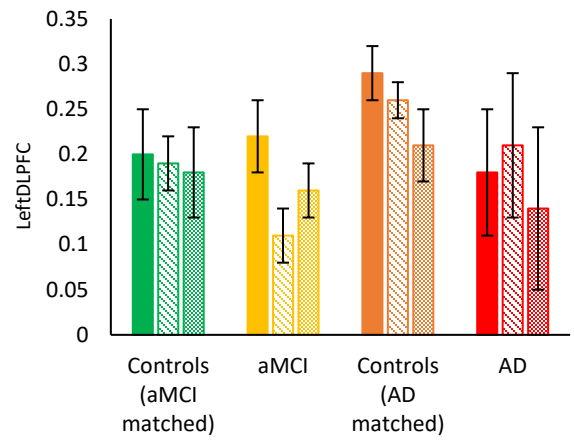
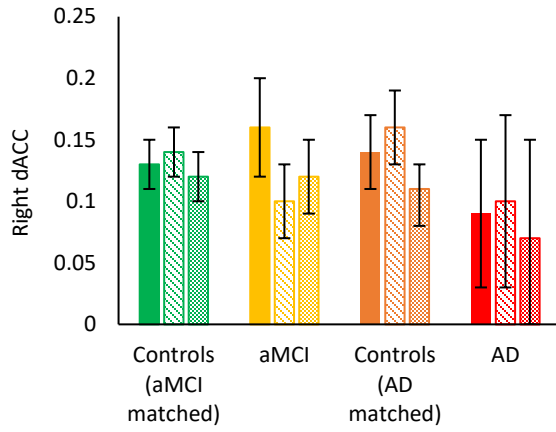
3.4.2 ROI Imaging Results from the fMRI Stroop Task

Participants in the aMCI-control group had significantly higher activity in the right parietal ROI during neutral trials compared to participants with aMCI $t(24) = 2.33$, $p < .05$ (Figure 3.2). A similar pattern was approaching significance in the left ACC (neutral) ROI $t(24) = 1.99$, $p = .058$. The left parietal ROI during congruent trials also showed greater activity in the aMCI-control group $t(24) = 2.12$, $p < .05$. There were no significant differences in activity during incongruent trials between aMCI and aMCI-controls across all the 11 ROIs. Within-group analyses revealed several differences in activity across the three conditions in the aMCI group. Activity in the right ACC showed differences across conditions $F(2, 24) = 6.60$, $p < .01$. Pairwise comparisons showed that activity was higher during incongruent trials versus congruent trials ($p = .021$) and

marginally greater in incongruent versus neutral trials ($p = .066$). A similar pattern was found in the left IFG $F(2, 24) = 5.38, p < .05$ (incongruent > congruent, $p = .005$; incongruent > neutral, $p = .079$). The left ACC was approaching significance $F(1.32, 15.83) = 3.27, p = .081$ (incongruent > congruent, $p = .063$), as was the DLPFC $F(1.34, 16.10) = 3.69, p = .063$ (incongruent > congruent, $p = .065$). No differences between activity in incongruent, congruent and neutral trials were identified in the aMCI-control group in any of the ROIs.

There were no statistically significant differences in the magnitude of BOLD response in any of the ROIs for all conditions between AD and AD-controls (Figure 3.2). Within the AD-controls, activity in the left IFG was trending towards being different across the three conditions $F(1.35, 21.57) = 3.53, p = .063$, with greater mean magnitude of BOLD response in the incongruent condition compared to the congruent condition ($p = .077$). Participants in the AD group showed no within group differences in activity during incongruent, congruent or neutral trials in any of the ROIs.





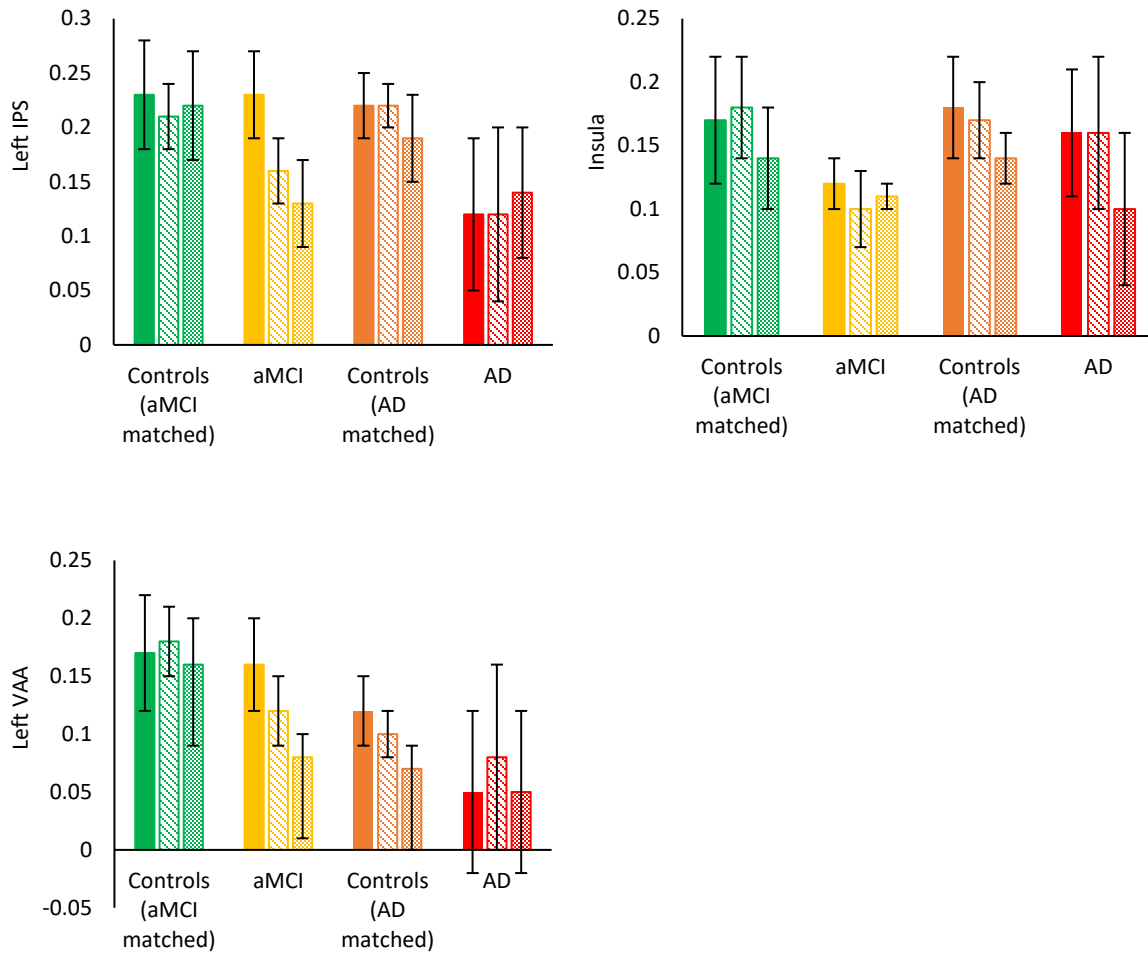


Figure 3.2 Average magnitude of BOLD responses within 11 ROIs for incongruent, congruent and neutral related conditions during the Stroop task. Significant between group comparisons (aMCI versus controls, and AD versus controls) are denoted by $p < .05$, and within-group differences for incongruent > congruent by #, $p < .05$.

3.4.3 Correlations Between ROIs and Stroop Behavior

In order to establish the relationship between neural activity and behavioral performance on the Stroop task Kendall's partial correlations were conducted between the magnitude of BOLD responses within the 11 ROIs during incongruent, congruent and neutral trials with behavioral measures from the Stroop task while controlling for years of education and age. Behavioral measures included reaction times for each trial type,

accuracy, Stroop effect and percentage of interference. Correlations were calculated within each of the groups: aMCI, AD and healthy controls, as well as all groups combined.

Average reaction times for incongruent ($\tau_b = .71$, $p = .007$) and neutral ($\tau_b = .72$, $p = .006$) stimuli correlated with the magnitude of the BOLD response during incongruent trials in the left parietal ROI in patients with AD. There were no significant correlations between the magnitude of BOLD responses and Stroop performance within the aMCI or aMCI-control groups, or the AD-control group.

With all groups combined, longer reaction times across the three stimulus types were associated with higher magnitude of BOLD responses within the insula (incongruent condition) and right parietal (congruent condition), although none remained significant after correcting for multiple comparisons. However, at an uncorrected p-value of $p < .05$, the magnitude of BOLD response in the insula during incongruent trials positively correlated with incongruent reaction time ($\tau_b = .28$, $p = .047$), neutral reaction time ($\tau_b = .30$, $p = .029$), as well as the insula during congruent trials with neutral errors ($\tau_b = .27$, $p = .049$). Neutral reaction times were also positively correlated with the magnitude of BOLD response within the right parietal during congruent trials ($\tau_b = .29$, $p = .035$). Stroop effect and percentage of interference was not related to magnitude of BOLD response in any of the ROIs in any individual group.

3.5 Discussion

The goal of this study was to investigate inhibitory control using a computerized trial-by-trial version of the Stroop task adapted for fMRI in people diagnosed with AD and aMCI as well as healthy older adults. Inhibitory control was analyzed using

behavioral data as well as the magnitude of BOLD responses from independently defined ROIs relating to the term “Stroop” from the Stroop task. Our aim was to ameliorate discrepancies in the literature as to how and if inhibitory control as measured by the Stroop task changes as a function of pathological aging. Overall, we found subtle changes between aMCI and the aMCI-control group, and minimal changes comparing the AD group with AD-controls.

3.5.1 Stroop Task Performance in AD and aMCI

We investigated behavioral measures from a computerized trial-by-trial version of the Stroop task in people with AD, aMCI and healthy controls. This included reaction times and error rates for incongruent, congruent and neutral trials, as well as the Stroop effect and percentage of interference. We found that participants with AD were prone to having longer response times when there was a higher conflict within the stimulus dimensions (color and word do not match) versus trials with low conflict (color and word match) compared to healthy elderly, and the opposite was found for healthy elderly. Increased incongruent errors as well as facilitation in individuals with mild AD relative to controls was also noted in a previous study, which the authors suggest could be due to more dependence on the word aspect of the stimuli, which happens to be the correct response for the congruent condition (Spieler, Balota, & Faust, 1996).

In our study, a direct comparison of reaction times for each condition as well as calculations for Stroop effect and percentage of interference yielded no significant difference between the groups. Stawarczy, Grandjean, Salmon, & Collette (2012) also reported no significant difference in reaction times between patients with AD and controls. Our results are somewhat in conflict with those of Coubard et al. (2011), in

which the authors suggest that attention changes differentially between healthy aging and AD. More specifically, the study by Coubard, et al. (2011) put forward the idea that AD does not affect inhibitory control as measured by the Stroop task, but instead AD has a negative impact on executive functioning and planning, or setting attention, preparing attention and task switching. However, calculations from the Stroop task were different from our study. In Coubard et al. (2011), a Stroop interference ratio was calculated by dividing the number of correct responses to an incongruent condition by correct responses to a control condition, which was naming the colors of crosses. Errors were calculated in a similar way. Implementation of color naming symbols and non-words has been used in previous studies as a control condition (MacLeod, 1991). One possibility for the lack of robust behavioral differences between participants with AD and controls is the stimulus type used, as participants may differ in their approach to symbols versus words and reading (Coubard, et al., 2011).

In terms of accuracy, participants with AD produced more errors overall compared to the control group, and more errors were committed during incongruent trials versus neutral and congruent. Increased errors in participants with AD, especially in the incongruent condition, have been reported in the literature (Stawarczy, Grandjean, Salmon, & Collette, 2012; Balota, et al., 2010; Bowes, Stroman, & Garcia, 2012). A retrospective study by Balota et al. (2010) investigated the potential for behavioral biomarker measures from a trial-by-trial computerized version of the Stroop task in 47 healthy older adults with a subsequent 12 that converted to AD. The authors found that incongruent errors were the strongest predictor of future conversion from healthy to AD. We also found that participants with AD tended to have a larger Stroop effect and

interference, although direct comparisons with healthy controls yielded non-significant results. This finding is somewhat supported by conclusions from a meta-analysis of patients with AD and the Stroop task state that the Stroop effect (incongruent – neutral reaction times) is significantly greater for people with AD compared to healthy adults (Ben-David, Tewari, Shakuf, & Van Lieshout, 2014). Perhaps the Stroop effect calculation in our study did not reach significance as a result of our limited sample size. In addition, the study by Ben-David, Tewari, Shakuf & Van Lieshout (2014) identified two additional contributing factors: decreased speed of processing due to generalized slowing beyond healthy aging and degradation of color perception leading to impaired color naming and reading. Since in our study reaction times were similar across groups, our results do not indicate an increase in generalized slowing above and beyond normal age-related slowing. Our participants were able to successfully identify the ink colors presented during the task. Furthermore, the Stroop effect, and more importantly percentage of interference, which helps control for generalized slowing, were not significantly different between groups. The participants with AD in our study were considered mild. It is possible that with greater disease severity there could be further slowing and increased interference effects (Nebes & Brady, 1992). Results from our study provide evidence that mild AD results in more incongruent errors, indicating an initial breakdown of inhibitory control.

Behavioral performance was similar between participants with aMCI and controls on all measures, which is in line with previous studies (Rosano, et al., 2005; Puente, Faraco, Terry, Brown, & Miller, 2014). There was however an interaction between group and accuracy for each stimuli type. More specifically, patients with aMCI produced more

errors to incongruent trials versus neutral trials compared to healthy controls, while controls made more congruent errors versus incongruent errors compared to the aMCI group. A study by Kaufmann et al. (2008) utilizing a numerical Stroop task also reported increased incongruent errors compared to controls. Thus, more errors pertaining to incongruent stimuli suggests that inhibitory mechanisms may begin to deviate from healthy aging during the aMCI stage.

3.5.2 fMRI Stroop Task in AD and aMCI

We found decreased activity in the aMCI group compared to healthy controls in the bilateral parietal ROIs, and marginally in the left ACC. In contrast, studies have described increased activity in participants with aMCI compared to controls, which has been interpreted as a compensatory mechanism to maintain behavioral performance (Kaufmann, et al., 2008; Li, Zheng, Wang, Gui, & Li, 2009). Our results conflict with a previous study that identified increased activity in the dACC, bilateral IFG, bilateral IPL, and bilateral insula in patients with MCI compared to controls during the Stroop task (Li, Zheng, Wang, Gui, & Li, 2009). A meta-analysis of the neural correlates underlying AD and MCI reported that dysfunction in the left inferior parietal lobe was associated with those that converted from MCI to AD (Schroeter, Stein, Maslowski, & Neumann, 2009). The ACC has been consistently implicated in inhibitory control and the Stroop task (MacLeod & MacDonald, 2000; MacLeod, 1991). This may suggest early deviations from healthy controls in brain areas related to the Stroop task in patients with aMCI.

Our investigations within the aMCI group revealed that participants with aMCI over-recruited the right ACC, left IFG and to a lesser extent the left ACC and DLPFC during incongruent trials versus congruent and neutral trials. One possible explanation for

this may be that when faced with stimuli containing a higher degree of conflicting information, participants with aMCI rely more on recruitment of the ACC and IFG to overcome interference and successfully inhibit the automatic reading response (Puente, Faraco, Terry, Brown, & Miller, 2014). A similar over-recruitment of the ACC during incongruent trials has been found in healthy young adults, with older adults showing an age-related additional increase in ACC activity to congruent stimuli (Milham, et al., 2002). However, this over-recruitment was not correlated with behavior. This falls in line with the dedifferentiation hypothesis discussed in Chapter 2, where there is increased activation to try and compensate for increasing pathological burden, but does not actually result in any performance benefit (Li, Lindenberger, & Sikström, 2001; Mathis, Schunck, Erb, Namer, & Luthringer, 2009). In our previous study of healthy aging and the Stroop task (see Chapter 2) we identified positive correlations between the ACC and parietal ROIs with age, with no correlations to behavior, indicating that the ACC may be susceptible to age-related changes. One study failed to report any between group differences in activations, however, found that when faced with interference (incongruent versus congruent) stimuli, a within-group analysis showed that people with aMCI recruited more voxels in the DLPFC, PCC and orbitofrontal cortex, which was absent in the control group (Puente, Faraco, Terry, Brown, & Miller, 2014). This can be related to our findings of within-group increases in the right ACC and left IFG and suggests that hyperactivation pertaining to greater interference as a form of compensation to maintain behavioral performance at a level similar to healthy controls. Furthermore, the degree of neural response to certain tasks may be impacted by disease severity (Celone, et al., 2006; Kaufmann, et al., 2008). For example, Celone et al. (2006) found that patients with

MCI with a higher Clinical Dementia Rating Scale sum-of-box score (CDR-SB), indicating more impairment, showed hypoactivation in memory related brain areas like that of patients with mild AD, whereas patients with MCI that were more mild (lower CDR-SB score), showed hyperactivation.

Interestingly, our results did not show differences in the magnitude of the BOLD response in any of the ROIs in the AD group versus controls. A study comparing patients with AD and healthy controls that implemented a block-version of the Stroop task also found similar activations between groups (Bowes, Stroman, & Garcia, 2012). This is contrary to previous findings that reported decreased activity associated with AD during the Stroop task in the dACC and DLPFC (Li, Zheng, Wang, Gui, & Li, 2009). However, our sample of participants with AD did perform quite well on the Stroop task, with behavioral performance comparable to controls apart from incongruent errors. This could suggest that our sample of patients with AD was high functioning or showing evidence of compensation by maintaining neural responses at a similar level to healthy older adults (Buckner, 2004). One study correlating Stroop measures with regional cerebral glucose metabolism (rCMglc) measured from positron emission topography (PET) in prefrontal areas found that in patients with AD, increased rCMglc was associated with worse Stroop performance (Yun, et al., 2011). When the AD group was further subdivided by disease severity, the correlations were not significant in the group with more severe AD. The authors suggest that correlations in the less severe group may represent a compensatory mechanism, which is not maintained as the disease progresses. Cognitively normal adults that were carriers of the APOE ϵ 4 allele, indicating risk of future development of AD, were found to have increased activity in frontal, parietal and hippocampal regions during

a memory task (Bookheimer, et al., 2001). This is in line with our findings from Chapter 2 in which our oldest adult group showed a lack of differentiation or specificity in neural responses across the three stimulus types, despite varying levels of interference. We found that within the AD group, more activity in the left parietal ROI during incongruent trials, or when faced with stimuli containing greater interference, was related to longer incongruent and neutral reaction times. This finding suggests that the parietal cortex may be negatively impacted by AD pathology (Buckner, 2004; Klunk, et al., 2004), and may help to explain the interaction we found between reaction time by stimulus time by group.

Another possibility for the lack of differences in magnitude of BOLD responses during the Stroop task in patients with AD compared to controls may be due to underlying genetic risk factors for development of late-onset AD. Genetic status, for example APOE ϵ 4, was unknown in our sample of participants. The influence of the APOE ϵ 4 allele has been shown to increase amyloid burden versus those without a copy of the allele (Rebeck, Reiter, Strickland, & Hyman, 1993). Therefore, it is possible that our participants consisted of a low proportion with the APOE ϵ 4 allele, or were mixed with carriers of the APOE ϵ 2 allele, which may actually reduce susceptibility of cognitive decline in those with AD (Martins, Oulhaj, de Jager, & Williams, 2005).

Correlations with all groups combined revealed a positive relationship between the insula and right parietal ROI with incongruent and neutral reaction times, and neutral errors. Interpretations must remain cautious since these partial correlations are reported at an uncorrected p-value of $p < .05$, however, it points to possible involvement of the insula and parietal lobes in the breakdown of inhibitory control. While both have been

implicated in the Stroop task, the insula, along with the dACC, has also been shown to be a hub of the salience network, which is a task positive network found to play a key role in inhibitory control (Seeley, et al., 2007).

One potential reason we found a decrease in aMCI magnitude of BOLD responses compared to controls but no significant difference in the AD group compared to controls may be due to a relatively small sample size. While we tried to ensure our sample of participants received an accurate diagnosis as AD and aMCI, the disease can be heterogenous. Increasing the sample size would help to form a more accurate interpretation of how inhibitory control may change as a function of the disease process and reduce the effects of possible outliers.

Another possibility is that compensatory mechanisms, such as recruitment of additional brain areas, was not captured by our study methods. We implemented an ROI approach to interrogate specific areas related to the Stroop task. Looking to the literature, it has been reported that patients with AD or aMCI may rely on the activation of additional brain areas during a cognitive task to aid in maintaining behavioral performance (Cabeza, Anderson, Locantore, & McIntosh, 2002; Bowes, Stroman, & Garcia, 2012).

It is also possible that the pathophysiology associated with AD does not overlap fully with the ROIs we selected from our Neurosynth meta-analysis, thus not revealing the full extent of possible dysfunction in patients with AD. The hallmark site for onset of AD pathology is the medial temporal lobes, which coincides with memory impairment, followed by progression to posterior and parietal areas and subsequently the frontal cortex (Braak & Braak, 1991). However, evidence suggests that frontal areas may be

affected by AD earlier on. More specifically, AD has been linked to a dysfunction of cholinergic neurons that project to frontal and parietal areas, including the ACC (Poudel & Park, 2022; Selden, Gitelman, Salamon-Murayama, Parrish, & Mesulam, 1998), which overlaps with brain areas related to attention and the Stroop task (Balota, et al., 2010; Nee, Wager, & Jonides, 2007).

3.5.3 Summary and Future Directions

There are several limitations to our study. The use of independently defined ROIs has some inherent limitations. While it controls for selection bias, this approach disregards individual differences in locations. This is of particular importance to consider in AD and aging in general since there is atrophy associated with both. Our small sample size is another limitation. Including a larger sample of patients would allow for a more precise representation of how inhibition, as measured by the Stroop task, may change as a function of aMCI or AD. Longitudinal studies would also allow for additional analysis to see neural differences in those that convert or do not convert from aMCI to AD or revert to normal functioning.

Future work could also benefit from the addition of other biomarkers. For example, one study investigated the relationship between common AD biomarkers with two attention tasks, including an fMRI Stroop task, in healthy adults and found that individuals with higher CSF levels of tau and phosphorelated tau (ptau) had over-activation in overlapping brain areas important for executive functioning and attention, including the DLPFC, ACC and lateral parietal cortex (Gordon, et al., 2015).

Overall, our study provides further insight into the underlying neural mechanisms involved in inhibitory control as measured by the Stroop task. While patients with AD showed a similar neural response within Stroop related ROIs to healthy controls, an increase in incongruent errors suggests an initial breakdown of inhibitory control. Our sample of patients with aMCI also showed subtle changes with errors compared to controls, in addition to decreased neural activity compared to controls, suggesting that inhibitory control may change very early on in the disease process. Due to the findings that incongruent errors seem to be particularly useful at discriminating patients with AD and even aMCI from healthy adults, future studies may benefit from investigating what brain areas correspond to accuracy. Our healthy control participants did not make enough errors for adequate power. Since task difficulty has been shown to be dependent on presentation style of the Stroop task (trial-by-trial versus card reading) (Spieler, Balota, & Faust, 1996), in order to investigate if difference exist in brain areas between AD and healthy adults underlying commission of errors, future studies may benefit from manipulating stimulus presentation rates to increase task difficulty and induce more errors.

Chapter 4

Resting State Functional Connectivity and Inhibitory Control in AD, aMCI and Healthy Elderly

4.1 Abstract

It is well known that in addition to cognitive changes, functional connectivity of various brain networks during resting-state functional magnetic resonance imaging (fMRI) are disrupted in Alzheimer's disease (AD) and amnesic mild cognitive impairment (aMCI). The purpose of this study was to further investigate inhibitory control in AD and aMCI using resting-state connectivity between brain areas found to be related to the Stroop task, a common measure of inhibitory control. Resting-state fMRI data were collected from 18 AD, 13 aMCI, and 31 age-matched healthy control participants. Seed based functional connectivity was conducted using brain areas defined independently using a meta-analysis with the search term "Stroop" in Neurosynth. The resulting 11 regions of interest (ROIs) were used as seeds and were comprised of the bilateral and dorsal anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), bilateral inferior frontal gyrus (IFG), bilateral parietal lobes, intraparietal sulcus (IPS), insula and visual association area (VAA). Contrasting both patient groups with their respective control groups revealed clusters of voxels showing significantly reduced connectivity in participants with AD compared to healthy elderly. Participants with AD had reduced functional connectivity between the left ACC seed and the left middle frontal gyrus (MFG) compared to AD-matched controls. Correlating the

functional connectivity between the left ACC and the left MFG with seeds of the DMN in patients with AD revealed that the more negative the DMN was, the greater the connectivity was in the left ACC and MFG. This finding may point towards initial changes between seeds within functionally connected networks in AD.

4.2 Introduction

Alzheimer's disease (AD) and the prodromal phase, amnesic mild cognitive impairment (aMCI), are associated with cognitive decline along with neuropathological changes that can be detected using various forms of neuroimaging, including task-based functional MRI (fMRI) and resting-state fMRI (rs-fMRI) (Badhwar, et al., 2017; Chhatawl & Sperling, 2012). In addition to memory impairments, AD also leads to dysfunction in attention and inhibitory control early in the disease progression (Spieler, Balota & Faust, 1996).

The Stroop task is a classic measure of inhibitory control where participants are required to identify the color of ink a word is written in while ignoring the written word or symbols (Stroop, 1935). Word stimuli can be congruent (color and word match, e.g., blue written in blue), incongruent (color and word do not match e.g., blue written in green), and neutral (non-color words or symbols) (MacLeod & MacDonald, 2000; Stroop, 1935). Reaction times in response to incongruent trials are typically slowest, which reflects the increased attentional demand required to inhibit the automatic reading response in order to identify the ink color (MacLeod & MacDonald, 2000; Stroop, 1935). The frontoparietal network has been attributed to supporting Stroop performance (Nee, Wager, & Jonides, 2007; Parris, et al., 2019; van Veen & Carter, 2005). More

specifically, the anterior cingulate cortex (ACC) has been reported as playing a major role in cognitive control during instances of conflicting stimuli, such as the Stroop task (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Nee, Wager, & Jonides, 2007).

Another approach to interrogate differences in brain function is through functional connectivity. Resting-state functional connectivity refers to the co-activation of brain areas in the absence of performing a task (van den Heuvel & Hulshoff Pol, 2010; Chhatawl & Sperling, 2012). Several networks have been consistently identified throughout the literature, and the two most relevant to the current study include the default mode (DMN) and salience networks (van den Heuvel & Hulshoff Pol, 2010; Fox & Greicius, 2010). It has been suggested that the salience network is related to inhibitory control and includes the dorsal anterior cingulate (dACC) and insula as major hubs, projecting to subcortical and limbic areas (Seeley, et al., 2007). Studies investigating the neural coupling of networks have shown that during moments requiring substantial attention, such as performing a cognitive task, the salience network is engaged, or more active, along with the simultaneous suppression of the DMN (Dosenbach, et al., 2006; Menon & Uddin, 2011). Thus, the salience network may be responsible for task switching and allocation of attention in the presence of salient stimuli (Menon & Uddin, 2011; Seeley, et al., 2007). Conversely, the DMN is more active while at rest (Greicius, Krasnow, Reiss, & Menon, 2003) and has been linked to episodic memory function through an increase in hippocampal activation and deactivation of the DMN (Chhatawl & Sperling, 2012). Complex cognitive functions appear to be dependent on the function of large-scale networks, which involve a balance of appropriate activation or deactivation (Chhatawl & Sperling, 2012). Previous work has shown support for the anticorrelated

relationship between the DMN and attentional networks using the Stroop task (Tam, Luedke, Walsh, Fernandez-Ruiz, & Garcia, 2015). Greater activity of brain areas comprising the DMN preceding stimulus onset was associated with longer reaction times irrespective of condition type, along with decreased activity of frontoparietal attention areas (Tam, Luedke, Walsh, Fernandez-Ruiz, & Garcia, 2015).

AD has also been viewed as a disconnection disorder, with the disruption of multiple functional networks (Wang, et al., 2015). More specifically, it has been well documented that resting-state networks including the DMN, salience and limbic networks are affected by AD and aMCI (see Badhwar et al., 2017 for a review). It is generally reported that patients with AD and aMCI show a decrease in DMN and may show an initial increase in the salience network early in the disease, followed by eventual impairment (Brier, et al., 2012). There are, however, inconsistencies in the literature as to whether the salience network is impaired in AD and aMCI. Research probing functional connectivity of the DMN in AD generally suggests that once a diagnosis of AD is reached there is substantial impairment, which may relate to the accumulation of amyloid beta in overlapping regions of the brain (Chhatawl & Sperling, 2012; Badhwar, et al., 2017). One study found no salience intranetwork differences between patients with aMCI and controls, nor significant gray matter atrophy within the salience network, but did report impaired salience network in patients with AD along with reductions in gray matter that correlated positively with MMSE scores (He, et al., 2014). However, another study found that in addition to reductions in DMN activity, patients with aMCI also showed reduced activity within the sensorimotor network, along with a decrease in internetwork connectivity between the sensorimotor and dorsal attention network (Wang,

et al., 2015). In addition, the authors did not find a difference in salience intranetwork connectivity between AD or MCI participants compared to healthy controls (Wang, et al., 2015). A longitudinal study by Zhan et al. (2016) identified a decrease in the salience network in early and more advanced MCI in the right insula and a decrease in the right putamen in later stage MCI. In contrast, Balthazar et al. (2014) reported increased activity between the right ACC and left middle frontal gyrus (MFG), along with a decrease in the precuneus (DMN) in patients with middle to moderate AD compared to controls. An increase in frontal salience network areas was associated with more neuropsychiatric symptoms, including disinhibition. In another example no difference in functional connectivity within the DMN was found in patients with aMCI, however patients with mild AD were found to have decreased functional connectivity between the ventromedial prefrontal cortex, medial parietal cortex, and inferior parietal lobe with the posterior cingulate cortex (PCC) seed of the DMN (Weiler, et al., 2014).

Studies linking Stroop performance and rs-fMRI in patients diagnosed with AD and aMCI are sparse. There are, however, several studies investigating the Stroop task and rs-fMRI in healthy older adults. One study found that poor performance on some measures of the Stroop task were related to reduced functional connectivity in the DMN and the salience networks in cognitively normal older adults (Duchek, et al., 2013). Furthermore, adults with higher cerebral spinal fluid (CSF) levels of amyloid beta ($A\beta_{42}$), a biomarker for AD, demonstrated a stronger negative relationship between functional connectivity in the DMN and Stroop performance. This suggests that the combination of resting-state fMRI and Stroop performance may be a valuable tool for assessing early AD and aMCI. Another study conducted in healthy adults aged 65+

probed the relationship between Stroop interference scores, age, and rs-fMRI connectivity of the salience network (Brewster, Smith Pasqualini, & Martin, 2022). According to the results from Brewster, Smith Pasqualini & Martin (2022), increasing age was associated with a decrease in functional connectivity within the salience network, and greater functional connectivity was associated with better performance (lower interference) on the Stroop task. The authors also failed to report a direct correlation between age and Stroop performance, suggesting that the salience network may play an important mediating role in inhibitory control with age (Brewster, Smith Pasqualini, & Martin, 2022). Here, we focus our investigation beyond networks alone and explore functional connectivity of specific brain areas related to the Stroop task in AD and aMCI.

Given the relative paucity of resting state functional connectivity and Stroop performance in AD and aMCI in the literature, the goal of this study was to determine if inhibitory control as measured by the Stroop task shows altered functional connectivity using rs-fMRI in people diagnosed with AD and aMCI compared to healthy controls. More specifically, we probed whether the seeds involved in the Stroop task, identified independently in Neurosynth (see Chapter 2 and 3), resulted in different functional connectivity maps in participants diagnosed with AD, aMCI as well as healthy adults, and if these changes correlated with Stroop performance. Since the Stroop seeds showed some overlap with main hubs of salience network, namely the ACC and insula, which are normally anticorrelated with the DMN, we also investigated if any functional connectivity differences between patient and control groups correlated with DMN activity.

4.3 Methods

4.3.1 Participants

This study was approved by the research ethics boards of Queen's University (#6004781) and Hotel Dieu Hospital. Before commencing the study, all participants were provided a letter of information detailing the study and gave written informed consent. Participants with aMCI and mild AD were recruited from a memory clinic in Kingston where they received a diagnosis according to the Peterson criteria (Petersen, et al., 2001) and the National Institute of Neurological Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) guidelines (McKhann, et al., 1984; Jack, et al., 2011). Thirteen participants with aMCI (7 females, mean age 64.8 ± 12.3 years) and 18 participants with probable mild AD (11 females, mean age 73.6 ± 6.9 years) were recruited and included in the study. In order to ensure adequate comprehension of the study and task, only patients with mild AD, as determined by a Montreal Cognitive Assessment (MoCA) score greater or equal to 18/30, were invited to participate in the study. While there is some overlap in MoCA scores between patients with AD and aMCI, generally the average MoCA score for AD is considered 16/30 (range 11-21) and 22/30 (19-25) for patients with aMCI (Nasreddine, et al., 2005). There was a total of 31 healthy adult community dwelling volunteers that were split into two control groups: 13 age and sex matched to the aMCI group (7 females, mean age 63.2 ± 11.4 years), and 18 age and sex matched to the AD group (11 females, mean age 73.4 ± 9.2 years) (Table 4.1). All participants had normal or corrected to normal vision, normal color vision, and followed all MRI safety standards.

	aMCI- matched Controls n = 13	aMCI n = 13	AD-matched Controls n = 18	AD n = 18
Demographics				
Age (years)	63.3 (3.5)	64.8 (3.4)	74.4 (1.8)	73.6 (1.6)
Sex (F/M)	7/ 6	7/6	11/7	11/7
YOE	17.9 (1.1)	15.6 (0.9)	16.8 (0.9)	14.4 (0.7)
MoCA (/30)	28.0 (0.4)	25.5 (0.6)	27.1 (0.6)	21.2 (0.6)
MMSE (/30)	29.5 (0.2)	27.8 (0.4)	28.4 (0.5)	24.3 (0.2)
fMRI Stroop Performance				
Incongruent (ms)	857.2 (60.8)	803.0 (27.1)	837.5 (36.4)	912.7 (36.3)
Congruent (ms)	694.3 (44.3)	630.4 (33.9)	654.3 (37.6)	623.5 (23.3)
Neutral (ms)	742.8 (51.8)	691.3 (22.3)	709.2 (33.5)	746.5 (25.3)
Stroop Effect (ms)	114.4 (16.1)	111.7 (12.5)	128.3 (14.3)	166.2 (21.2)
Interference (%)	15.5 (2.1)	16.3 (1.9)	18.7 (2.3)	22.3 (2.7)
INC Errors (%)	4.1 (1.0)	6.6 (1.7)	6.4 (1.5)	14.7 (1.8)
CON Errors (%)	0.9 (0.4)	0.2 (0.1)	0.8 (0.3)	0.3 (0.1)
NEU Errors (%)	1.5 (0.4)	0.3 (0.2)	1.7 (0.5)	1.8 (0.5)

Table 4.1. Summary of participant demographics and behavioral measures for average Stroop performance. INC = incongruent, CON = congruent, NEU = neutral, MoCA = Montreal Cognitive Assessment, MMSE = Mini Mental State Examination. Standard errors are shown in brackets.

4.3.2 Neuropsychological Testing

All participants completed a range of cognitive tests. Global cognition was assessed using the Montreal Cognitive Assessment (MoCA) (Nasreddine, et al., 2005), Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), and the Mattis Dementia Rating Scale version 2 (Mattis, 1988). Verbal memory was evaluated using the California Verbal Learning Test (CVLT) (Delis, Kramer, Kaplan, & Ober, 2000). Components of The Wechsler Memory Scale (WMS) Third Edition (forward/backward digit span and letter number sequencing) were used to test working memory (Wechsler, 1997). The Trail Making Test (TMT) (part B) (Reitan, 1958) and the

Wisconsin Card Sorting Test WCST) (Heaton, Chelune, Talley, Kay, & Curtiss, 1993) measured executive functioning. Participants also completed the paper version of the Stroop task (Stroop, 1935). In order to be included in the study as a control participant, all cognitive tests had to be within the normal range accounting for age, sex, and years of education. Participants with aMCI underwent the same test battery. Along with clinical guidelines, diagnosis of aMCI was considered based on performance on the CVLT with sub-scores below 1.5 standard deviations below the mean normalized for age, sex, and years of education, with all other cognitive tests within the normal range. The participants with AD completed a reduced version of the test battery, including the MoCA, MMSE, WMS, TMT, and the paper version of the Stroop task. Patients with AD had significantly lower MoCA $t(27) = -2.73, p < .05$ and MMSE $t(17.16) = -2.59, p < .05$ scores compared to AD-controls. Similarly, patients with aMCI also had significantly lower MoCA $t(24) = -3.61, p < .01$ and MMSE $t(19) = -3.92, p < .01$ scores compared to aMCI-control participants (Table 4.1).

4.3.3 Neuroimaging Data Acquisition

Each participant underwent an MRI session lasting approximately 1.5 hours. Participants were scanned using the Siemens 3T Magnetom Trio system (Siemens Medical Systems, Erlangen, Germany) with a 12-channel head coil at Queen's University. The protocol included a T1-weighted, 3D magnetization-prepared rapid gradient echo (MPRAGE) anatomical scan (single shot, ascending sequence in the sagittal plane with 176 slices, TR = 1760 ms, TE = 2.2 ms, flip angle = 9°, FoV = 256 mm, 1 mm isotropic voxels, spanning the entire brain), and eight functional runs of the

Stroop task (see Chapters 2 and 3). Each participant also underwent a 6-minute-long resting-state fMRI scan during which they were instructed to remain awake, keep their eyes open, and to not think of anything in particular. The rs-fMRI data were collected using functional T2*-weighted scans using echo-planar imaging covering the whole brain and acquired 180 volumes (32 slices acquired parallel to the anterior/posterior commissure (AC-PC) plane, TE = 30 ms, TR = 1970 ms, FoV = 211 mm, flip angle = 78°, 3.3 mm isotropic voxels).

4.3.4 fMRI Stroop Task

Each participant underwent 8 functional runs of the Stroop task (see Chapters 2 and 3). The fMRI Stroop task results provided trial-by-trial behavioral data to be used in subsequent analyses (see section 4.3.5). Participants were required to make verbal responses, which were recorded using an optical microphone (<http://www.magmedix.com>), as to the color each word was written in. The words were either congruent (i.e., the color word matches the color of ink it is written in), incongruent (i.e., the color word does not match the color of ink it is written in) or neutral words (i.e., common neutral words matched for letter frequency written in the four possible colors of ink: red, blue, yellow and green).

4.3.5 Behavioral Analysis

Verbal responses were analyzed outside of the MRI using Audacity (<http://audacity.sourceforge.net/>). All responses that were incorrect or inaudible were excluded from reaction time calculations. For each participant reaction times for individual trials of the congruent, incongruent and neutral conditions were calculated by subtracting the time at which the participant made their response with the time of

stimulus presentation and averaged across the three conditions. The averages for congruent, incongruent and neutral trials were then calculated for each group (AD, AD-controls, aMCI, and aMCI-controls). The Stroop effect was calculated by subtracting the average incongruent reaction time by the average congruent reaction time for each group. Percentage of interference was calculated by dividing the average Stroop effect by the average neutral reaction time for each participant to control for generalized slowing (Langenecker, Nielson, & Rao, 2004; Spieler, Balota, & Faust, 1996). The percentage of errors was calculated for each participant by dividing the number of errors for each condition by the total number of trials completed of each condition and averaged for each group.

Analysis of behavioral measures from the Stroop task was carried out using SPSS version 28.0 (SPSS, Chicago, IL, USA). Results from the Shapiro-Wilk test revealed that, in the aMCI group, neutral reaction times $W(12) = 0.83$, $p < .05$, and error rates for the neutral condition $W(12) = 0.45$, $p < .01$ and congruent condition $W(12) = 0.47$, $p < .01$ were significantly non-normal. Similarly, errors made during both the neutral $W(13) = 0.85$, $p < .05$ and congruent $W(13) = 0.76$, $p < .01$ conditions were non-normal for the aMCI-control group. The variances were equal for all behavioral measures except for error rates. Variances for incongruent errors $F(1, 23) = 7.56$, $p < .05$, neutral errors $F(1, 23) = 11.55$, $p < .01$, and congruent $F(1, 23) = 8.43$, $p < .01$ errors were significantly different between aMCI and aMCI-control groups.

As for the AD group, Stroop effect $W(17) = 0.87$, $p < .05$, neutral errors $W(17) = 0.83$, $p < .01$ and congruent errors $W(17) = 0.63$, $p < .01$ were significantly non-normal. The AD-control group also had several behavioral measures that were significantly non-

normal, including neutral reaction times $W(17) = 0.87, p < .05$, incongruent errors $W(17) = 0.79, p < .01$, neutral errors $W(17) = 0.79, p < .01$, congruent errors $W(17) = 0.67, p < .01$ and interference $W(17) = 0.86, p < .05$. The variances were equal between AD and AD-control groups for all behavioral measures except for congruent errors $F(1, 32) = 7.1, p < .05$.

Since some of the behavioral data showed non-normal distributions and unequal variances between the groups, the data was analyzed using non-parametric tests. The reaction time and error data were transformed using the Aligned Rank Transform procedure followed by a mixed ANOVA and post-hoc pairwise comparisons using ART-C (Wobbrock, Findlater, Gergle, & Higgins, 2011; Elkin & Wobbrock, 2021). We investigated if there was an interaction between condition type (incongruent, neutral and congruent) and group (patient versus control) on reaction time. The dependent variable was reaction time, and the independent variables were condition (incongruent, congruent and neutral) as the within-subjects factor and group (AD, aMCI and healthy control) as the between-subjects factor. In the instance that sphericity was violated, a Greenhouse-Geisser correction was applied. Group differences for Stroop effect and interference measures were carried out using the Mann-Whitney U test. The behavioral measures of Stroop performance including average reaction times for congruent, incongruent and neutral conditions, as well as Stroop effect, percentage of interference and percentage of incongruent and total errors were calculated for each group and used in subsequent analyses for correlations using Kendall's Tau between Stroop performance as a measure of inhibitory control and functional connectivity measures.

4.3.6 Preprocessing of Resting fMRI Data

Using Matlab version R2019a and SPM 12

(www.fil.ion.ucl.ac.uk/spm/software/spm12/) resting fMRI data were analyzed using the CONN toolbox (www.nitrc.org/projects/conn, Whitfield-Gabrieli & Nieto-Castanon, 2012). Each functional run was preprocessed using the default preprocessing pipeline in CONN that included slice scan time correction, head motion detection and correction and removal of linear and non-linear trends. In addition, BOLD signal from white matter and CSF masks were removed by linear regression. For each participant the resting functional run was co-registered to respective anatomical scans and transformed into Montreal Neurological Institute (MNI) space. Spatial smoothing was applied with a full-width at half maximum Gaussian kernel of 8 mm (Friston et al, 1995), and passed through a low-pass band-filter (0.01 – 0.1 Hz). In order to measure motion artifacts across all participants, the Artifact Detection Tool (ART) was used. One AD participant and two AD-matched controls were excluded due to number of invalid scans identified using ART based scrubbing.

Head motion estimates were evaluated in each participants resting functional run. Motion estimates included both translation (in mm) in the X, Y and Z plane, as well as rotation (in degrees) in three dimensions: pitch, yaw, and roll. Mean motion estimate was computed for each group (AD, aMCI, and their respective control groups). There were no significant differences in mean motion estimates between AD ($0.245 \pm \text{SD } 0.098$) and controls (0.232 ± 0.123), or between aMCI (0.233 ± 0.117) and controls (0.186 ± 0.072). However, motion estimates were controlled for in subsequent analyses using the realignment parameters defined by ART.

4.3.7 Seed Region Selection and Definition

Seeds were the same as regions of interest (ROIs) used in our previous studies (see Chapters 2 and 3), and were selected from the meta-analysis in Neurosynth showing regions involved in the Stroop task, and included the bilateral ACC, right dACC, left DLPFC, bilateral IFG, bilateral parietal lobes, left IPS, left insula and left VAA (Table 4.2) (Yarkoni, Poldrak, Nichols, Van Essen, & Wager, 2011). Spherical seeds were created with a 10 mm radius surrounding the peak of each cluster defined by our Neurosynth meta-analysis using MarsBaR (Figure 4.1) (Brett, Anton, Valabregue, & Poline, June 2-6, 2002). Each seed was input into CONN for functional connectivity analysis. The functional connectivity between each seed and the rest of the voxels in the brain was completed for each participant and compared between groups (AD versus AD-controls and aMCI versus aMCI-controls). The DMN was also assessed between groups. Seeds for the DMN were defined from CONN's independent component analysis (ICA) of 497 control subjects, and included the medial prefrontal cortex (MPFC), bilateral lateral parietal (LP), and posterior cingulate cortex (PCC) (Table 4.2).

Network	Hemisphere	ROI	MNI Coordinates (X, Y, Z)	
Stroop	Right	ACC	6, 22, 38	
	Left	ACC	-4, 32, 20	
	Right	dACC	16, 8, 42	
	Left	DLPFC	-42, 20, 28	
	Left	IFG	-40, 6, 32	
	Right	IFG	44, 14, 36	
	Left	Parietal	-32, -52, 38	
	Right	Parietal	34, -52, 32	
	Left	IPS	-22, -70, 42	
	Left	Insula	-34, 10, 16	
	Left	Visual	-22, -80, 8	
	DMN		MPFC	1, 55, -3
		Left	LP	-39, -77, 33
		Right	LP	47, -67, 29
		PCC	1, -61, 38	

Table 4.2. List of 11 ROIs from the Neurosynth meta-analysis using the search term “Stroop” and seeds from the DMN (default mode network). Montreal Neurological Institute (MNI) coordinates represent the peak of the activation. ACC = Anterior cingulate cortex, dACC = Dorsal anterior cingulate cortex, IFG = Inferior frontal gyrus, IPS = Intraparietal sulcus, DLPFC = Dorsolateral prefrontal cortex, MPFC = Medial prefrontal cortex, LP = Lateral parietal, PCC = Posterior cingulate cortex.

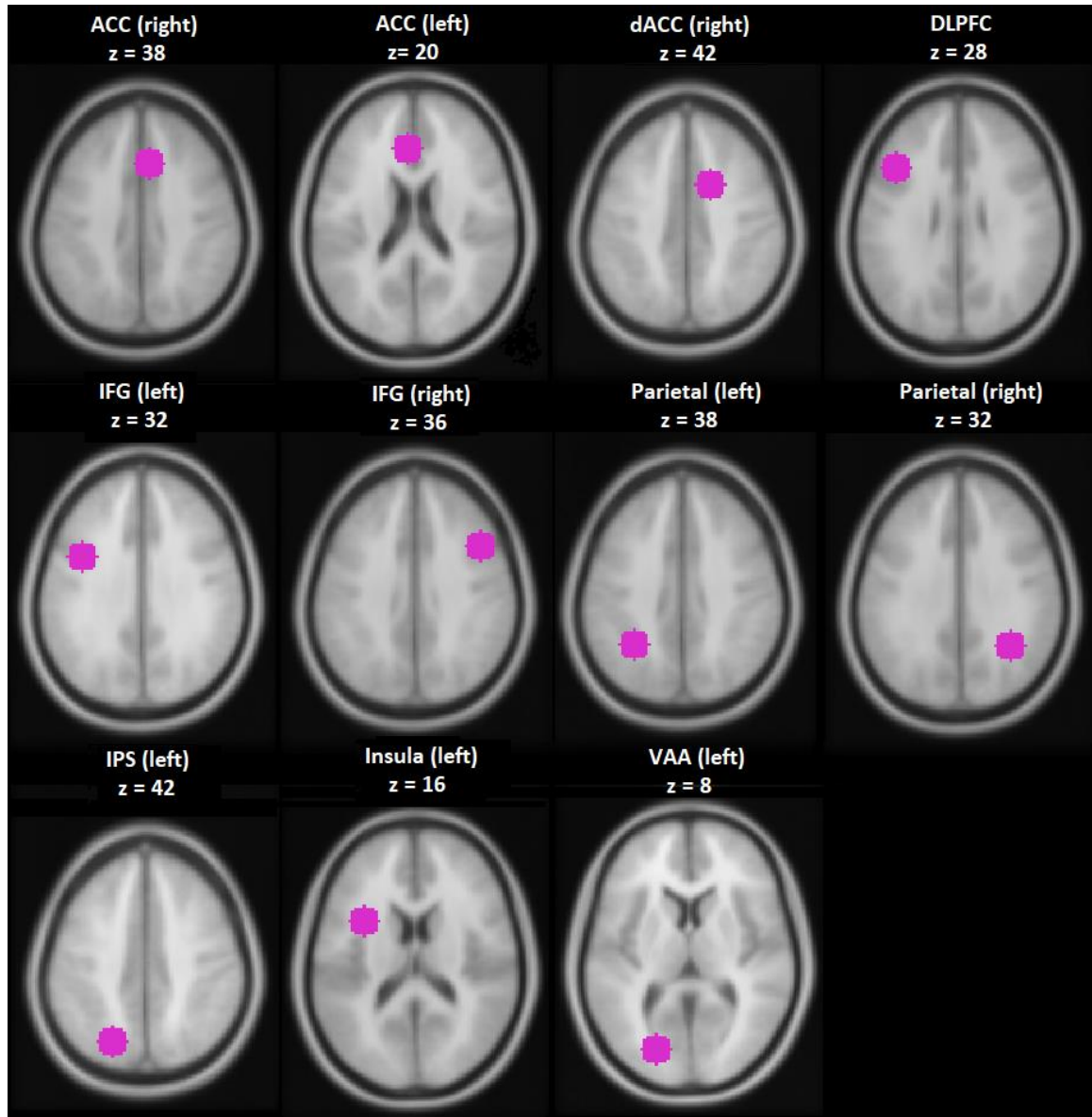


Figure 4.1. Visual representation of seeds regions used for functional connectivity analyses between patient and control groups. Spherical ROIs were drawn around the peak of each seed with a radius of 10 mm.

4.3.8 Resting fMRI Analysis

A seed-to-voxel analysis was conducted using seeds including the 11 ROIs identified from our Neurosynth analysis using the search term “Stroop” to investigate whether there were differences between patient and control groups in correlations

between the seeds and all other voxels in the brain. A seed to voxel analysis was also conducted using the DMN seeds.

The BOLD time courses of each of the seeds was extracted and correlated with voxels in the remainder of the brain to determine which areas were functionally connected to the seeds within each group. We used a two-sample t-test to investigate group differences (AD versus AD-matched controls and aMCI versus aMCI-matched controls) during rest. Functional connectivity maps were set at a height threshold of $p < 0.001$, and significant clusters were corrected at $q(\text{FDR}) < 0.05$. An additional Bonferroni correction was applied for the number of seed-to-voxel analyses, resulting in a significance value of $p < .003$ (15 seeds/.05). In the AD group, the Fisher transformed z-values representing functional connectivity strength from resulting significant clusters showing between group differences were extracted and correlated with behavioral measures from the Stroop task using Kendall's Tau correlation. Resulting between group functional connectivity differences from the Stroop seeds were correlated with functional connectivity within the DMN using Pearson correlation.

4.4 Results

4.4.1 Stroop Performance

For the aMCI and aMCI-control group, there was a significant main effect of condition on reaction time $F(1.27, 29.12) = 57.37$, $p < .001$, *partial* $\eta^2 = .71$ regardless of group. Pairwise comparisons revealed that incongruent reaction times were longer than both neutral ($p < .001$) and congruent trials ($p < .001$), and neutral trials were longer than congruent trials ($p < .001$). There was no significant main effect of group, nor was there a

significant group by condition interaction suggesting that both aMCI and aMCI-controls produced similar reaction times to each condition. There was also a significant main effect of condition on error rates $F(1.45, 33.35) = 25.19, p < .001, \text{partial } \eta^2 = .52$. Pairwise comparisons revealed that participants made more errors in response to incongruent trials versus neutral ($p < .001$) and congruent ($p < .001$) trials, and slightly more errors during neutral trials versus congruent ($p < .05$). There was no main effect of group, however the interaction between group and condition was approaching significance $F(1.38, 31.82) = 3.60, p = .054$. Therefore, there was a slight difference in errors produced between aMCI and aMCI-controls for each condition. Patients with aMCI made fewer neutral errors while producing similar error rates for incongruent and congruent trials. Conversely, participants in the aMCI-control group made less incongruent errors and more neutral and congruent errors.

There was also a significant main effect of condition on reaction times for the AD and AD-control groups $F(1.54, 49.17) = 143.33, p < .001, \text{partial } \eta^2 = .82$. Pairwise comparisons showed that incongruent trials took longer to respond to than neutral ($p < .001$) and congruent trials ($p < .001$), and neutral trials produced longer reaction times compared to congruent ($p < .001$). There was no significant main effect of group, indicating that reaction times were similar for both AD and AD-controls. However, there was a significant interaction between group and condition $F(1.56, 49.69) = 7.84, p < .01$. Patients with AD produced longer reaction times during incongruent and neutral trials compared to congruent trials, while participants in the AD-control group showed the inverse relationship with faster reaction times to incongruent and neutral trials compared to congruent. There was a significant effect of condition on error rates $F(2, 32) = 77.50, p$

<.001, *partial* $\eta^2 = .71$. Pairwise comparisons revealed that in accordance with reaction times, more errors were produced during incongruent trials versus neutral ($p <.001$) and congruent ($p <.001$) trials, and neutral versus congruent ($p <.05$) trials. There was also a significant main effect of group $F(1, 32) = 13.78, p <.001, \textit{partial} \eta^2 = .30$. Pairwise comparisons revealed that patients with AD made significantly more errors overall compared to AD-controls ($p <.001$), regardless of condition type. The interaction between group and error rates by condition was also significant $F(1.38, 44.13) = 25.18, p <.001$. Patients with AD made more incongruent errors compared to neutral and congruent, while participants in the AD-control group made more neutral and congruent errors compared to incongruent.

Both Stroop effect ($U = 75, p = .87$) and interference ($U = 73, p = .79$) calculations did not differ between aMCI and the aMCI-control group. Likewise, there were no significant group differences between the AD and AD-control groups for Stroop effect ($U = 101, p = .13$) or interference ($U = 99, p = .12$).

4.4.2 Group Differences in Resting fMRI

The AD-control group had significantly greater functional connectivity between the left ACC seed and the left middle frontal gyrus (MFG) (MNI: -38, 0, 58, 119 voxels) compared to patients with AD $T(34) = -5.68, p <.001$ (Figure 4.2). There were no differences in the functional connectivity between any of the Stroop seeds with all voxels in the brain between patients with aMCI and aMCI-controls. We also did not find differences between DMN seeds and all other voxels in the brain between AD and AD-controls or aMCI and aMCI-controls. In other words, the participants with AD had reduced functional connectivity within frontal areas compared to matched controls. In

order to see if the pattern of decreased connectivity in the patients with AD and increased connectivity in the AD-control group was a result of abnormal DMN activation, we correlated functional connectivity measures between the left ACC seed and MFG with functional connectivity within the DMN within the AD and AD-control groups.

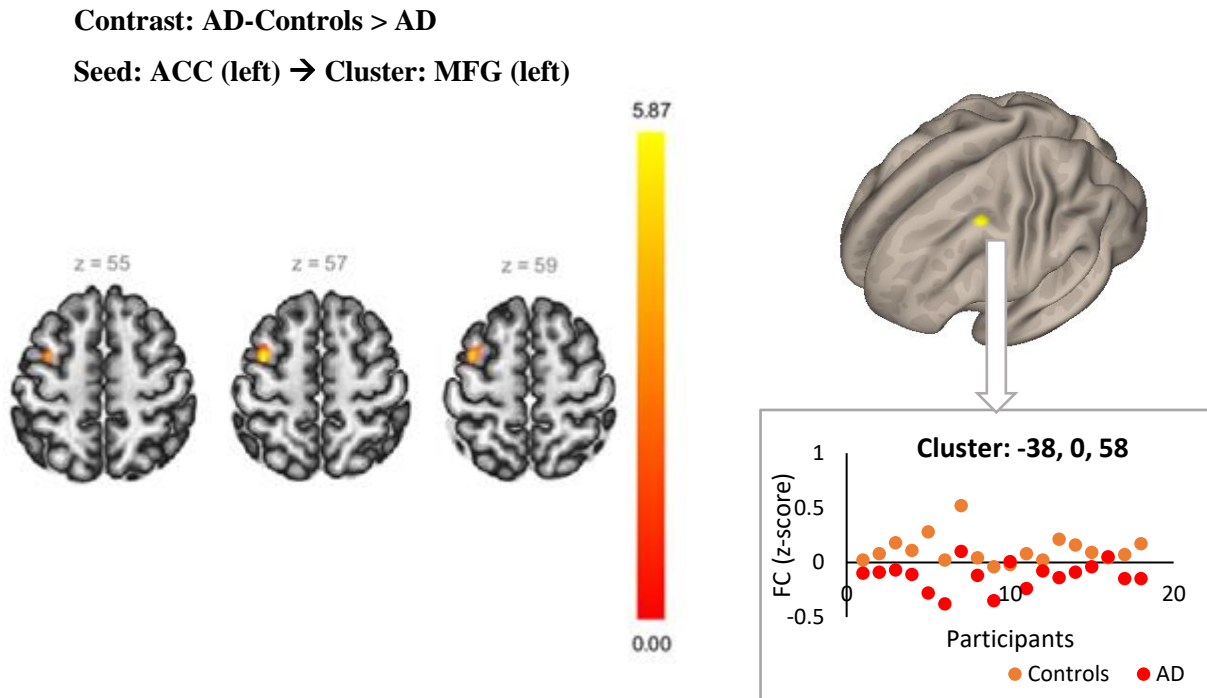


Figure 4.2. Significant functional connectivity differences between AD and AD-control in seeds related to the Stroop task. Scale on the left represents the T-statistic with yellow indicating greater between group difference in functional connectivity (FC) in the AD-Controls compared to patients with AD. Individual Fisher-z transformed correlation coefficients are plotted for the significant cluster (FDR corrected $p = .05$), indicating that patients with AD had lower functional connectivity between the left anterior cingulate cortex (ACC) seed and the left middle frontal gyrus (MFG) compared to AD-Controls.

4.4.3 Correlations Between Resting fMRI in Stroop and DMN Seeds in AD and AD-Controls

We assessed if the increased functional connectivity in the AD-controls and

decreased in the AD group between the left ACC seed from the Stroop task with the MFG was related to functional connectivity within the DMN. After correcting for multiple comparisons using Bonferroni correction, there remained one significant negative correlation in the AD group between the left ACC – MFG functional connectivity and the PCC – left frontal operculum (FO) (MNI: -42, 16, 6) $r = -.70$, $p = .001$ (Figure 4.3). There were no significant correlations in the AD-control group between functional connectivity from Stroop seeds and DMN.

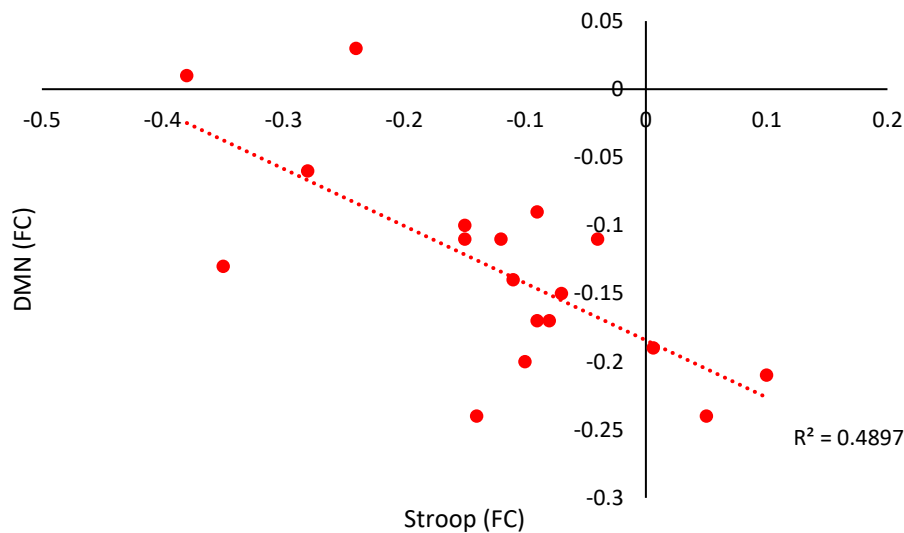


Figure 4.3. Correlation between the decreased functional connectivity difference found between patients with AD and controls in the Stroop related ROI seed (left ACC – MFG) with the DMN network (PCC – FO) in the AD group. ACC = Anterior cingulate cortex, MFG = Middle frontal gyrus, PCC = posterior cingulate cortex, FO = Frontal operculum, FC = Functional connectivity (z-score).

4.4.4 Correlations Between Resting fMRI and Stroop Behavior

Because we found significant differences in Stroop related seeds in the AD group, we investigated whether functional connectivity differences correlated with behavioral measures from the Stroop task, including average reaction times for incongruent,

congruent and neutral conditions, Stroop effect, percentage of interference and average error rates. More specifically, we conducted non-parametric Kendall’s Tau correlations between behavioral measures with the functional connectivity values in the significant clusters resulting from group differences and brain areas within the functional connectivity maps in the AD and AD-control groups. To control for multiple comparisons a Bonferroni correction was applied and thus significance was defined as $p < .006$. There was a significant positive correlation between functional connectivity and Stroop effect $\tau_b = .57$, $p < .006$ and interference $\tau_b = .51$, $p < .006$ in the AD-control group (Table 4.3). There were no significant correlations in the AD group between functional connectivity and any of the behavioral measures.

Seed: ACC (left) Cluster: MFG (left)	INC RT (ms)	CON RT (ms)	NEU RT (ms)	INC Errors (%)	CON Errors (%)	NEU Errors (%)	Stroop Effect (ms)	Interfer -ence (%)
AD - controls	.21	-.02	.08	.08	.12	-.21	.57*	.51*
AD	.01	-.11	.01	.02	.08	.12	.10	.07

Table 4.3. Kendall’s Tau correlation coefficients between behavioral measures and functional connectivity (Fisher z-transformed correlation coefficients) in ROIs from the Stroop task in AD and AD-controls. INC = incongruent, CON = congruent, NEU = neutral, RT = reaction time. Functional connectivity between the seed placed in the left ACC with the middle frontal gyrus (MFG). Significant correlations are denoted by * = $p < .006$.

4.5 Discussion

The goal of this study was to further characterize the relationship between inhibitory control in AD and aMCI using the Stroop task and intrinsic brain activity via resting-state fMRI. We found decreased functional connectivity in frontal areas in

patients with AD compared to controls that correlated negatively with seeds from the DMN. This finding suggests that in mild AD the expected anticorrelation between the DMN and frontal lobe areas important for attention and hubs within the salience network is altered.

We found that behavioral performance on the Stroop task was similar to our previous study (see Chapter 3). Participants with AD made more errors and produced longer reaction times when faced with trials that contained conflicting color and word information compared to controls. Participants with aMCI performed similarly to the aMCI-control group.

In terms of functional connectivity, our study provides support for decreased functional connectivity in AD (Zhan, et al., 2016), as only patients with AD showed a decrease when compared to their matched control group between the left ACC within the left middle frontal gyrus (MFG). According to the study by Brier et al. (2012) very mild AD was associated with an increase in the salience network, but as disease severity increased, the salience network showed signs of impairment and was decreased compared to controls. Furthermore, correlations between the seeds from the Stroop functional connectivity and seeds from the DMN in patients with AD revealed that the more negative the connectivity was between the PCC and left FO, the greater the functional connectivity was in the left ACC and MFG relating to the Stroop task. This finding represents a deviation from the normal anticorrelation between the two networks. While the correlation between the two is negative, according to studies of intrinsic functional connectivity at rest, the DMN is often reported as *more* active at rest (Greicius, Krasnow, Reiss, & Menon, 2003) followed by the suppression of the salience or central executive

network (CEN) (Dosenbach, et al., 2006). The opposite is found during tasks, with increased activity of the salience or CEN when attention is required with simultaneous suppression of the DMN. The frontal operculum is located near the anterior insula and has been linked to the salience network and modulating other brain areas involved in attention and cognitive control (Dosenbach, et al., 2006). Therefore, this finding represents a change associated with AD in areas relating to inhibitory control and may be linked to a disruption of other frontal areas including the salience network.

The middle frontal gyrus has been implicated in attentional control, while the left MFG specifically has been linked to tasks involving word reading and literacy (Koyama, O'Connor, Shehzad, & Milham, 2017). The left and right MFG have been shown to be correlated with each other in healthy young adults in their 20's (Japee, Holiday, Satyshur, Mukai, & Ungerleider, 2015). A case study involving a patient with resection of the right MFG found that during an attention task the left MFG also showed increased functional connectivity beyond that of controls, which the authors interpret as compensatory in nature (Japee, Holiday, Satyshur, Mukai, & Ungerleider, 2015). This may indicate a compensatory mechanism in our findings of increased left MFG connectivity in the healthy older adults. A study comparing participants with aMCI and controls found reduced gray matter volume in the frontoparietal network, including the MFG, as well as the insula and hippocampus in individuals diagnosed with aMCI (Xie, et al., 2014). Furthermore, participants with aMCI also showed reduced functional connectivity within the areas (seeds) showing reduced volumes. The authors found that, in addition to other areas of GM volume loss associated with AD pathology and reduced functional connectivity, a seed placed in the left MFG revealed reduced functional connectivity with

the right inferior parietal gyrus compared to controls. This is somewhat in line with our findings, except we identified functional connectivity changes in patients with mild AD as opposed to patients with aMCI. This difference may be due to the heterogenous nature of aMCI and AD, how patients were diagnosed, or perhaps our group of participants with aMCI was higher functioning. Additionally, functional connectivity accounted for reduced global cognitive scores, more than GM atrophy (Xie, et al., 2014). This reinforces the idea that functional connectivity can be a useful tool for understanding the neural underpinnings of cognitive decline in aMCI and AD.

Correlations between functional connectivity strength in significant clusters and behavioral measures from the Stroop task revealed that in the AD-control group, greater functional connectivity between the left ACC and the left MFG was associated with a larger Stroop effect and greater percentage of interference. Considering the usual anticorrelation between DMN and salience networks at rest, it would be expected that reduced functional connectivity in the ACC, a hub of the salience network (Seeley, et al., 2007), would correlate with better Stroop performance. Therefore, the increased functional connectivity in frontal areas could represent a detrimental change associated with aging, or an attempt at compensation but with no behavioral benefit (He, et al., 2014). In contrast, Duchek et al. (2013) identified a negative correlation between functional connectivity within both the salience and DMN with Stroop errors. They also found that the ex-Gaussian tau for Stroop reaction times, an indicator for a more positive skew to the right, or longer reaction times, was also negatively correlated with salience functional connectivity. More in line with our results, another study found a trend towards increased functional connectivity within the salience network with Stroop

interference in older adults, however this correlation was not significant ($p = .055$) (Brewster, Smith Pasqualini, & Martin, 2022). They also found that as opposed to a direct relationship between Stroop performance and age, resting-state connectivity from the salience network was a mediating factor, suggesting that functional connectivity of the salience may underlie age related changes to inhibitory control.

We did not identify any differences in functional connectivity between participants with aMCI and controls in any of the Stroop seeds or the DMN seeds. This finding is in line with the results from He et al. (2014), which also failed to report any intranetwork differences of the salience network between individuals with aMCI with controls. One possible reason for our lack of findings in the aMCI group is that our Stroop seeds may have been slightly different from other salience network seeds, and we used a seed-based approach compared to ICA or other network analyses. The location of the dACC identified in Brewster, Smith & Martin (2022) and Seeley et al. (2007) corresponded to our right ACC seed. Our lack of findings of differences between groups in the ACC and insula, known to be hubs within the salience network, may correspond to slight differences in locations, which could mean that the salience network was not evoked in our sample. Another possibility may be due to the heterogenous nature of aMCI. Studies that further classify aMCI by level of functioning (high performers vs. low performers) or those that convert to AD versus those that maintain aMCI diagnosis or revert to normal functioning may show differences in underlying brain function (e.g., hyper- or hypoactivation) (Puente, Faraco, Terry, Brown, & Miller, 2014; Celone, et al., 2006). Because aMCI is characterized by memory impairments, it is also possible that

differences between aMCI and healthy controls may not have shown up in our study probing inhibition, which is more susceptible later in the disease.

Somewhat surprisingly, we did not identify any differences in the maps of seeds from DMN with all other voxels in the brain between the AD and AD-control groups. This may be due to our limited sample size and including larger patient groups of both aMCI and AD may help to more accurately assess network differences compared to healthy older adults. Another reason could be because our sample of patients with AD were in the milder stage preceding wide-spread brain pathology and network disruption. Including other biomarkers such as cerebral spinal fluid amyloid beta ($A\beta_{42}$) or tau to correlate with functional connectivity and behavioral measures may give more insight into the relationship between these factors. A study following individuals diagnosed with MCI over a 3-year period identified significant declines in both DMN and salience networks in those with high amyloid burden compared to those with lower amyloid burden (Schultz, et al., 2020). In addition, genetic status may also be valuable, as a previous study found that healthy adults who were $A\beta_{42}$ positive had altered functional connectivity in the DMN (Duchek, et al., 2013). There has been a strong link between tau pathology and cognitive decline in AD (Bejanin, et al., 2017). Accumulation and spreading of tau pathology has also been linked to functional connectivity. A recent study combining rs-fMRI and tau positron emissions tomography (PET) imaging by Frotonzkowski et al. (2022) concluded that the frontoparietal network was susceptible to tau accumulation, especially in younger patients with AD. In younger AD patients increased tau accumulation in hub regions of networks also predicted faster cognitive decline and spread globally to other connected regions (Frontzkowski, et al., 2022). Yet

another possible reason for why we did not find differences between patients with AD and controls is due to genetic risk factors, such as APOE status, which was unavailable in our participant groups. In fact, even healthy adults that were negative for amyloid deposition but positive for the APOE ϵ 4 allele showed reduced functional connectivity of areas within the DMN (Sheline, et al., 2010). Therefore, it is possible that our sample of patients with AD were negative for the APOE ϵ 4 allele, or some may have even been carriers of the APOE ϵ 2, which has been shown to have protective effects to cognition (Martins, Oulhaj, de Jager, & Williams, 2005).

4.5.1 Summary and Future Directions

Our study expands on the current knowledge of functional connectivity and inhibition in AD and aMCI by investigating specific ROIs related to a task of inhibitory control. A recent study from Greene, Geo, Scheinost & Constable (2018) reported that functional connectivity of a behavioral task was better at predicting individual differences in behavioral traits than resting-state functional connectivity. To further this idea, one study found that aging resulted in changes to functional connectivity of a fluid reasoning task but not a memory task, indicating that aging or AD may have differential effects on specific tasks or networks (Varangis, Habeck, & Stern, 2020). On the other hand, one of the major draws of resting-state fMRI is the absence of a cognitive task, which allows for the inclusion of a wider range of participants with varying disease severity (Damoiseaux, et al., 2006). Nonetheless, future studies may benefit from investigating task-based functional connectivity, which may provide further insight into brain-behavior relationship changes in AD and aMCI, including inhibitory control and Stroop performance.

The DMN in AD has been well studied, which considering the overlap in locations of AD related pathology with the DMN it has received understandably a great deal of attention, however more work needs to be done on if and how other networks are affected by AD and aMCI (Badhwar, et al., 2017; Fox & Greicius, 2010; Chhatawl & Sperling, 2012). To further this idea, it is of interest to understand how tau pathology spreads and if it relates to impaired activation or deactivation of anticorrelated networks (Schultz, et al., 2020). Incorporating multiple biomarkers, such as tau pathology with a cognitive task to see how anticorrelated networks behave and change across healthy aging and with AD and aMCI to understand early disease changes (Chhatawl & Sperling, 2012).

Chapter 5

Discussion

5.1 Thesis Goals

The overarching goal of the three studies conducted in this thesis was similar; to see how inhibition, as measured by the Stroop task, may change as a function of healthy aging, and extend that to pathological aging, namely AD and aMCI. To investigate this, we implemented a multimodal approach including behavioral measurements and task-based and resting-state fMRI.

5.1.1 Summary of Studies

Our first study investigated inhibitory control in healthy aging by using a computerized version of the Stroop task adapted for fMRI in healthy young, middle-aged and older adults. We were able to collect behavioral measures of performance, including reaction times, error rates, and measures of interference along with the magnitude of BOLD responses during incongruent, congruent, and neutral trials of the Stroop task. The behavioral results from our study support the idea of generalized slowing with advancing age, as none of the age groups showed disproportionate reaction times to incongruent versus neutral trials. In terms of fMRI, we found evidence for both the compensatory (Cabeza, Anderson, Locantore, & McIntosh, 2002; Reuter-Lorenz & Cappell, 2008) and dedifferentiation (Li, Lindenberger, & Sikström, 2001) hypothesis of aging. Both older, and to a lesser extent middle-aged adults, showed increased activity during the Stroop task, and some of these increases did not seem to provide any cognitive benefit. Changes

to the underlying neural correlates of the Stroop task may begin to emerge in middle-age, even with the maintenance of behavioral performance, and become more marked after age 65.

Our second study extended the first by comparing patients diagnosed with aMCI and mild AD with healthy age-matched controls. A direct comparison of activity in ROIs relating to the Stroop task revealed no differences between patients with AD and controls, however, behavioral results from this study bolster the findings that inhibition as measured by the Stroop task is disrupted in AD. fMRI results also showed that patients with aMCI showed an over-recruitment of some areas when faced with increased interference, yet like our first study this provided no behavior benefit. A direct comparison to healthy controls showed a decrease in some ROIs in patients with aMCI, suggesting early neural changes associated with the disease process. Patients with aMCI also performed similarly to controls on the Stroop task, however, did produce slightly more incongruent errors, which falls in line with the findings from patients with AD.

Our last study built upon findings from our first two and expanded it by investigating the functional connectivity of areas identified in the Stroop task. The goal of this study was to further characterize the relationship between inhibitory control in AD and aMCI using the Stroop task and intrinsic brain activity via resting-state fMRI. We used ROIs identified to be relevant to the Stroop task in Neurosynth as seeds to test whether functional connectivity of these areas with all other voxels in the brain during rest was different in participants with AD and aMCI compared to healthy adults. Because the DMN is anticorrelated with the salience network, and some of our Stroop seeds overlapped with those of the salience network, we also tested whether intrinsic activity of

four DMN seeds with all voxels in the brain at rest correlated with any group differences in functional connectivity maps in the Stroop seeds. We found a decrease in functional connectivity in patients with AD compared to controls between the left ACC seed and the left MFG, which showed a negative correlation with seeds of the DMN. We did not identify any differences in functional connectivity between patients with aMCI and controls, which may indicate that functional connectivity of Stroop related areas may be spared early in the disease.

The findings from our studies extend the current knowledge of how the brain exerts inhibitory control, and suggest that it may be more susceptible to generalized slowing associated with aging as opposed to breakdown specifically due to brain changes with aging as well as in AD. Therefore, our computerized trial-by-trial version of the Stroop task was sensitive enough to identify differences between patients with AD and subtle changes in patients with aMCI compared to healthy older controls. We did not find major changes behaviorally in healthy aging, but our results suggest that error rates, especially during increased interference is the most sensitive measure in defining AD and aMCI. Our study points to a very gradual change to inhibition with aging and AD and may show more drastic changes with greater disease severity and inclusion of a wider age range and is further complicated by plasticity of the brain like compensation.

5.1.2 Limitations

The three studies outlined in this thesis share several limitations to consider when interpreting the results. The sample sizes used, particularly the patient groups, consisted of limited numbers. Our study was also cross-sectional in nature. Longitudinal studies would allow for an intra-subject design and the specific follow up to see who may

convert from healthy to aMCI, or from aMCI to AD or even back to normal functioning. Certain behavioral or fMRI markers could be extrapolated to see what combination is most likely to result in conversion to AD to provide earlier interventions.

Our approach to investigate specific ROIs also comes with limitations. ROI analysis is beneficial to circumvent both age and disease related brain changes. However, restricting our analysis to specific ROIs means that any potential recruitment of additional or bilateral brain areas in healthy aging or the patient groups may have not been captured by our methods. We chose to utilize the same ROI coordinates across studies and participant groups for consistency, however future studies may benefit from subject specific ROI placement or controlling for individual cortical thickness measures.

Another limitation to consider involves the heterogenous nature of dementias, including AD (Ryan, Fransquet, Wrigglesworth, & Lacaze, 2018). While all diagnoses conducted in the current studies followed current clinical guidelines for probable AD and aMCI, there is considerable overlap in symptoms across dementia. Therefore, without histopathological confirmation it is possible that there were misclassifications or mixed pathologies (e.g., tauopathy and synucleinopathy etc.). In addition, having genetic risk factor status, such as APOE, unknown may have contributed to the variability in our patient groups. General limitations of biomarkers in differentiating aMCI from AD involve the lack of standardized cut-off levels for normality, which vary across studies, as well as if cut-offs are determined from an independent sample or within the study group, which may result in bias (Jack et al., 2011; Rosén et al., 2013). In addition, longitudinal studies vary in follow-up lengths, which may contribute to variability in sensitivity and specificity rates (Rosén et al., 2013). Shifting from solely relying on clinical symptoms

that share similarities across many neurodegenerative diseases to in vivo biomarkers, including amyloid and tau imaging, may serve as the most successful way to classify AD (Ryan, Fransquet, Wrigglesworth, & Lacaze, 2018).

5.1.3 Recent Advancements in AD

Recent advancements in understanding the etiology of AD have been made since the conception of the studies presented here. Current theories suggest that dementia of the Alzheimer's type is classified as a metabolic syndrome (Livingston, et al., 2020; The Alzheimer Society of Canada, 2022). Factors indicative of metabolic syndrome include high blood pressure, blood glucose, serum triglycerides, and low serum high-density lipoprotein cholesterol and increased waist circumference (Livingston, et al., 2020). A recent study found that adults diagnosed with metabolic syndrome were 11.48 times more likely to develop AD compared to those without, and that smoking, high fasting glucose and previous stroke history were also associated with development of AD (Kim, et al., 2021). The authors also concluded that vascular dementia was differentially impacted by metabolic factors, as their analysis only found a link between increased glucose measures and previous stroke in development of vascular dementia, although other studies contradict this dissociation.

Modifiable lifestyle factors have been put forth and subdivided by age to delayed or prevent disease onset (Livingston, et al., 2020). Livingston et al. (2020) describe early-life as 45 years old or younger, mid-life as 45-65 years, and later-life as age 65+. The modifiable risk factors vary within these age boundaries, for example less education in early-life, hearing loss, hypertension, obesity, traumatic brain injury and excessive alcohol consumption in mid-life, and smoking, depression, social isolation, sedentary

lifestyle, diabetes and air pollution in later-life. While lifestyle factors are important factors to consider, Scheltens et al. (2020) proposed that genetic predispositions, as well as advancing age in general, contribute largely (60-80%) to and individuals risk factor for future development of AD.

It will be important to understand if and how metabolic syndrome, as it relates to AD, affects the brain. Healthy older adults classified as having amyloid ($A\beta$) accumulation at baseline through positron emission tomography (PET) imaging and the radiotracer (11)C-Pittsburgh compound-B (PiB +) and diagnosed with metabolic syndrome showed increased $A\beta$ accumulation in the bilateral superior parietal lobe and precuneus (Gomez, et al., 2018). Therefore, there may be overlap in brain areas impacted by AD pathology and metabolic syndrome. This in turn may relate to differences found in imaging studies of patients with AD and aMCI, for instance decreased functional connectivity found in patients with AD in the DMN since the precuneus is one of the major hubs of that network (Fox & Greicius, 2010).

5.1.4 Future Directions

As far as the Stroop task goes, our study was sensitive enough to detect differences in healthy aging, as well as comparing patients with AD and aMCI to controls. Errors during trials requiring the highest degree of inhibitory control were particularly useful at discriminating patients from controls, thus future work may consider looking at the neural correlates directly preceding incongruent errors. In relation to functional connectivity, errors may correspond to altered DMN activity, that is increased DMN activity during a task as opposed to the typical suppression, in patients

with AD or aMCI. Finding similar patterns in healthy adults may identify those at risk for future conversion to AD. Another future direction is to investigate task-based functional connectivity of the Stroop task. Studies have discovered age-related effects differentially impact task-based functional connectivity compared to resting-state connectivity (Archer, Lee, Qiu, & Chen, 2016), and type of cognitive task (e.g., memory versus fluid reasoning) may also show different age-related changes (Varangis, Habeck, & Stern, 2020).

Future studies may also benefit from investigating sex differences in the underlying neural correlates of the Stroop task. The sample size included in our studies did not allow for adequate analysis of potential sex differences in our patient groups, however recent numbers estimate a slightly disproportionate number of females diagnosed with dementia in Canada versus males (The Alzheimer Society of Canada, 2022). Whether sex differences are truly evident in the brains of females versus males is unclear, however a recent review suggests that there is some evidence of small but reliable differences (DeCasien, Guma, Liu, & Raznahan, 2022). The authors point to methodological challenges, including considering age, segmentation type and correction methods for whole brain volumes. A recent study probing sex differences in the Stroop task concluded that females tended to have greater performance, which may be due to enhanced color naming or verbal abilities as opposed to an advanced ability to exert inhibitory control (Sjoberg, Wilner, D'Souza, & Cole, 2022). Therefore, it would be interesting to determine if potential effects of sex are evident within brain regions underlying Stroop task performance across healthy aging as well as AD and aMCI.

Multimodal neuroimaging will be important moving forward to understand how different measures (network functions, fMRI, volumetry) relate to risk factors, both modifiable (e.g., lifestyle factors) and non-modifiable (e.g., age, genetic), and cognition to get an all-encompassing picture of AD and those at highest risk of developing AD. This is also of interest in terms of future treatment options, including non-pharmacological. One example of non-pharmacological intervention includes exercise. Exercise has been found to have positive effects in healthy older adults, including increasing brain volume, cognition and mood (Ruthirakuhan, et al., 2012; Meng, Lin, & Tzeng, 2020). Studies have found aerobic exercise to increase hippocampal volume (Erickson, et al., 2011) and blood flow to the ACC (Chapman, et al., 2013). Experiments implementing exercise programs in patients with AD are relatively sparse in comparison, however, evidence suggest that patients with AD may show similar benefits (Ruthirakuhan, et al., 2012). However, a review of the literature suggests there may be improvement in patients ADLs with no substantial evidence of enhancements to cognition or mood (Forbes, Forbes, Blake, Thiessen, & Forbes, 2015). A recent study investigated the predictive value of functional connectivity of the right anterior insula and ACC, areas involved in inhibitory control and goal directed behaviors, in future time spent sedentary in older adults without dementia assigned to an exercise group (walking, dancing, stretching) (Morris, et al., 2022). Combining functional connectivity with exercise studies may help identify those individuals at risk of living a more sedentary lifestyle to seek additional support (Morris, et al., 2022). The connection between exercise and brain health is promising, yet more work is needed, for example establishing recommendations for an exercise regime in a dose-dependent manner and evaluating how

long lasting the effects are. Attrition rates tend to be larger for patients with AD due to complexities of the disease itself, such as motivation, frailty, death and adequate study comprehension, especially in more advanced stages (Ruthirakuhan, et al., 2012; Meng, Lin, & Tzeng, 2020). It would be interesting to see how making positive strides towards modifiable risk factors in combination with an exercise regime would impact cognition, including inhibitory control, and brain function in AD, aMCI and older adults. Future studies employing a multifaceted healthcare approach to promote healthy aging will be vital for understanding healthy aging and prevention of age-related diseases. For example, the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability found that a multimodal approach that considered diet, exercise, cognitive training and vascular monitoring was beneficial in maintain cognitive functioning in older adults (Ngandu, et al., 2015). In a similar vein, providing community support and access to amenities to promote a healthy lifestyle would have a positive impact on individuals attempting to target modifiable risk factors (The Alzheimer Society of Canada, 2022). Ultimately, the combination of several biomarkers will provide the best discrimination and classification between aMCI and AD, as well as other potential diseases that share common clinical or pathological symptoms. Appropriate classification and staging are important in terms of administering treatments, evaluating new therapies, and for the patient and their families to understand the potential future outcomes of the disease.

It is crucial to establish the healthy, age-related changes to cognition as well as neural correlates to serve as the baseline for neurological diseases, especially ones associated with older populations including AD. This could in turn would allow for the

earliest detection of disease onset, thereby leading to early intervention. Considerable effort is being put towards prevention or delaying disease onset, in turn leading to a more positive outcome for individuals at risk for developing AD, their families and the healthcare system. Aside from identifying disease onset, understanding healthy aging is important in and of itself for older adults to maintain their ability to function independently (e.g., pay bills, administration of medication, grocery shopping, food preparation etc.) as well as overall well-being (Salthouse T. , 2012). Expanding on the knowledge of healthy aging also allows for the exploration of non-pharmacological interventions, including lifestyle changes, that may have a positive impact on cognition and the aging brain.

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