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Meditation Interventions in Subjects with Amnestic Mild Cognitive Impairment

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MEDITATION INTERVENTIONS IN SUBJECTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT

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ABSTRACT

The prodromal stage of Alzheimer's Disease, amnestic mild cognitive impairment, is characterized by subjective and objective memory impairment beginning with episodic memory. Few treatments have been identified to effectively slow disease progression to dementia. Meditation is an emerging novel treatment to improve deficits in subjects with these progressive cognitive impairments. Meditation and other novel treatments are critical for prolonging patients' independence, reducing caregiver burden, and healthcare costs. This study will examine the effectiveness of an eight-week intervention using two meditation methods and two control groups on cognition and mood in participants with amnestic mild cognitive impairment. The primary outcome is episodic memory. Secondary outcomes include verbal fluency, executive function, working memory, and mood symptoms. We believe meditation interventions are low-cost, safe, easily implemented interventions that could improve cognition and mood symptoms in patients with amnestic mild cognitive impairment through induced changes within the Default Mode Network.

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CHAPTER ONE-INTRODUCTION

I. Alzheimer's Disease

I.a Epidemiology

Alois Alzheimer first described Alzheimer's Disease in 1906 while presenting a 51 year old patient with progressive cognitive decline.[1] Now, the disease bearing his name affects 36 million people worldwide and is the most common cause of dementia.[2] This number is projected to increase to 115.4 million by 2050 due to the globally increasing population over 65. Additionally, it is suggested that half of people with AD are never diagnosed, so the scope is likely much greater than stated. AD most commonly presents in the seventh decade of life, and two-thirds of cases are in women. Earlier-onset cases are less common and are often due to familial genetic mutations.[1] Its prodromal state, amnestic mild cognitive impairment, is the target of many pharmaceutical and non-pharmaceutical interventions aimed at slowing cognitive decline and preserving independence. This study focuses on this population.

I.b Risk Factors

While age is the greatest risk factor for AD; family history and the presence of the APOE4 gene are other non-modifiable risk factors.[2] Known modifiable risk factors include cardiovascular disease, hypertension, hyperlipidemia, sedentary lifestyle, obesity, smoking, diabetes, insomnia, and fewer years of education. Growing evidence from the literature suggests depression, anxiety, and chronic stress are also risk factors for the disease.[3]

I.c Diagnosis of Cognitive Impairments

AD is a progressive neurodegenerative disease with hallmark neuropathology.[4] This includes β-amyloid protein fragments causing plaques to accumulate on neurons' exterior while tau proteins accumulate within neurons, leading to dysfunction, deregulation, and neuronal death. While these biomarkers of AD are well established and correlate to clinical severity, AD remains a clinical diagnosis, for which the National Institute on Aging and Alzheimer's Association proposed new diagnostic criteria in 2011.[2] The new guidelines divide AD into three separate stages that are described below: preclinical AD, mild cognitive impairment due to AD, and dementia due to AD. This study will adapt these criteria to the definition and diagnosis of aMCI.

I.c.i Preclinical AD

Preclinical AD features positive biomarkers including increased levels of tau or p-tau in cerebral spinal fluid, decreased cerebral spinal fluid ab 1-42 levels,[5] or changes on magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging.[2,6,7] However, the patient exhibits no subjective or objective memory complaints. The biomarkers have been found up to twenty years before a person exhibits memory symptoms.[2] Due to the lack of subjective or objective memory impairments, preclinical AD is largely a diagnostic category utilized by research studies.

I.c.ii aMCI

aMCI features subjective and objective impairments in one or more areas of memory, learning, and recall that do not preclude the person from functioning independently.[2] Of all aMCI cases, 50% progress to dementia within 3-4 years. While

symptoms vary, many individuals with aMCI first experience a loss of ability to remember newly learned information, often described as short term memory loss.[2] In this study, individuals with aMCI were selected because of the proposed effectiveness of disease modifying treatments administered before the cognitive decline has advanced into dementia.[1,2] Additionally, treatment in this group aims to prolong their high quality of life by preserving independent functioning.

I.c.iii Dementia due to AD

Dementia due to AD is diagnosed when clinical criteria for aMCI progresses to include impairments in daily functioning, such as the inability to manage finances or medication.[2] Disorientation to time and place, while retaining orientation to person is also common, as well as problems with planning, judgment, withdrawal, apathy, depression, and misplacing belongings. Eventually, incapacitation leads to development of secondary infections, such as aspiration pneumonia or falls, which can lead to death. In the United States, AD is the sixth leading cause of death across all ages.

I.d Treatment

The Food and Drug Administration has approved five medications for AD, but none can slow or stop the ultimate disease progression.[2] The efficacy of these medications has been based on delaying a patient's institutionalization or requirement of a full time aide by one year. Given that this is the benchmark for pharmaceutical effectiveness, there remains ample room for behavioral, non-pharmaceutical interventions, which may delay, or allow compensation for, the disease processes. Drug development cost, trial longevity, and the blood brain barrier are current barriers to more effective drug development. Studies have increasingly focused on the possibility of

delaying expression of AD clinical symptoms with non-pharmacologic therapies including meditation, exercise, sleep, yoga, and specialized nutrition.[8] These alternative therapies also primarily aim to delay the decline of cognitive function, preserve independence, and improve quality of life for patients and their caregivers.

II. Meditation

Meditation is thousands of years old practice traditionally used in religious or spiritual settings that has more recently been employed in Western cultures to enhance overall well-being.[3] While there are many variations and types of meditation, one uniting feature is the goal of focused, self-regulation of attention as well as open and accepting experience of the present moment. Meditation practice aims to engage the mind in goal-directed activity and regulation of attention and awareness while the body is in a hypometabolic state.[9] Meditation interventions have been studied in patients with chronic pain, fibromyalgia,[10] cancer,[11] coronary artery disease, and depression on a variety of outcomes including stress, cognition, attention, anxiety, and depression.[8,12] Meditation has recently been studied as a promising non-pharmaceutical intervention for subjective cognitive decline, aMCI, AD, and other dementias, though the pathophysiology behind its effects remains unclear.[2-4]

II.a Kirtan Kriya Meditation

Kirtan Kriya (KK) meditation is an established, safe, easy to learn, and effective meditation intervention studied in subjects with and without memory complaints, aMCI, and AD and other dementias.[3] It is the most common meditation intervention in the literature in subjects with aMCI and AD, and thus one of the meditation interventions for this study.

KK meditation is a simple chanting meditation performed over 12 minutes[13] (Appendix A). Chanting meditations focus on the repetition of speech, or mantras, to produce a meditative state. KK combines the mantra "saa, taa, naa, maa" with repetitive matched finger movements touching the thumb to the first, second, third, and fourth fingers in time with the words. The mantra is repeated spoken, whispered, and silently for two minutes each, then silently, whispered, and spoken for a total of 12 minutes of practice.

II.b Mindfulness Meditation

Another form of meditation, Mindfulness (MFL), teaches the subject to focus on the present, while they accept other stimuli that enter the mind, before dismissing them.[14] Practitioners focus on a physical element, such as breathing, to help maintain focus and control of thoughts moving through the body (Appendix B). Mindfulness Based Stress Reduction (MBSR) is an intensive intervention that requires longer periods of home meditation practice as well as group meetings.[8,15] MBSR has shown positive outcomes on wellbeing, acceptance of situations,[16] improved sleep,[17] decreased morning cortisol levels, and improved executive functioning and working memory[18] in subjects with and without cognitive impairment.

The effects of MBSR in AD and aMCI have been investigated, but the procedure of MBSR is much more intensive and too dissimilar from the KK protocol for this proposed study. Instead, this study's MFL intervention is a brief 12-minute intervention similar to the KK intervention. To our knowledge, this type of meditation intervention has never been studied in populations with aMCI. By including a MFL intervention similar to the KK meditation in time and duration, we aim to explore a possible role for

other types of brief meditation for people with aMCI. The MFL intervention will have subjects focus on their breathing, while they direct their thoughts in an L-shaped pattern through the top of their head and out of their mouth as they exhale.

III. Proposed Mechanism of Action

This study aims to expand on the current literature that has shown that meditation is an effective and safe intervention for people with aMCI. While this study will not pursue imaging, it draws from previous results to suggest a pathophysiology behind the hypotheses of meditation's impact on a brain with aMCI. Specifically, the default mode network (DMN) can be modified and strengthened through meditation.

The DMN is a group of anatomically separate regions that show functional interconnectedness through synchronized patterns of activation, especially at rest,[19,20] and when a person is not engaged in a goal-oriented task.[21] The proposed regions of the DMN include the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), anterior cingulate cortex (ACC), precuneus, inferior parietal cortex, and lateral temporal cortex.[22-24] The PCC is thought to be the posterior hub that communicates to areas important for memory encoding, retrieval, and formation like the hippocampus while the mPFC is thought to be the anterior hub of activity.[21]

Selective areas of DMN activation are associated with mind wandering, daydreaming, self-memory, self-reflection, prospective thought, and thinking in the perspective of others.[19-21] The DMN is also shown to be overall more active during tasks of episodic memory, such as autobiographical thought, which is compromised in aMCI and AD.[23] Concurrently, areas of the DMN are selectively deactivated during processes requiring attention and concentration. Research has found that experienced meditators

deactivate the regions of the DMN associated with mind wandering, and have better performance on measures of attention and concentration.[14,25,26] As many daily tasks involve aspects of simultaneous use of these functions, the DMN is normally partially, but selectively activated and deactivated.[22] Interruptions and deregulations of these patterns of activation and deactivation are thought to compose the cognitive disturbances that cause the aMCI and AD symptoms.[27]

III.a DMN Disruption

DMN disruption is found in AD, and is a target for pharmacological and alternative therapy interventions.[20,28] The patterns of disruption found in AD are so consistent and specific that it is a proposed biomarker for AD and aMCI studies.[19,21] Additionally, abnormal disruption and activation patterns of the DMN are found on functional-magnetic resonance imaging (fMRI) studies before objective or subjective memory symptom onset.[21,29] It is important to note that it is the deregulation of the DMN that is thought to cause the impairments of cognition, not just a global increase or decrease in activity of DMN function. For example, the areas associated with mind wandering and rumination show increased activation in AD and aMCI, while there is decreased activation in areas responsible for episodic memory retrieval.[23]

IV. Statement of the Problem

Currently there are few validated treatments for aMCI. Recent studies have focused on novel, non-pharmaceutical treatments to delay expression, and potentially progression of the disease towards AD. Meditation is one such novel intervention under investigation, with the aim to potentially improve cognitive function and delay institutionalization. The

current literature on meditation is limited in scope to KK meditation and MBSR in subjects with aMCI.

The studies reviewed were generally underpowered, used small sample sizes or limited to pilot or proof of concept studies, and lacked adequate control groups.[13,27,30-32] Several studies used active controls, engaging the subjects in a task of passive relaxation like music or book listening,[30,31] or psychoeducation classes.[32] While this strategy is helpful for separating effects unique to meditation compared to passive relaxation or active control, it does not allow comparison to an inactive control. This study will employ a wait list control as a way to control for the possible effects of music listening.[33]

V. Goals and Objectives

V.a Goals

The purpose of this study is to examine the effects of eight-week Kirtan Kriya and Mindfulness meditation interventions on percent change from baseline performance of pre- and post-measures of episodic memory, verbal fluency, working memory, executive function, anxiety, and depression, compared to an active (music listening) and inactive control. The primary outcome is episodic memory, specifically verbal memory, as it is classically impaired in aMCI.[34,35] The California Verbal Learning Test-II (CVLT-II),[36] a neuropsychological test, will operationalize verbal memory in this study with four dependent variables: semantic categorization, total learning (trials 1-5), long delayed recall, and cued long delayed recall.

Secondary outcomes of this study focus on other impairments common in aMCI including verbal fluency, working memory, executive function, and symptoms of depression and anxiety. Verbal fluency will be evaluated with phonemic fluency, using

the letters C, F, and L, as well as semantic (category-animal) fluency.[36] Working memory will be evaluated with the WAIS-IV Digit Span Test, where subjects must recall lists of numbers both forwards and backwards in sequence.[27] Executive function will be evaluated with the Trails Making Test (TMT) A and B, which evaluates attention span, task switching ability, and processing speed.[37] Two scales validated for use in elderly populations will be used for evaluation of mood symptoms. Depression symptoms will be evaluated by the Geriatric Depression Scale (GDS),[38] and anxiety symptoms with the Rating Anxiety in Dementia (RAID) Scale, which is additionally validated in elderly people with memory impairments and dementia.[39]

This study will compliment and expand the current literature by comparing KK and MFL meditation to two controls, one active and one inactive, on neuropsychological tests that reflect areas most affected by aMCI. Results will shed light on the relative effectiveness and feasibility of each meditation practice, which will provide impetus for future research on meditation in aMCI populations. Use of both an active and inactive control has not been attempted to our knowledge. Additionally, we aim to further validate meditation as an effective, safe therapy for aMCI and strengthen evidence for the role of the DMN in aMCI pathophysiology and treatment.

V.b Objectives

1. Compare KK meditation intervention to music listening and wait-list controls on measures of episodic memory using the CVLT-II.
2. Compare MFL meditation intervention to music listening and wait-list controls on measures of episodic memory using the CVLT-II.

3. Compare secondary outcomes of verbal fluency, working memory, executive function, depression, and anxiety using Animals Fluency, Letter C, F, and L Fluency, WAIS-IV Digit Span Test, TMT A and B, GDS, and RAID measures, respectively, amongst all groups.

VI. Hypothesis

Subjects with aMCI are known to have DMN dysfunction,[20,27] while meditation is known to improve DMN function. These improvements in DMN function have been linked to improve memory performance. Thus, we hypothesized that an eight-week KK or MFL intervention will improve scores, as determined by percent change from baseline, on measures of episodic memory, and secondary outcomes of verbal fluency, working memory, executive function, depression, and anxiety compared to music listening control and inactive control groups in subjects with aMCI, as a function of improved DMN functioning. We expect the effect to be greater between the meditation groups and inactive controls, compared to the effect between the meditation groups and active control.

VII. Definitions

A. KK—Kirtan Kriya; 12-minute meditation involving the repetitive movement of fingers to thumb and repetition of phrase “saa, taa, naa, maa” out loud, whispered, and silently in 2 minute intervals
B. MFL—Mindfulness; Meditation focused on creating and maintaining awareness of one’s surroundings, especially body, breath, and mind, and accepting thoughts and intrusions without judgment

C. CVLT-II—California Verbal Learning Test-II; neuropsychological test of episodic (verbal) memory, including semantic categorization, immediate recall, and delayed recall

D. RAID—Rating Anxiety in Dementia; Scale adapted for demented or cognitively impaired individuals to rate feelings and symptoms of anxiety in daily living

E. GDS—Geriatric Depression Scale; Scale for use in the elderly to rate feelings of depression, apathy, and risk for harm or self-harm

F. Animals Category Fluency—Measure of semantic fluency; subject names as many animals as they can in one minute

G. Letter C, F, L Fluency—Measure of phonemic fluency; subjects name as many words that begin with the letter C, F, or L as they can in separate one minute trials

H. WAIS-IV Digit Span Test—Measure of working memory, specifically mental flexibility and processing; subjects must recall lists of numbers forwards and backwards.

I. Trail Making Tests A and B—Measure of frontal executive function, including attention span, task switching ability, and processing

J. DMN—Default Mode Network; interconnected brain regions that show increased activity at rest. Includes posterior cingulate cortex, precuneus, anterior cingulate cortex, inferior parietal cortex, and lateral temporal cortex

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CHAPTER TWO—REVIEW OF THE LITERATURE

I. Introduction: Literature Search Criteria

A systematic literature review was conducted from August 2015 through May 2016 using Ovid (MEDLINE), PubMed@Yale, and relevant bibliographies with the following key words: mild cognitive impairment, amnestic mild cognitive impairment, Alzheimer's Disease, dementia, meditation, mindfulness meditation, mindfulness, Kirtan Kriya, default mode network, non-pharmaceutical intervention, non-pharmaceutical cognitive enhancement, neurodegenerative disease, semantic clustering, verbal fluency, episodic memory, semantic memory, California Verbal Learning Test-II, Trail Making Tests A and B, phonemic fluency, category fluency, anxiety and depression in MCI, GDS, RAID, and WAIS-IV Digit Span Test. Articles written in English, using human subjects, from 1985 until present were reviewed. Additional articles were sourced from relevant journals' bibliographies.

II. Review of Meditation Literature

In 2000, aMCI was formally established as the prodromal state of AD dementia.[1,2] aMCI has several characteristic impairments that are the focus of this study. First, episodic memory is affected, including poor acquisition, retention, or consolidation of information.[3] One part of episodic memory is verbal memory, the ability to process, retrieve, and recall words. Verbal memory deficiencies are characteristic of aMCI and AD,[4,5] and performance on measures of verbal memory can distinguish aMCI from other MCI subtypes with high specificity and sensitivity.[6] For example, people with AD and aMCI perform similarly on the CVLT-II compared to those with other subtypes of MCI

or healthy participants.[7] Verbal memory, operationalized by the CVLT-II (total learning trials 1-5, long delayed recall, and cued long delayed recall), is the primary outcome of this study. Changes in connectivity of various areas of the DMN are a hypothesized source of the cognitive changes seen in these people.[3]

Dunn et al. 2014 examined the differences between the DMN of 24 aMCI and 33 non-aMCI subjects using seed-based resting-fMRI.[3] The aMCI subjects showed impaired hippocampal and PCC connectivity when data were analyzed according to memory testing performance. The researchers found that resting patterns of DMN connectivity can distinguish between people with aMCI and non-aMCI. Additionally, they suggested that during episodic memory retrieval subjects with aMCI show dysfunction between the hippocampus, medial temporal lobe, and PCC, concluding that this dysfunction was partially responsible for episodic memory impairment in aMCI. This dysfunctional PCC connectivity was also seen by De Vogelaere et al. 2012[8] and Wang et al. 2013.[9] Collectively, these results suggest that DMN function correlated directly with participants' performance on neuropsychological testing. Concurrently, results of Garces et al. 2014[10] and Jin, Pelak and Cordes[11] also showed that DMN changes on imaging correlated with deficiencies of memory function, and are detectable before atrophy is noticeable on structural MRI.

The verbal memory impairments of aMCI are compounded by impairment of memory retrieval strategies, such as verbal fluency.[6] Verbal fluency tasks are measures of retrieval networks and are specific and sensitive to select for people with aMCI versus other MCI subtypes. Verbal fluency is operationalized here as timed word retrieval, and is comprised of phonemic (C,F,L) and category (animals) fluency. [6,7] Mueller et al. 2015

followed a cohort (n=283) of cognitively healthy people and people with aMCI for six years, and examined verbal fluency, phonemic switching, and semantic. Results suggested that individuals who developed aMCI over the following six years had significantly lower baseline scores on measures of verbal fluency, with most significant differences seen in phonemic and semantic fluency.

Gardini et al. 2015 conducted a prospective, randomized clinical trial to study the effect of aMCI-associated changes in the DMN on semantic memory.[12] Subjects were 21 people with aMCI and 21 demographically matched, healthy elderly controls. At baseline, they found that participants with aMCI had evidence of extensive semantic memory deterioration, which they attributed to increased, deregulated DMN connectivity as seen on fMRI. The study examined category fluency, word association tasks, confrontation naming tasks, definition tasks, and reading tasks. The researchers concluded that semantic and other impairments of aMCI can be correlated, and likely attributed to maladaptive reorganization of the DMN.

Working memory and executive function are also impaired in patients with aMCI,[13] and are secondary outcomes of this study. Deregulation of the DMN is thought to impair attention and concentration, leading to deficits in tasks of working memory and executive function.[9] Set shifting ability and working memory are processes modulated largely by a frontal neural network, and gray matter of the medial PFC and medial temporal cortex are amongst the regions most affected by MCI-associated atrophy.[14] Tsutsumimoto et al. 2015 found an association between gray matter volume and set-shifting ability as measured by the TMT A and B in 83 elderly people with MCI.[14]

Limitations of this study include its cross-sectional nature, which prevents attributing findings to causal relationships.

III. KK and MFL Meditation

While there are many forms of meditation practiced worldwide, this study focuses on mantra meditation (KK), and MFL meditation. Mantras are repeated phrases that are thought to have associated emotional or cognitive effects.[15] The KK intervention uses the Kundalini yoga mantra of "saa, taa, naa, maa," which represents beginning, life, death, and rebirth. While there is philosophical and spiritual meaning behind this mantra, we will not introduce this to our subjects. To evaluate whether a spiritual connection to a mantra or meditation is required to produce the described changes, Berkovic-Ohana et al. 2015 showed that repetitive speech, specifically the repetition of the Hebrew word for "one", can reduce fMRI blood-oxygen-dependent level (BOLD) activation primarily centered in areas of the DMN. These results suggest that even subjectively meaningless repetitive speech can elicit changes in DMN activity, and subjects do not necessarily need to spiritually relate to, or understand the mantra to exhibit cognitive changes.

III.a Kirtan Kriya—Independent Variable

Kirtan Kriya meditation is the most studied meditation intervention in people with AD and MCI. KK is a subtype of transcendental meditation, and involves the use of a mantra with synchronized finger-thumb touching. Newberg et al. 2010 was the first to use KK meditation in memory-impaired subjects compared to a music listening control group.[16] They used SPECT imaging to examine cerebral blood flow and neuropsychological tests to examine cognitive function in subjects with memory loss. Memory impairments ranged from mild and age-associated, to MCI, to diagnosed AD

dementia. After an eight-week KK intervention, practiced for 12 minutes per day, the KK meditation subjects showed improved verbal fluency, Trails B, and logical memory compared to music listening control, but only verbal fluency reached statistical significance, with 14% improvement from baseline scores for KK and 3% improvement from baseline scores for music listening.

On SPECT imaging during meditation, KK subjects with memory impairments had significantly decreased prefrontal cortex blood flow compared to the music listening group.[16] These results are similar to those of De Vogelaere et al. 2012,[8] who found that subjects with aMCI had increased prefrontal cortex activity at rest compared to cognitively healthy controls. Together, these results suggest that the aMCI DMN is more active at rest than in cognitively healthy people, while meditation enhances the ability to selectively decrease the activity of a brain at rest. Drawbacks of the Newberg et al. 2010 study include small sample size because it was a pilot study (total n=15).[16] The study also lacked an inactive control, and therefore effects of music listening cannot be excluded as a confounder, decreasing the effect size of the KK group outcomes. Finally, this study included participants with a range of memory impairments, from age related memory decline to known AD dementia, and therefore was not an optimally homogeneous, aMCI population.

III.b Mindfulness—Independent Variable

Two key tenets of MFL meditation are focusing on the present, and accepting one's present experience in a non-judgmental manner.[17] As interrupting thoughts enter the mind, the MFL meditator acknowledges them and returns his or her focus to the meditation object.[18] In this study, we employ concentration mindfulness, where the

subject focuses on their breath, with a goal on awareness of the present moment. Studies have shown mindfulness meditation to be effective in the treatment of pain, addiction, anxiety, and depression.[19]

Zeidan et al. 2010 used a brief MFL meditation intervention in 24 college students and controlled with a book-listening activity for 25 matched controls.[18] After four 20-minute sessions of MFL training (one per day over four days), the MFL group had significantly improved scores on Symbol Digit Modalities Test, verbal fluency, hit runs on n-back task, and measures of anxiety and fatigue. They did not find significant differences on mood scales of depression, anger, confusion, or vigor, forward or backward digit spans, or speed n-back task, and attributed this possibly to the short duration of intervention. While the mood scale scores improved, they did not reach significance. These results suggested that a brief MFL intervention successfully improved neuropsychological testing scores, but raised the possible confounder that the control book-listening group was perhaps equally relaxing to MFL meditation, and thus possibly prevented results from reaching statistical significance on other mood scores. Toneatto and Nguyen 2007 published a review article that supported these concerns, and suggested that MBSR techniques are not more effective than active control groups, such as relaxation, when compared to an inactive control.[20] The Zeidan et al. 2010 results are limited in applicability to our proposed study due to the average age of subjects (20 years old), and they are cognitively healthy. However, these results support our study in several ways.[18] First, they found significant effects over a short intervention period (four sessions) of training and reported no adverse events. Also, their suggestions further

support the use of our inactive and active control group as a way of controlling for the possible confounding effects produced due to a cognitively active control group.

A recent randomized trial by Smart et al. 2016 studied participants with (n=22) and without (n=14) subjective cognitive impairment.[21] MFL was operationalized similarly to MBSR protocols, using eight, weekly, two-hour group sessions and home meditation practice along to a CD. Psychoeducation served as the active control, and met five weekly two-hour sessions. In this group, participants learned about cognitive decline and aging. The researchers noted that these timelines were established protocols for MBSR and psychoeducation interventions, and thus the three-week difference should not cause confounding effects. One conclusion drawn from the study was that MFL participants had improved moment-to-moment attention. Improved attention was suggested to potentially help both cognitively health and subjects with cognitive impairment delay or slow future decline through better regulation of attention, which could possibly help retain newly learned information. One limitation to this study is that not all subjects with subjective cognitive impairment progress to aMCI or AD. The authors also cite restricted power due to small sample size, though the study was a pilot.

Several meditation studies have focused the effects of meditation on attention and concentration, which are crucial components of working memory and executive function. Tomasino et al. 2016 showed that an eight-week MFL, for 30 minutes, four times per week, intervention in 13 cognitively healthy adults had significant effects on activation of the right dorsolateral PFC, anterior insula, and left caudate; all areas important for focused attention.[22] Also, they found deactivation in DMN areas that are typically activated during mind-wandering, such as the rostral PFC and right parietal area 3b, with

increased activation in the dorsolateral PFC, an area important for attention and concentration. While these subjects were cognitively healthy adults, the results suggest meditation can reduce activity in areas associated with mind-wandering and improve concentration. Pre-intervention fMRIs allowed subjects in this study to serve as their own control, which is one limitation of this study, in addition to its small sample size of 13.

IV. Experienced Meditators

Garrison et al. 2015 showed that experienced meditators (n=20) have decreased activity on fMRI in DMN during meditation and rest, compared to meditation-naïve controls (n=26).[23] This study is limited in applicability to the proposed study because of younger mean subject age (44) without memory complaints. Brewer et al. 2011 also found that experienced meditators have decreased activation at rest of the posterior cingulate cortex and medial prefrontal cortex on fMRI.[17] These results collectively support the longevity of the effects of meditation on key DMN areas.

Pagnoni et al. 2008 studied differences in word and non-word identification between 12 experienced Zen meditators and 12 matched naïve meditators.[24] In between tasks, all subjects were instructed to focus on their breath, a task similar to mindfulness meditation. While there was no significant difference in reaction time or errors, meditators showed decreased BOLD fMRI signals, reaching even to below baseline between tasks. Pagnoni et al. suggested the experienced meditators are able to prevent mind-wandering that occurs between tasks compared to the controls. In concordance with prior literature, the researchers concluded that meditation practice allows for regulation of spontaneous mental activity. They suggest that experienced meditators are more efficient at refocusing their attention between tasks, and are therefore better at task switching than

non-meditators. The researchers point out that due to the difference in position (supine vs. seated) in the scanner and how a subject normally meditates could have prevented differences in reaction time and performance between groups. Limitations of this study include its cross-sectional design, and its small sample size despite being powered with the sample size it used. It is limited in generalizability to our study as well because the participants are cognitively healthy.

V. Meditation on Depression and Anxiety

The DMN modulates many intrinsic and self-related processes such as mind-wandering, day dreaming, and rumination, and is most active at rest and during autobiographical tasks in cognitively normal people.[3] Increased mind-wandering has been associated with decreased performance on cognitive tests, and rumination is linked to anxiety and depressive symptoms.[18] Depression, apathy, and social withdrawal are hallmark symptoms of AD, and negatively impact quality of life of the individual as well as the caregivers and family members.[25] In a study by Geda et al. 2004, more than half of people with aMCI scored significantly higher than age matched controls on measures of anxiety and depression.[26] Depressive symptoms are also found to be independent risk factors for both aMCI development and progression to AD. These and other mood symptoms occur significantly more in people with aMCI compared to age-matched controls with normal cognition. While research on the temporal relationship between depression and anxiety and aMCI is limited, higher depressive symptom scores have been associated with poorer baseline memory, and greater decline of memory testing scores in a five-year period amongst elderly participants.[27] These not only impact quality of life,

but also suggest that depression is related to poorer memory functioning. This exacerbation of cognitive impairment may hasten decline.

Feldman et al. 2004 found 59% of subjects with aMCI (n=1,010) suffer from neuropsychiatric symptoms, most commonly depression, anxiety, and irritability.[28] Additionally, the presence of these symptoms correlates with increased aMCI severity and functional impairment, and increased risk of progressing to AD. Decreasing symptoms of anxiety and depression in these patients through meditation would have important effects not only for their quality of life, but also potentially partially protect against disease progression.

Several studies have shown that meditation can improve anxiety and depressive mood symptoms. Goyal et al. 2014 conducted a meta-analysis of meditation literature in cognitively normal subjects and found MFL had moderate supporting evidence of improved anxiety, depression, and pain ratings after eight weeks of meditative practice.[19] This review lends support that an eight-week MFL intervention could show an effect that reaches significance on mood measures of anxiety and depression.

Moss et al. 2012 employed a small, randomized clinical trial using an eight-week KK meditation compared to a music listening control.[29] Fifteen subjects, with varying degrees of memory complaints, either meditated or listened to music for 12 minutes a day for eight weeks. Subjects were pre-imaged with SPECT to examine cerebral blood flow and given a neuropsychological battery was given of a category fluency test, Wechesler Adult Intelligence Scale Substitution Test, Logical Memory, and TMT A and B. After the intervention, subjects were reimaged and given the same neuropsychological tests. Additionally, they used the Profile of Mood States questionnaire to examine subjects'

feelings of tension, depression, anger, fatigue, and confusion. The meditation group showed improvement in all measures on the Profile of Mood States, but only fatigue reached significance. This study had several limitations, including small sample size of 15 and varying degrees of memory impairment, leading to a less homogeneous aMCI population. For example, seven of their subjects had age related memory impairment. A more homogenous population could have improved validity and potentially led to different results.

Lavretsky et al. 2013 examined the effects of an eight-week KK intervention on mental health, depressive symptoms, cognition, and telomerase activity in caregivers with mild depressive symptoms.[30] These 39 caregivers were all caring for people with aMCI, AD, or other dementia, and mean age was 60.3. After the eight-week KK intervention, they found an improvement in mental health ratings, specifically depression, as rated by the Hamilton Rating Scale for Depression (65.2% in KK group vs. 21.2% in relaxation group) and the Short Form (36) Health Survey (52.2% KK group vs. 18.7% relaxation group). They also found statistically significant improvements on the Trails B Test and Mini Mental Status Exam (MMSE) after KK intervention, supporting meditation's effect on executive function and overall cognition. Improvements were noted in the Trails A and CVLT tests mean change from baseline, but did not reach significance.

This study also examined immune cell telomerase activity, which correlated with decreased levels of depression and improved mental health score in the KK group.[30] They found 43.3% improvement in the KK group, reaching significance compared to 3.7% improvement in the relaxation group. Immune cell telomerase activity is inversely correlated with levels of chronic stress, and the researchers concluded that meditation is

able to specifically improve telomerase activity, cognition, and mental functioning, while significantly decreasing depressive symptoms. Limitations of this study include small sample size, and lack of cognitive deficits in the study population, which limits generalizability to our study's population.

VI. Music Listening as an Active Control for Meditation

Music listening will serve as this study's active control group, which is consistent with the majority of literature involving an active control. KK studies that do not use music listening often use audiotaped book listening as their control.[18] Because our primary outcome is verbal memory, we chose not to use book listening to prevent possible confounding effects attributed to listening to words.

The Moss et al. 2012 study compared meditation-naïve subjects undergoing an eight-week KK meditation intervention to subjects who listened to 12 minutes of Mozart violin concertos.[29] In the Newberg et al. 2010 study described above, a second comparison group was added (n=5) that listened to Mozart violin concertos for 12 minutes/day for eight weeks.[31] Neither study found significant differences in pre and post testing on measures of executive functioning or working memory in the music listening groups. However, they did not reach significance on several of their outcomes, which could be due to effects of music listening. For example, music therapy has been shown to improve verbal fluency in patients with dementia.[16] Newberg et al. 2010 suggested the future use of an inactive control group for the music listening group to mitigate these effects. The proposed study aims to mitigate these possible effects by adding a wait list control.

VII. Limitations with Current Literature

The purpose of this study is to compliment and expand the current literature on meditation in aMCI. Studies reviewed had small sample sizes, and thus were subject to underpowered results and null findings. Given the large number of subjects with aMCI in the US, we believe recruiting a larger sample size is feasible within the Yale Memory Clinic or Yale Alzheimer's Disease Research Center.

An additional limitation of the current literature surrounds the selected studies' control groups. Controls for the studies reviewed are either active (relaxation or book/music listening) or inactive, but to our knowledge no studies have used both. We aim to use both a music listening control and an inactive group to control for the possible benefits of music listening. For example, Ray and Mittleman 2015 found that two weeks of music therapy significantly improved depression and agitation symptoms in nursing home residents with dementia.[32] We believe that previous studies that did not include a waitlist control could have had their significance obscured or minimized by the effects of music listening, or other cognitively active control situations on the subjects, hence possibly obscuring effects of meditation.

KK and MBSR are the predominantly used meditation interventions in the literature. MBSR was not chosen for our study because it requires more hours of practice and a weekly group meeting,[33] making it too dissimilar to our KK intervention. A major conflict of interest exists with studies solely focusing on KK interventions, because the Alzheimer's Research and Prevention Foundation, which provides funding for many of these studies, promotes KK meditation. The Alzheimer's Research and Prevention Foundation sells KK meditation CDs, MP3 files, and offers free practicing material on

their website, and supported the Newberg et al. 2010, Lavretsky et al. 2012, Moss et al. 2012, Black et al. 2013 and other studies. This study aims to add the MFL group to potentially provide another meditation intervention for clinicians to introduce to patients. Finally, Newberg et al. 2010 suggested future studies employ larger samples, more groups, and a larger number of outcome measures,[22] which the proposed study aims to address.

VIII. Primary Outcome—Verbal Memory

The primary outcome variable is percent change from baseline performance on measures of verbal memory, operationalized by the CVLT-II, a well-validated and widely used test used to measure episodic memory in subjects with aMCI[22,34] and suggested by Albert et al. 2011 for use in diagnostic and research outcomes in populations with aMCI.[34] The CVLT-II is designed to test verbal episodic memory functions including encoding, delayed recall, and recognition.[35,36] It takes 20 minutes to administer followed by a delay of 30 minutes to examine delayed recall.[37] The encoding phase consists of reading a list of 16 words over five trials. Next, an interference list of words is read to the patient that they then recall followed by immediate and delayed recall of the first list and a yes/no recognition paradigm. Subjects will be randomly assigned to take the standard or alternate version to reduce practice effects.[9] Subjects will take the other version at eight-week follow up.

The CVLT-II is a well-validated and widely used test used to measure episodic memory in subjects with aMCI.[22,34] When compared with three other verbal memory tests using multivariate Cox regression analysis, CVLT-II is predictive of aMCI conversion to

AD.[7] Additionally, Wang et al. 2013 found a positive association between the CVLT-II and DMN dysregulation.[9]

IX. Secondary Outcomes

While the diagnosis of aMCI relies on subjects' impairments in episodic memory, aMCI is known to be associated with multiple domains of cognition.[3] Secondary outcome variables are designed to measure the effect, or lack of effect of these interventions on measures of verbal fluency, working memory, executive function, depression and anxiety. These will be measured with category fluency, letters C, F, and L fluency, TMT A and B, WAIS-IV Digit Span Test, RAID, and GDS. Percent change from baseline performance will be measured to determine differences in pre and post intervention testing. Albert et al. 2011 suggested these tests be used in populations with aMCI to examine deficits in cognitive domains outside of episodic memory.[34]

IX.a *Verbal Fluency*

Measures of phonemic (C, F, L) and semantic (animal) verbal fluency are validated and commonly used measures of verbal fluency in cognitively impaired adults including those with aMCI.[6,22] As discussed previously, Mueller et al. 2015 found that people with aMCI have lower scores on both of these measures compared to cognitively healthy subjects.[6] While diagnostically useful to differentiate normal aging from aMCI, these also represent a word finding deficit many patients with aMCI encounter from early stages of their disease process. Animal naming reflects semantic fluency while Letter C, F, and L naming reflects phonemic fluency. Semantic fluency is thought to be more impaired in subjects with aMCI due to greater impairment of semantic categorization compared to phonemic fluency.

IX.b Working Memory

The WAIS-IV Digit Span Test is a measure of working memory that includes attention, encoding, and mental flexibility.[3,14] It has been used as an outcome in meditation studies in people with aMCI previously.[16] Subjects repeat increasingly long sequences of digits beginning with practice trials increasing to nine digits forwards and backwards. For this study, the measure is determined by the difference between the scores for sequencing forwards and backwards digit spans.

IX.c Executive Function

Trail Making Tests A and B are measures of mental flexibility, and also examine set-shifting ability, both features of executive functioning.[3,14] Trail Making Test A requires subjects to connect circles with numbers 1-25 as fast as possible. Trail Making Test B is more difficult, and subjects must connect a sequence of numbers and letters in the following pattern: 1-A-2-B-3-C. This study will use the difference in time to completion of tests A and B as the outcome measure. This scoring technique for the Trail Making Tests has been found to have 64% sensitivity for predicting conversion of MCI to AD, and is therefore sensitive to the changes that are specific to aMCI.[38] Newberg et al. 2010 also found a correlation between improved Trails B time and increased PFC cerebral blood flow on SPECT imaging in the KK meditation group.[16]

IX.d Anxiety and Depressive Symptoms

The RAID and GDS are measures of anxiety and depressive symptoms, respectively, tailored for use in the elderly population. These measures are used as

screening tools to evaluate elderly subjects with anxiety or depressive symptoms,[10] and also as markers of mood symptoms as they change over time.[9]

X. Justification for Study Design

Study population, including exclusion and inclusion criteria, is sourced from the literature.[10] Our study population inclusion criteria will consist of patients aged (1) 65-85 with an MMSE score ≥ 26,[21] (2) who meet Petersen criteria for aMCI,[39] (3) have SPECT, PET, or MRI imaging suggesting or consistent with AD pathology,[34] (4) proficiency with English language,[24] and (5) have at least a high school diploma. Exclusion criteria includes (1) prior traumatic brain injury (TBI),[3,9] (2) prior cerebral vascular accident (CVA),[3] (3) comorbid psychiatric, medical, or neurological conditions,[9,10] (4) alcohol abuse,[13] (5) prior/current brain neoplasms,[10] (6) projected <1 year life expectancy, (7) prior meditation experience >1 year, (8) concurrent enrollment in another study. These criteria and projected power of the study aim to create a population that can be generalized to people living in the Northeast United States with aMCI, while reducing confounding variables that could affect outcome measures.

Our subjects will be randomized to the four groups. While other studies have used age- matched controls,[10] or matched we aim to reduce subject bias and enhance internal validity through randomization. Adherence will be ensured using a daily log of adherence to practice similar to the procedure of Lavretsky et al. 2012,[30] and an additional weekly phone call to check in on compliance for the MFL, KK, and music listening groups.

XI. Statistical Methods

XI.a Sample Size

Small sample size is a common limitation to the current literature on meditation interventions in cognitive impairment. This study's sample size calculation was derived from the Moss et al. 2012[30] and Newberg et al. 2010[16] studies described previously. Moss et al. 2012 used 15 subjects with varying degrees of memory problems in an eight-week KK intervention, and derived statistical significance on several parameters.[29] The Newberg study used three populations with differing levels of cognitive impairment, with total 14 subjects, also reaching significance on several outcomes.[22] Both studies cited small sample size as major limitations, and subjects groups had less than eight subjects per group. Data from the Newberg et al. 2010 study were used to determine effect size and sample size. Effect size (δ) was calculated at 11. The *PS: Power and Sample Size Version 3.1.2* software, an α-value of 0.05, power of 80%, a σ of 6.3 (conservative estimate), and m=1 for the independent t-test, generated an initial n=24 (Appendix C). After Bonferroni Correction, to control for multiple comparisons, the sample size was adjusted to n=40. A conservative 25% drop out rate,[22,30-31] increased the number to n=50. To make the groups even, the total number of participants was increased to 52, to make four groups of 13.

XI.b Statistical Tests

Outcomes will be measured as percent change from baseline, which is consistent with the Newberg et al. 2010 study.[16] Significance will be 0.05 for all tests. Consistent with several studies of cognition after meditation intervention, we will use a within-subjects repeated measures multivariate analysis of covariance (MANCOVA) for the

CVLT-II, phonemic and category naming, TMT A and B, WAIS-IV Digit Span Test, RAID, and GDS as dependent variables.[18,30] Age, gender, MMSE, and education level serve as covariates in this model for each outcome. A MANCOVA analysis will help protect against Type I errors, compared to multiple ANCOVA analyses. A paired T-test cannot be used, because the study is designed with more than two groups, unlike the Moss et al. 2012 and Newberg et al. 2010 studies, which used only two groups. While participants will be randomized, statistical corrections will be performed if baseline characteristics of age, gender, MMSE, and education level between groups significantly differ, which could affect the results. Post-hoc tests will be performed as needed to examine possible interactions from all model outcomes. IBM SPSS Statistics will be used with a general linear model for a within-subjects repeated measures analysis.

XII. References

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CHAPTER THREE—STUDY METHODS

I. Study Design

This study is a prospective, single center, randomized clinical trial to study two interventions, Kirtan Kriya and Mindfulness meditation, on measures of verbal memory, specifically semantic categorization, compared to music listening and wait list controls. Secondary outcomes include measures of verbal fluency, executive function, working memory, anxiety, and depression. Percent change from baseline scores within groups is the outcome measure. Participants will be computer randomized into KK, MFL, music listening, or wait list groups so baseline characteristics are not statistically different.

II. Study Population and Sampling

52 community-dwelling male and female patients will be recruited from Yale Memory Clinic and Yale Alzheimer's Disease Research Center. See Table 1 for inclusion and exclusion criteria. Randomization aims to create groups so that baseline characteristics between groups will not be statistically different, but if groups significantly differ, demographic variables (age, MMSE score, education level, gender) will be adjusted for in post-hoc statistical analysis.

Inclusion Criteria	Exclusion Criteria
Age: 65-85	Prior TBI[7,8]
Diagnosis of aMCI[1]	Prior CVA[3]
Imaging consistent with neuropathological findings of AD[2-6]	Comorbid conditions[7,9]
English proficiency	Concurrent enrollment in other study
MMSE score ≥ 26	>1 year meditation experience
High school education	Alcohol abuse[13]
	Prior/current brain neoplasms[9]
	<1 year life expectancy

Table 1. Inclusion and Exclusion Criteria

III. Subject Protection and Confidentiality

The Yale Human Investigation Committee (HIC) will approve the study, along with HIC approval. Prior to enrollment, volunteer participants will sign an informed consent form (Appendix D). This form contains information explaining the potential benefits, risks, and reasons for performing the study, as well as a timeline of the trial. It is clearly stated that participation is voluntary, and a participant is free to discontinue participation at any time. Participants will not be compensated for their participation, and will not be charged for clinician visits pertaining to the study. All employees involved in the study will complete HIPAA training certification and Human Subjects Protection training. Additionally, the Yale HIC will approve all study materials including consent forms and any study flyers or announcements.

All patient information will be used exclusively for this research study, and will be maintained according to guidelines set forth by HIPPA and the United States

Department of Health and Human Resources. Printed material will be secured in a filing cabinet at the researcher's office in New Haven, CT accessible only to study researchers. Digital information will be stored on an encrypted and password-locked computer that is only accessible to the Lead Investigator. Each patient will receive a computer generated random 10-digit code to replace his/her identifying information. The data will only be identifiable by code to investigators involved.

IV. Recruitment

Recruitment will last six months prior to initiation of the study. Patients will be recruited through clinician recommendation from the Yale Memory Clinic and self-referral through flyers and advertisements posted around Yale New Haven Hospital System (Appendix E). Clinician referral will attempt to decrease selection bias caused by self-referral through advertising. After a patient is referred, a research assistant will call the patient to explain the opportunity to participate in a research study. If interested, an appointment of approximately one hour will be scheduled to conduct baseline assessment following the six-month recruitment period and group randomization. At this appointment, participants will be formally enrolled, perform baseline testing, and learn their intervention if applicable.

We aim to yield a larger total study population to increase the power of our results, as lack of power has been a significant problem with previous studies exploring meditation in MCI and AD. While other studies of subjects with aMCI employed groups with an average total number of 13 participants, we aim to have 13 participants in each arm of the study, which is larger than other meditation studies exclusively focusing on subjects with aMCI.

V. Study Variables and Measures

We will employ two meditation interventions, one active control, and one wait list control. The meditation interventions will be KK and MFL. The active control will be music listening, and the wait list control will have no further intervention. Participants in the KK group will watch a ten-minute instructional video on how to practice KK meditation, and then show a researcher how to perform the meditation to demonstrate proficiency. They will be given Appendix A as a visual aid and a CD of guided KK meditation to listen to for 12 minutes/day for eight weeks at their convenience. Participants in the MFL group will watch a ten-minute instructional video on how to practice MFL meditation, and teach back the instructions to a researcher to demonstrate proficiency. They will return home with Appendix B and a MFL guided meditation CD to practice 12 minutes/day for eight weeks. Participants in the music control group will listen to Wolfgang Amadeus Mozart concertos for 12 minutes/day for eight weeks consistent with the protocols by Moss et al. 2012[1] and Newberg et al. 2010.[2]

The primary outcome is percent change from baseline scores on measures of episodic memory using the CVLT-II, specifically verbal recall (total, immediate, and delayed) and semantic categorization. Secondary outcomes include percent change from baseline scores on Animals Fluency and Letters C, F, L Fluency measuring semantic and phonemic fluency, respectively, as well as WAIS-IV Digit Span Test, TMT A and B, GDS, and RAID Scale to assess working memory, executive function, and symptoms of depression and anxiety.

VI. Blinding of Intervention/Outcome

Due to the nature of the interventions, the study will not be completely blinded. The researcher responsible for distributing the computer-generated randomizations will be blinded. A blinded researcher will conduct the pre and post neurocognitive test administration and subsequent data collection and analysis, identifiable only by the 10-digit code. Due to the nature of the study, the participants will not be blinded to their intervention. A non-blinded researcher will ensure intervention performance competence and call participants weekly. This researcher will also be responsible for answering the emails and calls of patients. This researcher will not be a part of data collection or analysis.

VII. Assignment of Intervention

Patients will be randomized to one of the four groups at the end of the 6-month recruitment period. Possible confounding baseline variables include age, gender, MMSE score, baseline test performance, and education level. An analysis will be performed to examine for group differences. If significant, these demographic variables will be adjusted for in the statistical analyses.

VIII. Data Collection

Following the recruitment and enrollment period, participants will perform baseline neuropsychological testing of the CVLT-II (total recall trials 1-5, immediate recall, delayed recall, semantic categorization), WAIS-IV Digit Span Test, Categories (Animals) and Letter C, F, and L Fluency, TMT A and B, GDS, and RAID scale. Next, they will undergo training for the intervention, followed by teach back for the meditation groups. At the eight-week follow up visit the tests will be re-administered. After intervention is complete, patients may keep the meditation or music CD. Data will be

identifiable only by the 10-digit code assigned to each participant. A blinded research assistant will enter the test scores into the encrypted computer database.

IX. Adherence

The participants will be provided with a daily diary to record the time they performed the intervention each day to ensure adherence. A researcher will call the patient within the first five days, and then weekly to ensure compliance with the intervention. Participants will be given a phone number and email address of the non-blinded investigator to contact with questions at any time. Wait list participants will not be contacted.

X. Monitoring Adverse Events

Clinicians not involved in the research will monitor for adverse events, however, these are atypical in meditation studies. Researchers will monitor for adverse or unexpected experiences, which could include depression, anxiety, boredom, confusion, and disorientation according to one study reporting the negative possible effects of meditation.[3] If an adverse experience occurs, the participant may withdraw from the study at any time.

While the study has several measures to ensure privacy and data protection, an unintended release of information or hack of medical information is still possible, and will be promptly managed with assistance from the Yale Information Technology Services. In such an event, participants will be notified promptly of the release of their medical or other sensitive information.

XI. Sample Size Calculation

Sample size calculation was performed using *PS: Power and Sample Size Version 3.1.2* software. Sample size calculation has been estimated to be n=52, with 13 participants per group. This number was based on the study by Newberg et al. 2010,[2] which also determined a power (δ) of 11 and standard deviation difference (conservative estimate, σ) of 6.3. Initially with a type 1 error of 0.05 (α), the calculation yielded six participants per group (total n=24), however the Bonferroni Correction estimate increased the calculation to total group size of 40. This number was adjusted to account for a 25% drop out rate, consistent with the literature,[2,4,5] ultimately yielding 13 participants per arm of the study, and total of 52 subjects. This study's sample size is larger than previously published similar studies whose results lacked power due to small samples.[1,6]

XII. Timeline

The study, including analysis, writing, and publication submission, will be completed within the 24-month deadline, starting June 30, 2016 with patient recruitment. The actual study, from enrollment to completion of follow up testing, will take nine months. See timeline below (Figure 1). Patients will be enrolled during the first appointment. Following the 6-month recruitment period, participants will be called back for baseline testing and to learn their intervention for the KK, MFL, and music listening groups. Wait list groups will be brought back for baseline testing only. The order of the tests will be: CVLT-II Short Recall, WAIS-IV Digit Span, Trail Making Test A and B, CVLT-II Delayed Recall, Animal Naming, and Letter C, F, L Fluency. Data collection and analysis will occur immediately following the post testing.

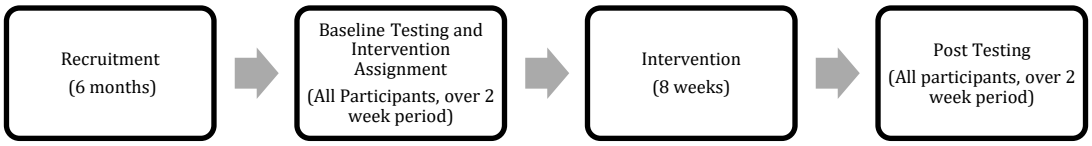

Figure 1. Timeline

XIII. Resources

We will have a secure computer for data entry and code generator to produce the patient's secure number code. The data will be stored on an external hard drive. The computer and external hard drive will be passcode protected. The passcodes must be changed every 6 months. The hard copies of the tests and baseline demographic sheet will be stored in folders identified with the patient's numerical code in a locked filing cabinet. All other identifying information will be removed. A secure office in the Yale Memory Clinic will be used to conduct the neuropsychological testing.

The instructional videos on the meditation will be delivered on a computer that can access YouTube. Compact discs will be given to the patients in the three active groups containing the audio recording of their guided meditation or music. Participants without a portable CD player and/or headphones will be given them. Funding for these materials will come from the grant.

XIV. References

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CHAPTER FOUR—CONCLUSION

I. Advantages and Limitations

Our study poses several advantages to previous studies, and aims to expand upon the current literature on meditation interventions in aMCI. First, we will use an inactive and active control group. Control group choice is a difficult aspect of research design in this field, and many studies have attempted to mitigate problems associated with one type of control group. For example, studies only using an active control could have trouble reaching significance because the control activity could also cause changes in cognition or brain activity on imaging. While studies using this control method cite this as a benefit to determine the unique cognitive effects of meditation, it is also a disadvantage, as we have previously described benefits of music listening in elderly people with memory complaints.

Studies that only use an inactive control group have difficulty attributing all of their findings to meditation alone, instead of the effect of introducing any intervention to a subject with aMCI. Known benefits of performing an intervention could include subject bias of expecting an effect after performing the intervention, but could be expanded to possible benefits gained by performing a daily routine, subjective feelings of purpose as a participant in a study, access to significant relationships, all found to improve quality of life in senior citizens.[1] For example, subjects in the MFL intervention and psychoeducation intervention in the Smart et al. 2016 both self-reported improved memory, cognition, and no significant difference on measures of depression between groups.[2]

Another advantage to our study is the specific inclusion and exclusion criteria to make a homogenous population. Previous studies used participants with varying degrees of cognitive impairment, or AD and aMCI, or had a diagnosis of aMCI that was only clinical. This study will solely focus on subjects with aMCI as diagnosed by clinical data[3,4] and neuroimaging suggestive of AD neuropathology.[5] We exclude a variety of confounders including prior stroke, alcohol abuse, malignancy, or prior traumatic brain injury. These exclusion criteria are consistent with the literature, and combined with the specific inclusion criteria will likely lead to a more homogenous aMCI sample. This sample population will also be much larger than previous studies, with the aim of achieving larger effect sizes.

The purpose of this study is to examine the effectiveness and feasibility of two interventions on cognitive outcomes, but it does not include neuroimaging as an outcome. In follow-up studies, we intend to use information gained from this study to design a study that would examine the effects of meditation interventions on the DMN with neuroimaging, as well as enlarge the neuropsychological testing battery. However, this study will require less funding because it does not include imaging, which is beneficial for a preliminary study. Another future direction could examine the longevity of the impact of meditation on the brain, and could provide more information on disease progression within the aMCI population throughout all of our participants.

Another limitation to this study is using a relatively large number of outcomes with several neuropsychological tests, which lends the possibility of finding effects reaching significance on chance alone, especially in the setting of our relatively small

sample size. The Bonferroni Correction was used to mitigate this effect, but it still poses a threat to validity.

This study will recruit participants partially through self-referral, which introduces the possibility of subject bias. We are hoping to mitigate this effect with randomization of participants and physician referral for recruitment, but the threat still exists. It is impossible to blind our participants; therefore knowledge of group could affect participants' subsequent testing and reporting of mood symptoms. We aim to address this by treating groups as equally as possible, and blinding the investigators involved with data analysis. We also have a dedicated researcher who is non-blinded who will perform the follow-up phone calls, and will not be a part of data analysis.

A final limitation to any study involving meditation is that it is inherently difficult to ensure true compliance. This study has several measures in place to ensure adequate compliancy, including a teach back for proficiency, weekly compliance phone calls, and a daily log to ensure proficiency and compliance, but ultimately it is difficult to ensure participants are putting effort into meditation during the practices. However, the voluntary nature of our study will hopefully protect against this, since participants are choosing to be involved.

II. Clinical and Public Health Significance

II.A Economic Impact

People with AD become increasingly dependent on others for basic tasks like driving and paying bills, but ultimately progress to relying on caretakers for all activities of daily living, including feeding, bathing, and dressing.[4] AD and other dementias are estimated to cost the United States $226 billion in 2015 for the hospitalization, long-term

facilities, and hospice care for people with dementia. In 2050, this cost is projected to increase to $1.1 trillion.

Before institutionalization, many people with AD are cared for in their home or a family member's home.[6] Often, their caregivers are family members, usually females (66%) with college degrees (40%). They are rarely compensated for their work, and cite caregiving as their primary barrier to gaining other employment. Last year, 15.5 million people provided 17.7 billion hours of unpaid care, valued at $220.2 billion.[4] These caregivers are unable to participate in the paid workforce, leaving them economically disadvantaged. The recently published 2016 Facts and Figures from the Alzheimer's Association reports that 48% of caregivers' families report the costs associated with providing for their loved one affects their ability to pay for basic needs.[4] While the burden is largely on spouses and daughters, it is also estimated that 250,000 children age eight to eighteen provide care for a family member with AD.

Importantly, a one-year delay of AD development from aMCI would result in nine million fewer cases by 2050.[4] This would also lessen caregiver burden, as it would increase the amount of time the patient is capable of performing tasks independently. This would have significant impacts on quality of life for the individual, and decrease costs and burden on the healthcare system, economy, and caregivers.

II.B Morbidity and Mortality

While most people diagnosed with AD die within a decade of diagnosis, many live for much longer after initial memory complaints emerge.[2] AD is ranked as the 12th most burdensome disease in respect to reducing a person's healthy and disability free years. This

number has increased drastically since 1990, when it was the 25th most burdensome disease.

The Alzheimer's Association estimates that 61% of people over the age of 70 with AD will die before age 80.[2] In contrast, people over the age of 70 without AD have a 30% risk of death before 80. Additionally, deaths attributed to AD, meaning they were unlikely to have occurred without the presence of AD, has increased 71% since 2000. While this increase could be related to an increase in citing AD as cause of death on death certificates, it is notable both in regards to disease burden and the incidence in the growing aging population.

III. Importance of the Proposed Study

Our proposed study targets a population with amnestic mild cognitive impairment, which is a frequently used group for interventions to slow cognitive decline and delay institutionalization.[6] As described previously, delay of institutionalization by only a year relieves an enormous economic burden on the health care costs within the country's rapidly growing elderly population.[2] Additionally, quality of life is closely associated with a person's independence, and perceived to decrease upon institutionalization.[1]

Meditation is a promising intervention studied in the context of many disease processes.[7,8] Materials, if any, are low cost, which benefits both the patient and insurer. Meditation is associated with minimal risks, and does not have any known negative side effects. After the practice is learned, meditation can be used at any time or place and requires no additional materials. Meditation is additionally advantageous compared to other non-pharmaceutical interventions especially in aging populations, because unlike

exercise or yoga, it requires none or very little physical abilities. This is important in the context of an aging population whose physical limitations advance with age and disease state. For KK meditation, one must only move their fingertips, while mindfulness does not require any purposeful movements. MFL provides an additional advantage, as it could be more appealing to an elderly demographic who could be averse to practicing a chanting meditation. From a caregiver's perspective meditation is an ideal intervention because the patient can perform it completely independently and at their leisure, with no supervision or set up required.

We designed this study to have a primary outcome on measures of verbal memory, a facet of episodic memory, because it is typically the first aspect of cognition impaired in people with aMCI.[9] We believe that if we focus on the first affected aspects of cognition, we will hopefully enable us to see the greatest improvements with an intervention. We aim to possibly help people slow their cognitive decline through meditation, build compensatory capabilities, reduce the expression of the disease and other domains of cognition including attention, processing speed, and executive processes, and possibly find a way to mitigate the DMN dysfunction found in aMCI.

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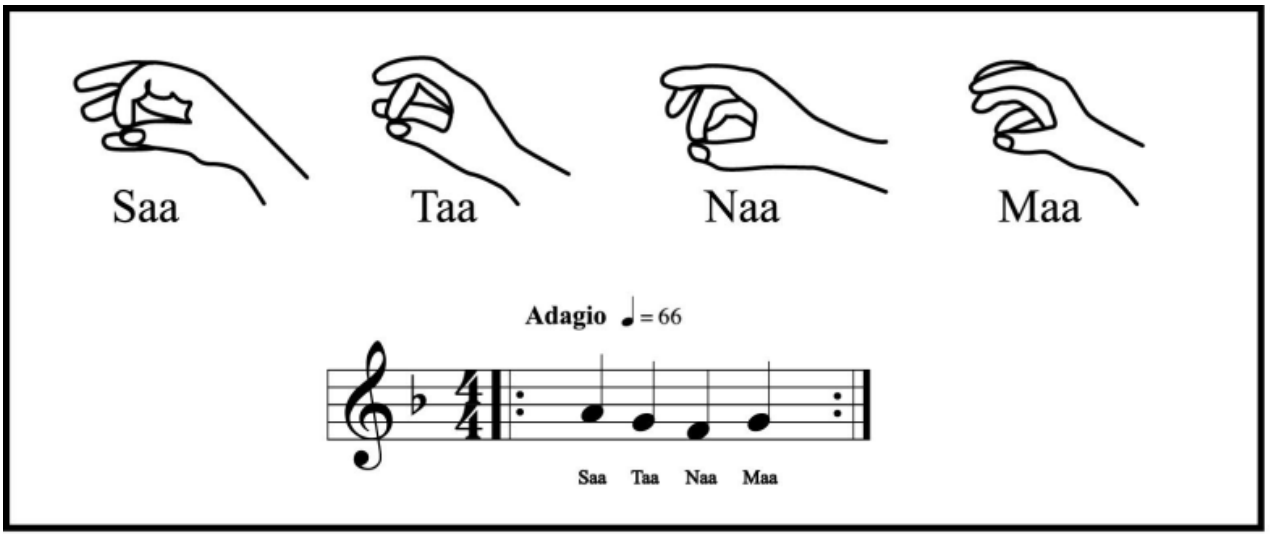
APPENDIX A: Kirtan Kriya Meditation Instructions

1. Repeat the Saa Taa Naa Maa sounds (or mantra) while sitting with your spine straight. Your focus of concentration is the L form, while your eyes are closed. With each syllable, imagine the sound flowing in through the top of your head and out the middle of your forehead (your third eye point). Start the CD when you are ready. It will cue your transitions.
2. For two minutes, sing in your normal voice.
3. For the next two minutes, sing in a whisper.
4. For the next four minutes, say the sound silently to yourself.
5. Then reverse the order, whispering for two minutes, and then out loud for two minutes, for a total of twelve minutes.
6. To come out of the exercise, inhale very deeply, stretch your hands above your head, and then bring them down slowly in a sweeping motion as you exhale.

The mudras, or finger positions, are very important in this kriya (see illustration below).



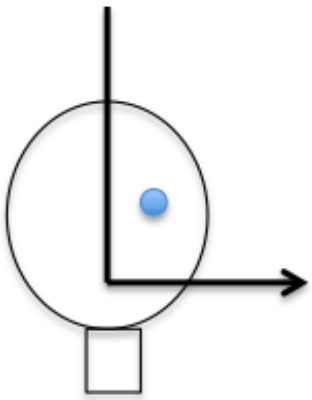
- On Saa, touch the index fingers of each hand to your thumbs.
- On Taa, touch your middle fingers to your thumbs.
- On Naa, touch your ring fingers to your thumbs.
- On Maa, touch your little fingers to your thumbs.

APPENDIX B: Mindfulness Meditation Instructions

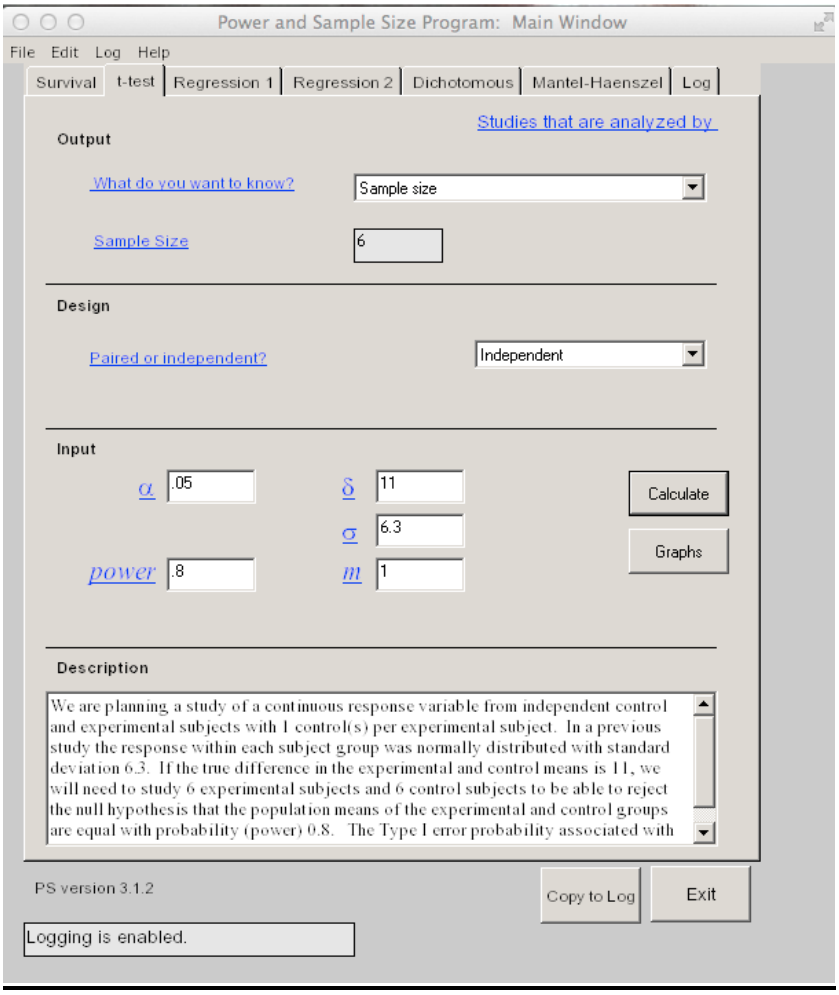
1. Sit in a comfortable position with spine straight.

2. Focus your concentration in an L form (see Illustration below) with your thoughts entering through the top of your head, and exiting through your mouth on expiration.

3. Begin the guided CD, which will lead your meditation for the next 12 minutes.



APPENDIX C: SAMPLE SIZE CALCULATION



Initial Sample Size: 6 x 4 groups = 24 total participants

- After Bonferroni Correction for multiple comparisons N= 10/group; total N=40
- Drop Out Rate (25%)= 40 x 0.25= 50
- 50/4=12.5→ 13 participants/group = **52 total participants**

APPENDIX D: INFORMED CONSENT FORM

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT
200 FR. 1 (2014-4)

YALE UNIVERSITY SCHOOL OF MEDICINE – YALE-NEW HAVEN HOSPITAL

Study Title: Meditation Interventions in Amnestic Mild Cognitive Impairment
Principal Investigator: *Emily K. Richards, PA-S; Emily Sharp, PhD.*
Funding Source: Yale School of Medicine

Invitation to Participate and Description of Project

You are invited to participate in a research study designed to examine the effect of a meditation intervention practiced over 8 consecutive weeks on cognitive outcomes and measures of anxiety and depression.

In order to decide whether or not you wish to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, and possible benefits. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

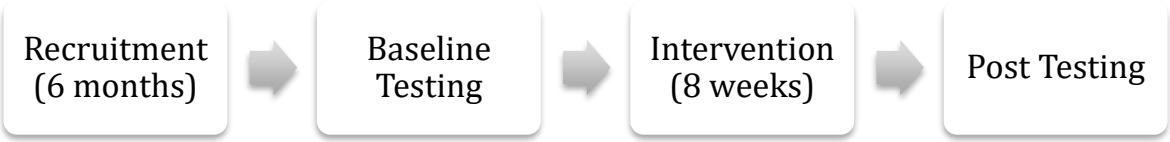
Description of Procedures

If you agree to participate in this study, you will be designated to an intervention group, an active control, or wait-list control group. Those in the intervention groups will learn one of two forms of meditation to be practiced for 12 minutes/day. The active control group will listen to a music CD for 12 minutes/day. Those in the intervention and active control groups will learn and perform their intervention on the same date as baseline testing. The interventions can be performed from home at a time of your choosing. Weekly phone calls will be made to your home to ensure compliance with performing the intervention and answer any questions you have. You will also be asked to record the time of your intervention on a daily log. At the end of the 8 weeks, you will return to the clinic for follow up testing. You may keep the materials given to you at the beginning of the program.

Content:
The cognitive tests will measure verbal memory, working memory, executive function, global cognition, and anxiety and depressive symptoms.

Participation time:
Total length of participation is up to 9 months, however six of those months include the total recruitment period before the possible intervention begins. If assigned to an intervention or active control, participation is 12 minutes/day for 8 weeks. The cognitive

testing is estimated to take 1 hour total, and will occur at the start and end of the 8 weeks. See Timeline below.



Risks and Inconveniences

There are no known physical risks associated with this study. However there is the possible risk of loss of confidentiality. Every effort will be made to keep your information confidential; however, this cannot be guaranteed.

Benefits

You may learn to perform an evidence-based meditation intervention. Meditation has been practiced for thousands of years and is associated with many measures promoting enhanced well-being. Participation in this study may allow researchers to identify a meditation intervention that may improve cognitive function in people with amnestic mild cognitive impairment, and potentially slow progression to dementia. This would allow clinicians to prescribe a cost effective, non-pharmaceutical adjunctive therapy for their patients that could benefit their function and quality of life as well as delay institutionalization.

Economic Considerations

No financial compensation is offered in this study. You may keep the materials provided to you.

Confidentiality

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. All participants will be identified to researchers as an identification number only. Information obtained throughout this study and any topics discussed via the online forum will be held strictly confidential and will be in compliance with the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Researchers will be required to successfully complete HIPAA training through Yale University and research information will only be accessible on encrypted devices that are also password protected. Any physical documentation that may be created throughout the course of the study will be identified by the assigned identification number only and will be stored in locked file cabinets in a secure room on the Yale University campus. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific consent for this activity is obtained.

Representatives from the Yale Human Research Protection Program, the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

Voluntary Participation and Withdrawal

Participating in this study is voluntary. You are free to choose not to take part in this study. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits). However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study. You do not give up any of your legal rights by signing this form.

If you do become a subject, you are free to stop and withdraw from this study at any time during its course. To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part. This will cancel any future appointments. The researchers may withdraw you from participating in the research if necessary. Developing any of the exclusion criteria may lead to removal from the study.

Withdrawing from the study will involve no penalty or loss of benefits to which you are otherwise entitled. It will not harm your relationship with your own doctors or with Yale-New Haven Hospital. If assigned to either control group, you will have the opportunity to obtain the meditation materials at the conclusion of the study if you so choose.

When you withdraw from the study, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to insure the integrity of the study and/or study oversight.

Questions

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the consent form carefully – as long as you feel is necessary – before you make a decision.

Authorization

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject:_____

Signature:_____

Date:_____

_____ _____
Signature of Principal Investigator Date

or

_____ _____
Signature of Person Obtaining Consent Date

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator Emily Richards PA-SII (717) 512-4617.
If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203)-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

THIS FORM IS NOT VALID UNLESS THE FOLLOWING BOX HAS BEEN COMPLETED BY THE HIC OFFICE

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_____.

INITIALED: _____

APPENDIX E: RECRUITMENT FLYER

Research Trial Opportunity

Do you, or someone you care, for have amnestic mild cognitive impairment? Or are you having concerns about your memory?

http://www.escapademagazine.com/meditation-more-than-a-trend/

We are recruiting healthy participants, ages 65-85 with amnestic mild cognitive impairment for a study on meditation

While there is no compensation, you have the opportunity to learn and practice a meditation for eight weeks in your home, at your convenience.

Meditation is known to help reduce symptoms of anxiety, depression, and improve thinking ability and concentration.

Contact your clinician to see if you are eligible for this study, or contact Emily Richards PA-SII —717-512-4617 for more information

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