Neural Structures Associated with Music Engagement
in Persons with Alzheimer's Disease

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Alzheimer's disease (AD) is the most common cause of dementia and a major public health issue resulting in decreased life expectancy of patients and high societal cost. Medical treatment of AD is palliative in nature as there is no definite cure. The symptoms can be alleviated through psychosocial interventions such as music-based rehabilitation. The aims of this small-scale study were to examine how AD affects the structure of brain areas associated with music engagement. Recently diagnosed patients with AD (N=5) were interviewed using Music Engagement Questionnaire (MusEQ) to acquire behavioral data relating to their musical activities. Using structural Magnetic Resonance Imaging (MRI) data acquired prior to this study with clinical indication, correlational Voxel-Based Morphometry (VBM) analyses were carried out evaluating the relationship between the neural architectures and MusEQ subtests and total score. Greater MusEQ total scores were associated with greater grey matter volume (GMV) in parietal and superior occipital areas, bilaterally. Greater GMV in frontal, limbic and inferior occipital areas were associated with higher scored both in Daily and Respond subtests. Higher scores in Emotion subtest were associated with greater GMV in the left parietal areas. The current results of the present feasibility-type study provide putative evidence on the relationship between music engagement and brain plasticity in persons with AD, and, if verified in a larger patient population, could contribute to personalized implementation of music-based therapeutic approaches for patients with memory impairment.

CONTENTS

1. INTRODUCTION

Functional imaging studies have revealed, which brain regions are critical for processing of music in healthy individuals (Särkämö et al., 2013). Musical training is known to induce plastic changes in the brain and may protect from progression of memory impairment into dementia (Groussard et al., 2010). The most common cause of dementia is Alzheimer's disease (AD), in which the histopathological alterations proceed to a relatively standard temporal pattern. The relationship of progressing AD histopathology to the brain networks responsible for processing music and to the patient's musical engagement are incompletely understood. A possible rationale to address this issue is to compare patients with AD at various stages of the disease in terms of musical engagement and musicality. The present thesis study reports on preliminary results of morphometric analyses of recently diagnosed patients with AD and relates them to assessment of musical engagement.

1.1 Human memory

The categorization of human memory is based on temporal dimensions and degrees of consciousness as well as anatomical localization. The classical modal model developed by Atkinson and Shiffrin has been used as a foundation for later research since 1968. Their model proposed three main modes of memory: sensory, short-term and long-term. Incoming sensory input is received and held by short-term memory, which has a two-way connection to long-term memory through encoding and retrieving. Sensory information held by short term-memory decays in a matter of seconds, if it is not attentively rehearsed and thus transferred permanently to long-term memory (Figure 1). Multi-modality of memory allows efficient storage of information as for example auditory information can be rehearsed in conjunction with visual information or a visualized mental representation. Even though the capacity and duration of long-term memories are seemingly endless, the access to them may deteriorate over time. A crucial brain structure involved in forming and retrieving long-term memories is the hippocampal region. (Atkinson and Shiffrin, 1977)
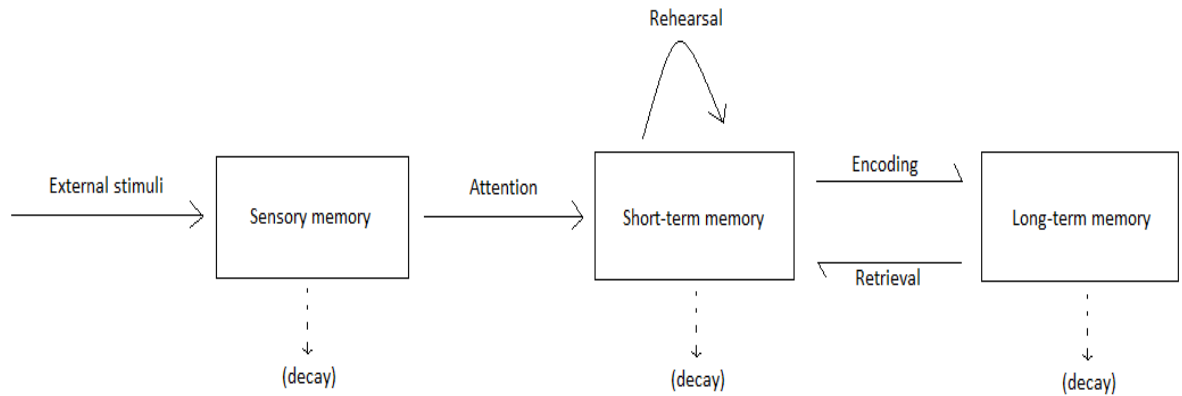
Figure 1 *The Atkinson & Shiffrin multi-store memory model.* Adapted from Atkinson and Shiffrin, 1977.

Short-term memory is divided into sensory memory, containing immediate perceptual information, and working memory, which is crucial for higher order cognition such as planning and learning. These goal-oriented tasks require conscious attention and are referred to as executive functions and they are processed in the prefrontal cortex. Long-term memory may be divided into declarative and non-declarative memory. Non-declarative long-term memory, or procedural memory, contains motor skills and learned habits and is central in classical conditioning. This information is stored in the striatum, amygdala and cerebellum. Declarative long-term memory, containing semantic and episodic memory, is processed by the thalamus and medial temporal lobe, which are interconnected with unimodal and polymodal association cortices. (Erkinjuntti et al., 2015)

Sensory information is transferred from periphery to central nervous system through thalamus to primary sensory cortex where it is processed and conveyed to secondary sensory cortex and their associative areas. Quickly decaying sensory memory is commonly divided into iconic, echoic and haptic memory, processing visual, auditory and tactile input, respectively. Iconic memory can retain information for a fraction of a second as it assembles visual perceptions into a coherent mental representation, involving the visual cortex and connecting to long-term semantic memory (Coltheart, 1980). Echoic memory, which has the longest duration of 3-10 seconds, encompasses different brain areas lateralized mostly to the left hemisphere: the primary auditory cortex, ventrolateral prefrontal cortex, including Broca's area responsible for verbal articulation, dorsal premotor cortex and posterior parietal cortex (Kwon et al., 2002). Haptic memory is composed of mechanoceptive and proprioceptive information stored for 1-2 seconds mainly in the right parietal lobule, parietal operculum and temporo-

parietal junction, also connecting to visual memory network (Gallace and Spence, 2009).

The most influential model for working memory has been Baddeley's and Hitch's model. In 1974 they proposed that working memory consists of the central executive system, which targets and regulates the information registered by its subsystems, the phonological loop and the visuo-spatial sketchpad (Figure 2). Later another subsystem, the episodic buffer, was proposed to act as a mediator between working memory and long-term memory through semantic representations, activating frontal and temporal lobes bilaterally (Baddeley, 2000). The phonological loop, lateralized to the left temporal and frontal lobes, is responsible for storing and retrieving verbal and non-verbal auditory memory traces. The visuo-spatial sketchpad, lateralized primarily to right occipital and superior parietal lobes, has two distinct pathways originating in the visual cortex: dorsal stream that detects spatial arrangements of observed objects and ventral stream that detects the size, shape and color of those objects (Denis et al., 2001).
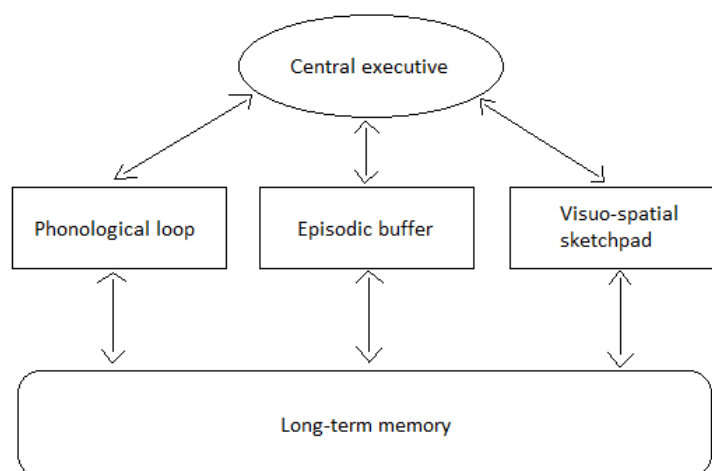
Figure 2 *Baddeley and Hitch's working memory model.* Adapted from Baddeley, 2000.

Working memory requires a balance between external perceptions and mental representations. The balance is regulated by dopaminergic neurons projecting from the tegmental area as well as by cholinergic neurons projecting from the basal forebrain and local glutamatergic NMDA-receptors. A large neural network is activated by the working memory processes connecting dorsolateral frontal cortex with posterior parietal cortex and subcortical areas (Erkinjuntti et al., 2015).

Information from sensory memory is encoded into working memory for a duration of seconds and it may then be consolidated into long-term memory. Memory traces are

retained in long-term memory through long-term potentiation (LTP), which is the process by which synaptic connections between neurons are strengthened with high-frequency activation. LTP, occurring in the hippocampus as well as in other parts of the brain, is tied to increased interneuronal glutamatergic activity as the amount of translated post-synaptic AMPA/NMDA-receptors is upregulated in response to increased pre-synaptic glutamate release. Consequently, lower frequency action potentials are sufficient for activation of these neurons. At resting potential, post-synaptic NDMA receptors are blocked by Mg2+. With strong enough depolarization they are expelled, opening the receptors to elevated influx of Ca2+ which leads to activation of intracellular protein kinases that insert additional AMPA receptors on the cell surface, thus increasing glutamate sensitivity (Figure 3). LTP leads to generation of new synapses and establishment of functional neuronal circuits, which is the basis of neuroplasticity (Cooke and Bliss, 2006). Non-REM sleep involving dreaming is known to enhance synaptic plasticity through memory consolidation by reactivating neural circuits in the hippocampus and cortical regions that have recently been involved in a learning task (Wamsley et al., 2010). LTP is counteracted by long-term depression (LTD) to maintain synaptic homeostasis through negative feedback and prevents neurons from becoming oversaturated, which would inhibit further encoding. LTD occurs in a reverse manner to LTP and selectively reduces glutamatergic activity between synapses (Massey and Bashir, 2007).
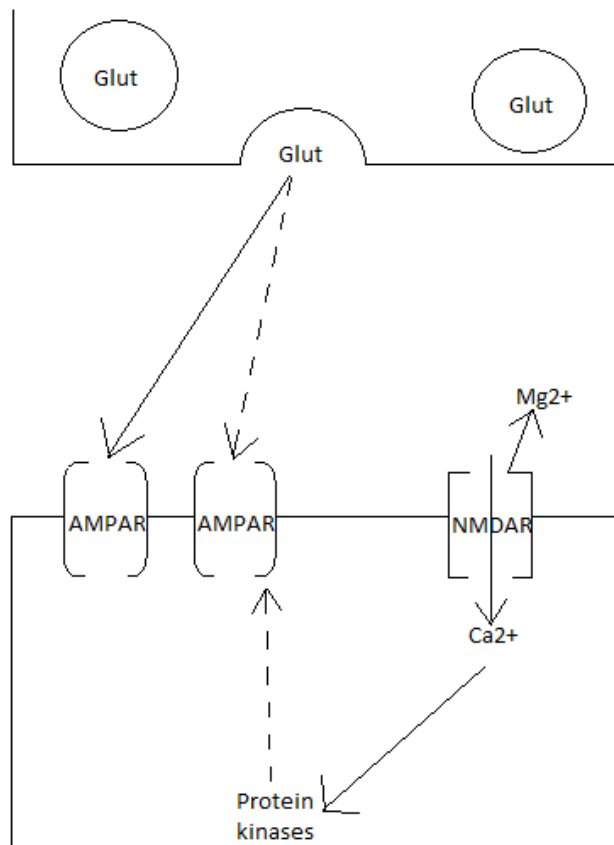
Figure 3 *LTP*. The depolarization of postsynaptic neuron and expulsion of Mg2+ from NMDA receptor triggers the influx of Ca2+ and subsequent synthesis of additional AMPA receptors by intracellular protein kinases. This leads to more efficient absorption of presynaptically released glutamate, strengthening the synaptic link. (Dotted lines represent changes in glutamatergic activity. Adapted from Purves et al., 2004)

Long-term memory is divided into different stores, which can be accessed implicitly or explicitly. Procedural memory is implicit as it does not require conscious control to function and it cannot be verbally described in detail. Procedural memory traces are mediated by the basal ganglia and enforced through practice to achieve automation of a certain motor skill. Basal ganglia contain evolutionary conserved motor nuclei and striatal pathways involved in motivation. Differing from thalamo-cortical glutamatergic circuits, striatal connections to basal ganglia and the limbic system are mainly modulated by dopamine and acetylcholine systems (Kreitzer, 2009).

Explicit memories are consciously accessible and can be categorized into semantic and episodic, referring to facts and events respectively. Main hub for explicit memory encoding and consolidation is the medial temporal lobe containing parahippocampal cortices: perirhinal cortex involved in familiarity-based recognition and entorhinal cortex connecting the hippocampus with neocortex (Aggleton, 2008).

Semantic memory, in contrast to episodic memory, is not context-dependent and previous semantic memories can be applied in learning new concepts. Another notable difference between semantic and episodic memory is that the former is not subject to normal age-related decline (Spaniol et al., 2006). Brain areas that are known to be distinctly involved in semantic memory processing include middle frontal gyrus and inferior temporal gyrus, whereas areas processing both semantic and episodic memories include dorsomedial and ventrolateral prefrontal cortex as well as the fusiform (medial occipitotemporal) gyrus (Burianova and Grady, 2007).

Semantic memory traces are distributed to various cortical areas according to their modularity. For example, retrieval of information about an object produces activation in the left temporal cortex, ventrally relating to color or form and laterally relating to motion, whereas retrieval of information on an object's size activates the parietal cortex and mental rotation of the object activates the premotor cortex. It has also been suggested that anterior regions of temporal cortices may hold non-perceptual representations such as verbal knowledge of semantic concepts (Thompson-Schill, 2003).

Episodic memory has been originally defined by Endel Tulving in 1972 to consist of subjective sense of time, connection to self and autonoetic consciousness (the ability to mentally place oneself in counterfactual situations). While the basic concept of episodic memory has been expanded with new research, the axiomatic understanding remains that episodic recollection is context-dependent and is characterized by self-awareness and spatio-temporal self-projection (Hassabis and Maguire, 2007). fMRI studies have verified the active role of dorsolateral prefrontal cortex and parietal regions as well as the hippocampus in episodic memory processing. Interestingly, it has been noted that hippocampal activation shifts from left to right hemisphere when comparing younger individuals to older ones (Maguire and Frith, 2003).

Episodic memory also contains autobiographical memories which are known to be prone to distortion. With age, autobiographical memories can be converted into semantic memories and thus become more preserved compared to episodic memory traces (Maguire and Frith, 2003). Episodic memory is interconnected with emotional memory which produces activation in amygdala, hippocampus and medial prefrontal cortex. The addition of an emotional component (positive or negative valence) to episodic memories enhances their recollections (Buchanan, 2007).

1.2   Alzheimer's disease

1.2.1   Pathophysiology and clinical diagnosis

Alzheimer's disease (AD) is a progressing neurodegenerative disease, which manifests as deteriorating cognitive skills, primarily memory loss, and leads to dementia in a few years. Brain atrophy in AD is a consequence of multiple pathological processes: formation of neurofibrillary tangles in neurites and amyloid plaques in the walls of cerebral blood vessels (amyloid angiopathy). Neurofibrillary tangles consist of hyperphosphorylated tau-protein, which destabilizes microtubules and the neuronal cytoskeleton. Amyloid angiopathy causes atherosclerosis in cerebral blood vessels which may result in ischemia and rupture of the blood vessels. The pathogenesis of amyloid plaques starts in the brain as amyloid precursor protein (APP) is cleaved into amyloid-beta-proteins (Aβ) of various lengths (36-43 amino acids) by enzyme secretase beta and gamma enzymes, the latter of which is combined with catalytic presenilin. Aβ-40 and Aβ-42 form plaques that accumulate in neurofibrillary tangles, disrupting axonal transport and neurotransmitter synthesis. The passage of electrical impulses between nerve cells decreases, resulting in synaptic loss. Consequently, this leads to hypometabolism and cerebral atrophy. (Erkinjuntti et al., 2015)

Animal studies on AD models have shown the effect of AD pathology on various transmitter systems and signaling molecules involved in memory processing. Chronic inflammation of hippocampi, mediated by Aβ-peptides, was observed to coincide with impaired LTD and disruption of postsynaptic glutamatergic activity, negatively altering synaptic plasticity (Min et al., 2009). Degeneration of cholinergic as well as histaminergic systems is also implicated in AD progression. Acetylcholine, synthesized by acetylcholine transferase, is involved in neurotransmission and regulation of numerous cognitive processes and has major implications in AD as cholinergic neurons in the basal forebrain degenerate. Acetylcholine acts through nicotinic and muscarinic receptors and is rapidly catabolized by acetylcholine esterase on the postsynaptic membrane. Cerebral histamine is synthesized in tuberomammillary nucleus within hypothalamus and histaminergic neurons project to widely over the cortex and the hippocampus (Dere et al., 2008). Oxidative stress and amyloid burden have also been

reported to contribute to hippocampal neuronal loss associated with downregulation of CREB (Cyclic-AMP-Response-Element-Binding-protein) (Pugazhenthi et al., 2011). CREB regulates BDNF (brain derived neurotrophic factor), which promotes neural growth and stimulates neuronal function. Knowledge of specific neurotransmitter systems involved in AD has led to development of disease-modifying medications. The two main types of AD medications are choline esterase inhibitors, such as donepezil, rivastigmine and galantamine, and memantine, which regulates glutamate activity by blocking NMDA receptors (Memory disorders: Current Care Guidelines, 2017).

The pathophysiology of AD is divided into six stages according to sequential localization of neurofibrillary tangles observed in post-mortem brain specimens: transentorhinal (I-II), limbic (III-IV) and neocortical (V-VI) (Braak and Braak, 1991). These changes reflect the clinical progression of AD which is divided into mild, moderate and severe stages, that are characterized by advancing decline in cognitive skills like impaired learning, memory deficits, language impairment and behavioral disturbances in later stages. Loss of grey matter (GM) begins in the medial temporal lobe and the limbic system and progresses to frontal and parietal lobes. The prefrontal cortex is the last brain region to lose its function (Thompson et al., 2003).

AD is diagnosed primarily by patient history, including questionnaires assessing memory function and other cognitive skills. The most common tests are MMSE (Mini-Mental State Examination) and CERAD (Consortium to Establish a Registry for Alzheimer's Disease). Brain imaging is routinely performed to obtain structural verification of hippocampal atrophy to exclude other causes of memory impairment (e.g. subdural hematoma, brain tumor, cerebrovascular disease). Laboratory examinations are performed to exclude treatable causes of memory problems other than AD, and they include blood count, electrolyte concentrations (Na, K, Ca), vitamin B12, thyroid, liver and kidney function analysis and Borrelia antibody screening. Brain imaging is preferably obtained by Magnetic Resonance Imaging (MRI), or by Computer Tomography (CT) in cases in which MRI is contraindicated (e.g. in patients with pacemakers or implanted cardioverter-defibrillators (Bovenschulte et al., 2012)). In unclear cases, Aβ and tau concentrations can be measured from cerebrospinal fluid (CSF) to detect elevated levels typical of AD. In selected cases, functional neuroimaging, such as and Positron Emission Tomography (PET), can be used to examine cerebral blood flow (CBF), glucose metabolism and accumulation of amyloid plaques. (Memory disorders: Current Care Guidelines, 2017)

MRI and CT can be used to quantitatively assess cortical and hippocampal atrophy, the latter of which is often seen in early stages of AD. Hippocampal atrophy can be assessed by using the four-step Scheltens-scale for evaluation of medial temporal lobe atrophy (MTA): Grade 0 - no atrophy, Grade 1 - widened choroid fissure, Grade 2 - widened temporal horn of the lateral ventricle and slight decrease in hippocampal height, Grade 3 - moderate loss of hippocampal volume, Grade 4 – substantial of loss of hippocampal volume, i.e. end-stage of the aforementioned observations. (Velickaite et al., 2017)

1.2.2 Risk and protective factors

Old age, high blood cholesterol level, hypertension, diabetes and certain gene polymorphisms such as apolipoprotein E4 -genotype and mutations in the genes coding for amyloid precursor protein (APP) and presenilin (PS1, PS2) are among the most common predispositions for the development of AD. In addition, low level of education is known to increase the risk of AD and other dementias. Failure to upkeep an adequate level of mental activity, including learning new things and maintaining previously learned skills, can lead to neural atrophy in the brain, which can also result from depression and a lack of social network. Some of the protective factors against AD and other dementias are regular physical exercise and a healthy diet that includes sources of omega-3 fatty acids and antioxidants. (Memory disorders: Current Care Guidelines, 2017)

Diagnosed mild cognitive impairment (MCI) which manifests as difficulties in tasks involving episodic memory, deduction and temporal orientation is also considered a significant risk factor and it can prognosticate the onset of AD in 15% of MCI patients. Vascular cognitive impairment (VCI) is a diagnostic category that covers a variety of symptoms, some resembling those of AD. VCI is caused by vascular degeneration in specific brain areas and it can affect both cortical and subcortical structures. In MRI scans, VCI shows up as white matter changes that can be graded on a four-step Fazekas-scale following the amount and confluence of these changes. VCI can progress to vascular dementia either independently or as a comorbidity of AD. (Erkinjuntti et al., 2015)

1.3   Music and brain

1.3.1   Neuroanatomical basis of music perception

Generally, the experience of music begins in the inner ear when an auditory stimulus travels in the form of an electrical impulse along the vestibulocochlear nerve through the brain stem and thalamus to auditory cortex. In addition to the auditory pathway, music activates several parts of the brain, e.g. the limbic system, which process emotions and the hippocampus, which process memories. The neuronal networks involved in musical processing are independent from those processing speech or ambient sound. Neuroimaging, such as fMRI and PET, has shown the lateralization of musical processing insofar as rhythm produces activation primarily in the right hemisphere. Harmony and melody produce activation primarily in the left hemisphere. Thus, there seems to be an anatomical duality regarding temporal and melodic aspects of musical cognition. Some evidence also supports the lateralization of musical memory. Learning and retaining unfamiliar melodies involves the right hemisphere and recognizing familiar melodies involves the left hemisphere. (García-Casares et al., 2011)

Acoustical basic features like frequency, duration and volume are processed in the auditory cortex, thalamus, brainstem and the inferior colliculus. Higher features such as harmony, rhythm and intervals are processed in medial prefrontal cortex, superior and inferior temporal gyri, premotor cortex, inferior parietal lobe and planum temporale. The process of following music through time activates the dorsal prefrontal cortex, inferior temporal gyrus, inferior parietal lobe and cingulate gyrus. Activation of episodic musical memory takes place in medial prefrontal cortex, inferior frontal gyrus, middle temporal gyrus, hippocampus, precuneus and angular gyrus. Musical motor functions activate the striatum, premotor and motor cortices, somatosensory cortex and the cerebellum. Feelings and pleasure arise from musical activation of orbitofrontal cortex, cingulate gyrus, basal ganglia, amygdala, insula, hippocampus and ventral tegmental area. (Särkämö et al., 2013)

Studies investigating semantic memory involvement in music processing have shown that recognition of familiar melodies activates bilateral orbital and medial frontal

regions, left angular gyrus, bilateral anterior temporal lobe, the parahippocampal gyrus, left temporal sulcus and left middle frontal gyrus (Johnson et al., 2011).

Based on Hickock and Poeppel's dual-stream model of speech processing which recruits the dorsal stream for mapping sound to articulation and the ventral stream for mapping sound to meaning (Hickock and Poeppel 2007), familiar songs that include sung lyrics are predicted to activate the ventral stream projecting ventrolaterally toward the posterior inferior temporal cortex (PITC) (Saito et al., 2012).

The left posterior inferior temporal region has been centrally implicated in multisensory representations of object-related information across visual, auditory and tactile modalities. A behavioral example of this sensory intersection could be the playing of an instrument or dancing with a partner. It has also been demonstrated that familiar melodies facilitate recall of lyrical material whereas sung songs and lyrics are recognized faster than just the melody and the specific activation produced by full-song recognition in PITC could explain the difference in recognition times. Common areas activated by songs and lyrics are located in the left anterior fusiform and left inferior occipital gyri as well as medial superior frontal and cingulate gyri. The left anterior fusiform gyrus, also referred to as visual word form area, is preferentially activated by voices of familiar people relative to those of unfamiliar people. Processing of semantic musical memories, in contrast to episodic musical memories, selectively activates the right temporal gyrus and sulcus as shown by familiarity decision tasks. Novel experimental auditory stimuli activate the bilateral temporo-occipital gyri together with the precuneus when they are compared to melodies stored in memory. Brain areas specifically activated by melody include the right frontoparietal regions such as the premotor and somatosensory areas. These have been reported to be involved in retrieval, imagery, working memory storage and rehearsal of melodies and singing. (Saito et al., 2012)

Musical pleasure is known to be transmitted by the dopaminergic mesolimbic system, which connects ventral striatum and the ventral tegmental area. Functional connectivity from nucleus accumbens, a key part of ventral striatum, has been observed to increase to superior temporal gyrus and inferior frontal gyrus has been observed to increase during a rewarding musical stimulus. Activity in dorsal striatum is increased during the anticipation of a rewarding musical stimulus and secretion of dopamine correlates with the intensity of positive emotional valence of the musical stimulus (Zatorre and Salimpoor, 2013).

In a PET study investigating changes in CBF relating to musical consonance/dissonance and perceived pleasantness/unpleasantness, activity in orbitofrontal, subcallosal cingulate and frontal polar cortices was correlated with increased consonance and perceived pleasantness (Blood et al., 1999). Activity in the parahippocampal gyrus and precuneus regions was correlated with increasing dissonance and perceived unpleasantness. The precuneus is known to be activated during memory-related tasks as well as during selective attention and emotional processes. The parahippocampal gyrus shares strong reciprocal connections with the amygdala, a central structure involved in detecting and generating fear-related negative emotions. However, activation of the amygdala was not detected with PET imaging. Increased activity in specific brain regions during positive emotions corresponded with decreased activity in regions that are activated during negative emotions. Affective responses to musical stimuli were shown to be lateralized to the right hemisphere in accordance to general dominance of the right hemisphere activity regarding emotional processing. Also, the regions activated by emotional musical stimuli differed from those activated by perceptual analysis of music. Perceived musical consonance or dissonance may result in various degrees of pleasantness or unpleasantness in different individuals.

An fMRI study investigating these findings also observed activation in the limbic region and temporal poles, as well as activation of the amygdala, in response to unpleasant (dissonant) music. Pleasant (consonant) music was shown to produce activation in Heschl's gyrus and Rolandic operculum. The Rolandic operculum, including Broca's area, is possibly implicated in a pre-motor circuitry for vocal sound production. The suggested circuitry involves parts of the reward system that also mediate motor functions, namely the ventral striatum and anterior superior insula. (Koelsch et al., 2006)

Studies investigating the effects of musical training on brain structure and functionality have discovered developmental differences between musicians and non-musicians (as reviewed by Groussard et al., 2010). Most notable differences were related to auditory, visual, motor and somatosensory cortices as well as in memory functions. Specific brain areas, such as the corpus callosum, planum temporale and anteromedial portions of Heschl's gyri are larger in musicians than in non-musicians, and differences in white matter are also present in corticospinal tracts. Long-term memory retrieval in non-musicians is known to produce functional activation of bilateral inferior frontal and superior temporal gyri and the right cerebellum. In musicians, familiar tunes produce

additional activation in bilateral anterior hippocampi, calcarine sulci, orbitomedial frontal gyri, Heschl's gyri, the medial cingulate cortex and the left cerebellum. These areas are known to be involved in mental imagery and self-referential processing. Both anatomical and functional evidence suggest that musical practice is an effective way of linking the neural networks that process semantic and autobiographical memories. The stronger involvement of hippocampi during familiarity judgement tasks as well as the increase of hippocampal grey matter density in musicians also suggests that developing specific memory abilities through musical training may contribute to a greater cognitive reserve when compared to non-musicians. This possibly buffers against age-related decline in memory processes in musicians.

1.3.2   Evidence for efficacy of therapeutic musical interventions

In terms of human evolution, music is thought to have preceded the development of spoken language and it has been used for therapeutic purposes in many ways throughout history. Therapeutic effects of music have been studied in relation to feelings and memory, among others, and music-based therapy has been used, for example, as a rehabilitation method for patients with dementia to enhance mood and cognition and to diminish neuropsychiatric disturbances. In post-stroke rehabilitation, music has been observed to promote the recovery of memory, attention and language skills (Särkämö et al., 2008). Listening to music promotes recovery in postoperative patients who have undergone major surgery by lowering the level of pain and anxiety as well as reducing serum cortisol levels and inhibiting cardiovascular stress. Sung music has been noted to have a greater therapeutic impact in neurological rehabilitation than instrumental music, especially in aphasic patients. Music-based interventions have also proven effective in improving motor performance in patients with Parkinson's disease, and some evidence suggests that music alleviates symptoms of epilepsy and multiple sclerosis (Sihvonen et al., 2017).

In patients with AD, musical memory has been noticed to be well-preserved and constituting a relatively independent part of memory. In these patients, brain areas associated with long-term musical memory, namely ventral supplementary motor area and caudal anterior cingulate gyrus, are preserved from notable cortical atrophy and hypometabolism (Jacobsen et al., 2015). These brain areas have also been associated

with executive functions, such as planning, learning and evaluation. Semantic and language deficits producing problems in communication that characterize the progression of AD may be bypassed through musical interactions. Patients with AD can retain the ability to distinguish between negative and positive emotional valence of music even if the patient's musical memory and cognition has been impaired. Especially upon hearing a familiar song, patients with severe AD can become animated to a degree which strongly contrasts the uncommunicative and unresponsive state preceding the musical intervention. Arguably, this can have its basis in the dissociation of emotional and perceptual (cognitive) neural networks for music processing (Gagnon et al., 2009). A recent study involving patients with mild to moderate AD and healthy controls has found that recognition of emotions conveyed by music does not significantly differ between these groups (Arroyo-Anlló et al., 2019). However, some emotions may be more easily recognized than others. For example, emotions such as joy and sadness are well recognized from the music pieces, while recognition of fear seems to be more elusive, possibly due to its relative complexity.

Therapeutic interventions utilizing individualized music-based approaches have demonstrated that listening to favorite music positively affects cognitive and behavioral symptoms of AD. The practice of evoking autobiographical memories through personally meaningful music can lead to notable improvements in patient's memory, orientation and language performance, as well as decrease in anxiety and depression (Leggieri et al., 2019). Listening to enjoyable music is generally considered a low-cost-high-gain way for enhancing well-being, which also applies to patients with AD and their caregivers, alleviating caregiver's burden.

1.3.3   Purpose of this study

Although it is well established that individualized music-based interventions have positive effects on several clinical parameters of AD, the relationship of musical background of AD patients to the potential benefit of music-based therapy is poorly understood. This feasibility study was undertaken to study the correlation of individual musical engagement of recently diagnosed AD patients with grey matter volume of specific brain regions. The results are expected to form the basis for further attempts to

investigate the role of musical engagement in protection from AD or slowing down the rate of its progression.

2. MATERIALS AND METHODS

2.1 Participants

Finnish-speaking patients who have been recently diagnosed with AD (N=5) were unselectively recruited from Turku University Central Hospital (Tyks). AD was diagnosed in the Neurological Outpatient Clinic of Tyks using the international guidelines based on NINCDS-ADRDA criteria (Dubois et al., 2007). Exclusion criteria were previously diagnosed other cognitive disorders, psychiatric illnesses and substance abuse. Demographic characteristics of the patients as well as medication, neuropsychological status and MRI scanner information is shown in Table 1. This patient group is somewhat younger than an average patient with AD at the time of diagnosis. None of the patients showed a family history of AD.

Patient ID	P1	P2	P3	P4	P5
Age	55	49	62	70	62
Sex	M	F	M	M	F
TFD (mos.)	3.6	7.5	11.9	12.76	13.33
Prescribed medication	escitalopram (5-10mg), rivastigmine (4.6mg)	donepezil (10mg)	galantamine (24mg)	memantine (10mg)	donepezil (10mg)
Latest MMSE-score	n/a[1]	27/30	25/30	8/30[2]	22/30
MRI scanner/ field intensity	Ingenia/ 1.5T	Ingenia/ 3T	Ingenia/ 3T	Ingenia/ 1.5T	Aera/ 1.5T
TR/TE (ms)	25.00/ 9.21	8.30/ 3.81	6.45/ 3.00	25.00/ 9.21	22.00/ 2.67
Flip angle/ voxel size	30°/ $1.0 \times 0.9 \times 0.9$ mm^3	8°/ $1.0 \times 1.0 \times 1.0$ mm^3	10°/ $1.2 \times 1.0 \times 1.0$ mm^3	30°/ $1.0 \times 0.9 \times 0.9$ mm^3	8°/ $1.0 \times 1.0 \times 1.0$ mm^3

Table 1 *Patient demographics, neuropsychological status, medication and MRI scanner information.* TFD=time from diagnosis (months), MMSE=Mini-Mental State Examination, n/a=not available, TR=relaxation time, TE=echo time.
[1] Cognitive status of P1 was assessed using Wechsler Memory Scale III.
[2] The spouse of P4 answered the MusEQ questions on the patient's behalf. At the time of diagnosis, the patient's MMSE score was 19/30.

2.2 Materials

Musical experience in daily life and its significance was assessed with Musical Engagement Questionnaire (MusEQ) that maps the use of music and its significance in their daily lives. MRI images acquired from Tyks database were used for evaluating the relationship between musical engagement and GM structures. Image analysis was performed by Voxel-Based Morphometry (VBM). Statistical methods were applied for correlating cerebral grey matter volumes with the scores from MusEQ.

2.2.1 Musical Engagement Questionnaire (MusEQ)

The patients were asked to fill a translated version of the Likert-type MusEQ questionnaire that was used to measure their engagement with music by themselves as well as in social contexts. This questionnaire, that contains 32 items pertaining to a patient's daily activities (Daily subtest), emotions (Emotion subtest), performance (Performance subtest), consumption (Consume subtests), responses (Respond subtest) and preferences (Prefer subtest) relating to music, has been tested as a self-report questionnaire for patients with AD and is also valid when filled by a caregiver (Vanstone et al., 2015). The Daily subtest assesses the responder's routine aspects of music use like listening to music during boring tasks and sharing favorite music with others. The Emotion subtest inquires about observable emotional behavior relating to favorite music such as playing a song over and over, pausing to listen music in the background or using certain types of music for relaxation. The Perform subtest pertains to activities like playing an instrument, making up own tunes and social aspects of music like discussing music with others. The Consume subtests assesses behavior regarding spending money on and seeking music (e.g. buying records or visiting concerts). The Respond subtests contains questions relating to singing or humming along when hearing a song, tapping the beat or engaging in singing with others. The Prefer subtests inquires about favorite and least favorite styles of music.

MusEQ data was collected by phone as the patient interviews were conducted during COVID-19 outbreak.

2.3 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive 3D imaging technique which is based on a phenomenon called Nuclear Magnetic Resonance (NMR) which describes the ability of atomic nucleus to absorb electromagnetic radiation at radio frequency when it is placed in a strong magnetic field. MRI utilizes hydrogen atoms which contain one proton and one neutron. Protons are surrounded by a small magnetic field that is produced by their random spinning motion around their axis at a certain frequency, which is called magnetic moment or nuclear spin. When protons are exposed to a strong external magnetic field (B0), their spin vectors align with the external field in z-plane, either in the same (positive) or opposite (negative) direction, the latter of which is a more stable and lower energy state than the former. Next the protons are targeted by a radiofrequency (RF) pulse that corresponds to their spinning frequency. After the RF-pulse is absorbed, each proton is excited to a higher energy state and aligns in the same direction with the outer magnetic field. This phase is called NMR and the spinning motion of the protons is termed precession, occurring at Larmor frequency.

Now, the magnetic field B1, generated by excited protons is perpendicular to the external field B0. When the RF-pulse is switched off, the atomic nuclei relax back to their lower energy states and emit photons at the same radiofrequency with which they were excited. This process is called Free Induction Decay (FID). The photons are captured by a detector coil and the signal can be converted to an image through Fourier transform.

Relaxation of the nuclei can occur longitudinally (z) with B0, as a result of energy transfer to surrounding tissues (spin-lattice), or transversely (xy), caused by dephasing nuclei and energy transferring to other nuclei (spin-spin). Relaxation times T1 and T2 describe the time it takes for the magnetization vector, which is comparable to proton density, to exponentially recover in z-plane (T1) or to exponentially decay in xy-plane (T2). Inhomogeneities of the external magnetic field also cause the nuclear spins to relax out-of-phase, depending on their location, which is termed T2*-relaxation.

In MRI, contrast is based on tissue properties, namely their different compositions, relaxation times, relative proton density, which affect how molecules behave inside the magnetic field. For example, the proton density of water is larger than that of fat, therefore the relaxation of hydrogen nuclei is slower in grey matter than in white matter. Contrast is visualized by assigning numerical values (0-255) to these differences in a greyscale image.

In the MRI scanner, magnetic fields are created by built-in gradient and radiofrequency coils and a superconducting magnet. A gradient magnet can be used to increase the strength of external field longitudinally or transversely. Along with localized variations in the strength of the magnetic field, the precession frequencies of protons vary accordingly. A targeted radio wave at a specific frequency can be used to select certain parts of the body where protons resonate and thus choose a slice for observation.

An MRI signal can be measured by using spin echo or gradient echo. Spin echo is produced by targeting spins with a 90° RF-pulse, with which the magnetization vector is flipped from z-plane to xy-plane and the spins begin to dephase through T2*-relaxation. Next, the spins are turned around the xy-plane with a 180° RF-pulse (refocusing), so that the spins that were spinning slower are now spinning faster and they recover at the same time (echo time, TE) to align back to z-plane. This sequence can be repeated with a specific time interval (repetition time, TR). By varying the durations of TE and TR, contrast can be produced. In gradient echo, refocusing is done by a bipolar readout gradient, which consists of a positive and a negative pulse. (Guy and Ffytche, 2005).

In AD diagnostics, T1-weighted scans are usually used to evaluate regional atrophy. T1-weighted scans show the signal coming from fat as brighter than from water, so for example, enlarged ventricles filled with cerebrospinal fluid (CSF) are seen as dark areas in T1-weighted images. FLAIR (Fluid Attenuated Inversion Recovery) is also a commonly used technique for imaging the brains of patients with AD (Erkinjuntti et al., 2010). It can suppress the signal of CSF, which fills the subarachnoidal space, ventricles, sulci and central canal. Hippocampus can be seen more clearly by suppressing the CSF signal coming from the temporal horn of lateral ventricle. FLAIR is based on a spin echo sequence preceded by a 180° RF-pulse, which turn the magnetization vector in the negative direction of z-plane and followed by a 90°-pulse. Unwanted signals can be selectively suppressed by varying the inversion time (TI) between these pulses (Mamourian, 2010).

The patients were scanned at the Medical Imaging Center of Southwest Finland to obtain structural 3D-T1-weighted images. Because clinical MRI data was used in this study, the participants were scanned with three different scanners (1.5T Siemens Aera, 1.5T Philips Ingenia and 3T Philips Ingenia) and with varying imaging sequences (T1W3D-ISO, T1W3D-TFE, T1-MPRAGE).

2.3.1   Voxel-Based Morphometry

VBM is a neuroanatomical technique for studying structural brain differences voxel-by-voxel from MRI images (Ashburner and Friston, 2000). Differing from manual ROI methods, VBM can be used as an automated whole-brain tool with better statistical accuracy and a higher throughput. The VBM sequence starts with spatially registering the origo of the individual T1 images to the anterior commissure. After that, the images are segmented into GM, white matter and CSF probability maps using unified segmentation with medium regularization (Ashburner and Friston, 2005). The probability maps are then spatially normalized into the MNI space (Montreal Neurological Institution) and modulated to preserve the original signal strength. Residual inter-individual variability was reduced by smoothing the probability maps using an isotropic spatial filter (FWHM = 6 mm). After these preprocessing steps, statistical methods of analysis can now be applied to the images to identify local or global variations in brain matter volume and to test the research hypothesis. Volumetric differences can be related to cortical thickness or folding (Mechelli et al., 2005).

2.3.2   Statistical analyses

Voxel-based morphometric analysis was carried out using Statistical Parametric Mapping software (SPM8, Wellcome Department of Cognitive Neurology, UCL) under MATLAB 8.4.0 (The MathWorks Inc., Natick, MA, USA, version R2014b). The preprocessed GM images were entered into a second-level analysis using one sample t-tests evaluating the relationship between the GM volume and each of the MusEQ subtests individually as well as the MusEQ total score separately. In total, seven one-sample tests were carried out. Results were thresholded at whole-brain uncorrected $p < 0.005$ threshold at the voxel level and FWE-corrected $p < 0.05$ at the cluster level with a cluster extent of more than 100 contiguous voxels (Lieberman and Cunningham, 2009). Neuroanatomical areas were identified using the Automated Anatomical Labelling Atlas (Tzourio-Mazoyer et al., 2002) included in the xjView toolbox (http://www.alivelearn.net/xjview8/). Due to the limited patient sample, no traditionally applied nuisance covariates were used (e.g. total intracranial volume).

3. RESULTS

Higher scores in MusEQ's Daily subtest were associated with greater GMV (grey matter volume) in three clusters comprising right anterior and right middle cingulum (R=.995, p<.001), right precentral and right superior frontal gyri as well as left lingual gyrus, left calcarine sulcus and left cuneus (R=.999, p<.001, for both) (Table 2, Figures 4, 5).
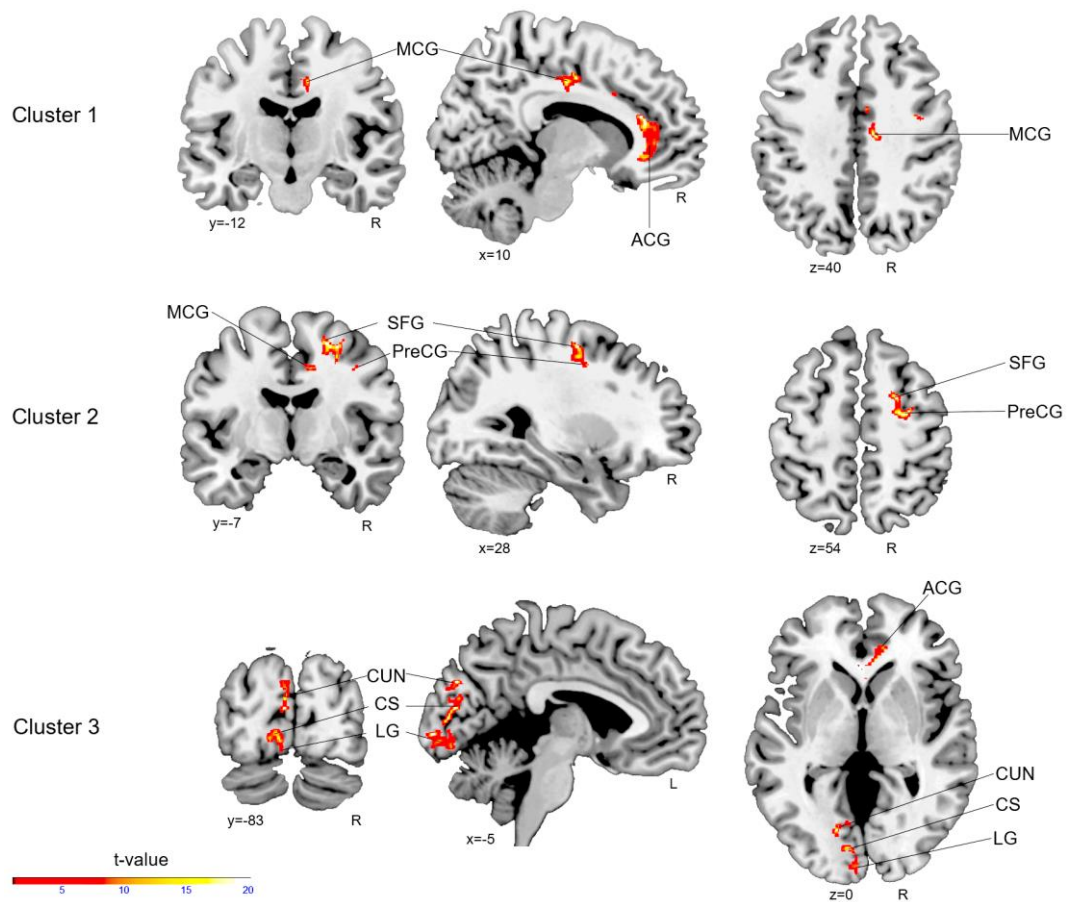

Figure 4 *Correlation of GMV and MusEQ 'Daily' subtest score.*
MCG=middle cingulate gyrus, ACG=anterior cingulate gyrus, PreCG=precentral gyrus, SFG=superior frontal gyrus, CUN=cuneus, CS=calcarine sulcus, LG=lingual gyrus.
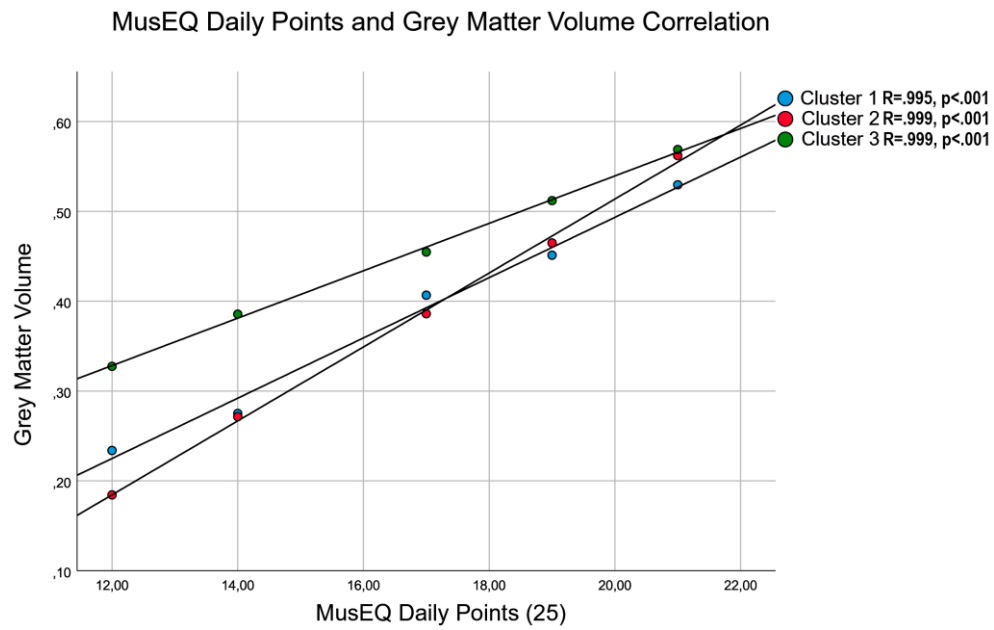
Figure 5 *Correlation of GMV and MusEQ 'Daily' subtest score.*

Higher scores in MusEQ's Emotion subtest were associated with greater GMV in one cluster comprising left calcarine sulcus and left cuneus (R=.994, p=.001) (Table 2, Figures 6, 7).
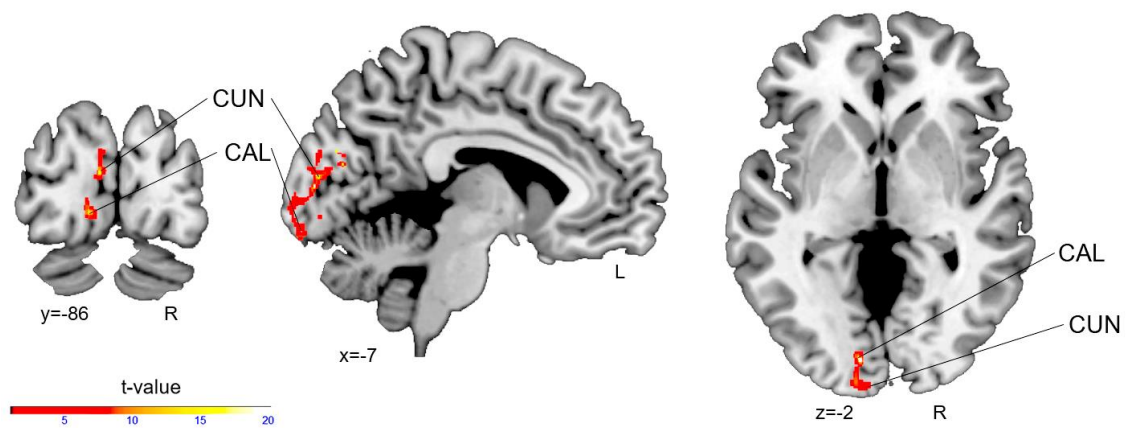
Figure 6 *Correlation of GMV and MusEQ 'Emotion' subtest score.*
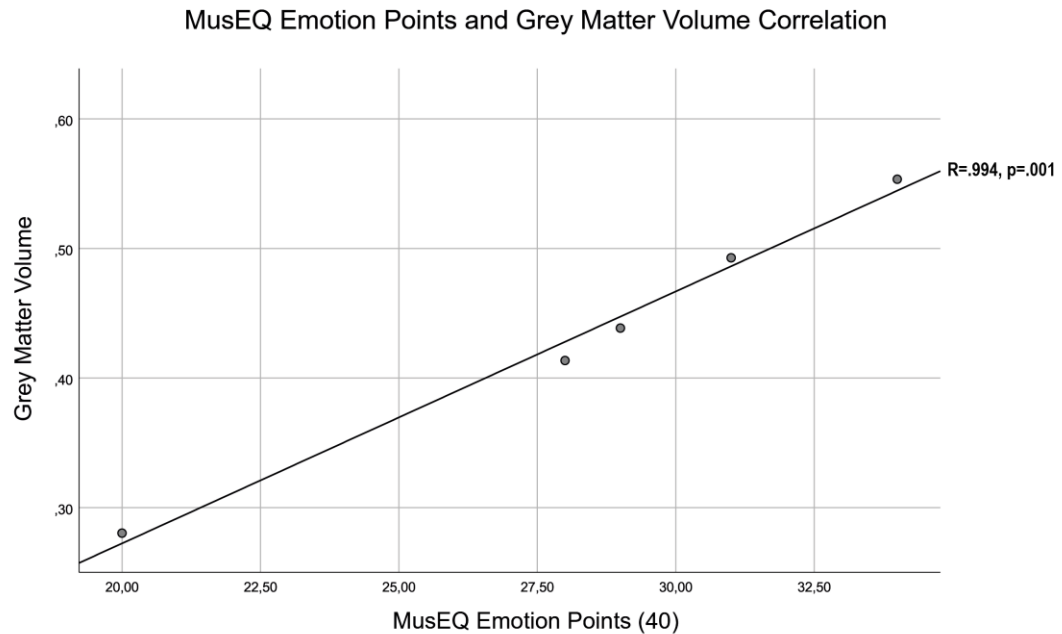CUN=cuneus, CAL=calcarine sulcus.

Figure 7 *Correlation of GMV and MusEQ 'Emotion' subtest score.*

Higher scores in MusEQ's Respond subtest were associated with greater GMV in eleven clusters comprising left precentral and postcentral gyri (R=.996, p<.001), right middle and bilateral anterior cingulum, right caudate nucleus and right putamen (R=.997, p<.001), right calcarine sulcus and right lingual gyrus (R=.996, p<.001), left superior and bilateral middle frontal gyri as well as right inferior frontal gyrus along with right Rolandic operculum, left middle and inferior temporal gyri. Notable correlations were also found in left superior temporal pole (R=.998, p<.001) and left superior, middle and medial frontal gyri (R=.999, p<.001), left thalamus and left calcarine sulcus as well as left lingual and parahippocampal gyri (R=.996, p<.001). Greater grey matter volume in the left (R.999, p<.001) and right (.994, p=.001) cerebellum was also associated with higher overall score in the MusEQ test (Table 2, Figures 8, 9).
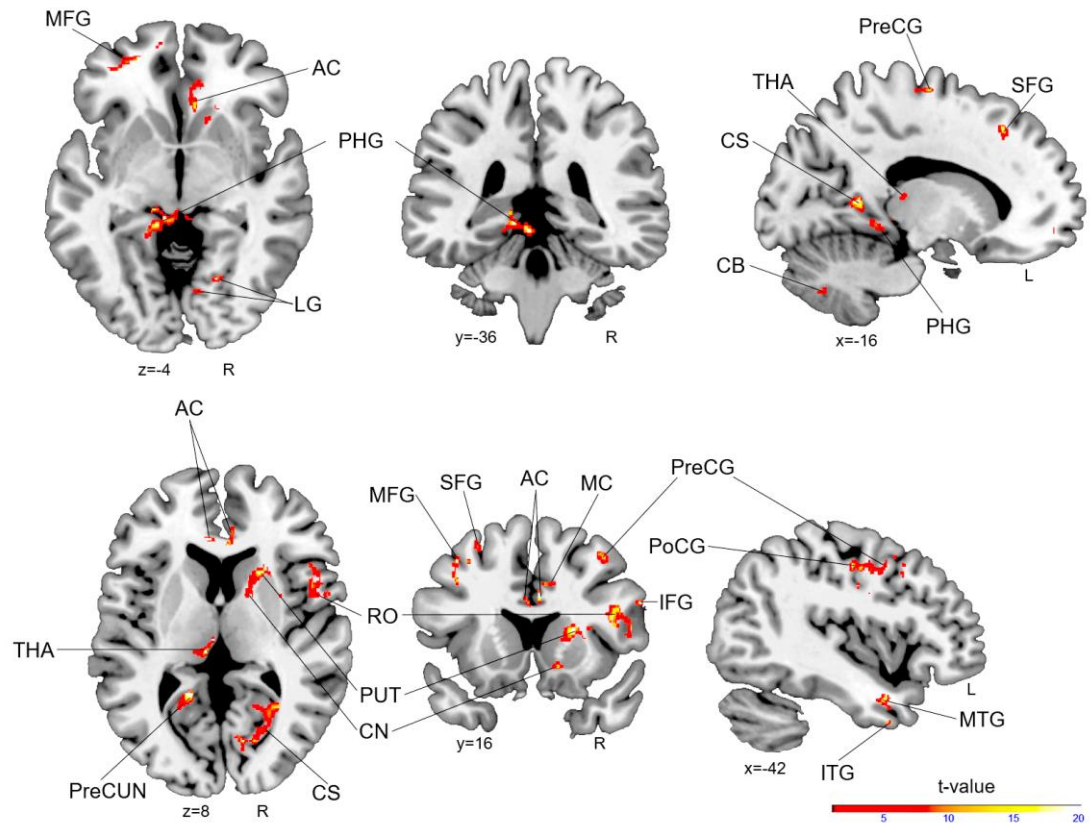
Figure 8 *Correlation of GMV and MusEQ 'Respond' subtest score.*
MFG=middle frontal gyrus, AC=anterior cingulum, MC=middle cingulum,
PHG=parahippocampal gyrus, LG=lingual gyrus, PreCG=precentral gyrus,
PoCG=postcentral gyrus, THA=thalamus, CS=calcarine sulcus, PreCUN=precuneus,
PUT=putamen, RO=Rolandic operculum, IFG=inferior frontal gyrus, SFG=superior
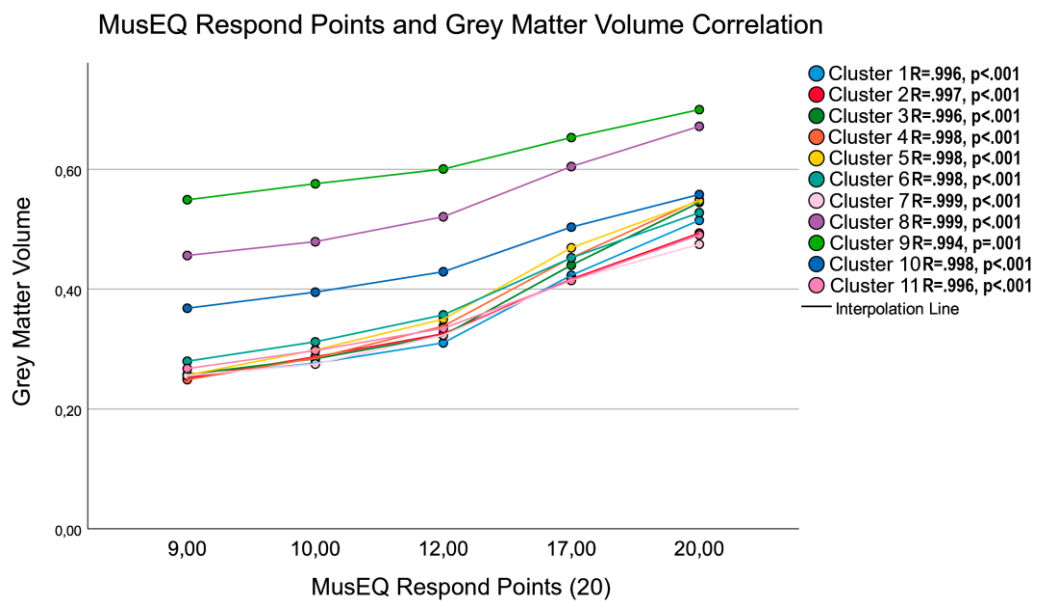frontal gyrus, MTG=middle temporal gyrus, ITG=inferior temporal gyrus,
CB=cerebellum.

Figure 9 *Correlation of GMV and MusEQ 'Respond' subtest score.*

Higher scores in MusEQ's Total points were associated with greater GMV in two clusters comprising superior occipital gyrus, calcarine sulcus and cuneus in the right (R=.998, p<.001) and left (R=.999, p<.001) hemisphere (Table 2, Figures 10, 11).
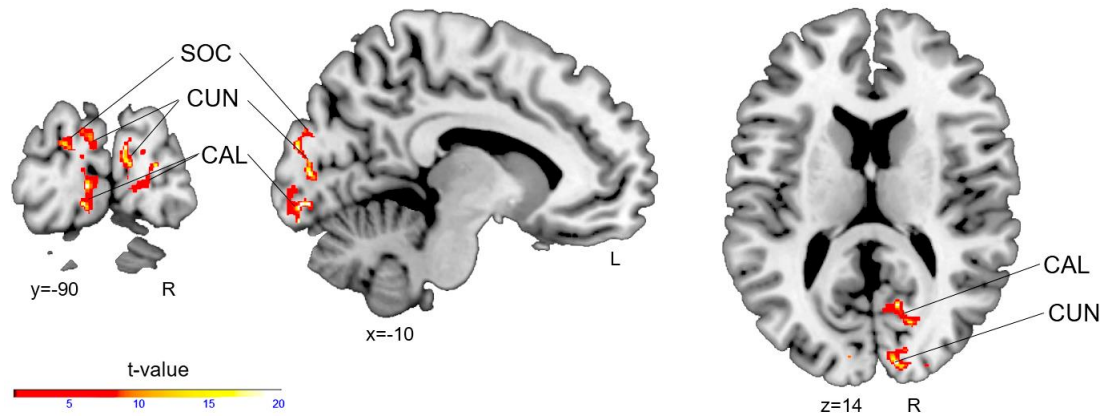

Figure 10 *Correlation of GMV and MusEQ 'Total' score.*
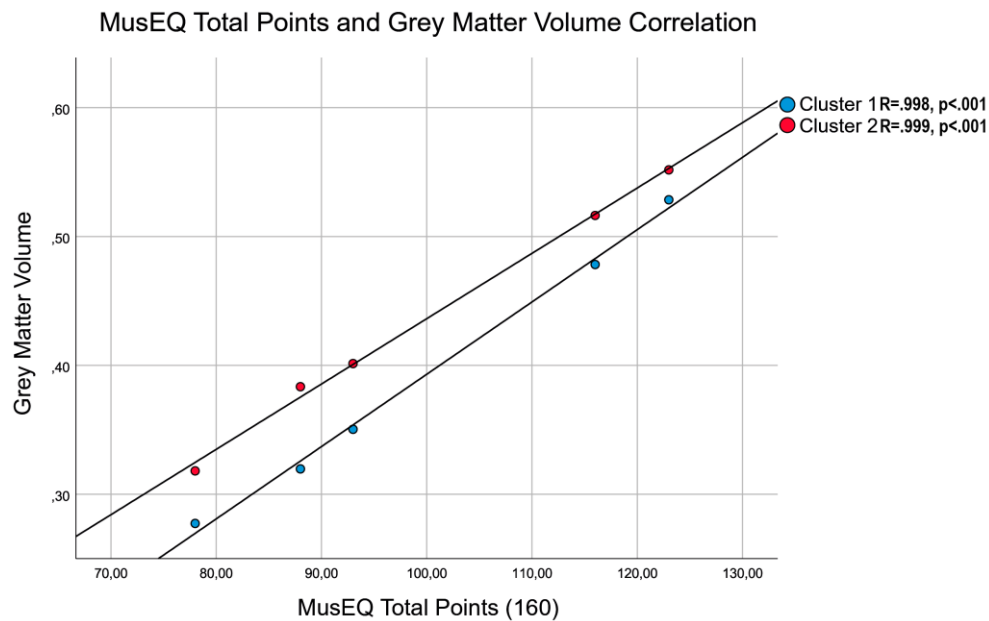SOC=superior occipital gyrus, CUN=cuneus, CAL=calcarine sulcus.

Figure 11 *Correlation of GMV and MusEQ 'Total' score.*

No significant correlations between MusEQ's Perform, Consume or Prefer subtests and greater GMV of any brain region studied were observed.

MusEQ subtest	Area name	Coordinates	Cluster size	t-value	z-value	Correlation
Daily	Right Middle Cingulum (BA 24)	12 -18 41	2115	87.46*	4.65*	R = 0.995, p < 0.001
	Right Anterior Cingulum (BA 32)	9 34 -6				
	Right Precentral Gyrus (BA 6)	29 -7 55	1212	82.97*	4.62*	R = 0.999, p < 0.001
	Right Superior Frontal Gyrus (BA 6)	20 -7 58				
	Left Lingual Gyrus (BA 17, 19)	-11 -84 2	2015	77.69*	4.58*	R = 0.999, p < 0.001
	Left Calcarine Sulcus (BA 17)	-6 -95 -6				
	Left Cuneus (BA 18)	-2 -84 24				
Emotion	Left Calcarine Sulcus (BA 17, 18)	-10 -90 3	1805	68.09*	4.49*	R = 0.994, p = 0.001
	Left Cuneus (BA 18)	-5 -87 17				
Respond	Left Precentral Gyrus (BA 6)	-40 -2 44	885	226.83*	5.21*	R = 0.996, p < 0.001
	Left Postcentral Gyrus (BA 6)	-42 -9 34				
	Right Anterior Cingulum (BA 24)	5 22 23	3294	97.83*	4.72*	R = 0.997, p < 0.001
	Left Anterior Cingulum (BA 24)	-4 32 0				
	Right Middle Cingulum (BA 24)	5 11 36				
	Right Putamen	23 16 8				
	Right Caudate Nucleus	21 14 11				
	Right Calcarine Sulcus (BA 18, 30, 31)	20 -70 11	1326	90.05*	4.67*	R = 0.996, p < 0.001
	Right Lingual Gyrus (BA 19)	20 -66 -2				
	Left Middle Frontal Gyrus (BA 8)	-35 23 46	2482	89.64*	4.67*	R = 0.998, p < 0.001
	Left Superior Frontal Gyrus (BA 6, 8)	-14 30 45				
	Right Middle Frontal Gyrus (BA 8)	37 17 49	997	83.13*	4.62*	R = 0.998, p < 0.001
	Right Rolandic Operculum (BA 44)	58 5 12	1406	82.75*	4.62*	R = 0.998, p < 0.001
	Right Inferior Frontal Gyrus (BA 44, 45, 46)	58 9 20				
	Left Superior Frontal Gyrus (BA 10, 11)	-28 50 -3	904	79.45*	4.59*	R = 0.999, p < 0.001
	Left Middle Frontal Gyrus (BA 11)	-26 52 -10				
	Left Medial Frontal Gyrus (BA 10)	-10 40 -9				
	Left Cerebellum	-11 -61 -44	2389	79.38*	4.59*	R = 0.999, p < 0.001
	Right Cerebellum	27 -50 -29	926	69.54*	4.51*	R = 0.994, p = 0.001
	Left Superior Temporal Pole (BA 38)	-47 7 -18	1288	69.4*	4.51*	R = 0.998, p < 0.001
	Left Middle Temporal Gyrus (BA 21)	-53 6 -26				
	Left Inferior Temporal Gyrus (BA 20)	-49 -2 -40				
	Left Thalamus	-10 -29 0	1309	58.65*	4.4*	R = 0.996, p < 0.001
	Left Calcarine Sulcus (BA 30)	-16 -53 6				
	Left Parahippocampal Gyrus (BA 30)	-16 -37 -8				
	Left Lingual Gyrus (BA 27, 30)	-13 -37 -4				
Total	Right Cuneus (BA 18, 19)	12 -93 16	1411	161.23*	5.02*	R = 0.998, p < 0.001
	Right Calcarine Sulcus (BA 17, 23, 31)	16 -76 9				
	Right Superior Occipital Gyrus (BA 18)	17 -90 18				
	Left Cuneus (BA 19)	-7 -85 26	1899	64.93*	4.46*	R = 0.999, p < 0.001
	Left Calcarine Sulcus (BA 17, 18)	-10 -86 4				
	Left Superior Occipital Gyrus (BA 18)	-17 -8 22				

*p < 0.005 FWE-corrected at the cluster level

All results are thresholded at a whole-brain uncorrected p < 0.005 threshold at the voxel level with a minimal cluster size set to 100 voxels.

Correlations are Pearson correlations (2-tailed p-value, FDR-corrected).

BA = Brodmann area

Table 2 *Correlation of GMV and MusEQ scores with statistical significance.*

4. DISCUSSION

The aim of this study was to explore how recently diagnosed patients with AD engage in musical activities in their daily lives and how this behavior correlates with regional GM volumes. Furthermore, our goal was to identify the brain areas involved in different types of musical activities as assessed by MusEQ's subtests as well as the total score. Higher scores on Daily, Emotion and Respond subtests showed significant association with greater GMV as did the Total points. The current results provide information on neural structures related to music engagement in persons with AD that could contribute to implementation of music-based therapeutic approaches for maintaining quality of life.

Higher total MusEQ score was associated with greater GMV in the cuneus, calcarine sulcus and superior occipital gyrus bilaterally. The cuneus-calcarine junction has been implicated in working memory (Neta and Whalen, 2011) and reward anticipation (Thomas et al., 2013). Left cuneus and calcarine sulcus were also associated with higher points on MusEQ's Emotion subscale suggesting that greater GMV in these areas may contribute to higher level of affectivity derived from musical activities. Supporting this, the cuneus has been identified in previously published literature to become activated in response to emotionally rewarding stimuli (Blood and Zatorre 2001).

Patients who scored higher points on the MusEQ's Daily and Respond subscales had greater GMV in areas of the limbic system, such as the cingulum and parahippocampal gyrus, suggesting that preserved cortical volume in those areas may contribute to greater frequency of musical activities and reflect greater meaning derived from music. Anterior part of the cingulum is known to be linked to emotion processes, while the middle part is connected to motor and premotor areas. The cingulum also connects to the hippocampus, integrating memory traces to other parts of the brain. It has been shown that damage to cingulum also damages the hippocampus and vice versa. This interaction plays a role in the development of MCI and signifies one of the earliest pathological changes in age-related dementia affecting verbal memory (Delano-Wood et al., 2012). The parahippocampal region is known to be critically important for episodic memory encoding and retrieval (Li et al., 2010).

Other brain areas associated with higher scores on Daily and Respond subtests included the superior frontal gyrus, which has been implicated in the process of self-awareness,

that also involves anterior parts of the cingulum, the posterior middle frontal gyrus, as well as posterior part of inferior frontal gyrus and the anterior insula. The ability to verbally describe one's daily music engagements and responsiveness to musical stimuli can be attributed to greater GMV in inferior frontal gyrus, which contains the Broca's area. Interestingly, it has been shown by an fMRI study that, while activated during introspection, activity in the superior frontal gyrus seems to be inhibited by auditory and visual sensorimotor tasks (Goldberg et al., 2006). Sensorimotor tasks have been shown to elicit activation in occipitoparietal and temporal cortices. Our findings support this as higher overall music engagement positively correlated with greater GMV in our patient's brain areas including the cuneus and calcarine sulci, precentral and postcentral gyri as well as bilateral lingual gyri along with left inferior and middle temporal gyri. The well-established concept of default-mode network (DMN) spanning across multiple brain areas helps explain the on-off switch regulating the focus of attention inwards and outwards. Reduced regional glucose metabolism caused by AD is known to disrupt the DMN and leads to impairments in cognitive functions, such as autobiographical memory retrieval (Buckner et al., 2008).

The proclivity to engage in singing and music-mediated movement as assessed by MusEQ's Respond subtest, correlated positively with GMV in the right Rolandic operculum, as also supported by previously published literature (Koelsch et al., 2006). Left thalamus was also logically implicated in these findings as it, among other functions, serves as a relay center for sensorimotor signals between the cortex and spinal cord. Increased GMV of basal ganglia, putamen and caudate nucleus, is also a logical finding under this condition, as they play a crucial role in movement and non-movement functions of music processing, respectively. In addition to motor functions. the caudate nucleus, being a part of the reward system, employs the dopaminergic pathway involved in learning and motivation. The putamen modulates various neurotransmitter systems, such as those using GABA or acetylcholine, the latter of which is centrally involved in AD pathology and treatment. Another interesting finding was the correlation observed in cerebellum. The cerebellum plays a key role in fine movements pertaining to dancing or playing a musical instrument, not only in actual motor activity, but presumably also in mental image of movement. Cerebellum may be important in relation to additional cognitive functions aside from the known learning-related functions (Rapoport et al., 2000).

The results of the present study should be considered with caution because of the following limitations. Due to small sample size, the effect of individual participant's focal GMV becomes more pronounced compared to studies with larger sample sizes. Moreover, as clinical MRI data was used in the study, the imaging was not carried out using a single MRI scanner, instead the clinical material was obtained using three scanners with slightly varying imaging sequences. Also, due to the small sample size, clinically relevant nuisance covariates (e.g. age, sex, adjustment for intracranial volume, MMSE) could not be implemented and only bivariate correlation (GMV with MusEQ) of the data could be obtained. While the analyses produced very high Pearson correlations and t-values, due to aforementioned limitations, the results could be interpreted as provisional and should be verified in a larger prospective study using a single scanner and fixed imaging parameters.

4.1 *Concluding remarks*

While this small-scale feasibility study has considerable limitations, it provides suggestive evidence that factors of musical engagement are associated with retained GMV of specific brain regions that are relevant to memory and music processing. In the future music engagement and perception as well as production in AD patients should be evaluated using a larger sample size in a longitudinal setting with multimodal MRI methods (e.g. DTI, fMRI). In addition, utilization of relevant covariates, such as clinical score of cognition, neuropsychiatric indices, prescribed medication and assessment of musicality as well as adjustments for head size is encouraged. Furthermore, the role of music as a protective factor or method of personalized treatment in AD patients warrants further research.

5. ACKNOWLEDGEMENTS

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