Microstructural changes related to dementia and music therapy in memory impaired patients measured with diffusion MRI: first insights of the ALMUTH trial

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ABSTRACT

Background: Dementia is a growing syndrome that requires many resources. Traditionally, its effects have been mitigated by pharmacological measures; however, new treatments such as music therapy are on the rise. In this study, underlying changes in brain microstructure associated to memory impairment and music therapy by means of diffusion are evaluated.

Methods: A cross-sectional and a longitudinal study were performed, in each of which a region of interest (ROI) analysis and a network analysis were carried out. 101 participants were selected for the short-term study (54 memory impaired participants and 47 healthy controls) and 31 for the long-term study (15 memory impaired participants in the music group and 16 memory impaired participants in the control group). All memory impaired subjects were recruited from the Alzheimer's and Music Therapy (ALMUTH) study. They underwent diffusion-weighted (DW) and T1 scans in the same way as healthy controls. Diffusion and connectomics metrics were computed in 16 regions of the brain and in the whole-brain, respectively. These metrics were compared between groups and related to active musical engagement, musical training and number of active singing lessons.

Results: A significant increase in fractional anisotropy (FA) in the right superior-temporal cortex, left medial-orbito-frontal cortex and right parahippocampal cortex regions was found when comparing pre and post scans in the ALMUTH music group. Also, FA and active musical engagement were found to be significatively correlated for healthy controls in three other regions: right insula cortex, left postcentral cortex and right postcentral cortex. Regarding mean diffusivity (MD), significant higher MD was encountered in ALMUTH participants than in healthy controls for the hippocampus and the parahippocampal regions. Likewise, a significant increase in MD in the entire hippocampus and in the left hippocampus was noticed when comparing pre and post scans in both the ALMUTH control and music groups, respectively. In relation to network metrics, significant higher local efficiency was obtained for healthy controls in comparison with ALMUTH participants.

Conclusion: The hippocampus and the parahippocampal regions are highly affected in dementia, but active musical engagement might prevent memory impairment in some regions. Also, the results suggest that a reduction in local efficiency can be a useful indicator for whole-brain memory decline. The findings regarding music therapy suggest it could have a positive effect on underlying microstructural changes, reversing the effects of memory loss in some regions.

Keywords: tractography, mean diffusivity (MD), fractional anisotropy (FA), network analysis, connectivity matrix, clustering coefficient, local and global efficiency, path length and memory impairment

I. INTRODUCTION

A. Background

Dementia is a syndrome of increasing prevalence worldwide [1] that involves the loss of cognitive functioning -thinking, remembering and reasoning- and behavioural and mood abilities to such an extent that it interferes with daily life and activities. For this reason, and because it requires a high volume of social and healthcare resources, was the prime research motivation for this study. Alzheimer's disease (AD) is the most common form of dementia in developed countries, accounting for between 60% and 70% of cases [2].

At present, the advance of cognitive deterioration is trying to be arrested with pharmacological measures [3]. However, this entails adverse effects and certain risks [4]. Thereupon, non-pharmacological measures such as music therapy are being considered. They have shown promising results regarding the improvement of cognition and behaviour.

Music therapy is the clinical use of music to accomplish individualized goals such as reducing stress, improving mood and self-expression. It is an evidence-based therapy well-established in the health community. In fact, many studies have reported physical, cognitive and psychological benefits in memory impaired subjects [5-6]. Nonetheless, its effect on brain structures has not yet been scientifically proven.

In the context of this work, the concepts brain plasticity, brain lateralization, myelin sheaths, partial volume effect (PVE) and stages of objective memory impairment (SOMI) are involved, which will be succinctly explained below.

Brain plasticity refers to the brain capability to change in response to environmental factors and it is based on assumption that the development of each person's brain is influenced by both physical and psychological experiences. The mechanisms involved in plasticity in the nervous system are thought to support cognition and some of these processes are affected during normal ageing [7]. However, recent studies suggest that better musical abilities in musicians are reflected in training-induced neuroplastic changes [8], which gives hope for the effectiveness of music therapy. In fact, research has shown that repeatedly practicing the association of motor actions with specific sound and visual patterns (musical notation), while receiving continuous multisensory feedback, will strengthen connections between auditory and motor regions as well as multimodal integration regions. Plasticity in this network may explain some of the sensorimotor and cognitive enhancements that have been associated with music training [9].

The lateralization of brain function is the tendency for some neural functions or cognitive processes to be specialized to one side of the brain or the other. The medial longitudinal fissure separates the human brain into two distinct cerebral hemispheres, connected by the corpus callosum. Although the macrostructure of the two hemispheres appears to be almost identical, different composition of neuronal networks allows for specialized function that is different in each hemisphere.

Myelin is an insulating layer, or sheath that forms around nerves, including those in the brain and spinal cord. It is made up of protein and fatty substances. This myelin sheath allows electrical impulses to transmit quickly and efficiently along the nerve cells. If myelin is damaged, these impulses slow down.

Partial volume effects occur where multiple tissues with different diffusion profiles contribute to a single voxel, resulting in a blurring of intensities at tissue boundaries. If PVE changes, the analysis and estimation of bundle-averaged diffusion metrics will change.

The SOMI framework provides a neuropathologically validated staging system for episodic memory impairment in the AD continuum and identifies four predementia stages and two dementia stages. SOMI was developed based on literature mapping of performance in the Free and Cued Selective Reminding Test to clinical outcomes and to biomarkers in longitudinal aging studies [10].

B. Objectives

The aim of this work is, on the one hand, to study the underlying microstructural changes that occur in memory impaired people and, on the other hand, to test whether music therapy induces any microstructural change in these subjects. Research shows that music can influence structural plasticity [11-13].

For this purpose, a cross-sectional and a longitudinal study were conducted. In each of them, two different analyses were carried out: a region of interest (ROI) analysis and a network analysis.

II. METHODS

A. Data

Magnetic resonance imaging (MRI) data was acquired from the Alzheimer’s and Music Therapy (ALMUTH) study which was conducted at the University of Bergen, Norway [14]. Moreover, healthy controls (HC) were also selected. Participants gave their informed consent and filled out a demographic questionnaire and 19 music-related questions from the Goldsmiths Musical Sophistication Index (Gold-MSI) [15]. Data acquisition and pre-processing were previously performed and are beyond the scope of this report.

The ALMUTH study is a randomized controlled trial, which started in April 2018 and is ongoing. Subjects were randomised to either a music intervention (singing lessons), a non-musical intervention (physical activity) or no intervention (control group) for 12 months of weekly intervention sessions. The participants were adults with mild AD, mild cognitive impairment (MCI) or who claimed to have subjective memory complaints that were also validated by a close family member, recruited through advertisements and collaborating institutions.

The Gold-MSI is a psychometric tool for the measurement of musical attitudes, behaviours and skills. It comprises a self-report questionnaire as well as a suite of music psychological tests measuring different musical skills. The purpose of the self-report questionnaire as a psychometric instrument is to quantify the amount of musical engagement and behaviour of an individual in its many possible facets and to record the self-assessed level of various musical skills [15]. There are six dimensions measured by the Gold-MSI: active engagement, perceptual abilities, musical training, emotion, singing abilities and general musical sophistication. In this project, two full subscales of the Gold-MSI questionnaire were considered: active engagement and musical training.

B. Participants

Inclusion criteria for the ALMUTH study was to be an adult with a diagnosis of mild AD or MCI due to AD, or with memory complaints without any other neuropsychiatric pathology. Also, participants needed to be able to complete questionnaires in Norwegian, undergo MRI and attend assessments and weekly intervention in the Bergen area in Norway. Further, exclusion criteria were having other dementia type, vascular disorders, traumatic brain injury, neurological illness, severe auditory impairments, physical immobility, severe psychiatric disorders, metal in the soft tissue of the body and moderate or severe AD [16].

The table below displays the means and standard deviations of age and gender for the subjects in each of the studies carried out in this report.

		Cross-sectional study		Longitudinal study	
		ALMUTH Participants (n = 54)	Healthy Controls (n = 47)	ALMUTH Music (n = 15)	ALMUTH Control (n = 16)
AGE	mean	71.22	71.28	68.60	71.88
	standard deviation	7.75	7.03	12.30	10.61
GENDER	males	24	15	6	9
	females	30	32	9	7

Table 1: Means and Standard Deviations of Age and Gender for the participants in the different studies

In this work, a total of 101 aged-matched participants were included in the cross-sectional study and a total of 31 in the longitudinal one. It should be noted that subjects in the ALMUTH exercise group were not contemplated in this investigation due to lack of continuity in the weekly intervention sessions during COVID-19 global pandemic. Unlike in the physical activity group, participants in the music intervention group continued online and over the telephone with their singing lessons during lockdown. Furthermore, participants with low quality scans due to motion artifacts or with incomplete data were also not considered in these studies.

C. Image Acquisition

All MRI scans were acquired using a gradient echo (GE) Discovery™ MR750 3T scanner with a 32 channel head coil. T1-weighted (T1-w) images were acquired using a spoiled gradient recalled echo pulse sequence with the following parameters: 162 axial slices of 1mm without gap, field of view (FOV) of (AP x RL x FH) 256 x 256 x 256 mm^3, voxel size of 1 x 1 x 1 mm^3, echo time (TE) of min full, inversion time (TI) of 450ms and flip angle of 12°, for a total scan duration of 9 minutes.

Diffusion-weighted imaging (DWI) was performed using a single-shot spin-echo echo-planar imaging sequence with the following parameters: 60 axial slices of 2.4mm without gap, FOV of 220 x 220 x 220 mm^3, acquisition matrix of 128 x 128, reconstructed voxel size of 1.72 x 1.72 x 2.4 mm^3, repetition time (TR) of 14000ms, TE of 93ms, flip angle of 90°, 30 diffusion directions (and 6 non-diffusion-weighted scans) and b-value of 1000 s/mm^2, for a total scan duration of 8 minutes.

D. Image Processing

The analyses and processing of the diffusion scans were conducted with FreeSurfer [17] and ExploreDTI [18]. The code from the ExploreDTI GUI interface was automated in MATLAB (R2020b) [19]. The complete image processing pipeline is summarized in figure 1.

i. Pre-processing

T1-w images were pre-processed with FreeSurfer to obtain regions of interest (ROIs) segmentations in native space for each subject. The output was checked visually by analysing the subcortical segmentation and the white surface overlaid on coronal, sagittal and axial T1 slices. The resulting files containing the segmented ROIs and the T1 volumes were converted to the nifti file format.

Diffusion-weighted (DW) images were pre-processed with ExploreDTI. Data was corrected for signal intensity drift, Gibbs ring artifacts, subject motion, eddy current induced distortions and susceptibility distortions [20]. Susceptibility-distortion correction was conducted based on the coregistered T1-w images, consequently transforming the processed DWI scan into the coregistered T1-w space. It should be noted that any rotation of the DWI was followed by realigning the B-matrices [21].

ii. Modelling and tractography

Further processing of the DW images consisted of estimating a diffusion model tensor: Diffusion Tensor Imaging (DTI) model. Although Constrained Spherical Deconvolution (CSD) model was considered because it better reproduces neural pathways such as crossing fibers, DTI was finally selected to facilitate further comparisons with present literature and, also, because the given data was acquired with few gradient directions and with a b-value of just 1000 s/mm^2.

Based on the DTI model, whole brain tractography was performed. The default parameters in ExploreDTI were selected: fractional anisotropy (FA) track range [0.2 1], angle deviation 30°, step size 1mm, seed point resolution [2 2 2]mm and fiber length range [50 500]mm.

iii. ROI analysis

Diffusion metrics for 16 ROIs -related to memory impairment and brain plasticity- were computed for each subject. In particular, mean diffusivity (MD) and FA values were obtained in the following regions of both hemispheres: insula, parsopercularis, superiortemporal, medialorbitofrontal, hippocampus, parahippocampal, postcentral and precentral.

Firstly, to obtain the FA and MD measures, a mask was created for each anatomical ROI with FreeSurfer. Next, the average FA and MD of all the voxels from the same ROI were calculated in those masks using ExploreDTI. It should be noted that this was possible because the mask was located in the same image space as the diffusion data.

Other diffusion metrics, like axial and radial diffusivity, were studied but dismissed as MD already represents the overall magnitude of water diffusion independent of anisotropy.

iv. Network analysis

The network analysis was performed based on individual parcellations of the T1-w images using FreeSurfer. In this work, 85 ROIs from the FreeSurfer segmentation were combined with the white matter streamline sets resulting from the tractography to construct connectivity matrices, from which connectivity metrics were calculated. The selected ROIs comprised all cortical ROIs from the Desikan-Killiany atlas [22] as well as the thalamus proper, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area and ventral diencephalon (all bilateral) and brainstem [23].

An extensive literature research was carried out to find the connectivity matrices and metrics most often used in connectomics when studying memory impairment [23-26]. Accordingly, binary and FA weighted connectivity matrices were constructed and used to calculate clustering coefficient, global and local efficiency and path length, using the Brain Connectivity Toolbox as defined in Rubinov et al. [27].

E. Statistical Analysis

Each diffusion and connectivity metric, obtained from these analyses, was compared between the ALMUTH participants and the healthy controls, in the cross-sectional study, and between the ALMUTH music and control groups, in the longitudinal study. Also, these metrics were related to the active engagement and musical training subscales of the Gold-MSI questionnaire, in the short-term study, and to the number of active singing lessons, in the long-term study.

The comparisons between groups were tested by means of a Student t-test, using a p-value of 0.05 as the threshold for statistical significance. An independent t-test for the cross-sectional study and a paired t-test for the longitudinal one. It should be noted that p-values were uncorrected for multiple comparisons.

Moreover, the relations between the metrics and active engagement, musical training and number of active singing lessons were inspected using Spearman's correlation. This type of correlation was chosen because categorical variables were involved. In addition, the Kolmogorov-Smirnov test was performed to confirm that the data did not follow a standard normal distribution; therefore, Pearson's correlation could not be employed.

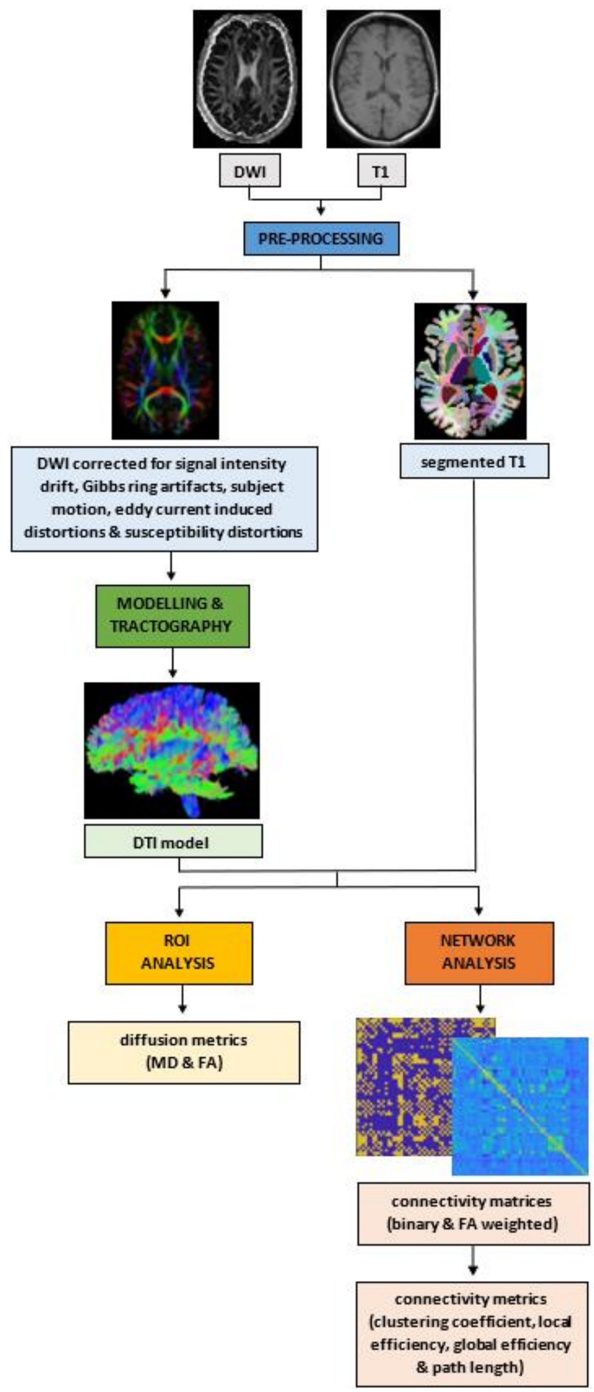

Figure 1: Graphical overview of the image processing pipeline

III. RESULTS

The results obtained are presented below. Firstly, ROI analysis will be addressed for both cross-sectional and longitudinal studies. Secondly, network analysis will be tackled for both studies as well. It should be mentioned that "ctx" is the abbreviation for "cortex", "lh" for "left hemisphere" and "rh" for "right hemisphere".

A. ROI Analysis

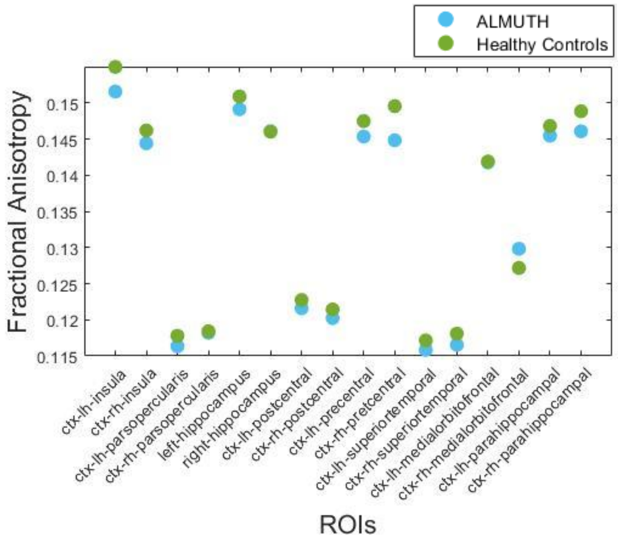

Figure 2: Fractional Anisotropy for ALMUTH Participants and Healthy Controls in every ROI

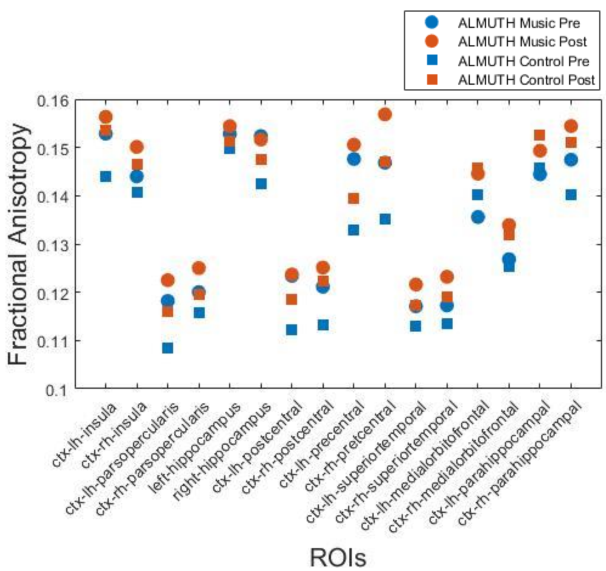

Figure 3: Fractional Anisotropy for ALMUTH Music and Control groups for Pre and Post scans in every ROI

To begin with, the FA metric will be evaluated. As can be observed in figure 2, ALMUTH participants have a slightly lower FA than healthy controls, meaning that the diffusivity is more isotropic. Looking at figure 3, an increase in FA for post scans of both ALMUTH groups -music and control- can be noticed.

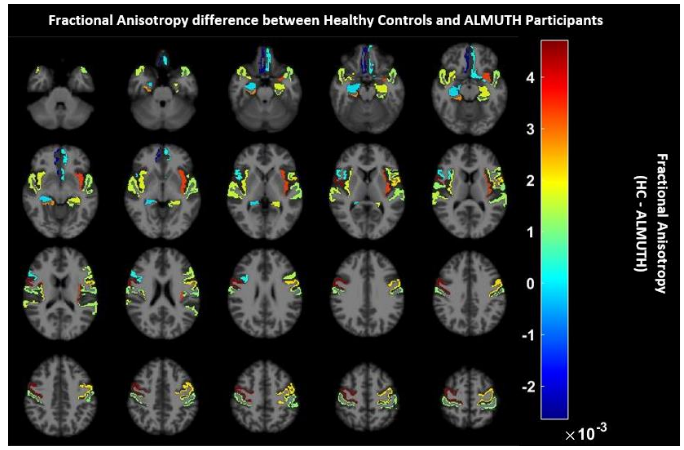

Figure 4: Fractional Anisotropy difference between Healthy Controls and ALMUTH Participants

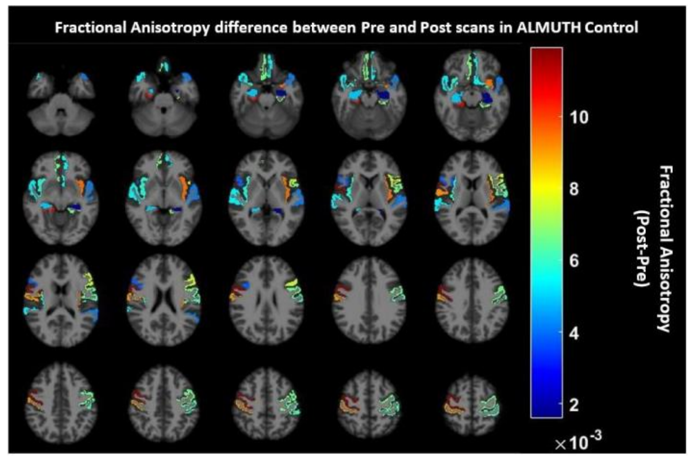

Figure 5: Fractional Anisotropy difference between Pre and Post scans in ALMUTH Control

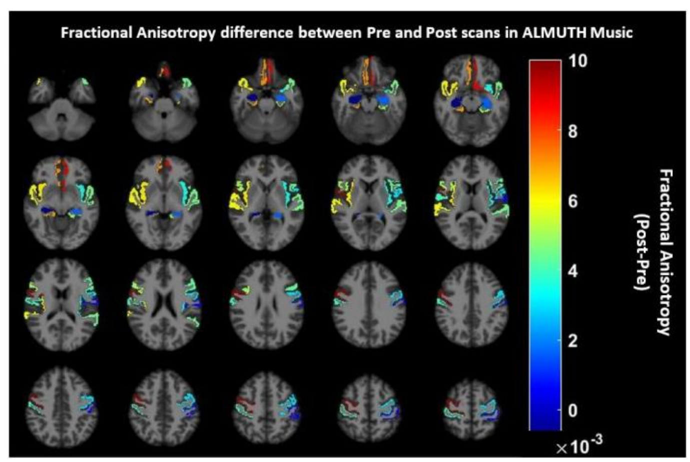

Figure 6: Fractional Anisotropy difference between Pre and Post scans in ALMUTH Music

From figure 4, it can be corroborated that healthy controls have higher FA values than ALMUTH participants in almost every region, as the range of values is mostly positive. Similarly, figures 5 and 6 show that all brain regions increase in FA for both ALMUTH groups after 12 months of weekly intervention sessions.

Another aspect to consider is brain's laterality. All regions, except the medial-orbito-frontal cortex, have higher FA in the right hemisphere (left side in the image).

In order to examine the results from the previous plots, an independent t-test was conducted for the cross-sectional study and a paired t-test for the longitudinal one. Likewise, correlations between FA and active musical engagement and musical training were carried out for the cross-sectional study and between FA and the number of active singing lessons for the longitudinal one.

Using a p-value of 0.05 as the threshold for statistical significance, no relevant ROI was found either when studying the difference between ALMUTH participants and healthy controls nor when studying the differences between scans in the ALMUTH control group. Nonetheless, the increase of FA in post scans compared to pre scans in the ALMUTH music group turned out to be significant in three ROIs: right superior-temporal cortex ($t_{14} = -2.69$, $p = 0.02$), left medial-orbito-frontal cortex ($t_{14} = -2.79$, $p = 0.01$) and right parahippocampal cortex ($t_{14} = -2.37$, $p = 0.03$).

With regard to the correlations, FA and active musical engagement were found to be significantly correlated for healthy controls in three other ROIs: right insula cortex ($r_{45} = 0.37$, $p = 0.01$), left postcentral cortex ($r_{45} = 0.31$, $p = 0.04$) and right postcentral cortex ($r_{45} = 0.33$, $p = 0.03$). No correlation was found with respect to musical training or the number of active singing lessons.

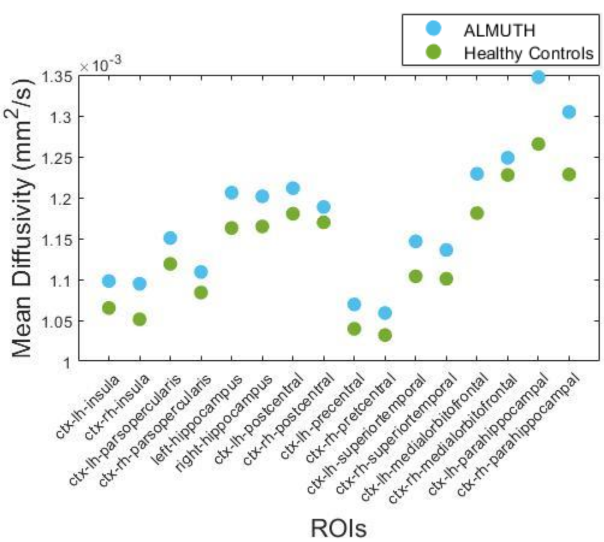

Figure 7: Mean Diffusivity for ALMUTH Participants and Healthy Controls in every ROI

Figure 7 displays that ALMUTH participants have higher MD than healthy controls, implying the amount of diffusion is higher in the ALMUTH group. Moreover, figure 8 shows a general trend for lower MD in the post scans, in comparison with the pre scans, for both ALMUTH groups.

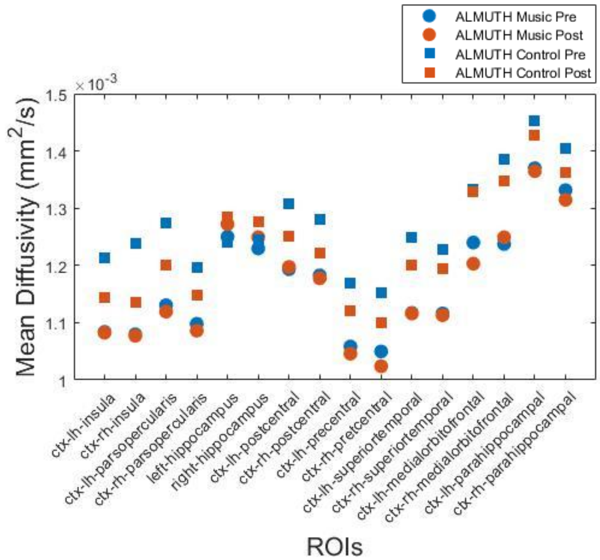

Figure 8: Mean Diffusivity for ALMUTH Music and Control groups for Pre and Post scans in every ROI

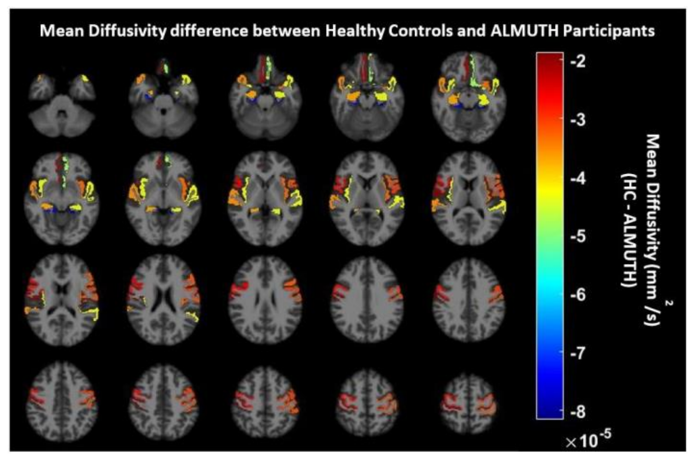

Figure 9: Mean Diffusivity difference between Healthy Controls and ALMUTH Participants

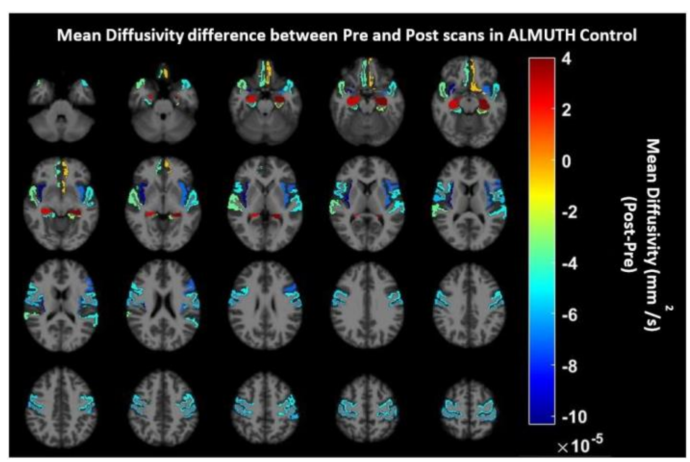

Figure 10: Mean Diffusivity difference between Pre and Post scans in ALMUTH Control

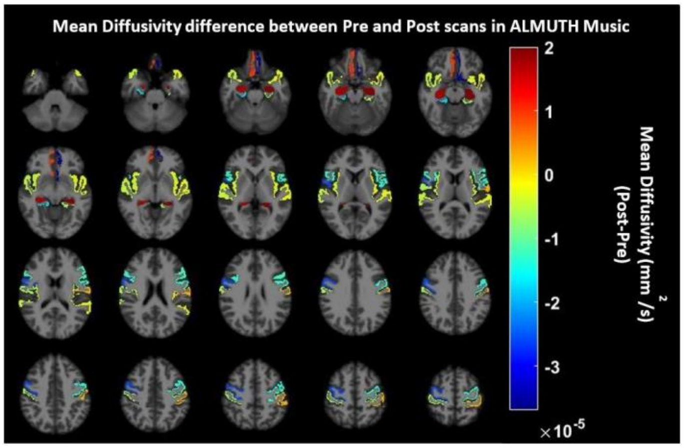

Figure 11: Mean Diffusivity difference between Pre and Post scans in ALMUTH Music

Figure 9 further supports that ALMUTH participants have higher MD than healthy controls in every of the 16 studied brain regions, as all the range of values in the colormap is negative. Nevertheless, figures 10 and 11 display both positive and negative values. Therefore, some ROIs increase in MD and others decrease after 12 months of weekly intervention sessions.

It should be noted that in the ALMUTH control group the differences are larger than in the ALMUTH music group.

With the purpose of inspecting the aforementioned results, the same t-tests and correlations as in the FA metric were conducted.

When carrying out the independent t-test for the cross-sectional study, the higher values of MD for ALMUTH participants compared to healthy controls turned out to be significant in four ROIs: left hippocampus (t_{99} = 2.34, p = 0.02), right hippocampus (t_{99} = 2.10, p = 0.03), left parahippocampal cortex (t_{99} = 2.34, p = 0.02) and right parahippocampal cortex (t_{99} = 2.53, p = 0.01).

For the longitudinal study, the paired t-test indicated that the increase of MD in post scans compared to pre scans in the ALMUTH music group was significant in the left hippocampus (t_{14} = -2.47, p = 0.02). In addition, the increase of MD in post scans in the ALMUTH control group was significant in the left hippocampus (t_{15} = -3.64, p = <0.01) as well as in the right hippocampus (t_{15} = -2.61, p = 0.02).

Regarding the correlations, MD and active musical engagement were found to be significantly correlated for healthy controls in the left medial-orbito-frontal cortex region (r_{45} = -0.30, p = 0.04). Therefore, the higher the active musical engagement, the lower the MD. Likewise, MD and musical training turned out to be significantly correlated for healthy controls in four ROIs: left insula cortex (r_{45} = -0.30, p = 0.04), right insula cortex (r_{45} = -0.41, p = <0.01), left postcentral cortex (r_{45} = -0.30, p = 0.04) and left superior-temporal cortex (r_{45} = -0.30, p = 0.04). No correlation was found with respect to the number of active singing lessons.

B. Network Analysis

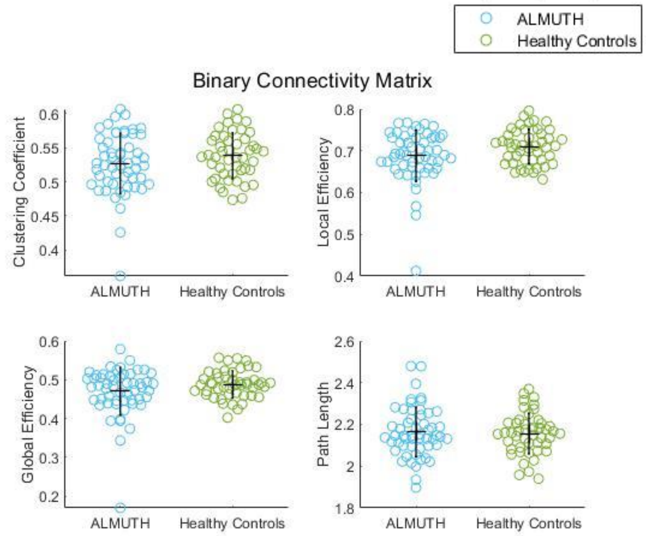

Figure 12: Scatter plots of connectomics metrics (clustering coefficient, local efficiency, global efficiency and path length) calculated from the Binary Connectivity Matrix for ALMUTH Participants and Healthy Controls

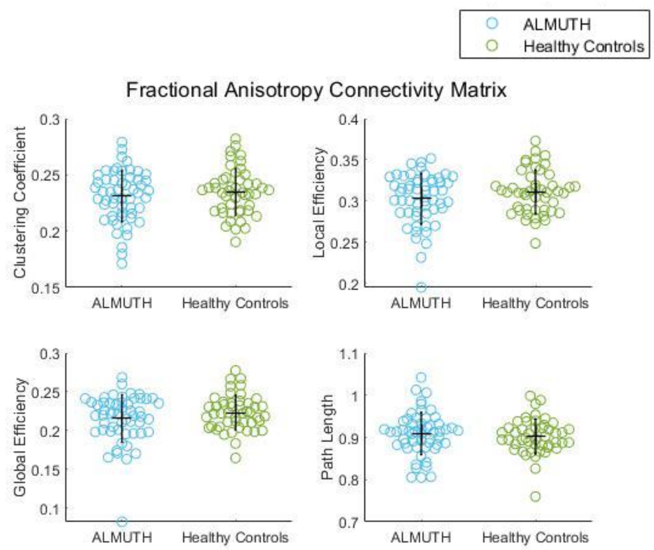

Figure 13: Scatter plots of connectomics metrics (clustering coefficient, local efficiency, global efficiency and path length) calculated from the Fractional Anisotropy Connectivity Matrix for ALMUTH Participants and Healthy Controls

With the aim of having a first overview of the metrics obtained from the network analysis, scatterplots are displayed in figures 12-15. The range of values for all these measures was validated through comparison with the results reported in the study by Kok et al. [23].

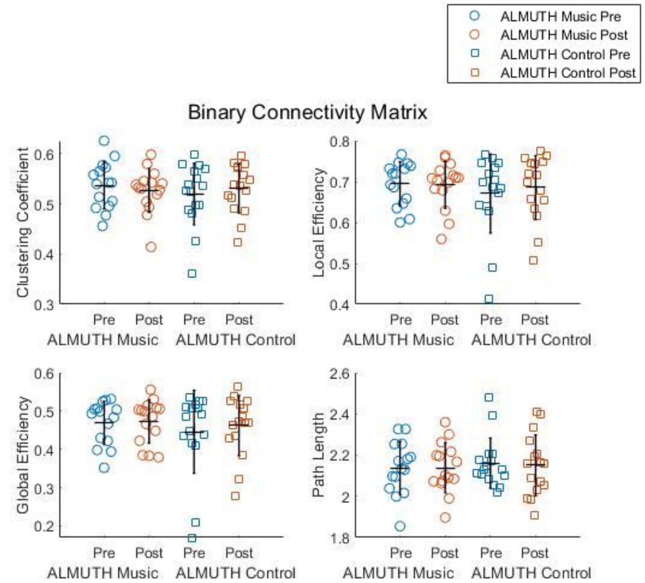

Figure 14: Scatter plots of connectomics metrics (clustering coefficient, local efficiency, global efficiency and path length) calculated from the Binary Connectivity Matrix for ALMUTH Music and Control groups

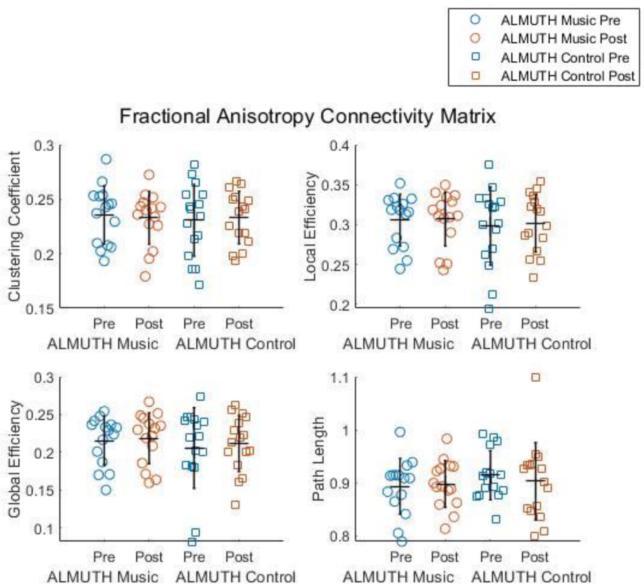

Figure 15: Scatter plots of connectomics metrics (clustering coefficient, local efficiency, global efficiency and path length) calculated from the Fractional Anisotropy Connectivity Matrix for ALMUTH Music and Control groups

At first glance, both the cross-sectional study (figures 12 and 13) and the longitudinal study (figures 14 and 15) show no differences between the medians of the groups studied in each case.

Moreover, in the longitudinal study, it was investigated if any trend between pre and post scans existed. The figures A-D, attached in the appendix section, confirm that no tendency was found in any connectomics metric between these two points in time.

To confirm the lack of significant differences between groups in both cross-sectional and longitudinal studies, an independent and a paired t-test were conducted, respectively. Unexpectedly, for the cross-sectional study, the local efficiency obtained from the binary connectivity matrix turned out to be significantly different ($t_{99} = -2.10$, $p = 0.04$). Looking at the values, higher local efficiency was obtained for the healthy controls rather than for the ALMUTH participants.

Furthermore, no correlations were found between any connectivity metric and active musical engagement, musical training or the number of active singing lessons.

IV. DISCUSSION

For the ROI analysis, the FA metric will be discussed first. The fact that ALMUTH participants have lower FA than healthy controls might be because in ALMUTH participants neurons could have damaged myelin sheaths, which are the insulating layers. This damage could make diffusion more isotropic and thus could decrease the FA. Following with the same hypothesis, the increase in FA in the post scans for both ALMUTH groups demonstrates that the underlying tissue has changed, which suggests that neural substrate has changed. In particular, it suggests that new myelin sheaths have been created or at least reinforced, as higher FA means a preferred direction of diffusion which implies improved fiber integrity, since water flows more freely along the axons compared with through the cell wall. The statistically significant increase of FA in the post scans of the ALMUTH music group in the right superior-temporal cortex, left medial-orbito-frontal cortex and right parahippocampal cortex regions corroborate this hypothesis based on the existence of the brain's plasticity. These findings suggest that music therapy may have had a neuroplastic effect on fiber tracts in three particular ROIs, potentially by reinforcing the myelin sheaths of the neurons, causing an increase in FA. Moreover, studies such as the one by Chaddock-Heyman et al. [28] support the efficacy of music therapy on the brain. In their study, it was demonstrated that musical training was positively and significantly associated with the volume of the inferior frontal cortex and parahippocampus. Furthermore, Andrews et al. [29] revealed that, when studying music as a field that increases structural connectivity, increased FA was found in aging musicians in bilateral superior longitudinal fasciculi and bilateral uncinate fasciculi.

According to the difference maps in figures 4-6, higher FA in the right hemisphere hints at the brain's laterality in terms of neural changes. The right side of the brain is suggested to be the more artistic and creative hemisphere [30-31]. It is responsible for creative thinking, imagination, intuition, insight and musical awareness, among others. The musical awareness explains why the difference in FA is higher in the right hemisphere in the ALMUTH music group. However, the same occurs in the ALMUTH control group. A possible justification might be that, despite AMUTH control participants did not take weekly active singing lessons, they continued with their daily activities, many of which could be related to the right hemisphere as well.

The significant correlations for healthy controls between FA and active musical engagement in the right insula cortex, left postcentral cortex and right postcentral cortex regions, although not very strong, might still indicate the importance of active musical engagement to prevent memory impairment. Recent findings by Speranza et al. [32] support this idea. They report how music changes the chemistry of the brain by inducing the release of neurotransmitters and hormones and activates the reward and prosocial systems. Furthermore, effects of brain plasticity have been demonstrated in professional musicians where lifelong musical practice has also been associated with reduced risk of dementia and mild cognitive impairment [33]. Likewise, anatomical brain differences between musicians and non-musicians when analysing the effects of musical training even after only a few months of intensive practice have been shown [34].

The finding that ALMUTH participants have higher MD values than healthy controls may be due to the PVE [35]. It is known that atrophy in the brain is related to memory impairment [36-37]. Therefore, in the ALMUTH subjects, the smaller brain structures are closer to the cerebrospinal fluid which has a higher diffusion and, hence, a higher PVE. Continuing with the same theory, the general trend for lower MD in the post scans for both ALMUTH groups suggests that PVE could be driving this observation. The size of these brain structures might be larger in the post scans and thus the PVE gets smaller, making the MD decrease. The statistically significant difference in MD between ALMUTH participants and healthy controls in the hippocampus and parahippocampal regions was to be expected, as these areas are intimately related to memory [38-41]. The expected deterioration of these structures in ALMUTH participants explain the higher MD, in comparison with healthy controls, due to PVE.

Besides, the significant increase of MD in the post scans of the ALMUTH control group in the hippocampus substantiate this hypothesis, since higher MD can be explained due to further atrophy of the hippocampus and thus a higher effect of the PVE. However, contrary to expectations, a significant increase of MD in the post scans of the ALMUTH music group was also found in the left hippocampus, suggesting active singing lessons could not temper the atrophy in this region. This could be explained due to the fact that the hippocampus is a region intimately related to memory, hence music therapy might take longer to show its beneficial effects in this ROI. Also, as discussed before, the right side of the brain is responsible for musical awareness, thus its effect on the left hemisphere are less noticeable.

The significant negative correlation between MD and active musical engagement for healthy controls in the left medial-orbito-frontal cortex region implies that the higher the active musical engagement, the lower the MD. This suggests that musical engagement may reduce the PVE, as the underlying microstructures in the brain would grow. Likewise, the significant negative correlations between MD and musical training for healthy controls in the left insula cortex, right insula cortex, left postcentral cortex and left superiortemporal cortex areas advocate the same theory. In fact, other studies have shown that musical engagement and musical training are a good practice to prevent brain atrophy and consequently avoid cognitive decline. For instance, Van't Hooft et al. [42] observed a considerable overlap in atrophy patterns associated with frontotemporal dementia and functional activation associated with music perception. In addition, Chaddock-Heyman et al. [28], reported that musical training was positively associated with volume of the inferior frontal cortex, parahippocampus, posterior cingulate cortex, insula, and medial orbitofrontal cortex. Furthermore, Zhu et al. [43], confirms that musical experience reduces the risk of dementia and decrease the rate of cognitive decline.

Continuing with the network analysis, statistically significant higher local efficiency calculated from the binary connectivity matrix for the healthy controls in comparison with the ALMUTH participants indicates that nodes in healthy brains tend to effectively share information within their immediate local communities. Therefore, this finding indicates that a reduction in local efficiency might be an important indicator of memory impairment. The literature regarding changes in local efficiency is not consistent. While some reported an increase in healthy controls [44-45], some other studies showed a decrease [25] [46].

V. LIMITATIONS

A. COVID-19

The effects of the COVID-19 global pandemic are another cause that may have influenced the results. Although the participants of the ALMUTH musical group continued with their active singing lessons, they were not as enthusiastic and at ease, as the classes were online. Also, other aspects such as lockdown, fear, stress or the death of loved ones may have had an effect on the brain microstructure of each subject in different and unpredictable ways.

B. SOMI stages

This project aimed to contrast healthy versus memory impaired and to study how weekly musical interventions for 12 months affected the memory impaired. However, the ALMUTH sample has a variation of participants in different SOMI stages which are related to Braak staging [47] of biomarker pathology. Braak staging refers to a method used to classify the degree of pathology in AD by performing an autopsy of the brain. Braak stages I and II are used when neurofibrillary tangle involvement is confined mainly to the transentorhinal region of the brain, stages III and IV when there is also involvement of limbic regions such as the hippocampus, and V and VI when there is extensive neocortical involvement.

According to Grober et al. [48], the SOMI stages align with the Braak stages. As shown in figure 16, the prevalence of storage and retrieval impairment (defined by SOMI 3 and higher stages) increased as Braak stage increased: 30% of Braak stage 0, I, II, 24% of Braak stage III, 69% of Braak stage IV, 80% of Braak stage V, and 95% of Braak stage VI cases had storage and retrieval impairment.

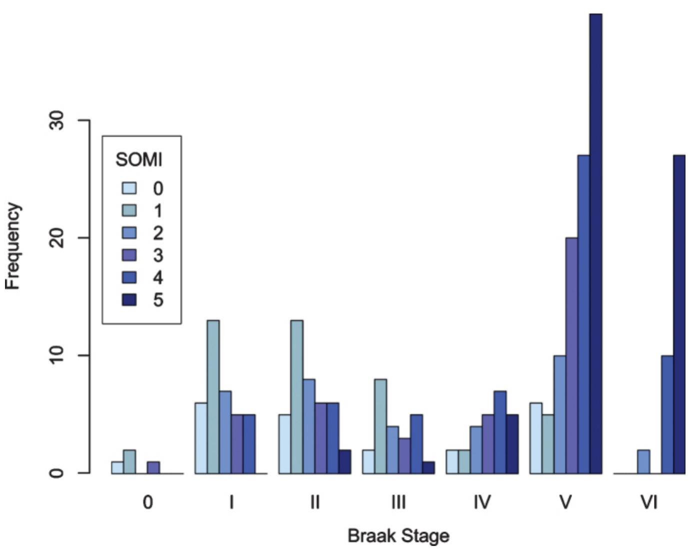

Figure 16: SOMI stage as a function of Braak stage. Source: (Grober et al., 2021)

During the past decade, a conceptual shift occurred in AD area considering the disease as a continuum, as shown in Grober et al. and evidenced by other studies [49-50]. They report that the disease begins decades before the presentation of clinical symptoms. Nevertheless, memory impairment is a nuanced subject since subjective memory complaints can be due to many other factors. For instance, in Maeshima et al. [51] memory impairment is reported to be related to depressive episodes. A further example can be found in the meta-analysis carried out by Shields et al. [52] where it is stated that stress disrupts some episodic memory processes and enhances others.

For the reasons outlined above, all future studies about memory impairment should be conducted by splitting the memory impaired group into subgroups corresponding to the stages of objective memory impairment.

C. Gender differences

Studies like Mehrabinejad et al. [53] show that associations between fiber integrity and both active musical engagement and musical training are different between males and females. As they report, these findings could potentially account for distinctive mechanisms related to musical perception and musical abilities across genders. This would explain why the present research did not reveal as many correlations as the aforementioned study.

VI. CONCLUSIONS

In this report, changes in the brain microstructure related to dementia and music therapy were evaluated by means of diffusion MRI in two different studies and through two different types of analysis. The results indicate that the hippocampus and the parahippocampal regions are highly affected in dementia, but active musical engagement might prevent memory impairment in some regions of the brain. Likewise, the outcomes suggest that a reduction in local efficiency can be a useful indicator for whole-brain memory decline. The findings regarding music therapy suggest it could have a positive effect on underlying microstructural changes, potentially reversing the effects of memory loss in some ROIs.

VII. REFERENCES

[1] M. Prince, R. Bryce, E. Albanese, A. Wimo, W. Ribeiro y C. Ferri, *The global prevalence of dementia: A systematic review and metaanalysis*, 2013.

[2] H. Niu, I. Álvarez-Álvare, F. Guillén-Grima y I. Aguinaga-Ontoso, *Prevalence and incidence of Alzheimer's disease in Europe: A meta-analysis*, Barcelona, 2017.

[3] J. Conde-Sala, J. Garre-Olmo, J. Vilalta-Franch, J. Llinàs-Reglà, O. Turró-Garriga, M. Lozano-Gallego, M. Hernández-Ferràndiz, I. Pericot-Nierga y S. López-Pousa, *Cognitive decline in Alzheimer's disease. Follow-up of more than three years of a sample of patients*, 2013.

[4] J. Vilalta-Franch, S. López-Pousa, J. Garre-Olmo, A. Turon-Estrada y I. Pericot-Nierga, *Mortality in patients with Alzheimer's disease treated with atypical neuroleptics*, 2008.

[5] M. Gómez-Romero, M. Jiménez-Palomares, J. Rodríguez-Mansilla, A. Flores-Nieto, E. Garrido-Ardila y M. González López-Arza, *Benefits of music therapy on behaviour disorders in subjects diagnosed with dementia: a systematic review,* Barcelona, 2017.

[6] M. Gómez Gallego y J. Gómez García, *Music therapy and Alzheimer's disease: Cognitive, psychological, and behavioural effects,* Barcelona, 2017.

[7] S. Burke y C. Barnes, *Neural plasticity in the ageing brain,* 2006.

[8] A. Olszewska, M. Gaca, A. Herman, K. Jednoróg y A. Marchewka, *How Musical Training Shapes the Adult Brain: Predispositions and Neuroplasticity,* Frontiers in Neuroscience, 2021.

[9] G. Schlaug, *Musicians and music making as a model for the study of brain plasticity,* Progress in brain research, 2015.

[10] E. Grober, A. Veroff y R. Lipton, *Temporal unfolding of declining episodic memory on the Free and Cued,* 2018.

[11] M. Piccirilli, P. D'Alessandro y S. Elisei, *Music Training as a Potential Neuroprotective Agent,* Psychiatria Danubina, 2021.

[12] D. Irvine, *Plasticity in the auditory system,* Hearing research, 2018.

[13] E. Altenmüller y G. Schlaug, *Apollo's gift: new aspects of neurologic music therapy,* Progress in brain research, 2015.

[14] S. Koelsch, U. Faerovik y C. Gold, *ALzheimer and MUsic THerapy: Randomised trial of Singing Lessons versus Exercise or No Treatment on Brain Age and Depression Symptoms in People with Alzheimer Disease (ALMUTH),* 2018.

[15] D. Müllensiefen, B. Gingras, L. Stewart y J. Musil, *Goldsmiths Musical Sophistication Index (Gold-MSI): Technical Report and Documentation,* 2013.

[16] A. Matziorinis y T. Sudmann, *Study protocol for the Alzheimer and Music Therapy (ALMUTH) Study: a 12-month, Randomised Controlled Trial to Compare the Efficacy of Music Therapy and Physical Activity on Brain Plasticity, Depressive Symptoms, and Cognitive Decline,* 2022.

[17] B. Fischl, *FreeSurfer,* 2012.

[18] A. Leemans, B. Jeurissen, J. Sijbers y D. Jones, *ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data,* 2009.

[19] Natick, Massachusetts: The MathWorks Inc., *MATLAB. (2020). version R2020b.*

[20] J. Andersson, J. Xu, E. Yacoub, E. Auerbach, S. Moeller y K. Ugurbil, *A Comprehensive Gaussian Process Framework for Correcting Distortions and Movements in Diffusion Images,* 2012.

[21] A. Leemans y D. Jones, *The B-matrix must be rotated when correcting for subject motion in DTI data,* 2009.

[22] M. Irfanoglu, L. Walker, J. Sarlls, S. Marenco y C. Pierpaoli, *Effects of image distortions originating from susceptibility variations and concomitant fields on diffusion MRI tractography results,* NeuroImage, 2012.

[23] J. Kok, A. Leemans, L. Teune, K. Leenders, M. McKeown, S. Appel-Cresswell, H. Kremer y B. de Jong, *Structural Network Analysis Using Diffusion MRI Tractography in Parkinson's Disease and Correlations With Motor Impairment,* Frontiers in Neurology, 2020.

[24] F. Hou, C. Liu, Z. Yu, X. Xu, J. Zhang, C. Peng, C. Wu y A. Yang, *Age-Related Alterations in Electroencephalography Connectivity and Network Topology During n-Back Working Memory Task,* Frontiers in Human Neuroscience, 2018.

[25] S. Afshari y M. Jalili, *Directed Functional Networks in Alzheimer's Disease: Disruption of Global and Local Connectivity Measures,* IEEE, 2016.

[26] X. Hong, Y. Liu, J. Sun y S. Tong, *Age-Related Differences in the Modulation of Small-World Brain Networks during a Go/NoGo Task,* Frontiers in Aging Neuroscience, 2016.

[27] M. Rubinov y O. Sporns, *Complex network measures of brain connectivity: Uses and interpretations,* 2010.

[28] L. Chaddock-Heyman, P. Loui, T. Weng, R. Weisshappel, E. McAuley y A. Kramer, *Musical Training and Brain Volume in Older Adults,* Brain sciences, 2021.

[29] E. Andrews, C. Eierud, D. Banks, T. Harshbarger, A. Michael y C. Rammell, *Effects of Lifelong Musicianship on White Matter Integrity and Cognitive Brain Reserve,* Brain sciences, 2021.

[30] M. Bosnar-Puretić, M. Roje-Bedeković y V. Demarin, *The art: neuroscientific approach,* Acta clinica Croatica.

[31] V. Demarin, M. Bedeković, M. Puretić y M. Pašić, *Arts, Brain and Cognition,* Psychiatria Danubina.

[32] L. Speranza, S. Pulcrano, C. Perrone-Capano, U. di Porzio y F. Volpicelli, *Music affects functional brain connectivity and is effective in the treatment of neurological disorders,* Reviews in the neurosciences, 2022.

[33] J. Verghese, R. Lipton, M. Katz, C. Hall, C. Derby, G. Kuslansky, A. Ambrose, M. Sliwinski y H. Buschke, *Leisure activities and the risk of dementia in the elderly,* The New England journal of medicine, 2003.

[34] K. Hyde, J. Lerch, A. Norton, M. Forgeard, E. Winner, A. Evans y G. Schlaug, *Musical Training Shapes Structural Brain Development,* Society for Neuroscience, 2009.

[35] S. Vos, D. Jones, M. Viergever y A. Leemans, *Partial volume effect as a hidden covariate in DTI analyses*, Elsevier, 2011.

[36] V. Planche, V. Bouteloup, J. Mangin, B. Dubois, J. Delrieu, F. Pasquier, F. Blanc, C. Paquet, O. Hanon, A. Gabelle, M. Ceccaldi, C. Annweiler, P. Krolak-Salmon, M. Habert, C. Fischer, M. Chupin, Y. Béjot, O. Godefroy, D. Wallon, M. Sauvée, I. Bourdel-Marchasson, I. Jalenques, F. Tison, G. Chêne y C. Dufouil, *Clinical relevance of brain atrophy subtypes categorization in memory clinics,* Alzheimer's & dementia : the journal of the Alzheimer's Association, 2021.

[37] S. Ahmed, I. Baker, M. Husain, S. Thompson, C. Kipps, M. Hornberger, J. Hodges y C. Butler, *Memory Impairment at Initial Clinical Presentation in Posterior Cortical Atrophy,* Journal of Alzheimer's disease, 2016.

[38] J. Huijgen y S. Samson, *The hippocampus: A central node in a large-scale brain network for memory,* Revue neurologique, 2015.

[39] Y. Köhncke, S. Düzel, M. Sander, U. Lindenberger, S. Kühn y A. Brandmaier, *Hippocampal and Parahippocampal Gray Matter Structural Integrity Assessed by Multimodal Imaging Is Associated with Episodic Memory in Old Age,* Cerebral cortex, 2021.

[40] E. LaFlamme, H. Waguespack, P. Forcelli y L. Malkova, *The Parahippocampal Cortex and its Functional Connection with the Hippocampus are Critical for Nonnavigational Spatial Memory in Macaques,* Cerebral cortex, 2021.

[41] H. Eichenbaum, *On the Integration of Space, Time, and Memory,* Neuron, 2017.

[42] J. Van't Hooft, Y. Pijnenburg, S. Sikkes, P. Scheltens, J. Spikman, A. Jaschke, J. Warren y B. Tijms, *Frontotemporal dementia, music perception and social cognition share neurobiological circuits: A meta-analysis,* Brain and cognition, 2021.

[43] Q. Zhu, A. Bao y D. Swaab, *Activation of the Brain to Postpone Dementia: A Concept Originating from Postmortem Human Brain Studies,* Neuroscience bulletin, 2019.

[44] K. Supekar, V. Menon, D. Rubin, M. Musen y M. Greicius, *Network Analysis of Intrinsic Functional Brain Connectivity in Alzheimer's Disease,* PLOS Computational Biology, 2008.

[45] S. Tumati, J. Marsman, P. de Deyn, S. Martens y A. Aleman, *Functional network topology associated with apathy in Alzheimer's disease,* Journal of affective disorders, 2020.

[46] L. Wang, H. Li, Y. Liang, J. Zhang, X. Li, N. Shu, Y. Wang y Z. Zhang, *Amnestic mild cognitive impairment: topological reorganization of the default-mode network,* Radiology, 2013.

[47] H. Braak y E. Braak, *Neuropathological stageing of Alzheimer-related changes,* Acta neuropathologica, 1991.

[48] E. Grober, Q. Qi, L. Kuo, J. Hassenstab, R. Perrin y R. Lipton, *Stages of Objective Memory Impairment Predict Alzheimer's Disease Neuropathology: Comparison with the Clinical Dementia Rating Scale–Sum of Boxes,* Journal of Alzheimer's disease, 2021.

[49] B. Dubois, H. Hampel, H. Feldman, P. Scheltens, P. Aisen, S. Andrieu, H. Bakardjian, H. Benali, L. Bertram, K. Blennow, K. Broich, E. Cavedo, S. Crutch, J. Dartigues, C. Duyckaerts, S. Epelbaum, G. Frisoni, S. Gauthier, R. Genthon y A. Gouw, *Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria,* The journal of the Alzheimer's Association, 2016.

[50] A. Sanford, *Mild Cognitive Impairment,* Clinics in geriatric medicine, 2017.

[51] H. Maeshima, B. Hajime, E. Satomura, T. Shimano, M. Inoue, S. Ishijima, T. Suzuki y H. Arai, *Residual memory impairment in remitted depression may be a predictive factor for recurrence,* 2016.

[52] G. Shields, M. Sazma, A. McCullough y A. Yonelinas, *The effects of acute stress on episodic memory: A meta-analysis and integrative review,* Psychological bulletin, 2017.

[53] M. Mehrabinejad, P. Rafei, H. Moghaddam, Z. Sinaeifar y M. Aarabi, *Sex Differences are Reflected in Microstructural White Matter Alterations of Musical Sophistication: A Diffusion MRI Study,* Frontiers in neuroscience, 2021.

VII. APPENDIX

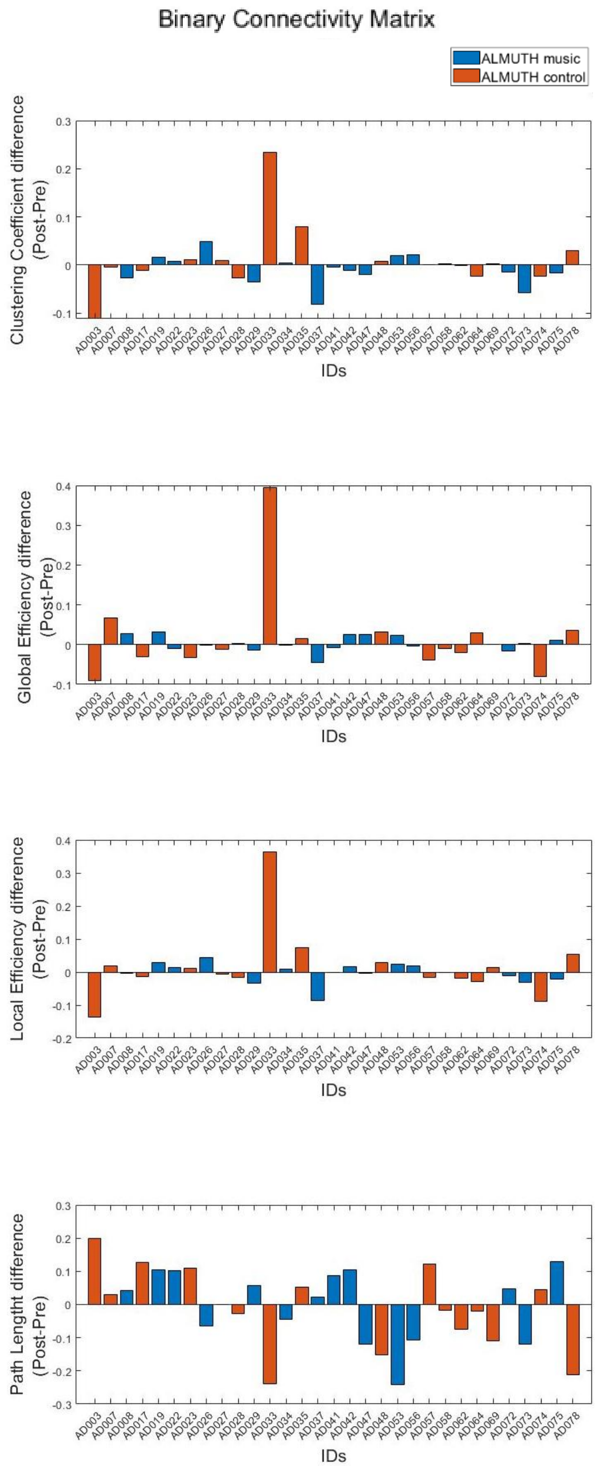

Figure A: Difference between Pre and Post Connectivity Metrics calculated from the Binary Connectivity Matrix for the Longitudinal Study

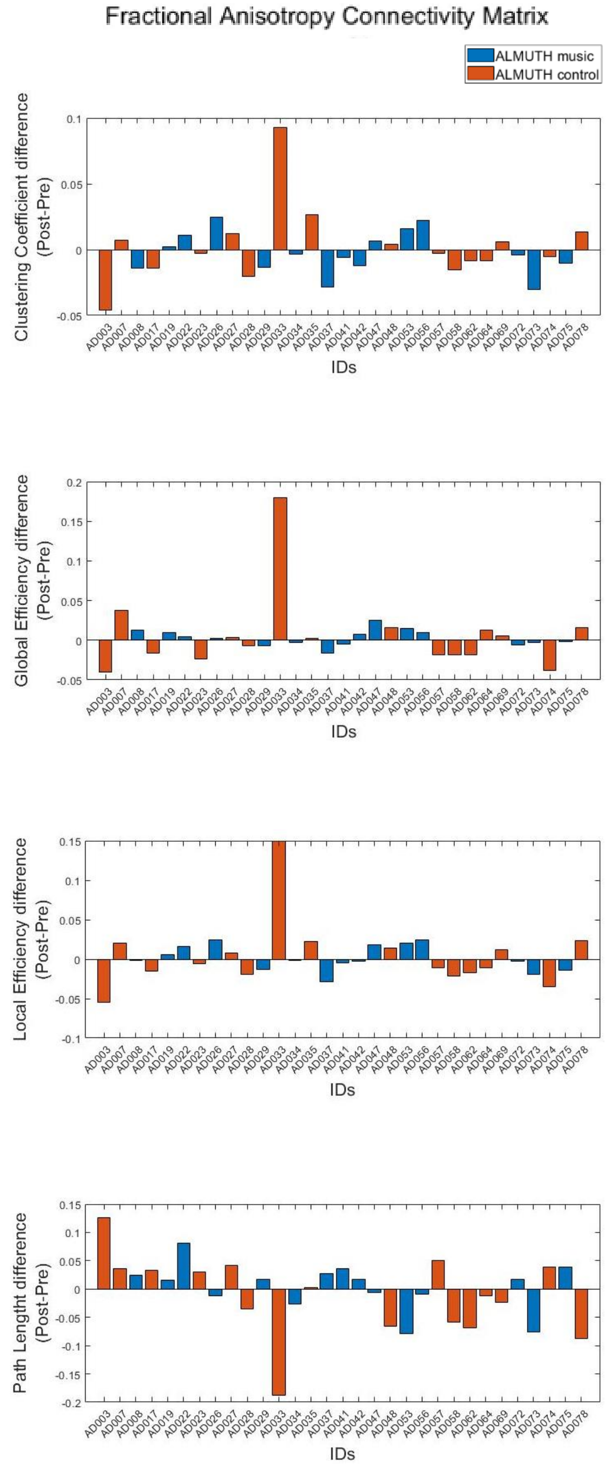

Figure B: Difference between Pre and Post Connectivity Metrics calculated from the Fractional Anisotropy Connectivity Matrix for the Longitudinal Study

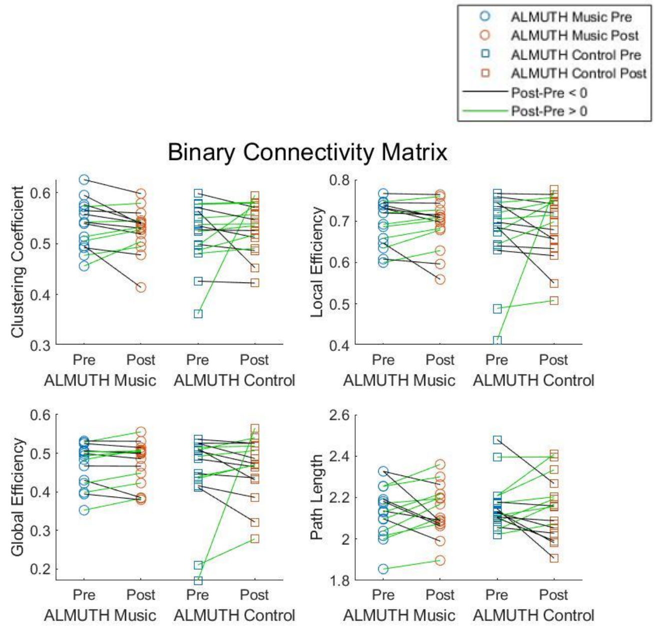

Figure C: Tendency of Post-Pre Connectivity Metrics calculated from the Binary Connectivity Matrix for the Longitudinal Study

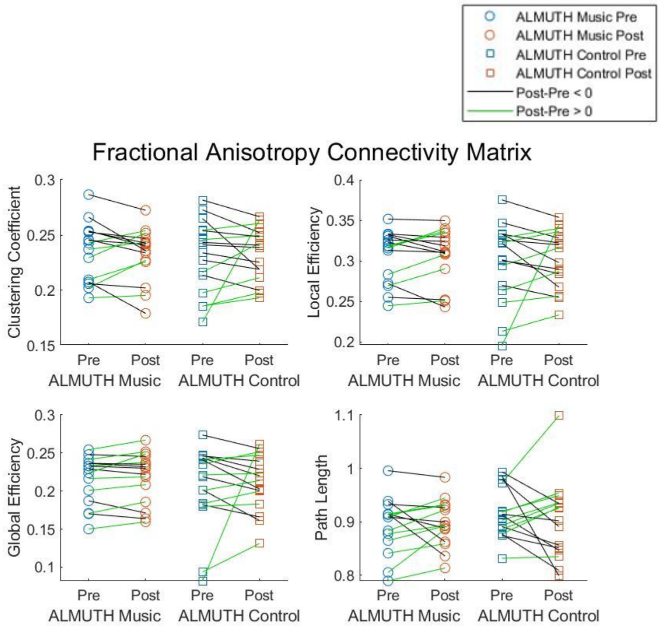

Figure D: Tendency of Post-Pre Connectivity Metrics calculated from the Fractional Anisotropy Connectivity Matrix for the Longitudinal Study