Graph theory application with functional connectivity to distinguish early mild cognitively impaired and mild cognitive impaired patients from healthy controls

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Abstract:

The structural and functional organisation of the brain in an Alzheimer's (AD) patient is altered. The focus of recent research on AD and MCI patients investigates how the small-world network properties of different resting-state networks are altered and if alterations to the default mode network can act as an early marker for AD pathology. Very few studies focus on Early Mild Cognitive Impairment (EMCI) and Mild Cognitive Impairment (MCI), which have a high probability of progressing to AD. We utilised resting-state fMRI data of 30 EMCI patients, 30 MCI patients and 30 Cognitively Normal subjects (CN) aged 60-69 from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to model the brain as a graph-based network using 53 selected ROIs involved in memory and cognitive functions to study the topological properties of the brain network. Moreover, we also extensively analysed the functional connectivity (FC) across 164 ROIs between groups of EMCI, MCI, and CN subjects. Similar to previous functional connectivity studies, we saw no significant FC differences when we compared CN subjects with MCI patients. Although, we saw an increased FC between the sensorimotor network and different local brain lobules in the EMCI patients compared to MCI patients. In addition, the FC between the hippocampus, thalamus and other brain lobules was higher in the CN group compared to the EMCI group. Surprisingly, all cortical networks formed by the EMCI, MCI, and CN groups exhibited small-world properties. Among the three groups, the small-world measures of the MCI group exhibited intermediate values. MCI patients showed decreased nodal centrality compared to CN subjects, mainly in the inferior temporal gyrus, superior parietal lobule, angular gyrus, thalamus, and hippocampus. In addition, nodal centrality increase was observed in the default mode network's medial prefrontal cortex and the lateral parietal. These results suggest that theoretical graph analysis may be a helpful approach in studying the node-wise/region-wise

characteristics of the brain and the complexity of the networks formed by these nodes/regions.

Introduction:

The aetiology of AD, a progressive neurological illness characterised by cognitive decline, memory loss, language and reasoning with a high incidence rate, is unknown. The available therapeutic drug treatments for AD, such as human antibodies or immunotherapy, are rather insubstantial. Conflicting views on whether drug-based treatments involving acetylcholinesterase inhibitors can help lower the rate of this progression (Diniz et al., 2009; Loy & Schneider, 2006; Petersen et al., 2005; Raschetti et al., 2007) was one of the motivators for us to study the neural underpinnings of memory and cognitive impairment of individuals in the early stages of AD.

Recent research has identified transitional states between normal ageing and AD, known as Early Mild Cognitive Impairment (EMCI) and Mild Cognitive Impairment (MCI), with a high conversion rate of 10-15% every year to AD. EMCI refers to a cognitive impairment that falls between 1 and 1.5 SD below the normative mean on a standard test (Aisen et al., 2010). Aside from mild cognitive deficits, EMCI patients have amyloid deposition, functional network breakdown, and brain density alterations (Hua et al., 2016). MCI is a disease affecting which patients suffer a deterioration in their mental functions, with objective evidence of impairment in conventional memory or other cognitive tests, but without the functional impairments in everyday tasks seen in dementia (Farahani et al., 2019; Petersen et al., 2001). EMCI can persist for 2-4 years, whereas in some people, this progressive transition phase state of MCI does not advance to clinical AD and may not even have AD neuropathology (Dickerson et al., 2007; Petersen et al., 2006).

An increasing amount of evidence (Coleman et al., 2004; Selkoe, 2002) states that changes in synaptic functions occur very early in the disease process, potentially well before the development of clinical symptoms and even significant neuropathology. Functional magnetic

resonance imaging (fMRI) based on blood-oxygen-level-dependent (BOLD) has proven to be a powerful non-invasive tool and a candidate biomarker for detecting changes in brain function that may occur very early in the course of disease (Celone et al., 2006; Dickerson et al., 2005; Johnson et al., 2006; Sandstrom et al., 2006; Vandenbulcke et al., 2007). Resting-state fMRI (rs-fMRI) and task-based fMRI studies have identified disruptions in functional connectivity (FC) within the default mode network (DMN) and sensorimotor network (SMN) brain regions in AD and MCI patients over the last decade (Agosta et al., 2011; Agosta et al., 2010; Buckner et al., 2008) with rs-fMRI's low-frequency fluctuation phenomenon in BOLD signals being vital for a better understanding of human brain function because extremely excessive energy consumption appears within the regions showing high metabolisms (Raichle et al., 2001).

Task-based FC studies have been prominent in detecting multiple cognitive domain failures as AD progresses. Buckner (2004) reports that in addition to the hippocampus and medial temporal lobe, a set of cortical regions, also known as the DMN, typically deactivates during memory encoding and other cognitively demanding tasks focused on processing external stimuli. Converging evidence suggests that episodic memory, executive function and other cognitive functions affected in AD depend on the integrity and inter-play of large-scale networks (Mesulam, 1990; Mesulam, 1998). However, evidence that crucial components of the DMN, particularly the posteromedial cortices, are strongly connected to the hippocampal memory system even in resting-state fMRI (rs-fMRI) studies were found by Kobayashi & Amaral (2007). rs-fMRI studies by Greicius et al. (2004) and Wang et al. (2000) indicate that impaired connectivity between the hippocampus and posterior cingulate cortex could lead to reduced posterior cingulate cortex (PCC) activity.

On the other hand, FC analysis has limited ability to characterise the dynamic reconfiguration and regional activity of complex brain networks. Despite the apparent cognitive impairment,

the physiological changes in the prodromal or early phases of AD can occasionally be difficult to detect (Grieder et al., 2018).

Graph-theory-based approaches model the brain as a complex network represented graphically by a collection of nodes and edges. In the virtual graph, nodes indicate anatomical elements, i.e., brain regions, and edges represent the relationship between the nodes, i.e., the connectivity (Wang et al., 2010). We used graph theory to investigate how the topological properties of the brain, i.e., the intra- and inter-subnetworks, are altered during rest and to address the dynamic change in network properties from EMCI to MCI. While studies were conducted on whole brain networks using a graph theoretical approach to AD and MCI populations (He et al., 2007; Rombouts et al., 2005; Stam et al., 2007), very few studies have attempted to construct a network using ROIs responsible for memory and cognitive function based on the disassociation hypothesis and examine the constructed network characteristics in EMCI and MCI patients.

We based our theory on previous research that showed the brain to be a small world network (Strogatz, 2001). Small world-ness indicates the brain's ability to integrate information functionally across different regions rapidly and specialised processing within highly interconnected brain regions. Several studies have found a loss of small-world attributes in whole brain networks in AD and MCI patients (Binnewijzend et al., 2012; He et al., 2008; He et al., 2007; Li et al., 2020; Liu et al., 2012; Rombouts et al., 2005; Seo et al., 2013; Sorg et al., 2007; Stam et al., 2007).

In the graph-based network constructed using 53 selected ROIs, we hypothesised that EMCI and MCI patients might have topological abnormalities compared to cognitively normal (CN) subjects. In contrast, MCI patients may have them on a large scale. After modelling the brain as a network, we calculated the various graph-theoretical metrics such as average path length,

average clustering coefficient, global efficiency, degree and betweenness centrality. Recent research has revealed a decrease in functional connectivity and a shift in nodal centrality in the hippocampus of AD and MCI patients (Sanz-Arigita et al., 2010; Sohn et al., 2014; Sperling et al., 2010; Wang et al., 2007; Wang et al., 2006; Xue et al., 2019). We hypothesised that EMCI and MCI patients would have lower functional connectivity than CN subjects. We also hypothesised that MCI patients would have lower functional connectivity than EMCI patients. In addition, we expect a reduced nodal centrality in the hippocampus in MCI and EMCI patients compared to CN subjects and in MCI patients compared to EMCI patients.

Materials and Methods:

Data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

This study included 30 early MCI (EMCI), 30 MCI, and 30 Cognitively Normal (CN) subjects. In the ADNI project, MCI diagnostic criteria had 1) Mini-Mental State Examination (MMSE) scores between 24 and 30, 2) a memory complaint, objective memory loss measured by education adjusted scores on the Wechsler Memory Scale Logical Memory II, 3) a Clinical Dementia Rating (CDR) of 0.5, and 4) absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living and an absence of dementia. As shown in the ADNI project, the detailed diagnostic criteria of EMCI are characterised by evidence of AD biomarker abnormalities, with EMCI patients showing milder cognitive deficits. Regarding neuropsychological criteria, EMCI is defined as a performance 1–1.5 SD below the mean in one episodic memory test, identifying an intermediate level of subtle memory impairment between normal cognition and MCI. Further, structural MRI, and rs-fMRIs of the 90 subjects of the three different study groups, EMCI, MCI, and CN group patients, are analysed (see Table 1).

Table 1

Mean, standard-deviation, and statistically significant values of group differences.

Groups	Gender (M/F)	Age (years)	Age (mean ± SD)	p-value (age)
MCI (N=30)	15M/15F	60-69	65.56 ± 2.44	MCI and CN = 0.05
EMCI (N=30)	15M/15F	60-69	66.33 ± 1.76	EMCI and CN = 0.05
CN (N=30)	15M/15F	60-69	65.56 ± 2.44	EMCI and MCI = 0.05

Note. The values were calculated using a posthoc analysis using G-power.

Data Acquisition:

The resting-state fMRI data were collected using 3T scanners from different manufacturers as a part of a large-scale collaborative effort on the ADNI project (GE Medical System, SIEMENS, and Philips systems). At the same time, patients were advised to keep their eyes open, according to the ADNI protocol (http://adni.loni.usc.edu/). T1-weighted structural images were acquired as follows: repetition times (TR) were 6.8ms (MPRAGE, Philips Medical Systems), 2300ms (accelerated MPRAGE, SIEMENS) and 7.4ms (accelerated IRF-SPGR, GE Medical Systems); echo time (TE) varied from 3.0s to 3.2s; flip angle = 9.0 degree; acquisition matrices were 256 x 256 (MPRAGE, Philips Medical Systems), 240 x 256 (accelerated MPRAGE, SIEMENS), and 256 x 256 (accelerated IRF-SPGR, GE Medical Systems); and slice thickness = 1.2mm. Blood oxygenation level-dependent (BOLD) signal was acquired using an echo planar imaging (EPI) sequence with a repetition time (TR) varying from 2250ms to 3001ms across the three groups; echo time (TE) = 30ms; flip angle (FA) = 90; slices = 9600. The imaging resolution varied from 3.2 mm to 3.4 mm in the X and Y directions, and the slice thickness was consistently 3.4 mm. Whilst some parameters change, the ones most crucial for the quality of BOLD signal measurement, i.e., TE and FA,

remain consistent, so we do not expect between-group comparisons to be confounded by acquisition parameters.

Pre-processing:

Resting state-fMRI data pre-processing was performed using software MATLAB 2020a (MathWorks, Inc, https://www.mathworks.com) and Connectivity toolbox for Matlab (CONN, https://web.conn-toolbox.org/home) and Statistical Parametric Mapping software (SPM12) package (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). We used CONN's default pre-processing pipeline using default values for performing the following steps: functional realignment and unwarp; the slice-timing correction was skipped as the scans were obtained on different acquisition systems; outlier identification, where acquisitions with framewise displacement above 0.9mm or global BOLD signal changes above 5 S.D. were flagged as potential outliers; direct segmentation and normalisation, where the data was normalised into the standard Montreal Neurologic Institute (MNI) space and segmented into grey matter, white matter, and CSF tissue class using SPM12; and spatial smoothing was performed using an 8mm Gaussian kernel and linear trends were eliminated. Following smoothing, the physiological noise was corrected using the component-based noise correction method (COMPCOR) inbuilt into the CONN toolbox. The white matter and cerebrospinal fluid signals were regressed out of the BOLD signal by applying them as nuisance covariates (Behzadi et al., 2007).

Functional Network Construction:

We constructed a functional connectivity network on 164 region-of -interests (ROIs) employed as seeds for experimental purposes. 132 ROIs were cortical and subcortical atlases from the FSL software library (www.fmrib.ox.ac.uk/fsl), Harvard-Oxford atlas and cerebellar areas from the automated anatomical labelling (AAL) atlas. 32 ROIs were network atlases

(i.e., 32 ROIs across eight networks: the sensorimotor, optical, salience, dorsal attention, default mode, frontoparietal, language network, and cerebellar network).

Functional connectivity between a seed/ROI and each voxel or location in the brain, as well as ROI-to-ROI correlation matrices, was calculated where each element in an ROI-to-ROI correlation matrix (RRC) is the fisher-transformed bivariate correlation coefficient between a pair of ROI BOLD time-series. We performed ROI-based analyses for all subjects' data with a general linear model (GLM) test to determine significant resting-state connections at the individual level (1st level). Based on 1st level results, correlation coefficients were converted into standard scores, and an unpaired t-test was used with a threshold set at $p < 0.05$ false discovery rate (FDR) corrected to determine significant connections for between-group comparisons between EMCI and MCI, EMCI and CN, and MCI and CN. Furthermore, graph theory calculated the global efficiency, local efficiency, degree, cost, clustering coefficient, and average path length of the network constructed.

Graph Theory Network Construction:

We computed ROI-level graph measures using user-defined non-directional graphs with ROIs as nodes and connections between the different ROIs as suprathreshold edges. A graph adjacency matrix A was calculated for each subject by dividing the associated ROI-to-ROI Correlation (RRC) matrix r by a cost threshold (cost<0.15) (Nieto-Castanon, 2020; Whitfield-Gabrieli & Nieto-Castanon, 2012). To avoid scale-free networks, i.e., networks in which a few nodes have more edges than others, the cost threshold, which also displays network sparsity, was set to <0.15 (Maslov & Sneppen, 2002; Milo et al., 2002; Salvador et al., 2005). We set the p-FDR threshold for the analysis at 0.05. After that, several measures were computed from the resulting graphs (see Table 2) to address the properties of each ROI within the network and the entire network constructed using the ROIs.

Table 2

Graph Theory Measures.

Graph Theoretical Metrics	Definition
Global Efficiency	The average of inverse distances between the node pairs in the graph network.
Local Efficiency	Represents the efficient node communication transfer between the direct neighbours of a particular node.
Clustering Co-efficiency (CC_o)	Represents the degree of interconnectedness among all nodes within a node neighbouring subgraph. A ratio of the number of triangles formed by a node and the total number of triangles. It also represents the ability for specialised processing to occur within densely interconnected groups of brain regions.
Cost	It is the ratio of all existing connections in an actual network by all possible connections between the network nodes.
Degree and Average Degree (d and k)	The degree is the total number of connections from/to each node, and the average number of connections per node is called the average degree.
Betweenness Centrality (BC_i)	Describes the number of shortest path lengths passing through the node. Betweenness centrality provides information on whether the node is a central node and gives the node's influence in the network.
Average Path Length (L_o)	It is the average distance between a selected node and all other nodes. It is a measure of functional integration in the brain. The ability to rapidly combine specialised information from distributed brain regions is derived from average path length.

ROI Utilised in Graph Theory Analysis:

Out of the 164 ROIs described above, we took into consideration 53 ROIs (see Table 3) that are functionally responsible for memory, and cognitive functioning (Mesulam, 1990; Mesulam, 1998) and a network was constructed to help us understand each ROI node-wise and its efficiency in the network. Based on the disconnection hypothesis, we selected 53 ROIs, supported by neuropathological AD data, showing that the distribution of pathological changes in AD patients' brains does not appear in a random or widespread uniform fashion but is rather selective and restricted to some brain areas or even some laminae within those

areas. These data highlight that the degeneration in AD is not global but rather specific to areas related to corticocortical connections (Lafleche & Albert, 1995; Lakmache et al., 1998; Pantel et al., 1999; Teipel et al., 1999). Moreover, neuroimaging AD data also shows a decrease in the size of a few brain regions as the disease progresses (Delbeuck et al., 2003; Friston et al., 1999; Mesulam, 1990; Mesulam, 1998). Progression of the disease, i.e., in EMCI and MCI, may also support this conclusion since areas affected at the onset of the disease are the "associative areas" that integrate information, such as the hetero modal cortex and sensory or motor areas (Uylings & de Brabander, 2002).

Table 3

53 ROIs selected to form a graph-network

S.No	ROIs Selected for Graph Theoretical Analysis
1.	DMN – Medial Prefrontal Cortex
2.	DMN – Lateral Parietal (R)
3.	DMN – Lateral Parietal (L)
4.	DMN – Posterior Cingulate Cortex (PCC)
5.	SMN – Lateral (R)
6.	SMN – Lateral (L)
7.	SMN – Superior
8.	Visual Network – Medial
9.	Visual Network – Occipital
10.	Visual Network – Lateral (R)
11.	Visual Network – Lateral (L)
12.	DAN – Frontal Eye Fields (R)

13.	DAN – Frontal Eye Fields (L)
14.	DAN – Intraparietal Sulcus (R)
15.	DAN – Intraparietal Sulcus (L)
16.	Precentral Gyrus (R)
17.	Precentral Gyrus (L)
18.	Superior Temporal Gyrus – anterior division (R)
19.	Superior Temporal Gyrus – anterior division (L)
20.	Superior Temporal Gyrus – posterior division (R)
21.	Superior Temporal Gyrus – posterior division (L)
22.	Inferior Temporal Gyrus – anterior division (R)
23.	Inferior Temporal Gyrus – anterior division (L)
24.	Inferior Temporal Gyrus – posterior division (R)
25.	Inferior Temporal Gyrus – posterior division (L)
26.	Inferior Temporal Gyrus – temporooccipital division (R)
27.	Inferior Temporal Gyrus – temporooccipital division (L)
28.	Postcentral Gyrus (R)
29.	Postcentral Gyrus (L)
30.	Superior Parietal Lobule (R)
31.	Superior Parietal Lobule (L)
32.	Angular Gyrus (R)
33.	Angular Gyrus (L)
34.	Lateral Occipital Cortex – superior division (R)
35.	Lateral Occipital Cortex – superior division (L)
36.	Lateral Occipital Cortex – inferior division (R)

37.	Lateral Occipital Cortex – inferior division (L)
38.	Frontal Orbital Cortex (R)
39.	Frontal Orbital Cortex (L)
40.	Temporal Occipital Fusiform Cortex (R)
41.	Temporal Occipital Fusiform Cortex (L)
42.	Occipital Fusiform Gyrus (R)
43.	Occipital Fusiform Gyrus (L)
44.	Occipital Pole (R)
45.	Occipital Pole (L)
46.	Thalamus (R)
47.	Thalamus (L)
48.	Hippocampus (R)
49.	Hippocampus (L)
50.	Amygdala (R)
51.	Amygdala (L)
52.	Medial Frontal Cortex
53.	Precuneus

Note. ROIs were selected based on the disconnection hypothesis.

DMN – Default Mode Network

SMN – Sensorimotor Network

DAN – Dorsal Attention Network

(R) – Right

(L) – Left

Analysis of small world-ness:

We further calculated the small world characteristics for EMCI, MCI and CN groups. To determine whether the networks formed using 53 ROIs by EMCI, MCI, and CN groups have small-world attributes; we compared the original network to a random network constructed with the same number of nodes and average degree as the original network but from different ROIs (Albert & Barabási, 2002). Random networks with a gaussian degree distribution will have a clustering co-efficiency given by $CC_r = k / N$ (where *k* is the average degree of nodes, and *N* is the total number of nodes) (Albert & Barabási, 2002). $L_r = \log(N) / \log(k)$ (where *k* is the average degree of the network, and *N* is the total number of nodes) is the average path distance of a random network (Albert & Barabási, 2002). A real network is said to have a small world topology if it meets the criteria $\gamma = CC_o/CC_r > 1$ and $\lambda = L / L_r \sim 1$ (γ, λ represent normalised clustering coefficient and normalised path length, respectively) (Watts & Strogatz, 1998).

Betweenness Centrality:

The CONN toolbox gives us a measure of Betweenness Centrality (BC) which measures a node's influence in a network, defined as the number of shortest pathlengths passing through the node (Freeman, 1977). To understand the influence of the 53 selected nodes, i.e., 53 ROIs, in the network formed and in between-group differences, we normalised the betweenness $b_i = B_i/B$ (where B_i is the betweenness of the defined node and B is the average betweenness of the network formed) as (He et al., 2008 & Liu et al., 2012), for brain regions to qualify as hubs of the network they had to have high values of b_i.

Statistical Analysis:

Post hoc statistical analysis was performed for the sample sizes across the different group comparisons using GPower 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html) which gave us a significant value of $p \approx 0.05$.

In CONN, for ROI-to-ROI level analyses, we performed a customised one-sided and two-sided cluster-level p-False Discovery Rate (p-FDR) Multivariate pattern analysis omnibus test for the group comparisons across the EMCI, MCI and CN groups. The connection and cluster threshold was set to $p<0.05$.

The graphs constructed were unweighted and non-directional to avoid statistical complications. This cost threshold ensures that the networks produce the same number of edges across all the subjects. Since there is no gold standard for setting the cost threshold, we kept it fixed at 0.15, the default value suggested by CONN. A one-way ANOVA test using SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) was performed for all the six calculated graph theory metrics to check for statistical significance among the EMCI, MCI and CN group-comparisons.

Results:

In this study, we performed an extensive ROI-to-ROI level analysis (p-FDR corrected < 0.05, two-sided) of 13,366 connections among 164 hierarchically clustered ROIs comparing the patient groups EMCI and MCI to CN subjects and a between-patient group comparison between EMCI and MCI. In addition, we also computed graph theory measures for the selected 53 ROIs based on the disassociation hypothesis.

ROI-to-ROI Level Results:

We did not observe any significant differences in gender and age across the group comparisons. Although we observed substantial FC differences between the two group comparisons, CN and EMCI groups and EMCI and MCI patient groups (see **Fig.1 & Fig. 2**). However, we observed no significant FC differences between MCI and CN groups.

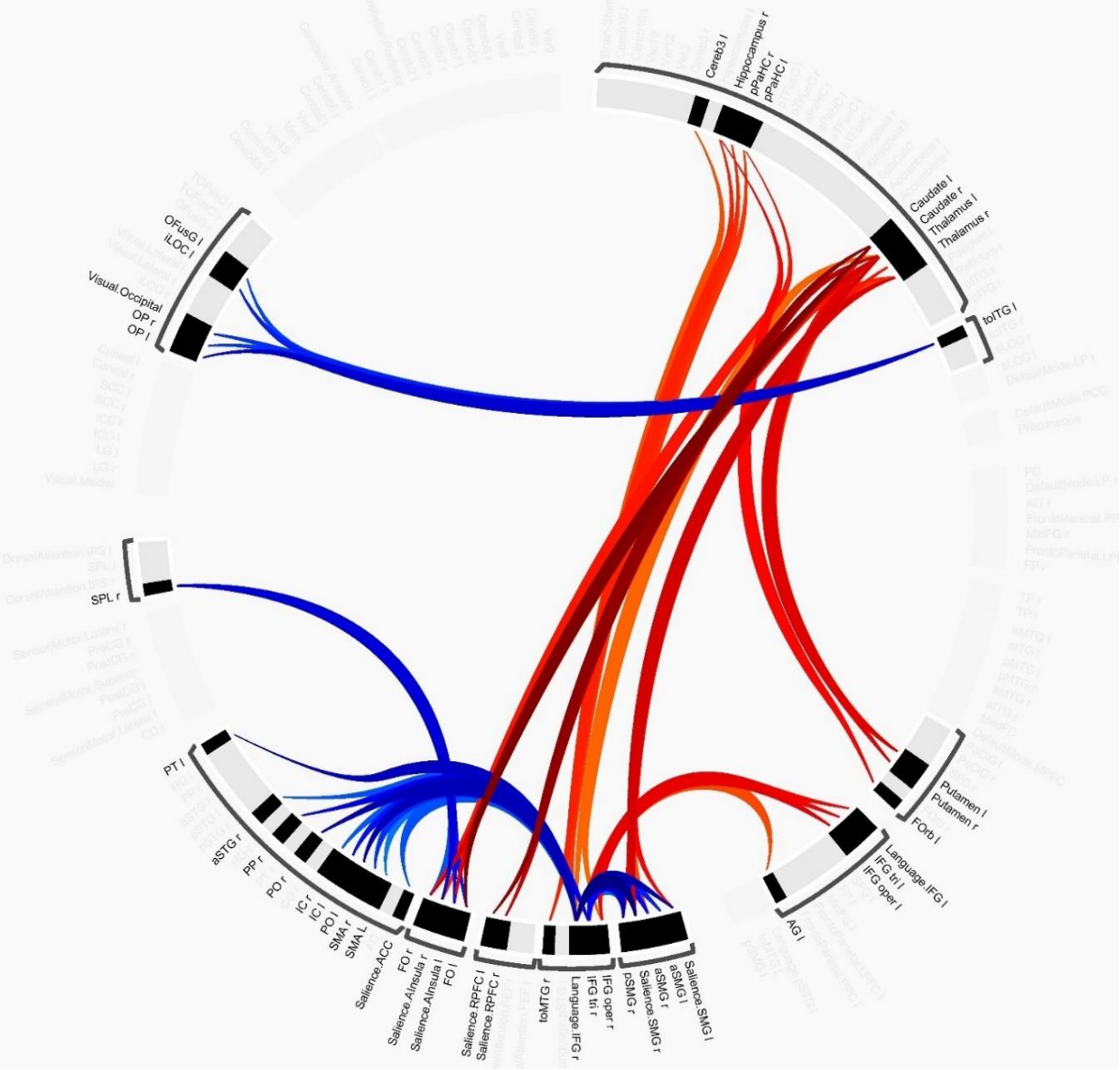
In the EMCI and CN group comparison (**Fig. 1**), the following regions showed higher FC compared to the EMCI population; Caudate (R) showed high FC with the salience network's anterior insula (L), rostral prefrontal cortex (L, R), language network's inferior frontal gyrus (R), supramarginal gyrus (anterior division right and posterior division right), inferior frontal gyrus (posterior division right), frontal operculum cortex (R), and the putamen (L, R). The hippocampus had strong FC with the frontal orbital cortex (L) and middle temporal gyrus (temporooccipital cortex right). In contrast, the thalamus (L, R) had strong FC with the salience network's supramarginal gyrus (L, R) and the frontal operculum cortex (R).

In the EMCI and MCI group comparison (**Fig. 2**), SMN ROIs showed higher FC with each other in EMCI than in the MCI group. Moreover, we also saw that the SMN ROIs highly correlated with the central opercular cortex (L), planum temporal (L), planum temporal (R), superior temporal gyrus (posterior division, L), heschl's gyrus, insular cortex (L), insular cortex (R), parietal operculum cortex (R). In contrast, the hippocampus in the MCI group had

high FC with the inferior Frontal gyrus (pars opercularis right), superior temporal gyrus (posterior division right), parietal operculum cortex (R), heschl's gyrus (L), superior temporal gyrus (posterior division, left), planum temporal (L), and the language network's posterior superior temporal gyrus. In addition to the hippocampus in the MCI group, we also see the salience network's ROIs strongly correlated with the cerebellum (4 5, right), Vermis (4 5), and Thalamus (R). The SMN having a weaker FC in the MCI population compared to the EMCI population goes hand in hand with the previous literature (Agosta et al., 2010; Ahmadi et al., 2020).

Figure 1

Significant functional connectivity differences when comparing the EMCI group to the CN group



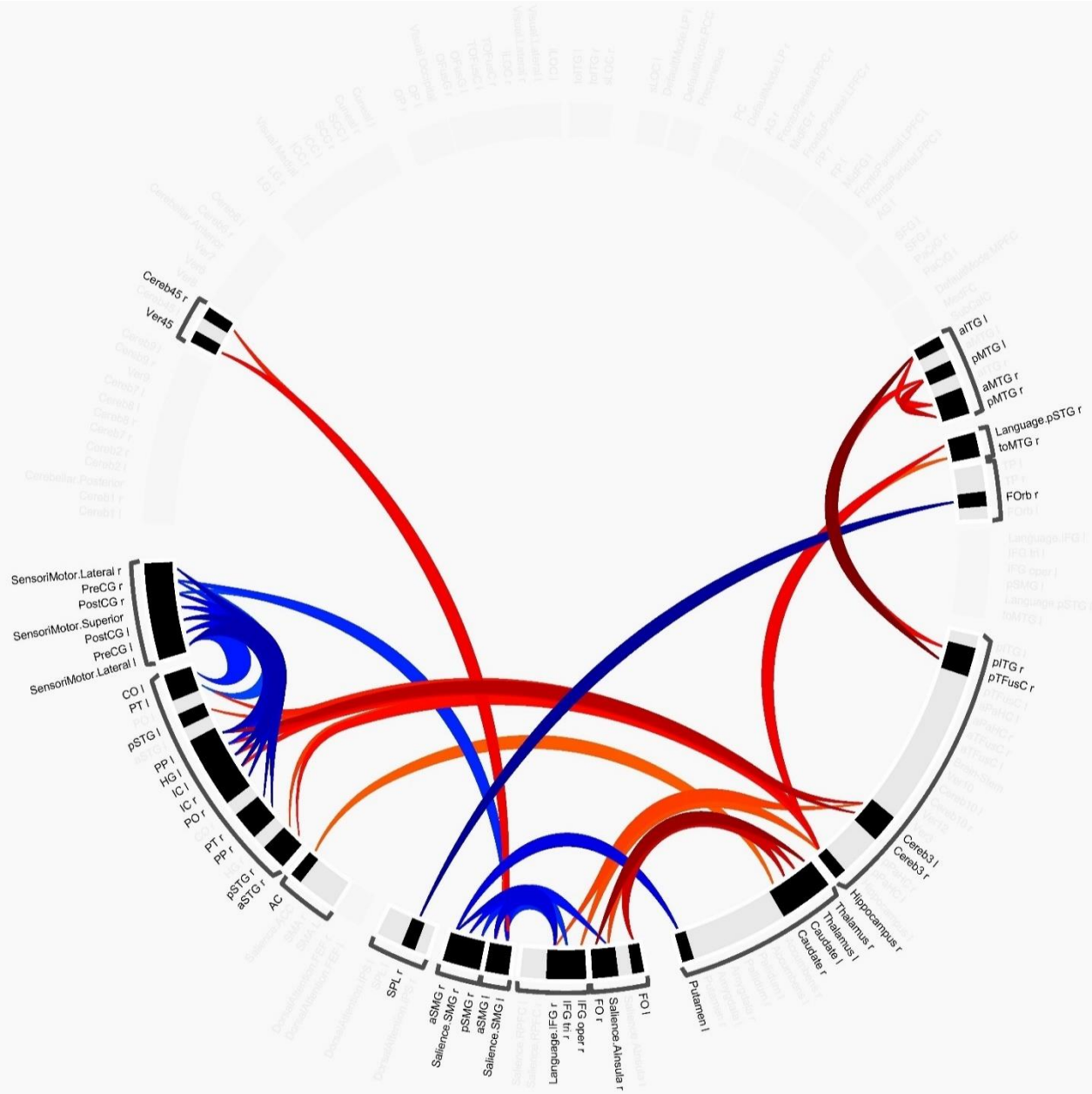
Note. Connectome (result from the CONN toolbox) presents significant functional connectivity differences in comparing EMCI and CN groups.

Red (positive) indicates greater connectivity in CN compared to EMCI

Blue (negative) indicates greater connectivity in EMCI patients compared to CN

Figure 2

Significant functional connectivity differences when comparing EMCI group to the MCI group



Note. Connectome (result from the CONN toolbox) presents significant functional connectivity differences in comparing EMCI and MCI groups.

Red (positive) indicates greater connectivity in MCI patients compared to EMCI.

Blue (negative) indicates greater connectivity in EMCI patients compared to MCI.

Graph-Theory Results:

We examined different graph theory attributes and the small world attributes of the 53 ROIs in EMCI patients, MCI patients and age-matched controls (see **Table 4**). Compared with random networks, all networks demonstrate small-world architectures as they all had almost identical characteristic path lengths ($\lambda = 1$) and a higher clustering co-efficiency locally ($\gamma > 1$), which is consistent with previous literature that found the human brain to be an efficient neural architecture (Bullmore & Sporns, 2009).

Table 4

Graph theory measures EMCI, MCI, and CN groups.

Graph Theory Metrics	EMCI	MCI	CN	p-significance (p<0.05) (ANOVA)	EMCI vs MCI (p<0.05) (ANOVA)	EMCI vs CN (p<0.05) (ANOVA)	MCI vs CN (p<0.05) (ANOVA)
Betweenness Centrality (BC_i)	0.0325	0.04	0.04	0.974	0.874	0.949	0.805
Degree (d)	8.2564	7.7924	7.7924	<0.001	<0.001	<0.001	1
Avg. Path Distance (L_o)	2	3	3	0.939	0.720	0.860	0.862
Clustering Co-efficiency (CC_o)	0.6214	0.6023	0.6356	0.071	0.177	0.314	0.029
Global Efficiency (GE)	0.39836	0.39962	0.39938	0.994	0.919	0.937	0.985
Local Efficiency (LE)	0.7411	0.7237	0.7560	0.102	0.153	0.494	0.039
Cost (C)	0.14985	0.14985	0.14985	1	1	1	1

Note. ANOVA testing (set at p<0.05) was conducted to test the statistical significance of the graph theory metrics.

Table 5

Small world-ness calculations from the above values in Table 4 for EMCI, MCI and CN groups

Group	Average Path Distance (k)	CC_o	L_o	$CC_r = k / N$	$L_r = \log N/\log k$	$\gamma = CC_o / CC_r$	$\lambda = L_o/L_r$	$\sigma = \gamma/\lambda$
EMCI	8.2564	0.6214	2	0.155	1.880	4.009	1.063	3.771
MCI	7.7924	0.6023	3	0.147	1.933	4.097	1.551	2.641
CN	7.7924	0.6356	3	0.147	1.933	4.323	1.551	2.787

Note.

CC_o – Clustering co-efficiency of the original network

CC_r – Clustering co-efficiency of the random network

L_r – Average path distance of a random network

γ – Normalised clustering co-efficient

λ – Normalised path length

σ – Small world-ness

In table 4, we first calculated the individual statistical significance of each metric when we compared it across the EMCI, MCI and CN groups. Among the seven metrics calculated, we see that only degree and clustering co-efficiency show significant differences between groups.

We calculated the average path distance (L_o) and the clustering coefficients (CC_o) to further study the alterations in the small-world networks in the group differences. We used an ANOVA test to test for statistical significance in between-group differences. We only

considered the L_o and the CC_o values as they represent the ability for specialised processing to occur within densely interconnected group regions.

While the CC_o was not statistically significant when we compared the EMCI group against the MCI and CN, the CC_o was statistically significant when we compared MCI against CN ($p<0.05$). In addition to the clustering coefficient, the local efficiency when we compared MCI against the CN group was also statistically significant ($p<0.05$). We found no statistically significant differences in L_o when we compared EMCI and MCI to CN and EMCI to MCI. We reported no loss of small-world attributes in the three group comparisons, i.e., EMCI to CN, MCI to CN and EMCI to MCI (see **Table 5**). The networks formed by MCI and CN may have the ability for specialised processing to occur within densely interconnected group regions. The ROI-to-ROI analysis for the MCI and CN group comparisons did not yield any significant FC changes that support this theory.

Nodal Centrality Characteristics:

We constructed a network at a cost threshold < 0.15, which ensured that all the subjects produced the same number of edges and that no subject had edges beyond the threshold. To check if the brain region identified as the network's hub, we tested each ROI and set the threshold at $b_i > 1.5$, signifying that the node's betweenness value is 1.5 times the average betweenness of the network. The differences observed in b_i may tell us the topological changes in the patient groups.

Across the different groups, the lateral occipital cortex – superior division (R) and lateral occipital cortex – superior division (L) were common brain regions which were significant and were hubs in the network. In addition, MCI had the three DMN ROIs that formed significant hubs in the network: the DMN – the medial prefrontal cortex, DMN – lateral parietal (L), and DMN – lateral parietal (R). EMCI and CN had DMN's lateral parietal (R) and visual lateral (R) as the common regions forming hubs in the network (see **Table 6**).

We then proceeded to look at the differences between groups; we compared CN to EMCI and MCI groups to check for any changes in betweenness centrality as it addresses the question of which is the most critical node in the network and if we see any abnormalities in the 53 selected ROIs responsible for memory and cognitive function (see **Tables 7 & 8**). The differences between EMCI and CN and EMCI and MCI were significant (see **Table 4**). However, we do not observe any decrease or increase in the betweenness centrality (see **Tables 8 & 9**). MCI patients showed a reduction in betweenness centrality compared to CN in inferior temporal gyrus – anterior division (R), inferior temporal gyrus – anterior division (L), inferior temporal gyrus – posterior division (R), superior parietal lobule (L), angular gyrus (R), thalamus (L), hippocampus (R). In contrast, we observed increases in visual – lateral (R), visual – lateral (L), the lateral occipital cortex – superior division (R), the lateral

occipital cortex – superior division (L), DMN – lateral parietal (L), DMN – the medial prefrontal cortex, and visual network– medial (see **Table 7**).

Table 6

Nodes/brain regions that have $b_i > 1.5$ for MCI, EMCI and CN groups.

Regions	Normalised betweenness (b_i)
Group 1 – MCI	
DMN – Medial Prefrontal Cortex	2.425
DMN – Lateral Parietal (L)	2.37
DMN – Lateral Parietal (R)	1.6
Lateral Occipital Cortex – superior division (R)	2.475
Lateral Occipital Cortex – superior division (L)	1.897
Group 2 - EMCI	
Visual Lateral (R)	2.105
Lateral Occipital Cortex – superior division (R)	2.833
Lateral Occipital Cortex – superior division (L)	2.45
DMN – Lateral Parietal (R)	1.61
Group 3 – CN	
Lateral Occipital Cortex – superior division (R)	2.15
Lateral Occipital Cortex – superior division (L)	2.02
DMN – Lateral Parietal (R)	1.7
Visual – Lateral (L)	1.7
Visual – Lateral (R)	1.52

Note.

DMN – Default Mode Network

(R) – Right

(L) – Left

Table 7

Nodes/brain regions that have $b_i > 1.5$ for MCI and CN group comparison.

Brain Region	MCI	CN
Visual – Lateral (R)	2.062	0.225
Visual – Lateral (L)	2.06	1
Lateral Occipital Cortex – Superior Division (R)	2.50	0.81
Lateral Occipital Cortex – Superior Division (L)	2.50	0.68
DMN – Lateral Parietal (L)	1.84	1.55
DMN – Medial Prefrontal Cortex	1.63	1.727
Visual – Medial	1.15	1.63
Inferior Temporal Gyrus – anterior division (R)	0.71	1.65
Inferior Temporal Gyrus – anterior division (L)	0.71	1.63
Inferior Temporal Gyrus – posterior division (R)	0.65	1.78
Superior Parietal Lobule (L)	0.65	2.04
Angular Gyrus (R)	1.66	2.045
Thalamus (L)	0.406	1.863
Hippocampus (R)	0.34	2.06

Note.

DMN – Default Mode Network

(R) – Right

(L) – Left

Table 8

Nodes/brain regions that have $b_i > 1.5$ for EMCI and CN group comparison.

Brain Regions	EMCI	CN
DMN – Lateral Parietal (L)	2.33	2.34
Visual Network – Lateral (R)	1.53	1.52
Visual Network – Lateral (L)	1.67	1.68
Precentral Gyrus (R)	1.52	1.53
Lateral Occipital Cortex – superior division (R)	2.850	2.871
Lateral Occipital Cortex – superior division (L)	2.240	2.254
Lateral Occipital Cortex – inferior division (L)	1.55	1.564

Note.

DMN – Default Mode Network

(R) – Right

(L) – Left

Table 9

Nodes/brain regions that have $b_i > 1.5$ for EMCI and CN group comparison.

Brain Regions	EMCI	MCI
DMN – Medial Prefrontal Cortex	1.59	1.57
DMN – Lateral Parietal (L)	1.961	1.95
Visual Network – Lateral (R)	1.625	1.625
Visual Network – Lateral (L)	2.154	2.142
DAN – Intraparietal Sulcus (L)	1.552	1.54
Superior Parietal Lobule (R)	1.551	1.551
Lateral Occipital Cortex – superior division (R)	2.855	2.857
Lateral Occipital Cortex – superior division (L)	2.312	2.315

Note.

DMN – Default Mode Network

DAN – Dorsal Attention Network

(R) – Right

(L) – Left

Discussion:

Several studies have used graph theory in the last decade to model the brain's function as a network in AD and, in addition, have also performed functional connectivity analysis (Liu et al., 2012; Zamani et al., 2022; Xiang et al., 2013; Agosta et al., 2010; Ahmadi et al., 2020; Grieder et al., 2018). Very few studies focus on the transitional phases of AD, the EMCI and MCI stages. Studying these transitional phases helps us understand the risk of progression across the phases of AD and help us determine if an EMCI or MCI patient will develop AD. Here we used rs-fMRI to construct a network using a graph theoretical approach to understand the node-wise/region-wise properties of the network built and to study differences in functional connectivity between three groups, EMCI, MCI, and CN. The study's main findings were:

1. No significant FC differences between MCI and CN,
2. Significant FC differences were observed between EMCI and CN and between the two patient groups, EMCI and MCI,
3. The three groups, EMCI, MCI, and CN showed similar small-world attributes.

The lack of any observable difference in functional connectivity when comparing MCI and CN could be because network deterioration and compensatory mechanisms offset each other. A decrease in functional connectivity may represent the deterioration of the neural network by the loss or weakening of synaptic connection and, later, neuronal or axonal degeneration (Bondareff et al., 1989; Rodrigue et al., 2009; Sohn et al., 2014). Increased connectivity may reflect the recruitment of other elements for compensation by enhancing the synaptic efficiency of pre-existing connections or forming new connections (Buckner, 2004).

For better neural efficiency, the human brain must have a globally minimum wiring length, i.e., a short average path length and a higher clustering co-efficiency. The metabolic cost of

neural connections should be minimised wherever possible (Kaiser & Hilgetag, 2006). It is also essential to quantify network anatomy because structure influences function (Strogatz, 2001). For example, the topology of social networks influences the spread of information and disease, and the power grid's topology influences the power transmission's robustness and stability (Strogatz, 2001; Watts & Strogatz, 1998). Likewise, in our case, it may be possible that the network constructed using 53 ROIs can help us understand the spread of disease in EMCI and MCI, i.e., to know how far the transitional phase has progressed (Liu et al., 2012; Strogatz, 2001; Watts & Strogatz, 1998). Recent studies have also shown that memory decline in patients with AD is coupled with losses of small-world attributes (Fleisher et al., 2009; Li et al., 2020). Our focus was more on the average path length and clustering co-efficiency of the network formed by the 53 selected ROIs as they depict a measure of functional integration in the brain, i.e., the ability to combine specialised information from distributed brain regions rapidly, and the ability for specialised processing to occur within densely interconnected groups of brain regions.

In this study, we detected that the average path length and the clustering co-efficiency were more or less equal across the three groups. An increase of L and a decrease of CC_o across the groups would indicate disruption of specialised information processing from the distributed brain regions and the ability for specialised processing to occur (Liu et al., 2012; Seo et al., 2013; Stam et al., 2007; Strogatz, 2001). In recent studies, the increase of L and CC_o indicates disrupted information processing among distant brain regions across the whole brain, supporting the disconnection theory of AD (Delbeuck et al., 2003). Although, since CC_o is significant and higher in the MCI group in the MCI and CN group comparison, and we also observe a higher average path distance in MCI groups, MCI groups may present disrupted specialised processing in the network formed using the 53 ROIs. However, we

found no significant differences in L and CC_o when we compared EMCI and MCI groups and EMCI and CN groups.

The criteria to understand if the brain region was the hub in the network, we set $b_i > 1.5$. Across the different groups, the lateral occipital cortex – superior division (R) and lateral Occipital Cortex – superior division (L) were common brain regions which were significant and were hubs in the network. In addition, MCI had the three DMN ROIs that formed significant hubs in the network: the DMN – the medial prefrontal cortex, DMN – lateral parietal (L), and DMN – lateral parietal (R). EMCI and CN had DMN's lateral parietal (R) and visual lateral (R) as the common brain regions forming hubs in the network.

We then looked at the group differences; we compared CN to the EMCI and MCI groups to check for abnormalities in betweenness centrality in the 53 brain regions that formed a network. No significant differences were found when we compared EMCI, MCI, EMCI and CN populations. Significant decreases were found in MCI populations with inferior temporal gyrus (ITG), superior parietal lobe, angular gyrus (AG), and hippocampus (R) brain regions as compared to the CN population. In contrast, an increase in BC was observed in the visual areas and the DMN regions. Chetelat et al. (2002, 2005) mapped grey matter volume in the hippocampus using voxel-based morphometry and found a significant reduction in the grey matter volume. Delbueck et al. (2003) suggest that the hippocampus is affected in the early stages of patients with AD and is likely to show abnormalities before other regions. These abnormalities could indicate that these brain regions showing nodal centrality abnormalities could cause memory and cognitive decline (Sanz-Arigita et al., 2010; Sohn et al., 2014; Sperling et al., 2010; Wang et al., 2007; Wang et al., 2006; Xue et al., 2019).

A possible limitation of this study could be neuropsychological tests used in determining the EMCI and MCI phases of AD. The definition of EMCI provided by ADNI, ADNI GO, and

ADNI 2 is still debatable (Landau et al., 2012; Qiu et al., 2014). It is critical to provide an even more comprehensive framework for neuropsychological testing that can aid in detecting cognitive alterations in the pre-MCI stages of AD. Among neuropsychological measures, composite scores (obtained by normalising and aggregating standardised z-scores) have demonstrated greater sensitivity than single scores at cognitive tests in detecting changes over time within the preclinical AD stages (Amariglio et al., 2012; Buckley et al., 2016; Langbaum et al., 2014; Lim et al., 2016; Mormino et al., 2017; Papp et al., 2017; Rentz et al., 2013). As a result, they have been used as outcome measures in prevention trials (Amariglio et al., 2015; Dubois et al., 2016). In addition to this, the data obtained was on different imaging systems. Whilst this is not thought to affect the data quality or between-group comparisons massively, it might contribute to differences. Thus, identical acquisition methods should be used in the future. Furthermore, it is unknown how the topological organisation of brain networks changes as the disease progresses, but we attempted to understand this by limiting structural complexity and connection diversity (Achard et al., 2006; Albert & Barabási, 2002; Bullmore & Sporns, 2009; Strogatz, 2001). We also propose combining multi-modal neuroimaging data where the data can be separately recorded and analysed but interpreted together in the same template space to comprehensively understand the transitional stages of AD (Chen et al., 2022; Chételat, 2018; Wu et al., 2022). Multi-modal neuroimaging data integration can yield important insights into brain processes and structures in addition to spatiotemporal resolution, including a comprehensive physiological view of brain processes and structures, quantification, generalisation and normalisation, and availability of biomarkers such that there exists an added benefit compared to analysing and interpreting each dataset separately.

Finally, this study used an ROI-to-ROI level FC and graph theoretical approach to build a network of 53 ROIs in EMCI, MCI and CN groups aged 60-69. Our findings suggested that,

while the small world property was present in all three groups, MCI patients had abnormal nodal centralities compared to the age-matched CN population. However, large-scale brain networks in neurological disorders can have drastically different region-to-region connectivity, making it challenging to understand the progression of these neurological disorders. Longitudinal studies will aid in our understanding of this problem. To characterise brain networks and understand the patterns of interconnections that occur in complex networks, we could use the network MOTIF algorithms proposed by (Milo et al., 2002). The MOTIFs method could reveal functionally important patterns but not statistically significant ones, which would help us understand the essential structural elements unique to the 53 ROIs' small-world networks. In addition, we must attempt to find a new imaging-based biomarker of AD in the future.

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Appendix - 1

MSc Dissertation

Student report

Student ID	2350663
Title of dissertation	Graph Theory Application with Functional Connectivity to distinguish Early Mild Cognitively Impaired and Mild Cognitively Impaired patients from Healthy Controls.
Word count: Introduction + methods+ results + discussion + supplementary materials	1034 + 1656 + 1046 + 1428 = 5164 words.
Target journal	JNeurosci
Justify journal choice	Being a multidisciplinary journal with a focus on the working of the nervous system, I decided to choose this journal.
Required format included sections	Introduction, Methods and Materials, Results, and Discussion.
Journal specified word limit	3,000-5000 words preferred limit. To not exceed 8000 words.
Journal citation style	Any style was recommended in the journal but the style has to be consistent. Thus. APA-style 7th edition was used in this dissertation report.
Description of supplementary material – if applicable	None.
Description of appendices	None.

Please explain who contributed what to the project

Projects parts	Individual/team work with other master students/supervisor (inc PhD, postdoc)/others/NA	comments
Define the research question	Individual	
Design the study	Individual	
Create the stimuli	-	
Program the experiment	Individual	
Collect the data	Individual	
Create analyses scripts	Individual	
Pre-process/clean the data	Individual	
Statistical analysis	Individual	

Create data summaries (figures/charts)	Individual	

Other comments that you feel should be considered regarding your contribution to the project and the quality of work – please describe in details (e.g., you had to learn new skills, the project radically changed at very late stage, the supervisor (their lab) did not have the expertise to support you, part of the project was very challenging and took longer than expected to resolve):

Details:

1. Project had minor changes in the last stage as the analysis took a very long time.
2. Had to learn new computational techniques: Graph Theory
3. Graph Theory proved to be challenging and took longer than expected to resolve the problem at hand.
4. Had to learn a new software named CONN for analysis purposes.