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Exercise Training and Functional Connectivity Changes in Mild Cognitive Empairment and Healthy Elders

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Abstract:

Background: Effective interventions are needed to improve brain function in Mild Cognitive Impairment (MCI), an early stage of Alzheimer's disease (AD). The posterior cingulate cortex (PCC)/precuneus is a hub of the Default Mode Network (DMN) and is preferentially vulnerable to disruption of functional connectivity in MCI and AD.

Objective: We investigated whether 12 weeks of aerobic exercise could enhance functional connectivity of the PCC/precuneus in MCI and healthy elders.

Methods: Sixteen MCI and 16 healthy elders (age range = 60-88) engaged in a supervised 12-week walking exercise intervention. Functional MRI (fMRI) was acquired at rest; the PCC/precuneus was used as a seed for correlated brain activity maps.

Results: A linear mixed effects model revealed a significant interaction in the right parietal lobe: the MCI group showed increased connectivity while the healthy elders showed decreased connectivity. In addition, both groups showed increased connectivity with the left postcentral gyrus. Comparing pre to post intervention changes within each group, the MCI group showed increased connectivity in 10 regions spanning frontal, parietal, temporal and insular lobes and the cerebellum. Healthy elders did not demonstrate any significant connectivity changes.

Conclusion: The observed results show increased functional connectivity of the PCC/precuneus in individuals with MCI after 12 weeks of moderate intensity walking exercise training. The protective effects of exercise training on cognition may be realized through the enhancement of neural recruitment mechanisms, which may possibly increase cognitive reserve. Whether these effects of exercise training may delay further cognitive decline in patients diagnosed with MCI remains to be demonstrated.

Keywords: Alzheimer's disease, connectivity, cognitive disorders, aging, default mode network, posterior cingulate, precuneus, exercise interventions, resting state fMRI

Introduction

Neuropathological changes associated with Alzheimer's disease (AD) occur many years before the onset of clinical symptoms.[1] Older adults who have declined cognitively but who do not meet criteria for a diagnosis of AD are often classified as having Mild Cognitive Impairment (MCI),[2,3] and more than half of these individuals progress to an AD diagnosis within five years.[4] There is an urgency to identify biomarkers for preclinical detection of neuropathology prior to the onset of symptoms in order to inform treatment strategies and to aid in the understanding of AD progression.[5] Resting state functional connectivity is emerging as a viable biomarker and predictor of future conversion to AD[6-9] and as an indicator of treatment efficacy.[10,11]

Resting state functional connectivity in this paper is based on the correlations of spontaneous blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signals during the absence of an external task. It is assumed to reflect the underlying anatomy of the neuronal architecture[12] through direct and indirect neural networks consisting of monosynaptic and polysynaptic connections.[13-15] Temporal correlations of spatially distinct brain regions indicate either direct or indirect neuronal connections, and resting state functional connectivity has been found to predict performance on higher order cognitive tests.[16-18] Higher order cognitive processes require the integration of several segregated, domain-specific neural processing pathways,[12] and these diverse pathways intersect in regions of the brain called 'hubs', characterized by a disproportionately high number of functional, and often concurrently anatomical, connections.[19] These hubs, while few in number, may limit the large metabolic cost of neural communication by integrating otherwise disparate networks[20] and play an important role in information flow.[21] The posterior cingulate (PCC) and precuneus regions together constitute a key hub of the default mode network (DMN). This hub fosters efficient communication between the DMN and the medial temporal lobe (MTL) network, a network with an important role in memory processes[22] that is highly vulnerable to AD pathology.[23] The PCC/precuneus is also an area associated with the accumulation of amyloid-β (Aβ) plaque, a hallmark of AD pathology.[12] The PCC/precuneus exhibits reduced functional connectivity in MCI, early AD,[24,25] and in clinically normal older adults that test positive for brain amyloid burden.[26,27] Changes in the functional connectivity of the PCC/precuneus have also been associated with accelerated atrophy and other preclinical pathological changes associated with AD,[24,28,29] underscoring its potential role as a predictive biomarker. Thus, alterations in resting state functional connectivity, while concurrently associated with cognitive decline, may also precede measureable cognitive changes.

Prior to evidence of cognitive decline in AD, the PCC/precuneus exhibits increased connectivity with frontal and parietal brain regions that do not show AD pathology until very late disease stages,[30] and there is growing evidence that increased recruitment of these frontal

Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

regions in older adults is a compensatory response to aging.[31] Although this compensatory response is associated with neuronal damage,[32] it is also thought to be indicative of maintaining cognitive function.[33,34] In individuals diagnosed with MCI and AD, declines in connectivity have been noted in brain areas affected early in the progression of AD, such as the MTL.[23] Interventions or treatments that preserve and/or increase the connectivity of the PCC/precuneus with available frontal and parietal resources in older adults may help promote cognitive stability.

It is well established that both leisure time physical activity and exercise training help to improve and maintain cognitive function in healthy older adults,[35,36] even in those at increased risk for AD.[37-40] Aerobic training in healthy elders appears to increase the functional connectivity within the DMN[41] and hippocampal networks.[42] Furthermore, although local neuronal networks exhibit deterioration in healthy elders, high levels of physical activity have been shown to protect these networks.[43] Exercise training in individuals diagnosed with MCI has been shown to improve cognition[4,44-46] and increase neuronal efficiency during a semantic memory retrieval task,[46] but it is not known if exercise training results in changes to neural network functional connectivity in older adults diagnosed with MCI. If exercise training does increase the connectivity of hubs enhancing network recruitment in this population, it may indicate a gain of cognitive reserve that would help to preserve cognitive abilities and possibly delay cognitive decline. Cognitive reserve is a concept that explains the typically nonlinear relationship between cognitive performance and neuronal damage or brain disruption,[31] and is associated with the ability to recruit additional brain networks when the primary networks are disrupted[47] as in MCI. Greater levels of cognitive reserve proxies, such as education, have been associated with increased functional connectivity,[48] and physical activity (PA) also has been implicated as one of the factors that increases cognitive reserve.[49]

The current study extends this literature with evidence that aerobic exercise training may stimulate functional connectivity of the PCC/precuneus in individuals diagnosed with MCI. This is a continuation of our previously published paper that reported a 12-week walking intervention resulted in decreased fMRI activation in

several cortical regions during a semantic memory related task.[46] We hypothesized that healthy elders and individuals diagnosed with MCI would demonstrate *increased* connectivity between the PCC/precuneus and frontal-parietal cortices from before to after the intervention, indicating enhanced network recruitment capabilities. We expected increased connectivity to the MTL in the MCI group, as the MTL is particularly vulnerable to AD progression.[23]

Materials and Methods

Participants and pre-screening

This study used resting state fMRI data from participants (17 MCI and 18 healthy elders) described in previous work,[46] except that resting state fMRI data were missing for one MCI and two healthy elder participants. Community dwelling older adults, ages 60 to 88, were recruited through physician referrals, local newspaper advertisements, and in-person informational sessions at retirement communities and recreational centers. Interested volunteers who were still eligible after a phone interview met face-to-face with a study team member to review all procedures, expectations, possible risks, and a physician approval form for moderate intensity exercise was obtained. A neurological evaluation completed the eligibility evaluation. Informed consent was obtained from all individual participants included in the study. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Inclusion and exclusion criteria

In order to maximize the effect of exercise training, all study participants indicated they engaged in only light physical activity two or fewer days/week for the past six months. Participants were excluded if they reported a history or evidence of: 1) medical illnesses or conditions that may affect brain function (including glaucoma, chronic obstructive pulmonary disease, and untreated hypertension); 2) neurological illnesses or conditions (including cerebral ischemia,

Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

vascular headache, head trauma with loss of consciousness (>30 min), epilepsy, carotid artery disease, cerebral palsy, brain tumor, normal-pressure hydrocephalus, chronic meningitis, pernicious anemia, multiple sclerosis, Huntington's disease, Parkinson's disease or HIV infection); 3) current untreated Axis I psychiatric disturbance meeting DSM-IV Axis I criteria (including substance abuse or dependence and severe depressive symptoms); 4) exclusion criteria specific to MR scanning (such as pregnancy, history of claustrophobia, weight inappropriate for height, and ferrous objects within the body); 5) any unstable or severe cardiovascular disease or asthmatic condition; 6) left-handedness (laterality quotient [LQ] <50);[50] 6) current use of psychoactive medications, except stable doses of antidepressants; and 7) history of transient ischemic attack or >4 on the modified Hachinski ischemic scale. Participants were also excluded if they scored >15 on the Geriatric Depression Scale (GDS)[51] or showed relatively impaired activities of daily living (ADL) using the Lawton and Brody Self-Maintaining and Instrumental Activities of Daily Living Scale.

Neuropsychological test battery and clinical criteria for MCI

The Neuropsychological test battery included the Mini-Mental State Exam,[52] Mattis Dementia Rating Scale-2 (DRS),[53] Rey Auditory Verbal Learning Test (AVLT),[54] Logical Memory and Letter-Number Sequencing subtests of the Wechsler memory Scale-III,[55] Symbol-Digit Modalities Test,[56] Controlled Oral Word Association Test,[57] animal fluency, and the Clock Drawing Test.[58] This comprehensive battery was administered before and after the exercise intervention, and alternate forms of the AVLT and DRS were used at each testing session.

Cognitive status of the participants was determined using the core clinical criteria set by the NIH-Alzheimer's association workgroup on MCI due to AD.[7] MCI was defined as a subjective concern regarding a change in cognition supported by an informant, impairment in one or more cognitive domains (defined as 1.5 standard deviations below age and education matched means on delayed recall on the AVLT), and intact activities of daily living. Three neuropsychologists (including K.A.N.) reached a consensus on impairment. A neurologist ruled out all other possible etiologies. Healthy elders had no specific cognitive

complaint, intact cognitive performance in all domains, and intact activities of daily living.

Exercise test

Participants completed a submaximal exercise test on a motorized treadmill (General Electric, Milwaukee, WI) to estimate peak aerobic capacity ($\dot{V}O_{2peak}$) before and after the exercise intervention. The exercise test used a modified Balke-Ware protocol of 2.0 miles/hr beginning with a 0^0 grade and increasing 1^0 per minute.[59] Concentrations of oxygen and carbon dioxide in expired air were collected every 15 seconds by a metabolic measurement system (ParvoMedics, Sandy, UT). Each test included measurements of heart rate, blood pressure (every 2 minutes), and ratings of perceived exertion (RPE; each minute). Test termination criteria included reaching 85% of age-predicted heart rate max, a diastolic blood pressure greater than 110 mmHg, or the participant's desire to stop. The peak rate of oxygen uptake ($\dot{V}O_{2peak}$) was estimated from the highest $\dot{V}O_2$ value achieved during the test (expressed as ml/kg/min at STPD).[59] Additional details have been described by Smith et al.[46]

Exercise intervention

A qualified personal fitness trainer or an exercise physiologist supervised the participants in the 12-week intervention at fitness centers located near the participants' homes or within their communities. The exercise intensity, session duration, and weekly frequency were increased during the first four weeks until the participants were walking for 30 minutes, four times a week, at approximately 50-60% of HRR (heart rate reserve). Each session began and ended with 10 minutes of light walking and flexibility exercises. Participants wore a Polar® heart rate monitor and provided subjective RPE's using the Borg 6-20 RPE scale throughout each exercise session at minutes 5, 15, and 30.[60,61] The treadmill grade and/or speed were modified to moderately challenge the participant based on the heart rate and perception of effort (not more than 15 on the Borg scale). This is considered a moderate intensity exercise for older adults.[62]

Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

MRI acquisition procedures

Prior to the first MRI acquisition using a General Electric (Waukesha, WI) 3.0 Tesla scanner, participants were familiarized with the MRI environment by lying in a mock scanner. During MRI acquisition, participants were instructed to lie as still as possible and foam padding was used to limit movement and improve comfort. Anatomical and resting state sequences were run during the scanning session. High-resolution, three-dimensional spoiled gradient recalled at steady-state (SPGR) anatomic images were acquired (TE = 3.9ms; TR = 9.6ms; inversion recovery (IR) preparation time = 450ms; flip angle = 12°; number of excitations (NEX) = 1; slice thickness = 1.0mm; FOV = 240mm; resolution = 256 x 224). During the resting state scan, participants were instructed to keep their eyes open and to look at a fixation cross. A gradient-echo, echo-planar pulse sequence sensitive to blood oxygenation level-dependent (BOLD) contrast were acquired (TE = 25ms; TR = 2000ms; flip angle = 77°; NEX = 1; 36 axial slices; 4.0 mm isotropic voxels; FOV = 240mm; resolution = 64 x 64; duration 6 minutes).

MRI preprocessing

Preprocessing steps were done using tools from the Analysis of Functional NeuroImages (AFNI) software package.[63] During the initial preprocessing and analysis, the researcher (TC) was blind to each participant's group classification. Time series and anatomical images were aligned and skull-stripping, slice time correction, and motion correction procedures were performed. The first 3 TRs were removed, a 0.005 to 0.10 Hz bandpass filter was applied, and the following sources of noise were regressed out: six-parameter rigid body head motion, ventricle signal, white matter signal, mean global signal, and the derivatives of the motion parameters, white matter signal, and ventricle signal. The time series data were smoothed using a 4mm full-width at half-maximum Gaussian blur and normalized to Montreal Neurological Institute (MNI) space.

Seed based analysis

The seed based analysis was also conducted using AFNI. The seed region of interest (ROI) was defined using a 5mm spherical mask surrounding the MNI coordinates -2, -50, 36, the peak voxel coordinates of the PCC/precuneus reported by Buckner and colleagues.[12] The time course in the ROI was extracted, and seed correlation maps for all participants at each testing session were formed by correlating the seed ROI with all other voxels in the brain. A Fisher's r to z transformation was implemented to normalize the correlation coefficients. Group and Time differences were analyzed using linear mixed effects[64] and cluster-based analysis (after interpolation to 2 mm^3 voxels, 105 voxels or more with primary threshold = 0.01; cluster based statistic $p < 0.05$, FWER controlled). This allows for sensitivity (minimizing Type II errors), while maintaining some spatial specificity. The AFNI 3dLME command was used to run the linear mixed effects model using age as a covariate. This is an ideal analysis for repeated measures analyses because it allows for random intercepts; thus initial variability in the correlations are taken into account. F-statistics indicate main effects and interactions, and we also conducted post hoc paired sample t-tests within each group to assess changes from baseline to post-intervention.

Results

Participant and baseline characteristics

Usable resting state fMRI data were available for 16 healthy elders and 16 participants diagnosed with MCI. As shown in Table 1, the healthy elders and individuals diagnosed with MCI did not significantly differ in sex, age, education, depression or activities of daily living. As expected, the MCI group exhibited poorer baseline performance than the healthy group on all but two neuropsychological subtests (DRS Attention and DRS Construction). Participants' baseline characteristics are presented in *Table 1*.

Exercise intervention fidelity

The mean (SD) number of exercise sessions attended, adherence rate, exercise intensity and perceived exertion over the first 4 weeks and during weeks 5-12 of the intervention did not differ between groups. Out of a total of 44 sessions, 42.3 (2.2) were completed resulting in a 96.1 (5.0) % adherence rate. During weeks 1-4 and weeks 5-12, the mean intensity was 46.9 (7.1) %HRR and 54.7 (11.0) %HRR, respectively. RPEs were most closely associated with the verbal descriptor "light" at 10.6 (1.8) and 10.8 (2.0) during the first 4 weeks and last 8 weeks, respectively. There was also a mean increase over the 12-week intervention in $\dot{V}O_{2peak}$ by 2.0ml/kg/min – an approximate 10.6% increase in cardiorespiratory fitness. More details regarding the change in cardiorespiratory fitness can be found in Smith et al.[46]

Neuropsychological test performance

Neuropsychological test results for the entire sample (35 participants) have been previously reported.[46] The results reported here are consistent with those reported by Smith et al. (2013), and reflect the slightly smaller sample size (32 participants) in the current study. A repeated measures ANOVA revealed a significant effect of Time in Trial 1 of AVLT ($p = .013$), where both groups demonstrated improvement from baseline to post-intervention (mean (SD): MCI pre: 3.75 (2.02), MCI post: 4.81 (1.97)); healthy elders pre: 5.50 (2.00) healthy elders post: 6.38 (1.57)). The Group by Time interaction for Trial 1 was not significant ($p = 0.800$), and there were no significant changes in the other Rey AVLT indices.

Seed based functional connectivity: Group by Time interaction

Connectivity results are based on the correlation maps of the mean BOLD time course from the PCC/precuneus seed ROI and the remaining voxels in the brain. One significant Group by Time interaction was observed and is presented in *Figure 1A* and *Table 2*. The MCI group showed an increased correlation between the

PCC/precuneus and right inferior parietal lobe (IPL). The healthy elders showed a decreased correlation with the cluster in the right IPL.

Seed based functional connectivity: Time main effects

A Time main effect, reflecting significant changes from before to after the intervention on average collapsed across both groups, was found in the left postcentral gyrus. There was an increased correlation of the PCC/precuneus with this cluster, and the region is presented in *Figure 1B* and *Table 2*.

Seed based functional connectivity: Post-hoc t-tests: Changes within each group

Significant changes in the MCI group are identified in *Table 3* and *Figure 2A.* The MCI group exhibited increased correlations after the exercise intervention in ten regions. Clusters had peak voxels in the right MFG, superior frontal gyrus, postcentral gyrus, PHG, and claustrum. Clusters were also found in the left IPL and bilateral precentral gyrus (two clusters in the left precentral gryrus) and culmen. No significant clusters demonstrating changes in connectivity across time were found in the healthy elders group.

Discussion

We investigated the effects of a 12-week walking intervention on the functional connectivity of the PCC/precuneus in individuals diagnosed with MCI and healthy elders. We hypothesized that both the MCI group and healthy elders would show increased connectivity with frontal and parietal regions, suggestive of enhanced recruitment of preserved brain regions. We also hypothesized that the intervention would increase PCC/precuneus connectivity with MTL regions in the MCI group. We did find the hypothesized changes in functional connectivity of the PCC/precuneus with frontal, temporal, and parietal brain regions in the MCI group, but only one region, the left postcentral gyrus, showed increased connectivity in both groups. Additionally, a group by time interaction in the right IPL revealed that the MCI group showed the expected increased connectivity while the

healthy elders demonstrated decreased connectivity in this region. These results, in conjunction with the findings from our previously published paper on the same subjects,[46] may indicate that exercise training has divergent effects on neural compensation and neural efficiency in the MCI group compared to healthy elders.

In developing our hypotheses for this study, we focused primarily on neural compensation as demonstrated by increased connectivity of the PCC/precuneus with preserved brain regions as a mechanism to increase cognitive reserve. After exercise training, the MCI group, presumed to be on the AD continuum, did demonstrate increased synchrony between compensatory networks and the PCC/precuneus, a region preferentially targeted in AD pathology. We expected, but did not observe, a similar increase in connectivity after exercise training in the healthy elders. This prediction was based on a previous report by Voss and colleagues,[41] which found trends of connectivity changes in the DMN after 6 months of exercise training. However, these effects did not reach statistical significance in their study until after the 12-month intervention. As our intervention lasted only 3 months, it is possible that a longer exercise intervention is needed to observe connectivity changes in cognitively healthy older adults.

Another hypothesized mechanism to increase cognitive reserve is augmenting neural reserve – an indicator of neural efficiency.[65] In our previous paper utilizing the same subjects,[46] we found group differences in activation changes in the precuneus and PCC during a famous name discrimination task. While both groups maintained equal task performance, the activation intensity decreased in the healthy elders after the exercise intervention, while there were no changes in the MCI group. This suggests increased neural efficiency in the healthy elders (as found in several other regions of the semantic memory network), and a need to maintain a compensatory response in the MCI group, as this region is targeted by AD pathology. Unfortunately, we were not able to measure levels of amyloid deposition or neurological damage in either group, so this possibility needs to be further explored. However, our current results are consistent with this interpretation and suggest that the MCI group demonstrated neural compensation through increased connectivity of the PCC/precuneus,

Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

while the healthy elders, who appear to not have reached a critical threshold for age related changes, did not require neural compensation.[65] Rather, exercise training may have resulted in increased neural reserve (or efficiency) of the PCC/precuneus, as indicated by our previously published findings of reduced activation during memory retrieval.[46] While the participants diagnosed with MCI did not differ in education from the healthy elders group (both were highly educated), the presence of cognitive impairment indicates a critical threshold was reached through combined age-related and AD processes. Thus, in those diagnosed with MCI, our findings support the idea that exercise training may stimulate increased cognitive reserve through enhanced recruitment of compensatory networks, such as increased function connectivity with a key neural hub in the PCC/precuneus.

As a first attempt to examine changes in resting state functional connectivity in MCI after an exercise intervention, we conducted *a priori* post hoc analyses to further explore the functional connectivity changes within this group. Results showed that the MCI group demonstrated increased connectivity of the PCC/precuneus with frontal and parietal regions from pre to post intervention. These effects suggest improved coordination of intrinsic activity of PCC/precuneus and several network regions including the fronto-parietal network (bilateral precentral gyrus and right middle frontal gyrus), the somatosensory network (right postcentral gyrus), and DMN regions (right superior frontal gyrus and left IPL). There was also increased connectivity between the PCC and the right parahippocampal gyrus, a region that links the DMN to the medial temporal lobe system.[66] The increased connectivity suggests possible enhancement of posterior-anterior connections vulnerable to aging.[18,31] Our findings raise the possibility that these increased compensatory connections across networks connected to the PCC/precuneus may in part explain the neural protective effects of physical activity in MCI. This pattern of stronger connectivity with the PCC/precuneus after exercise training is a stark contrast to the typical progression of connectivity disruptions with the emergence of clinical symptoms. As AD progresses there has been shown to be an initial decrease in functional connectivity within the DMN, followed by compensatory hyperconnectivity in the frontal/parietal network regions, and ultimately, with severe dementia,

an overall loss of connectivity.[32] The increased functional connectivity after exercise training observed in the MCI group between the PCC/precuneus and the other 10 regions suggests a possible reversal of the expected progression of connectivity decrements in MCI and, furthermore, enhancement of recruitment mechanisms that would increase cognitive reserve and possibly delay cognitive decline. Future randomized controlled trials should test this hypothesis.

Interestingly, the MCI group showed increased connectivity with regions in the insular lobe and cerebellum after the walking intervention. Connectivity in insular networks are reported to correspond to cognitive performance in individuals with amnesic MCI and cognitively healthy older adults.[67] Additionally, while we focused on the recruitment of frontal and parietal regions by the PCC/precuneus as an example of increased compensation to protect the DMN, the cerebellar region identified in our study corresponds to regions identified to be functionally related to the DMN.[68] Our results may indicate that the insular lobe and cerebellum are additional resources of reserve for the DMN. These results should be interpreted with some caution due to lower signal to noise ratio in these regions and particularly in the cerebellum.

Future research on the effects of an exercise intervention on functional connectivity in MCI and healthy elders should address potential mechanisms for these effects. Candidate measures that have been linked to exercise training would be BDNF, which has been found to modify functional connectivity,[69] cerebral blood flow,[42] hippocampal brain volume[70,71] white matter integrity,[72] and Aβ plaque burden.[73] Given the evidence that exercise may oppose the actions of acetylcholinesterase in the hippocampus and cerebral cortex of rats,[74] the effects of exercise training on the cholinergic system should also be considered. We have also recently reported, in this same cohort, that increased cardiorespiratory fitness after the exercise intervention was positively correlated in both groups with increased cortical thickness in regions including the precuneus, posterior cingulate cortex, pre-central and post-central gyri, and the medial and middle frontal gyri;[75] regions that partly overlap with the areas that showed changes in resting state connectivity in the current analysis. Future studies should focus on multimodal imaging techniques to understand

the mechanisms of exercise on neural plasticity in older adults and if this changes by disease state.

The lack of a non-exercise control group is a limitation of this study, and some caution is warranted in the interpretation of these effects. We cannot rule out the possibility that the walking intervention and its social context (most participants exercised alone under the supervision of a certified personal trainer) combined to produce changes in connectivity. However, the passage of time does not seem to be a plausible explanation for the changes we observed, as a longitudinal study in healthy older adults (ages 49 to 79) found functional connectivity within the DMN to be stable over a period of six years.[76] We observed that several regions showed bilateral increases in functional connectivity, and the fact that the effects were more pronounced in those diagnosed with MCI argues against a generalizable influence of the experimental context.

Many longitudinal studies have shown that the risk of cognitive decline is reduced in older adults who are physically active[77,78] and cognition is protected in individuals with MCI who have greater physical activity.[44,45] Our results indicate that these protective effects may manifest in individuals with MCI through the enhanced recruitment of the PCC/precuneus, an important hub for higher order cognitive processes, and the preservation of posterior-anterior resting state functional connections. These connections are vulnerable in normal aging, and when these aging effects are combined with AD, the results are even more devastating to cognition.[31] The pathological processes of AD and normal aging have divergent effects on brain networks,[79,80] and the differential effects of exercise training on functional connectivity in our study suggest that exercise-induced neural plasticity may vary based on AD progression and available cognitive reserve. While it remains to be conclusively demonstrated that exercise training may delay the conversion of individuals diagnosed with MCI to AD, or delay the onset of MCI among the cognitively intact, these results further underscore the complexity and pleiotropic nature of exercise as a potential intervention to modify neural network connectivity along the AD continuum.

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Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

Table 1. Demographic data and baseline characteristics of the participants diagnosed with mild cognitive impairment (MCI) and the healthy elders (HE).

Variables	MCI (n=16) Mean (SD)	HE (n=16) Mean (SD)	Group Difference p-value
Demographics			
Age (y)	79.6 (6.8)	76.1 (7.2)	0.167
Education (y)	15.6 (3.1)	16.6 (2.1)	0.322
Sex*	6M, 10F	3M, 13F	0.238
Depression Symptoms and Activities of Daily Living			
GDS	4.9 (4.1)	3.8 (2.8)	0.386
Lawton ADL	4.7 (0.5)	4.8 (0.5)	0.705
Neuropsychological Testing			
Logical Memory IR	27.1 (12.7)	43.1 (6.6)	<0.001
Logical Memory DR	15.9 (10.4)	25.9 (5.8)	0.002
Logical Memory Recognition	22.9 (3.7)	26.0 (1.8)	0.005
DRS Total	128.1 (13.3)	140.3 (2.5)	0.001
LNS Total	6.9 (2.7)	9.4 (1.9)	0.005
BDS	17.1 (2.1)	18.8 (0.5)	0.004
COWS FAS	29.8 (12.1)	41.9 (9.4)	0.003
Category Fluency - Animals	12.4 (6.5)	20.4 (4.2)	<0.001
Clock Drawing Test	2.6 (1.2)	1.4 (0.9)	0.003

Logical Memory, Wechsler Memory Scale-III subtest: IR, immediate recall; DR, delayed recall; DRS, Mattis Dementia Rating Scale-2; LNS, Wechsler Adult Intelligence Scale-III Letter Number Sequencing; BDS, Behavioral Dyscontrol Scale; COWA, Controlled Oral Word Association Test; GDS, Geriatric Depression Scale (scores were available for 14 MCI, 15 HE); ADL, activities of daily living. *p-value based on chi-sq.

Journal of Alzheimer's Disease, Vol 57 (2017): pg. 845-856. DOI.

Table 2. Regions that showed a significant Group by Time interaction and Time main effect for functional connectivity changes with the PCC/Precuneus from before to after a 12-week exercise training intervention in older adults diagnosed with mild cognitive impairment (MCI; n = 16) and healthy elders (HE; n = 16).

Region	BA	k	Peak Voxel			F	MCI		HE	
			x	y	z		Pre (r)	Post (r)	Pre (r)	Post (r)
Group by Time interactions										
Parietal Lobe										
R IPL	39	110	48	-70	38	36.66	0.31[a]	0.46[a]	0.49[b]	0.28[b]
Time main effects										
Parietal Lobe										
L Postcentral Gyrus	40	213	-44	-34	50	24.77	0.01	0.12	-0.14	0.01

Common superscript within region indicates significant difference, $p<0.01$. BA: Brodmann Area; k: cluster size; r and F: correlation and statistic of the peak, respectively; MCI: mild cognitive impairment; HE: healthy elders; L: left hemisphere; R: right hemisphere; IPL: inferior parietal lobule. xyz: MNI coordinates. Pre indicates baseline; Post indicates after exercise intervention. Shown in *Figure 1.*

Table 3. Regions that showed a significant change in functional connectivity with the PCC/precuneus from before to after a 12-week exercise training intervention in older adults diagnosed with mild cognitive impairment (MCI; n = 16).

Region	BA	k	x	y	z	t-statistic
MCIpost>MCIpre						
Frontal Lobe						
R Middle Frontal Gyrus	6	187	30	-4	64	4.41
R Precentral Gyrus	6	193	48	-4	38	5.00
R Superior Frontal Gyrus	10	170	32	56	2	4.87
L Precentral Gyrus	6	177	-24	-16	74	4.59
	6	147	-44	-12	32	4.43
Parietal Lobe						
R Postcentral Gyrus	3	215	48	-22	44	4.74
L IPL	40	167	-50	-34	46	3.93
Temporal Lobe						
R PHG	30	139	12	-48	2	3.94
Insular Lobe						
R Claustrum	13	128	36	-2	4	4.33
Cerebellum						
L Culmen		173	-32	-48	-36	4.83

BA: Brodmann Area including the peak voxel; k: cluster size; xyz: peak voxel MNI coordinates; MCI: mild cognitive impairment. L: left hemisphere; R: right hemisphere;

IPL: inferior parietal lobule; HP: hippocampus; PHG: parahippocampal gyrus. Pre indicates baseline; Post indicates after exercise intervention. Shown in *Figure 2.*

Figure Legends

Figure 1. Statistically significant (family-wise error corrected, $p < .05$) Group by Time interactions and main effects of Time for functional connectivity of the PCC/precuneus in response to a 12-week walking exercise intervention in older adults diagnosed with mild cognitive impairment (MCI; n = 16) and healthy elders (HE; n = 16). The mean correlation coefficients, MNI coordinates, and cluster size for each region are shown in Table 2. Panel A: A significant Group by Time interaction was found in the right inferior parietal lobule (IPL), where functional connectivity with the PCC/precuneus increased after exercise training in the MCI group and decreased in the HE group. Panel B: Functional connectivity significantly increased between the PCC/precuneus and the postcentral gyrus after exercise training in both the MCI and HE groups.

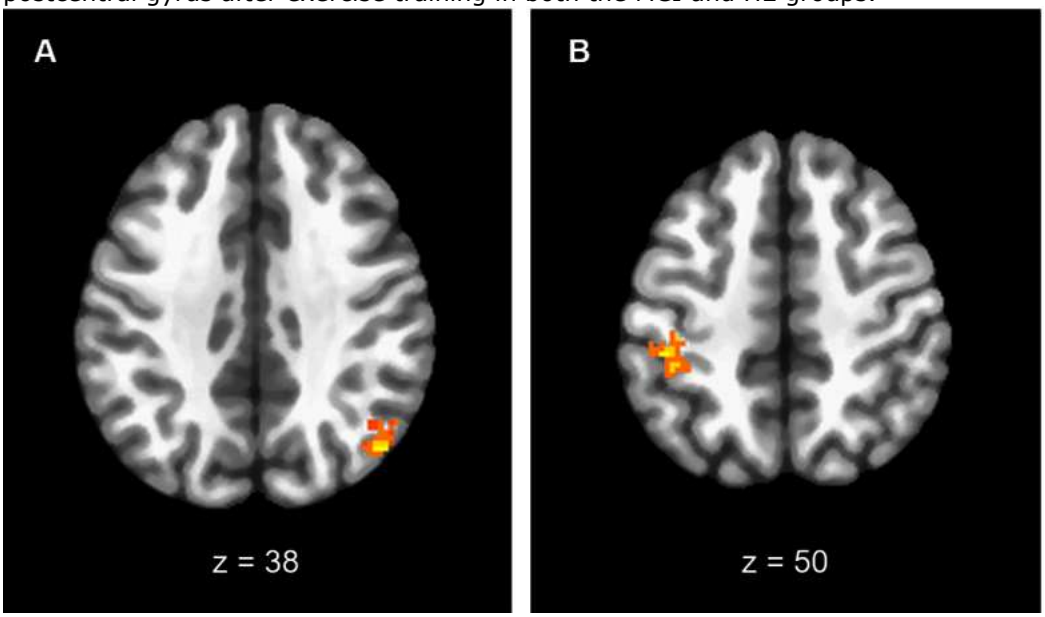

Figure 2. Resting state functional connectivity changes with the PCC/precuneus seed region in response to a 12-week walking exercise intervention in older adults diagnosed with mild cognitive impairment (MCI; n = 16).. All 10 highlighted brain regions indicate increased functional connectivity with the PCC/precuneus from before to after exercise training (familywise error corrected, $p < .05$). The MNI coordinates and cluster sizes for each region are shown in Table 3.