Cholinergic Enhancement of Functional Networks in Older Adults with Mild Cognitive Impairment

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Objective: The importance of the cholinergic system for cognitive function has been well documented in animal and human studies. The objective of this study was to elucidate the cognitive and functional connectivity changes associated with enhanced acetylcholine levels. We hypothesized that older adults with mild memory deficits would show behavioral and functional network enhancements with an acetylcholinesterase inhibitor treatment (donepezil) when compared to a placebo control group.

Methods: We conducted a 3-month, double-blind, placebo-controlled study on the effects of donepezil in 27 older adults with mild memory deficits. Participants completed a delayed recognition memory task. Functional magnetic resonance imaging (fMRI) scans were collected at baseline prior to treatment and at 3-month follow-up while subjects were on a 10mg daily dose of donepezil or placebo.

Results: Donepezil treatment significantly enhanced the response time for face and scene memory probes when compared to the placebo group. A group-by-visit interaction was identified for the functional network connectivity of the left fusiform face area (FFA) with the hippocampus and inferior frontal junction, such that the treatment group showed increased connectivity over time when compared to the placebo group. Additionally, the enhanced functional network connectivity of the FFA and hippocampus significantly predicted memory response time at 3-month follow-up in the treatment group.

Interpretation: These findings suggest that increased cholinergic transmission improves goal-directed neural processing and cognitive ability and may serve to facilitate communication across functionally-connected attention and memory networks. Longitudinal fMRI is a useful method for elucidating the neural changes associated with pharmacological modulation and is a potential tool for monitoring intervention efficacy in clinical trials.

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The neurotransmitter acetylcholine (ACh) plays a critical role in cognition, supporting perception, attention, learning, and memory. Animal studies have revealed the role of ACh in attention and memory through local manipulation of cholinergic function. For instance, sustained attention tasks in rodents have been associated with upregulation of ACh and enhanced activity in cortical sensory and prefrontal cognitive control regions.[1,2] In contrast, lesions to cholinergic input to the prefrontal cortex and other neocortical regions in animals have demonstrated severe impairments in stimulus detection, working memory, and spatial localization tasks.[3–5]

Human neuroimaging studies have also shown behavioral and neural effects of cholinergic upregulation, with reports of both enhancements and decrements.[6,7] A recent meta-analysis by Bentley and colleagues reviewed several pharmacological–neuroimaging studies, highlighting key differences in experimental design.[8] They reported that relative changes in neural activity were often related to task goals, such that attended stimuli resulted in

increased activity, whereas passively viewed or irrelevant stimuli commonly resulted in no change or decreased activity. In addition, the differences in reported findings varied based on drug type, brain region, study population, and drug duration, such that acute exposure of ACh upregulation had a greater impact on neural activity than prolonged exposure.[9] Furthermore, whereas healthy subjects with normal cholinergic function showed detrimental effects, hypocholinergic populations, such as individuals with Alzheimer's disease (AD), showed beneficial effects with increased ACh levels. In AD patients, prefrontal, occipital, and hippocampal regions showed increases in regional blood oxygenation level–dependent (BOLD) activity during a face-encoding task after 6 days of galantamine administration.[10] Additionally, increased fusiform gyrus activity was observed during face encoding after acute exposure to rivastigmine in AD patients.[11]

Previous studies of patients with mild cognitive impairment (MCI), a preclinical stage of AD, have demonstrated cognitive and neural improvements after undergoing cholinergic treatments, including improved task accuracy and response time (RT), enhanced neuropsychological test performance, and increased task-related brain activity.[11–13] MCI patients showed increased regional activity in prefrontal cortex during an n-back working memory task after 6 weeks of donepezil treatment when compared to a healthy control group.[13] Neuroimaging studies of ACh upregulation in MCI patients have mostly examined changes in regional whole-brain activity, leaving the possibility of changes in functional network connectivity largely unexplored. A recent study in rodents found that pharmacological manipulation using medetomidine, an α_2 adrenergic agonist, modified large-scale brain networks in a dose-dependent manner, while not influencing regional neural activity.[14] Thus, functional network connectivity may be a more sensitive approach for detecting neuropharmacological changes due to the effect of cholinergic upregulation on spatially distinct but functionally related networks.[8,15,16]

The present study aimed to elucidate cholinergic interactions associated with attention and memory by examining changes in functional network connectivity in a donepezil treatment group relative to a placebo control group. All participants were older adults with mild memory impairment and represent a vulnerable population at risk for further cognitive decline. The inclusion of a placebo control group offered additional insight into the naturally occurring changes that take place over time without an intervention, thus serving as an important control comparison. We hypothesized that increased ACh concentration through acetylcholinesterase inhibition (donepezil) would enhance behavioral performance and functional magnetic resonance imaging (fMRI) task-related functional network connectivity in a treatment group when compared to the placebo control group. Using a delayed recognition memory task, we interrogated functional connectivity in prefrontal cortex, parietal lobe, and the hippocampus, areas responsive to ACh upregulation during attentionally demanding stimulus encoding.[8,15–18]

Patients and Methods

Recruitment

Twenty-seven older adults with mild memory deficits between 50 and 80 years old were recruited for this study from the University of California, San Francisco Memory and Aging Center or through community screening. All participants gave written informed consent to participate, which was approved by the University of California, San Francisco Committee for Human Research. Participants were screened and diagnosed after a comprehensive neurological and neuropsychological evaluation. Screening for depression was done using the 30-item Geriatric Depression Scale (self-report). Diagnosis of mild memory impairment was determined by consensus between the neurologist and neuropsychology specialist. Participants met the core clinical criteria for MCI due to AD published by Albert and colleagues as a part of the international workgroup convened by the Alzheimer's Association and the National Institute on Aging. These criteria include (1) concern regarding a change in cognition, (2) impairment in 1 or more cognitive domains, (3) preservation of independence in functional abilities, and (4) not being demented.[19] Participants endorsed subjective memory decline over the past year and demonstrated objective memory impairment ($\geq$1 standard deviation below age- and education-matched normative values) on verbal or visual memory testing. Participants were excluded if they met criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders, 4th edition), a history of a neurological disorder, current psychiatric illness, head trauma with loss of consciousness >10 minutes, severe sensory deficits, or substance abuse. All participants were also screened for medical contraindications and were ineligible if they used donepezil, N-methyl-D-aspartate antagonists, antidepressants, antianxiety medication, narcotics, anticonvulsants, or antipsychotic agents.

Neuropsychological Testing

Participants were administered a comprehensive screening battery of neuropsychological tests assessing memory, executive function, and visuospatial ability. Tests included the California Verbal Learning Test II (20-minute delayed recall),[20] modified Wechsler Memory Scale III logical memory (15-minute delay),[21] Benson figure (copy and 10-minute delayed recall),[22] modified trail-making test A and B (time to complete),[23] design fluency (number of unique designs in 60 seconds),[24] modified Stroop interference (number correct in 60 seconds),[23] letter fluency (D words in 60 seconds),[23] forward and backward digit span (longest length), and digit symbol (number correct in 60 seconds).[25]
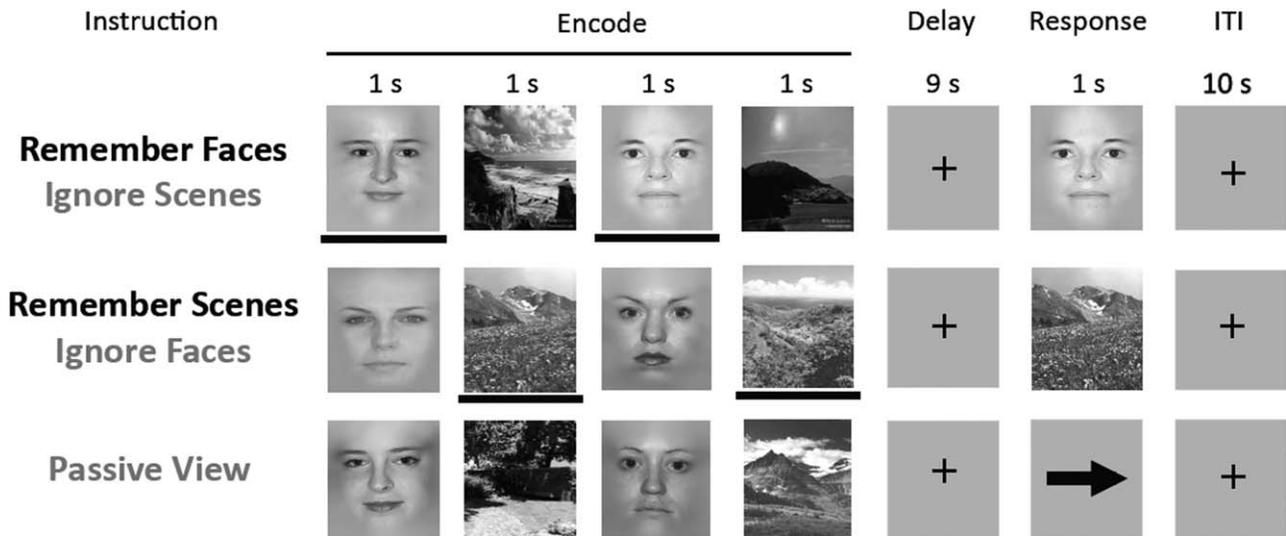
FIGURE 1: Experimental paradigm. Participants were instructed to remember faces or scenes over a delay period, followed by a matched forced choice yes/no response. Reaction time and accuracy were recorded as performance measures. A passive view condition was included to control for bottom-up effects associated with treatment. ITI = intertrial interval.

Experimental Paradigm

The experiment consisted of 3 conditions; participants were instructed to (1) remember faces and ignore scenes, (2) remember scenes and ignore faces, and (3) passively view the faces and scenes (as a control condition). In all conditions, participants viewed 4 sequentially presented novel, grayscale images (2 faces and 2 scenes presented in a randomized order). Each image was presented for 800 milliseconds, with a 200-millisecond blank-screen interstimulus interval. Presentation of stimuli was followed by a 9-second delay period and a 1-second face or scene probe stimulus. Participants pressed a button to indicate whether the stimulus matched 1 of the previously presented stimuli. In the passive view task, an arrow was presented, and participants pressed a button to indicate the direction of the arrow (Fig 1). Of note, in the passive view condition the same perceptual information was presented in the absence of an overt task, creating 2 important controls.[26–34] There was a physiological/neural control, which was the BOLD response during the passive face/scene viewing (encoding period), used as a baseline for encoding-selective activity in the other tasks. There was also an RT control, which was the time to respond to the direction of an arrow presented during the retrieval period. This was used as a behavioral control of RT for the other tasks. As a simple discrimination task, it served as a baseline to indicate whether treatment-induced changes in RT during the delayed-recognition tasks were selective or simply due to a generalized increase in RT. There were a total of 9 sessions (3 per condition) for each scan, lasting 4.5 minutes. Each session contained 10 trials of 1 task condition. Conditions were presented in a counterbalanced order across the study. Each participant practiced the task prior to the fMRI scanning session. Grayscale images of faces and natural scenes were 225 pixels wide and 300 pixels tall (14 × 18cm), subtended 3° of visual angle from fixation, and were presented foveally. All cue images were novel throughout the fMRI experiment and across visits.

Scanning and Drug Dose Schedule

Eligible participants were randomly assigned in a double-blind manner to either a placebo or treatment group. The randomization list was computer-generated, and a research assistant who was not affiliated with the study gave the assignments. All participants were given a deidentified subject number, and the association between subject number and group designation was stored in a sealed envelope. Participants completed a baseline fMRI scan prior to treatment. Following the scan, participants in the placebo group received a bottle of sugar pills, and participants in the treatment group received a bottle of 5mg donepezil pills. This dose was to be taken daily for 1 month for drug stabilization and titration, consistent with current standard of care for donepezil treatment. A second fMRI scan was completed at this time (1 month post-baseline). Participants were then titrated to 10mg of donepezil or placebo to be taken daily for 2 months, and a third fMRI scan was completed (3 months post-baseline). The study lasted for a total of 3 months, and the goal of the study was to examine baseline and 3-month follow-up differences at 10mg of donepezil.

Behavioral Performance Analysis

Participants performed a forced choice yes/no recognition response for each trial, to indicate whether the probe matched 1 of the previously viewed stimuli (or the direction of an arrow during the passive view condition). These responses resulted in 2 behavioral measures: RT for the memory probe and accuracy (ACC) for stimulus recognition. To control for bottom-up effects due to treatment (eg, elevated arousal), we used the passive view condition as an internal control within each subject. Thus, for the RT behavioral measure, the computed values for each participant were "faces RT − passive view RT" or "scenes RT − passive view RT." ACC was the raw accuracy score computed as percentage correct. To explore the effect of treatment over time, we conducted a 2-way repeated measures analysis of

variance (ANOVA) with 2 groups and 2 time points, resulting in a 2 × 2 factorial design to examine the interaction of group by visit. The 2 × 2 ANOVA was completed to directly assess the differences between baseline and 3-month follow-up. Possible titration effects of 5mg of donepezil or placebo after 1 month of treatment were also assessed for interactions for baseline versus 1-month follow-up and 1-month versus 3-month follow-up, as secondary analyses.

fMRI Data Acquisition
All fMRI data were collected on a Siemens (Erlangen, Germany) 3T MAGNETOM Trio. Echo planar imaging data were acquired (fractional anisotropy = 77°, echo time = 28 milliseconds, repetition time = 2 seconds) with 29 interleaved axial slices and 1.8 × 1.8 × 3mm voxels (field of view = 23cm; 128 × 128 matrix). All preprocessing of the data was conducted in SPM5. Raw BOLD data were corrected for slice-timing acquisition and motion artifacts. A 5mm isotropic Gaussian smoothing kernel was applied prior to modeling the data. High-resolution T1 magnetization-prepared rapid gradient-echo images were also acquired (1mm³ voxels).

Separate regressors were modeled for each stage of the trial (encode, delay, and probe) and convolved with a canonical Gaussian hemodynamic response function. Each session was modeled separately and included regressors for the encode, delay, and probe stages, which were modeled separately for correct and incorrect trials. In addition, there were 6 motion regressors for the X, Y, and Z directions and pitch, roll, and yaw rotations to control for possible subject movement. There were a total of 108 regressors in the general linear model for the univariate analysis. The instruction period at the start of each block was removed from the analysis. Correct trials were subjected to further analysis.

fMRI Localizer Scan
To allow us to independently localize stimulus-selective regions in each participant's visual association cortex, participants performed a brief functional localizer task before beginning the main experiment. Participants were presented with alternating 16-second blocks of face stimuli, scene stimuli, and rest periods (7 blocks of each type) and were instructed to attend to the stimuli and to indicate with a button press whenever they noticed an immediate (1-back) repeat of a stimulus. Face-selective regions of interest (ROIs) were defined within the fusiform face area (FFA) of the fusiform gyrus,[35] and scene-selective ROIs were defined within the parahippocampal place area (PPA) of the parahippocampal/anterior lingual gyrus[36] for each participant. Each participant's native space ROI was defined as the cluster of 35 contiguous voxels within each anatomical region with the highest cluster t value on a face–scene contrast for FFA and scene–face contrast for PPA.[31,37–40] The FFA and PPA ROI clusters were based on 35 contiguous voxels identified at $p < 0.01$ to reach a whole-brain cluster extent correction of $p < 0.05$.

fMRI Functional Connectivity Analysis
Functional connectivity maps of the regions of interest were generated by extracting beta values for every stage of every trial and correlating these values using the beta series correlation method.[41,42] The beta series correlation method was a functional connectivity analysis approach that utilized trial-by-trial variability to measure covariance in activity between spatially disparate regions, and offered a powerful tool for assessing network interactions during substages of a trial.[26,37–39,41–45] Single-subject z-transformed maps were normalized to the Montreal Neurologic Institute (MNI) space (2mm³ voxels) and Gaussian smoothed (5mm full width at half maximum) for the group analysis. Functional network connectivity during the encode stage was examined within the prefrontal cortex, parietal lobe, and hippocampus. To account for possible nonspecific perfusion effects of donepezil, an additional analysis using primary motor cortex (M1) as a control region was conducted. The peak MNI coordinates (−37, −21, 58) from a large meta-analysis of 126 motor studies[46] was used to form a 4mm sphere (36 voxels) around the peak. Monte Carlo simulations of 1,000 permutations were conducted to control for multiple tests, and a minimum cluster size of 24 voxels was accepted for a cluster-corrected significance of $p < 0.05$ for each ROI (WFU Pickatlas toolbox; Wake Forest University School of Medicine, Winston-Salem, NC).[47] To explore the neural effects of treatment over time specific to the cognitive task, a 3-way ANOVA with 2 groups (placebo, treatment), 2 time points (baseline, follow-up), and 2 conditions (face/scene, passive view) was performed, resulting in a 2 × 2 × 2 factorial design with main effects for group, visit, and condition to examine the interaction of group by visit by condition.

Results

Demographics and Neuropsychological Screening Tests
Our groups did not differ on any demographic or neuropsychological screening test. As expected, all participants performed significantly lower on tests of memory, with respect to age- and education-matched normative values, as indicated in the Table. There were no other significant differences. One participant was excluded from the study and analysis due to poor drug tolerability at 10mg of donepezil. All other participants reported study compliance.

Behavioral Performance
For RT on the face memory task, there was a significant group-by-visit interaction for baseline versus 3-month follow-up ($F_{1,24} = 6.86$, $p < 0.04$, Cohen d [d] = 1.07). As a secondary analysis to explore for possible titration effects at 1-month follow-up, additional 2 × 2 ANOVAs were computed for each pairwise comparison. There was no significant interaction for baseline versus 1-month follow-up ($F_{1,24} = 2.55$, $p = 0.12$, $d = 0.52$) or 1-month versus 3-month follow-up ($F_{1,24} = 1.28$, $p = 0.27$, $d = 0.39$). For RT on the scene memory task, there was a significant group-by-visit interaction for baseline versus 3-month follow-up ($F_{1,24} = 4.76$, $p < 0.04$, $d = 0.89$;

TABLE . Demographics and Neuropsychological Testing

Subject Demographics	Placebo	Treatment	Group Difference
No. (M/F)	13 (7/6)	13 (8/5)	0.69
Age, yr	69.2 [8.2]	63.8 [7.4]	0.10
Handedness, left/right	2/11	1/12	0.54
Education	16.3 [2.0]	16.9 [2.5]	0.50
Geriatric Depression Scale	4.5 [3.9]	7.3 [6.1]	0.16
Neuropsychological tests			
Global			
MMSE, max 30	28.4 [1.9]	28.3 [0.9]	0.89
Memory			
CVLT long delay free recall, max 16	5.4 [3.5][a]	6.9 [3.2][a]	0.26
Delayed Benson figure recall, max 17	7.9 [3.7][a]	9.4 [4.1][a]	0.36
Logical memory immediate, max 25	9.4 [3.8][a]	10.5 [3.2][a]	0.47
Logical memory delayed, max 25	6.6 [3.6][a]	8.9 [3.8][a]	0.18
Digit span backward	5.3 [1.3]	4.5 [1.1]	0.12
Attention/processing speed			
Digit span forward	6.7 [1.1]	6.7 [0.8]	0.99
WAIS-III digit symbol, 90 seconds	42.5 [8.7]	45.6 [11.9]	0.65
Number sequencing, max 150 seconds	36.4 [12.1]	30.8 [10.3]	0.27
Executive function			
Modified trail-making test B, max 300 seconds	83.4 [36.5]	84.8 [21.3]	0.92
Stroop interference, No. correct in 60 seconds	41.3 [10.2]	40.7 [11]	0.92
Verbal fluency, D words in 60 seconds	14.9 [4.5]	13.5 [5.1]	0.49
Visuospatial			
Copy of Benson figure, max 17	15.3 [1.2]	15.0 [1.3]	0.52

There were no significant group differences in demographics or neuropsychological tests (as shown by p values). As expected based on diagnostic criteria, the memory scores for both the placebo and treatment group were significantly lower than normative values. Values are presented as mean [standard deviation]. The p values of group differences were computed from a 2-sample t test or chi-square test.
[a]Significant within-group differences from age- and education-matched normative values at $p < 0.05$.
CVLT = California Verbal Learning Test; F = female; M = male; max = maximum; MMSE = Mini-Mental State Examination; WAIS = Wechsler Adult Intelligence Scale.

Fig 2). When probing the pairwise comparisons with 1-month follow-up, again there was no significant interaction for baseline versus 1-month follow-up ($F_{1,24} = 0.18$, $p = 0.68$, $d = 0.15$) or 1-month versus 3-month follow-up ($F_{1,24} = 2.99$, $p = 0.10$, $d = 0.62$).

RT change, calculated as baseline − 3-month follow-up, revealed an RT gain for the treatment group of 107 milliseconds for faces (baseline = 796 milliseconds, follow-up = 689 milliseconds) and 102 milliseconds for scenes (baseline = 643 milliseconds, follow-up = 541 milliseconds), whereas there was an RT loss for the placebo group of −91 milliseconds for faces (baseline = 625

milliseconds, follow-up = 714 milliseconds) and −93 milliseconds for scenes (baseline = 586 milliseconds, follow-up = 679 milliseconds). The change in RT for the treatment group was significantly different from that for the placebo group for faces ($t_{24} = 2.62$, $p < 0.02$, $d = 1.07$) and scenes ($t_{24} = 2.18$, $p < 0.04$, $d = 0.89$). Interestingly, these results revealed that the placebo group actually became slower 3 months later, presumably reflecting that MCI is a vulnerable population for further cognitive decline. Of note, RT on a simple discrimination task, used as a control task, was not different between the baseline and 3-month follow-up in the
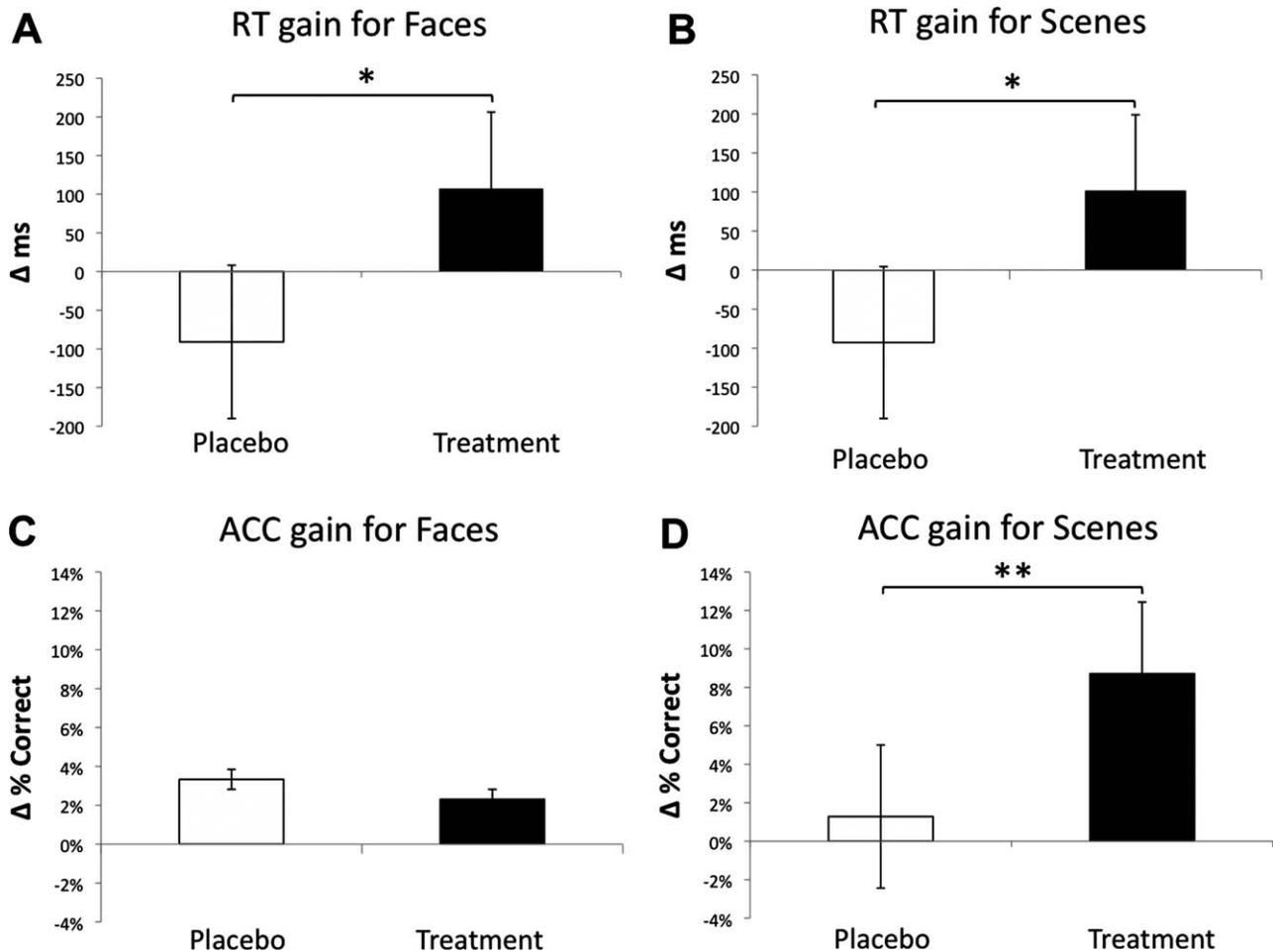
FIGURE 2: Response time (RT) and accuracy (ACC) change scores. Behavioral data show the difference between baseline and follow-up for RT and ACC in the 2 groups. Two-way repeated measures analysis of variance was used to assess group-by-visit interactions for (A, B) RT and (C, D) ACC for faces and scenes. Gain scores represent the behavioral improvement between baseline and follow-up. *Significant interaction ($p < 0.05$), **nonsignificant trend ($p < 0.10$). The error bars represent standard error of the mean.

treatment ($t_{12} = -0.78$, $p = 0.45$, $d = 0.45$) or placebo ($t_{12} = 0.65$, $p = 0.53$, $d = 0.38$) groups. Additionally, there were no significant RT differences between groups at baseline for any condition.

There were no significant 2 × 2 interactions for group by visit for ACC of face stimuli. There was a trend for a group-by-visit interaction for ACC of scene stimuli in the analysis of baseline versus 3-month follow-up ($F_{1,24} = 2.96$, $p = 0.10$, $d = 0.63$) and baseline versus 1-month follow-up ($F_{1,24} = 3.40$, $p = 0.08$, $d = 0.76$). The ACC gain (calculated as follow-up − baseline) suggested that the treatment group showed a trend to perform better after treatment. Similar to RT, there were no significant ACC differences between groups at baseline for any condition.

We next assessed within-group changes in performance over time in post hoc pairwise comparisons. For the treatment group, there was a significant improvement in

RT for faces ($t_{12} = 1.97$, $p = 0.04$, $d = 1.14$) and scenes ($t_{12} = 1.73$, $p = 0.05$, $d = 1.00$), in addition to a significant improvement in ACC for scenes ($t_{12} = -3.16$, $p = 0.004$, $d = -1.82$). There was no difference in ACC for faces. For the placebo group, there was a significant RT slowing for faces ($t_{12} = -1.73$, $p = 0.05$, $d = 1.00$). There was no significant change in RT for scenes or ACC for faces or scenes in the placebo group.

As a secondary RT analysis, we investigated the difference in RT across all participants at baseline for correctly remembered versus incorrectly remembered trials. The RT for correctly remembered trials was significantly faster than incorrectly remembered trials ($t_{25} = -8.23$, $p < 0.0001$, $d = 3.29$). This supports that RT reflects memory performance for the delayed recognition task.[48,49]

Overall, the treatment group showed significant enhancement in performance on 2 behavioral measures (RT for faces and RT for scenes), and the placebo group
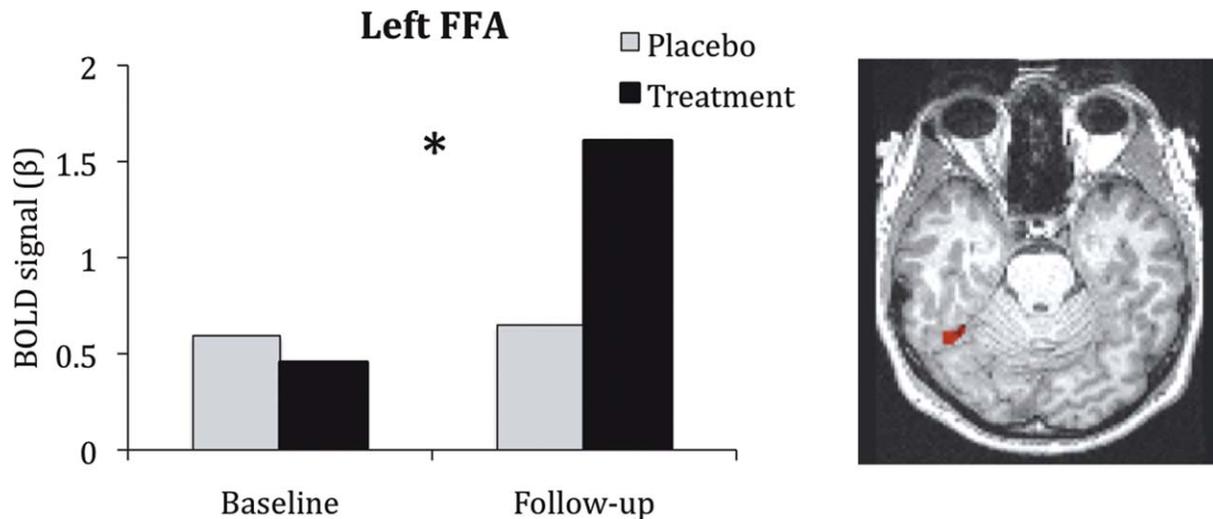
FIGURE 3: Increased activity in the left fusiform face area (FFA) region of interest (ROI) for face encoding. The blood oxygenation level–dependent (BOLD) signal in the left FFA ROI was defined by an independent localizer task. The left FFA showed a *significant group-by-visit interaction, in which the BOLD signal increased for the treatment group and remained constant in the placebo control group, relative to baseline. This ROI displayed on a representative native T1 image was used as a seed for functional connectivity analysis to explore network-based changes associated with acetylcholine upregulation.

showed a significant decrement on 1 behavioral measure (RT for faces). It is important to note that the RT gain in the treatment group was obtained with values corrected by an independent measure of RT in the discrimination control task (passive view); thus, the findings represent changes in cognitive-specific memory performance. Furthermore, there was no change in control task RT from baseline to follow-up in the treatment (or placebo) group, suggesting that treatment did not result in general motoric enhancements or arousal.

FFA and PPA BOLD signal

To investigate the neural effects associated with the significant behavioral changes, we assessed group-by-visit interactions for the FFA and PPA for baseline versus 3-month follow-up (herein, denoted as "baseline" and "follow-up," respectively). To account for physiological changes associated with bottom-up effects of cholinergic enhancement, we used BOLD measures during the stimulus-viewing period of the passive view condition as a control for the encoding-period of the face and scene memory conditions (eg, activity for face memory − activity for passive view). There were no main effects of group or visit on univariate activity in the FFA or PPA. We found a significant group-by-visit interaction for the left FFA ($F_{1,24} = 5.47$, $p < 0.03$, $d = 0.85$; Fig 3). The right FFA did not show a significant change associated with treatment ($F_{1,24} = 1.30$, $p = 0.27$, $d = 0.39$). In addition, the left and right PPA did not show a significant change associated with treatment ($F_{1,24} = 0.53$, $p = 0.48$, $d = 0.29$ and $F_{1,24} = 0.67$, $p = 0.42$, $d = 0.32$,

respectively). To guide the network connectivity analysis based on these findings, we subjected the left FFA ROI to functional connectivity analysis.

Functional Network Connectivity

Using the FFA as a seed region, we performed a ROI analysis in the prefrontal cortex, parietal lobe, and hippocampus using a 3-way ANOVA of group (treatment, placebo) × visit (baseline, follow-up) × condition (face memory [FM], passive view [PV]) to explore neural changes during the encode stage after treatment. Functional connectivity in these regions was analyzed if they survived correction for multiple comparisons and showed no group differences at the baseline visit. No regions in the parietal lobe survived permutation testing to correct for multiple comparisons in which there were no group differences at baseline.

For the prefrontal cortex analysis, the right inferior frontal junction (IFJ) showed a significant 3-way interaction ($F_{1,24} = 7.38$, $p = 0.01$, $d = 0.68$). To explore this 3-way interaction further, the FM and PV conditions were analyzed separately. There was a significant group-by-visit interaction for FM, such that functional connectivity increased in the treatment group after 3 months when compared to the placebo group ($F_{1,24} = 9.56$, $p < 0.005$, $d = 1.25$; Fig 4A). The group-by-visit interaction for PV was not significant ($F_{1,24} = 0.33$, $p = 0.57$, $d = 0.24$), nor were the main effects of group or visit significant, suggesting that the enhanced connectivity with the IFJ was specific to face memory processing.

For the hippocampal analysis, the right hippocampus showed a significant 3-way interaction ($F_{1,24} = 5.17$,
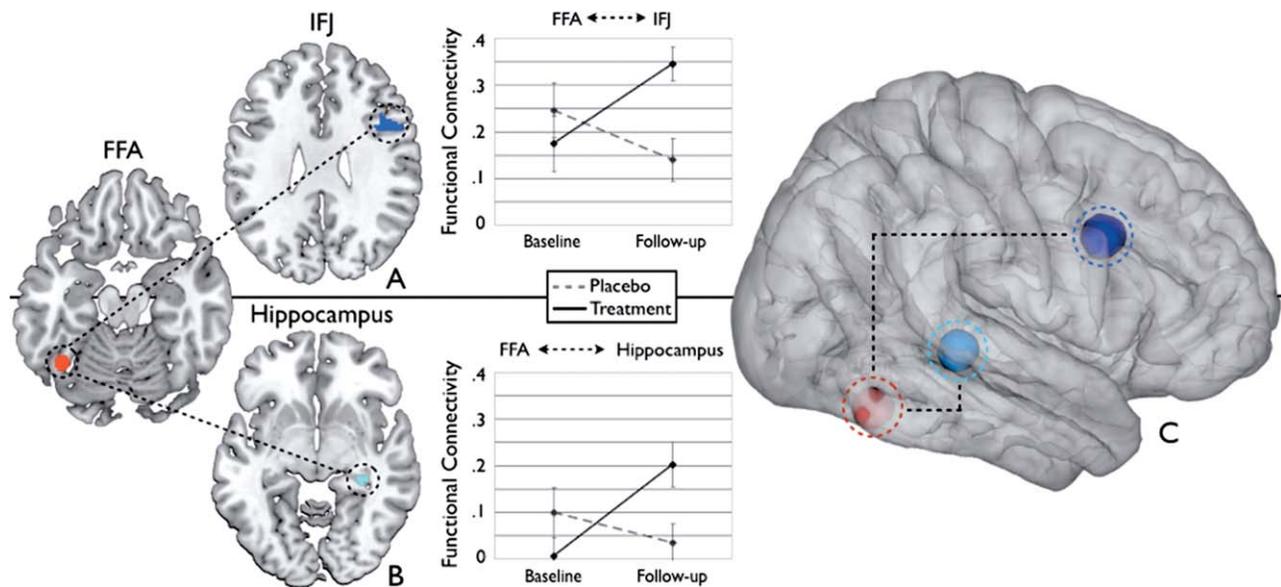
FIGURE 4: Enhanced network connectivity in the hippocampus and inferior frontal junction (IFJ) with fusiform face area (FFA). The FFA network connectivity to the hippocampus and IFJ revealed significant enhancements after treatment *(solid lines)* when compared to the placebo group *(dashed lines; p<0.05)*. These regions have been implicated in memory and cognitive control networks with known cholinergic projections from the medial septal nuclei and nucleus basalis of the basal forebrain, respectively. Increases in the functional connectivity of (A) FFA and IFJ and (B) FFA and hippocampus from baseline to follow-up in the treatment group are shown. (C) Schematic of the network connectivity of memory and attention systems with the FFA. The regions of interest are illustrated using 3-dimensional (3D) spheres around the peak voxel rendered onto a 3D brain.

$p = .03$, $d = 0.55$). To explore this further, the FM and PV conditions were analyzed separately. There was a significant group-by-visit interaction for FM, such that functional connectivity increased after treatment when compared to the placebo group ($F_{1,24} = 6.67$, $p < 0.02$, $d = 1.01$; see Fig 4B). The group-by-visit interaction for PV was not significant ($F_{1,24} = 0.59$, $p = 0.45$, $d = 0.31$), nor were the main effects of group or visit significant, also suggesting that the enhanced connectivity in the hippocampus was specific to face memory processing.

An M1 control region did not show a significant group-by-visit interaction ($F_{1,24} = 0.03$, $p = 0.86$, $d = 0.06$) or main effect of group ($F_{1,24} = 0.11$, $p = 0.74$, $d = 0.13$) or time ($F_{1,24} = 1.13$, $p = 0.26$, $d = 0.47$), further suggesting that the effects reported for FFA connectivity with the IFJ and hippocampus are task-specific and not a product of generalized global enhancement. Figure 4C illustrates the 3-dimensional (3D) projections of the IFJ, hippocampus, and FFA regions rendered on a 3D brain.

Although we found a significant interaction in the ANOVA for the behavioral and functional connectivity data analyzed separately, we also assessed a direct relationship between the 2 measures to strengthen our interpretation that the changes in functional connectivity were related to task performance. Across-participant

correlation analyses revealed that there was a significant relationship between the RT for faces and the functional connectivity of the FFA and hippocampus in the treatment group at the follow-up visit ($r = -0.68$, $p = 0.01$, $d = -1.85$), which was not found in the placebo group ($r = 0.16$, $p = 0.62$, $d = 0.32$; Fig 5). Both correlations were controlled for baseline RT for faces. These data suggest that the relationship between faster face RT and increased functional connectivity to the hippocampus is associated with cholinergic treatment.

Discussion

The goal of the present study was to elucidate the neurobiological changes associated with cholinergic modulation in older adults with mild memory impairment after a 3-month treatment period. Our findings revealed cognitive and functional network enhancements after donepezil treatment, which were not shown in the placebo control group. The treatment group had improved RT and enhanced functional network connectivity of the hippocampus and inferior frontal junction with the FFA. This result was specific to attentionally demanding face memory processing, as there was no observed effect when passively viewing the same stimuli without an overt memory task. The treatment group demonstrated a significant group-by-visit interaction and neurobehavioral correlation at the 3-month visit, whereas the placebo group did not
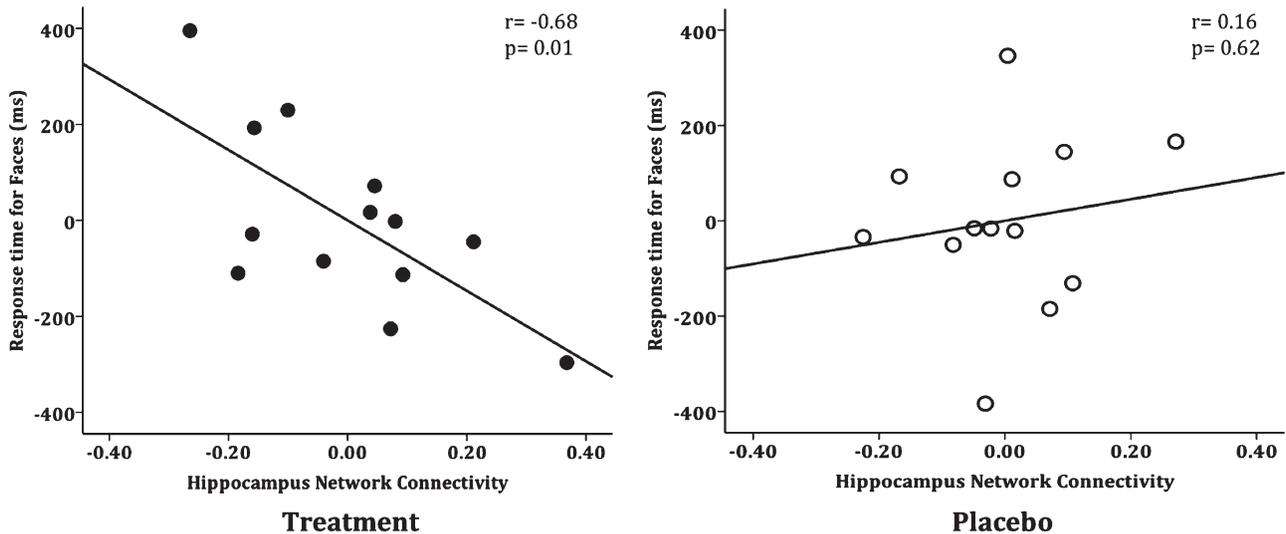
FIGURE 5: Enhanced functional connectivity of fusiform face area (FFA) and hippocampus predicts performance. The treatment group demonstrated a significant neurobehavioral relationship between the functional connectivity of the FFA and hippocampus and response time for faces at the follow-up visit ($r = -0.68$, $p = 0.01$; solid dots). The placebo control group did not show a significant relationship ($r = 0.16$, $p = 0.62$; open dots). Displayed partial regression plots are controlled for face response time at baseline. These data suggest that enhanced communication between the functionally connected regions of the FFA and hippocampus predicts better performance.

show a significant correlation. The placebo group demonstrated signs of cognitive decline on the memory task. This is the first study to show that ACh upregulation remediates functional network connectivity to the hippocampus relative to baseline function and that the reinstated function predicts improved delayed recognition performance after donepezil treatment.

Previous studies of the effect of cholinergic modulation in MCI patients have shown increased regional brain activity in several areas, including visual association cortex, frontal cortex, and the hippocampus.[10,13,50] Some studies identified a neural change after treatment in lieu of a cognitive effect,[11] reported a cognitive or neural benefit without a predictive relationship,[10] or produced significant within-group treatment effects without a placebo control.[13,51] The present study extends the existing body of literature on ACh upregulation in older adults with mild memory deficits by demonstrating functional network enhancements and associated cognitive benefits in a donepezil treatment group compared to a placebo control group over a 3-month period. Importantly, the placebo control group, which was comprised of older adults with mild memory deficits similar to the treatment group at baseline, actually demonstrated a slowing of RT on the delayed recognition task after 3 months. Thus, the treatment not only prevented decline in performance that was exhibited if there was no treatment, but also resulted in significantly faster responses on the task. This revealed the robust influence of ACh upregulation that could not have been demonstrated without a placebo

control group. The study design also allowed for investigation of goal-directed effects of ACh upregulation. Our findings showed both increased regional activity (ie, FFA) and enhanced functional connectivity (FFA to hippocampus, IFJ) during the task-relevant condition of face memory and not during the passive view condition. This is consistent with previous studies that documented the specificity of neural changes associated with task-relevant goals, such that attended stimuli resulted in increased activity, whereas passively viewed stimuli resulted in no change.[8]

The role of the hippocampus in face encoding has been shown in single-cell recordings and electroencephalography studies of nonhuman primates[52,53] and human functional neuroimaging studies.[54,55] The activity of the hippocampus during encoding differs in patients with memory deficits, such that individuals with early memory dysfunction have a hyperactive neural signal (ie, MCI), whereas those with more severe memory impairment have a hypoactive neural signal (ie, AD).[56,57] The role of the hyperactive signal is still under investigation, as some believe it is compensatory for degradation in task performance, whereas others view it as an indicator of neuronal dysfunction. A recent study demonstrated that reducing hippocampal activity to a control level using antiepileptic levetiracetam treatment improves task performance.[58] Our data support that reestablishing functional connectivity between the hippocampus and stimulus-selective visual cortex relates to improved cognition. A study by Goveas and colleagues also provides

interesting evidence for increased intrinsic (resting state) hippocampal connectivity with the prefrontal cortex in AD patients after 12 weeks of donepezil treatment, which correlated with improved cognition on the AD Assessment Scale cognitive subscale (ADAS-cog).[59] This study was open-label and did not have a placebo control group, so the possibility of a placebo or learning effect is unknown. Another study by this group also reported similar increases in intrinsic connectivity between the medial prefrontal cortex and middle and posterior cingulate gyri in AD patients, which again correlated with ADAS-cog.[60] Ricciardi and colleagues conducted a recent study examining the effects of physostigmine in healthy young adults during a task-on selective attention task.[61] They reported decreased connectivity between task-specific ventral visual regions and prefrontal and parietal regions. These findings are consistent with previous studies, which concluded that cholinergic upregulation in healthy populations results in decreased BOLD signal due to enhanced neural efficiency.[8] Together, these studies support the importance of understanding what neural mechanisms may be involved in ACh upregulation and highlight potential differences in at-risk populations with memory impairment.

The role of the cholinergic system in cognitive control tasks supported by regions of the middle frontal gyrus has been shown in animal studies on distraction and sustained attention.[1,62] In humans, the right IFJ has been implicated in similar cognitive control tasks of attentionally mediated perceptual and memory processing.[63] Strikingly, a study by Zanto and colleagues[44] demonstrated the causal role of IFJ in goal-directed perceptual processing, such that perturbation of the IFJ activity resulted in impaired task performance.

The inferior frontal junction receives projections from the nucleus basalis, whereas the hippocampus receives cholinergic innervations from the medial septal region of the basal forebrain. This anatomical division of basal forebrain projections to separate cortical regions may support distinct cognitive networks for memory and attention.[8] Our findings support this model, as we attribute the FFA–hippocampus network to face memory encoding and FFA–IFJ connectivity to cognitive control mechanisms of face encoding. Moreover, the recruitment of cognitive control regions for enhanced memory encoding has been suggested to combat performance decline or maintain task rules in the presence of distraction.[64]

Changes in cholinergic function may have an impact on the pathogenesis of age-associated or pathological memory impairment. Tonic increases in cholinergic signaling are implicated in supporting top-down control of attention, and attentional effort (for a review, see Hasselmo and Sarter[65]). In its role as a neuromodulator, ACh increases gain functions by causing postsynaptic changes in neuronal excitability, and coordinates the firing of groups of neurons.[66–69] The basal forebrain undergoes degeneration with age,[70] and degeneration of cholinergic neurons of the basal forebrain is among the earliest pathological events in AD.[71–76] Age-related cognitive impairment may be caused by loss of cholinergic modulation that normally supports downstream neuronal excitability and enhances synchronized activity across populations of neurons.

Possible hemodynamic confounds are a consistent concern in pharmaco-fMRI, as it is still unclear how perfusion influences functional neuroimaging measurements (ie, BOLD signal).[8] However, although donepezil can increase cerebral blood flow,[77] we believe it is unlikely that the effects we currently describe are a result of these confounds. Our critical predictions regarding ACh rely on condition $\times$ drug and region $\times$ drug interactions, not main effects.[78] The influence of donepezil on functional connectivity is also unlikely to arise from hemodynamic confounds because these measures rely on correlated activity and not overall activity changes. Furthermore, the lack of a treatment effect in our M1 control region supports that our conclusions are not likely due to global perfusion effects associated with ACh upregulation.

Additional longitudinal studies would be useful in examining the persistence of treatment benefits. Exploring the changes in brain network communication associated with treatment could be a sensitive approach to disease monitoring, treatment efficacy, or proof of concept preclinical trials. Longitudinal task fMRI is a potential tool for tracking cognitive and functional network enhancements associated with any therapeutic intervention.

Acknowledgment

Potential Conflicts of Interest

A.G.: consultancy, stock/stock options, travel expenses, Akili Interactive Labs; patents, "Enhancing Cognition in the Presence of Distraction and/or Interruption."

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