

# Donepezil Improves Episodic Memory in Young Individuals Vulnerable to the Effects of Sleep Deprivation

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**Study Objectives:** We investigated if donepezil, a long-acting orally administered cholinesterase inhibitor, would reduce episodic memory deficits associated with 24 h of sleep deprivation.

**Design:** Double-blind, placebo-controlled, crossover study involving 7 laboratory visits over 2 months. Participants underwent 4 functional MRI scans; 2 sessions (donepezil or placebo) followed a normal night's sleep, and 2 sessions followed a night of sleep deprivation.

**Setting:** The study took place in a research laboratory.

**Participants:** 26 young, healthy volunteers with no history of any sleep, psychiatric, or neurologic disorders.

**Interventions:** 5 mg of donepezil was taken once daily for approximately 17 days.

**Measurements and Results:** Subjects were scanned while performing a semantic judgment task and tested for word recognition outside the scanner 45 minutes later. Sleep deprivation increased the frequency of non-responses at encoding and impaired delayed recognition. No benefit of donepezil was evident when participants were well rested. When sleep deprived, individuals who showed greater performance

decline improved with donepezil, whereas more resistant individuals did not benefit. Accompanying these behavioral effects, there was corresponding modulation of task-related activation in functionally relevant brain regions. Brain regions identified in relation to donepezil-induced alteration in non-response rates could be distinguished from regions relating to improved recognition memory. This suggests that donepezil can improve delayed recognition in sleep-deprived persons by improving attention as well as enhancing memory encoding.

**Conclusions:** Donepezil reduced decline in recognition performance in individuals vulnerable to the effects of sleep deprivation. Additionally, our findings demonstrate the utility of combined fMRI–behavior evaluation in psychopharmacological studies.

**Keywords:** Sleep deprivation, cholinergic system, episodic memory, prefrontal cortex, parietal cortex, fusiform gyrus, fMRI

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SLEEP AND MEMORY ARE STRONGLY INTERLINKED. SLEEP IS VITAL TO MEMORY CONSOLIDATION<sup>1-3</sup> AND SLEEP DEPRIVATION RESULTS IN THE IMPAIRMENT OF short-term as well as long-term memory.<sup>4</sup> In theory, the impact of sleep deprivation on memory might be reduced by directly influencing its component processes, encoding, consolidation and retrieval, or indirectly by enhancing arousal and/or attention.

The best-known countermeasures, caffeine, amphetamines and modafinil, all boost arousal and vigilant attention<sup>5</sup> and to date, the more direct approach has not yielded much success. For example, while CX717, an ampakine, could theoretically improve memory through glutaminergic mechanisms, promising results obtained with primates<sup>6</sup> were not similarly realized in a human study.<sup>7</sup>

Here, we evaluated the enhancement of cholinergic transmission with the long acting cholinesterase inhibitor, donepezil, as a means of ameliorating memory deficits in sleep deprivation, using a combination of behavioral and functional magnetic resonance imaging (fMRI) measures. Augmenting cholinergic transmission could benefit cognition in sleep-deprived individuals via multiple mechanisms: promoting wakefulness,<sup>8</sup> through top-down increases in attention,<sup>9</sup> increasing the signal-to-noise

ratio of processing within visual sensory cortex,<sup>10,11</sup> and enhancing LTP in hippocampal circuits.<sup>12</sup> Additionally, cholinesterase inhibitors secondarily influence dopaminergic and noradrenergic neurotransmission.<sup>13</sup> fMRI provides a noninvasive means of identifying the neuroanatomical locus of drug action that could help discern the functional relevance of these putative mechanisms.<sup>14</sup>

The neurobehavioral effects of manipulating cholinergic transmission in the context of non-sleep deprived, healthy adults, performing tasks tapping attention and memory, have been well characterized in several functional imaging studies.<sup>15-21</sup> In these studies, increasing cholinergic transmission generally improved attention and *short-term memory* whereas antagonists like scopolamine induced decline in long-term, episodic memory. Behavior-only studies using donepezil have yielded positive<sup>22</sup> and negative<sup>23</sup> results.

Reflecting the multiple mechanisms through which acetylcholine can modulate cognition across different tasks,<sup>24</sup> the locus of drug effect(s) has varied across different studies. Drug induced modulation of neural activity has been reported in top-down control regions such as the frontal<sup>17</sup> and parietal lobes,<sup>15,20</sup> regions involved in visual processing such as the extrastriate visual cortex<sup>17,25</sup> and the fusiform cortex<sup>19</sup> as well as brain regions involved in memory encoding such as the hippocampal formation<sup>19,21</sup> and the lateral prefrontal cortex.<sup>19</sup>

Another source of the varied results in these psychopharmacological-imaging studies is the point along the neurotransmitter signaling continuum that a subject lies. Recent studies involving cholinergic agents in elderly volunteers,<sup>15,23</sup> sleep-deprived volunteers,<sup>25</sup> as well as a pharmacogenetic study in

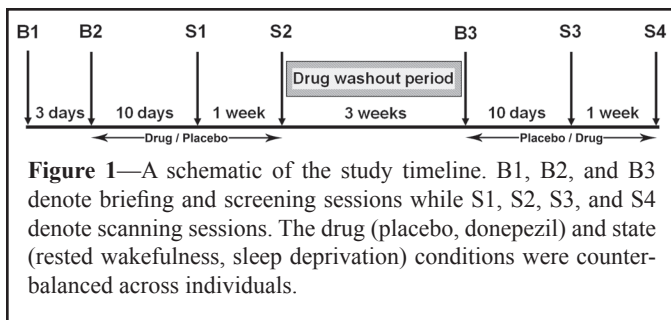
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volving modafinil in sleep-deprived persons,<sup>26</sup> have reinforced the notion that there exists a bell-shaped response curve to neurotransmitter augmentation.<sup>27,28</sup> This is particularly relevant to the present study because the cognitive deficits experienced following sleep deprivation reportedly show trait-like inter-individual variation.<sup>29,30</sup> One can reasonably postulate that inter-individual variation in reduced cholinergic drive might mediate vulnerability to sleep deprivation and that there would be a corresponding range of response to exogenous cholinergic augmentation (Supplementary Figure 1).

In this report, we evaluated two hypotheses. Firstly, in accordance with a sister study<sup>25</sup> that evaluated visual short-term memory, we anticipated that donepezil would benefit episodic memory performance in persons vulnerable to the effects of sleep deprivation, but not otherwise. Secondly, we predicted that we would find correlation between donepezil-induced modulation of brain activation and memory improvement in left lateral prefrontal cortex, a region that participates in successful word encoding. This would be in line with the thesis that while cholinergic neurons have widespread projections in the cerebral cortex, modulation in brain activation can occur in a task-specific manner.<sup>14,31</sup>

In consideration of these goals, we conducted a double-blind, placebo-controlled, cross-over functional imaging study (Figure 1) that involved 26 healthy young adult participants. In this within-subject design, volunteers were scanned 4 times, twice following a normal night's sleep and twice after 24 hours of total SLEEP DEPRIVATION. During scanning, participants encoded words while they performed a semantic judgment task. A 5 mg dose of donepezil was compared to placebo. Delayed recognition memory (reflecting episodic memory), non-responses (reflecting attentional lapses), and judgment accuracy at encoding were the main behavioral measures of interest. Task-related activation associated with successfully encoded words was the main imaging variable of interest. As we studied the same subjects in both the present and previous report, we minimized the likelihood that between-subject anatomical variation would confound the localization of drug-induced fMRI signal modulation.

## METHODS

### Participants

Forty-three healthy young adults were recruited through web-based advertisements and were screened by questionnaire and an interview. Prospective participants had to: (1) be right-handed, (2) be between 18 and 35 years of age, (3) have

habitual good sleeping habits (sleeping  $\geq 6.5$  h each night for the past month), (4) have no history of sleep disorder, (5) have no history of any psychiatric or neurologic disorders, (6) have normal color vision, (7) drink  $< 3$  cups of coffee a day, (8) not smoke cigarettes, and (9) score  $\leq 22$  on a modified Morningness-Eveningness scale.<sup>32</sup> Only 12.2% of our potential subjects were excluded on the basis of this criteria (estimated based on 500 individuals who completed our internet-based questionnaire; 1.2% were definitely morning types and the remaining 11% were moderately morning types). Filtering of subjects on the basis of chronotype was intended to reduce this as a source of variance in our study. As recently shown, chronotype can influence fMRI findings.<sup>33</sup> The sleeping habits of qualifying participants were monitored using wrist actigraphy and only those who had good sleep habits (i.e., they usually slept no later than 01:00 and woke no later than 09:00) were eligible for brain imaging. All participants were screened for color blindness, and cleared blood and urine biochemistry tests prior to enrollment.

The final sample consisted of 26 participants (13 males, mean age = 22.19 years, SD = 1.02 years). Fifteen did not complete the study: 4 were medically unsuitable; 3 were unsuitable for MR scanning (claustrophobia, large braces); 4 experienced gastrointestinal side-effects; and 4 were unable to maintain a regular sleep-wake schedule. Technical error caused the loss of data for another subject, and one subject was rejected because of an extremely low rate of correct rejects during delayed recognition (range = 0 to 3.29%).

Participants were financially compensated for their time.

### Design and Procedure

The experimental protocol was approved by the Singapore General Hospital IRB. This double-blind, placebo-controlled, crossover study involved 7 visits to the laboratory over a period of about 2 months (Figure 1). The experiment was conducted minutes after experiments that evaluated visual short-term memory and visual perception.<sup>25</sup>

Three of the visits were briefing sessions. The 4 remaining sessions were test/scanning sessions. Two of these test sessions were conducted following a night of normal sleep (or rested wakefulness) and the remaining 2 sessions followed a night of sleep deprivation. Scanning always took place at fixed times for both rested wakefulness and sleep deprivation sessions. The order of the scanning sessions (rested wakefulness followed by sleep deprivation and vice versa) was counterbalanced across all subjects. A pharmacist who was not involved in data collection or analysis dispensed the tablets (5 mg donepezil/matched placebo) in a counterbalanced manner across all subjects. Compliance to the drug schedule was checked daily by telephone and further verified with tablet counts that were conducted before every scanning session.

During Briefing Session 1 (B1), participants were provided with detailed information about the aims and requirements of the study and gave informed consent before undergoing a physical examination and blood tests.

Briefing Session 2 (B2) took place approximately 3 days after B1 and involved subjects who passed the medical and lab test screens. Participants' sleeping patterns were verified by sleep diary and wrist actigraphy. At the end of this session, par-

ticipants were given tablets (either 5 mg donepezil or placebo) with instructions to take one a day at 09:00 and to record this. Participants took the tablets daily for an average of 10 days prior to their first scan session and continued taking the same tablets for another week until the end of the second scanning session. There was a 3-week washout period between each drug phase.

Briefing Session 3 (B3), post washout, took place about 3 weeks following the second scanning session. Participants collected tablets and their compliance with the sleep schedule was verified. During the second drug phase, subjects took either donepezil or placebo daily for an average of 10 days prior to the third scan and continued taking the same tablets daily for a week until their fourth scan.

### **Rested Wakefulness Sessions**

Subjects arrived at the lab at approximately 07:45. Adequate sleep was verified by checking the volunteer's sleep diary and wrist actigraph. Compliance to the medication schedule was checked. Subjects were questioned on their health and well-being and for any possible side effects from the tablets. Intake of other medications was also documented. All participants verified that they had not smoked, consumed any medications, stimulants, alcohol or caffeine for at least 24 h prior to scanning. Subjects also completed a 10-min trial on the psychomotor vigilance task (PVT).<sup>34,35</sup>

Participants in this study also took part in the experiment that evaluated visual short-term memory and visual perception. After that experiment was completed, within the same session, volunteers were taken out of the scanner and took a 10-min break before participating in the current study. Prior to re-entering the scanner, they practiced the word classification (encoding) task. Subjects were informed that there would be a delayed recognition component but were instructed to focus on classifying the words. Self-ratings of sleepiness were taken using the Karolinska Sleepiness Scale (KSS) prior to scanning, after each in-scanner run and prior to the recognition component. Scanning for this task took place at approximately 09:00.

### **Sleep Deprivation Sessions**

Subjects were required to report to the laboratory no later than 19:00 on the test night. Sleep habits, compliance with tablet intake, intake of other medications, possible side effects, and general health were checked as described for the rested wakefulness session. Participants were monitored throughout the night and were only allowed to engage in non-strenuous activities such as reading, working on a computer and conversing. Every hour from 20:00 to 05:00, participants also completed a 10-min PVT trial as described earlier. Subjects verified that they had not taken any medications, stimulants, alcohol and caffeine for 24 h prior to scanning. Practice trials on the in-scanner task and the KSS were administered in the same manner as described for the rested wakefulness session. Scanning for the episodic memory task took place at approximately 06:30, around the circadian nadir for most persons. While this time is not identical to the one used during rested wakefulness scans, most of the effects of interest in this study accrue from extended wakeful-

ness rather than circadian phase. Further, carefully conducted studies have shown that the difference in sustained attention performance between 06:00 and 09:00 after a night of sleep deprivation are small.<sup>36</sup> Finally, we chose this time because most vehicular accidents following SLEEP DEPRIVATION occur most frequently at 02:00 and 06:00.<sup>37</sup>

### **Experimental Task**

Participants were scanned while performing a semantic judgment task in an event-related fMRI experiment. Recognition memory was tested out of scanner 45 min after completion of scanning.

The test set comprised 720 English, concrete nouns obtained from the MRC Psycholinguistic Database ([http://www.psy.uwa.edu.au/mrcdatabase/uwa\\_mrc.htm](http://www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm)). Ninety target words were used at each testing session. There were equal numbers of low, medium, and high frequency words. These words were matched for concreteness, familiarity, and imageability across all 4 scanning sessions. An additional set of 90 new words was used as foils during the delayed recognition component of the task. Each word was used only once throughout the experiment. Experience with this task and with this word database in our laboratory has been previously reported.<sup>38-40</sup>

At encoding, participants decided if a presented word depicted a living or non-living entity, and responded by pressing a "yes" or "no" button. Each word appeared for a maximum of 2 s and was replaced with a fixation cross immediately after a response. Stimulus onset asynchronies (the time interval between the onset of successive stimuli) of 2.5, 5, or 7.5 s were used. Participants had up to 2.5 s to respond on each trial. There were 2 encoding runs, and each encoding run involved the acquisition of 101 functional scans. Ninety different words were presented across the 2 runs, with 45 words in each run. Performance was continuously monitored, and participants were verbally prompted if they failed to respond to 2 consecutive words.

The delayed recognition component of the task was conducted outside the scanner approximately 45 min after encoding. Participants were shown 90 target words and 90 foils and asked to respond according to 3 response categories: (1) confident that the word was old (seen previously during encoding), (2) thought the word was old, but not confident, and (3) that the word was new (not seen previously during encoding).<sup>41,42</sup>

### **Imaging Procedure**

Stimuli were projected onto a screen using a LCD projector (Epson EMP 7250, Epson Corp, Japan) and viewed through a rearview mirror. Participants responded using a button box held in the right hand. A bite bar and foam padding were used to reduce head motion. Images were acquired on a 3T Siemens Allegra system (Siemens, Erlangen, Germany). A single-shot gradient-echo, EPI sequence was used (TR: 2500 ms; TE: 30 ms; flip angle 90°; FOV: 192 × 192 mm; Matrix: 64 × 64). Thirty-two oblique axial slices (3-mm thick; 0.3-mm inter-slice gap) approximately parallel to the AC-PC line were acquired. High-resolution T1 coplanar anatomical images were also obtained. For the purpose of image display on Talairach space,

**Table 1**—Classification Accuracy During Encoding According to State, Drug and Recognition Success (N = 26)

	Non-responses (%)	Classification Accuracy (%)			
		Overall	HC Hits	LC Hits	Miss
Placebo					
RWP	1.41 (2.05)	88.45 (0.06)	88.93 (6.46)	93.57 (7.73)	86.68 (15.26)
SDP	9.62 (9.60)	91.03 (6.80)	92.82 (5.52)	88.82 (11.54)	83.33 (20.66)
Donepezil					
RWD	2.22 (3.85)	91.81 (6.85)	92.68 (5.30)	88.79 (22.71)	87.11 (17.16)
SDD	8.50 (12.09)	86.78 (9.03)	87.83 (8.38)	81.58 (23.56)	87.41 (15.00)

Percentage of non-responses was computed as a percentage of all trials during encoding (i.e., 90). Trials on which subjects made advances (i.e., RT < 100 ms) were also classified as non-responses. Overall classification accuracy was computed as a percentage of trials to which subjects made a valid response during encoding (i.e., RT ≥ 100 ms). Classification accuracy for hits and misses was computed as a percentage of trials to which subjects made valid responses of that category during recognition. Standard deviations are reported in parentheses. RWP: rested wakefulness placebo; RWD: rested wakefulness donepezil; SDP: sleep deprivation placebo; SDD: sleep deprivation donepezil; HC: high confidence; LC: low confidence.

further 3D high-resolution anatomical reference images were acquired using a T1-weighted MPRAGE sequence.

### Data Analysis

#### Behavioral data

Number of non-responses, accuracy of classification and response time were measured during encoding (Table 1). Accuracy of classification was assessed on trials where subjects made a valid response (reaction time ≥ 100 ms). Reaction time was averaged across all valid trials.

Delayed recognition was indexed using corrected recognition (Hit Rate - False Alarm Rate).<sup>41,43,44</sup> *Only trials responded to during encoding were analyzed for delayed recognition.* All trials with response times < 100 ms were considered invalid. Corrected recognition was determined using only high confidence responses.<sup>41,42</sup> Such responses discriminated previously presented words from foils ( $P < 0.001$  for all test sessions) whereas with low confidence responses, there were more false alarms than hits in all 4 conditions (Table 2).  $A^2$ , a nonparametric measure of recognition memory<sup>45</sup> is another commonly used measure of delayed recognition. The 2 measures correlated highly within each test session ( $r = 0.96$  to  $0.99$ ), and we arbitrarily used the former metric.

The effects of state and drug on behavior (encoding and recognition) were explored using 2 (state: rested wakefulness, sleep deprivation) by 2 (drug: placebo, donepezil) repeated-measures ANOVA conducted using SPSS 13.0 (SPSS, Chicago, IL). (RWP: rested wakefulness placebo; RWD: rested wakefulness donepezil; SDP: sleep deprivation placebo; SDD: sleep deprivation donepezil). To determine if vulnerability to sleep deprivation affected response to donepezil, participants were divided into 2 groups (median split), according to how their performance declined following sleep deprivation when unmedicated (i.e., SDP-RWP). The ANOVA described above was repeated with vulnerability as a between-subjects factor. This analysis was performed separately for non-responses and correctly recognized words so that it would be possible to determine how lapses of attention and episodic memory might be affected differently by sleep deprivation.

### Imaging Data

Functional images were preprocessed and analyzed using Brain Voyager QX version 1.10.3 (Brain Innovation, Maastricht, The Netherlands). Data preprocessing included correcting for inter-slice timing differences using trilinear sinc interpolation, linear trend removal, and temporal high-pass filtering of period 83 s to remove low frequency non-linear drifts of 3 or fewer cycles per run. Three-dimensional rigid-body motion correction across runs was performed using the first image of the second functional run as the reference image. Spatial smoothing was performed using an 8-mm Gaussian kernel (full-width at half-maximum). Functional slices were co-registered to the MPRAGE anatomical volume and transformed into Talairach space.

Group statistical maps were obtained using a 2-level, multi-subject general linear model (GLM) approach. At the first level, the stimulus-related BOLD response was estimated for every individual using a finite-impulse-response (FIR) model with 35 predictors, 7 predictors for each of 5 conditions. The first 4 conditions (RWP, RWD, SDP, SDD) included only valid, high confidence hit trials. This was to avoid taking into consideration trials where a volunteer could have been asleep. The last condition contained all other events (i.e., low confidence hits, misses, and non-response trials).

To account for baseline drifts across runs and between experimental sessions, z-transformation of the signal time-courses for each run was performed. The obtained  $\beta$  values for each predictor in every individual served as the input for a second-level, random effects analysis.

Task-relevant regions were identified from voxels that showed significant signal change relative to baseline, for both RWP and RWD conditions (conjunction of 2  $t$ -contrasts). Voxel-wise  $\beta$  values were subjected to a 2 (state) by 2 (drug), within-subject ANOVA to identify regions that showed main effects of state, drug and their interaction. These analyses involved time-points around the empirically determined peak of activation (7.5 and 10 s).

To control for type I error, voxels were processed using an iterative cluster size thresholding procedure<sup>46</sup> that considered the spatial smoothness of functional imaging data when generating activation maps based on a corrected cluster threshold ( $P < 0.05$ ). This still yielded voxels above threshold and addition-

**Table 2**—Delayed Recognition Performance According to State, Drug and Recognition Success (N = 26)

	Delayed Recognition (%)						CR
	Targets			Lures			
	Hhits	Lhits	Miss	HFA	LFA	Crej	
Placebo							
RWP	75.26 (11.14)	9.14 (9.99)	15.60 (10.36)	12.17 (12.16)	21.17 (18.43)	66.66 (22.71)	0.63 (0.16)
SDP	64.68 (18.44)	15.05 (14.21)	20.27 (16.07)	15.99 (12.57)	24.52 (19.61)	59.48 (22.44)	0.49 (0.19)
Donepezil							
RWD	75.34 (13.13)	10.33 (11.72)	14.33 (11.81)	12.02 (10.86)	22.70 (21.29)	65.28 (26.13)	0.63 (0.17)
SDD	68.55 (17.65)	12.50 (12.25)	18.94 (15.91)	15.88 (9.22)	24.22 (19.95)	59.89 (22.46)	0.53 (0.17)

Ninety targets and 90 lures were present delayed recognition. However, only words that were responded to during encoding were treated as valid trials. Performance in each condition was computed as a percentage of the number of valid trials. Standard deviations are reported in parentheses. RWP: rested wakefulness placebo; RWP: rested wakefulness placebo; RWD: rested wakefulness donepezil; SDP: sleep deprivation placebo; SDD: sleep deprivation donepezil; Hhits: high confidence hits; Lhits: low confidence hits; Crej: correct rejections; NR: non-responses; HFA: high confidence false alarms; LFA: low confidence false alarms; CR: corrected recognition (high confidence hit rate – high confidence false alarm rate).

ally, a voxel level threshold of  $P < 0.0001$  (uncorrected) for  $t$  maps and  $P < 0.005$  (uncorrected) for  $F$  maps was applied.

As no region showed a main effect of drug, we performed region-of-interest (ROI) based analyses on areas that showed significant task-related activation in both RWP and RWD conditions. We examined activation magnitude in these ROI for significant correlations between state and drug-driven changes in behavior and the corresponding changes in activation. Only correlations that remained significant at  $P < 0.05$  after the removal of influential outliers ( $n = 1$ ) was reported (i.e.,  $N = 25$ ).

## RESULTS

### Behavioral Findings

#### Encoding

As might be expected, we observed very few *non-responses* during encoding at rested wakefulness. These increased significantly following sleep deprivation,  $F_{1,25} = 17.01$ ,  $P < 0.001$  (Table 1). As observed with the same subjects previously,<sup>25</sup> there was neither a main effect of drug,  $F_{1,25} = 0.02$ ,  $P = 0.88$ , nor an interaction between state and drug,  $F_{1,25} = 1.91$ ,  $P = 0.18$ .

Critically, when vulnerability was included as a between-subjects factor (vulnerability to sleep deprivation here refers to an increase in non-responses), there was a significant interaction between state, drug, and vulnerability,  $F_{1,24} = 11.60$ ,  $P = 0.002$  (Figure 2A). This interaction was driven by the number of non-responses following sleep deprivation,  $F_{1,24} = 8.73$ ,  $P = 0.007$ , but not at rested wakefulness,  $F_{1,24} = 0.39$ ,  $P = 0.54$ . Paired  $t$ -tests showed that donepezil reduced non-responses in sleep-deprived subjects who would otherwise deteriorate if untreated,  $t_{12} = 3.79$ ,  $P = 0.003$ . This benefit was not present in the more sleep deprivation resistant half of the subjects,  $t_{12} = 1.26$ ,  $P = 0.23$  (Figure 2A). Neither subgroup showed treatment effects at rested wakefulness (smaller  $P = 0.17$ ).

Accuracy at encoding was not significantly reduced following sleep deprivation,  $F_{1,25} = 1.97$ ,  $P = 0.17$  (Table 1). Hence, when volunteers were not lapsing, they seemed to perform the task accurately. There was no main effect of drug,  $F_{1,25} = 0.20$ ,  $P = 0.66$ .

Reaction time increased following sleep deprivation,  $F_{1,25} = 26.12$ ,  $P < 0.001$  (Supplementary Table 1). There was no significant effect of drug or interaction between state by drug (smaller  $P = 0.73$ ). No additional effects emerged when vulnerability was added as a between-subjects factor.

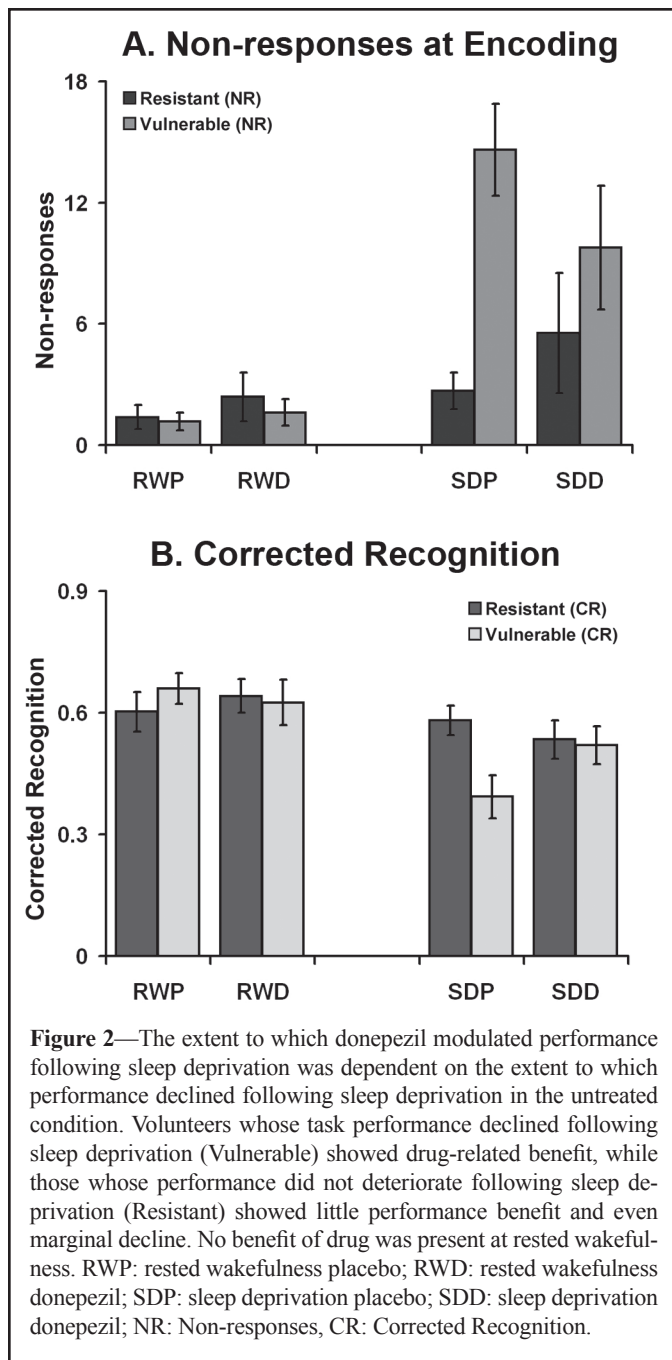
#### Recognition

Sleep deprivation resulted in significant decline in delayed recognition,  $F_{1,25} = 31.24$ ,  $P < 0.001$  (Table 2). There was no main effect of drug  $F_{1,25} = 0.74$ ,  $P = 0.40$ , and no interaction between state and drug,  $F_{1,25} = 0.82$ ,  $P = 0.37$ .

When vulnerability (defined as the drop in corrected recognition following sleep deprivation) was included as a between-subjects factor, there were significant interactions between state and vulnerability,  $F_{1,24} = 10.09$ ,  $P = 0.004$  and between state, drug, and group,  $F_{1,24} = 13.10$ ,  $P = 0.001$  (Figure 2B). Separate ANOVA for each state revealed significant interaction between drug and vulnerability in the sleep-deprived condition,  $F_{1,24} = 6.16$ ,  $P = 0.02$ , but not at rested wakefulness,  $F_{1,24} = 2.34$ ,  $P = 0.14$ . Post hoc  $t$  tests showed that, as was the case for non-responses, sleep deprivation vulnerable subjects showed improved recognition when on donepezil following sleep deprivation,  $t_{12} = 3.50$ ,  $P = 0.004$ , whereas the sleep deprivation resistant half did not benefit,  $t_{12} = 0.78$ ,  $P = 0.45$ .

#### Dissociation of State and Drug Effects of Donepezil

State-related shifts in non-response and corrected recognition scores across subjects in the untreated condition (i.e., RWP-SDP) were not correlated ( $r = -0.32$ ,  $P = 0.11$ ). However, donepezil-mediated shifts in the corresponding scores in the sleep-deprived condition (i.e., SDP-SDD) were significantly correlated even after the removal of an influential outlier ( $r = -0.46$ ,  $P < 0.02$ , for  $N = 25$ ). These findings suggest that sleep deprivation can have separable effects on lapses in attention (non-responses) and episodic memory (corrected recognition). However, in the context of sleep deprivation, donepezil affects both performance measures to a similar extent in any given individual.



### Relationship Between Subjective Sleepiness and Performance

Subjective sleepiness was significantly higher in sleep-deprived subjects at both encoding,  $F_{1,25} = 224.60$ ,  $P < 0.001$ , and delayed recognition,  $F_{1,24} = 142.45$ ,  $P < 0.001$  (Supplementary Table 2) but there were no significant effects of drug. There were no significant correlations between the change in KSS scores and changes in either behavioral measure (non-responses or corrected recognition) across state (i.e., RWP-SDP; smallest  $P = 0.14$ ). There were also no significant correlations between these variables following donepezil treatment in the sleep deprivation condition (i.e., SDD-SDP; smallest  $P = 0.15$ ).

Thus increases in subjective sleepiness did not correlate with performance change, regardless of whether a subject was on drug or placebo. Hence, while donepezil may modify cognitive

performance, it does not appear to influence subjective feelings of sleepiness.<sup>25</sup>

### Neuroimaging Findings

#### Areas Activated for High Confidence Hits

Following a normal night of sleep, high confidence hits in both rested wakefulness conditions elicited activation of the inferior frontal gyrus, pre-supplementary motor area, inferior and superior parietal cortex (all left hemisphere predominant). The thalamus, fusiform gyrus, and ventral occipital cortex were activated bilaterally. Additionally, there was significant deactivation in the posterior cingulate/precuneus bilaterally and in the right temporal parietal junction (Figure 3, Supplementary Table 3). These findings concur with the results of prior fMRI studies on episodic memory.<sup>39,41,42,47-49</sup>

#### State-Related Change in Activation

Sleep deprivation attenuated task-related activation within the left inferior frontal gyrus, insula, inferior temporal region, intraparietal sulcus and the fusiform gyri bilaterally (Figures 4 and 5, Supplementary Table 4).

#### Relationship Between Sleep Deprivation Related Increase in Non-Responses and Activation in Areas Associated with High Confidence Hits

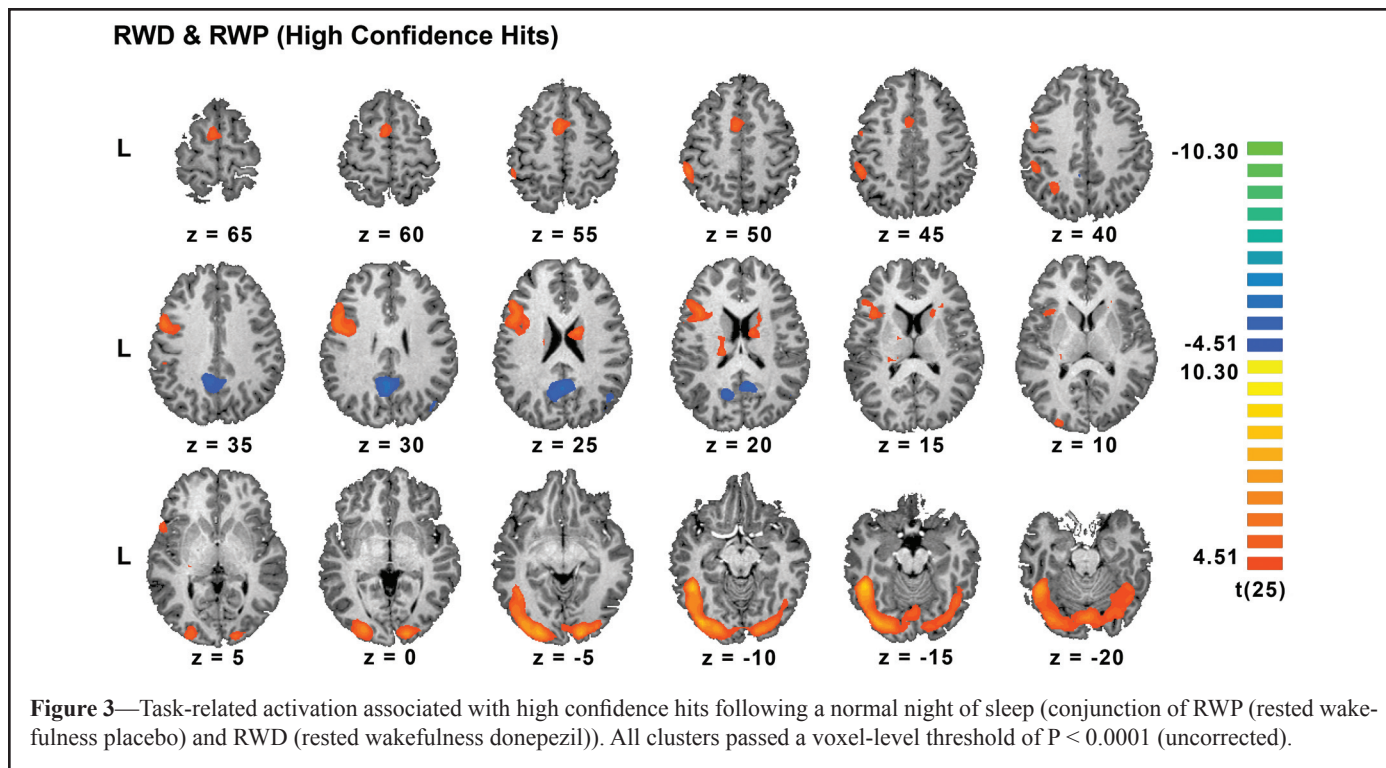
Previous studies suggest that cognitive decline following sleep deprivation may be contributed by processes that also increase lapses in attention. This led us to examine the relationship between sleep deprivation induced increase (SDP-RWP) in non-response rates and activation magnitude in regions associated with high confidence hits. We found significant correlation between behavioral and imaging metrics in the left middle and inferior frontal gyrus, the intraparietal sulcus, bilateral fusiform gyrus, and the pre-supplementary motor area (Figure 5).

#### Donepezil Did Not Modulate Brain Activation in Every Sleep-Deprived Subject

In concert with the behavioral findings, ANOVA revealed no main effect of drug on hit-related brain activation. Additionally, the influence of donepezil in each state evaluated by paired comparisons (RWD vs. RWP as well as SDD vs. SDP) also yielded null results. These findings raised 2 possibilities. Either there was no relationship between drug effect and task-related brain activation, or the relationship was masked by inter-individual responses such that an increase in activation in donepezil responders was negated by decreased activation in non-responders.

#### Donepezil Modulated Activation According to How a Subject Performed When Sleep Deprived

To test this, we evaluated the correlation between activation change associated with the SDD-SDP contrast and corresponding changes in behavior. This analysis was conducted separate-



ly for non-responses at encoding and corrected recognition. The analyses were performed on regions known to be involved in successful encoding after a normal night of sleep to ensure that they would be functionally meaningful. Further, in analyzing only successful encoding trials, we reduced the likelihood of including trials where the volunteer was momentarily asleep.

### Non-Responses

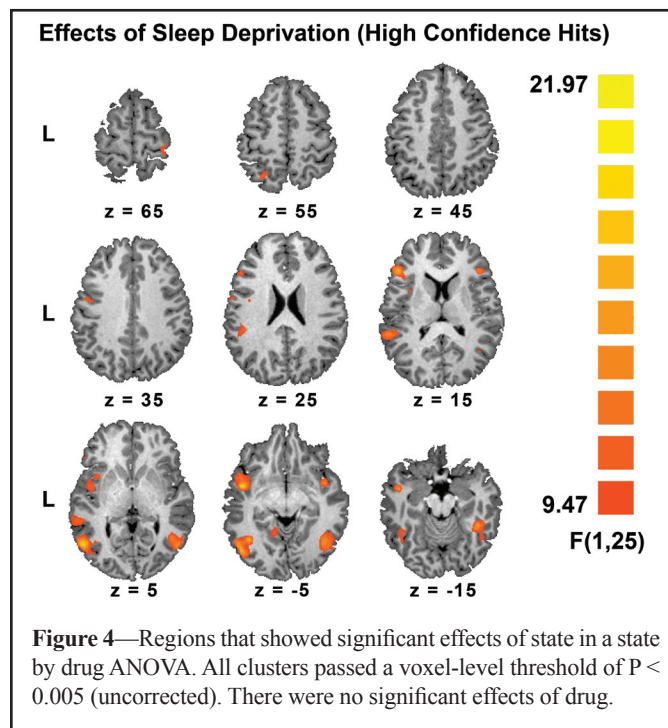
Following the removal of a significant outlier (i.e.,  $N = 25$ ), we found significant correlations between donepezil-induced changes (SDD-SDP) in non-responses and donepezil-related alterations in activation within the left superior parietal cortex (Figure 6A), right fusiform gyrus (Figure 6B), left fusiform gyrus and left ventral occipital cortex.

### Corrected Recognition

Corresponding significant associations between drug-related improvements in corrected recognition were found in the left middle and inferior frontal gyri (Figure 7A and B). These regions overlap with regions that show a subsequent memory effect in a fixed effects analysis (Supplementary Figure 2). Activation of the right fusiform (Figure 7C) also correlated with donepezil-mediated changes in corrected recognition. There was a marginal, nonsignificant correlation with the left superior parietal ROI mentioned above ( $r = 0.37$ ,  $P = 0.07$ ).

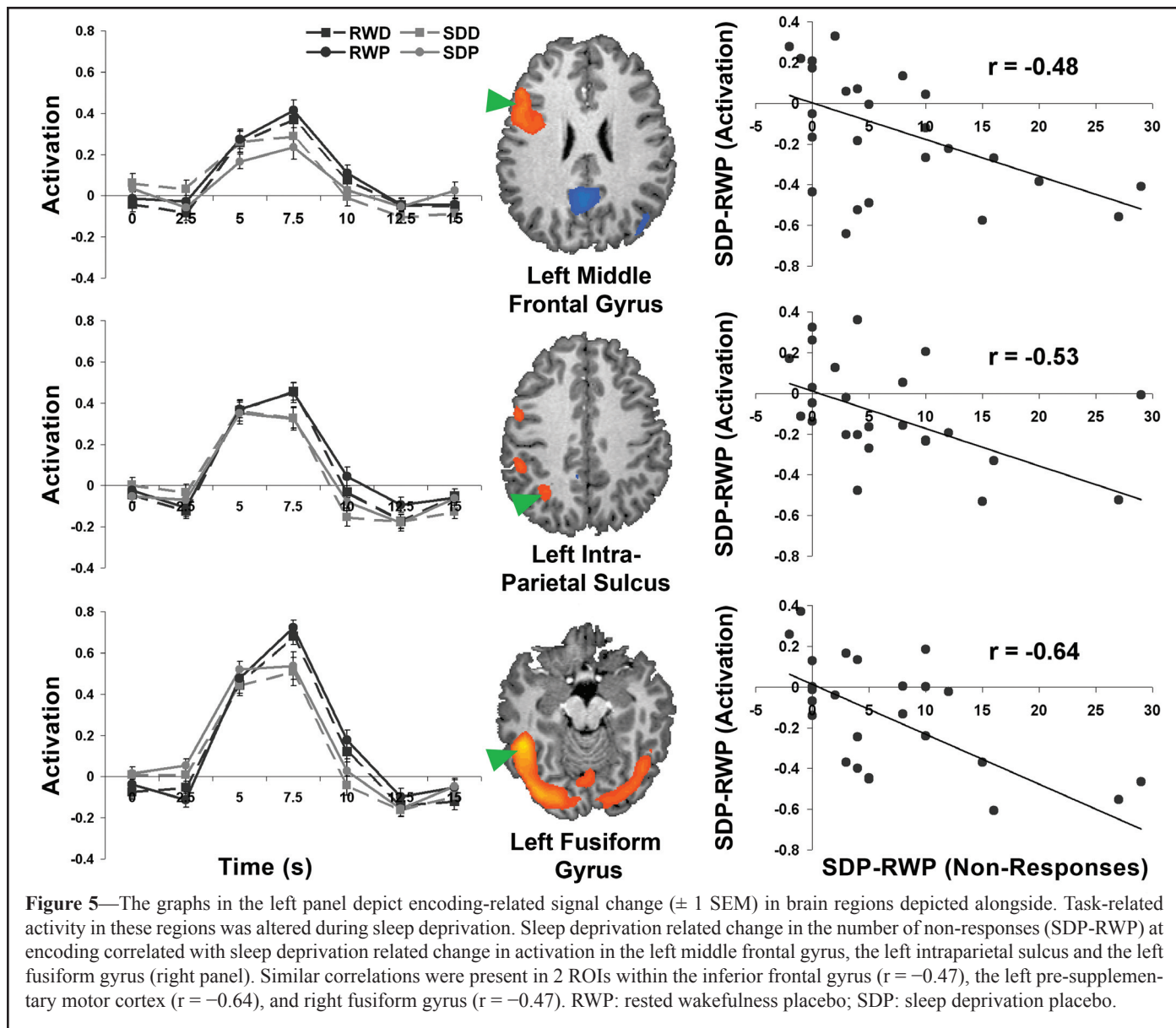
### Absence of Effects in RW

Similar analyses were conducted for possible donepezil-related alterations in activation at RW (RWD-RWP). None of the aforesaid regions showed significant behavior/ activation correlations of the type described for the sleep deprived state.



## DISCUSSION

There were three main findings in this present study. The first was that donepezil attenuated decline in verbal episodic memory in accordance with an individual's vulnerability to cognitive impairment following sleep deprivation. Donepezil did not alter episodic memory following a night of habitual sleep. Secondly, treatment-related changes in behavior correlated with corresponding alterations in encoding-related activity within task-relevant regions, suggesting that fMRI can be a function-



ally relevant imaging probe that tracks drug-related changes in activation. Thirdly, donepezil-related improvement of cognitive performance in the context of sleep deprivation may be attributed to both reduced lapses of attention as well as improved encoding but not to reduced subjective sleepiness.

### Vulnerability to Sleep Deprivation Influences the Behavioral Effects of Donepezil

Donepezil improved performance in persons who declined in performance when sleep deprived but not in those who were relatively unaffected by this manipulation. Vulnerable individuals might benefit from exogenous cholinergic augmentation because of the effects of reduced cholinergic transmission arising from sustained wakefulness (Supplementary Figure 1, middle panel). In contrast, persons with relatively preserved behavior despite sleep loss may generate elevated (“compensatory”) task-related activation when sleep deprived<sup>50,51</sup> that may relate to an endogenous elevation of cholinergic transmission. As such additional exogenous augmentation would result in little benefit

or even slight behavioral decline<sup>27,28</sup> (Figure 6; Supplementary Figure 1, bottom panel).

Concern may be raised regarding the absence of donepezil effects in rested wakefulness. However, prior studies on healthy, non-sleep deprived adults have reported both negative<sup>23</sup> and positive<sup>22</sup> effects on episodic memory. The latter study argued that it takes at least three weeks of continuous administration for donepezil to elicit behavioral benefit and this could contribute to the null effect in rested wakefulness observed here.

It is noteworthy that while enhancing cholinergic transmission is the primary mode of action of donepezil, cholinesterase inhibitors also influence noradrenergic and dopaminergic transmission<sup>13</sup> that are relevant in maintaining wakefulness as well as performance in sleep-deprived persons.<sup>26,52</sup> Neurotransmission involving these other substances is altered following sleep deprivation and could explain the aforementioned state differences in donepezil effect in some individuals (see ref. 52 in relation to dopamine). Further, the various cholinergic agonists and antagonists used in functional imaging studies to date affect these transmitters to differing degrees.<sup>13</sup> As such, while there is

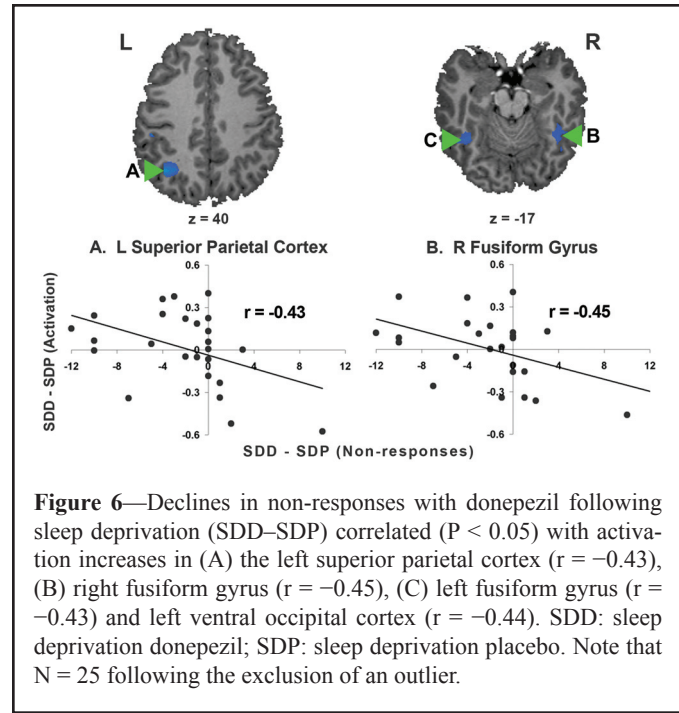
considerable evidence concerning how cholinergic augmentation can benefit attention and memory,<sup>53-55</sup> some caution is in order in attributing the observed effects in the current studies solely to the cholinergic system.

### Functionally Relevant Relationships Between fMRI Signal and the Effects of State and Drug Highlight Its Value as a Functional Probe

The within-subject design of this study controlled for confounds that could arise from inter-individual variation in response to sleep deprivation, donepezil or both these manipulations. Additionally, imaging the same volunteers as they performed tasks from different cognitive domains allowed us to differentiate task-independent and task-dependent effects on brain activation.

We found brain regions where donepezil modulated behavior and brain activation in a consistent manner across visual short-term memory, visual perceptual control,<sup>25</sup> and episodic memory tasks. These were the intraparietal sulcus that mediates attentional control and bilateral extrastriate regions involved in visual sensory processing. The effect of donepezil on attention (measured by the non-response rate) was reproduced across different tasks (correlation with non-response rate between the present experiment and previously reported tasks were:  $r = 0.81$ ,  $P < 0.001$  for the short-term memory task and  $r = 0.58$ ,  $P = 0.002$  for the visual perceptual control task).

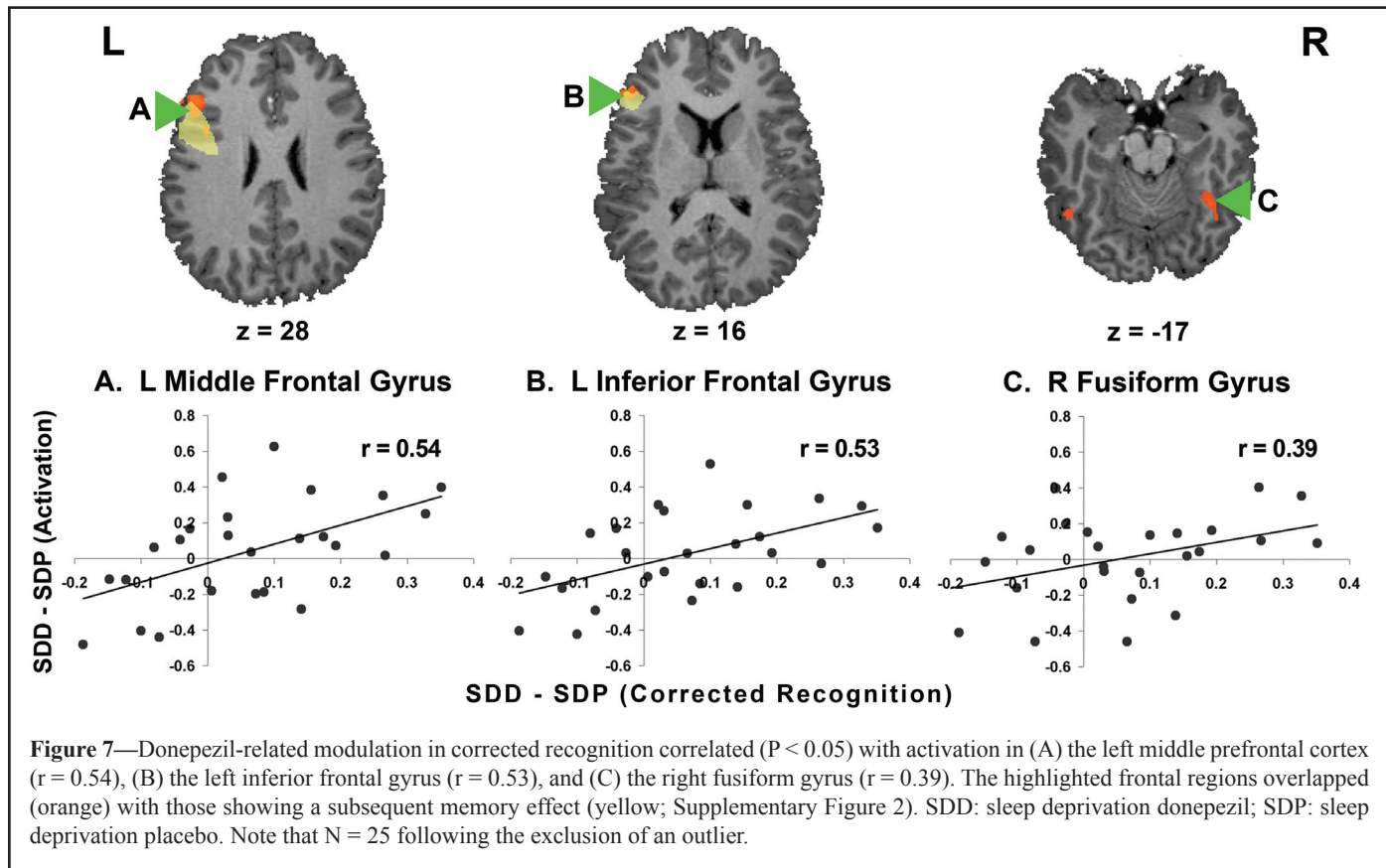
In the current study, we also found significant correlation between left inferior prefrontal activation at encoding, and recognition memory that was not previously observed with the visual short-term memory and perceptual control tasks. This repre-



**Figure 6**—Declines in non-responses with donepezil following sleep deprivation (SDD-SDP) correlated ( $P < 0.05$ ) with activation increases in (A) the left superior parietal cortex ( $r = -0.43$ ), (B) right fusiform gyrus ( $r = -0.45$ ), (C) left fusiform gyrus ( $r = -0.43$ ) and left ventral occipital cortex ( $r = -0.44$ ). SDD: sleep deprivation donepezil; SDP: sleep deprivation placebo. Note that  $N = 25$  following the exclusion of an outlier.

sents a task-dependent correlation between brain activation and behavior that may relate specifically to episodic memory.

Our findings support the notion that neurotransmitter modulation can alter brain activation and behavior in a task-specific manner,<sup>14,16,31,56</sup> even though neurotransmitter systems mediating wakefulness and arousal, such as the cholinergic system, have very widespread representation throughout the cerebral cortex. Further, the neuroanatomically and psychologically plausible



**Figure 7**—Donepezil-related modulation in corrected recognition correlated ( $P < 0.05$ ) with activation in (A) the left middle prefrontal cortex ( $r = 0.54$ ), (B) the left inferior frontal gyrus ( $r = 0.53$ ), and (C) the right fusiform gyrus ( $r = 0.39$ ). The highlighted frontal regions overlapped (orange) with those showing a subsequent memory effect (yellow; Supplementary Figure 2). SDD: sleep deprivation donepezil; SDP: sleep deprivation placebo. Note that  $N = 25$  following the exclusion of an outlier.

correlations between drug-dependent *change* in activation and behavior strengthen the case for fMRI serving as a dependable probe in psychopharmacological studies.<sup>27,57</sup>

### Donepezil May Benefit Memory in Sleep-Deprived Persons Through Improvements in Attention and Memory Encoding

The locus at which donepezil altered brain activation in the sleep-deprived state was dependent on whether trials relating to non-responses or recognition were analyzed, yielding clues concerning the underlying mechanism of drug benefit. Activation-behavior correlation for non-responses during encoding was found in the intraparietal sulcus, which mediates attentional control, and bilateral visual extrastriate regions involved in visual sensory processing.

Attention during encoding strongly influences later memory performance<sup>58</sup> and numerous functional imaging studies attest to the importance of the parietal cortex in mediating the control of multiple aspects of visual attention.<sup>59-61</sup> The downstream effects of attention manifest in the form of greater activation (as well as increased neuronal firing) in the ventral visual cortex in response to attended stimuli<sup>59,62,63</sup> and reduced neuronal firing<sup>64</sup> in response to ignored stimuli. As such, the effect of donepezil on the aforesaid regions could be to counteract sleep deprivation mediated diminution of activation in the same regions (Figures 4, 5). The current findings together with those relating to the visual short term memory and visual perceptual control tasks<sup>25</sup> support the hypothesis that attention and sensory processing are task-independent mechanisms by which cholinergic augmentation could improve cognitive performance.<sup>65,66</sup>

On the other hand, the finding of activation-behavior correlation in the left lateral prefrontal region that coincidentally showed subsequent memory effects (Figure 7 and Supplementary Figure 2), suggests that donepezil may elicit a task-dependent benefit on episodic memory. However, this surmise should be interpreted cautiously as current opinion is divided as to whether acetylcholine has direct effects on memory<sup>22,67</sup> or if these are mediated via indirect effects on attention<sup>24</sup> or some combination of attention and memory.<sup>68,69</sup>

### Significance of Reduced Encoding-Related Activation During Sleep Deprivation

Although the left inferior prefrontal signal associated with word encoding was lower following sleep deprivation relative to rested wakefulness, the subsequent memory effect on activation was present in both states (Supplementary Figure 2). Had we not excluded invalid encoding trials, it might be argued that reduced activation could simply have been a consequence of the subject falling asleep. However, this was not the case. A general reduction in blood flow is also unlikely as we have previously shown that the visual cortex responds to alternating check patterns normally under sleep deprivation conditions similar to the current experiment.<sup>70</sup>

To account for the reduced “successful encoding signal” following sleep deprivation, we speculate that the reduced activation represents some processing elements going “off-line” under the condition of sleep deprivation in a manner that, while not affecting tested memory performance, may compromise en-

coding so as to affect long-term consolidation. Some effects on memory consolidation may take longer than the experimental period (up to months) to reveal.<sup>71,72</sup> A useful metaphor arises from the concept of “safety factor” in neuromuscular transmission.<sup>73</sup> Transmission remains effective under various physiological conditions because the amount of transmitter released per nerve impulse is greater than that required to minimally trigger a muscle action potential. Along this line of reasoning, we posit that some of the “redundant” activation observed in association with task performance after a normal night of sleep could serve to ensure robust encoding into long-term memory.

### Conclusion

Donepezil reduced decline in recognition performance in individuals vulnerable to the effects of sleep deprivation by improving both attention and enhancing encoding. Additionally our findings demonstrate the utility of combined fMRI-behavior evaluation in psychopharmacological studies.

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