OXFORD

Cerebral Cortex, August 2019; 3445–3456

doi: 10.1093/cercor/bhy214
Advance Access Publication Date: 6 September 2018
Original Article

ORIGINAL ARTICLE

Anticholinergic Amnesia is Mediated by Alterations in Human Network Connectivity Architecture

Jasmeer P. Chhatwal[1,2,3], Aaron P. Schultz [1,3], Trey Hedden[3,4,5], Brendon P. Boot[2,6], Sarah Wigman[1], Dorene Rentz[1,2,3], Keith A. Johnson[1,2,3,4,5] and Reisa A. Sperling[1,2,3,4]

[1]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA, [2]Department of Neurology, Brigham and Women's Hospital, Boston, MA 02115, USA, [3]Harvard Medical School, Boston, MA 02115, USA, [4]Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA 02129, USA, [5]Department of Radiology, Massachusetts General Hospital, MA 02114, USA and [6]Voyager Therapeutics, Cambridge, MA 02139, USA

Address correspondence to Jasmeer P. Chhatwal, Massachusetts General Hospital, Martinos Center for Biomedical Imaging, 149 13th St, Room 2669, Charlestown, MA 02129, USA. Email: chhatwal.jasmeer@mgh.harvard.edu

Abstract

Disrupted cholinergic neurotransmission plays a central role in Alzheimer's disease, medication-induced memory impairment, and delirium. At the systems level, this suggests anticholinergic drugs may alter the activity and interplay of anatomically distributed neural networks critical for memory function. Using a network-sensitive imaging technique (functional connectivity MRI) and a double-blind, crossover design, we examined the consequences of anticholinergic drug administration on episodic memory and functional network architecture in a group of clinically normal elderly. We observed that low-dose scopolamine (0.2 mg IV) decreased episodic memory performance and selectively decreased connectivity strength in 3 of 7 cortical networks. Both memory and connectivity effects were independent of β-amyloid burden. Drug-induced connectivity changes within the Default and Salience networks, as well as reductions in the strength of anticorrelation between these 2 networks, were sufficient to fully statistically mediate the effects of scopolamine on memory performance. These results provide experimental support for the importance of the Default and Salience networks to memory performance and suggest scopolamine-induced amnesia is underpinned by disrupted connectivity within and between these 2 networks. More broadly, these results support the potential utility of fcMRI as tool examine the systems-level pharmacology of psychoactive drugs.

Key words: Alzheimer's disease, amnesia, amyloid, cholinergic, networks

Introduction

Anticholinergic medications are common precipitants of delirious and amnestic states (particularly in older individuals), and reductions in cholinergic neurotransmission are thought to play a key role in the emergence of memory loss in Alzheimer's disease (AD) and normal aging (Bartus et al. 1982; Kopelman and Corn 1988; Christensen et al. 1992). Anticholinergic medications have been used in human and animal studies to model AD and delirium, as well as to test experimental therapeutics aimed at improving memory storage and recall (Christensen et al. 1992; Sperling et al. 2002). Conversely, medications that increase the half-life of acetylcholine are associated with improved cognitive performance, and represent the most commonly used class of medications to treat dementia symptoms (Ashford 2015).

Cortical cholinergic innervation is derived mainly from projection neurons originating in the nucleus basalis of Meynert (NBM), a brainstem region known to be profoundly and preferentially degraded early in AD (Bartus et al. 1982). The amnestic effects of anticholinergic medications and the recognition that the NBM is an early site of AD neurodegeneration (Sassin et al. 2000) provided support for the hypothesis that degraded cholinergic neurotransmission may be partly responsible for AD-related memory loss (Coyle et al. 1983).

Muscarinic and nicotinic acetylcholine receptors (AChRs) are widely distributed in cortical regions known to be critical for memory function (e.g., hippocampus and parietal cortical regions), but also in many cortical regions not classically associated with mnemonic function (Cortés et al. 1987; Davies and Verth 1977). At a cellular and local circuit level, changes in both muscarinic and nicotinic receptor-mediated neurotransmission are known to alter synaptic plasticity, neuronal excitability, and the balance between inhibitory and excitatory neurotransmission (Hasselmo 2006). At the level of larger scale neural circuits, neurophysiological studies using pharmacologic and genetic approaches indicate AChR activation affects the oscillatory activity of large collections of neurons, including the modulation of hippocampal gamma oscillations (Fisahn et al. 1998; Betterton et al. 2015).

Cholinergic dysfunction has also been tied to the presence and production of pathologic amyloid-beta species. Activation of muscarinic AChRs has been shown to increase the release of amyloid precursor protein from affected cells (Nitsch et al. 1992). More recent work suggests that the presence of amyloid peptides can disrupt cholinergic signaling, and that certain AChRs (esp. the alpha-7 nicotinic subtype) directly bind amyloid beta 1–42 (Aβ42) fragments (Wang et al. 2000; Dziewczapolski et al. 2009), supporting a link between the cholinergic and amyloid hypotheses of AD pathophysiology.

Though a variety of cholinergic modulating drugs are available, many studies have opted to use the muscarinic antagonist scopolamine in modeling memory loss. Scopolamine is commonly used in clinical settings for motion sickness, nausea treatment, and to control excess secretions, and has a well-known safety and pharmacokinetic profile (Renner et al. 2005). Studies using intravenous or subcutaneous administration of scopolamine have consistently demonstrated decreases in memory performance, including on word-list learning (e.g., the Buschke Selective Reminding Task [SRT]; Buschke 1973), delayed nonmatching to sample, and spatial memory tasks (Broks et al. 1988; Potter et al. 2000; Sperling et al. 2002; Lim et al. 2015). Similar studies also suggest that many motor, attention, and language tasks that do not require delayed recall are comparatively less affected by scopolamine, especially when given at lower doses (Beatty et al. 1986; Huff et al. 1988; Christensen et al. 1992). Functional imaging studies suggest that scopolamine-induced changes in the blood-oxygen level dependent (BOLD) signal during task performance are observable and may have relevance to drug-induced changes in cognition and experience-dependent plasticity (Thiel et al. 2002; Thiel 2003; Dumas et al. 2010). Additionally, scopolamine models of amnesia have been extensively used as platforms from which to test nootropics or potential symptomatic treatments for memory loss (Fishkin et al. 1993; Snyder et al. 2005; Rutten et al. 2006).

Converging evidence from imaging, physiological, and genetic studies suggests that anatomically distributed brain regions organize into large-scale neural networks that share a predictable anatomy across individuals (Fox et al. 2005; Yeo et al. 2011; Mueller et al. 2013; Richiardi et al. 2015). Resting-state functional connectivity MRI (rs-fcMRI) is a noninvasive imaging technique that can be used to assess the strength of the coordinated activity across nodes within these anatomically distributed networks. Studies of resting-state networks suggest that they are somewhat specialized for particular types of cognitive, motor, and social behaviors, and that variations in connectivity strength within these networks are reflected in variations in behavioral performance (Fox and Greicius 2010; Lehmann et al. 2013). Specifically with respect to memory, elegant recent work suggests that increased connectivity within the Default network predicts better memory performance, and that memory training exercises led to increases in Default network connectivity that mirrored improvements in post-training memory performance (Dresler et al. 2017). Rs-fcMRI measurements are also being used as biomarkers in neurologic and psychiatric disease states (Fox et al. 2005), and recent work suggests that neurodegenerative conditions may be distinguished based on the intrinsic connectivity networks they target (Seeley et al. 2009; Lehmann et al. 2013). In AD, prior work suggests that the degradation of cognitive networks (particularly the Default, Dorsal Attention, Salience, and Frontoparietal Control networks) is progressive across the disease course, such that decreased connectivity in these networks is associated with worsening cognitive impairment (Greicius et al. 2004; Thomas et al. 2014; Jones et al. 2016).

To elucidate the systems-level mechanisms that underlie anticholinergic induced amnesia, we measured scopolamine-induced changes in memory performance and coordinated network activity using rs-fcMRI in cognitively normal elderly individuals using a double-blind, placebo-controlled, counterbalanced, crossover design. Leveraging data on amyloid burden in scopolamine-treated participants, we also tested the hypothesis that elevated amyloid burden may potentiate scopolamine-induced memory loss or changes in network strength.

Materials and Methods

Participants

Participants were drawn from the Harvard Aging Brain Study (HABS), a community-based longitudinal study of cognitive aging and preclinical AD based in Boston, MA. Participants in the HAB study were judged by consensus to be clinically normal at study entry with Mini-Mental Status Exam within the normal range, global Clinical Dementia Rating of zero, and Logical Memory scores within the normal range for age. Baseline data from the first year of the HABS were used in cross-sectional analyses. Prior to any study procedures, all participants provided written informed consent using study protocols and consent procedures approved by the Partners Healthcare Institutional Review Board. All study procedures were carried out at Massachusetts General Hospital (MGH) or Brigham and Women's Hospital. Baseline data from individuals who later participated in the scopolamine substudy were not included in the cross-sectional dataset.

MR Imaging and Processing

Participants underwent eyes-open resting state fMRI using a 12-channel head coil on one Siemens Trio TIM 3 T scanner at the MGH/Martinos Center for Biomedical Imaging. Cross-sectional rs-fcMRI data were acquired in 2 imaging runs, each lasting approximately 6 min (total of 124 volumes) using the following parameters: 3000 ms, echo time: 30 ms, flip angle: 85°,

matrix: 72 × 72, 3 by 3 by 3 mm voxels. Data were processed as previously described using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/; version r4290; Schultz et al., 2014). Briefly, each run was slice-time corrected, realigned (INRIAlign; http://www-sop.inria.fr/epidaure/software/INRIAlign/), normalized (MNI 152 EPI template; Montreal Neurological Institute, Montreal, Canada), and smoothed with a 6 mm FWHM Gaussian kernel. To reduce the effect of movement artifacts on connectivity measurements, movement estimates from the realignment step and their first derivatives, were regressed out prior to connectivity analyses. A temporal band-pass filter was used to focus the analysis on frequencies ranging from 0.01 to 0.08 Hz.

Network templates for the 7 cortical networks examined (Default, Salience, Dorsal Attention, Control, Primary and Extrastriate Visual, and Motor) were derived from an outside sample of 675 young individuals imaged as part of the Brain Genomics Superscript Project (http://neuroinformatics.harvard.edu/gsp/). Derivation, reproducibility, and application of these network templates to determine connectivity measurements (Template Based Rotation or TBR) has previously been described in detail (Schultz et al. 2014; Shaw et al. 2015). Analytic code and template maps used are publicly available at mrtools.mgh.harvard.edu. Connectivity maps for each participant were generated for each network template. For scopolamine substudy participants, separate subject-level maps were generated for each experimental day. Whole network measurements represented the average connectivity strength for the voxels represented in each subject-level network map.

Nodal connectivity analyses have also previously been described in detail (Schultz et al. 2014; Shaw et al. 2015). Briefly, voxel sets representing nodes for each network were identified by separating noncontiguous voxel clusters in each network template map, and thresholding each cluster to include only voxels with within-network connection strengths ≥60% of the peak correlation strength within each cluster. The resulting node regions are irregularly shaped, contiguous clusters of voxels centered on regions of high connectivity in each whole network map (Fig. 6A, Supplementary Fig. S3). Node-to-node connectivity strengths represent the average correlation strength between the BOLD time courses for each voxel in each node region. The node maps used in the present report are publically available at mrtools.mgh.harvard.edu.

Positron Emission Tomography Methods

Amyloid positron emission tomography (PET) was performed using Pittsburgh Compound B (PiB) using a Siemens ECAT EXACT HR+ imaging system at the MGH using previously described protocols (Johnson et al. 2007; Hedden et al. 2009; Sperling et al. 2009). The FreeSurfer (version 5.1, (http://surfer.nmr.mgh.harvard.edu)) defined cerebellar gray matter ROI was used as a reference region for PiB imaging. As in prior reports from the HAB sample, we report a summary measure of aggregate PiB binding in frontal, parietal, lateral temporal and retrosplenial cortices (FLR; Johnson et al., 2007).

Scopolamine Substudy Experimental Design

Participants

In total, 34 participants were recruited from HABS to participate in the scopolamine substudy. The physicians responsible for recruitment were blinded to the potential participant's biomarker status and prior neuropsychological test performance. Participants in the scopolamine substudy were all in their third or fourth year of HABS. Of the 34 participants in the scopolamine substudy, 32 participants carried a consensus diagnosis of clinically normal at the HABS neuropsychological visit closest to the date of their first scopolamine substudy visit. Two scopolamine substudy participants were assessed as clinically normal at the time of HAB study entry but received a consensus diagnosis of Mild Cognitive Impairment (MCI; 1 amnestic, 1 nonamnestic MCI) at the HABS neuropsychological visit closest to their scopolamine substudy visit. Analyses examining drug effects on cognitive testing including and excluding these MCI participants were extremely similar. Accordingly, all 34 individuals treated with scopolamine are included in the analyses presented.

Demographic characteristics of the 34 clinically normal older individuals enrolled in the scopolamine study were similar to the overall HAB cohort, with the exception of significantly higher average amyloid burden in the scopolamine substudy (Table 1). To identify candidate networks that may be mediating drug effects in the scopolamine challenge, we first assessed cross-sectional relationships between connectivity and memory performance in HABS participants who did not participate

Table 1 Participants

Dataset	Cross-sectional Mean (SD)	Scopolamine substudy Mean (SD)	χ^2 P-value
Number of participants	209	34	
Age	73.9 (6.1)	73.8 (6.7)	0.84
Sex (% female)	58.8	55.9	0.70
Years of education	15.7 (3.1)	15.8 (2.8)	0.99
Verbal IQ	120.5 (9.3)	122.0 (8.0)	0.41
Amyloid burden (PiB FLR SUVR)	1.16 (0.2)	1.25 (0.2)	0.009
% Amyloid positive	22.5%	47.1%	0.006
ApoE4 ε4 carrier (%)	27.3%	29.4%	0.95
Cognitive performance			
MMSE	29.0 (1.1)	28.8 (1.0)	0.33
Selective reminding task			
Long-term storage	30.5 (14.3)	25.8 (13.7)	0.09
Delayed free recall (5 and 30 min)	10.6 (6.2)	10.8 (5.4)	0.94
Multiple choice recall (5 and 30 min)	21.9(2.9)	21.4 (4.7)	0.37

in the scopolamine substudy (cross-sectional dataset, n = 209; Table 1).

Experimental Design

On the first experimental day, participants were administered scopolamine (0.2 mg IV, slow push) or a matched volume of saline immediately prior to imaging and neuropsychological testing. All participants were brought back for a second experimental day 1–3 weeks after the first, and received scopolamine or saline, whichever was not given on the first experimental day. Accordingly, all participants received scopolamine on only one visit in a counterbalanced order. A research pharmacist was responsible for randomization and provided drug or placebo for administration in 1 mL of total volume. Study physicians, nurses, testers, and participants were blinded to drug condition on each experimental day. To minimize practice effects and recall between days, alternate versions of Buschke SRT were used on experimental days 1 and 2. Vital signs, including heart rate and oxygen saturation, were monitored at set intervals over the course of each testing day.

Rs-fcMRI imaging in the scopolamine substudy was acquired in 3 imaging blocks, each lasting approximately 5 min. Scanner and imaging parameters were identical to those in the cross-sectional dataset. Drug or saline was administered immediately prior to MR imaging.

Behavioral Testing

Cognition was assessed through a short battery of tests performed immediately after completing MR imaging. The present report focused on the Buschke SRT. During the SRT, participants are presented with 12 semantically unrelated words, and then asked to repeat back the word list from memory in an untimed fashion. In subsequent encoding trials, participants are reminded of only words they did not recall correctly (selective reminding). This process proceeds for a total of 6 encoding/reminding trials, minimizing the effects of inattention at any one trial on delayed recall. As demonstrated in prior studies, a measure of consistent trial-to-trial recall during these initial 6 trials can be used to estimate successful memory storage (SRT storage) (Buschke 1973, 1974). Following the last encoding/reminding trial, delayed recall of the words is assessed at 5 and 30 min. At each delayed recall session, participants are first asked to freely recall as many words as possible (delayed free recall), and then asked to recognize each word from among 4 choices (multiple-choice or recognition memory). Correctly recalled items at the 5- and 30-min delays were added together to yield the SRT Delayed Free Recall and SRT Multiple-Choice Recall measures used in the analysis phase of the study. Alternate versions of the SRT were used on experimental days 1 and 2, such that each version was tested with an equal number of saline- and scopolamine-treated participants.

In addition to the SRT memory task, standard procedures were used to administer the Trails Making (TMT), Visual Form Discrimination, category word generation (semantic fluency), and F-A-S word generation (phonemic fluency). As in prior reports, TMT performance was assessed as the difference between versions A and B of the task. Semantic and phonemic fluency performance was the aggregate of words generated during one minute following presentation of a category or letter cue, with 3 cues presented for each task.

Statistical Approach

All participants who were randomized as part of the scopolamine substudy are included in the presented analyses. Linear and linear mixed effects modeled implemented in R Version 3.2 using the nlme package were used to examine the cross-sectional dataset and repeated measures data from the scopolamine substudy. A model-based approach for causal mediation analyses was used, employing the lme4 and mediation packages (Tingley et al. 2014).

Nominal P-values are shown for all analyses, including for exploratory (cross-sectional) analyses. Building on the results of our exploratory analyses, our primary analyses in the scopolamine substudy focused on memory performance and its relationship to network connectivity in a subset of networks. However, though we focus on memory tasks and their relationships to Default, Salience, and Control network connectivity on- and off-scopolamine, we report statistical results for a wider set of behavioral tests and networks to provide context for the specificity of scopolamine-induced changes in behavior and connectivity.

Connectivity measurements represented the average of connectivity measurements across each block on a given imaging day (two 6-min blocks for cross-sectional data, three 5-min blocks on each experimental day from the scopolamine substudy). To minimize the effects of differential movement and number of unusable volumes (defined as mean movement >0.2 mm/volume) on connectivity measurements, mean movement and the number of unusable volumes were regressed from each connectivity measurement prior to further analysis.

The time of each imaging block relative to the time of injection was recorded (time-from-injection; TFI) and examined as a potential covariate in subsequent analyses. Similarly, we examined TFI as a potential covariate with respect to performance on cognitive tests. No differential effect was observed in the relationship between TFI and whole network connectivity in the on- versus off-drug state, arguing against a time-varying effect of scopolamine on connectivity. TFI also was not observed to be a significant covariate in models predicting scopolamine-induced changes in behavioral performance. As TFI was not a significant predictor in models assessing drug-connectivity relationships, connectivity values from the 3 resting state imaging blocks were averaged together prior to inclusion in statistical models, and TFI was not included in subsequent statistical models.

Additionally, we examined whether the order of administration (i.e., Scopolamine then Saline vs. Saline then Scopolamine; order) impacted behavioral results. We observed that individuals who received saline on day 1 had 1) trend level higher performance on the SRT long-term storage measure as compared with those receiving scopolamine on day 1 (effect of order: $F_{(1,32)} = 3.23$, $P = 0.082$); and 2) modestly higher performance on the SRT delayed free recall measure ($F_{(1,32)} = 7.18$, $P = 0.012$). No effect of order was seen in any of the other behavioral tests. Accordingly, order of drug administration was included as a covariate in models examining drug effects on SRT performance.

Results

Demographics and Experimental Design

Using baseline behavioral and connectivity data on participants included in the cross-sectional dataset, we performed a series of exploratory analyses to assess relationships between

connectivity and memory performance. We focus mainly on the Buschke SRT, a challenging verbal memory task in which participants are presented with twelve words to remember (Buschke 1973, 1974). The SRT (Buschke 1973; Masur et al. 1989) and similar verbal list learning tests (Petersen et al. 1999; Sarazin et al. 2007) have been extensively used in studies of AD-related mnemonic dysfunction, and have high reproducibility in both mildly impaired and unimpaired older populations. Additionally, prior work from our group identified SRT performance as the largest single contributor to an episodic memory composite score derived from a data-driven factor analysis of cognitive testing from the HAB sample (Shaw et al. 2015).

Cross-Sectional Relationships Between Connectivity and Memory Performance

Of the 7 networks examined, 3 cortical networks showed significant relationships with performance on the SRT across the HABS cohort, in agreement with prior reports from HABS (Shaw et al. 2015). Connectivity within the Default, Salience, and Control networks was positively correlated to SRT storage, as well as delayed free recall at 5 and 30 min (r = 0.15–0.23, Fig. 1). As performance and correlation with connectivity was very similar at 5 and 30 min, the number of recalled words at each delay was added together in all subsequent analyses to yield a summary of SRT delayed free recall and multiple-choice performance. Notably, connectivity within another cognitive network, the Dorsal Attention Network, was not correlated with SRT performance, nor was connectivity in the Motor, Primary Visual, and Extrastriate Visual networks.

A second analysis was performed to assess if amyloid burden interacted with connectivity in the Default, Salience, or Control networks to predict SRT performance. Individuals with higher amyloid burden (measured using PiB PET) showed significantly lower performance on SRT delayed free recall ($F_{(1,197)}$ = 7.0, P = 0.009) and long-term storage ($F_{(1,197)}$ = 1.2, P = 0.008). However, the interaction of connectivity and amyloid burden (used continuously) was not predictive of SRT performance (all connectivity by amyloid interactions $P > 0.3$). Applying a previously derived cutoff for amyloid positivity in this sample (Mormino et al. 2014), we observed that the relationships between connectivity and SRT performance were similar in amyloid positive and negative individuals, suggesting that amyloid burden does not alter the relationship between connectivity and SRT performance (Supplementary Table S1).

Though the focus of the present study was on memory performance, to contextualize the specificity of network connectivity for memory performance and facilitate comparison with the cognitive battery used in the anticholinergic challenge experiment, we also correlated connectivity in cortical networks to performance in 4 nonmemory tasks. Default network connectivity was positively correlated with performance on semantic fluency (category task; r = 0.15), but not phonemic fluency (FAS task), Visual Form Discrimination task (VFDT), or Trails Making task (TMT) performance. In addition to SRT performance, increased Control network connectivity was associated with better performance on semantic fluency and TMT (Fig. 1). Connectivity within the Salience network did not show significant correlations with any of the 4 nonmemory tasks (Fig. 1).

Connectivity Changes Following Anticholinergic Challenge

Low-dose scopolamine induced significantly decreased connectivity in the Default ($F_{(1,33)}$ = 20.3, P = 0.0001), Salience ($F_{(1,33)}$ = 17.8, P = 0.0002), and Control ($F_{(1,33)}$ = 15.2, P = 0.0004) networks (Fig. 2A–C). As a percentage, 79% (27 of 34) of participants showed numerically lower Default network connectivity following scopolamine versus saline, as compared with 76% (26 of 34) and 70% (24 of 34) for Salience and Control network connectivity, respectively (Fig. 2A–C). Nonsignificant, numeric increases in connectivity were seen in the Dorsal Attention ($F_{(1,33)}$ = 2.5, P = 0.12), Extrastriate Visual ($F_{(1,33)}$ = 2.4, P = 0.13), Primary Visual ($F_{(1,33)}$ = 1.4, P = 0.24), and Motor ($F_{(1,33)}$ = 1.4, P = 0.24) networks (Fig. 2D–H) following scopolamine administration. The average connectivity across all networks was not significantly changed following scopolamine challenge ($F_{(1,33)}$ = 0.2, P = 0.68; Fig. 2H).

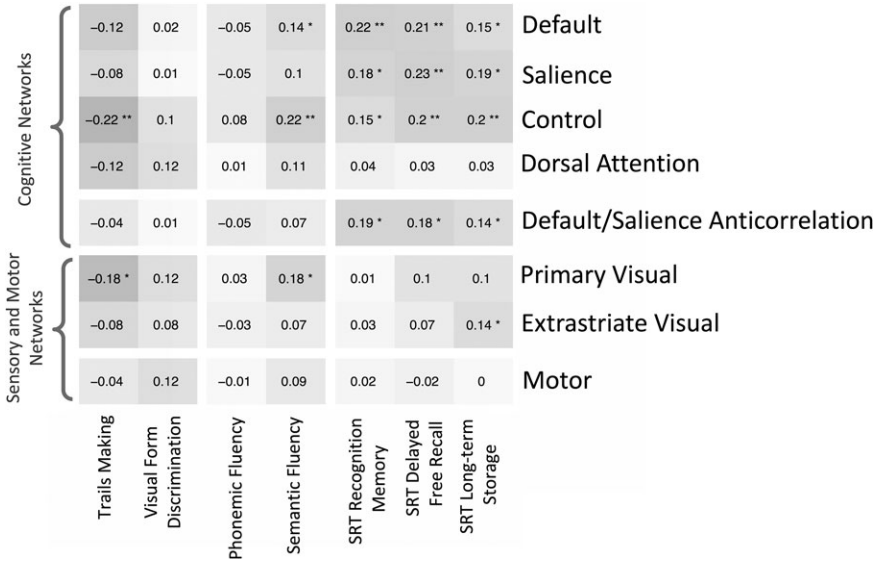

Figure 1. Cross-sectional relationships between network connectivity and cognitive performance. Network connectivity in 7 cortical networks was compared with performance in the selective reminding task (SRT), and 4 nonmemory tasks. The average anticorrelation between voxels within Default and Salience nodes is also shown (Default/Salience Anticorrelation). *$P \leq 0.05$ and **$P \leq 0.005$.

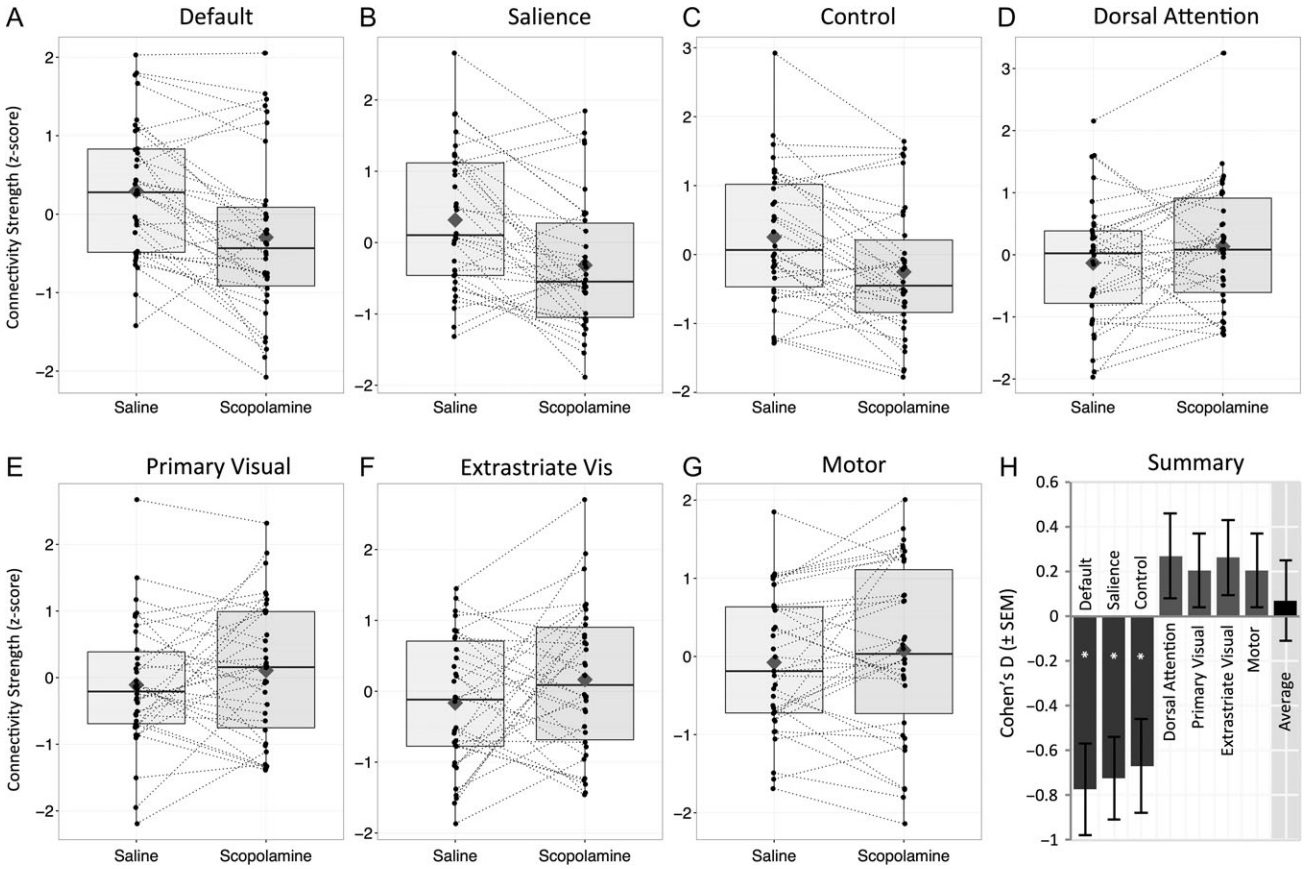

Figure 2. Changes in whole network connectivity following administration of low-dose scopolamine. Overall, 34 clinically normal elderly received scopolamine (0.2 mg IV) or saline using a double-blind, counterbalanced, crossover design. Scopolamine administration led to significant decreases in Default (*A*, *H*), Salience (*B*, *H*), and Control (*C*, *H*) network connectivity. No significant changes in connectivity were observed in other networks (*D*–*H*), and the average connectivity across all 7 networks was not altered (*H*, right). Gray diamond indicates mean connectivity for each drug condition, $^{*}P \leq 0.005$.

Behavioral Changes Following Anticholinergic Challenge

A behavioral battery consisting of the SRT, semantic fluency, phonemic fluency, VFDT and TMT was given following the blinded administration of scopolamine or placebo. The largest behavioral changes were seen in the SRT, where measures of storage and delayed free recall were significantly decreased following low-dose scopolamine administration (Fig. 3; storage: $F_{(1,32)} = 10.21$, $P < 0.005$; delayed recall: $F_{(1,32)} = 12.9$, $P < 0.005$). In contrast, only a trend level decrease in SRT recognition memory (multiple choice delayed recall) was observed following anticholinergic challenge ($F_{(1,32)} = 3.95$, $P = 0.055$). Compared with performance off-drug, scopolamine treated participants recalled an average of 2.9 words fewer (24.4%) during SRT delayed free recall and 1.0 fewer words during multiple choice recall (4.5%). Compared with performance off-drug, the SRT storage estimate was reduced by an average of 28.7% following scopolamine administration.

Aside from the SRT, modest but significant scopolamine-induced decreases in semantic fluency ($F_{(1,33)} = 5.0$, $P = 0.033$) performance were observed. TMT performance was also significantly decreased ($F_{(1,33)} = 4.8$, $P = 0.037$), but this effect appeared driven by 2 individuals with relatively large increases in TMT completion time on- versus off-drug (Fig. 3). No significant changes in VFDT or phonemic fluency were observed ($P > 0.2$).

Relationships Between Drug-Induced Changes in Connectivity and Performance

Default, Salience, and Control network connectivity strength were correlated with memory performance in the cross-sectional dataset, and both memory performance and connectivity in these 3 networks was diminished following scopolamine administration. To examine the relationship between changing connectivity and changing memory performance, we examined the extent to which diminished connectivity in these 3 networks predicted diminished performance on the SRT memory task.

Drug-induced decreases in Default network connectivity were correlated with decreases in SRT delayed free recall ($r = 0.43$, $P = 0.01$; Fig. 4*B*) and storage ($r = 0.55$, $P < 0.001$; Fig. 4*A*), but not recognition memory ($r = -0.1$, $P = 0.58$; Fig. 4*C*). A similar pattern was seen with respect to Salience network connectivity, where decreased connectivity predicted decreased SRT delayed recall ($r = 0.55$, $P < 0.001$; Fig. 4*E*) and storage ($r = 0.64$, $P < 0.001$; Fig. 4*D*), but not multiple-choice recall ($r = 0.046$, $P = 0.80$; Fig. 4*E*). Relationships between connectivity and memory performance were similar on- and off-scopolamine (Supplementary Fig. S1), suggesting that scopolamine decreased both connectivity and performance, and did not alter the relationship between connectivity and memory task performance. Additionally, similar relationships between connectivity and memory performance were seen in the cross-sectional datasets and in

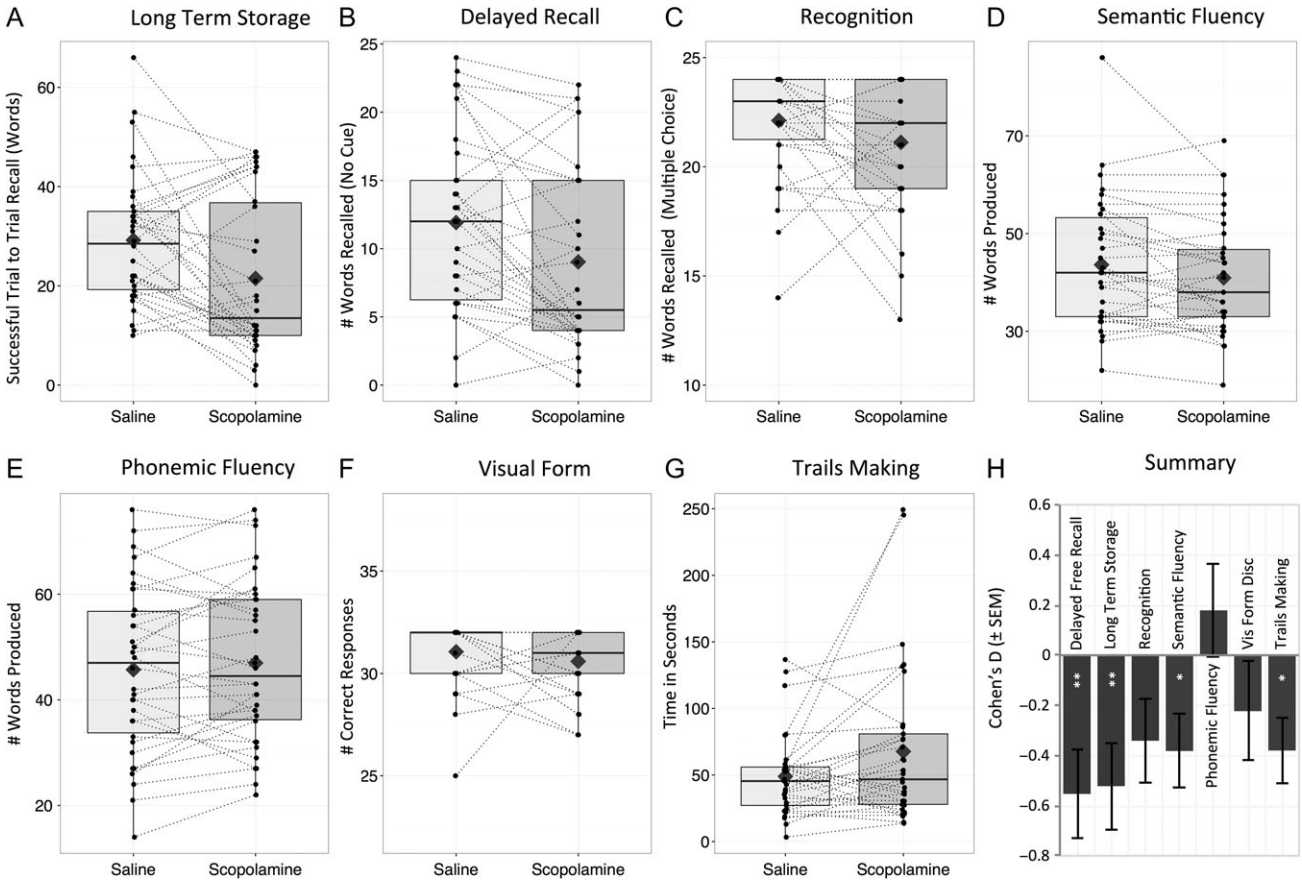

Figure 3. Changes in cognitive performance following administration of low-dose scopolamine. Participants were administered a cognitive battery following administration of low-dose scopolamine or saline. Scopolamine administration led to significantly decreased performance in the SRT memory task measures of long-term storage (*A*) and delayed free recall (*B*). A trend towards decreased recognition memory (multiple choice recall; *C*) in the SRT task was observed. Drug-induced decrements in semantic fluency (*D*) and trails making (*G*) performance were also observed. Gray diamond indicates mean performance for each drug condition, $^{*}P \leq 0.05$, $^{**}P \leq 0.005$.

the scopolamine substudy (Supplementary Fig. S1), further suggesting that scopolamine administration did not alter the relationship between connectivity and memory task performance. No significant main or interaction effects of amyloid burden were observed with respect to SRT performance in this sample (main effect of amyloid on SRT delayed recall, $F_{(1,32)} = 1.03$, $P = 0.32$, SRT storage: $F_{(1,32)} = 0.24$, $P = 0.63$; interaction of drug condition with amyloid burden on SRT delayed recall: $F_{(1,32)} = 1.05$, $P = 0.31$, SRT Storage: $F_{(1,32)} = 0.16$, $P = 0.70$; Supplementary Fig. S2).

Decreased Control network connectivity did not correlate with changes in SRT performance, outside of a trend-level association with the SRT storage estimate ($r = 0.29$, $P = 0.1$). Changes in the Dorsal Attention, Motor, and Visual network connectivity were not associated with changes in SRT performance (all $P > 0.2$). Scopolamine-induced changes in semantic fluency and TMT performance did not significantly correlate with changes in network connectivity, though a trend-level relationship between decreased Salience network connectivity and poorer semantic fluency performance was observed ($r = 0.32$, $P = 0.060$).

Separate analyses were performed to assess whether changing connectivity in the Default and/or Salience networks may mediate the effects of scopolamine on memory performance in the SRT. Default and Salience network connectivity were significantly related to SRT performance in the scopolamine-treated sample (Delayed recall: Default $F_{(1,32)} = 30.2$, $P < 0.0001$; Salience $F_{(1,32)} = 35.9$, $P < 0.0001$; Storage: Default $F_{(1,32)} = 33.2$, $P < 0.0001$; Salience $F_{(1,32)} = 36.4$, $P < 0.0001$), and scopolamine led to significant decreases in SRT delayed recall and long-term storage (reported above). Drug condition was no longer predictive of SRT delayed recall or long-term storage if either Default or Salience network connectivity was included in the model (effect of drug: $P > 0.2$ for all comparisons), indicating a full statistical mediation of scopolamine's effect on SRT performance by connectivity in either of these 2 networks (Fig. 5). Models including both the Default and Salience network connectivity as predictors of SRT performance resulted in models statistically similar to those including one network alone, suggesting a large degree of shared variance between these 2 networks (Supplementary Table S3).

This simple mediation analysis was supplemented with a causal mediation approach (Tingley et al. 2014). Connectivity in the Default network accounted for 61.1% (95% CI: 0.25–1.44; $P < 0.001$) and 59.3% (95% CI: 0.26–1.41 $P < 0.001$) of scopolamine's effect on SRT delayed recall and long-term storage, respectively. Similar results were obtained using Salience network connectivity as a mediator, with Salience network connectivity accounting for 64.8% (95% CI: 0.28–1.50; $P < 0.001$) and 64.1% (95% CI: 0.29–1.48, $P < 0.001$) of scopolamine's effect on SRT delayed recall and long-term storage, respectively. In all models

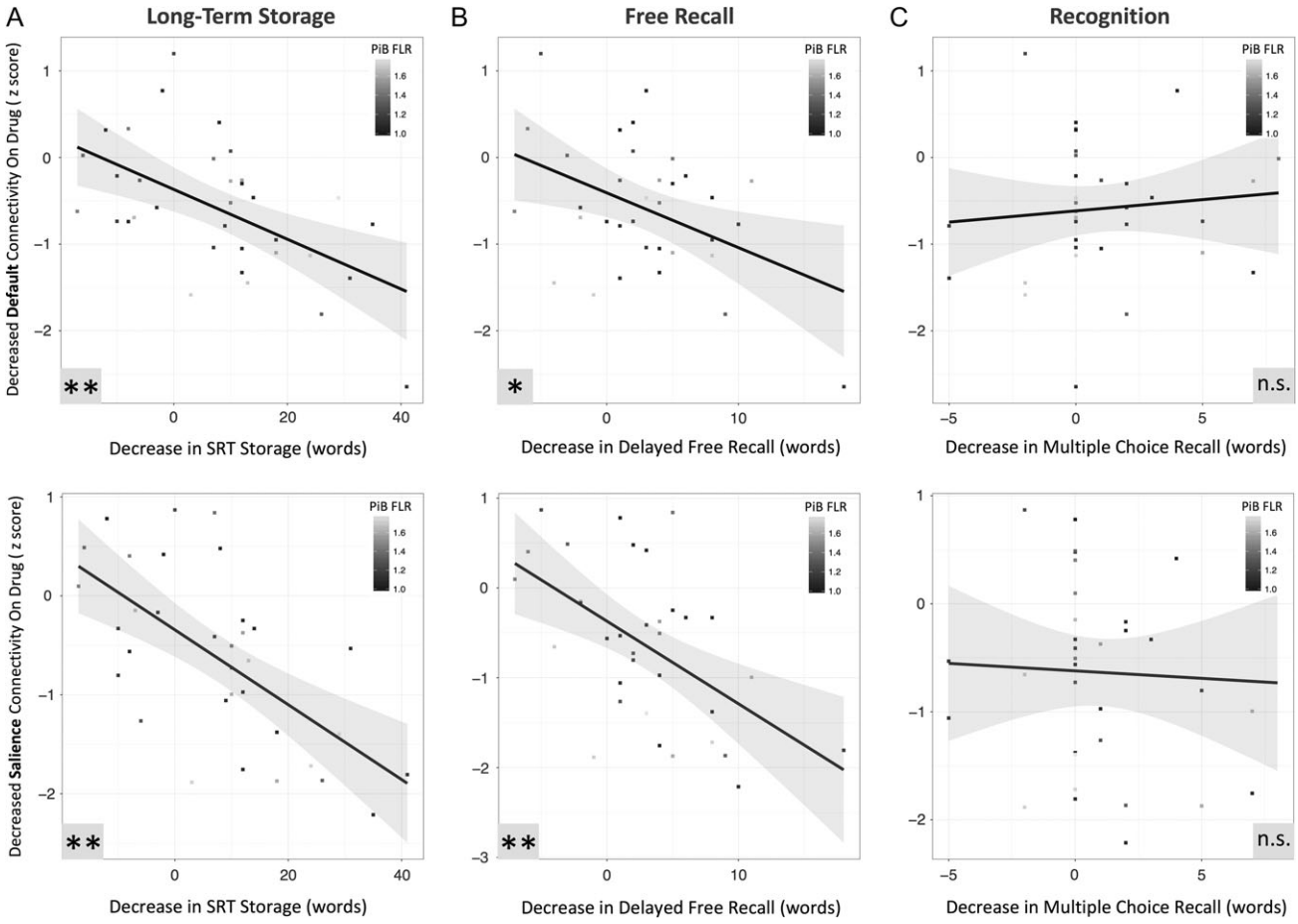

Figure 4. Correlations between drug-induced change in connectivity and change in memory performance. Scopolamine-induced changes in Default (*A*–*C*) and Salience (*D*–*F*) Network Connectivity were significantly correlated with changes in memory performance on the SRT storage (*A*, *D*) and delayed recall (*B*, *E*) measures, but not with recognition memory (multiple choice recall; *C*, *F*). *$P \leq 0.05$, **$P \leq 0.005$, n.s. corresponds to nonsignificant.

including Default or Salience network connectivity, the average direct effect (i.e., nonmediated effect) of scopolamine on memory performance was nonsignificant (P = 0.19–0.26; Supplementary Table S4), corroborating the simple mediation analyses and indicating that the majority of scopolamine's effect on memory performance can be accounted for by drug-induced decreases in Default or Salience network connectivity.

Nodal Connectivity Analyses

To better examine the anatomy and focality of altered within- and between-network connectivity induced by scopolamine, we decomposed each cortical network into its constituent nodes and assessed node-to-node connectivity on- and off-scopolamine. The 4 examined cognitive networks were split into 29 node regions, and correlated activity across each node-to-node pair was assessed (Fig. 6).

Connectivity patterns off-drug were as expected, with a high number of positive correlations ($r > 0$; correlations) and no negative correlations ($r < 0$; anticorrelations) between node pairs residing within the same parent network. Correlations between node pairs that did not share network membership (internetwork connections) were disproportionately negative ($r < 0$; anticorrelations), also as expected. Such anticorrelations were particularly prominent between nodes of the Salience and Default networks. This is consistent with prior studies demonstrating a high degree of anticorrelation between these networks and with the related generalization that Salience network connectivity increases during task performance (a "task-positive" network) whereas Default network connectivity decreases (a "task-negative" network).

Most aspects of network architecture were similar between scopolamine and placebo administrations (Fig. 6*B*,*C*), with robust positive correlations within networks, and mainly anticorrelations between networks. Quantitative comparison of connection strength on- and off-drug demonstrated weaker within-network correlations in both Default and Salience network nodes following scopolamine administration (Fig. 6*D*, outer ring), consistent with the foregoing results showing decreased whole network connectivity following scopolamine administration. Intriguingly, many internetwork connections were also disrupted following scopolamine administration (Fig. 6*D*, inner), particularly anticorrelations between Default and Salience network nodes. This pattern suggests that scopolamine may weaken the usual juxtaposition of default and salience network connectivity (reduced internetwork anticorrelation), in addition to decreasing coordinated activity within each network. Averaging across all connections between the default and salience networks, anticorrelation strength was itself predictive of memory performance (delayed recall: $F_{(1,33)} = 37.1$, $P < 0.0001$; LTS: Default $F_{(1,33)} = 41.79$, $P < 0.0001$; Fig. 7). Also similar to the whole network measurements, drug-

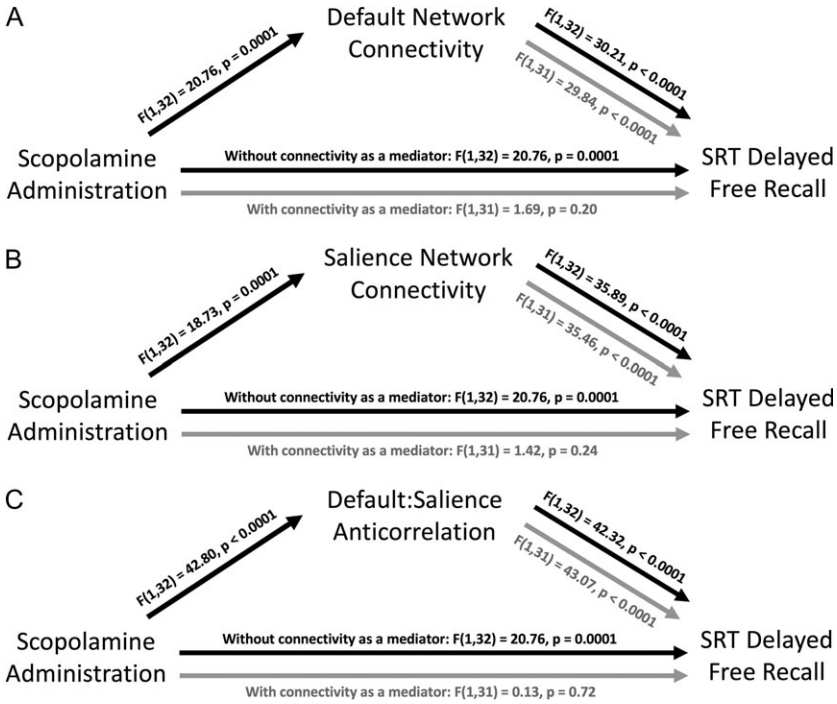

Figure 5. Mediation models of scopolamine induced decreases in connectivity mediating decreases in memory performance on the SRT. Network connectivity in the Default (*A*), Salience (*B*), or anticorrelation strength between the Default and Salience Networks (*C*) show complete statistical mediation of the effect of scopolamine on memory performance (SRT Delayed Free Recall), as no significant effect of drug (saline vs. scopolamine) is present when any of these 3 connectivity measures is included as a mediator.

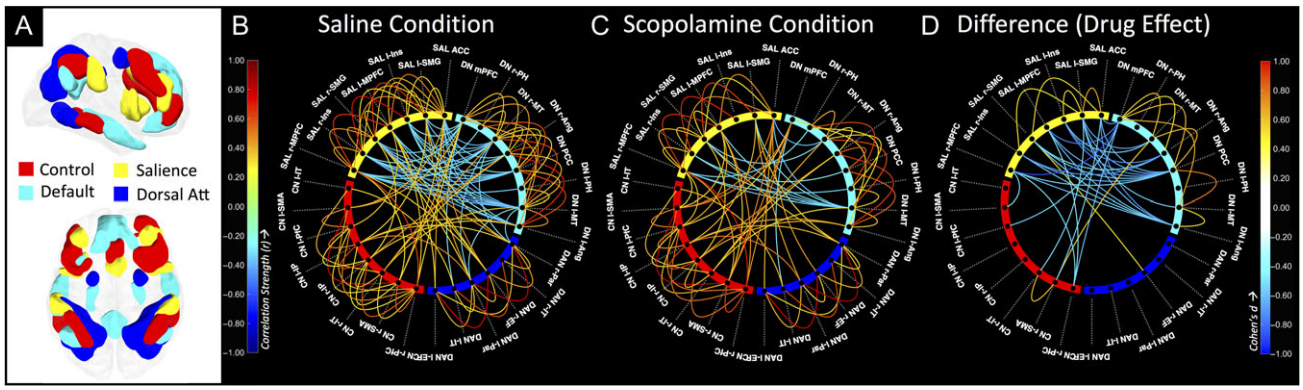

Figure 6. Nodal connectivity analyses on- and off-scopolamine. Panel (*A*) depicts node locations, colored by network assignment. Connectivity across nodes comprising 4 cognitive cortical networks was assayed following IV administration of saline (*B*) or low-dose scopolamine (*C*), using a one sample *t*-test (map threshold of $P < 10^{-6}$). Values shown are correlations in the BOLD signal across nodes, with positive correlations shown in warm colors, and anticorrelations in cool colors. Panel (*D*) depicts a paired *t*-test across saline and drug conditions, with colors scaled to resulting Cohen's *d* values (map threshold at $d > 0.5$, corresponding to $P = 0.007$). A full list of abbreviations and node locations can be found in Supplementary Figure S3.

induced changes in anticorrelation strength between the default and salience networks also predicted drug-induced changes in SRT performance (delayed recall: $r = 0.55$, $P = 0.0001$; LTS: $r = 0.61$, $P < 0.0001$), and statistically mediated the effects of scopolamine on SRT performance (Figs 5C and 7A,B).

Based on these results, we also examined Default to Salience anticorrelation as a predictor of cognitive performance cross-sectionally in the larger HABS cohort. Consistent with what was observed in the scopolamine challenge experiment, we observed a modest but significant positive relationship between SRT performance and Default to Salience anticorrelation (Storage, Free Recall, and Recognition all $P < 0.05$; Fig. 1). No significant correlations were observed between Default to Salience anticorrelation and the performance on TMT, VFDT, semantic, or phonemic fluency performance.

Discussion

We examined the hypothesis that administration of scopolamine, a commonly used antagonist of muscarinic AChRs, would alter connectivity within and between a subset of anatomically distributed cortical networks, and that these changes in connectivity would bear on drug-induced changes in memory performance. We observed that administration of

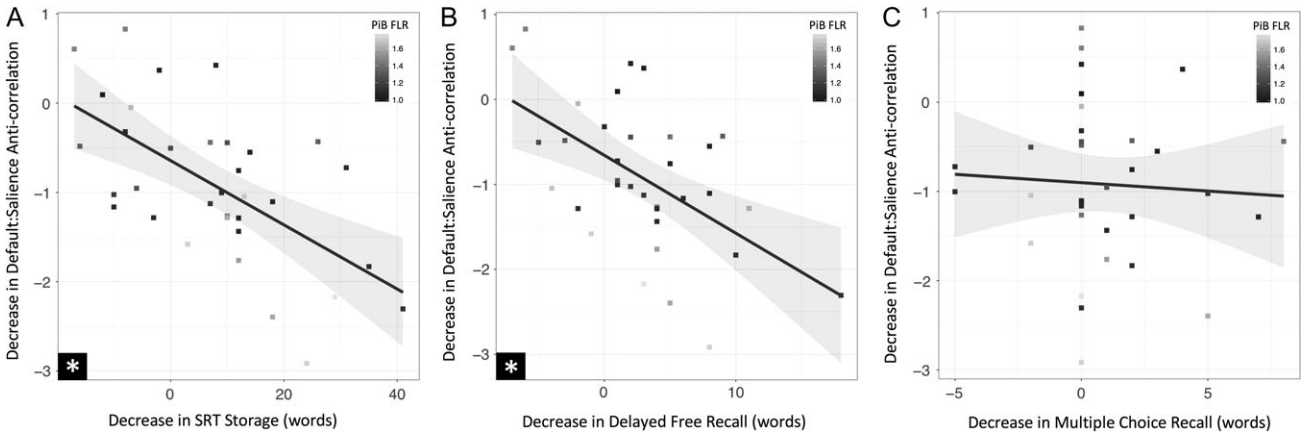

Figure 7. Relationships between default and salience network anticorrelation strength and memory performance. Average connectivity between all nodes of the Default and Salience networks was calculated on- and off-scopolamine. Change in anticorrelation with scopolamine is shown plotted against change in SRT performance with scopolamine (*A–C*). Drug-induced changes in SRT storage (*A*) and delayed free recall (*B*) correlated with drug-induced changes in anticorrelation strength between the Default and Salience networks. This same relationship was not present for multiple choice recall (*C*). *$P \leq 0.005$.

low-dose scopolamine to clinically normal elderly led to decreased connectivity in 3 of 7 examined cortical networks (Default, Salience, and Control), without altering the average connectivity strength across all 7 networks. In agreement with a rich literature on anticholinergic induced amnesia, scopolamine administration led to decreased performance on a challenging word-list memory task, especially on measures that required free recall of remembered items. Connectivity strength in the Default and Salience networks (both networks known to be degraded in AD; Greicius et al., 2003; Thomas et al., 2014; Jones et al., 2016), was predictive of memory performance both on- and off-drug and in a separate cross-sectional dataset. The extent to which scopolamine decreased connectivity in the Default or Salience networks was highly predictive of decrements in memory performance. Using mediation analyses, we show that changes in connectivity in either network could account for decreased memory performance following scopolamine administration, with no significant drug-induced direct effect on memory after controlling for connectivity in either the Default or Salience networks. Together these data support the critical involvement of the Default and Salience networks in mnemonic function and suggest that altered connectivity in these networks is a systems-level mechanism underlying anticholinergic amnesia. More broadly, these studies highlight the potential of rs-fcMRI as a tool to study the systems neuropharmacology of psychoactive drug action and the neural underpinnings of complex behavior.

Given the similarities between connectivity in the Default and Salience networks with respect to anticholinergic sensitivity and memory performance, we also examined whether the internetwork relationship between these 2 cognitive networks was altered by scopolamine. Consistent with prior work, we observed that the constituent nodes of the Default and Salience networks were highly anti-correlated at rest, and that this anticorrelation structure was present both on- and off-scopolamine. However, quantitative examination demonstrated that scopolamine administration led to significantly decreased anticorrelations between these 2 networks. Similar to the foregoing results with Default or Salience connectivity, the magnitude of decreased anticorrelation between the Default and Salience networks was highly predictive of altered memory performance and could serve as a full statistical mediator of scopolamine-induced decrements in memory performance. Though further work is needed to examine this issue, this novel finding suggests that cholinergic neurotransmission may be critical in mediating the anticorrelation between the Default and Salience networks, and that the maintenance of this internetwork anticorrelation structure may itself be important for successful memory performance. Prior work at the cellular and local circuit level suggest that modulating cholinergic tone can have profound effects on oscilliatory activity across neural ensembles (Fisahn et al. 1998; Betterton et al. 2015), suggesting a potentially plausible mechanism by which anticholinergic drug administration may modulate internetwork connectivity at the level of large-scale neural systems.

Prior studies suggest that administration of scopolamine may be useful as a means of unmasking amyloid positivity in preclinical AD, leveraging the hypothesis that subtly decreased cholinergic neurotransmission may already be present in very early stages of the disease (Román et al. 2014; Lim et al. 2015). However, we found little evidence in the present study to suggest that individuals with high amyloid burden were more susceptible to scopolamine-induced changes in memory performance or network connectivity. Additionally, no interaction between amyloid burden and connectivity in predicting memory performance was seen in the cross-sectional or scopolamine substudy datasets, indicating that elevated amyloid burden does not alter the relationship of network connectivity to memory performance. The result here largely agrees with a recent report (Lim et al. 2015) demonstrating that individuals with high amyloid burden treated with scopolamine showed similar peak impairments in a spatial associative memory task to their amyloid negative counterparts (albeit with significantly slower recovery in amyloid positive individuals at 5–8 h post drug administration). However, given the relatively small sample size of the present study and the potential for dose- and time-dependent interactions between cholinergic tone and amyloid burden, the lack of a simple interaction between amyloid and scopolamine in predicting memory performance observed here should be further tested in experiments that vary the doses and timing of the anticholinergic exposure.

Consistent with prior work in HABS (Shaw et al. 2015), connectivity in the Control network was significantly correlated with memory performance in the cross-sectional data. However, as demonstrated by the anticholinergic challenge, drug-induced changes in Control network connectivity were

not significant predictors of drug-induced changes in memory performance. Given that Control network connectivity is correlated with both Default and Salience network connectivity, this result may indicate that the Control network has a relationship to memory performance only to the extent that it relates to Default and Salience network connectivity.

That scopolamine induced decreases connectivity in Default and Salience network connectivity were readily observed in a relatively small sample of individuals suggests that rs-fcMRI may be a useful tool in understanding the systems-level pharmacology of psychoactive medications and in the development of experimental therapeutics aimed at reversing or preventing memory loss. In the present study, scopolamine-induced changes in Default and Salience network connectivity were generally more consistent and of similar or greater magnitude than drug-induced changes in behavior, hinting that rs-fcMRI has the potential to serve as a biologically relevant, intermediate measure of drug-behavior relationships in proof-of-concept trials. Importantly, scopolamine administration did not alter the relationship between connectivity and memory performance, but rather served to stretch the range of connectivity and memory performance in a manner that facilitated identification of networks closely tied to memory function in a relatively small sample (e.g., Supplementary Fig. S1). Accordingly, the results here suggest that pharmacologic manipulation of resting state networks may be a useful tool in determining the specialization of particular networks for particular complex behaviors.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Funding

Data collection for this project was supported by the Harvard Aging Brain study (HABS; P01AG036694, Sperling and Johnson PIs) and the BrightFocus Foundation. J.P.C. is grateful for fellowship support from the American Brain Foundation/American Academy Neurology, the BrightFocus Foundation, and from the National Institute on Aging (K23 AG049087). T.H. acknowledges the support of the National Institute on Aging (K01 AG040197). This research was carried out in part at the Athinoula A. Martinos Center for Biomedical Imaging at the Massachusetts General Hospital, using resources provided by the Center for Functional Neuroimaging Technologies, P41EB015896, a P41 Biotechnology Resource Grant supported by the National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health. This work also involved the use of instrumentation supported by the NIH Shared Instrumentation Grant Program and/or High-End Instrumentation Grant Program; specifically (Grant numbers S10RR023401, S10RR019307, S10RR019254, and S10RR023043). The project described was also supported by (Grant number 1 UL1 TR 001102 and 8 UL1 TR000170-05 and 1 UL1 RR025758-04), Harvard Clinical and Translational Science Center, from the National Center for Advancing Translational Science.

Notes

We gratefully acknowledge the altruism and dedication of our participants and their families, as well as the efforts of the HABS research staff for their contributions to this study. *Conflict of Interest*: None declared.

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