Acetylcholine modulates the temporal dynamics of human theta oscillations during memory

Tamara Gedankien[1], Ryan J. Tan[2], Salman E. Qasim[3], Joshua Jacobs[1,4,*], Bradley C. Lega[2,*]

[1]*Department of Biomedical Engineering, Columbia University, New York, NY, 10027, USA*
[2]*Department of Neurological Surgery, University of Texas Southwestern, Dallas, TX, 75390, USA*
[3]*Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA*
[4]*Department of Neurological Surgery, Columbia University, New York, NY, 10027, USA*

Summary

The cholinergic system is essential for memory. While degradation of cholinergic pathways characterizes memory-related disorders such as Alzheimer's disease, the neurophysiological mechanisms linking the cholinergic system to human memory remain unknown. Here, combining intracranial brain recordings with pharmacological manipulation, we describe the neurophysiological effects of a cholinergic blocker, scopolamine, in the human hippocampal formation during episodic memory. We found that the memory impairment caused by scopolamine was coupled to disruptions of both the amplitude and phase alignment of theta oscillations (2–10 Hz) during encoding. Across individuals, the severity of theta phase disruption correlated with the magnitude of memory impairment. Further, cholinergic blockade disrupted connectivity within the hippocampal formation. Our results indicate that cholinergic circuits support memory by coordinating the timing of theta oscillations across the hippocampal formation. These findings expand our mechanistic understanding of the neurophysiology of human memory and offer insights into potential treatments for memory-related disorders.

[*]These senior authors contributed equally. Correspondence: Joshua Jacobs (joshua.jacobs@columbia.edu) and Bradley C. Lega (bradley.lega@utsouthwestern.edu).

Introduction

Cholinergic pathways, which widely innervate the hippocampal formation, play a critical role in memory. Extensive research has shown that experimentally induced cholinergic blockade disrupts memory in both animals (Hamburg, 1967; Tang et al., 1997; McGaughy et al., 2005) and humans (Drachman and Leavitt, 1974; Ghoneim and Mewaldt, 1975; Atri et al., 2004). Further, patients with memory-related disorders such as Alzheimer's disease (AD) exhibit disruptions in cholinergic pathways (Bowen et al., 1976; Whitehouse et al., 1981), including brain-wide decreased density of cholinergic innervation (Aghourian et al., 2017). The effectiveness of cholinesterase inhibitors, which promote cholinergic function and help attenuate memory deficits in patients with AD, further underscores the importance of cholinergic circuits to human memory and the pathophysiology of dementia (Summers et al., 1986; Hampel et al., 2018). However, the specific neurophysiological mechanisms by which acetylcholine supports memory and cognition remain poorly understood in humans. Our limited knowledge creates an obstacle to the development of new therapeutic strategies for treating AD and the broader range of brain disorders related to cholinergic dysfunction (Terry, Jr., 2008; Müller and Bohnen, 2013; Karvat and Kimchi, 2014).

An established strategy for investigating the functions of cholinergic circuits involves measuring physiological changes following the administration of cholinergic antagonists, such as scopolamine. Scopolamine, which blocks muscarinic acetylcholine receptors, strongly impairs memory during spatial and visual memory tasks in animals (Savage et al., 1996; Pilcher et al., 1997; Huang et al., 2011; Melamed et al., 2017). In animals, administration of cholinergic antagonists disrupts the generation and tuning of hippocampal theta oscillations (Kramis et al., 1975; Teitelbaum et al., 1975; Monmaur et al., 1997; Asaka et al., 2000) whereas cholinergic agonists induce theta activity (Konopacki et al., 1987; Huerta and Lisman, 1993). Specifically, in rodents, cholinergic blockade inhibits immobility-related slow theta oscillations (4–7 Hz) but spares movement-related fast theta (7–12 Hz), revealing a distinction between cholinergic-sensitive slow theta ("Type 2") and cholinergic-resistant fast theta ("Type 1") (Kramis et al., 1975; Dunn et al., 2021). This link between cholinergic modulation and theta oscillations is important because extensive research has shown that hippocampal theta oscillations contribute critically to memory processing (Buzsáki, 2002; Burgess et al., 2002; Colgin, 2013; Orr et al., 2001), specifically by coordinating spike timing (O'Keefe and Recce, 1993; Lisman and Idiart, 1995; Rutishauser et al., 2010). Together, these results suggest that a mechanism by which cholinergic antagonists disrupt mnemonic behavior in animals is by impairing theta oscillations and associated neural processes (Newman et al., 2013).

In humans, the main behavioral effect of scopolamine is a disruption in the ability to encode new episodic memories (Ghoneim and Mewaldt, 1975; Petersen, 1977; Grober et al., 1989). Importantly, the effects of scopolamine are specific to memory encoding and not retrieval, as there is minimal impact for recalling items learned prior to drug administration (Atri et al., 2004; Huang et al., 2011). A separate line of research in humans shows that theta oscillations are critical for memory encoding, supporting the notion that the memory impairment from cholinergic blockade in humans could also result from changes in theta. Theta power in the human hippocampus reliably increases for successful episodic memory encoding (Sederberg et al., 2003; Lega et al., 2012). Further, previous studies show that inter-trial phase locking in the theta band predicts successful memory (Rizzuto et al., 2006; Fell et al., 2008; Kleen et al., 2016; Lin et al., 2017; Kota et al., 2020; Cruzat et al., 2021), and that theta networks synchronize during memory processing (Eichenbaum, 2000; Solomon et al., 2017; Gruber et al., 2018; Choi et al., 2020). Computational models and associated experiments further suggest that acetylcholine promotes the shifting of hippocampal theta networks between encoding and retrieval states that preferentially occur at different phases of theta (Hasselmo et al., 2002; Douchamps et al., 2013; Newman et al., 2013). Together, these findings support a hypothesis by which the cholinergic system regulates theta power and phase dynamics that underlie successful memory encoding.

Administering cholinergic drugs offers a direct intervention to understand the physiological effects of cholinergic tone in the human brain and examine its potential link to theta oscillations. Here, combining intracranial brain recordings, pharmacological manipulation, and behavioral experiments, we provide the first account of the neurophysiological effects of an anticholinergic drug in the human hippocampal formation during episodic memory encoding. We administered a single dose of scopolamine (or saline, in a placebo condition) to twelve epilepsy patients prior to a verbal episodic memory task. Based on animal models, we hypothesized that scopolamine would

disrupt theta oscillations in the human hippocampal formation. As such, we analyzed three physiological phenomena linked to theta oscillations and memory in humans: oscillatory power, phase reset and synchrony. We found that scopolamine impaired memory for every subject and this impairment was accompanied by a selective disruption in slow (2–4 Hz) theta power during encoding. We also found that scopolamine disrupted the phase reset of theta oscillations at the time of encoding, and that the magnitude of this disruption correlated with memory impairment. Lastly, we demonstrated that scopolamine disrupted theta-band synchrony within the hippocampal formation. These findings demonstrate that cholinergic blockade significantly influences oscillatory dynamics of theta power and phase in the hippocampal formation, suggesting potential strategies to restore memory in diseased states via selective modulation of theta oscillations.

Results

Scopolamine impairs episodic memory encoding. To investigate the neurophysiological effects of cholinergic modulation on memory, we asked twelve patients with surgically implanted electrodes to perform a verbal episodic memory task after double-blind administration of a cholinergic blocker, scopolamine, or a placebo (saline). The subjects in these experiments were neurosurgical epilepsy patients who had intracranial electroencephalographic (iEEG) electrodes implanted throughout their brain for seizure mapping. The electrode coverage was extensive across the hippocampal formation, including bilateral anterior and posterior hippocampus, and entorhinal cortex (Table 1). We recorded iEEG signals during both placebo and scopolamine sessions. In each trial of the episodic memory task, subjects learned a list of words and then, after a short math distractor, tried to recall as many words as possible (Fig. 1A). To assess how the disruption of cholinergic processes affects cognition, we compared subjects' performance during free recall and the math distractor task between the scopolamine and placebo conditions.

Subjects' memory performance significantly decreased in the presence of scopolamine. Every subject individually showed a significantly lower recall probability in the scopolamine condition compared to placebo (all p's < 0.05, χ^2 tests), with the magnitude of this decrease ranging from approximately 20 to 100% (Fig. 1C). At the group level, the disrupted memory performance from scopolamine was significant, with subjects remembering an average of 31.2% ± 3.27% of items with saline, and only 10.3% ± 3.56% with scopolamine (mean ± SEM; $t(12) = 6.19$, $p < 10^{-4}$, paired t-test) (Fig. 1B). Across subjects, the magnitude of the scopolamine-related memory disruption was not explained by variations in patients' weight (Fig. S1B), age ($r = 0.101$, $p = 0.754$, Pearson's correlation), or the interaction between weight and age ($p > 0.05$, linear mixed effect (LME) model). The effects of scopolamine were sustained throughout the experiment, as there were no significant changes in mean recall probability across trials within sessions (Fig. S2C). These results, showing the robust disruption of human episodic memory encoding following scopolamine administration, are consistent with previous experiments (Ghoneim and Mewaldt, 1975; Petersen, 1977; Grober et al., 1989).

To better understand how scopolamine impacted the mechanisms of memory encoding, we examined whether it affected memory for item order during recall (Murdock, 1962). We found that, while memory was universally altered by scopolamine, there were no differences in the temporal dynamics of memory performance, such as the serial position of recalled items and the recall order (Fig. 1E–G, two-way ANOVA tests). There was also no significant difference in the rate of subjects recalling items from previous lists (list intrusions, see Fig. S2B). These results are consistent with notion that scopolamine specifically affects encoding-related processes (Atri et al., 2004; Huang et al., 2011), insofar as when encoding succeeded, recall dynamics were unaffected.

We found additional evidence that scopolamine selectively affects episodic memory encoding by analyzing subjects' performance in the math distractor task. At the end of each word list, subjects performed a distractor task in which they solved a series of math problems. Notably, performance on this math task did not significantly change due to scopolamine. Accuracy on the math task was consistently high in both scopolamine and control conditions (placebo: 91.9% ± 8.0%, scopolamine: 91.6% ± 7.4%, $t(12) = 0.42$, $p = 0.68$, paired t-test) (Fig. 1D). Consistent with earlier work (Higgs et al., 2000), reaction times were slower with scopolamine ($t(12) = -3.04$, $p = 0.01$, paired t-test) (Fig. S2A). Overall, these results indicate that the cognitive effects of scopolamine are selective, most strongly disrupting episodic memory encoding.

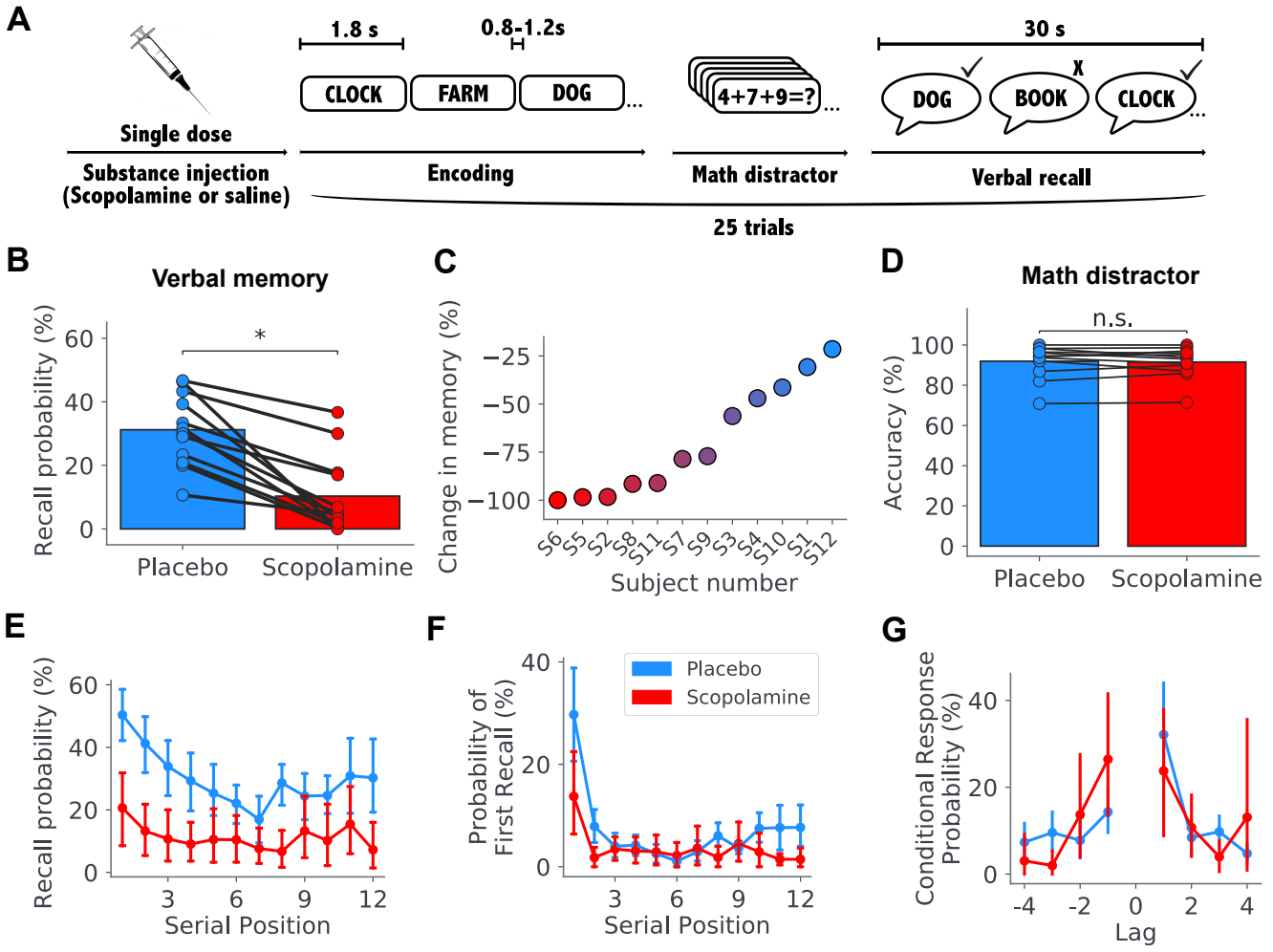

Figure 1: **Scopolamine impairs episodic memory encoding. A.** Diagram of the task structure: prior to the task, subjects received a single dose of scopolamine (or saline, in a placebo condition). During the encoding period of the task, subjects are presented with a sequence of twelve words. Then, after completing a math distractor consisting of simple algebra equations, subjects are asked to verbally recall as many words as possible from the encoding period. **B.** Bar plot showing differences in recall probability between the placebo and scopolamine sessions for all subjects ($t(12) = 6.19$, $p < 10^{-4}$, paired t-test). **C.** Percent decrease in recall probability with respect to the placebo session for all subjects. **D.** Bar plot showing differences in accuracy in the math distractor between the placebo and scopolamine sessions ($t(12) = 0.42$, $p = 0.68$, paired t-test). **E.** Recall probability by word serial position during encoding. Error bars indicate the 95% confidence interval. **F.** Probability of first recall (PFR) by word serial position during encoding. Error bars indicate the 95% confidence interval. **G.** Conditional response probability (CRP). Error bars indicate the 95% confidence interval.

Scopolamine disrupts memory-evoked slow theta oscillations and elicits broadband neural activity. We next examined the power of neuronal oscillations during memory encoding in the presence of scopolamine, building off work showing that theta oscillations in the human temporal lobe exhibit increased power during successful memory encoding (Sederberg et al., 2003; Lega et al., 2012; Staudigl and Hanslmayr, 2013; Kota et al., 2020). We used spectral analysis (see *Methods*) to examine the power of neuronal oscillations at each electrode after item presentation during the encoding phase of the task. For each electrode, we measured power at frequencies between 2 and 32 Hz, and normalized the measured power relative to the mean and standard deviation of the baseline period 500 ms before word onset at each session, electrode, and frequency (Fig. 2A–C). We then compared encoding-related shifts in theta power between scopolamine and control sessions.

As expected from earlier work (Sederberg et al., 2003; Lega et al., 2012; Staudigl and Hanslmayr, 2013; Kota et al., 2020), in the placebo condition theta oscillations across the hippocampal formation showed increased theta power during memory encoding. However, following scopolamine administration, this power increase was

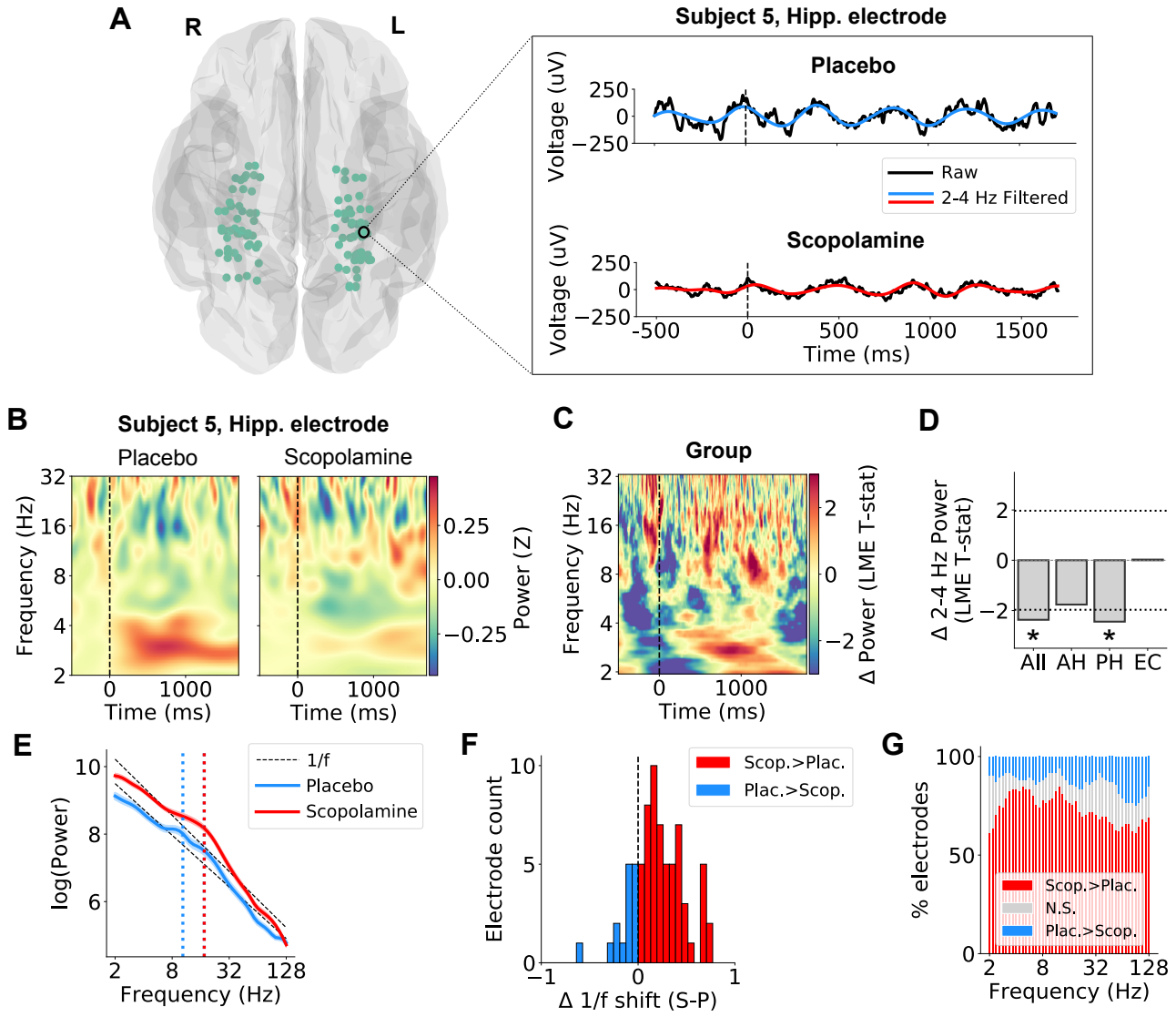

Figure 2: **Scopolamine disrupts memory-evoked slow theta oscillations and elicits broadband neural activity. A.** Left: anatomical locations of all electrodes analyzed in this study. Right: example trials from an hippocampal electrode from subject 5, showing onset of slow theta oscillations following encoding cue (t=0). **B.** Time–frequency spectrogram showing memory-evoked normalized power for the same example electrode across all trials. The increase in 2–4 Hz slow theta power seen during the placebo session is not present during the scopolamine session. **C.** Group-level time–frequency spectrogram showing differences in normalized power between the placebo and scopolamine conditions. Contrast denotes t-statistics from linear mixed effect (LME) models computed at every frequency and timepoint. **D.** Plot showing statistical differences in time-averaged 2–4 Hz normalized power between placebo and scopolamine for electrodes within all hippocampal formation subregions combined (All), anterior hippocampus (AH) only, posterior hippocampus (PH) only, and entorhinal cortex (EC) only. Dashed lines indicate 95% confidence intervals. Asterisks denote statistical significance ($p < 0.05$, LME model, multiple-comparison corrected). **E.** Power spectral density (PSD) for an example hippocampal electrode from subject 2 during encoding. Shading denotes ± SEM. **F.** Distribution of offset change values (scopolamine − placebo) across all electrodes. **G.** Percentage of electrodes showing a significance increase in power at each frequency across trials for each condition ($p < 0.05$, Wilcoxon rank-sum test).

significantly lower (Fig. 2A,B). To assess the robustness of this pattern, we implemented a linear mixed effects (LME) model that tested for differences in the time-averaged power during encoding for slow (2–4 Hz) and fast (4–10 Hz) theta frequency bands separately. We found that power was significantly lower during scopolamine compared to placebo, and this effect was restricted to the 2–4-Hz slow theta band (Fig. 2C–D, Fig. S3). Notably, this 2–4-Hz band is the same range where theta power was linked to episodic memory encoding in earlier human studies (Lega et al., 2012; Staudigl and Hanslmayr, 2013; Jacobs, 2014). This effect was strongest in the posterior hippocampus (PH: $t(23) = -2.44$, $p < 0.05$, LME model, multiple-comparison corrected) (Fig. 2D), and it was

present when including all encoding trials as well as forgotten trials only (PH: $t(23) = -2.82$, $p < 0.05$, LME model, corrected). Across subjects, encoding-related theta power changes did not correlate with scopolamine concentration ($r = 0.091$, $p = 0.790$, Pearson's correlation) or with the magnitude of memory impairment induced by scopolamine (Fig. S11A).

In addition to examining the effect of scopolamine on narrowband oscillations, we measured broadband neural signals, which capture aperiodic activity in the brain (Miller et al., 2009) and reveal the balance between excitation and inhibition in local neural circuits (Gao et al., 2017; Manning et al., 2009). We measured broadband power (2–120 Hz) throughout memory encoding (Donoghue et al., 2020) (see *Methods*), and compared the magnitude of broadband power between the scopolamine and placebo conditions. Across all trials, broadband power was significantly higher in the scopolamine condition compared to placebo. An example of this effect can be seen in Fig. 2E, where the measured power for one electrode was greater at all frequencies for scopolamine compared to placebo. Because this effect was present at all frequencies, the difference primarily reflects a broadband pattern rather than narrowband oscillations. This effect was robustly present at the population level (Fig. 2F,G), where the group of hippocampal electrodes showed significantly greater broadband power at all frequencies in the presence of scopolamine (p's< 0.05, Wilcoxon rank-sum tests).

These results indicate that scopolamine changes the power of iEEG activity during memory processing by disrupting encoding-related narrowband slow theta oscillations within a background of increased broadband activity. These results are consistent with a model by which cholinergic blockade leads to a disruption in rhythmicity among neuronal populations, thus resulting in a decrease in the amplitude of theta oscillations and a simultaneous increase in aperiodic neuronal excitation.

Scopolamine disrupts the phase reset of theta oscillations. Drawing upon evidence that the phase of ongoing neuronal oscillations supports memory processes (Fell et al., 2008; Gupta et al., 2012; Jutras et al., 2013; ter Wal et al., 2021), we examined how scopolamine modulated the phase dynamics of theta oscillations and whether those changes were linked to behavior. First, to ensure we were measuring robust theta oscillations, we implemented an established oscillation-detection procedure (Hughes et al., 2012) (see *Methods*). Next, we computed the inter-trial phase coherence (ITPC) during memory encoding to test for phase reset of theta oscillations following stimulus presentation (Tallon-Baudry et al., 1996; Van Diepen and Mazaheri, 2018). For a given electrode, the ITPC quantifies the consistency of the phase alignment of neuronal oscillations at particular timepoints across trials (Fig. 3A). We found that theta oscillations in the placebo condition showed significant phase reset following stimulus presentation, with individual electrodes resetting to various phases (Fig. S6). However, the magnitude of theta phase reset was significantly lower in the scopolamine condition. This disruption of theta phase reset by scopolamine can be seen robustly in signals from individual electrodes (Fig. 3B) as well as at the group level (Fig. 3D, see also Fig. S6) ($n = 69$, $p < 0.05$, cluster permutation test).

Because the magnitude of memory impairment varied across subjects (Fig. 1C), we considered the possibility that changes in phase reset vary with intersubject differences in memory performance. To test this hypothesis, we computed the correlation between the mean change in phase reset and changes in memory performance caused by scopolamine across subjects. We found a significant correlation between the magnitude of the change in theta-band phase reset and the severity of memory impairment ($r = 0.739$, $p = 0.015$, Pearson's correlation) (Fig. 4B). Thus, subjects who showed more severe memory impairments from scopolamine exhibited a greater disruption of theta phase reset. This effect was stronger in the fast theta band (4–10 Hz) (Fig. 4B), but it was also present in the slow theta band (2–4 Hz) ($r = 0.658$, $p = 0.039$, Pearson's correlation) (Fig. S4).

We considered the possibility that scopolamine's apparent disruption of theta phase reset reflected a disruption of an evoked signal or changes in power rather than the phase of an ongoing oscillation. To evaluate these possibilities, we first examined whether changes in event-related potentials (ERPs) were related to changes in power (Van Diepen and Mazaheri, 2018). We did not find significant correspondence between the magnitude of power changes and ERP differences (Fig. S7A,B), and we also did not find a significant correlation between peak ERP amplitude changes and time-matched power changes ($r = -0.117$, $p = 0.330$, Pearson's correlation) (Fig. S7C). Also, changes in phase reset did not significantly correlate with changes in either slow or fast theta power (p's > 0.4) (Fig. S5). Consistent with suggested best practices for these analyses (Sauseng et al., 2007; Van Diepen and Mazaheri, 2018), these results

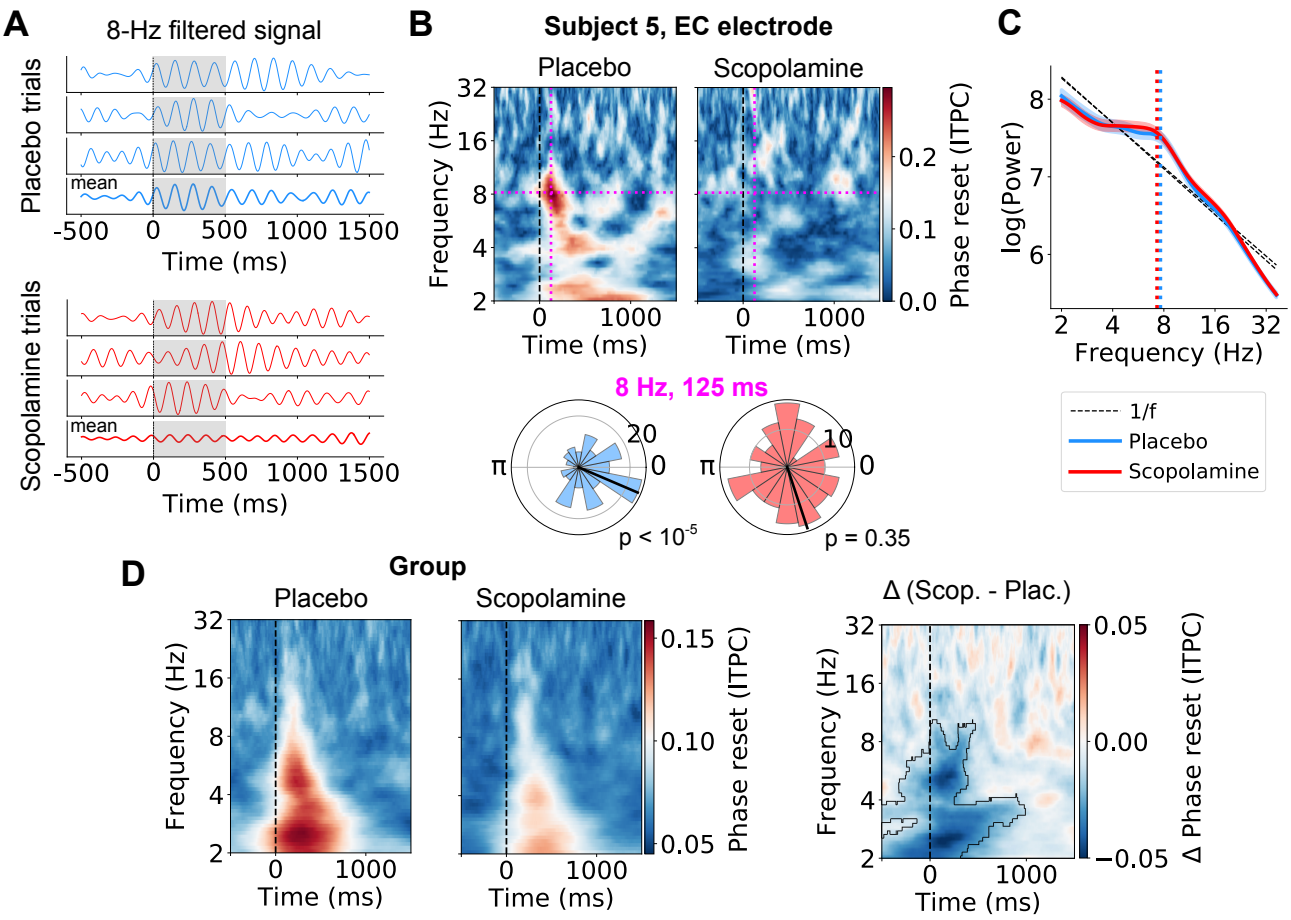

Figure 3: **Scopolamine disrupts the phase reset of theta oscillations. A.** 8-Hz-filtered EEG from an example entorhinal cortex (EC) electrode from subject 2 for selected trials. Unlike the placebo condition, the 8-Hz filtered oscillations in the scopolamine condition do not show phase alignment across trials. **B.** Top: Inter-trial phase coherence (ITPC, or phase reset) during encoding for the same example electrode for the placebo and scopolamine conditions (0 ms denotes time of encoding cue). The increase in 8-Hz theta phase reset seen during the placebo session is not present during the scopolamine session. Bottom: circular histograms showing phase distributions at 8 Hz and 125 ms following encoding cue for placebo (blue) and scopolamine (red). **C.** Power spectral density (PSD) between 2–32 Hz for the same example electrode. Black dashed line indicates power that exceeds the 1/f baseline. Blue and red dashed lines indicate the presence of an 8-Hz oscillation for both the placebo and scopolamine conditions, respectively. Shading denotes ± SEM. **D.** Left: group-level mean phase reset across all hippocampal formation electrodes for the placebo and scopolamine conditions. Right: group-level mean change in phase reset between placebo and scopolamine. Black outline indicates significant clusters of change in phase reset ($n = 69$, $p < 0.05$, cluster permutation test).

indicate that the observed phase reset cannot be explained by changes in ERPs or power, and instead more directly reflect changes in the dynamics of theta phase.

Scopolamine disrupts phase synchrony in the theta band. Previous studies showed that theta oscillations synchronize neuronal activity across brain regions during memory processing (Eichenbaum, 2000; Kaplan et al., 2014; Solomon et al., 2017; Clouter et al., 2017). We therefore tested whether scopolamine impacted phase synchronization within the hippocampal formation during memory encoding.

To measure the synchrony of the activity between different electrode locations, we first measured the phase of the theta oscillations at each timepoint throughout the recordings. Next, for all pairs of electrodes within a subject's hippocampal formation, we measured oscillatory synchrony by comparing the differences in the instantaneous phase between the electrodes. If two electrodes were synchronized then their phase difference should be approximately constant over extended periods of time. Figure 5A shows two illustrative examples of this phenomenon in the data. In one sample trial from the placebo session (Fig. 5A, left panel), a pair of hippocampal electrodes showed a

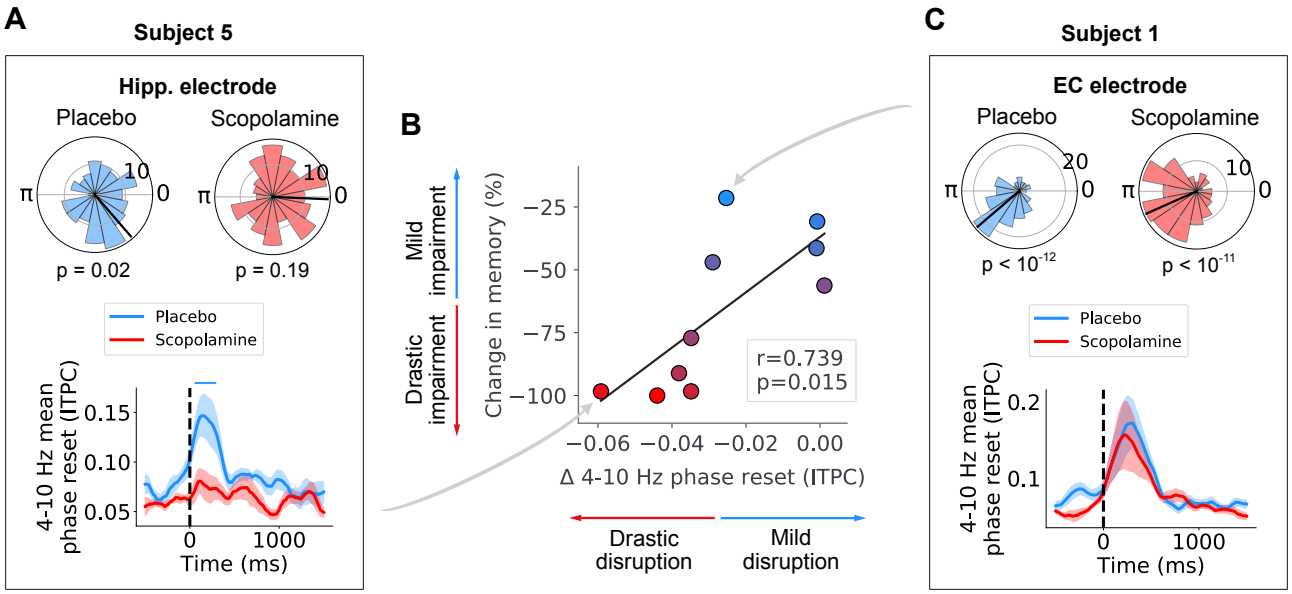

Figure 4: **Theta phase reset disruption correlates with memory impairment. A.** Top: circular histograms from example hippocampal electrode from subject 5, displaying significant preferred phase during placebo but not during scopolamine conditions ($p = 0.02$ and $p = 0.19$, respectively, Rayleigh tests). Bottom: line plot showing mean phase reset across all electrodes for subject 5. Shading denotes ± SEM. Bar denotes statistical significance ($p < 0.05$, Wilcoxon rank-sum test). **B.** Correlation between subjects' mean phase reset changes in fast theta during encoding and subjects' percent change in memory ($r = 0.739$, $p = 0.015$, Pearson's correlation). Subjects with greater memory impairment following scopolamine showed greater disruption in phase reset. **C.** Top: circular histograms from example entorhinal cortex (EC) electrode from subject 1, displaying significant preferred phase during placebo and scopolamine conditions ($p < 10^{-12}$ and $p < 10^{-11}$, respectively, Rayleigh tests). Bottom: line plot showing mean phase reset across all electrodes for subject 1. Shading denotes ± SEM.

consistent phase difference of $\sim \frac{\pi}{2}$ rad for a 6–8 Hz-filtered theta oscillation. In a trial from the scopolamine session (Fig. 5A, right panel), this same electrode pair showed a wide range of phase differences for a theta oscillation of the same frequency range. Thus, whereas there was strong synchrony between the signals measured across this electrode pair when the subject had taken a placebo, this synchrony was disrupted in the presence of scopolamine.

To quantify this phenomenon systematically, we measured the strength of phase synchrony between all electrode pairs by computing the phase locking value (PLV; see *Methods*) both in the placebo and scopolamine conditions Lachaux et al. (1999). The PLV provides a quantitative measure of the phase alignment between two electrodes, ranging between 0 (no alignment) and 1 (perfect alignment). For each electrode pair, we tested whether the mean PLV significantly shifted between placebo and scopolamine conditions.

Overall, many electrode pairs showed significant decreases in PLV following scopolamine administration, relative to placebo. This effect can be seen in different narrowband frequencies within the theta band, with the precise band often varying between subjects (e.g., 6–8 Hz in Fig. 5B, and 2–3 Hz in Fig. 5C). At the group level, a significant number of electrode pairs exhibited significant decreases in the level of theta-band (2–10 Hz) synchrony for the scopolamine condition compared to placebo ($z = -4.24$, $p < 10^{-4}$, two-proportion z-test) (Fig. 5D). This effect was driven by an increase in theta synchrony during memory encoding in the placebo condition, as this increase was not present during the non-memory baseline period (dotted lines, Fig. 5D). Together these results indicate that scopolamine disrupts connectivity within the hippocampal formation by impairing the phase synchrony of theta-band oscillations during encoding. Notably, this effect was present for intra- as well as inter-hemispheric electrode pairs (Fig. S10A), suggesting that cholinergic pathways have a role in coordinating the timing of hippocampal activity both within and across hemispheres.

To test whether changes in synchrony might relate to the patterns of phase reset described above, we repeated this synchrony analysis after excluding the first 500 ms following word onset, which was the interval that showed the strongest phase reset). Here, we also found decreased theta-band synchrony following scopolamine compared to placebo ($z = -3.94$, $p < 10^{-4}$, two-proportion z-test) (Fig. S8B). Additionally, we did not find a significant

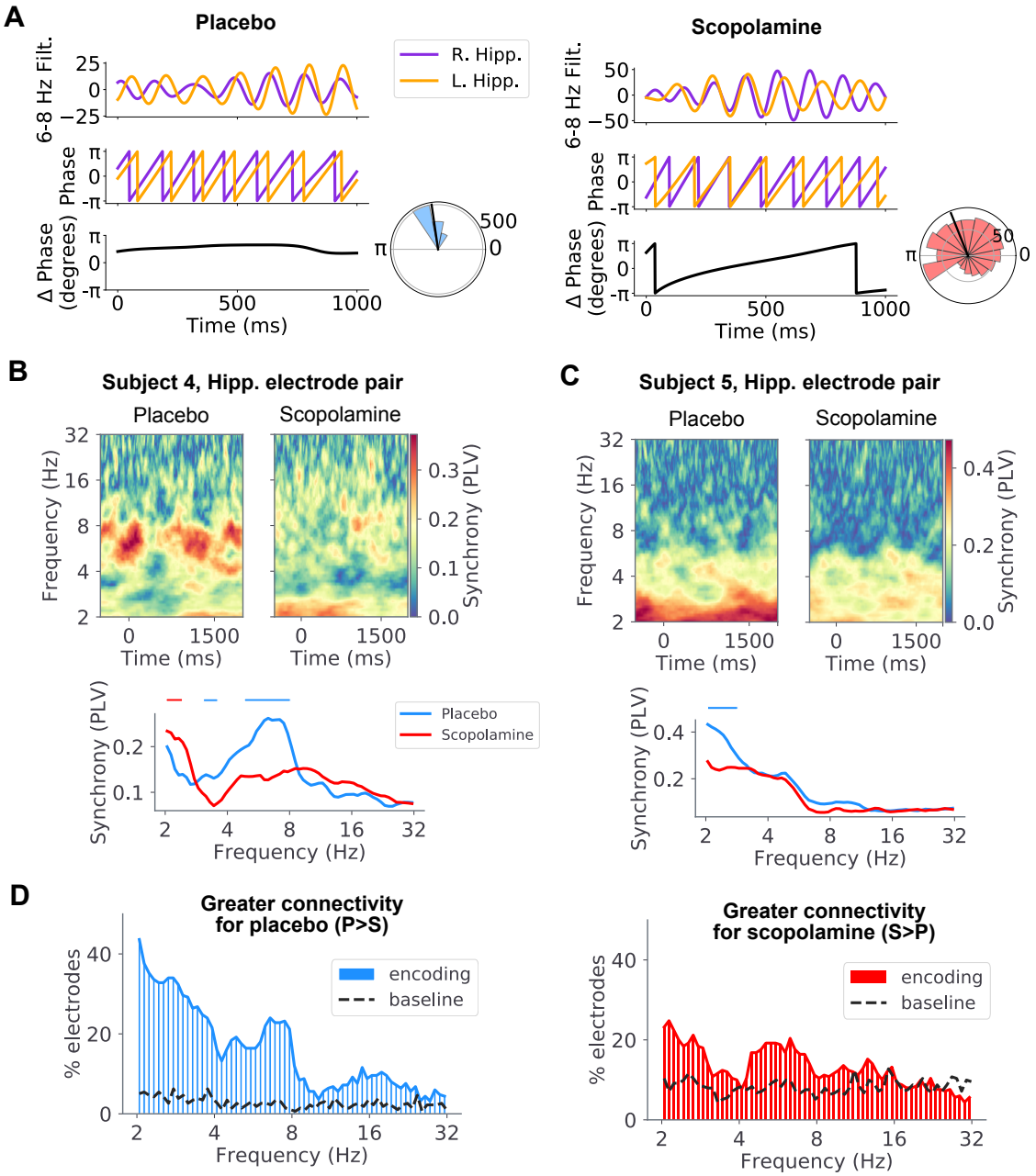

Figure 5: **Scopolamine disrupts phase synchrony in the theta band. A.** Top-down: 6-8Hz filtered traces, phases, phase differences, and circular histograms showing the phase differences between a right and left hippocampal electrode pair during an example trial in the placebo and scopolamine conditions for subject 4. **B.** Top: Time-frequency spectrogram showing the phase-locking values (PLVs) for an hippocampal electrode pair from subject 4 during encoding for the placebo and scopolamine sessions. Warmer colors indicate greater PLV (synchrony). Bottom: Mean PLV over time for the time-frequency spectrogram shown above. Bars indicate frequency windows where statistical significance was present (blue = Plac. > Scop., red = Scop. > Plac., $p < 0.05$, Wilcoxon rank-sum test). **C.** Top: Time-frequency spectrogram showing the PLVs for an hippocampal electrode pair from subject 5 during encoding for the placebo and scopolamine sessions. Warmer colors indicate greater PLV (synchrony). Bottom: Mean PLV over time for the time-frequency spectrogram shown above. Bars indicate frequency windows where statistical significance was present (blue = Plac. > Scop., red = Scop. > Plac., $p < 0.05$, Wilcoxon rank-sum tests). **D.** Percentage of electrodes showing significant increases in synchrony at each frequency for placebo (left; blue) and scopolamine (right; red) conditions ($p < 0.05$, Wilcoxon rank-sum test). Dotted lines indicate the percentage of electrodes showing significant increases in synchrony during the baseline period (see *Methods*).

correlation between changes in synchrony and changes in power ($r = -0.057$, $p = 0.373$, Pearson's correlation) (Fig. S8A) or a significant difference in the peak frequency of the oscillations present in each conditions ($p > 0.05$, Wilcoxon rank-sum test) (Fig. S9), indicating that differences in power do not explain our observations of differences in phase synchrony.

Overall, these findings show that scopolamine decreases connectivity, as measured by theta phase synchrony, across the hippocampal formation. Taken together with our analyses of oscillatory power and phase reset, our findings suggest that cholinergic circuits critically underlie the generation and synchronization of theta oscillations in the hippocampal formation.

Discussion

Despite extensive evidence of the importance of the cholinergic system for episodic memory encoding, the neurophysiological mechanisms that underlie this relationship remain poorly understood in humans. Here, using rare intracranial recordings, we described the physiological effects of cholinergic blockade on the human hippocampal formation during episodic memory. We demonstrated that scopolamine administration suppressed the amplitude of oscillations in the slow theta band (2–4 Hz) (Fig. 2), and interrupted the resetting of theta phase following stimulus presentation (Fig. 3). Across subjects, this disruption of phase reset correlated with the magnitude of memory impairment caused by scopolamine (Fig. 4). Scopolamine also disrupted the synchrony of theta oscillations across the hippocampal formation (Fig. 5). These findings suggest that one role for cholinergic processes in memory is to coordinate the temporal dynamics of neuronal activity, in particular by allowing network patterns related to theta rhythms to flexibly change their timing to match behavioral events. Because theta oscillations coordinate the timing of single-neuron spiking across the brain (O'Keefe and Recce, 1993; Jacobs et al., 2007; Rutishauser et al., 2010), our results suggest that scopolamine may impair memory by disrupting the theta-dependent temporal coordination of neuronal activity across the hippocampal memory network.

Our behavioral findings build on a line of studies that examined the behavioral effects of cholinergic blockers in animals (Savage et al., 1996; Tang et al., 1997; Pilcher et al., 1997; McGaughy et al., 2005; Huang et al., 2011; Cai et al., 2012; Okada et al., 2015; Melamed et al., 2017) and humans (Ghoneim and Mewaldt, 1975; Petersen, 1977; Grober et al., 1989; Atri et al., 2004). In humans, cholinergic blockade disrupts episodic memory encoding (Ghoneim and Mewaldt, 1975; Petersen, 1977; Atri et al., 2004). Our behavioral results replicate these findings, because we found that scopolamine significantly impaired verbal episodic memory encoding during free recall, without impairing performance in a non-memory control task (solving math problems). Our results also demonstrated that key features of the behavioral dynamics of recall, such as recall order, were preserved under scopolamine. These findings suggest that scopolamine primarily impairs the overall efficiency of memory encoding, as measured by the number of items remembered, rather than changing higher-order memory processes that supports recall dynamics.

In addition to behavioral studies in humans, research in animals has focused on the physiological effects of cholinergic antagonists. In rodents, cholinergic antagonists ablate slow theta oscillations ("Type 2") but spare movement-related fast theta oscillations ("Type 1") (Kramis et al., 1975). Our human results are consistent with these findings, as we found that during memory encoding scopolamine disrupted the amplitude of slow theta (2–4 Hz) but not fast theta (> 4 Hz) oscillations. However, we also found key differences in our data compared to animals, which suggests physiological differences between humans and animals (Jacobs, 2014). In contrast to rodents, where there is a strict dissociation between cholinergic-sensitive slow theta and cholinergic-insensitive fast theta oscillations (Kramis et al., 1975; Jeewajee et al., 2008), here we found scopolamine-related phase and synchrony disruptions at a range of theta-band frequencies. Based on earlier intracranial recording studies in humans, our findings of cholinergic effects across a broad range of theta oscillations is perhaps unsurprising, because memory-related activity was previously observed across the 2–10-Hz spectrum (Sederberg et al., 2003; Lega et al., 2012; Staudigl and Hanslmayr, 2013). Thus, our findings are most consistent with a model by which cholinergic modulation causes band-specific disruptions to theta power and phase, which may support distinct aspects of memory processing.

Computational models have proposed a number of mechanisms for how cholinergic processes support memory, including the notions that acetylcholine enhances encoding by modulating synaptic excitability and enhancing theta-band oscillations (Hasselmo, 1999, 2006). Consistent with these models, we found that scopolamine affected

slow theta oscillations following item presentation during memory encoding—notably, this is the specific time period that shows memory-related changes in theta power in earlier studies (Sederberg et al., 2003; Lega et al., 2012). Acetylcholine release modulates synaptic excitability (Picciotto et al., 2012), thereby changing resonance properties of neuronal populations. Our findings support the hypothesis that scopolamine disrupts theta generation by modulating the engagement of separate theta-generating neurons and causing them to oscillate more independently from their neighbors. The consequences of disrupting this rhythmicity, especially in populations of interneurons (Alger et al., 2014), are diminished amplitude of theta oscillations and simultaneous increase in aperiodic neuronal excitation. In human intracranial brain recordings, the mean level of neuronal excitation can be estimated from the mean broadband power across all frequencies (Miller et al., 2009; Manning et al., 2009). Consistent with this model, we show that broadband power increased in the presence of scopolamine (Figure 2E–G). These hypothesized effects of cholinergic blockade on hippocampal neuronal populations could also help explain the disrupted phase patterns we observed following scopolamine administration. Scopolamine may disrupt the ability of local oscillators to synchronize with projecting inputs (Ermentrout and Kleinfeld, 2001), thus causing local oscillators that generate theta-band rhythms to function more independently with decreased coupling to neighboring networks. This decreased oscillatory coupling may explain our finding of decreased bilateral theta synchrony following scopolamine, as well as our finding of decreased theta phase reset, in which hippocampal theta rhythms normally reset following perceptual inputs (Mormann et al., 2005). Taken together, our findings support a link between neuron–level changes in oscillatory activity observed in rodent models and large–scale changes in the human hippocampal formation.

Theta oscillations are known to be important for coordinating spike timing, and this may explain why the theta disruption from scopolamine causes memory impairment. In animals and humans, item and context representations encoded by hippocampal neurons require coordination with local theta oscillations via phase precession (O'Keefe and Recce, 1993; Qasim et al., 2021) and phase locking (Rutishauser et al., 2010; Yoo et al., 2021). Based on this literature, our results suggest that the way in which cholinergic blockade impairs memory performance is by disrupting theta-related temporal dynamics, and thus disrupting the normally organized timing of single-neuron spiking. One study in rodents is consistent with this view, showing that scopolamine in rodents disrupted hippocampal neurons' phase precession while sparing rate coding (Venditto et al., 2019). Given the role of theta phase in modulating the timing of neuronal spiking (Siegel et al., 2009; Rutishauser et al., 2010), we hypothesize that reset of theta phase that occurs after stimulus presentation is important because it causes particular phase-coupled neuronal assemblies to activate at precise timepoints during stimulus processing. When phase resetting is disrupted by scopolamine, it therefore impairs the optimal timing patterns of neuronal spiking that normally occur. Further, our connectivity analysis revealed that scopolamine impaired regional connectivity by disrupting theta-band synchrony within and between bilateral brain regions. Together, these findings suggest that cholinergic blockade may impair memory encoding by disrupting the precise timing of neuronal activity across the bilateral hippocampal network that is normally coordinated by theta oscillations.

Since scopolamine was applied intravenously, we cannot exclude the possibility that the impact of cholinergic blockade on non-mnemonic circuits indirectly affected our obsevations of impaired memory encoding. A known side effect of scopolamine is pupil dilation, which leads to decreased visual contrast sensitivity and impaired visual recognition (Aigner and Mishkin, 1986; Sherman et al., 2003), which could impair memory. However, we think it is unlikely that vision impairments generated our results because in our task words were presented in a large and legible font, and no subjects reported issues with reading and comprehension. A second potential limitation of our study is that we could not rule out effects of scopolamine on other cognitive functions beyond memory, such as those related to attention. However, because we found the preservation of performance during our math control task, it also suggests that an effect on attentional system would be secondary to that on memory systems. Consistent with view, a number of prior studies show that the effects of scopolamine were focused on the hippocampal formation (Teitelbaum et al., 1975; Monmaur et al., 1997; Heys et al., 2012; Asaka et al., 2000) and demonstrate that scopolamine specifically impairs episodic memory encoding, which is a behavior known to be hippocampally dependent (Tulving and Markowitsch, 1998). Additionally, previous studies in implant patients demonstrate that the human hippocampus expresses muscarinic acetylcholine receptors (Ayhan et al., 2021), which are directly and specifically targeted by scopolamine (Klinkenberg and Blokland, 2010), supporting the view that scopolamine acts directly on memory systems.

Our results suggest a number of important questions for future research to clarify the specific mechanisms by which acetylcholine-dependent theta oscillations support the computational processes underlying memory. For example, it will be important to determine whether acetylcholine and theta are differentially vital for encoding versus retrieval, as suggested previously (Hasselmo and McGaughy, 2004), as well as for the encoding of familiar versus novel items (McGaughy et al., 2005). Another area of future investigation includes administering cholinergic agonists to intracranial patients, and test whether the theta disruption described here can be reversed to cause memory enhancement via upregulation of theta phase and power.

In summary, our findings provide evidence that the cholinergic system is closely involved in the oscillatory dynamics of theta power and phase in the human hippocampal formation, and support a model by which cholinergic-sensitive theta oscillations in 2–10-Hz range support complementary aspects of memory formation. More broadly, our findings suggest that cholinergic circuits play a role in regulating the temporal dynamics of theta-band activity and allowing it to vary with behavior to support episodic memory formation. Going forward, our results provide a roadmap for using pharmacological modulation as a tool to identify the specific physiological mechanisms of human cognition, in particular dissociating how slow and fast theta oscillations differentially support aspects of memory processing. Lastly, our findings suggest that improving the temporal coordination of neuronal activity, specifically those involving theta oscillatory dynamics, may provide a novel route to treating memory-related disorders such as AD, as well the broader range of brain diseases related to cholinergic dysfunction.

Methods

Participants. The study included twelve subjects who were undergoing intracranial electroencephalographic (iEEG) monitoring as part of their treatment plan for medication-resistant epilepsy. The patients had intracranial depth electrodes that were surgically implanted for the purpose of seizure localization. Participants came from the University of Texas Southwestern (UTSW) epilepsy surgery program across a time span of two years.

Scopolamine administration. Participants received a 0.5-mg dose of scopolamine or saline (placebo) via an intravenous line. Intravenous scopolamine starts taking effect about five minutes after administration, and has a half-life of approximately sixty-nine minutes (Renner et al., 2005). Subjects completed the free-recall task approximately fifteen minutes following injection. A second session using the alternate agent (scopolamine or saline) took place the following day or at least four half-lives after the first session. The test administrator and participant were blinded to the drug/placebo randomization.

Ethics and safety. The protocol was approved by the UTSW Institutional Review Board on Human Subjects Research prior to data collection, and all participants provided informed written consent. Physiologic monitoring of blood pressure, electrocardiogram (EKG), oxygen saturation, and mental status was performed just prior to and for one hour after scopolamine injection. Intravenous scopolamine was administered by the participant's attending nurse, and a board-certified anesthesiologist was present at the time of injection and remained available throughout the duration of the experiment to treat any potential adverse reactions. Low doses of scopolamine (such as 0.5 mg) are commonly administered in clinical settings and generally do not pose major risk factors (Renner et al., 2005). There were no reports of adverse events from substance administration in either the drug or placebo conditions.

Memory task. Each participant completed two sessions (drug and placebo) of a delayed free-recall task. During encoding subjects were presented with 12 words, one at a time, and later during retrieval they were asked to verbally recall as many words as possible from the list. Each word was displayed in capital letters for 1800 ms, followed by an 800–1200 ms blank interstimulus interval (ISI) to decorrelate physiological responses from successive word presentations. Words were selected at random, without replacement, from a pool of nouns (http://memory.psych.upenn.edu/WordPools). Following each list presentation, subjects were given a series of simple arithmetic problems in the form A + B + C = ?, where A, B and C were random positive integers. Next, a row of asterisks accompanied by a 300-ms, 60-Hz auditory tone was presented to indicate the start of the recall period. During the 30-s recall period, subjects were instructed to recall as many words as they could (in any order) from

the most recent list. Each session included 25 lists of 12 words, for a total of 300 encoded words (Fig. 1A). At the beginning of each list, there was a baseline period in which subjects stared at a 10-s countdown on the screen.

Intracranial recordings. Patients were implanted with either Ad-Tech Medical or PMT depth electrodes with 0.8 mm in outer diameter, forming 10 to 14 recording contacts arrayed at 4–5 mm intervals along the shaft of the electrode (Ad-Tech: 10 electrodes with variable spacing; PMT: 10 to 14 electrodes with uniform spacing, depending upon the overall depth of insertion). Intracranial EEG was sampled at 1 kHz on a Nihon–Kohden 2100 clinical system under a bipolar montage with adjacent electrodes as reference. Electrodes from hemispheres with radiographic abnormalities including temporal sclerosis or previous neurosurgery were excluded from analysis. A kurtosis algorithm (with a maximum amplitude threshold of 4) was used to exclude abnormal events and interictal activity. One subject (S8) was excluded from the neural analyses due to excessive noise. Our hippocampal formation electrode coverage included contacts in the anterior hippocampus (AH; n=40), posterior hippocampus (PH; n=26), mid-hippocampus (n=8), and entorhinal cortex (EC; n=12).

Anatomical localization. Participants had intracranial depth electrodes implanted at locations specified by the neurology team. Electrodes were laterally inserted into the specified regions with robotic assistance. Final electrode localization in the hippocampal formation subregions was determined by post-operative expert neuroradiology review.

Power analyses. For the memory-evoked power analysis, spectral power was extracted using a continuous Morlet wavelet transform (wave number = 6) across 64 logarithmically spaced frequencies from 2 to 32 Hz. The analysis included all 1800-ms encoding periods following word onset, and 3000-ms buffers to avoid edge artifacts. Power measures at each session, electrode and frequency were normalized relative to the mean and standard deviation of the baseline period 500 ms before word onset. To establish significant differences between the conditions, a linear mixed effects (LME) model was implemented using time-averaged power measures during encoding and setting condition (scopolamine/placebo) as fixed effects and electrodes as a random effects. We used this LME framework because it accounts for intersubject differences in power measures, which are common for human hippocampal theta (Goyal et al., 2018). For the broadband power analysis, we extracted spectral power across 2–120-Hz and parameterized the aperiodic activity of the power spectral density (PSD) of each electrode (Donoghue et al., 2020). This method involves recursively fitting the aperiodic background curve (1/f) to the observed smoothed spectral signal, resulting in two parameters: an offset (or intercept) and a slope (or exponent). We assessed statistically significant differences in broadband power in two ways. First, we computed the distribution of the aperiodic offsets for all electrodes in each condition. Second, for each electrode and at each frequency we computed the difference of the PSD between the two conditions using Wilcoxon rank-sum tests.

Phase reset analysis. In order to ensure phase values reflected true oscillations, we first implemented an established oscillation-detection routine ("Better Oscillation Detection" method, or BOSC) (Hughes et al., 2012), which detects both sustained and transient oscillatory activity using thresholds for power and duration. Next, we extracted phase measures using a continuous Morlet wavelet transformation (wave number = 6) across 64 logarithmically spaced frequencies from 2 to 32 Hz. We computed the phase reset of oscillations at word onset using the inter-trial phase coherence (ITPC) measure (Tallon-Baudry et al., 1996; Van Diepen and Mazaheri, 2018) as follows:

$$ITPC(f,t) = \frac{1}{N}\left|\sum_{k=1}^{N} e^{i\varphi^k(f,t)}\right| \tag{1}$$

where f is the frequency for a given time t, N is the number of trials, and and $e^{i\varphi^k}$ is the polar representation of the phase angle φ. This measure was computed separately for individual electrodes, and averaged at the subject-level for the group analysis. To assess significant differences in ITPC between conditions, we used an electrode-level nonparametric cluster permutation-based approach to correct for multiple comparisons across time and frequency (Maris and Oostenveld, 2007). To avoid spurious phase measures, we only computed the ITPC for electrodes that showed spectral peaks in the theta frequency range (2–10 Hz) (Donoghue et al., 2020). Since ITPC calculations are

highly susceptible to the number of observations, we excluded one subject (S7) who had fewer than sixty total trials in the scopolamine condition. Phase reset differences used in all correlations were based on measurements at the timepoint of the maximal phase reset difference between conditions (± 150 ms).

Phase synchrony analysis. To assess connectivity within the hippocampal formation, we computed the phase-locking value (PLV) for all possible electrodes pairs within a subject for each condition (Lachaux et al., 1999). The PLV is a continuous measure between 0 and 1 that indicates how synchronized two electrodes are across trials. A low PLV means that the electrode pair is completely unsynchronized (i.e., they possess varying phase differences over time), whereas a high PLV indicates that the electrodes are highly synchronized (i.e., they always show a constant phase difference). The PLV is computed as follows:

$$PLV(t) = \frac{1}{N}\left|\sum_{k=1}^{N} e^{j(\phi_1(t,k)-\phi_2(t,k))}\right| \tag{2}$$

where $\phi_1(t,k) - \phi_2(t,k)$ is the phase difference between electrodes. Significant differences between conditions were assessed using Wilcoxon rank-sum tests of the time-averaged PLVs for each electrode pair.

Event-related potential analysis. To test whether phase reset measures were driven by event-related potentials (ERPs), we assessed whether changes in ERP significantly matched changes in power (1–10 Hz). Significant differences between conditions were assessed using Wilcoxon rank-sum tests at each timepoint -500 ms prior to and 1500 ms following encoding cue for each electrode. To compute the correlation between ERP and power changes, we used the timepoint of maximal ERP difference between conditions (± 150 ms) and its corresponding power measures in time.

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Author contributions statement

B.L. conceived the study and performed surgical procedures; T.G., S.E.Q., J.J., and B.L. designed the data analyses; T.G. and R.J.T. implemented the data analysis; and T.G., J.J., and B.L. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Subject ID	Gender	Weight (lb)	Seizure onset zone (SOZ)	Electrode coverage			
				AH	PH	MH	EC
S1	M	220	R. Amygdala	5	3	0	2
S2	M	198	L. Temporal / Parietal	4	3	0	0
S3	M	209	R. Orbital Frontal	2	0	0	3
S4	M	343	L. Orbital Frontal, R. Hippocampus	4	4	0	4
S5	F	166	R. Prefrontal	5	0	0	1
S6	F	104	L. Hippocampus, R. Fusiform Gyrus	2	1	3	2
S7	M	180	R. Hippocampus	5	5	5	0
S8	F	208	L. Posterior Insula, L. Temporal Operculum	4	3	0	4
S9	F	195	Bi. Heterotopia	4	2	0	0
S10	F	150	R. Posterior Hippocampus	3	3	0	0
S11	M	260	L. MFG Prefrontal, SFG Mesial Cortex	1	2	0	0
S12	M	207	R. Frontal Operculum	5	3	0	0

Table 1: **Subject demographics and electrode coverage.** L: Left, R: Right, Bi: Bilateral, MFG: Middle Frontal Gyrus, SFG: Superior Frontal Gyrus, AH: Anterior Hippocampus, PH: Posterior Hippocampus, MH: Mid-Hippocampus, EC: Entorhinal Cortex.

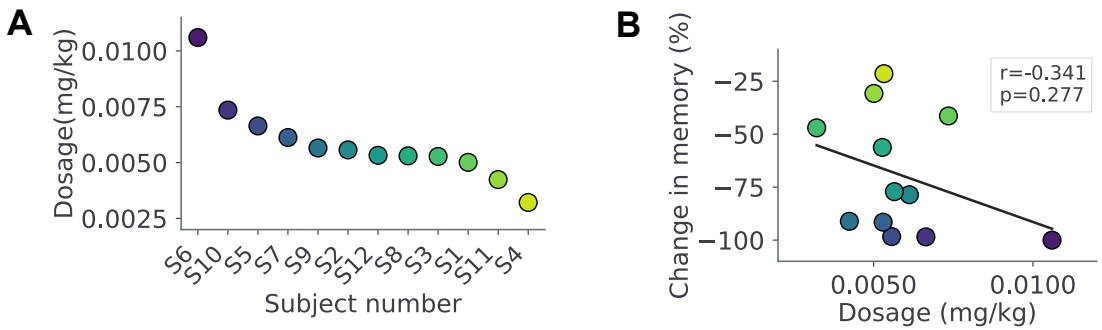

Figure S1: **Effect of scopolamine concentration on memory. A.** Scopolamine concentration (i.e., scopolamine dosage divided by subject's weight) for each subject. **B.** Subject-level correlation between scopolamine concentration and change in memory performance. Change in memory performance is not significantly correlated with scopolamine concentration, although there was a trend for subjects with higher concentrations to have higher degrees of memory impairment.

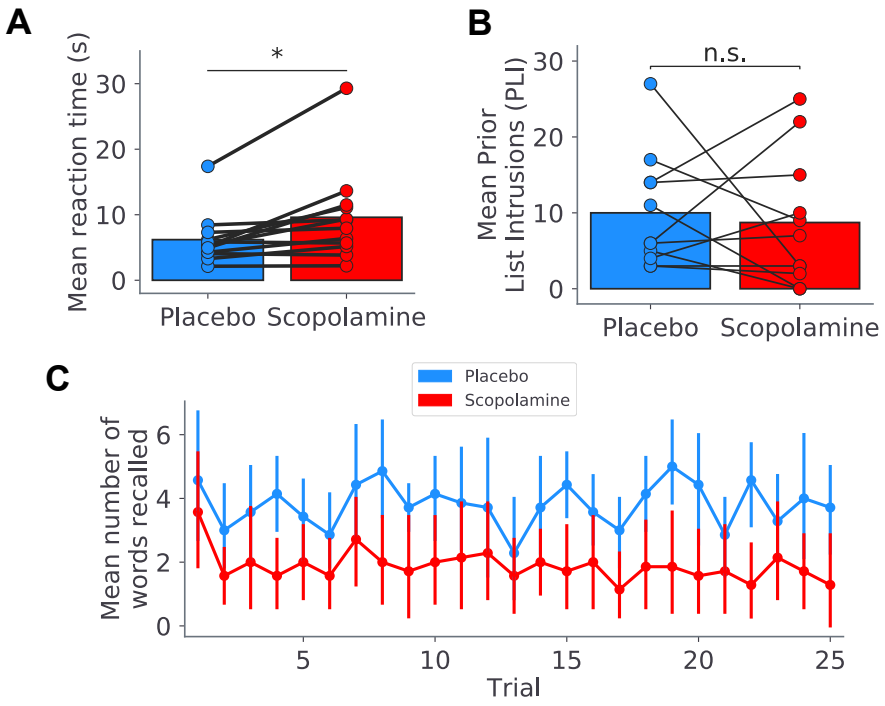

Figure S2: **Additional analyses on the behavioral effects of scopolamine. A.** Bar plot showing subject-level mean reaction time during the math distractor for each condition ($t(12) = -3.04$, $p = 0.01$, paired t-test). **B.** Bar plot showing subject-level mean number of prior list intrusions (PLI) for each condition ($p > 0.05$, paired t-test). **C.** Mean number of words recalled across trials. Each session was made up of 25 trials of 12 words each.

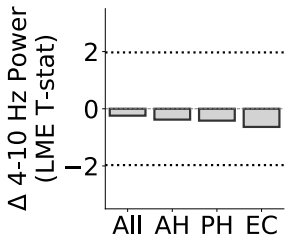

Figure S3: **Changes in fast theta power between placebo and scopolamine.** Plot showing the t-statistics of a linear mixed effects (LME) model comparing power changes between placebo and scopolamine for electrodes across all hippocampal formation subregions combined (All), anterior hippocampus (AH) only, posterior hippocampus (PH) only, and entorhinal cortex (EC) only. Dashed lines indicate 95% confidence interval.

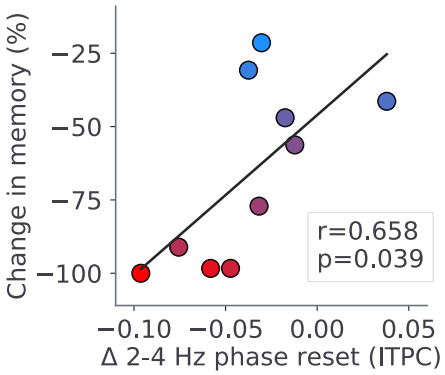

Figure S4: **Relation between slow theta phase reset and severity of memory impairment from scopolamine.** Correlation between changes in slow theta (2–4 Hz) phase reset and changes in memory (r = 0.658, p = 0.039, Pearson's correlation).

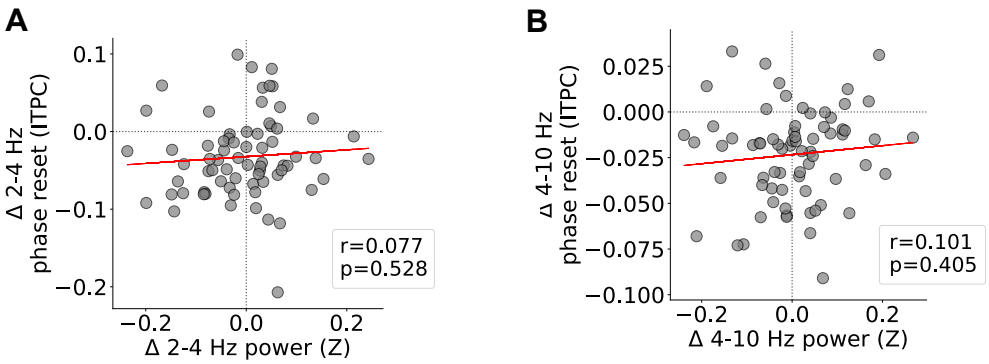

Figure S5: **Changes in power do not explain changes in phase reset. A.** Correlation between changes in slow theta (2–4 Hz) power and changes in slow theta phase reset across all electrodes (r = 0.077, p = 0.528, Pearson's correlation). **B.** Correlation between changes in fast theta (4–10 Hz) power and changes in fast theta phase reset across all electrodes (r = 0.101, p = 0.405, Pearson's correlation).

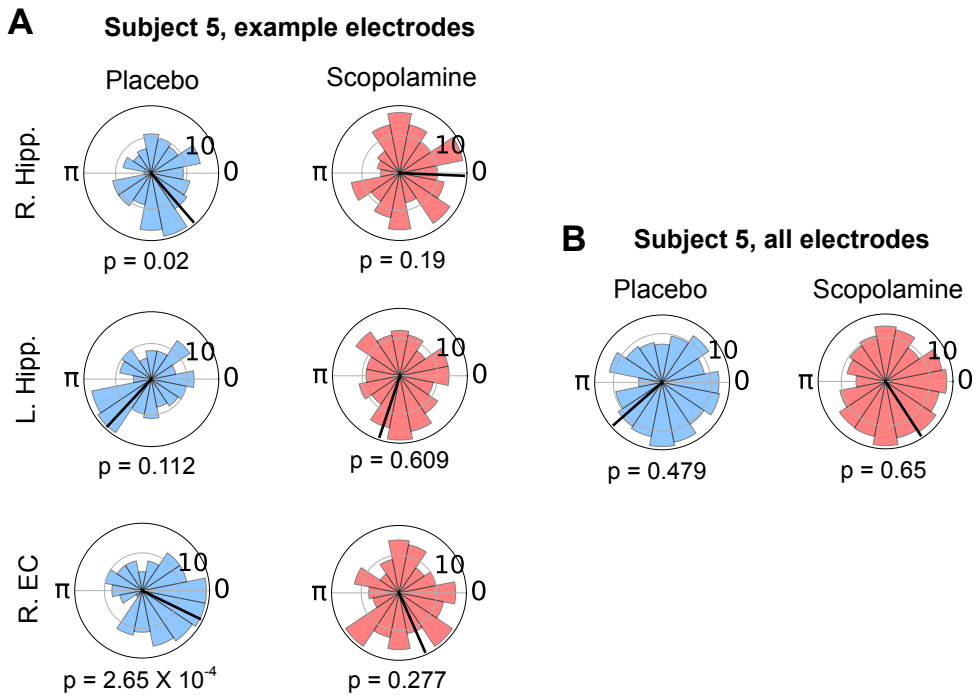

Figure S6: **Individual electrodes reset to different preferred phases. A.** Circular histograms of three example electrodes from subject 5, displaying phase distribution of a 8-Hz oscillation at 100 ms following encoding cue during placebo and scopolamine conditions (Rayleigh tests). Each electrode resets to a different preferred phase angle. **B.** Circular histograms showing the phase distribution across all electrodes for subject 5. Because individual electrodes reset to different preferred phase angles, the phase distribution at the subject-level is nearly uniform.

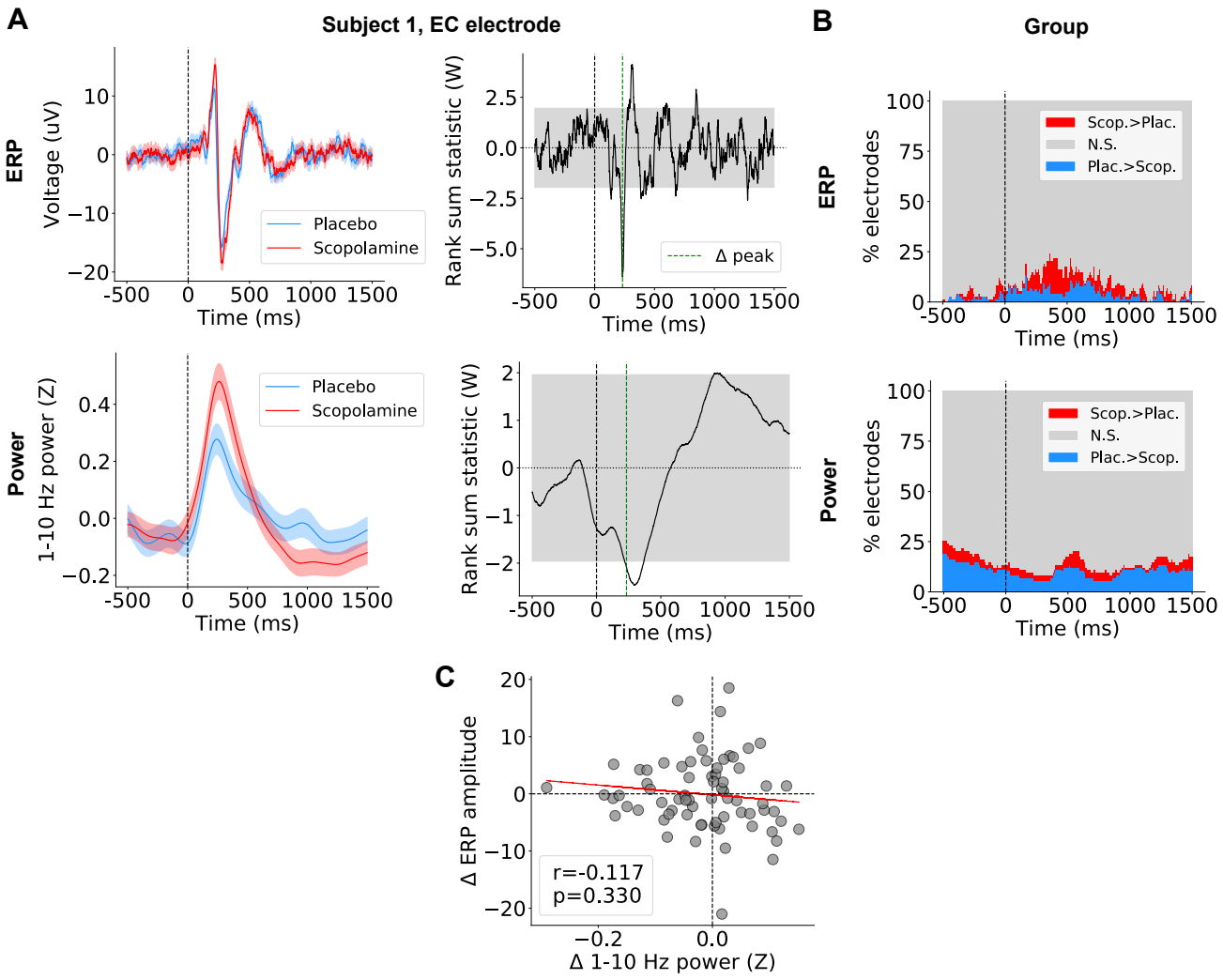

Figure S7: **Event-related potentials (ERPs) do not explain changes in power. A.** Top: mean ERP for an example electrode (left) and corresponding mean ERP differences between placebo and scopolamine (Wilcoxon rank-sum test) (right). Bottom: mean 1–10-Hz power for the same example electrode (left) and corresponding mean power differences between placebo and scopolamine (Wilcoxon rank-sum test) (right). Dashed green line denotes timepoint of maximal ERP difference between conditions. **B.** Group-level percentage of electrodes showing significant increase for placebo (blue = Plac. > Scop.), scopolamine (red = Scop. > Plac.), or no significant changes (gray = N.S.) for ERP measures (top) and power measures (bottom). The distribution of significant differences between placebo and scopolamine differs between ERP and power measures. **C.** Correlation between changes in ERP amplitude (measured at the point of maximal ERP difference between conditions, with a 150-ms buffer on each side) and changes in 1–10-Hz power at those same points in time. Peak amplitude changes in ERP do not significantly correlate with changes in power ($r = -0.117$, $p = 0.330$, Pearson's correlation).

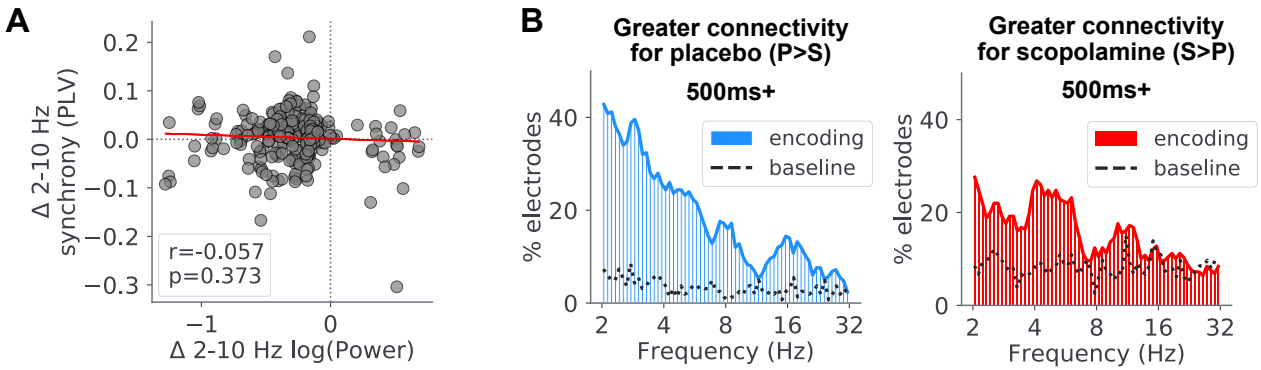

Figure S8: **Changes in power and phase reset do not explain changes in synchrony. A.** Correlation between changes in normalized power and changes in synchrony (PLV) across all electrode pairs ($r = -0.057$, $p = 0.373$, Pearson's correlation). **B.** Percentage of significant electrodes showing increase connectivity for placebo (left; blue) and scopolamine (right; red) after excluding the first 500 ms following word onset where the phase reset effect is strongest ($z = -3.94$, $p < 10^{-4}$, two-proportion z-test). Dotted lines indicate the percentage of electrodes showing significant increases in synchrony during baseline.

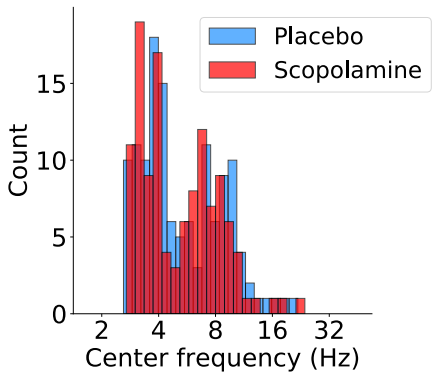

Figure S9: **Distribution of peak frequencies does not differ between conditions.** Distribution of non-baseline-corrected power peak frequencies for placebo and scopolamine ($p > 0.05$, Wilcoxon rank-sum test).

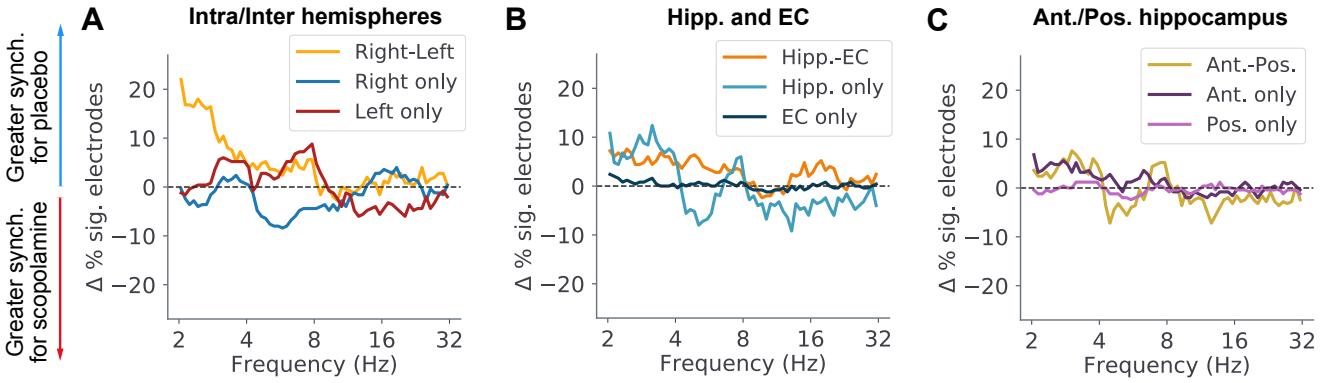

Figure S10: **Connectivity disruptions within and across hemispheres and different subregions. A.** Change in percentage of electrode pairs showing significantly greater connectivity for placebo and scopolamine, separated by inter-hemispheric (Right-Left) and intra-hemispheric (Right only and Left only) electrode pairs. **B.** Change in percentage of electrode pairs showing significantly greater connectivity for placebo and scopolamine, separated by hippocampus-entorhinal cortex (Hipp.-EC), hippocampus only (Hipp. only), and entorhinal cortex only (EC only) electrode pairs. **C.** Change in percentage of electrode pairs showing significantly greater connectivity for placebo and scopolamine, separated by anterior-posterior hippocampus (Ant.-Pos.), anterior hippocampus only (Ant. only), and posterior hippocampus only (Pos. only) electrode pairs.

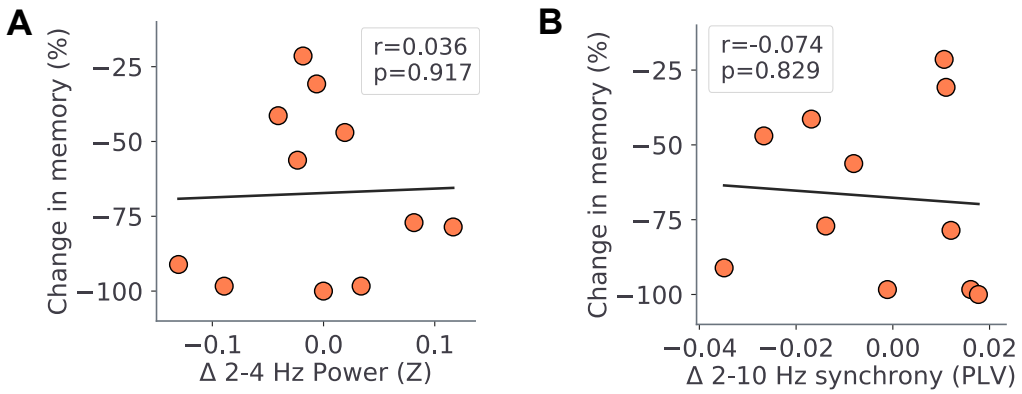

Figure S11: **Effects of memory on power and synchrony. A.** Subject-level correlation between changes in 2–4 Hz normalized power and changes in memory ($r = 0.036$, $p = 0.917$, Pearson's correlation). Changes in power measures do not explain changes in memory following scopolamine. **B.** Subject-level correlation between changes in 2–10 Hz synchrony measures and changes in memory ($r = -0.074$, $p = 0.829$, Pearson's correlation). Changes in synchrony measures do not explain changes in memory following scopolamine.

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