



Acetylcholine modulates human working memory and subsequent familiarity based recognition via alpha oscillations



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ABSTRACT

Working memory (WM) can be defined as the ability to maintain and process physically absent information for a short period of time. This vital cognitive function has been related to cholinergic neuromodulation and, in independent work, to theta (4–8 Hz) and alpha (9–14 Hz) band oscillations. However, the relationship between both aspects remains unclear. To fill this apparent gap, we used electroencephalography (EEG) and a within-subject design in healthy humans who either received the acetylcholinesterase inhibitor galantamine (8 mg) or a placebo before they performed a Sternberg WM paradigm. Here, sequences of sample images were memorized for a delay of 5 s in three different load conditions (two, four or six items). On the next day, long-term memory (LTM) for the images was tested according to a remember/know paradigm. As a main finding, we can show that both theta and alpha oscillations scale during WM maintenance as a function of WM load; this resembles the typical performance decrease. Importantly, cholinergic stimulation via galantamine administration slowed down retrieval speed during WM and reduced associated alpha but not theta power, suggesting a functional relationship between alpha oscillations and WM performance. At LTM, this pattern was accompanied by impaired familiarity based recognition. These findings show that stimulating the healthy cholinergic system impairs WM and subsequent recognition, which is in line with the notion of a quadratic relationship between acetylcholine levels and cognitive functions. Moreover, our data provide empirical evidence for a specific role of alpha oscillations in acetylcholine dependent WM and associated LTM formation.

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Introduction

Neural oscillations in the theta and alpha frequency band provide a physiological mechanism for working memory (WM) functions (Klimesch et al., 2010). For instance, electroencephalography (EEG) and magnetoencephalography (MEG) recordings in humans revealed increases in power (Jensen and Tesche, 2002; Onton et al., 2005) or a reset of phase (Tesche and Karhu, 2000) of frontal midline theta oscillations (~5–8 Hz, here “high theta”) during WM tasks. Similarly, power in the low theta (2–4 Hz) range increased during WM in the human hippocampus and at fronto-central EEG electrodes (Lega et al., 2012; Mizuhara and Yamaguchi, 2011; Vugt et al., 2010). Finally, the alpha band (9–14 Hz), was also shown to be modulated during WM maintenance in a similar fashion as the theta band (Gevins et al., 1997; Jensen and Tesche, 2002).

While WM is critical for immediate adaptive behavior, there is also a strong link to subsequent long-term memory (LTM) formation. For instance, neural activity during WM maintenance in the prefrontal cortex, occipital cortex, hippocampus and parahippocampal structures is predictive of LTM (Blumenfeld and Ranganath, 2006; Ranganath et al., 2005; Schon et al., 2004). Importantly, electrophysiological studies could show enhanced alpha and theta oscillations during WM for remembered items in a subsequent LTM task (Khader et al., 2007). Therefore, these and other studies (Axmacher et al., 2010; Davachi et al., 2001) demonstrated a functional and anatomical overlap for WM and LTM functions, with alpha and theta oscillations being particularly important for successful memory encoding.

Despite this evidence, the precise neural mechanisms underlying the link between theta/alpha oscillations, WM and associated LTM formation still remain unclear. One little explored avenue involves the role of acetylcholine (ACh) (Picciotto et al., 2012) – a key neuromodulator in learning and memory (Bentley et al., 2011; Hasselmo, 2006). While cholinergic antagonists impair WM (Aigner and Mishkin, 1986) and encoding of novel information in explicit memory tasks (Sherman et al., 2003), cholinergic agonists can have opposite effects (Buccafusco et al., 2005). Importantly,

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there is also evidence in favor of an inverted u-shaped relationship between ACh levels and performance in a variety of tasks (Bentley et al., 2011; Newhouse et al., 2004). As a consequence, pro-cholinergic drugs do not necessarily improve behavior and increase associated activity in high functioning cortical regions of healthy subjects but may have detrimental effects.

Physiologically, the cholinergic system (Hasselmo and Sarter, 2011; Mesulam, 2004; Picciotto et al., 2012) originates in the basal forebrain (including the medial septum) and projects to the medial temporal lobe (MTL, including the hippocampus and surrounding structures), where it generates and sets the pace of theta oscillations (Lee et al., 1994). However, there are also cholinergic projections to the entire neocortex, which nicely fits to the notion of the basal forebrain being involved in modulating theta, alpha, beta and gamma oscillations within and between the frontal cortex, dorsal hippocampus and central amygdala (Sanchez-Alavez et al., 2014). Although these studies point towards a critical role of the cholinergic system in neural theta and alpha oscillations, its relationship to WM maintenance and subsequent LTM in humans remains unclear.

To investigate this issue, healthy human subjects received the cholinesterase inhibitor galantamine (8 mg) or placebo on two different test-days, and subsequently performed a Sternberg WM task including complex scene stimuli that were presented sequentially in three different load conditions (two, four, six items) (Eckart et al., 2014). During the WM task, brain activity was measured using scalp EEG recordings, and recognition memory for the presented images was tested one day later. On the basis of previous work (see above), we hypothesized that stimulating the healthy cholinergic system leads to changes in WM and LTM. These behavioral effects were expected to be paralleled by effects on neural oscillations in the low theta (2–4.5 Hz), high theta (5–7.5 Hz) or alpha (8–14 Hz) band.

Materials and methods

Participants and procedures

Thirty healthy volunteers were recruited for this study but four were excluded due to technical issues during EEG recordings or bad signal quality. Thus, the final group consisted of 26 subjects (10 females, age range: 19–33 years, mean = 25.7, SD = 3.7). All were right-handed, had normal or corrected-to-normal vision and reported no history of medical, neurological or psychiatric disorders. The study was approved by the local Ethics Committee (Medical Association Hamburg) and all subjects gave written informed consent.

The study followed a randomized double-blind within-subject protocol similar as described in (Eckart et al., 2014). Subjects participated in two study blocks that were separated by 12 to 20 days (mean = 14.2 days, SD = 1.6). Each study block comprised two parts taking place on two consecutive days. On the first day, participants received placebo or galantamine (8 mg) in randomized fashion after a physician checked exclusion criteria. We only included healthy (and not pregnant) subjects with no current or recent medical treatment; subjects with known intolerance to levodopa or galantamine were excluded. Smokers were not excluded in this experiment; however, only two subjects smoked one cigarette or less per day; two smoked 7.5 cigarettes per day on average according to self-disclosure.

Galantamine is licensed for the treatment of mild to moderate Alzheimer's disease. It develops cholinergic effects by inhibition of cholinesterase and by allosteric activation of nicotinic acetylcholine receptors. Thereby, it enhances the activity of hippocampal CA1 neurons through action on both nicotinic and muscarinic cholinergic receptors (Oh et al., 2006). Therefore, galantamine has stimulating effects on cholinergic neurotransmission.

On the first of the two study days (i.e. when drug or placebo was administered and EEG was recorded during the WM task), participants arrived in the lab, filled in a rating scale and questionnaire to control for

potential side effects (see below) and received either galantamine or placebo. Secondly, the electrode cap was placed on the participant's head and EEG recordings were prepared. Thirdly, around 60 min after drug administration, subjects again filled in the subjective rating scale to document any potential change in their well-being and thereafter completed the WM task while EEG was recorded. Finally, the subjective rating scale was filled in a third time after the EEG recording finished.

As mentioned above, to control for potential side effects, subjects filled in a rating scale and questionnaire at three time points: directly before drug intake, ~55 min after drug administration and after the EEG measurement (~130 min after drug administration). Moreover, blood pressure and heart rate were monitored at each time point. A series of 3×2 analyses of variance (ANOVA) with the factor drugs (galantamine, placebo) and time points (three) on blood pressure (systolic, diastolic, pulse), side effects (dry mouth, dry skin, blurred sight, fatigue, nausea, dizziness, headache) and subjective well-being (alert/drowsy, calm/excited, strong/feeble, clear-headed/muzzy, well-coordinated/clumsy, energetic/lethargic, contented/disconnected, tranquil/troubled, quick-witted/mentally slow, relaxed/tense, attentive/dreamy, proficient/incompetent, happy/sad, amicable/antagonistic, interested/bored, gregarious/withdrawn, secure/insecure) revealed no statistically significant interactions ($p > 0.05$) except for "gregarious/withdrawn" ($p = 0.03$; uncorrected for multiple comparisons). Thus, galantamine had no substantial effects on blood pressure (including heart rate) or subjective well-being, and it did not induce any side effects arguing against a global effect of galantamine. After completing the WM task, all subjects performed a spatial attention task of about 15 min – these results will be reported elsewhere.

On the next day, LTM was tested for images presented during the WM task. Here, no drug or placebo was administered and no EEG was recorded. Participants were informed about the LTM testing at the beginning of the experiment.

Experimental tasks

As in our previous work (Eckart et al., 2014), the WM task followed a Sternberg paradigm (Sternberg, 1966) with 90 delayed match-to-sample trials in three different load conditions (Load2: two pictures, Load4: four pictures, Load6: six pictures). In any given trial, gray-scaled indoor and outdoor pictures were serially presented for 1500 ms each and separated by a fixation cross (1500 ± 100 ms; encoding). The temporal jitter was used to avoid correlations between ongoing oscillations and the structure of the task. During encoding, subjects were uncertain about the length of the picture sequence. Subsequent to the last of the encoded pictures, a green fixation cross indicated a retention phase of 5 s (maintenance). Then, one final picture (i.e. the probe) was presented and subjects were required to indicate via button press whether it was novel or part of the preceding sequence (retrieval). The probe was novel in 50% of all trials. During the WM tasks, each picture was trial-unique unless it was used as probe in the WM task. Note that these repeated images were not used in the LTM test, so that every picture tested in the LTM task was only presented once the day before. See Fig. 1 for an illustration of the WM task.

WM tasks have a long history and can take many forms (D'Esposito and Postle, 2015). This particular task was chosen on the basis of our previous work and studies by others investigating the relationship between WM load and neural oscillations. In particular, Cashdollar et al. (2009) could show that variations in WM load (one, three or five scene images) are associated with a change in theta coupling between frontal and temporal sensors. Scene images were used here as stimulus material instead of simpler items, such as colored squares or letter configurations, since we were particularly interested in the relationship between neural activity during WM maintenance and subsequent LTM.

Similarly to our previous work (Eckart et al., 2014), LTM for the presented images was tested on the subsequent day using a "remember/know" paradigm (Tulving, 1985). Here, 120 images (30 scenes of each

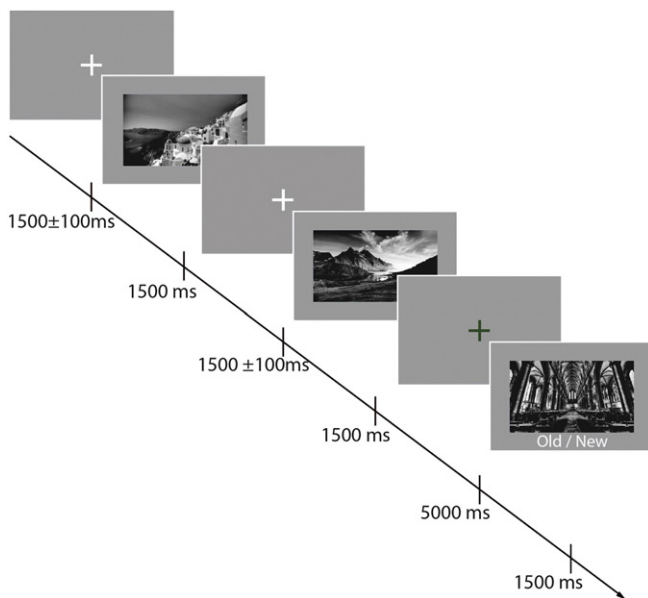


Fig. 1. Experimental design: two, four or six gray-scaled indoor/outdoor pictures were serially presented for 1500 ms each and separated by a fixation cross (1500 ± 100 ms). Subsequent to the last of the encoded pictures, a green fixation cross indicated a retention phase of 5 s. Then, one final picture, called probe, was presented and subjects indicated via button press whether it was a novel or a previously shown item.

load condition and 30 new distractors) were presented in random order for 1500 ms. Subjects first made an “old/new” decision to each individually presented picture. Following a “new” decision, subjects indicated whether they were confident (“certainly new”) or unsure (“guess”). Following an “old” decision, subjects indicated whether they were able to remember something specific about seeing the scene at study (“remember” response), just felt familiarity without any recollective experience (“familiar” response), or were unsure that the picture was an old one (“guess” response). Subjects had 3 s to make each of both judgments and they could pause for 40 s after 40 pictures.

During both tasks (Sternberg and LTM task), all subjects were asked to respond as quickly and as accurately as possible. All stimuli were gray-scaled and normalized to a mean gray value of 127 and a standard deviation of 75 (8-bit gray scale, 0–255).

Behavioral data

Behavioral measures were calculated separately for each load and drug condition. For the WM task, hit rates and false alarm rates were analyzed as a measure of accuracy; medians of reaction times (RT) across correct responses were used as a measure of retrieval speed.

Recognition memory performance was analyzed according to the assumption that recollection and familiarity are two independent recognition processes (Yonelinas, 1995). The probability of recollection for each condition was calculated by subtracting the proportion of false alarms (i.e. incorrect remember judgments for new items, Fa R) from the proportion of hits (i.e. correct remember judgment for studied items, R) $[R - Fa R]$. Familiarity was estimated as the probability of ‘know’ responses (K) corrected for the probability of making ‘know’ responses to new items (Fa K) and corrected for the fact that ‘know’ responses were given in the absence of recollection: familiarity = $(K - Fa K) / (1 - R)$ (Yonelinas, 2002).

EEG acquisition and processing

EEG signals were recorded with a 60-channel elastic cap with Ag/AgCl electrodes positioned according to the 10–20 system (Acti cap,

Brain Products GmbH, Munich, Germany) and BrainVision Recorder (Brain Products GmbH, Munich, Germany). Vertical and horizontal eye movements were recorded with four electrooculogram (EOG) electrodes. Active electrodes' impedances were maintained below 20 kΩ using conduction gel. Electrodes were referenced to FCz and grounded on the right mastoid. Recordings were digitized at 500 Hz sampling frequency with 16-bit resolution and band-pass filtered at 0.1–1000 Hz.

EEG data were pre-processed off-line in EEGLab version 13.1 (Delorme and Makeig, 2004) running in Matlab R2013b (The MathWorks, USA). First, continuous recordings were band-pass filtered between 1 and 120 Hz. Second, EEG recordings were epoched from 1000 ms before to 6000 ms after the onset of the retention phase (presentation of the green fixation cross) to avoid edge effects in the time-frequency analysis. Third, epochs that contained large artifacts were rejected automatically when they contained EEG activity exceeding three standard deviations (SDs) from the mean at a specific channel and five SDs from the mean over all channels. Up to three bad channels per session were visually identified. This data selection was followed by an independent component analysis (ICA) to remove eye blinks and cardiac components from the EEG. After first decomposition and elimination of bad components, a second round of ICA filtering was performed as proposed in Onton et al. (2006). The choice of bad components was based on visual inspection of the spatial and temporal patterns of every component. Finally, cleaned EEG signals were re-referenced to the common average for further analysis.

EEG spectral analysis

The artifact-free signals were selected for further processing. Spectral decomposition was applied on epoched signals with 0.5 Hz frequency resolution from 2 to 45 Hz using Morlet wavelet decomposition with 4 cycles and a sliding time window of 20 ms. Because of an edge effect, the final time window ranged from 120 to 4800 ms. Importantly, due to the specific experimental design and interest in differences in oscillatory power between load conditions, baseline correction was not applied (Eckart et al., 2014). At the time directly before the retention phase, the presented images were already in the WM buffer and correcting for pre-stimulus baseline might remove some of the important changes of brain activity we were interested in. Instead, it would be possible to use a baseline without WM-related activity taking the time period directly before the first image presentation; however, this solution is not appropriate for this specific design since there are significant differences in loads' presentation time (~4.5 s before the beginning of the retention phase in Load2 and ~16.5 s in Load6). Finally, spectral power of each condition was averaged over all trials and analyzed in separated frequency bands: low theta (2–4.5 Hz), high theta (5–7.5 Hz) and alpha (8–14 Hz).

Statistical analysis

For each frequency band of interest, statistical analysis was performed on the data averaged across the entire maintenance since we had no time-specific hypotheses. Nonparametric permutation tests (Blair and Karniski, 1993) were applied for statistical comparison between conditions (see below). At first, initial two-tailed t-tests were computed for each electrode site. In the next step, the estimates of the participants belonging to each condition (drug, load) were permuted. After each random permutation ($n = 1000$), a t-test was computed and the result used to create the permutation distribution that served to estimate the p-value of a cluster ($p < 0.05$) (Eckart et al., 2014). We only considered effects that were clustered at three or more neighboring electrodes to avoid Type I errors (Eckart et al., 2014; Maris and Oostenveld, 2007).

After identifying significant electrode clusters, we extracted the power information (averaged across all significant electrodes within the cluster and the whole time window) to further investigate potential

drug effects and the nature of particular relations (i.e. linear vs. quadratic) that could not be explored with the initial t-test. Calculation and interpretations of subsequent 2×3 repeated measure ANOVAs (drug status \times WM load) were identical to our behavioral analyses (see Results).

In case of significant drug effects, Pearson correlations were calculated to test for direct relationships between power information and behavioral measures. More specifically, we correlated drug-related changes in power (i.e. power in the OFF condition minus power in the ON condition) and behavior (i.e. performance in the OFF condition minus performance in the ON condition) averaged over all three load conditions (reflecting a main effect of drug) as well as for each load condition separately.

Results

Behavior

WM data (including hit rates, false alarm rates and RT) and LTM data were analyzed using 3×2 ANOVAs with the within-subject factor load (2, 4, 6 items) and the within subject-factor drug (galantamine, placebo).

WM task

Behavioral data show that participants performed the WM task with high accuracy (Table 1) and no significant differences between galantamine and placebo condition, i.e. there was no main effect of drug ($F(1,25) = 1.29, p = .27$) and no significant interaction between drug and load ($F(2,50) = 1.30, p = .28$) on hit rates. However, there was an effect of load ($F(2,50) = 14.08, p < .0005$), that was linear ($F(1,25) = 20.06, p < .0005$) but not quadratic ($F(1,25) = .02, p = .88$). Hit rates decreased as a function of WM load and pairwise post-hoc comparisons showed significant differences between Load6 vs. Load2 ($p < .0005$), Load6 vs. Load4 ($p = .02$) and Load4 vs. Load2 ($p = .03$). Analyses of false alarm rates revealed a similar pattern with no main effect of drug ($F(1,25) = 2.00, p = .17$), no interaction between drug and load ($F(2,50) = 1.47, p = .24$), but a highly significant effect of load ($F(2,50) = 12.69, p < .0005$), that was linear ($F(1,25) = 18.53, p < .0005$) but not quadratic ($F(1,25) = .08, p = .78$). Again, significant differences were present between all load conditions (Load6 vs. Load2: $p = .001$, Load6 vs. Load4: $p = .02$, Load4 vs. Load2: $p = .04$). Detailed information is presented in Table 1.

Analysis of reaction time (RT, Fig. 2) revealed a significant main effect of drug ($F(1,25) = 5.95, p = .02$) that was driven by slower responses in the galantamine condition. Direct paired t-tests for each load condition revealed that performance in the galantamine condition differed significantly from performance in the placebo condition for Load4 ($p = .05$) and Load6 ($p = .04$) and on a trend level for Load2 ($p = .09$). Furthermore, there was a highly significant effect of WM load ($F(2,50) = 20.03, p < .0005$) that was both linear ($F(1,25) = 30.36, p < .0005$) and quadratic ($F(1,25) = 6.22, p = .02$); response times were slower with increasing load. Pairwise post-hoc comparisons

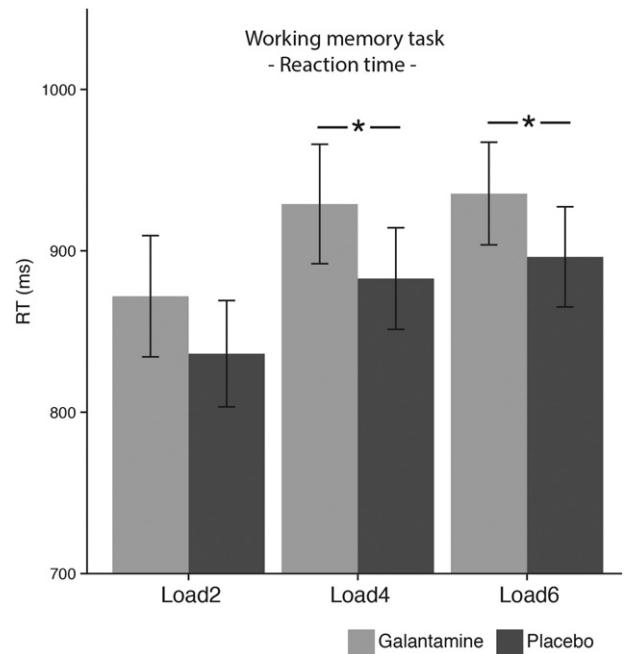


Fig. 2. RT during the WM task. RT increased as a function of load. Following cholinergic stimulation, subjects responded slower to the probe especially when pictures were presented in the Load4 and Load6 conditions (note that there was no significant interaction between load and drug, see text). Error-bars represent standard errors and asterisks indicate statistically significant post-hoc comparisons ($p < 0.05$).

showed significant differences between Load4 vs. Load2 ($p < 0.0005$) and Load6 vs. Load2 ($p < 0.0005$), but no significant difference between Load4 vs. Load6 ($p = 0.97$). There was no significant interaction between drug and load ($F(2,50) = .14, p = .87$).

LTM task

For LTM accuracy, a significant main effect of drug ($F(1,25) = 4.58, p = .04$) was revealed for corrected familiarity rates (CHR-know, Fig. 3), which was driven by decreased recognition memory in the galantamine condition. As expected based on the RT effects during encoding (see above), separate post-hoc comparisons for all three load conditions showed marginally significant differences (Load2: $t(25) = -1.87, p = .0035$, Load4: $t(25) = -1.78, p = .045$, Load6: $t(25) = -1.86, p = .04$; one-tailed). There was no main effect of load ($F(2,50) = .27, p = .76$) and no interaction between drug and load ($F(2,50) = .04, p = .96$) on CHR-know responses. For corrected recollection rates (CHR-rem) there were no significant main effects of drug ($F(1,25) < .005, p > .99$) or load ($F(2,50) = 1.28, p = .29$) and no interaction between drug and load ($F(2,50) = .63, p = .54$). See Table 1 for memory performance in the LTM task.

Table 1

Response accuracy (hit rates and false alarm rates), reaction times (ms) and memory performance in the WM- and LTM-task. Numbers in brackets indicate standard deviations.

	Placebo			Galantamine		
	Load2	Load4	Load6	Load2	Load4	Load6
<i>WM task</i>						
Hit rates	.93 (.07)	.92 (.07)	.89 (.08)	.93 (.05)	.89 (.08)	.88 (.10)
False alarm rates	.07 (.07)	.08 (.07)	.11 (.08)	.07 (.07)	.12 (.09)	.13 (.10)
Median RT (ms)	836.25 (168.09)	882.83 (160.47)	896.25 (158.16)	871.85 (191.52)	929.02 (188.66)	935.48 (162.11)
<i>LTM task</i>						
CHR know	.11 (.13)	.10 (.15)	.11 (.15)	.05 (.15)	.03 (.14)	.04 (.12)
CHR remember	.17 (.13)	.19 (.11)	.17 (.11)	.18 (.13)	.19 (.12)	.16 (.09)

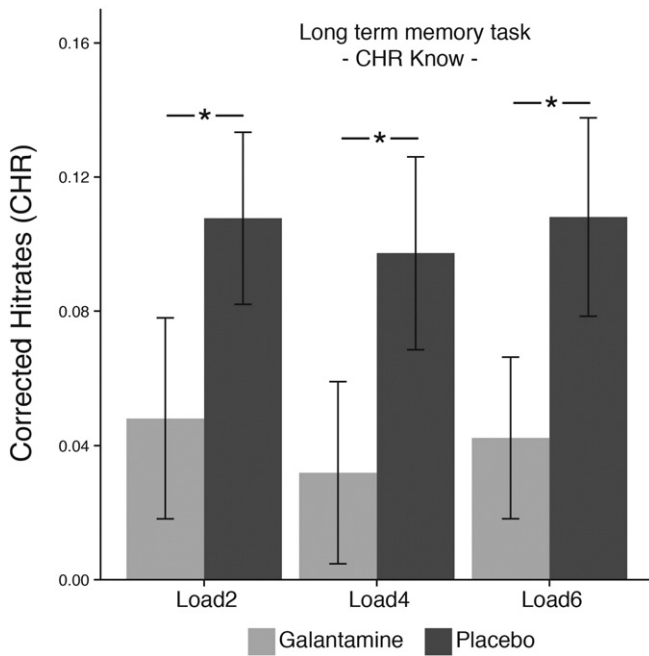


Fig. 3. Galantamine administration before encoding (WM task) reduced subsequent familiarity responses in the LTM task (main effect of drug, see text). Error-bars represent standard errors and asterisks indicate significant post-hoc comparisons ($p < 0.05$, one-tailed).

EEG-data: spectral power changes

Load effect

To study the relationship between WM load and neural oscillations, we first performed a statistical comparison between Load4 vs. Load2 averaged across galantamine and placebo (Eckart et al., 2014). As expected (Eckart et al., 2014), this contrast revealed significant effects in the low and high theta as well as in the alpha band. Although the effect covered the entire scalp, most powerful differences were visible at frontal and occipital sensors (Fig. 4). Note that the comparison Load2 vs. Load6 revealed a similar activation pattern, and there was no statistically significant difference between Load4 vs. Load6.

To further characterize the effects, we performed an ANOVA on the extracted raw power values from significant clusters (Fig. 4). Importantly, this analysis confirmed that activity from these electrodes was modulated by WM load in low theta ($F(1,6,41.0) = 5.16$, $p = .01$; linear: $F(1,25) = 1.01$, $p = .33$, quadratic: $F(1,25) = 16.58$, $p < .0005$; post hoc comparisons: Load2 vs. Load4: $p = .003$, Load2 vs. Load6: $p = .98$, Load4 vs. Load6: $p = .11$), high theta ($F(2,50) = 12.60$, $p < .0005$; linear: $F(1,25) = 6.98$, $p = .01$, quadratic: $F(1,25) = 20.20$, $p < .0005$, post hoc comparisons: Load2 vs. Load4: $p < .0005$, Load2 vs. Load6: $p = .04$, Load4 vs. Load6: $p = .04$) and alpha range ($F(2,50) = 11.86$, $p < .0005$; linear: $F(1,25) = 7.34$, $p = .01$, quadratic: $F(1,25) = 19.90$, $p < .0005$, post hoc comparisons: Load2 vs. Load4: $p < .0005$, Load2 vs. Load6: $p = .04$, Load4 vs. Load6: $p = .10$). However, within these clusters there was no main effect of drug in any of the studied frequencies: low theta ($F(1,25) = .14$, $p = .72$), high theta ($F(1,25) = .07$, $p = .80$) and alpha range ($F(1,25) = 2.26$, $p = .15$); and no interaction of drug and load: low theta ($F(2,50) = .50$, $p = .61$), high theta ($F(1,6,40.7) = 2.16$, $p = .14$) and alpha range ($F(2,50) = 1.02$, $p = .37$).

Drug effect

To identify a neural correlate that resembled the behavioral effect of galantamine, we averaged the mean power over WM loads (because the behavioral effect of drug was independent of load) and compared placebo vs. galantamine. This contrast revealed specific effects at frontal and central electrodes specifically in the alpha band (Fig. 5A). Post-hoc

analyses on the extracted power values from the significant clusters revealed that galantamine intake induced significant ($p < 0.05$) decreases in alpha power (see Fig. 5B). More specifically, a 2×3 ANOVA showed a main effect of drug ($F(1,25) = 11.39$, $p = .002$) as well as a main effect of load ($F(2,50) = 11.92$, $p < .0005$), that was both quadratic ($F(1,25) = 17.89$, $p < .0005$) and linear ($F(1,25) = 8.27$, $p = .008$). Further comparisons between galantamine vs. placebo revealed significant drug differences for Load2 ($p = .01$), Load4 ($p = .004$) and Load6 ($p = .002$). However, there was no significant interaction between drug and load ($F(2,50) = 1.15$, $p = .32$). Note that the effects at these sensors were specific to alpha, since no drug effects emerged for low theta (drug: $F(1,25) = .58$, $p = .45$; drug \times load: $F(2,50) = .50$, $p = .61$) or high theta (drug: $F(1,25) = 1.35$, $p = .26$; drug \times load: $F(2,50) = 2.12$, $p = .13$).

To investigate the relationship between drug-related alpha power decrease and drug-related increases in RTs during the WM task, Pearson correlations were calculated (see Materials and methods section). No direct relationship between changes in power and behavior was revealed for the average over all load conditions ($r(26) = -.25$, $p = .22$) and for Load2 ($r(26) = .02$, $p = .91$) or Load4 ($r(26) = -.17$, $p = .41$). However, under highest memory load (Load6), drug-related decreases were significantly related to increases in reaction times ($r(26) = -.51$, $p = .009$).

Furthermore, for electrodes showing a significant drug effect during the WM task, there was no direct relationship between drug-related alpha power decrease (alpha power ON minus OFF) and decreased CHR know (CHR know ON minus CHR know OFF; $r(26) = -.04$, $p = .86$). Finally, given the relatively small number of trials per WM load that was tested in the subsequent LTM test (30 per condition), we did not perform a “DM-analysis” (Paller et al., 1987).

Data re-analysis excluding four smokers

Chronic nicotine consumption may have various effects on cognition and the central nervous system including increased numbers of acetylcholine receptors (Swan and Lessov-Schlaggar, 2007). Therefore and to strengthen our findings, four smokers from the initial sample were excluded for a re-analysis of our data. As a result, there was no significant change in the overall pattern of hit rates and false alarm rates. For RTs, however, the main effect of drug lost power but remained significant on a trend level ($F(1,21) = 3.00$, $p = .098$). Importantly, the main effect of drug on CHR-know ($F(1,21) = 5.24$; $p = .03$) and the main effect of drug on alpha oscillations remained statistically significant.

Discussion

The aim of this study was to investigate the role of cholinergic neuromodulation in theta and alpha driven WM as well as subsequent LTM. As a main finding, we can show that both theta and alpha oscillations scale as a function of WM load resembling the typical performance decrease with WM load. Importantly, cholinergic stimulation slowed down retrieval speed during WM and reduced associated alpha power. At LTM, this pattern specifically translated to reduced familiarity based recognition suggesting a functional relationship between acetylcholine, alpha oscillations, WM performance and subsequent familiarity.

According to the Yerkes-Dodson law (Yerkes and Dodson, 1908) cognitive performance is best when individuals operate on an optimal level of neurotransmitters; aberrations of this optimum – either lower or higher levels – decrease performance. On the basis of rich empirical work, recent reviews (Bentley et al., 2011; Newhouse et al., 2004) suggest that this inverted u-shape relationship also applies to the cholinergic system. More precisely, cholinergic stimulation most readily exerts beneficial effects on cognitive performance or brain activation patterns in clinical populations with generally reduced ACh levels, e.g. patients suffering from mild cognitive impairment (White and Levin, 2003), Alzheimer’s disease (White and Levin, 1999) or schizophrenia (Jacobsen et al., 2004). By contrast, many of these studies show decreases in

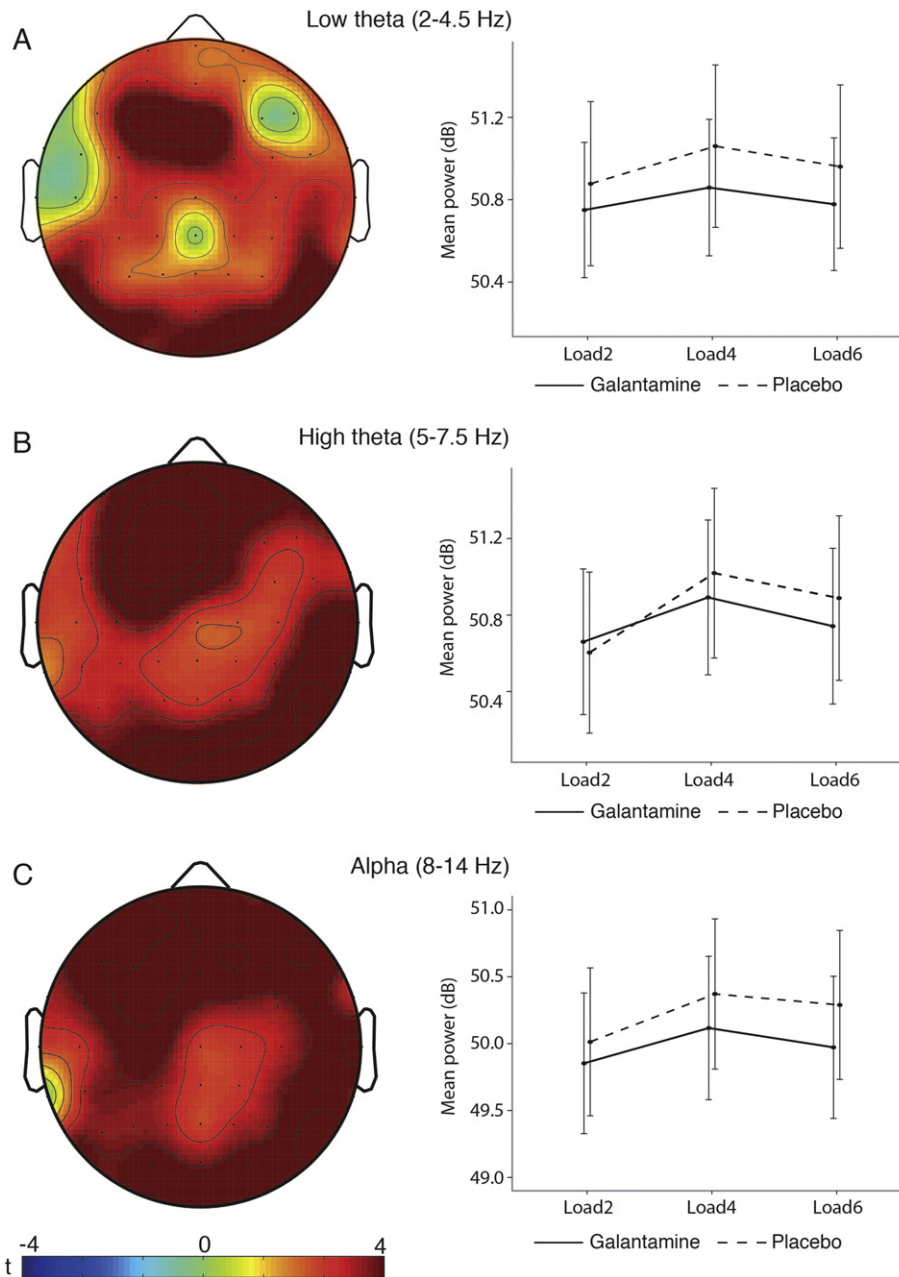


Fig. 4. Load dependent changes in oscillatory power. The scalp maps represent the statistics of the permutation tests for significant differences ($p = .05$) of WM related oscillatory activity (Load4 vs. Load2) for low theta (A), high theta (B) and alpha (C). Post-hoc analyses showed linear ($p = .02$) and quadratic ($p < .0005$) changes in all frequency bands. Error-bars represent standard errors.

cognitive performance or brain activation when healthy controls are tested with the same drugs (Bentley et al., 2011; Newhouse et al., 2004). For instance, nicotinic stimulation reduces task performance in a 2-back WM task in healthy humans, and this effect was associated with changes in activity of a network of brain regions including the anterior cingulate cortex and bilateral thalamus (Jacobsen et al., 2004). In schizophrenia patients, on the other hand, the same treatment had opposite effects (Jacobsen et al., 2004). Thus, pro-cholinergic agents may be beneficial in states when cognitive performance is particularly low – as in aging, disease or sleep-deprivation – but it may reduce performance in healthy humans with optimal behavior and ACh levels (Chuah and Chee, 2008; Knott et al., 2015; Kukulja et al., 2009).

Our results resonate well with these previous findings. In fact, our subjects performed very well in the WM task (indicated by overall high hit rates and low false alarm rates), which suggests that they operated at an optimal cognitive and ACh level. In line with the assumption

of an inverted u-shape relationship, acute stimulation of this healthy cholinergic system by galantamine had deleterious effects on retrieval speed during WM, subsequent familiarity (i.e. cognition) and accompanying neural activity.

At the neural level, we could replicate our previous findings (Eckart et al., 2014) by showing changes of prefrontal high theta and alpha with WM load. In fact, the power of both frequency bands increased with memory load (linear effect), reaching a maximum peak for Load4, and then showing a trend to decrease in Load6 (quadratic effect). As suggested previously (Eckart et al., 2014), this demonstrates that increases in WM load do not necessarily go hand in hand with theta or alpha power increases, but they start to drop after a certain load is exceeded. One possible explanation for this pattern is that different memory systems support WM maintenance depending on the nature of the task (Eckart et al., 2014). While initial models proposed a physiological and functional separation between WM and LTM (Baddeley and

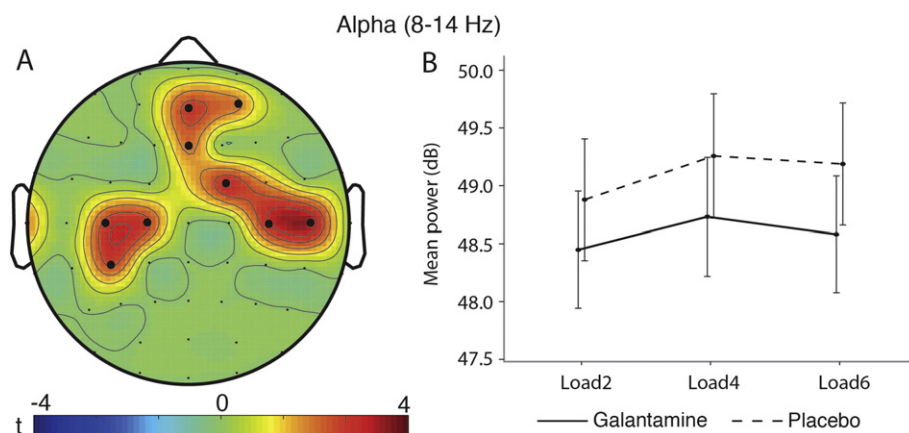


Fig. 5. Effects of galantamine on oscillatory power in the alpha band. (A) The scalp map represents the statistics of the permutation test for the contrast placebo vs. galantamine averaged across WM load conditions ($p < 0.05$). (B) Post-hoc analysis confirmed the main effect of drug on alpha (but not theta) oscillations and revealed linear and quadratic load effects. Error-bars represent standard errors.

Warrington, 1970; Squire and Wixted, 2011), there is growing evidence that both memory systems interact to drive cognition (Ranganath and Blumenfeld, 2005). One prominent view is that prefrontal WM is supported by MTL-dependent LTM if WM capacity is exceeded (Cashdollar et al., 2009; Jensen and Squire, 2012). Our data are in line with this notion since they show that WM maintenance of six items is behaviorally possible but prefrontal theta and alpha only increased for up to four items. Thus, prefrontal WM capacity of relatively complex scene images seems to be limited to around four items and further load increases may recruit other mechanisms (Cashdollar et al., 2009). Whether this involved the MTL, as indicated by recent studies (Axmacher et al., 2008), remains speculative due to the low spatial resolution of scalp EEG recordings.

Furthermore, as in our previous work (Eckart et al., 2014), high theta and alpha power not only showed very similar response profiles (linear and quadratic effects) but also similar topographic representations suggesting common functional properties. This is in line with a study by Cohen (2011) demonstrating that individual peak frequencies during WM maintenance are highly variable between subjects, ranging from 2 to 13 Hz. However, as a main finding of this study, cholinergic stimulation had a specific effect on alpha band oscillations (Fig. 5) arguing in favor of different underlying functional mechanisms associated with both frequency bands. Indeed, Bauer et al. (2012) combined MEG and psychopharmacology in humans (administration of the cholinergic agonist physostigmine) to show that ACh modulates alpha in a visuospatial attention task. This concurs to the idea (Jensen et al., 2014) that alpha oscillations are part of a computational mechanism involved in organizing the temporal coding of items in sequential task such as ours.

Our specific effects of galantamine on alpha power also fit to a recent network model proposing that neural oscillations provide a mechanism for the different operations involved in WM (Dipoppa and Gutkin, 2013). More precisely, it suggests a close relationship between alpha oscillations and cholinergic neuromodulation, whereas theta oscillations might be more closely related to dopamine. Specifically, Dipoppa and Gutkin (2013) argue that the PFC flexibly controls alpha oscillations via PFC-thalamic loops; theta oscillations, on the other hand, might be controlled by PFC-mesolimbic interactions. Importantly, according to this model, only the interplay of both frequency bands and underlying loops guide WM performance with theta being particularly important for WM maintenance while alpha oscillations prevent distracting stimuli to intrude the WM buffer (Roux and Uhlhaas, 2014). Our current findings and previous work (Eckart et al., 2014) (where dopaminergic stimulation had a specific effect on low theta oscillations during WM) give empirical evidence to the view of a physiological dissociation between the role of acetylcholine (alpha) and dopamine (theta) in WM (Dipoppa and Gutkin, 2013).

Cholinergic stimulation during WM had a specific effect on subsequent familiarity but spared recollection memory. This observation supports previous work indicating a critical overlap between WM and LTM functions, with alpha and theta oscillations being particularly relevant for successful memory encoding (Axmacher et al., 2010; Khader et al., 2007). Importantly, our findings extend this work by showing that stimulating the cholinergic system during encoding impairs WM possibly via alpha oscillations and this effect specifically translates into familiarity. According to dual process models, information can be remembered based on recollection and familiarity (Yonelinas, 2002). While recollection refers to the retrieval of contextual information of the episode, familiarity is based on recognition in absence of any contextual information. Physiologically, recollection closely relates to the integrity of the hippocampus and familiarity to the surrounding anterior parahippocampal gyrus (Diana et al., 2007) – see also (Squire et al., 2007). Importantly, cholinergic receptors are distributed across the entire neocortex (Mesulam, 2004), which nicely fits to the observation of the basal forebrain being involved in modulating various frequency bands including alpha (Sanchez-Alavez et al., 2014).

From a more mechanistic point of view, impairments of familiarity can be explained by effects of galantamine on memory consolidation. In fact, human and animal studies suggest that stimulating the cholinergic system after learning reduces memory consolidation possibly through interference (see e.g. Gais and Born, 2004; Winters et al., 2006). Since galantamine, as administered in our study, has a relatively long half-life of ~7–8 h and the encoding of experimentally relevant items ended ~2.5 h after drug intake, it seems possible that consolidation was impaired by the increased acetylcholine levels.

Taken together, our results confirm that theta and alpha band oscillations play a critical role in WM maintenance. Importantly, stimulating the cholinergic system specifically reduced alpha power, WM retrieval speed and subsequent familiarity based recognition. This observation is in line with the notion of a quadratic relationship between ACh levels and WM performance, and it provides new insights into the functional significance of alpha oscillations for ACh dependent cognitive functions.

Conflict of interest

None.

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