



Dopaminergic stimulation facilitates working memory and differentially affects prefrontal low theta oscillations



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ABSTRACT

We used electroencephalography (EEG) together with psychopharmacological stimulation to investigate the role of dopamine in neural oscillations during working memory (WM). Following a within-subjects design, healthy humans either received the dopamine precursor L-Dopa (150 mg) or a placebo before they performed a Sternberg WM paradigm. Here, sequences of sample images had to be 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. Behaviorally, L-Dopa improved WM and LTM performance as a function of WM load. More precisely, there was a specific drug effect in the four-load condition with faster reaction times to the probe in the WM task and higher corrected hit-rates in the LTM task. During the maintenance period, there was a linear and quadratic effect of WM load on power in the high theta (5–8 Hz) and alpha (9–14 Hz) frequency range at frontal sensors. Importantly, a drug by load interaction – mimicking the behavioral results – was found only in low theta power (2–4 Hz). As such, our results indicate a specific link between prefrontal low theta oscillations, dopaminergic neuromodulation during WM and subsequent LTM performance.

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Introduction

Animal studies suggest that coordinated theta oscillations (~4–10 Hz) between the hippocampus and prefrontal cortex (PFC) provide a central mechanism underlying working memory (WM; Benchenane et al., 2010; Jones and Wilson, 2005). Physiologically, the PFC is densely innervated by dopaminergic neurons (Goldman-Rakic, 1995) and dopamine injections into the rats' PFC increase hippocampal–prefrontal theta coherence (Benchenane et al., 2010). This indicates that the WM-dependent interplay between hippocampus and PFC may be modulated by dopamine (Dash et al., 2007; Goldman-Rakic, 1995). Importantly, dopaminergic neuromodulation of the PFC (Williams and Goldman-Rakic, 1995) and hippocampus (Chowdhury et al., 2012) does not follow a linear but inverted u-shaped function. Accordingly, WM performance is optimal in a relatively narrow range of dopamine activity, while too much or too little dopamine results in a decline of memory performance and associated neural activity (Bertolino et al., 2008; Chowdhury et al., 2012).

In humans, electroencephalography (EEG) or magnetoencephalography (MEG) recordings revealed increases in power (Gevins et al.,

1997; Moran et al., 2011; Onton et al., 2005; Sauseng et al., 2010) or a reset of phase (e.g. Tesche and Karhu, 2000) of frontal midline theta oscillations (~5–8 Hz, here called “high theta”) during WM tasks. In line with animal findings, these oscillatory patterns seem to be linked to dopaminergic neurotransmission. For instance, stimulation with dopamine agonists increases the duration of high theta in the resting state (Mizuki et al., 1997) and enhances its amplitude during WM maintenance (Moran et al., 2011).

However, in some WM studies, high theta oscillations are weak, absent, or even decreased (Bastiaansen et al., 2002; Mitchell et al., 2008), particularly when the tasks were controlled for attention or difficulty (Griesmayr et al., 2010; Missonnier et al., 2006; Sauseng et al., 2010). At least two possible explanations have been discussed in this context. First, WM functions rely not only on high theta oscillations but also on other frequency bands. Specifically, power in the low theta (2–4 Hz) range increases during semantic (Lega et al., 2012) and working (Axmacher et al., 2010; Mizuhara and Yamaguchi, 2011; van Vugt et al., 2010) memory tasks in the human hippocampus (Axmacher et al., 2010; Lega et al., 2012; van Vugt et al., 2010) and at fronto-central EEG electrodes (Mizuhara and Yamaguchi, 2011). Furthermore, oscillations at the upper edge of the theta band, namely the alpha band (9–14 Hz), are modulated during WM maintenance (Bastiaansen et al., 2002; Gevins et al., 1997; Jensen and Tesche, 2002; Krause et al., 2000). Second, the classical view of WM being strictly separated from long-term memory (LTM; Baddeley, 1992) has recently been challenged by

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suggestions that WM and LTM interact during item maintenance depending on the quality of the task and stimulus material. More specifically, WM – i.e. the retention of information for short time periods – was long thought to rely mainly on the PFC (Goldman-Rakic, 1995). However, this view was recently challenged by several reports of hippocampal contributions to WM performance (Bertolino et al., 2008; Karlsgodt et al., 2005). In fact, it has been suggested that PFC-dependent WM might be supported by LTM structures (e.g. in the medial temporal lobe), particularly when stimuli are complex, difficult or abstract or when the number of items exceeds WM capacity (Cashdollar et al., 2009; Fuentemilla et al., 2010; Ranganath and Blumenfeld, 2005; Ruchkin et al., 2003).

We conducted a within-subject EEG study using L-Dopa (150 mg, 37.5 mg Benserazide) vs. placebo. The employed Sternberg WM task included complex scene stimuli that were presented sequentially in three different load conditions (two, four, six items). Additionally, recognition memory for the images was tested one day after the WM task. EEG data analysis focused on neural oscillations in three frequency bands: high theta (5–8 Hz), low theta (2–4 Hz) and alpha (9–14 Hz). We predicted improved WM (Moran et al., 2011) and LTM (Lisman and Grace, 2005) performance after L-Dopa administration and a close link of these effects with neural oscillations in either of the three frequency bands.

Materials and methods

Subjects and procedure

21 subjects participated in the study but three were excluded for technical reasons during EEG recordings. Thus, the final sample consisted of 18 healthy subjects (9 males, age range: 19–32 years, mean = 26.06, $SD = 3.57$). 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). All subjects gave written informed consent.

The study followed a randomized double-blind within-subject protocol. Subjects participated in two study blocks that were separated by 9 to 21 days (mean = 14.00 days, $SD = 3.60$). Each study block comprised two parts taking place on two consecutive days. On day one, participants received L-Dopa (LV; 150 mg, 37.5 mg Benserazide) or placebo (PL) in a randomized fashion. L-Dopa is licensed for the treatment of Parkinson's disease and provokes only little to no side-effects if taken in low dosages. It has been used in previous human imaging studies showing that 150 mg results in drug-related differences in memory performance (Chowdhury et al., 2012) and electrophysiological activity (Aptiz and Bunzeck, 2013; Eckart and Bunzeck, 2012). To control for potential side effects, subjects filled in a rating scale and questionnaire at three time points: 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. No drug-related changes were observed for potential side effects, mood, blood pressure or heart rate. 60 min after drug administration, subjects completed the WM task (see below) while EEG was recorded.

On day two, LTM was tested for images presented during the WM task. Here, no LV or PL was administered and no EEG was recorded. Participants were informed about the LTM testing at the beginning of the experiment. All but one subject (who missed the second LTM testing) completed all four appointments. This participant was excluded from the LTM analyses.

Experimental tasks

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; *encoding*).

Here, 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). 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 a previously shown item (*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 – these repeated images were not used in the LTM test. See Fig. 1 for an illustration of the WM task.

LTM was tested on the subsequent day using a 'remember/know' paradigm (Tulving, 1985). Here, 120 images (30 scenes of each 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.

All stimuli were gray-scaled and normalized to a mean gray value of 127 and a standard deviation of 75 (8-bit grayscale, 0–255).

Analyses of behavioral data

Behavioral measures were calculated separately for each load condition. For the WM task D -prime (d') was calculated as a measure of retrieval accuracy by subtracting the standardized false alarm (FA) rates (i.e. distractors that have been classified as 'old') from standardized hit rates (i.e. correct 'old'/'new'-decisions). Reaction times (RT) across correct responses were averaged as a measure of retrieval speed.

For the LTM task, recognition memory performance was analyzed based on corrected hit-rates (CHR) for 'remember' and 'know' responses. Hit-rates were calculated by dividing the number of correctly retrieved items by the total number of pictures. Then, CHRs were obtained by subtracting the respective FA-rates (i.e. 'remember' or

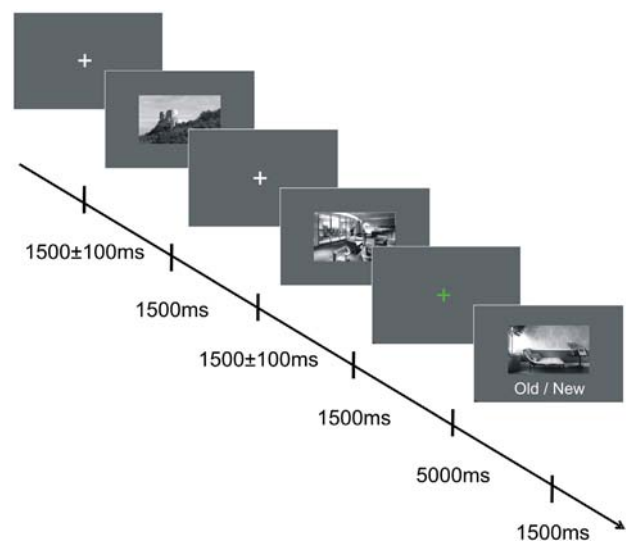


Fig. 1. Experimental design: two, four or six gray-scaled indoor/outdoor pictures were serially presented for 1500 ms each and separated by a fixation cross (1500 ± 100 ms). Subsequent to the last of the encoded pictures, a green fixation cross indicated a retention phase of 5 s. Then, one final picture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

'know' responses following a distractor). Furthermore, mean RTs during the LTM task for correct 'old'/'new' and 'remember'/'know' decisions were calculated. For RT analyses (both WM and LTM tasks), individual trials with outlying values (i.e. more than 2.5 SDs above the subject-specific mean RT) were excluded from the analysis (i.e. the mean).

Statistical analyses were based on 2×3 repeated measure analyses of variance (ANOVAs) including drug status (LV, PL) and WM load (Load2, Load4, Load6) as within-subject factors. Huynh–Feldt corrected statistics were interpreted if the assumption of sphericity was violated (at least at the trend level, $p < .10$). In case of significant effects, the precise nature of the effect was clarified using repeated (for drug) and polynomial (for WM load) within-subject contrasts. Pairwise post-hoc comparisons (i.e. between load conditions) were corrected for multiple comparisons using Bonferroni correction. To further investigate the nature of interaction effects (i.e. drug status \times WM load), the drug-related increase or decrease in RT or CHR (L -Dopa minus placebo, $\Delta LV/PL$) was calculated for each subject. Plasma levels of L -Dopa depend on body weight (Zappia et al., 2002) and the cognitive effects of psychopharmacologic drugs are dose-dependent (Goldman-Rakic et al., 2000; Knecht et al., 2004). Thus, we considered "mean-centered" body-weight as a covariate in all statistical models to control for potential inter-individual differences in body-weight dependent drug dose. However, we also repeated all analyses without including this covariate. It did not change our main results but two minor effects lost significance: the main effect of load in the low theta band (2–4 Hz) and the post-hoc test 'Load2 vs. Load6' in the high theta band (5–8 Hz).

EEG recording and preprocessing

EEG activity was recorded using a 60-channel system positioned according to the extended 10–20 system using acticap (Brain Products GmbH, Munich, Germany) and the BrainVision Recorder (Version 1.03.0003). Electrodes were referenced to FCz and grounded on the right mastoid. Active electrodes were kept below the impedance level of 20 k Ω . Electrooculogram (EOG) activity was recorded from 2 pairs of leads to register horizontal and vertical eye movements. EEG and EOG signals were continuously digitized at a sampling rate of 500 Hz, high-pass filtered at .1 Hz and low-pass filtered at 1000 Hz. The electrode CP6 had to be excluded from data analyses due to technical issues.

EEG data was preprocessed and analyzed using EEGLab (Delorme and Makeig, 2004). Continuous data was re-referenced to an average reference and filtered between .5 Hz and 120 Hz. Trials were segmented from 1000 ms before the beginning of the retention phase (i.e. presentation of the green fixation cross) until 1000 ms after the maintenance period (presentation of the probe) to avoid edge effects in the time-frequency decomposition. Trials with severe artifacts were rejected automatically when they contained EEG activity that exceeded 3 SDs from the mean at a specific channel and 5 SDs from the mean over all channels. Blinks and eye movements were removed via independent component analysis (Delorme and Makeig, 2004). Subsequently, all trials were visually inspected and rejected if they still contained artifacts. After preprocessing, an average number of 21.45 ($SD = 1.90$) trials per subject and run remained (Load2: $M = 21.56$, $SD = 3.16$, Load4: $M = 20.67$, $SD = 3.03$, Load6: $M = 22.14$, $SD = 2.21$).

Time–frequency (TF) analysis

Spectral decomposition was applied at a trial-by-trial level in 1 Hz steps from 2 to 45 Hz using Morlet wavelets (Percival and Walden, 1993) with 4 cycles and a sliding time window of 20 ms. After accounting for edge effects, a time window from 120 ms to 4880 ms remained. It should be noted that baseline correction was not performed, since our focus was on differences in oscillatory power between load conditions. Furthermore, correcting for a pre-stimulus baseline might impose some important confounds to our analyses: relying on the time span directly before the retention phase might eliminate neural activity of

interest, since the presented images were already in the WM buffer at that time and thus retained in WM. Alternatively, using a baseline without WM-related activity (e.g. the time span directly before the first image was presented) might introduce systematic differences in the timing of the baseline (i.e. ~ 4.5 s before the beginning of the retention phase in Load2 but ~ 16.5 s in Load6). Finally, TF-power across all trials was averaged for each condition.

To identify electrodes showing load effects, we averaged both drug conditions and compared Load4 with Load2. We did not choose Load6 for this contrast since it might not rely on prefrontal theta oscillations but other LTM-related mechanisms (e.g. Cashdollar et al., 2009; see Introduction). To identify electrodes reflecting the behavioral drug effects (see Results), we subtracted the power spectrum in Load2 (as a 'baseline') from the power in Load4 and compared this difference measure between drug conditions (L -Dopa vs. placebo). Three frequency bands of interest were chosen for the analyses: low theta (2–4 Hz), high theta (5–8 Hz) and alpha (9–14 Hz) – see Introduction.

For statistical comparison, permutation tests (Blair and Karniski, 1993) were calculated (data was averaged over time as no specific time effects were expected). Here, initial two-tailed t -tests were run for each electrode site. Then, data was randomized and split into two pseudo conditions on which t -tests were run again. This step was iterated 1000 times to create a distribution of pseudo t -values, which was used as a reference distribution for the actual t -statistic (with a p -value of .05). The procedure was conducted for 59 electrodes and would thus lead to a Type I error on 3 electrodes ($59 \times .05$). Therefore, we only considered effects that were clustered at three or more neighboring electrodes (Maris and Oostenveld, 2007). Furthermore, clusters were rejected if they were located exclusively at the extreme outer edge of the scalp.

After identifying significant electrode clusters, we extracted the power information (averaged across all 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.

Results

Behavior

WM task

Subjects performed the task with high accuracy as reflected in a mean D -prime of 5.19 ($SD = 2.15$) in the placebo condition and 4.53 ($SD = 1.68$) in the L -Dopa condition. D -prime varied as a function of load ($F(2,32) = 4.01$, $p = .03$): the higher the WM load, the poorer the performance. This relationship was linear, $F(1,16) = 6.96$, $p = .02$, but not quadratic ($p = .69$). Subsequent pairwise post-hoc comparisons revealed significant differences between Load2 and Load6 ($p = .05$), but not between Load2 and Load4 ($p = .39$) or Load4 and Load6 ($p = .64$). No main effect of drug and no interaction between drug and load emerged ($p > .05$) – see Table 1.

Reaction time analysis revealed a highly significant effect of WM load ($F(2,32) = 54.88$, $p < .0005$) that was driven by slower responses with higher load. The effect was both linear ($F(1,16) = 96.71$, $p < .0005$) and quadratic ($F(1,16) = 6.48$, $p = .02$). Indeed, pairwise post-hoc comparisons revealed significant differences between all three load conditions, i.e. Load2 vs. Load4 ($p < .0005$), Load4 vs. Load6 ($p = .01$) and Load2 vs. Load6 ($p < .0005$). There was no main effect of drug ($p > .05$). However, a significant interaction between drug and load, $F(1.8,28.6) = 3.72$, $p = .04$ (Huynh–Feldt corrected), indicated that L -Dopa modulated the speed of 'match/not-match' decisions, depending on how many pictures had to be maintained in WM. This interaction was quadratic, $F(1,16) = 4.85$, $p = .04$, but not linear ($p = .13$). A 1×3 ANOVA on the drug-dependent RT increase/decrease ($\Delta LV/PL$)

Table 1
Response accuracy (D-prime), reaction times and memory performance.

	OFF status			ON status		
	Load2	Load4	Load6	Load2	Load4	Load6
<i>WM task</i>						
D-prime	7.15 (1.87)	5.77 (2.58)	5.86 (2.35)	6.42 (1.99)	6.11 (2.49)	4.97 (2.26)
Reaction time in ms	792.70 (118.93)	862.80 (134.74)	867.52 (123.97)	780.52 (97.24)	833.24 (101.08)	879.08 (107.23)
<i>LTM task</i>						
CHR remember	.22 (.12)	.18 (.10)	.20 (.09)	.20 (.13)	.25 (.13)	.20 (.11)
CHR know	.18 (.15)	.18 (.15)	.16 (.14)	.16 (.17)	.12 (.14)	.11 (.17)
RT remember	686.23 (215.68)	643.65 (207.16)	635.74 (206.66)	703.19 (249.63)	695.40 (217.01)	657.99 (203.20)
RT know	629.28 (255.86)	717.24 (208.46)	753.06 (232.60)	741.99 (224.68)	677.39 (258.30)	751.66 (318.20)

revealed that L-Dopa accelerated RTs when ‘match/not-match’ judgments were made on Load4 but not Load2 or Load6 pictures (Fig. 2 and Table 1).

LTM task

Regarding ‘remember’ responses, no main effect of drug or load was found and the interaction between drug and WM load did not reach significance either, $F(1.8,26.3) = 2.48$, $p = .11$ (Huynh–Feldt corrected). However, since our WM analysis revealed differential drug effects on pictures in Load4, we calculated planned pairwise t -tests (placebo vs. L-Dopa) for memory performance in each load condition. Indeed, L-Dopa intake enhanced the recollection of Load4, $t(16) = -2.23$, $p = .04$, but not Load2, $t(16) = .38$, $p = .71$, or Load6 pictures, $t(16) = .12$, $p = .91$ (Fig. 2). However, there was no significant correlation between drug effects on RTs in the WM task and response accuracy in the LTM task ($p > 0.05$).

No other significant effects emerged for recollection or familiarity responses in the LTM task (all $p > .10$). See Table 1 for memory performance and reaction times in the LTM task.

EEG spectral power

Load effects

Statistical comparison between Load4 and Load2 across LV and PL revealed significant differences in all three frequency bands of interest (Fig. 3). In the low theta band (2–4 Hz) higher power in Load4 than in

Load2 emerged over right posterior scalp sites. Extraction of power data revealed that activity from these electrodes was indeed modulated by WM load, $F(2,32) = 3.19$, $p = .05$. This effect was quadratic, $F(1,16) = 9.17$, $p = .008$, but not linear, $F(1,16) = .20$, $p = .66$. However, when corrected for multiple comparisons none of the direct post-hoc comparisons reached significance (Load2 vs. Load4: $p = .11$, Load2 vs. Load6: $p > .99$, Load4 vs. Load6: $p = .07$). At these electrodes there was no main effect of drug and no drug \times load interaction ($p > .05$).

In the high theta band (5–8 Hz), higher power in Load4 was identified in a large cluster of frontal and occipital electrodes. Again, the EEG oscillations in this frequency band were modulated by WM load, $F(2,32) = 6.54$, $p = .004$, following a linear, $F(1,16) = 7.16$, $p = .02$ and quadratic, $F(1,16) = 6.06$, $p = .03$, relationship. Post-hoc testing revealed significant power differences in Load2 vs. Load4 ($p = .03$) and Load2 vs. Load6 ($p = .05$), but not in Load4 vs. Load6 ($p = .70$). At these electrodes there was no main effect of drug and no drug \times load interaction ($p > .05$).

Analysis of the alpha band (9–14 Hz) revealed a similar picture: a power increase over frontal and temporal electrodes was modulated by WM load, $F(2,32) = 9.00$, $p = .001$. Again, this relationship was linear, $F(1,16) = 9.38$, $p = .007$ and quadratic, $F(1,16) = 8.67$, $p = .01$ and post-hoc comparisons revealed differences between Load2 vs. Load4 ($p = .006$) and Load2 vs. Load6 ($p = .02$), but not between Load4 vs. Load6 ($p = .65$). At these electrodes there was no main effect of drug and no drug \times load interaction ($p > .05$).

Drug effects

To identify a neural correlate of L-Dopa on behavior, we compared a difference measure (Load4 minus Load2) between drug conditions (L-Dopa vs. placebo) in every frequency band of interest (see Materials and methods section). Interestingly, a significant power increase over frontal electrodes in the L-Dopa group was revealed only in the low theta band (2–4 Hz) but not in the high theta or alpha band ($p > .05$). Further analysis of the extracted raw data confirmed a significant interaction between drug and WM load at these electrodes, $F(2,32) = 4.98$, $p = .01$. Importantly, in line with our behavioral results, this effect was quadratic, $F(1,16) = 7.70$, $p = .01$, but not linear, $F(1,16) = 2.90$, $p = .11$, and driven by increased power in Load4 but not Load2 or Load6 (Fig. 4). However, there was no significant correlation between the drug effects on power (in the low theta range) and RTs in the WM task and CHRs at LTM retrieval.

There were no other main effects of drug or load and no interactions between both factors (on the difference measure) for any of the three frequency bands.

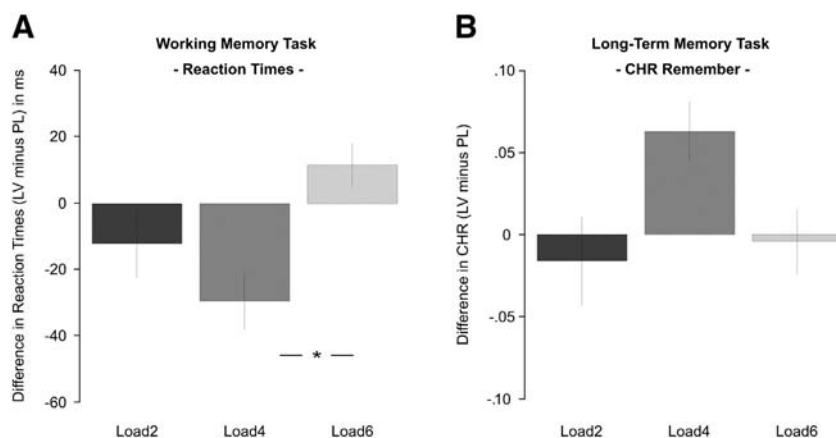


Fig. 2. L-Dopa improved memory performance as a function of WM load. Following dopaminergic stimulation subjects responded faster to the probe during the WM task (A) and remembered more pictures during the LTM task (B), but only when pictures were presented in the Load4 condition. Since we calculated within-subject ANOVAs, error bars were corrected for subject-specific variance (Cousineau, 2005). Asterisks indicate significant post-hoc comparisons (Bonferroni-corrected).

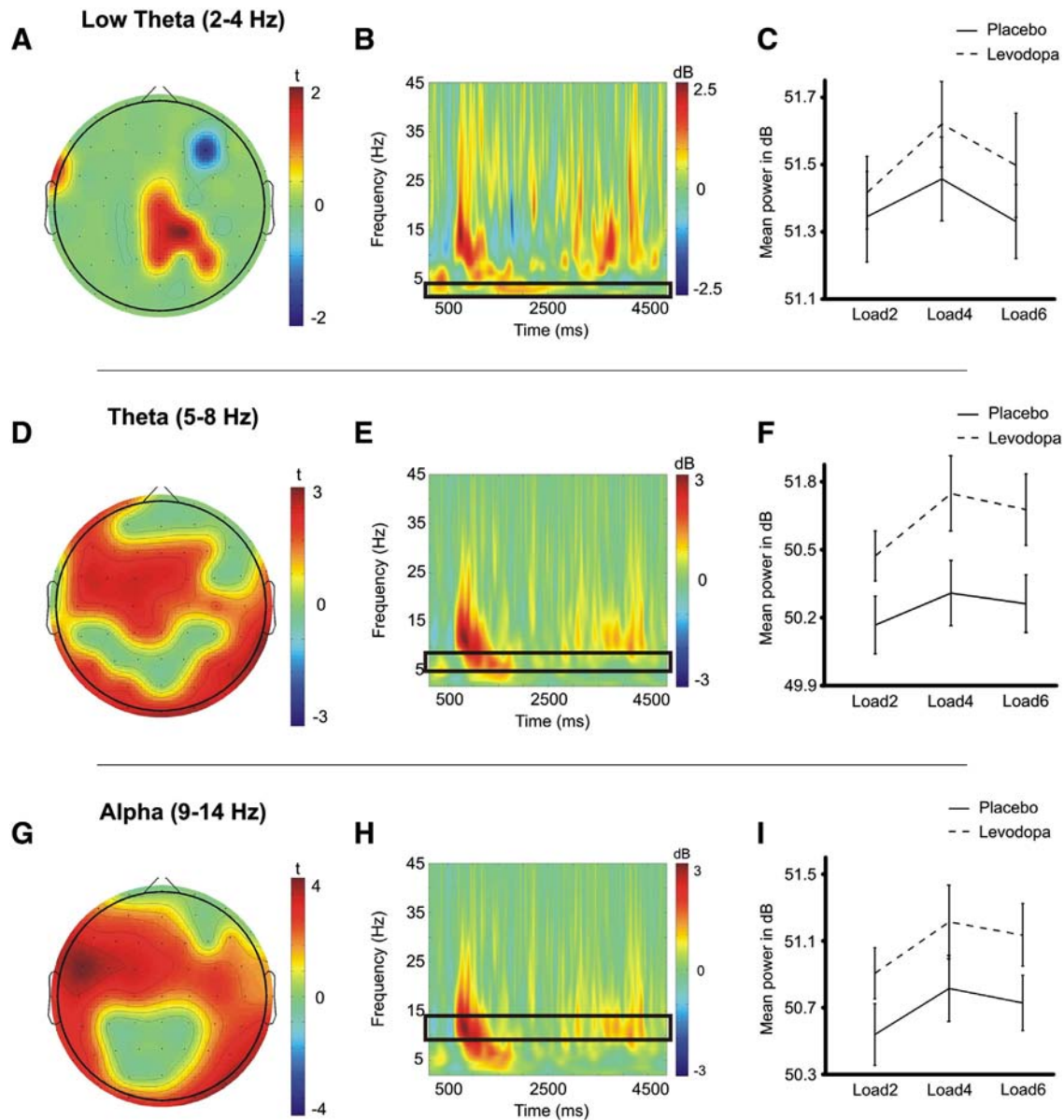


Fig. 3. An increase in WM load was associated with increased oscillatory power in low theta, high theta and alpha power. Statistical scalp maps (A, D, G) represent the direct comparison between the retention of four items (Load4) vs. two items (Load2). Maps show the statistics of the permutation tests where significant differences were found ($p = .05$). The time–frequency plots (B, E, H) display this difference during the retention phase (120–4880 ms) averaged over all significant electrodes. The load-dependent power increase was quadratic in the low theta band (C), but linear and quadratic in the high theta (F) and alpha (I) bands. Since we calculated within-ANOVAs, error bars were corrected for subject-specific variance (Cousineau, 2005).

Discussion

We used EEG in combination with psychopharmacology in healthy humans to investigate the link between WM, neural oscillations and dopaminergic neuromodulation. As a main finding, we can show that L-Dopa accelerated WM retrieval and enhanced LTM-based recollection as a function of WM load. At the neural level, this effect was mimicked by specific increases in the power of low theta (2–4 Hz) but not high theta (5–8 Hz) or alpha (9–14 Hz) oscillations over frontal electrodes. As such, our data indicate dissociating roles of low theta, high theta and alpha-related frequency bands in dopamine-dependent working and subsequent long-term memory.

During WM maintenance, prefrontal theta (5–8 Hz) has often been demonstrated to increase with load (Gevins et al., 1997; Jensen and Tesche, 2002; Moran et al., 2011; Onton et al., 2005; Sauseng et al., 2010). However, in some studies, this effect was weak, absent or even reversed (Bastiaansen et al., 2002; Mitchell et al., 2008) questioning

the role of theta in WM maintenance. Our data help to disentangle these seemingly diverging findings by showing that theta oscillations can be modulated by WM load in a linear and quadratic fashion (Fig. 3). Specifically, while theta power increased from two to four items, there was no further increase from load four to six – instead, it even tended to decrease. This suggests that, if WM load exceeds a certain threshold theta power decreases, which helps to explain why increases in load do not necessarily go hand in hand with theta power increases.

It is important to note that our results do not conflict with previous reports showing only a linear effect of WM load on oscillatory power (Jensen and Tesche, 2002; Onton et al., 2005). In these studies, relatively simple stimuli (digits or letters) were used and the traditional limits of WM capacities (7 ± 2 items; Miller, 1956) were rarely exceeded. Here, we used more complex stimuli – black and white scene images – for which WM capacity is thought to be lower (4 ± 2 items). Accordingly, the retention of six scene images might exceed this capacity and recruit other mechanisms than theta oscillations.

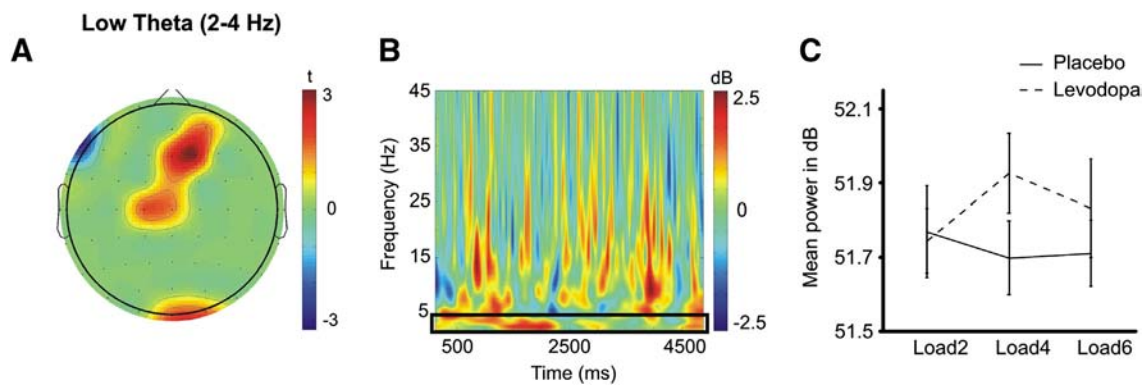


Fig. 4. L-Dopa administration led to increased oscillatory power in the low theta band. The statistical scalp map (A) represents the direct comparison between L-Dopa and placebo in WM-related oscillatory activity (power spectrum for four items (Load4) minus power spectrum for two items (Load2)). Maps show the statistics of the permutation tests for significant differences ($p = .05$). The time–frequency plot (B) displays this difference averaged over all significant electrodes during the retention phase (120–4880 ms). Comparable to our behavioral results, the interaction between drug and load was quadratic (C). Since we calculated within-subject ANOVAs, error bars were corrected for subject-specific variance (Cousineau, 2005).

Indeed, the quadratic effect of WM load on prefrontal high theta (and low theta after dopaminergic stimulation) can be reconciled on the notion that different memory systems support WM maintenance depending on the nature of the task. While initial models claimed a physiological and functional separation between WM and LTM (Baddeley, 1992), there is mounting evidence that both memory systems interact to drive cognitive functions (Ranganath and Blumenfeld, 2005; Ruchkin et al., 2003). One prominent view is that prefrontal WM is supported by medial temporal lobe (MTL)-dependent LTM if WM capacity is exceeded (Cashdollar et al., 2009; Jeneson 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 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.

The behavioral benefits of L-Dopa on WM concord with previous findings (e.g. Moran et al., 2011) suggesting a prominent role of prefrontal dopamine in WM maintenance (Goldman-Rakic, 1995). Importantly, in our study they were accompanied by a specific enhancement of low but not high theta or alpha power. Together with distinct topographic representations of low theta vs. high theta and alpha, this indicates a functional dissociation between these frequency bands. Low theta oscillations in the human hippocampus (Axmacher et al., 2010; Lega et al., 2012) and over prefrontal sensors (Cohen, 2011; Mizuhara and Yamaguchi, 2011) have already been shown to play a role during memory tasks. However, our finding of a modulation of this oscillation pattern by dopaminergic stimulation is, to our knowledge, an important new insight. It resonates well with the baseline firing rates of midbrain dopamine neurons of ~2–5 Hz (Hyland et al., 2002). Moreover, in rats, WM-related neural activity in the PFC and ventral tegmental area (VTA) is also coordinated by oscillations at 2–5 Hz and this was coupled to hippocampal high theta (5–8 Hz; Fujisawa and Buzsáki, 2011). Accordingly, PFC-dependent WM might be orchestrated by two complementary theta bands: the low theta band (2–4 Hz), originating in the VTA, and the high theta band (5–8 Hz), originating in the hippocampus (Fujisawa and Buzsáki, 2011). Our results support this assumption in humans by demonstrating a selective effect of dopaminergic stimulation on low prefrontal theta that was paralleled by improved WM performance. Whether this link reflects a causal relationship or is mediated by other factors remains to be addressed in future studies. The absence of a significant correlation between power increase in the low theta range and the behavioral effects of L-Dopa on RTs in the WM task and CHRs during LTM retrieval argues against a linear relationship. However, it might also be due to

other factors such as differences in noise levels of the behavioral measures and power increase.

Another way to interpret the quadratic drug effects on behavioral and electrophysiological data is based on the inverted u-shaped function of dopaminergic action (Cools and D'Esposito, 2011; Williams and Goldman-Rakic, 1995). Using psychopharmacological manipulations, such effects have been reported for PFC-dependent working memory (Williams and Goldman-Rakic, 1995) and hippocampus-based episodic memory (Chowdhury et al., 2012). However, the amount of dopamine availability is also determined by endogenous factors, such as genetic polymorphisms (Bertolino et al., 2008), and might be expressed e.g. in baseline dopamine levels (Cools and D'Esposito, 2011). Furthermore, task properties also drive endogenous dopamine levels. Specifically, WM activity per se stimulated extracellular dopamine release in the PFC of monkeys (Watanabe et al., 1997) and rats (Phillips et al., 2004). Furthermore, stimulation with amphetamine decreased cortical efficacy during a WM task in carriers of the met/met allele of the catechol O-methyltransferase gene (which tend to have above-average baseline dopamine levels) but only when WM load was high (Mattay et al., 2003). Thus, the higher WM load in our Load6 condition might have resulted in higher endogenous dopamine release and the corresponding decline in WM/LTM performance and prefrontal low theta activity could be the result of supra-optimal dopaminergic activity due to the additive effects of the exogenous (drug intake) and endogenous (task-related release) dopaminergic stimulations. Future studies could use, for instance, positron emission tomography (PET) to directly test this hypothesis.

Our interpretation of improved WM by L-Dopa is based on faster response times during WM retrieval (Vinkhuyzen et al., 2010). Importantly, this effect was specific for the probe when four items had to be encoded. That means, there was no global acceleration of response times by L-Dopa (during encoding or WM retrieval), arguing against a general effect on vigor, which has previously been linked with dopaminergic neurotransmission (Beierholm et al., 2013; Niv, 2007; Pessiglione et al., 2007).

Apart from low and high theta, the alpha band is another 'slow' oscillation associated with WM maintenance (for a review see Jensen et al., 2002). However, similar to theta, the exact role of alpha in WM is still controversial (Jensen and Tesche, 2002; Klimesch, 1999). In particular, given that high theta and alpha often show similar topographic representations and functional properties, it remains unclear whether both frequency bands serve different or similar functions. This is further supported by the fact that individual peak frequencies during WM maintenance are highly variable between subjects, ranging from 2 to 13 Hz (Cohen, 2011). In line with these similarities, we did not observe distinct oscillatory patterns between high theta and alpha activity. Instead, both frequency bands were modulated by WM load (i.e. linear

and quadratic effects) at similar electrodes and there was no effect of ι -Dopa. Thus, our findings argue against a functional separation between high theta and alpha during WM maintenance of complex visual scenes.

As expected, ι -Dopa administration improved subsequent LTM as expressed in higher corrected remember rates. This finding is in agreement with previous work (Chowdhury et al., 2012; Knecht et al., 2004) suggesting a functional link between dopaminergic midbrain regions and the MTL. According to a model by Lisman and Grace (2005), the MTL, including hippocampus, generates a novelty signal if incoming information is classified as new (as during the WM task). This novelty signal is sent to the SN/VTA, which releases dopamine back to the MTL where it drives synaptic plasticity and learning (Lisman et al., 2011; Lisman and Grace, 2005). Although our data further support a role of dopamine in MTL-dependent learning, it remains unclear why subsequent long-term memory was specifically enhanced for load four rather than all three load conditions. One possibility is that facilitating effects of ι -Dopa on subsequent long-term memory require the involvement of the PFC during encoding. This hypothesis is supported by the notion that the PFC might be a key player in regulating the hippocampal-SN/VTA loop (Lisman and Grace, 2005) and our observation of strongest prefrontal theta oscillations in the load four condition.

Finally, we would like to acknowledge that oscillations below 4 Hz have often been referred to as ‘delta’ oscillations. Here, we followed recent work and labeled them as ‘low theta’ (Lega et al., 2012) for two reasons. First, oscillations in this range (2–4 Hz) are known to be modulated by memory load (van Vugt et al., 2010), and second, they play a role in spatial navigation (Watrous et al., 2013). Thus, it shares some important features with conventional theta oscillations (5–8 Hz) and might be functionally related to it.

Taken together, our data give new insights into the functional properties of theta and alpha band oscillations during dopamine dependent WM maintenance. While high theta and alpha power showed similar functional properties and topographic representations, they were not affected by dopaminergic stimulation. In contrast, low prefrontal theta power increased following ι -Dopa administration and this effect was paralleled by enhanced WM and LTM performance. This, in turn, might indicate functional interactions between WM and LTM systems as well as differential properties of low vs. high theta and alpha band oscillations.

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Conflicts of interest

The authors declare no competing financial interests.

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