THE EFFECT OF SCOPOLAMINE ON DECLARATIVE LEARNING AND FRONTAL MIDLINE THETA ACTIVITY

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ABSTRACT

The role of theta oscillations in and around the frontal midline have been well documented in working memory and sustained attention related processes. However, the role of these oscillations in relation to learning processes, such as encoding and consolidation, have not yet been thoroughly studied in humans. In animal studies, scopolamine has been shown to hinder conditioning as well as decrease the hippocampal neural response and amount of theta. Some studies have also reported scopolamine induced disruption in hippocampal activity in humans. Using a double-blind setting, the present study used 1mg/72h transdermal scopolamine to hinder learning and memory functions in half of the sample while measuring memory performance with WMS-R logical memory and word pairs tasks, and learning performance in a trace eyeblink classical conditioning setting while measuring a frontal midline EEG activity. Scopolamine caused a decrement in encoding performance in WMS-R logical memory and word pairs tasks, and a slower rate of acquisition in trace-EBCC. Conditioned stimulus evoked frontal midline theta activity was more prominent in the control group. Groups did not differ based on alpha activity during any of the measured segments. The findings of the present study provide leeway for another interpretation of frontal midline theta activity besides its prevalence observed during working memory load and sustained attention.

Keywords: scopolamine, frontal midline theta, learning, declarative memory, EEG, classical conditioning, EBCC

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Frontomediaalisen alueen theta-oskillaatioilla on todettu olevan merkitsevä yhteys työmuistiin sekä tarkkaavuuden ylläpitoon liittyviin prosesseihin. Näiden oskillaatioiden yhteyttä oppimiseen, kuten mieleen painamiseen ja konsolidaatioon, on tutkittu melko vähän ihmisillä. Eläintutkimuksissa skopolamiinin on osoitettu haittaavan ehdollistumista ja alentavan hippokampuksen neuraalista toimintaa sekä theta-aktiivisuuden määrää. Joidenkin tutkimusten mukaan skopolamiini aiheuttaa hippokampuksen häiriintynyttä toimintaa myös ihmisillä. Tässä tutkimuksessa, noudattaen kaksoissokkoasetelmaa, koehenkilöille annettiin 1mg/72h skopolamiinilaastari, jolla heikennettiin oppimiseen ja muistiin liittyviä toimintoja. Puolet koehenkilöistä altistuivat skopolamiinille, ja puolelle koehenkilöistä annettiin plasebolaastari. Oppimis- ja muistisuoriutumista mitattiin silmäniskuehdollistamiskokeen sekä WMS-R -testistön loogisen muistin tehtävien ja sanaparitehtävien avulla. Ehdollistamiskokeen aikana mitattiin frontomediaalisen alueen EEG-aktiivisuutta. Skopolamiini heikensi mieleenpainamista sanapari- ja loogisen muistin tehtävissä sekä alensi ehdollisen vasteen esiintymistodennäköisyyttä ehdollistamiskokeessa. Ehdollisen ärsykkeen aiheuttama frontomediaalisen alueen theta-aktiivisuus oli merkitsevästi suurempaa kontrolliryhmällä. Alfa-aktiivisuudessa ei ollut eroa ryhmien välillä. Tämän tutkimuksen tulokset laajentavat näkökulmaa frontomediaalisen theta-aktiivisuuden yhteyksistä kognitiivisiin toimintoihin.

Avainsanat: skopolamiini, frontomediaalinen theta, oppiminen, deklaratiivinen muisti, EEG, klassinen ehdollistaminen, EBCC

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INTRODUCTION

The present study investigates the effects of scopolamine on human cognitive functions and neurophysiology. Scopolamine, also known as hyoscine, is an anticholinergic medication mainly used to treat motion sickness. Simpraga et al. (2017) applied imaging with EEG to discover biomarkers related to scopolamine. By using the data measured with EEG, they were able to compose 14 biomarkers. Based on those biomarkers it was possible to differentiate healthy subjects from those who suffered from Alzheimer disease (AD). Therefore, scopolamine could produce similar cognitive deficiency related electroencephalogram alterations as AD. In this study, WMS-R and eyeblink classical conditioning (EBCC) were used to measure the effect scopolamine has on learning and declarative memory. EEG was used to image evoked brain activity in the frontomedial region during EBCC.

Scopolamine and the hippocampal cholinergic system

Acetylcholine is a central neurotransmitter in the hippocampus (Taepavarapruk & Song, 2010; Drever et al., 2011; Micheau & Marighetto, 2011). Medial temporal lobe, of which the hippocampus is a part of, has an essential role in the formation of declarative memory trace (e.g. Squire, 1992; Tulving, & Markowitsch, 1998). Furthermore, it is known that AD produces alterations specifically on the functioning of the medial temporal lobe (Braak & Braak, 1995).

Because of the centrality of acetylcholine in the hippocampus, scopolamine might produce memory and learning related cognitive deficits via its antagonistic mechanism on the cholinergic system. Scopolamine interacts with the functioning of the cholinergic system by blocking the binding sites of acetylcholine on the cell membrane. More specifically, scopolamine interacts with muscarinic acetylcholine receptors (mAChR) in the nervous system. Muscarinic receptors are G protein (heterotrimeric [α, β & γ-subunits] GTP-binding proteins) coupled receptors (GPCR). Muscarinic receptors are divided into five subunits from M_1 to M_5 that differ based on their location in the nervous system. M_1, M_3 and M_5 are G_q protein-coupled receptors, whereas M_2 and M_4 are G_i protein-coupled receptors. G_i protein-coupled receptors act by a cyclic-ATP cascade (activation results in the inhibition of protein kinase A release [cf. G_s]), while G_q protein-coupled receptors act through IP3/GAD signal pathway. (Caulfield, 1993.) Even though there are findings pointing to scopolamine's

indirect specificity for M_1-receptors (Burke, 1986**),** scopolamine is still widely considered a nonspecific antimuscarinic.

Literature into scopolamine's effects on cognition demonstrates that scopolamine causes decrements in memory performance (Sperling et al., 2002; Koller et al., 2003; Sherman et al., 2003; Schon et al., 2005; see Graef et al., 2011 for review on cholinergic effects on cognition). In their study, Bahro et al. (1999) exposed ten young females to scopolamine. They then measured blood circulation of the brain during an EBCC experiment. They noticed that a decline on the conditioning to a stimulus was also perceptible as alterations on the blood circulation of the brain. More elaborately, it increased blood circulation in posterior cingulate gyrus and lateral temporo-occipital cortex. The blood circulation decreased significantly in the cerebellar cortex and the insula. These findings were congruent with earlier findings (Molchan et al., 1994; Schreurs et al. 1997). There were also bilateral alterations in occipital cortex, medial temporo-occipital cortex, thalamus, putamen and the right side of the cerebellar/brain stem. The evidence from the study demonstrates how scopolamine influences brain functions.

Central to the present study is the effect scopolamine has on the hippocampus. Previous research has revealed some of these effects in both human and animal studies. Asaka et al. (2000) found that when injecting a microinfusion of scopolamine into the medial septum of rabbits, their conditioning was hindered, alongside a decreased hippocampal neural response to conditioning and a decreased amount of hippocampal theta. Antanova et al. (2011) also showed that scopolamine disrupts hippocampal activity in humans. In addition, scopolamine has also been shown to hinder consolidation when directly injected to dorsal hippocampus (intra-CA1) (Jamali-Raeufy et al., 2011) and encoding when directly injected into the CA3 (Rogers & Kesner, 2003) in rats. These findings from animal studies as well as studies done on humans point towards scopolamine having a significant impact on memory and learning related hippocampal activity.

A discussed aspect in the research of scopolamine's effects on memory derive from a difficulty to separate memory impairments from decrements in arousal, vigilance or attention. Therefore, some previous studies have been subject to criticism for their inability to disassociate scopolamine’s memory impairing effects from its effects on arousal. This makes literature on the cognitive effects of scopolamine equivocal. Some previous research has found connections between scopolamine and impairments in attention (Graef et al., 2011) and vigilance (Koller et al., 2003). However, some research points to scopolamine having an intrinsic effect on memory that is not caused by a reduction in arousal (Curran et al., 1998). Other previous studies have also failed to associate scopolamine with impaired attention (Sherman et al., 2003). The equivocal nature of the findings into scopolamine's cognitive effects calls for more multifaceted and strictly controlled studies as well as a critical

evaluation of the current understanding on the matter. One aim of this study is to also contribute towards a clearer picture of the cholinergic modulation of cognition.

Frontal midline theta activity

Execution of complex cognitive functions demands coordination between separate neural circuits. Brain rhythms, or oscillations, enable the co-operation between different circuits. Oscillations offer a mechanism to link requisite areas in the brain to accomplish the required function. The theta rhythm is one of the largest rhythms in the brain and it is also the most sinusoidal rhythm (Colgin, 2013). Theta is a slow rhythm with an assigned frequency of about ~3–12 Hz (e.g. Nokia et al., 2008; Colgin, 2013) in animal studies and ~4–8 Hz in human studies (e.g. Gevins et al., 1997; Jensen and Tesche, 2002). Theta activity in and around hippocampus relates essentially to learning. Theta oscillations occur especially in the input areas of the hippocampus: in the dentate gyrus and in the hippocampal fissure (Buzsáki, 2002; Waselius et al., 2018).

Relevant to the present study is frontal midline (FM) theta activity. FM theta is especially linked to memory functions (Mitchell et al., 2008; Hsieh & Ranganath, 2014). More specifically, increased theta activity in frontal medial regions is associated with increased working memory (WM) load (Maurer et al., 2015). Many studies have found that the frequency range of 5–7 Hz is especially associated with this increase in WM load (e.g. Gevins et al., 1997; Hsieh et al., 2011; Maurer et al., 2015), and some studies have localized the origins of these oscillations to the anterior cingulate cortex (Gevins et al., 1997).

Some of the more deviant findings came from Jensen and Tesche (2002), who found that a retention of visually presented digits increased 7–8.5 Hz frontal theta response in relation to how much information was retained. The more digits the participants had to retain, the more theta activity they had. In another study, Melzer et al. (2008) found mixed results after conducting intracranial EEG recordings with epilepsy patients who performed Sternberg tasks. Along with increases, some patients also showed a decrease in theta power (mean 6.5 Hz) during Sternberg task induced WM load. This deviance from scalp EEG findings could be explained with the more precise measuring methods, but also by the fact that the patients had a possibly altered cognition due to epilepsy and pain medication. Additionally, FM theta has been shown to increase during successful recognition and retrieval (Gruber et al., 2008), suggesting a more multifaceted role for FM theta in different memory and learning related processes.

The WM load associated increase of FM theta activity might also be due to increase in attentional engagement. Therefore, FM theta activity might reflect an attentional instead of memory related neural processes. Some previous studies (including some of the studies regarding FM theta in relation to WM load mentioned in the previous paragraph) have found increases in FM theta in relation to increases in a task's attentional demand (Aftanas & Golocheikine, 2001; Asada et al., 1999). Sauseng et al. (2007) specifically showed an increase in FM theta without inducing demands for WM, paving the way for the hypothesis that FM theta is more closely related to attentional instead of memory related processes. Nevertheless, the notion that FM theta is associated with cognitive demand is consistent with the findings regarding WM load and sustained attention.

There are suggested connections between FM theta and hippocampal theta (Mitchell et al., 2008). This is in line with assumptions about the possible connections between the cholinergic system, AD symptoms (learning and memory) and FM theta activity. Currently there is not an established literature based around the relation between FM theta and learning (for an exception, see Laukka et al., 1995). Therefore, one aim of the present study is to extend the literature regarding FM theta and its possible connection to learning.

Eyeblink classical conditioning

Eyeblink classical conditioning (EBCC) is a method where a subject is exposed to initially meaningless stimulus, e.g. noise, followed by a biologically meaningful stimulus (e.g. air puffs aimed at the corner of the eye). In trace-EBCC, learning of the relation between former stimulus, CS (conditioned stimulus), and latter stimulus, US (unconditioned stimulus), demands conscious perception and learning. In delay-EBCC, the conscious component is not necessary. In the trace method, there is pause between CS and US, but in the delay method, the CS continues until the US occurs. (e.g. Clark & Squire, 1998.)

The cerebellum is substantial in EBCC, whereas the hippocampus modulates the acquisition of a conditioned response (CR). Therefore, by manipulating the hippocampal cholinergic system, it is possible to affect to a level of learning (Woodruff-Pak et al., 1996). Hippocampal activity is related to the acquisition of a CR in the EBCC. Instead of trace-EBCC, the delay paradigm where CS and US are interlaced does not require hippocampus at all. Consequently, learning can occur even when the hippocampus is removed, as Schmaltz & Theios (1972) and Solomon & Moore (1975) noticed already in the 1970´s. Furthermore, humans with hippocampal lesions or amnesia are still capable to

learn CR in the delay-EBCC paradigm (see Daum et al., 1989; Daum et al., 1991; Gabrieli et al. 1995). Thus, the intact hippocampus and declarative memory are essential for acquisition of the CR in the trace-EBCC paradigm, but not in the delay-EBCC paradigm.

In a study by Moore et al. (1976), where rabbits received an injection of scopolamine hydrobromide subcutaneously, suggested that scopolamine decreases the rate of acquisition of conditioned response in EBCC paradigm. Later, Solomon et al. (1993) demonstrated in their study that scopolamine caused similar effects in humans too. They compared the effects of scopolamine hydrobromide and peripheral cholinergic blocker, glycopyrrolate, to elicit the detrimental effect of scopolamine on cholinergic system in a central nervous system (CNS). Only the group administered with scopolamine hydrobromide showed decreased learning rate in EBCC paradigm. Consequently, it is presumable that scopolamine could have effect on learning and declarative memory via cholinergic system in the CNS.

Research aims

The aim of the present study is to examine the effects of scopolamine on learning, declarative memory and FM theta during a learning task. Additionally, because alpha activity has generally been associated with psychophysiological states before falling asleep, drowsiness and mental fatigue (e.g. Cantero & Atienza, 2000; Shigihara et al., 2013; Gharagozlou et al., 2015), which in turn are assumed to hinder learning, the significance of FM theta in learning mechanisms is supported with inspections of group differences in alpha activity.

Previous literature on FM theta has focused primarily on working memory and sustained attention, without much emphasis on processes such as encoding, storing (consolidation) and retrieval. Another aim of this study is to fill the lack of literature on the relationship between FM theta and learning in general.

The research questions of the present study are:

1. Does scopolamine affect memory performance in WMS-R logical memory and word pairs tasks?
2. Does scopolamine affect the rate of acquisition during a trace-EBCC setting?
3. Are there significant group differences in evoked frontal midline theta activity?
4. Are there significant group differences in ongoing frontal midline theta activity?

We hypothesize a decremental effect of scopolamine on memory tests as well as on the learning rate of the conditioned response in a trace-EBCC setting. We hypothesize a decrease in evoked frontal midline theta activity in the experimental group during the trace-EBCC test. We also hypothesize no group differences in spontaneous, ongoing frontal midline theta activity between groups.

METHODS

Participants

The sample of this study consisted of two sets of data which included participants from years 2016 (N=27, male=12, female=15) and 2017 (N=21, male=3, female=18). Participants were aged from 19 to 31, with a mean age of 22.1. All participants were university students who volunteered to participate in the study. Participants were recruited from lectures and via email lists or social networks. The first dataset consisted of the data gathered in 2016 and 2017. This dataset was used for all behavioral analyses. The second dataset consisted only of the participants from 2016. The second dataset was used for the EEG analysis. All participants signed an informed consent.

Procedure

After recruitment, the participants were assigned randomly to either control or experimental groups. The participants were informed about the procedure of the study and were provided with an informative label about the scopolamine product (transdermal scopolamine, product name "Scopoderm", 1mg/72h). The scopolamine or a placebo patch was personally delivered (by a person not interacting with the participant during the next morning's experimental procedures) to every participant during the day before the actual experiment. Participants were instructed to place the patch behind their ear at around 20:00, and not to remove it until the end of the experiment.

A maximum of two measurements were carried out on each experiment day, and a measurement began either at 8:00 or 9:00. Because of a double-blind procedure, the researchers doing the measurements did not know which group the participants belonged to. A small tour of the measuring

space was first shown to every participant upon their arrival. After the tour, two WMS-R (Wechler Memory Scale-Revised) tests, logical memory and word pairs, were performed.

After the WMS-R memory tasks, the participants underwent a trace eyeblink classical conditioning setting that lasted approximately 25 minutes. Scalp EEG was measured from all participants during trace-EBCC. After the EBCC experiment, the second part of the logical memory and word pairs tasks were performed to examine delayed recall performance. All participants were rewarded with a movie ticket after their experiment ended.

Electrophysiological recordings

EEG measurements were carried out using a passive 64-channel elastic cap (EasyCap GmbH; Ag/AgCl electrodes). External amplifiers (NeurOne, MegaElectronics, Kuopio) were used to amplify the signals from the electrodes, and the signal was recorded with a sample rate of 1000 Hz. NeurOne-software (MegaElectronics, Kuopio) was used to record EEG. The channels were recorded with a maximum impedance of 5 kOhm. EMG was recorded with a pair of disposable electrodes attached under the right eye, aimed to measure the electrophysiological activity of the orbicularis oculi -muscle.

Tests for declarative memory

Two WMS-R test parts, the logical memory task and the word pairs task, were used to examine declarative memory. Both tasks were performed in the beginning and in the end of a research session. The logical memory task involved two stories told separately. After hearing one story, the participant was asked to verbally repeat every detail they could recall of it. The procedure was identical with the second story. Delayed logical memory recall was examined at the end of the research session by asking the subject to recall as many details from the stories as they could without hearing the stories again. The logical memory task was scored from 0 to 50 points, 25 points from each story in both phases (immediate or delayed recall).

The second task, word pairs, involved a researcher presenting eight pairs of words. The participant was then told only the first word of the pairs, to which they had to recall the pair of that word. Four

out of the eight word pairs were congruent and four were incongruent. If a subject was not able to recall the pair, the right answer was told to him/her. The list of eight word pairs was repeated at least three times to all subjects. The word pairs were in different order when they were repeated. Even if a subject was able to recall all the pairs, the list of pairs was still repeated three times. The achieved points from three repeats were written down. If a subject was not able to recall word pairs after three repeats the word pairs were repeated six times at maximum. Repeating was stopped if a subject was able to recall all word pairs. Repeats four to six did not accumulate achieved points. In the end of a research session the word pairs were asked again to measure delayed recall. The participant was told the first word of the pairs, to which they had to recall the pair of that word. The pairs were asked only once. The word pairs task was scored from 0 to 24 points in the immediate recall phase, and from 0 to 8 points in the delayed recall phase.

Behavioral analysis

EMG data was used to decipher differences in learning between groups. First, the EMG data was high-pass filtered (30 Hz), rectified and then low-pass filtered (50 Hz). An eyeblink within 450-850ms after the CS was counted as a CR. Eyeblinks before or beyond that range were counted as non-CRs. An eyeblink was considered to have occurred when the EMG activity exceeded the baseline by at least 4 standard deviations. Comparisons between participants were carried out by dividing a frequency of each individual´s CRs with the total amount of CSs within each block, i.e. a probability to exhibit the conditioned response was calculated and analyzed.

The WMS-R logical memory test, i.e. two stories, had a maximum score of 50 points in both, immediate and delayed, recall phases. The raw scores achieved from the test were used in the analysis to examine possible group differences in logical memory performance. The WMS-R word pairs test scores were analyzed as percentages, because the delayed word pairs task had a maximum score of only 8 points instead of the 24 points in the immediate recall task. The GLM (general linear model) repeated measures (SPSS Statistics, version 24, IBM) were used to carry out the statistical analyses with all behavioral variables.

EEG Analysis

The raw EEG data was analyzed with BrainVision Analyzer (version 2.1.0.327, professional edition, Brain Products GmbH). First, the EEG channels that had visually noticeable interferences, were removed from further analysis. A new reference for the EEG channels was calculated based on the averages of all the EEG channels. Independent component analysis (ICA) was used to eliminate artefacts caused by blinking and eye movements (intervals used for ICA: Start 100 s, Length 120 s; channels: 26). Components representing stereotypical artefacts (eyeblinks and lateral eye movements) were removed based on visual inspection. A total of 50 channels were removed from the data so that 14 frontomedial channels were included in further analysis.

Three segmentations were carried out based on CS (conditioned stimulus, sound cue) and US (unconditioned stimulus, air puff) marker positions. First segmentation, named ”pre-CS”, consisted of 5000ms before the CS. Second segmentation, named ”CS”, consisted of 600ms after the CS. The final segmentation, named ”US”, consisted of 1000ms of data from 400ms to 1400ms after the US. The final segmentation begun 400ms after the US to eliminate the effect of the artefact caused by UR (unconditioned response, eyeblink).

A Fast Fourier transform (FFT) was used to extract the spectral power of the segments, which were then divided into blocks (each representing 9 trials). The data was further pooled with 8 channels representing the region of interest (see. Figure 1). Finally, the area information (μV *x* Hz) from 4 to 6 Hz (theta) and 8 to 12 Hz (alpha) was extracted for statistical analysis. The theta range of 4–6 Hz was specifically selected for further analysis by visually inspecting the spectral power and determining the theta range where groups differed most noticeably (see Figure 2). To test for differences between groups in theta and alpha power, the GLM repeated measures was carried out using SPSS Statistics (version 24, IBM).

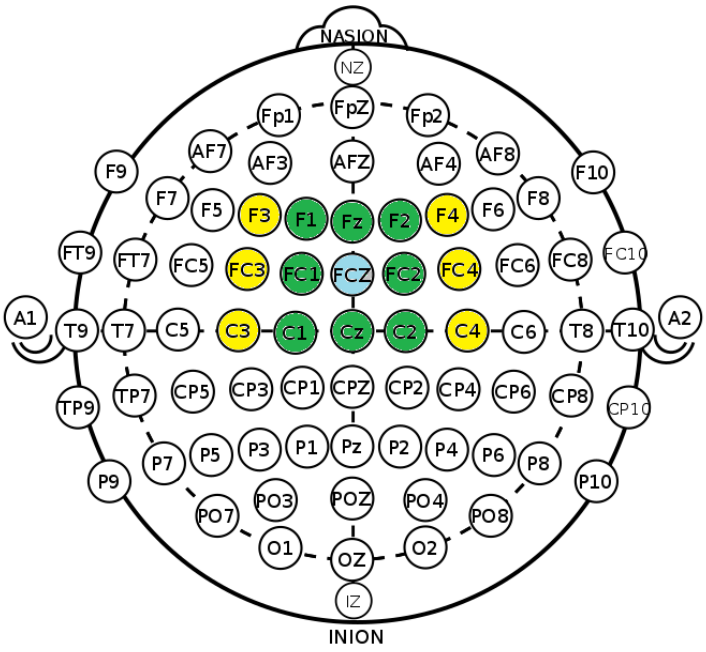

Figure 1. Frontomedial channels. Blue channel (Fcz): implicit reference. Yellow channels: removed from the final analysis. Green channels: included in the final analysis.

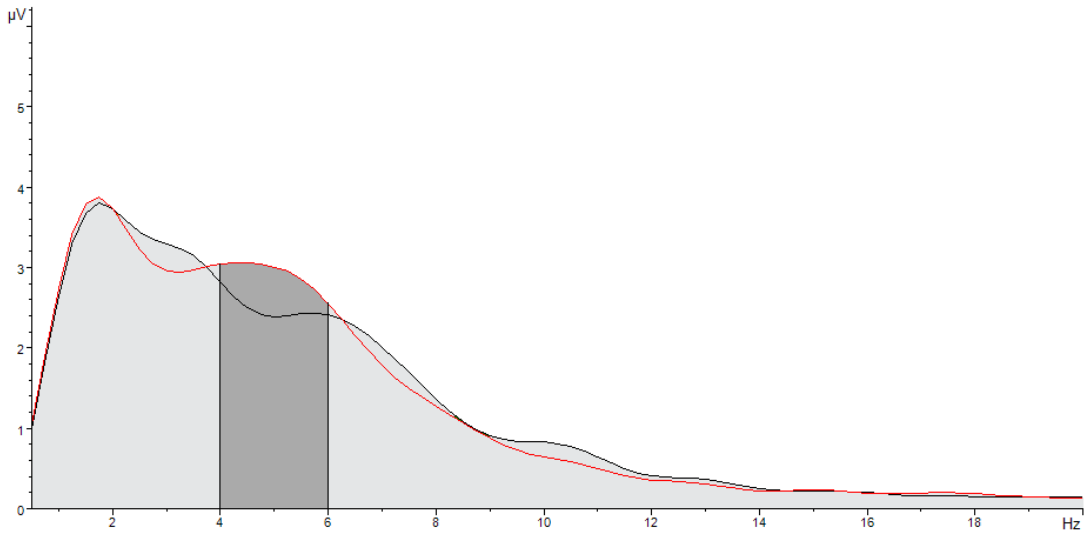

Figure 2. Frequency distribution (Fast Fourier transform) of neural oscillations 0-600ms after CS in early-block. Black line = experimental group, red line = control group. Theta band (4–6 Hz) selected for further analysis is highlighted with darker grey.

The data (consisting of 8 blocks with 9 trials within each) were further divided into four blocks: the first block, named ”intro”, consisted of first nine trials which contained only the CS, the second block, named ”early”, consisted of the next 36 trials which contained both the CS and the US, the third block, named ”late”, consisted of the next 36 trials and was otherwise identical with the second block, and the final block, named ”extinction” consisted of only 9 CSs.

The same statistical tests were performed for theta and alpha power in response to the different segmentations (pre-CS, CS and US) with and without the first block. The rationale behind the exclusion of the first block in some of the statistical tests is to determine whether inclusion of only blocks that involve learning elements (acquisition and extinction) leads to more significant difference in FM theta between groups. The underlying assumption is that if the tests which include only blocks that involve learning processes lead to more statistically significant results (in terms of theta activity) compared to the tests that also involve the first block (which consisted of 9 CS-alone trials), then further conclusions about the role of FM theta in learning can be made.

RESULTS

Behavioral results

The GLM repeated measures revealed that there was a significant difference between groups in term of probability to exhibit a CR in the trace-EBCC experiment ($F(1, 43) = 4.41$, $p = .042$, ηp2= .093). There was also a significant difference in interaction (block*group) ($F(2.05, 88.30) = 5.34$, $p = .006$, ηp2= .111) (The Greenhouse-Geisser correction was used because the Mauchly´s test of sphericity was significant). See also Figure 3A. See Table 1 (section *CR probability*) for means and standard deviations.

The GLM repeated measures discovered that the performance in WMS-R subtests were different between groups. The control group performed significantly better in logical memory test ($F(1, 42) = 5.43$, $p = .025$, ηp2= .115) and in word pairs test ($F(1, 42) = 6.14$, $p = .017$, ηp2= .128). The interaction was not significant in the logical memory test ($F(1, 42) = 0.60$, $p = .444$, ηp2= .014) or in the word pairs test ($F(1, 42) = 0.94$, $p = .337$, ηp2= .022). Thus, the change between immediate and delayed recall tasks were not significantly different between groups in either test. See also Figure 3B and 3C.

See also Table 1 (sections *WMS-R logical memory* and *WMS-R word pairs*) for means and standard deviations.

		control group		exp. group	
	phase	$\bar{x}$	*SD*	$\bar{x}$	*SD*
CR probability					
	intro	0.15	0.13	0.16	0.17
	early	0.57	0.28	0.36	0.31
	late	0.57	0.31	0.33	0.32
	extinction	0.17	0.14	0.12	0.18
WMS-R logical memory					
	immediate	27.76	6.94	23.57	6.05
	delayed	25.71	6.90	20.87	6.43
WMS-R word pairs					
	immediate	86.51	13.30	74.28	21.01
	delayed	96.43	8.96	88.59	15.03

Table 1. Means and standard deviations used in the behavioral analyses. exp. group = experimental group; *phase* column in CR probability = intro, early, late or extinction block; *phase* column in WMS-R sections = immediate or delayed recall; $\bar{x}$ = mean; *SD* = standard deviation; Means and SDs for WMS-R word pairs are presented in percentages (because of the difference in maximum scores between immediate and delayed recall).

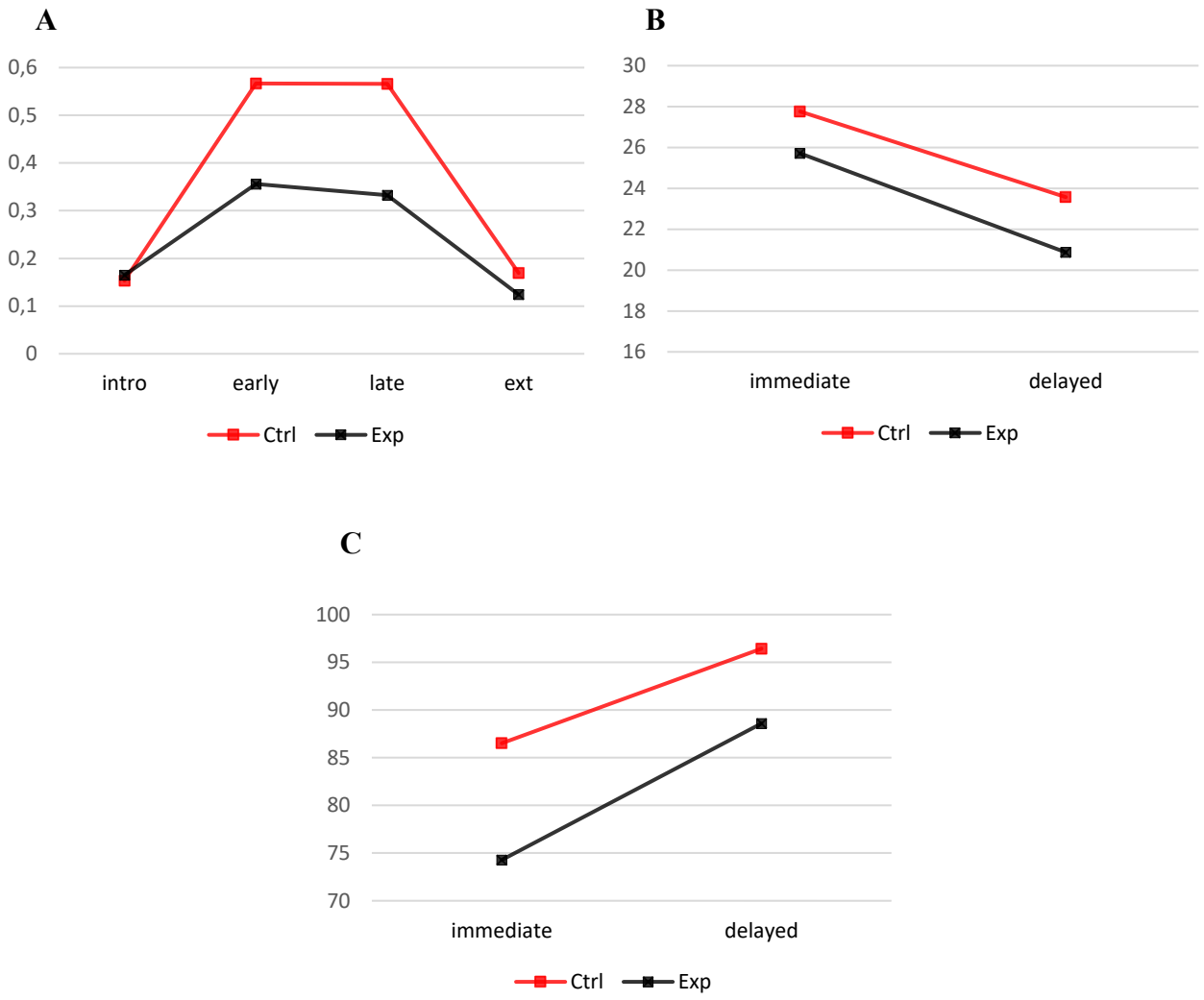

Figure 3. Charts for behavioral data. (A) The groups' probability of showing a conditioned response in trace-EBCC setting in each block. (B) The change in scores between immediate and delayed recall in logical memory tasks by groups. (C) The change in scores between immediate and delayed recall in word pairs tasks by groups (the ascending trend is caused by the different pointing system between immediate and delayed recall phases)

EEG results

The GLM repeated measures revealed that only theta in CS segment was significantly different between groups (when the intro block was excluded). The examination for interactions (block*group)

revealed that there was not any significant interaction in any case (alpha, theta; pre-CS, CS, US; with or without the intro block). For the F-values, *p*-values and partial eta squared -values for group differences attained from the statistical analyses, see Table 2. See Table 3 for means and standard deviations. See also Figure 4. The Greenhouse-Geisser correction was used in some cases (interactions for alpha, in cases of pre-CS (both three and four blocks) and CS (only in a case of three blocks)) because the Mauchly´s test of sphericity was significant.

	blocks	*F(1, 25) = x.xx*	*p*	*ηp2*
Theta 4–6 Hz, between groups				
pre-CS	4	0.71	.407	.028
pre-CS	3	0.75	.396	.029
CS	4	4.01	.056	.138
CS	3	5.63	*.026	.184
US	2	0.28	.604	.011
Theta 4–6 Hz, interactions (block*group)				
pre-CS	4	0.93	.430	.036
pre-CS	3	1.48	.237	.056
CS	4	1.24	.302	.047
CS	3	0.13	.878	.005
US	2	0.02	.900	.001
Alpha 8–12 Hz, between groups				
pre-CS	4	0.11	.917	.000
pre-CS	3	0.004	.951	.000
CS	4	1.70	.204	.064
CS	3	2.24	.147	.082
US	2	0.19	.667	.008
Alpha 8–12 Hz, interactions (block*group)				
pre-CS	4	0.74	.501	.029
pre-CS	3	1.22	.298	.047
CS	4	0.65	.587	.025
CS	3	0.35	.657	.014
US	2	0.39	.539	.015

* $p < 0.05$

Table 2. Statistics attained from the analyses. *blocks* = number of blocks included in the analysis, 4 blocks = intro, early, late and extinction blocks; 3 blocks = early, late and extinction blocks; 2 blocks = early and late blocks. *x.xx* = F-value, *p* = p-value, *ηp2* = partial eta squared. There are only two blocks (early and late) in the US segmentation, because intro and extinction blocks involved only the CS (and pre-CS).

		control group		exp. group	
	block	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Theta 4–6 Hz					
pre-CS	intro	3.21	0.71	3.01	0.76
pre-CS	early	2.99	0.74	2.80	0.71
pre-CS	late	2.95	0.81	2.79	0.74
pre-CS	extinction	3.14	0.85	2.76	0.72
CS	intro	5.23	2.00	4.96	2.10
CS	early	6.36	1.96	5.10	1.43
CS	late	5.77	2.55	4.17	1.37
CS	extinction	5.91	1.84	4.44	1.96
US	early	2.50	0.85	2.36	0.76
US	late	2.52	0.81	2.34	1.10
Alpha 8–12 Hz					
pre-CS	intro	4.71	2.62	4.96	2.83
pre-CS	early	5.22	2.81	5.03	2.75
pre-CS	late	5.42	3.11	5.40	2.97
pre-CS	extinction	5.41	3.23	5.85	3.31
CS	intro	2.24	1.59	2.46	1.26
CS	early	2.78	1.39	3.28	1.49
CS	late	2.97	1.53	3.72	1.63
CS	extinction	2.78	1.61	3.79	1.83
US	early	3.53	1.96	3.74	2.52
US	late	3.66	2.76	4.23	2.35

Table 3. Means and standard deviations used in the analyses. exp. group = experimental group; *block* = intro, early, late or extinction block; $\bar{x}$ = mean; *SD* = standard deviation. There are only early and late blocks in the US segmentation, because only those blocks included the US.

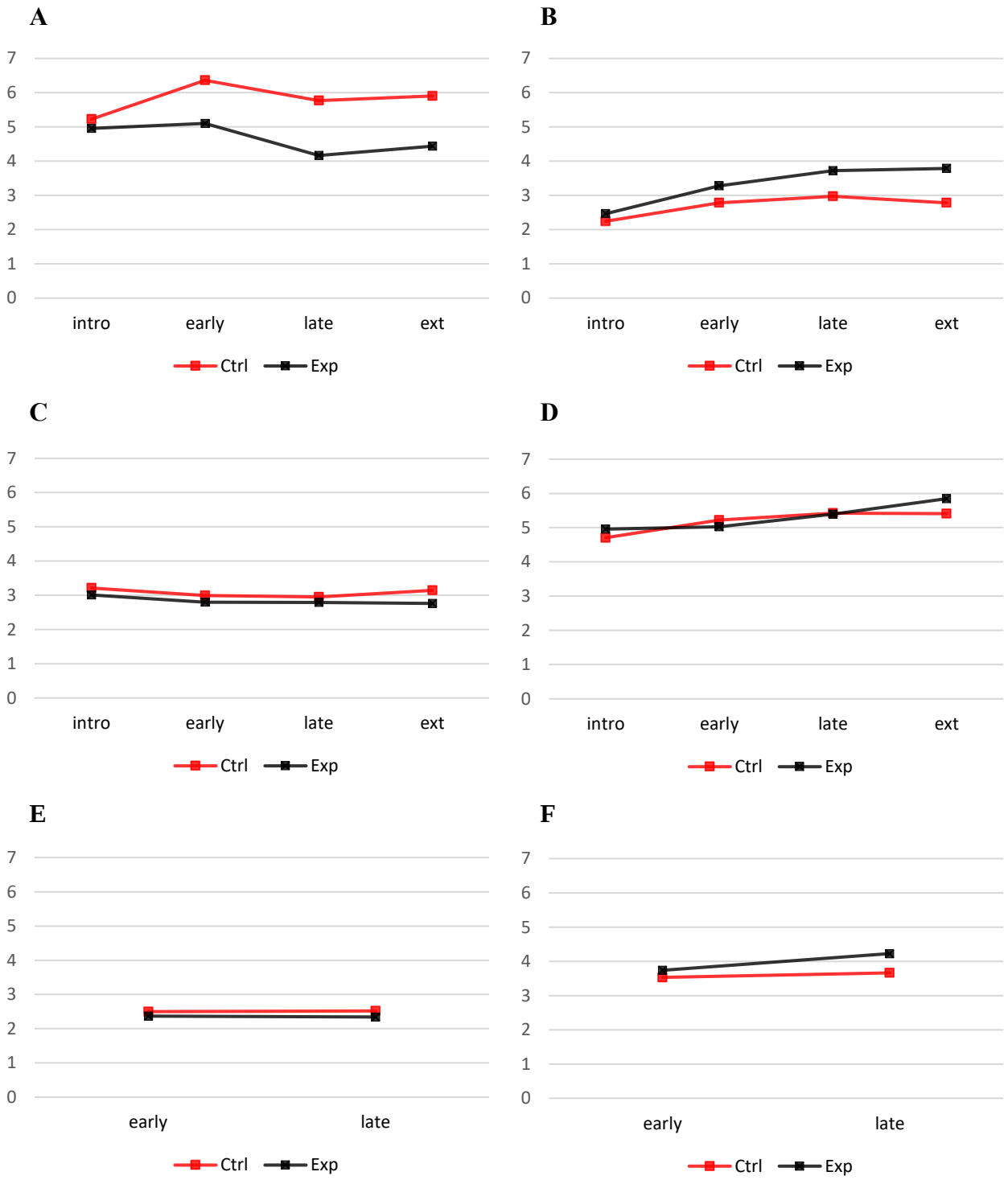

Figure 4. Charts indicating the values for area information of 4–6 Hz theta and 8–12 Hz alpha separated by groups. (A) Theta 0–600ms after the CS. (B) Alpha 0–600ms after the CS. (C) Theta 5000ms before the CS. (D) Alpha 5000ms before the CS. (E) Theta 400–1400ms after the US. (F) Alpha 400–1400 after the US.

DISCUSSION

The present study investigated the effect of transdermal scopolamine on learning and declarative memory, and on frontal midline theta activity during a trace-EBCC setting. Scopolamine was shown to cause decrements in performance in memory tasks using WMS-R logical memory and word pairs tasks. Scopolamine also decreased the rate at which the conditioned response was acquired during the trace-EBCC. CS evoked 4–6 Hz frontal midline theta activity was significantly more prominent during the trace-EBCC in the control group when only trials that involved learning were included, and almost statistically significant ($p=0.056$) when all blocks were included. There were no significant group differences in spontaneous, ongoing theta activity. The interactions were not significant in any case. The level of theta power stabilizes quite quickly after the CS has been presented and will not change over time (see also Figure 4 A). These results are suggesting that the power of theta might act as a mediator or moderator of learning, or that scopolamine could have effect on both, learning and the power of theta. The experimental design of this study could not reveal the exact mechanism.

The findings regarding the memory performance in the WMS-R tasks reflect an effect on encoding instead of consolidation (see Figure 3B and 3C), as groups did not have an interaction with the time of measurement. In other words, both groups forgot a relatively equal amount of information between the immediate and delayed recall tasks. This finding points towards the interpretation that scopolamine has either a direct or an indirect decremental effect on encoding processes.

How, then, might scopolamine directly or indirectly hinder encoding? An example of an indirect effect on encoding would be an effect on attentional processes. It is reasonable to assume that if scopolamine hinders attentional processes, then it will indirectly hinder learning processes as well. Nevertheless, scopolamine might also have a direct effect on the neurophysiological processes responsible for encoding. As mentioned before, acetylcholine plays a central role in the hippocampus (Taepavarapruk & Song, 2010; Drever et al., 2011; Micheau & Marighetto, 2011), and the medial temporal lobe has an essential role in the formation of declarative memory trace (e.g. Squire, 1992; Tulving & Markowitsch, 1998). These neurophysiological findings are in line with the interpretation that scopolamine indeed directly affects the neurophysiological processes of encoding. It should also be noted, however, that a decremental effect on attention and a decremental effect on encoding might not be mutually exclusive.

In support of the indirect effect are previous findings concerning the effect of sustained attention on increases of frontal midline theta. Theta has been associated with cognitive demands and

attentional modulation in the anterior cingulate gyrus (e.g. Sauseng et al., 2007), and theta activity as measured from the frontal medial cortex might also reflect activity in anterior cingulate cortex and/or other adjacent areas, as noted by Gevins et al. (1997). Previous literature also supports the notion that FM theta is closely tied to WM (working memory) processes (e.g. Maurer et al., 2015), which are not mutually exclusive with processes of sustained attention. Therefore, FM theta itself might only indicate an allocation of attentional resources to sustain WM processes. The theta activity itself might not be reflective of learning, but only of the emergence of cognitive processes that underlie learning.

However, the present study provides a relatively strong counter to this interpretation. The methods employed in the present study, especially the trace-eyeblink conditioning paradigm, enable deductions about the role of FM theta in learning outside of cognitive demand, which, as earlier discussed, has been associated with increases in FM theta. It might be reasonable to assume that the trace eyeblink conditioning does not put as much cognitive strain on WM or sustained attention as tasks such as those employed in previous studies of FM theta, such as n-back or Sternberg. However, even if FM theta reflected learning performance in a simple learning task such as trace-EBCC, the FM theta might still only reflect a sort of mental orienting to stimuli instead of being directly connected to some physiology of learning.

Another observation in support of the interpretation that FM theta directly relates to learning processes is that previous findings related to WM load and sustained attention have found effects in the 5–7 Hz theta range (e.g. Gevins et al., 1997; Hsieh et al., 2011; Maurer et al., 2015), whereas the present study found that FM theta at 4–6 Hz was more prominent during learning. Different theta frequencies as measured from the frontal medial cortex might therefore reflect different cognitive functions. Additionally, groups did not differ in their levels of alpha activity, which indicates that any learning related observation cannot be explained away with oscillatory activity on the 8 to 12 Hz range.

Whether FM theta relates solely on encoding processes cannot be comprehensively determined in light of the findings in the present study. As can be seen from the Figure 4A, the amount of FM theta seems to decrease as the conditioning experiment goes on (less theta activity in the late bock compared to the early block). This opens a possible topic for further research while simultaneously offering preliminary support for the notion that FM theta relates to learning. The amount of FM theta seems to increase again during extinction (which arguably involves relearning a [lack of] connection between two stimuli), which, once again, is in line with the assumption that FM theta and learning are connected. FM theta's role in storing of new information, or consolidation, cannot be properly addressed considering the findings in the present study. Although, an interesting phenomenon can be seen during the conditioning experiment. As can be seen in Figure 3A, the experimental group's

probability of exhibiting a conditioned response seems to be consistently lower during the acquisition phase (blocks “early” and “late”). While this might reflect lowered alertness or a failure in encoding, it is noteworthy that it might also reflect a failure in storing.

A key implication of the present study is that even a relatively small dose of scopolamine can cause a neurophysiologically observable disruption in learning and memory functions. The observed effects of scopolamine were achieved using a transdermal patch available from pharmacies. However, it is noteworthy that the decrements in memory and learning achieved with the dose used in the present study are not subjectively noticeable.

Noteworthy shortcomings of the present study were mostly tied to the experimental design. The trace-EBCC setting used in the present study included relatively simple learning. The association between the conditioned and unconditioned stimulus is likely to form during the early phases of the experiment. If that is the case, then having an experimental design that lasts longer than is necessary to capture the phenomenon of interest might end up skewing the statistical analyses. Localization of observed brain activities are also difficult to pin down using an EEG. Using an MEG, or a combination of locally and temporally accurate imaging techniques, would mitigate the spatially lacking accuracy of the methods used in the present study. Methodologically, the learning related results obtained through the use of trace-EBCC could be subject to other effects, like changes in motor functioning, which were not addressed in this paper.

Future research could focus on different forms of learning to get a clearer picture of the role of FM theta in learning. Learning is a complex neuropsychological phenomenon, and simpler learning processes such as acquisition in a trace-eyeblink conditioning paradigm might not reflect or demand the same cognitive processes as more complex forms of learning related to, for example, language or mathematics. However, experimenting with other forms of simpler learning, like memorizing simple and often repeated visual or auditory patterns, that do not put much strain on cognition, could also clarify the findings of the present study about how FM theta and learning are related. Future studies could also attempt to clarify the connection between FM theta and learning by subjecting humans to learning tasks that involve new information as well as already known information, while controlling for the cognitive demand necessitated by the material to be learned.

In sum, these findings alongside the findings of previous studies support the assumption that FM theta is closely related to the processing of information, for either encoding or short-term memory purposes. While FM theta’s role in consolidation cannot be entirely dismissed, the present study did not find any conclusive connections between the two.

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