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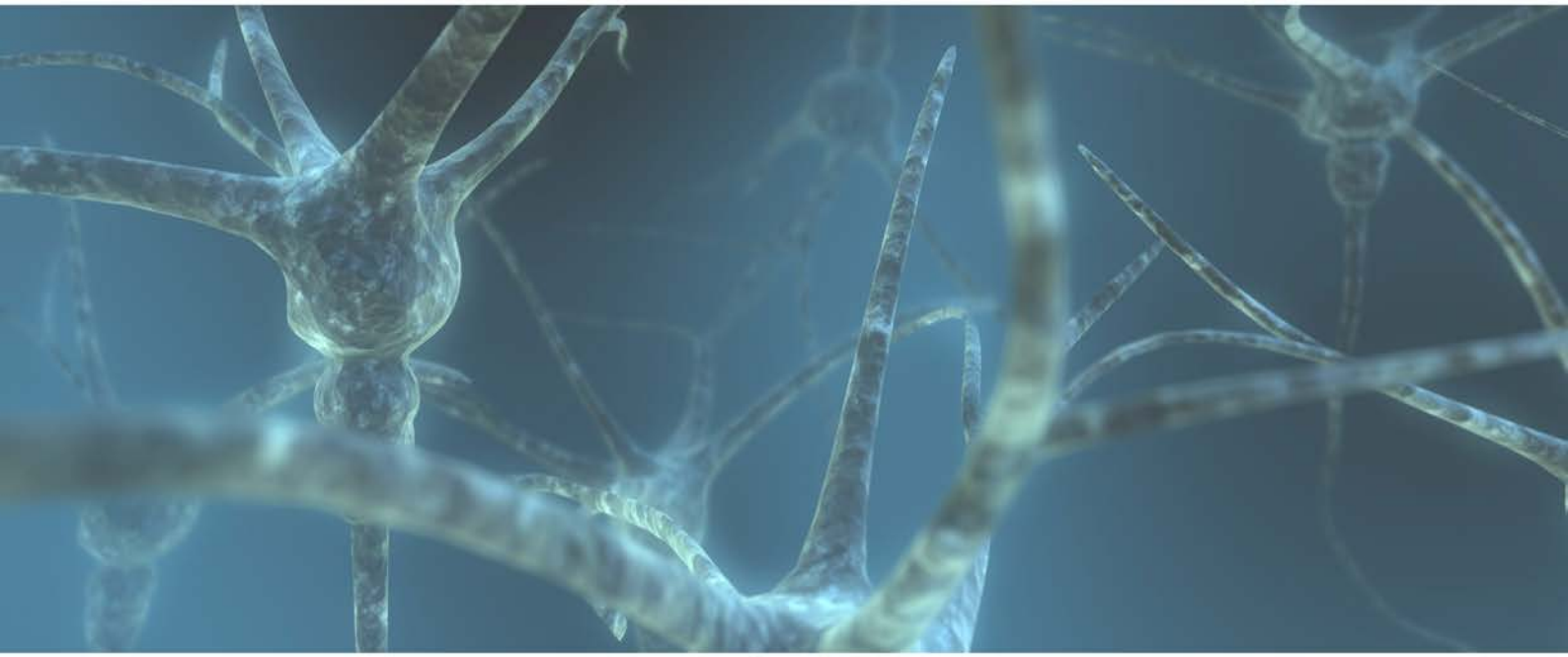

Instituut voor Interdisciplinaire Studies

UNIVERSITY OF AMSTERDAM

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Editorial Board of the ABC Journal

Editorial

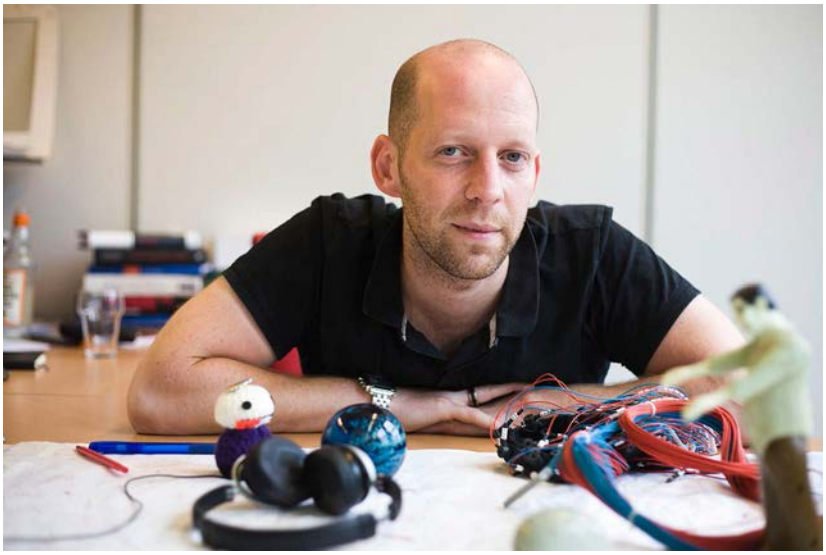
Dear Reader,

Science is an international large-scale effort, and although individual scientists work to make their own achievements and discoveries, the boundaries of human knowledge are pushed forward by the collective input and output of scientists all over the world. Thus, developing theories, running experiments, and analyzing data are only part of a scientist's contribution to science. The other part of this contribution is communicating ideas and findings, and the main capsule for the communication of science is the peer-reviewed journal article. Some argue that there is too much pressure on getting publications (do those same people also argue that there are too many books in libraries?), but on the other hand, a lifetime of amazing scientific discoveries is wasted if no one learns of them. Like any other skill in science, writing a journal article is one that must be honed and practiced. In short, communication is critical in science, and aspiring young scientists need practice to optimize their communication skills.

This brings us to the ABC Journal. The purpose of this Journal (a.k.a. academic archive) is two-fold. First and foremost, this Journal will showcase the impressive empirical science being done by research masters students in our program. Masters students make important contributions to the ongoing research of their supervisors, and, in many cases, the masters students directly contribute to and improve the quality of the research. Their accomplishments deserve recognition, and we feel that the ABC Journal is an excellent mechanism to highlight those accomplishments.

Second, the purpose of this Journal is to introduce masters students to the peer-review publication process, and to offer them the experience and training they need to help them hone their scientific communication skills. Having a paper accepted in the Journal involves the same peer-review and revision process (though a bit easier) that takes place in professional scientific journals.

It has been both a pleasure and an inspiration to work with a highly motivated group of students to bring this Journal from inception to fruition, and I look forward to continue working with them.



Michael X Cohen
Editor in Chef
Amsterdam Brain and Cognition Journal

Editorial

Dear Reader,

Two years ago the idea arose to create the Amsterdam Brain and Cognition Journal, an academic archive for the research of students of the Master for Brain and Cognitive Sciences. The objective was to give students the opportunity to have their work professionally peer-reviewed by scientists in their field. This would provide students with firsthand experience of the peer-review process, an integral and vital part of the scientific method.

With this thought in mind the first group of editors started to promote the journal and try to get students to submit their manuscript. This was a bumpy road with lots of ups and down, the project was almost dropped once, but the staff decided that it was too valuable an addition to not persist. Through a final effort and much appreciated help from the newly appointed editors of the journal, the first issue of the Amsterdam Brain and Cognition Journal has arrived. Acknowledgments should be made to Wouter Boekel and Mareen Engel for their help during the first phases of the startup.

Our hope for the future of the journal is that it will function as both an academic archive and a forum for discussing academic publishing. The aim is that through online and offline discussion, workshops and lectures learning about the various aspects of academic publishing will be fostered and that these aspects of research will eventually become integrated into the Brain and Cognitive Sciences Master.

The new editorial staff, consisting of Natalia Rivera, Koen Seignette, Tineke Slotegraaf, Siméon Lahaije and David Iñaki Lopez already contributed a great deal to this issue and they are ready to take the Amsterdam Brain and Cognition journal to the next level. For now, we hope that you enjoy this first issue.

Steven Raaijmakers & Cato Drion
Founding Editors
Amsterdam Brain and Cognition Journal

Medial frontal theta oscillations and sustained cognitive control

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University of Amsterdam

ABSTRACT

In this study we investigated the role of medial frontal theta (MF-theta) oscillations in sustained cognitive control. In past research, bursts of theta activity in medial frontal areas have been observed when cognitive control was required for short periods. For this study, we developed a new tracking task to research whether sustained cognitive control can be associated with sustained theta activity. Medial frontal theta power showed only phasic increases, but sustained synchronization of theta oscillations was observed between medial frontal and left lateral prefrontal sites. The results suggest that medial frontal theta activity is mainly related to temporary cognitive control demands, such as error processing while functional connectivity between medial frontal and lateral prefrontal cortex is related to both sustained and phasic cognitive control processes.

INTRODUCTION

In the past, medial frontal activity in the theta frequency range (4-8 Hz) has been linked to cognitive control. An increase in medial frontal theta activity (MF-theta) has been associated with a range of mental processes such as performance monitoring and adjustment, feedback and error processing, response conflict, action selection, affective evaluation and sustained attention (Mitchell et al., 2008; Ridderinkhof et al., 2004; Ridderinkhof et al., 2004). Although these processes require cognitive control and show considerable overlap in terms of location and frequency range, it remains unclear whether these processes rely on identical neural mechanisms.

The sources of MF-theta oscillations are thought to be located in central frontal areas that fall within the rostral cingulate zone (Ridderinkhof et al., 2004). The main anatomical structures in this zone are the anterior cingulate cortex (ACC), the supplementary motor area (SMA) and the pre-supplementary motor area (pre-SMA). Neural activity in these areas has been associated with the cognitive control processes mentioned above, further supporting the relation of these processes to MF-theta (M. X. Cohen, Elger, & Fell, 2009; Hanslmayr et al., 2008; Luu, Tucker, & Makeig, 2004; Mitchell et al., 2008; Nigbur, Ivanova, & Stuermer, 2011; Trujillo & Allen, 2007; Ullsperger & von Cramon, 2003; Wang et al., 2005).

Next to medial frontal sites, the lateral prefrontal cortex is also thought to be part of the neural network for cognitive control. Activity in this area has been linked to response conflict, performance adjustment and action selection, among others (Hester et al., 2007; Marco-Pallares et al., 2008). In addition, phase synchronization in the theta range has been demonstrated between medial frontal and lateral prefrontal cortex during cognitive control situations, such as error processing, response conflict and post-error adaptation (Cavanagh, Cohen, & Allen, 2009; M. X. Cohen & Cavanagh, 2011; M. X. Cohen, 2011; Hanslmayr et al., 2008; Kerns et al., 2004).

Phase coherence may be used as a measure for functional connectivity. Fries (2005) has suggested that oscillations, and specifically their phase synchronization, may form a mechanism for long-range communication between brain areas. By tuning their neuronal activity onto the same rhythmic pattern of excitation

and inhibition, distant areas could create similar time-windows of effective input processing on one hand, and the production of output on the other hand. In this way, the transfer of information between them can be facilitated, while being reduced for areas that do not fire in synchrony. Thus, phase coherence between medial frontal and lateral prefrontal sites may indicate that communication is taking place between these areas, allowing them to form a neural network that is recruited whenever there is need for cognitive control.

As mentioned before, the exact function of each area within this general neural circuitry for cognitive control remains unclear. Research has indicated that at least some of the cognitive control processes differ in terms of underlying neural mechanisms. For example, error or conflict detection has been dissociated from the selection of post-error adaptive actions, with the ACC being more involved in the detection of the need for cognitive control and the DLPFC mainly implementing the cognitive control itself (Hester et al., 2007; Marco-Pallares et al., 2008). Other researchers state that the primary function of the ACC is not error or conflict detection, but the general monitoring of performance in order to avoid losses (Magno et al., 2006; Ridderinkhof et al., 2004).

Another possible division is based on the difference between reactive and proactive control. Chen, Scangos & Stuphorn (2010) found that the medial frontal cortex, in particular the pre-SMA and SMA, can recruit a fast, reactive system to respond to sudden external changes, but also a complementary, slow, proactive system, that can adjust the motor responsiveness level based on internal expectations. According to these results, processes such as selective attention and performance adjustment would fall under the category of internally driven mechanisms exerting proactive control, while processes such as error or conflict detection and subsequent action selection would rely on externally driven mechanisms exerting reactive control.

Still, several of these cognitive control processes can be initiated by the same area. Some studies indicate that the ACC produces ongoing theta oscillations, which either increase in power or become phase-locked when an error is detected, thus resulting in the well-known error-related negativity (ERN) phenomenon (Luu et al., 2004; Trujillo & Allen, 2007). It is possible that the ongoing theta oscillations in the ACC represent one mechanism, capable of both tracking performance in the long range and signalling errors when needed, just by changing the phase or amplitude of these oscillations.

Nevertheless, ongoing MF-theta oscillations have not been studied widely. One reason is that in most experiments, researchers make use of tasks containing trials, that require only brief moments of cognitive control. These tasks generally result in bursts of theta power that last a few milliseconds. Therefore, the focus of this study was to design a novel task requiring sustained cognitive control, to examine the possibility of sustained theta activity, and of a dissociation between ongoing and momentary cognitive control processes.

In the task, participants need to track a moving line on the screen continuously. In the proactive condition, they can anticipate the movement of the line, while in the reactive condition, they can only adjust their actions after the onset of the line movement. If ongoing theta oscillations are indeed linked to sustained cognitive control processes, the expectation is that they will be present in both conditions, and even more pronounced in the proactive condition, since additional continuous anticipatory control will be exerted there. Another hypothesis is that MF-theta is mainly involved in error detection, which will result in brief post-error theta power increases. Finally, it is possible that the lateral prefrontal cortex is responsible for the initiation of cognitive control and performance adjustments, in which case phase coupling with medial frontal sites should be observed.

METHODS

Participants

Twenty-one right-handed healthy adults (twelve females) were recruited for this experiment, all with normal or corrected-to-normal vision, their age ranging from 20 to 33 years (M = 24.7; SD = 3.3). Participants were current or former university students and they all gave their informed consent to take part in the study. The experiment was conducted according to a protocol approved by the Ethical Committee of the University of Amsterdam. To increase motivation, subjects received course credits or a monetary reward of 10,50 euro and the participant with the highest score on the experimental task received an additional 7 euro. Two male participants were eventually excluded from the analysis due to a large number of EEG artefacts.

Task

For this experiment we developed a new computer task named ‘operation task’, programmed in Presentation software (Neurobs.com). Participants had to track a moving line on the screen with a ball. They could control this trackball by moving their finger on a mouse pad. Whenever the trackball was not placed on the moving line, its color would turn from green to red. The line was oriented in a horizontal position and the trackball could move in the vertical plane only. During the task, the size of the trackball would adjust to the level of performance; depending on the relative amount of time in which the line was green during the last few seconds of the game, the size of the ball would increase or decrease by 20%, thus making the tracking easier or more difficult, respectively. The score was calculated by combining the amount of errors (falling off the line) and error duration (staying off the line).

The task included two conditions, a proactive and a reactive one. In the proactive condition (fig.1), the line would remain in the center of the screen, while continuously moving in a smooth, snake-like pattern, resulting from an addition of sinus-waves that changed randomly but gradually throughout the experiment. In the reactive condition (fig.2), the line would shift up and down randomly, but remain flat. Thus, in both conditions, participants had to move the ball up and down in a random way in order to keep track of the moving line. In the proactive condition though, they could anticipate the position of the line by looking at the preceding sinusoid waves,

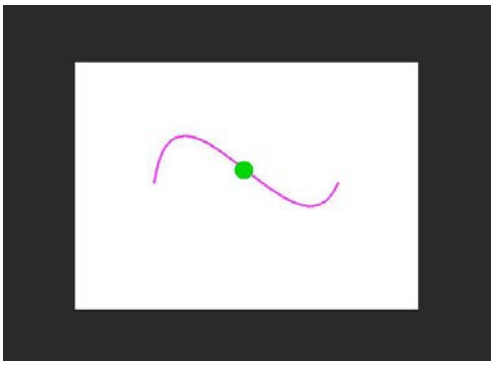

Figure 1. Proactive condition

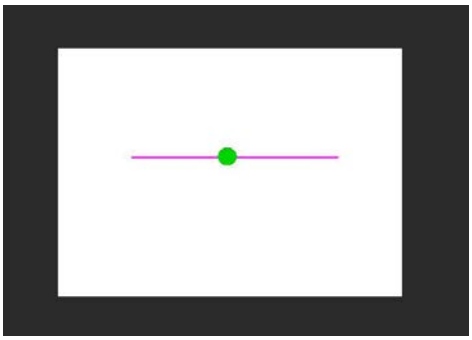

Figure 2. Reactive condition

while in the reactive condition they could only react to the movements of the line after their onset.

After data collection, the proactive condition was divided in two separate conditions, based on the type of errors that participants made. The proactive sustained condition would contain the regular sustained errors, in which error- as well as correct-events had a prolonged duration. In all participants though, sometimes the error- and correct-events would follow each other up very rapidly, as the subjects tried to stay on the line but kept on adjusting their position for some time in order to succeed. Instead of treating these extremely short events as individual errors, we decided to group them together as one 'flutter error' whenever the inter-event time was less than 390 ms., based on visual inspection. We placed these errors in a separate 'proactive flutter condition' when they occurred in the proactive condition. Flutter errors in the reactive condition hardly ever occurred, therefore those were not taken into account in the analysis. Our hypothesis for the proactive flutter condition was that it would show similar results to the proactive sustained condition, although perhaps with MF-theta more spread out over time, since the subject is trying to correct the error for a longer period of time.

Procedure

Subjects sat on a comfortable office chair with armrests, with their eyes on a distance of approximately 90 cm or 35 ½ inches from the computer screen. The mouse pad was attached to the right armrest of their chair. After receiving instructions, they practiced the task for 16 minutes (16 blocks of one minute each) during the placement of the EEG cap and electrodes. Then, the task was conducted in dim light during 48 minutes (12 blocks of four minutes each). The blocks alternated in condition, with the condition of the first block being chosen randomly by the computer. After each block, participants had the chance to pause and were presented with their score for that block and their total score so far. In the end of the experiment, participants filled in a brief questionnaire about their mood, vigilance level and subjective experience of the task, to check for abnormalities.

EEG recording

EEG was recorded from 64 sites according to the international 10-20 system, using Ag/AgCl surface electrodes of the Active-Two BioSemi system (BioSemi, Amsterdam, The Netherlands). Two additional electrodes were placed on the earlobes as a reference and four periocular electrodes were used for the vertical and horizontal electro-oculogram (EOG). Electrode impedances were kept below 50 KΩ and the signal was sampled at 512 samples per second.

Pre-processing

All analysis was conducted with code written in Matlab software (Mathworks). The data were downsampled to 256 Hz and re-referenced offline with the earlobe electrodes as reference. A high-pass filter of 0.5 Hz was applied to minimize slow drifts. Epochs ranging from -1.5 to 2.5 seconds were created around each error- and correct-event (falling off the moving line and getting back on it) for every condition. Correct-events were included in the analysis for comparison with error-events, to test the hypothesis that MF-theta is more closely related to error detection rather than error correction.

Linear baseline correction was applied with -200 to 0 ms as the baseline period. Trials containing incidental artefacts were removed by visual inspection. Eye blinks and continuous noise artefacts were removed using independent component analysis (ICA) with the EEGLAB toolbox. All data were current source density (CSD) transformed with the CSD toolbox to increase spatial resolution (Kayser & Tenke, 2006).

Time-frequency analysis. To gain phase and power information, the pre-processed data were fast-Fourier-transformed and convolved with a series of complex Morlet-wavelets, which result from the convolution of a series of sine waves with a Gaussian:

$$e^{-1*2\pi*f*t} * e^{-t^2/(2s^2)}$$

where t is time, f is frequency (ranging from 1 to 50 Hz in 30 logarithmically spaced steps) and s defines the width of the Gaussian, therefore the amount of wavelet-cycles for each frequency. For optimal comparison between frequencies, the data were converted to a decibel (dB) scale.

Inter-site phase synchrony was calculated with the following formula:

$$\left| \frac{1}{n} * \sum_{t=1}^{n} e^{i[\phi_{jt} - \phi_{kt}]} \right|$$

where t is time, n is the number of events, j and k are the seed and the target electrode, and ϕ is the phase angle of these electrodes.

Statistical analysis. We conducted planned ANOVA's for electrode Cz to test the difference in theta power between proactive, reactive and flutter condition, and between error- and correct-locked data. We also tested synchronization effects in the theta band for sites F3 and F5 and Cz as a seed electrode with planned ANOVA's for all conditions. Although specific electrodes for synchronization analysis were chosen after visual data inspection, the selection was highly restricted to the DLPFC-area, based on our pre-existing hypothesis of MFC-DLPFC connectivity.

RESULTS

Behavioral results

The mean amount of errors was 7226, with a standard deviation of 1.56. On average, subjects committed 48.6% of the errors in the proactive condition and 51.4% in the reactive condition. Of the proactive errors, 67.1% were sustained errors and 32.9% were flutter errors. The amount of errors in the proactive and the reactive condition do not differ significantly. Although flutter errors occurred in all subjects, the smaller relative amount of flutter errors, added to the variety of this relative amount between subjects, has most likely led to less powerful results compared to the other conditions.

Besides the errors, an indication of each subject's performance was given by average ball size (m = 6.79; s = 0.21) and total score (min = 8.300; max = 12.442; m = 10.156; s = 1.11). Ball size and score showed a significant negative correlation (r = -.87; p<.01) but they did not correlate with the total amount of errors, since they also took the duration of each error into consideration. These performance measures are based on random task parameters and are mainly interesting for their relative value in terms of the EEG data, which are reported below.

Theta power

Figures 3 and 4 show topographical maps for the reactive condition, with time zero being error and correction time, respectively. The power is averaged for the theta frequency (4-8 Hz) and all subjects. At 150 ms post-error, an increase of theta power is visible at midfrontal sites, concentrated around electrode Cz (fig.3). As correction takes place, midfrontal theta power gradually diminishes and returns to baseline values.

A time-frequency plot for electrode Cz is shown on figure 5, averaged for all subjects. A strong theta power increase takes place after error onset in the reactive condition, lasting approximately 500 ms and decreasing after correction onset. In the proactive sustained condition, this effect is similar, but theta power increase is moderate and it seems to take place slightly later in time. The effect is hardly observable in the proactive flutter condition, which may be due to the fact that this condition contained relatively less data.

The ANOVA for theta power (4-8 Hz) in electrode Cz and a time window of 150 to 400 ms revealed a significant effect for the comparison between proactive-flutter-reactive conditions ($F(2,17) = 67.230$, $p < .000$), a significant effect for the comparison between error-locked and correct-locked data ($F(1,18) = 82.357$, $p =< .000$) and a significant effect for the interaction between condition and error/correct-locked data ($F(2,17) = 9.227$, $p = .002$).

Figure 6 illustrates some pixel-based statistical differences between error-locked and correct-locked data. For each condition, the correct-locked value of each time-frequency point has been subtracted from the error-locked value of the same time-frequency point. This results in a new time-frequency plot, with the red colors depicting differences caused by a higher error-locked value and the blue colors depicting differences caused by a higher correct-locked value. Statistically significant (p<.001) differences are present in the theta frequency range for the proactive, the flutter and the reactive condition, mainly within a time-window of 150 to 400 ms. For the reactive condition, significant differences even extend to other time-windows and further up to the beta frequency.

Inter-site phase synchronization

Figure 7 shows the difference in inter-channel phase synchronization values between error-locked and correct-locked data for each condition (proactive, flutter and reactive). Data are limited to the theta frequency range and a time window of 150 to 400 ms. Electrode Cz is used as a seed. In this way, error-specific phase

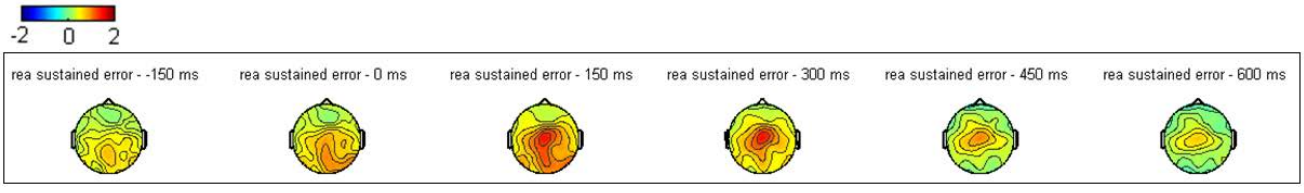

Fig.3: Topographical maps showing theta activity (4-8 Hz) for the reactive error condition, in a time course of -150 to 600 ms.

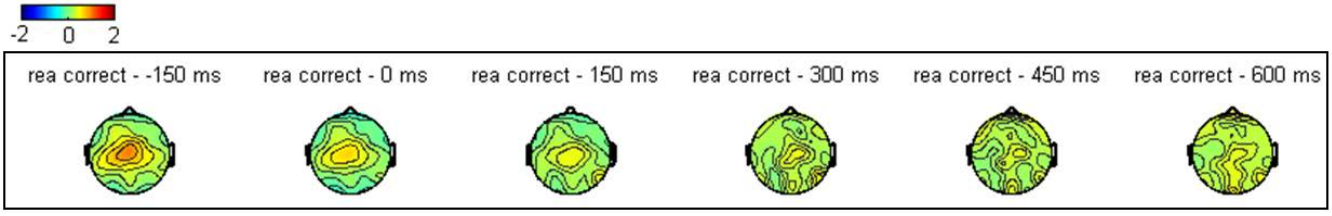

Fig.4: Topographical maps showing theta activity (4-8 Hz) for the reactive correct condition, in a time course of -150 to 600 ms.

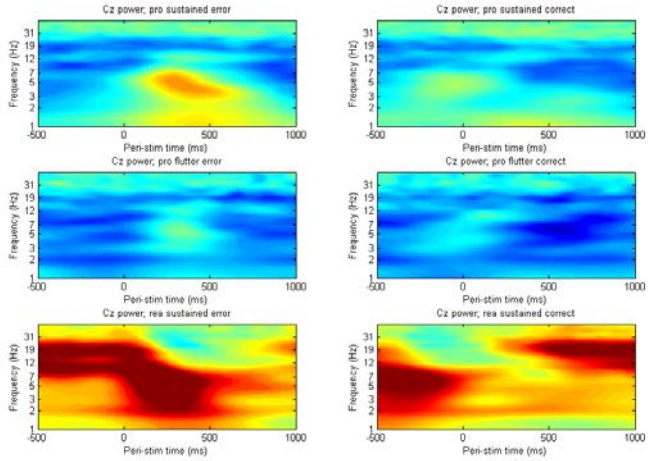

Figure 5: Time-frequency plot for power, electrode Cz, all subjects. Time zero is error for left column and correction for right column. Upper row: proactive condition. sustained condition; middle row: proactive flutter condition; lower row: reactive

synchronization between medial frontal cortex and other brain areas is depicted. High difference values are observable for the reactive condition between electrodes Cz (medial frontal cortex) and F5 (left lateral prefrontal cortex). This effect is not present in the proactive sustained and proactive flutter condition.

Time-frequency plots for inter-channel synchronization between electrodes Cz and F5 are shown on figure 8. In the reactive condition, an increase of synchronization in the theta frequency takes place in a time window of 150 to 400 ms after error onset. This effect diminishes again after correct onset, where synchronization returns to pre-error values. Some continuous synchronization is visible throughout the full time window in the theta frequency for every condition.

To compare error-specific inter-channel synchronization in the theta frequency (4-8 Hz) for electrodes Cz and F5, first a time window of 150 to 400 ms was chosen, because in this time window the main error-locked effect was visible for the reactive condition. The ANOVA showed that the difference between proactive sustained, proactive flutter and reactive condition was marginally significant (F(2,17) = 3.204, p =< .066). Results were significant for the difference between error and correct-locked data (F(1,18) = 13.089, p =< .002) and for the interaction between condition and error/correct-locked data (F(2,17) = 12.845, p = .000).

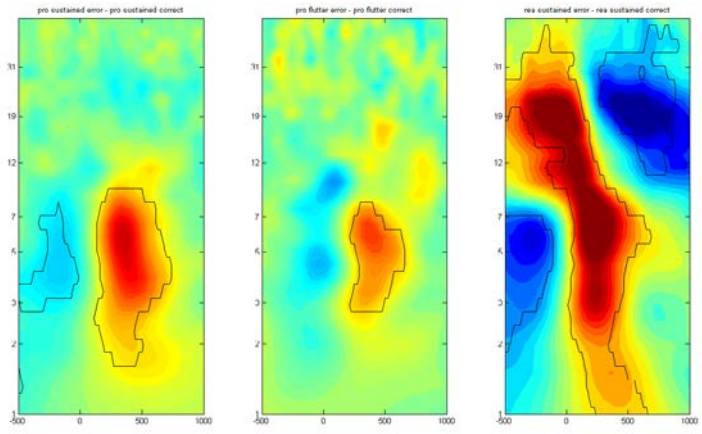

Figure 6: Pixel-based statistics for power from electrode Cz, for proactive (left), flutter (middle) and reactive condition (right). Each time-frequency plot depicts error-locked data minus correct-locked data. Significant values are bordered by a black line (p<.001).

By looking at error-locked and correct-locked data separately (i.e. without subtracting one from another) during the full time course of -500 to 1000 ms, it is possible to see phase synchronization that is not directly related to errors. Figures 9 and 10 show topographical plots of these data, with electrode Cz as a seed. On figure 9 on

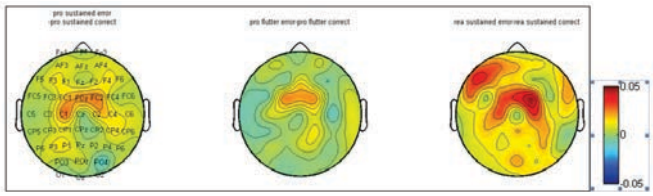

Figure 7: Inter-site phase synchronization differences between error-locked and correct-locked values for each condition (left = proactive sustained, middle = proactive flutter, right = reactive). Data are shown for the theta frequency range (4-8 Hz) with electrode Cz as the seed and averaged for the time period of 150 to 400 ms.

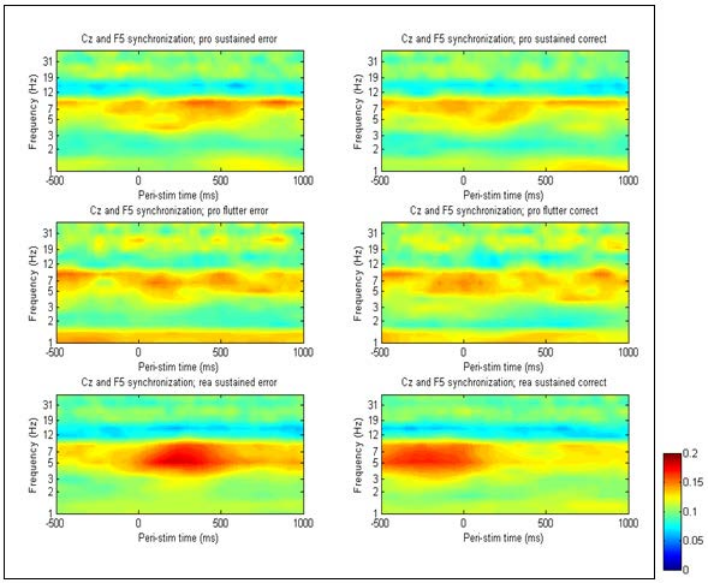

Figure 8: Time-frequency plot for inter-site phase synchronization between electrode Cz and F5. Time zero is error for left column and correction for right column. Upper row: proactive sustained condition; middle row: proactive flutter condition; lower row: reactive condition.

the left, data are depicted for the theta frequency (4-8 Hz). A clear red blob is visible in the upper left of every plot, representing increased synchronization between electrode Cz and F3. This result is specific for the theta-frequency. For comparison, similar series of topographical plots are presented on the right for the gamma frequency (20-30 Hz) (figure 10). Here, a tendency for increased synchronization towards the left upper corner is visible in every plot as well, but the localization of this increase is different, focusing more around electrodes F1 and Fc3.

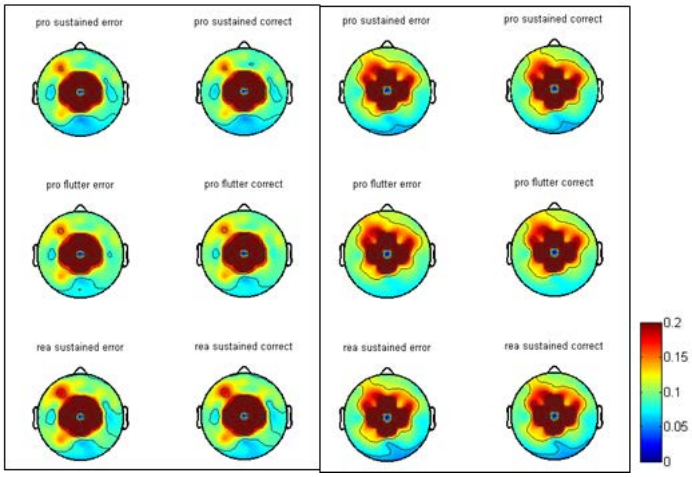

Figure 9 (left side): Topographical plots for inter-channel synchrony in the theta frequency range (4-8 Hz) for seed electrode Cz, averaged over the full time-window (-500 to 1000 ms). Upper row: proactive sustained, middle row: proactive flutter, bottom row: reactive condition.

Figure 10 (right side): same, for the gamma frequency range (20-30 Hz).

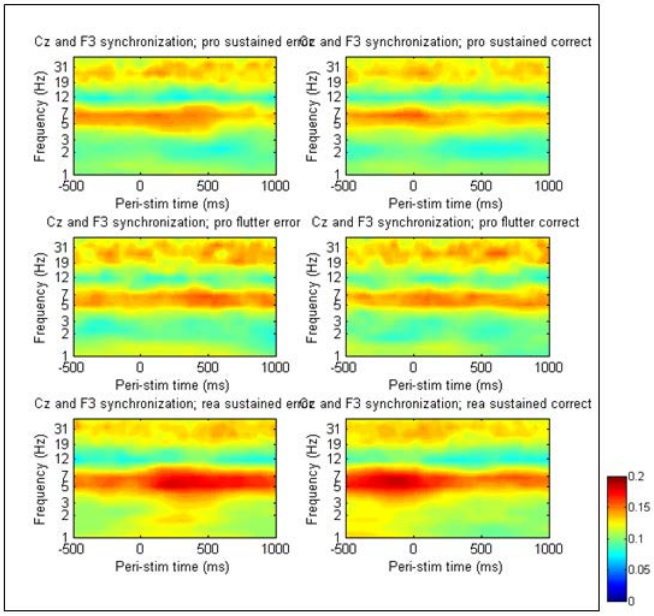

Figure 11: Time-frequency plot for inter-site phase synchronization between electrode Cz and F3. Time zero is error for left column and correction for right column. Upper row: proactive sustained condition; middle row: proactive flutter condition; lower row: reactive condition.

To look closer at the dynamics of non-error-specific phase synchronization in the theta frequency between electrode Cz and F3, time-frequency plots for every condition are shown in figure 11. Compared to figure 8, that presents the same data for electrode Cz and F5, some differences can be noticed. First, the post-error synchronization burst in the theta frequency is more concentrated in figure 8 and spread-out in figure 11. Second, the continuous synchronization in the theta frequency is more robust and sustained in figure 11.

To compare non-error-specific inter-channel synchronization in the theta frequency (4-8 Hz) for electrodes Cz-F3 and Cz-F5, the following method was used: for the error-locked data, only the pre-error time window of -500 to 100 ms was used for the analysis, the same for every condition. For the correct-locked data, only the late post-correct time window from 600 to 1000 ms was used for the analysis, again the same for every condition. The chosen time windows should contain only 'baseline' inter-channel phase synchronization values, that are not influenced by error-related activity. For the Cz-F5 synchronization, statistical comparison revealed no significant main effects for condition or error/correct-locked data, and no interaction effect. For the Cz-F3 synchronization, only a marginally significant main effect was found for condition ($F(2,17)=3.534$, $p=.052$).

Behavioral results and EEG data

Individual mean theta power (4-8 Hz), averaged over all conditions for the error-locked data and over a time window of 150 to 400 ms, showed a positive correlation with individual score values ($r = .53$, $p = .020$). Furthermore, it showed a marginal negative correlation with individual average ball size ($r = -.45$, $p = .053$). Thus, higher average theta power after the commission of errors is accompanied by higher score values and lower average ball size on the task. Individual mean phase synchronization values in the theta frequency for Cz-F5 or for Cz-F3, averaged over the full time window of -500 to 1000 ms, did not show any significant correlations with score or ballsize. They also did not significantly correlate with individual mean theta power in electrode Cz for the time window of 150 to 400 ms.

DISCUSSION

In this study, three hypotheses were tested. The main hypothesis was that sustained theta oscillations would occur during sustained cognitive control. Another hypothesis was that phase synchronization between medial frontal and lateral prefrontal cortex would take place during cognitive control demands. A final hypothesis was that proactive control would differ from reactive control in terms of neural activity. The general aim of this research was to gain more clarity about possible dissociations between the several processes that fall into the cognitive control category.

The first hypothesis was not confirmed by the data; sustained MF-theta oscillations were not observed whatsoever. On the contrary, MF-theta power showed a significant brief increase in response to errors, mainly in the reactive condition. These results suggest that theta activity in the medial frontal cortex is mainly related to cognitive control processes of a short duration, such as error detection and immediate action adjustment, and not as much in processes that require sustained cognitive control.

The phase synchronization in the theta frequency between medial frontal and lateral prefrontal was observed in twofold. First, post-error theta synchronization was observed between electrodes Cz and F5, but only in the reactive condition, following the trend of the post-error MF-theta power. Second, sustained theta synchronization occurred in all conditions between electrodes Cz and F3. For the sustained synchronization effect, no difference was found between proactive and reactive condition.

These results suggest that the lateral prefrontal cortex may be involved in at least two different mechanisms of cognitive control. A lateral area may establish a functional connection with the medial frontal cortex briefly, as soon as an error is made, perhaps to support the cognitive control network by providing an appropriate

plan for immediate action. A less lateralized site seems to maintain an ongoing functional connection with the medial frontal cortex. Possibly, this synchronization indicates that attention is being sustained during the tracking task, or that the task requirements are being kept in mind and compared continuously to the results of one's performance. Nevertheless, since all the data were collected during the tracking task, without having a non-tracking condition included in the experiment, it is possible that this ongoing functional connection is a general and not a task-specific phenomenon.

It is worth to notice that these medial frontal and lateral prefrontal areas synchronize specifically in the theta frequency. Phase coupling between medial frontal and lateral prefrontal cortex was also observed in the gamma frequency range, but the location of this prefrontal site was less lateralized. This small but clear difference in the location of the prefrontal area involved in the synchronization with the medial frontal cortex, may indicate that the observed gamma synchronization may represent yet another mechanism of cognitive control or interact with the theta activity.

The fact that the non-sustained, post-error synchronization does not correlate with post-error MF-theta power, may also indicate that these activations represent different cognitive control processes. For example, the strength of error signaling in the medial frontal cortex may be independent of the strength of relevant functional communication between medial frontal and lateral prefrontal cortex. Although researchers have already suggested that the two areas are involved in different processes (Hanslmayr et al., 2008; Marco-Pallares et al., 2008; Ridderinkhof et al., 2004), this result is slightly opposite to what is found in other studies (Hester et al., 2007; Kerns et al., 2004), were the strength of MF-theta power was directly related to the strength of theta power in lateral prefrontal sites. The discrepancy can be caused by differences in task requirements, applied techniques (in both studies, fMRI was used and not EEG), or by the fact that in these studies, power was quantified instead of phase synchronization.

Proactive and reactive control conditions differed mainly in terms of error-related activation and synchronization values in the theta frequency. While the reactive condition showed remarkable post-error increases, the proactive sustained and proactive flutter conditions showed only modest significant fluctuations. On average, MF-theta power was significantly higher in the reactive condition. Furthermore, proactive and reactive condition only differed marginally for the medial frontal-lateral prefrontal theta synchronization when the error-related increases were not taken into consideration, with the reactive condition showing the highest synchronization. These results do not support the prediction that MF-theta power or synchronization with lateral prefrontal cortex would be higher during proactive control. Instead, the results suggest that this is the case for reactive control, and that the possibility to anticipate one's actions as in the proactive condition requires less cognitive control in general.

With regard to the behavioral results, it is worth to notice that MF-theta power correlated with individual high score and ball size, but not with the amount of errors. As mentioned before, the score and ball size take also the time spent on the line into account, which is obviously more representative for the power of MF-theta. In other words, MF-theta power is not so much related to the initial commission of errors, but to how quickly errors were detected and corrected. Since the commission of an error is likely to be caused by a decrease in sustained attention, while error detection would occur right after the error is made, these results support the EEG data, were MF-theta power showed mainly post-error increases and was not linked to sustained cognitive control processes.

Overall, new insights in the neural dynamics of sustained and less sustained cognitive control mechanisms were provided in this study. The main finding was that MF-theta power is likely to be involved in transient, error-related cognitive control processes, while sustained neural activity was observed in the form of synchronization between medial frontal and lateral prefrontal sites. Future studies should be conducted to confirm the hypothesis that this form of synchronization is specifically related to cognitive control demands instead of a general ongoing neural mechanism. Finally, the possibility to exert proactive control seems to reduce the need for MF-theta activity compared to situations in which only reactive control can be used.

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Dynamic shift in low frequency oscillations and the role of the dopamine D1 receptor in the dorsal striatum during transition from goal-directed to habitual behavior

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ABSTRACT

Different cortico-striatal loops are found to be involved in distinct learning processes in instrumental behavior. A network involving the dorsomedial striatum (DMS) is found to be mainly implicated in initial goal-directed behavior, while the dorsolateral striatum (DLS) and its connections are found to be essential for habitual behavior. These processes are highly dependent on dopamine, which is one of the main input neurotransmitters to the dorsal striatum and plays an important role in long-term potentiation during learning of new skills. Because some oscillatory responses have been associated with reward and decision processes and specifically theta band (5-10 Hz) oscillations seem to reflect goal-directed processes, we investigated the differential oscillatory responses in the rat DMS and DLS along concurrent training of a habitual choice. Additionally we tested the effects of the dopamine D1 receptor antagonist SCH on behavior and oscillatory responses in the DMS and DLS during performance of the overtrained choice. Results indicate a dynamic shift in low-frequency (1-10 Hz) power during transition from goal-directed to habitual responding, with decreasing power in the DMS compared to the DLS over sessions. Furthermore, perfusion of SCH after habit development led to an increase in initial approximations to the preferred alternative, but left choice behavior unaffected. In addition, SCH perfusion increased low-frequency power in the DMS compared to the previous and following sessions. Our results suggest the involvement of a differential synchronized input pattern in the DMS and DLS during the transition from goal-directed to habitual behavior. In the final stages of habit learning this input pattern in the dorsal striatum seems to be modulated by D1 receptors.

INTRODUCTION

In our society we always seek ways to reduce our efforts, while maintaining the quality of our goals. Straightforward examples can be found in the technology surrounding us, making it easy to reach someone on the other side of the world, or perform complex calculations by pressing a few buttons on a keyboard. Systems to reduce efforts can also be found in our brains. Making decisions by assigning values to different options, weighing the options and updating the valuation process is costly in terms of energy resources. If we had to consider every step we take and every muscle movement we make in our lives, we would quickly be out of resources and our productivity would be much lower. Therefore, our brains have ways of processing a large amount of input and output in an unconscious, energy reducing manner, making it easy for us to use our resources for less steady environmental challenges in our daily life.

In instrumental behavior two distinct systems have been identified: a goal-directed and a habitual system (e.g. see Balleine et al., 2009). The goal-directed system evaluates the outcomes related to specific actions and facilitates these actions only when the outcomes are in line with the respective goal of the agent. In an environment that is fairly stable, goal-directed actions can lead to formation of habits; the actions in response to stimuli will

become valuable by themselves and will therefore be independent of changes in outcome. In a stable environment, formation of a more automatic habitual response is advantageous over the more cognitive demanding (Daw et al. 2005) goal-directed evaluation of the response and its consequences. The goal-directed system is a necessary requisite not only for the formation of habits in stable environments, but also to change behavior when the outcome or the goal changes.

The two systems involve different structures of the basal ganglia and (prefrontal) cortex, which are connected in a spiral from the striatum via the thalamus to the cortex and back to the striatum, going from the medial and ventral parts of the prefrontal cortex to the ventral striatum, from the anterior cingulate cortex to medial parts of the caudate and putamen in primates (dorsomedial and dorsolateral striatum in rodents, respectively) and from the dorsolateral prefrontal cortex to the more dorsal regions of the caudate and putamen (Haber, 2003; Knutson et al., 2009). The more associative cortices like the medial prefrontal cortex and its projections to the dorsomedial striatum (DMS) are more associated with goal-directed action, while the sensorimotor cortices and their connections to the dorsolateral striatum (DLS) seem to be more involved in habitual action (see Balleine et al. 2009)(see Fig. 1).

Several studies indicate an important role of the DMS and DLS in these two networks, with lesions of the DLS but not the DMS rendering previously habitual behavior goal-directed again and lesions of the DMS but not the DLS rendering behavior insensitive to outcome devaluation (Balleine et al., 2009; Ragozzino, 2007; White, 2009; Yin 2004, 2005; Yin and Knowlton, 2006). Also neuronal activity in the dorsomedial striatum has been associated with goal-directed behavior (Kimchi and Laubach, 2009a, 2009b; Thorn et al., 2010; Yin et al., 2009) while activity in the dorsolateral striatum has been associated with motor behaviors (Belin and Everitt, 2008;Kimchi et al., 2009; Yin et al., 2009). Several studies indicate the importance of the integrity of the network rather than activity in the DLS or DMS rendering behavior habitual or goal-directed, respectively (Lingawi & Balleine, 2012; Stalnaker et al., 2010).

In order to assess both systems, in this study we trained rats in a concurrent choice task that would lead to a stable, overtrained, habitual choice for one of the alternatives. At the beginning of the training a more goal-oriented mechanism is expected to lead behavior, while at the end of the training, habitual processes are expected. We measured local field activity in the DLS and DMS simultaneously during habit formation to study the relative involvement of these two areas in goal-directed and habitual behavior. Finding out the exact contribution of the DMS and DLS in goal-directed and habitual behavior is important for understanding the mechanisms behind our everyday automatic behavior and could in particular be relevant for understanding the core mechanisms underlying addictive behavior and impulsive action.

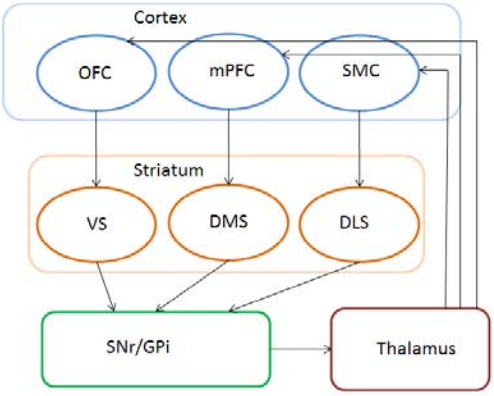

Figure 1. Cortico-striatal circuits. OFC: orbitofrontal cortex, mPFC: medial prefrontal cortex, SMC: sensorymotor cortex, VS: ventral striatum, DMS: dorsomedial striatum, DLS: dorsolateral striatum, SNr: substantia nigra pars reticulate, GPi: globus pallidus. Dopaminergic projections are not shown.

Local field dynamics in the dorsal striatum

Local field potentials find their main origin in the summation of postsynaptic potentials and can therefore be seen as the reflection of input to a group of neurons in the vicinity of the recording electrode.

Oscillations in the delta band are correlated with motivational processes and craving, and is found in several areas of the brain reward system, like the nucleus accumbens, ventral tegmental area and medial prefrontal cortex (Knyazev, 2007). Theta oscillations are found in a large number of areas and systems, including the thalamus, hippocampus, neocortex and striatum and seem to play an important role in task related integration of information between areas and recurrent activity within areas involved in decision making processes (see Womelsdorf et al., 2010 for a review). Several studies show presence of theta oscillations at decision points (Johnson and Redish, 2007), with theta synchronization between the hippocampus and prefrontal cortex (Benchenane et al., 2010) and between the hippocampus and dorsal striatum (DeCoteau et al., 2007b) correlating with task accuracy. Furthermore, van Wingerden and colleagues (2010) found that during correct anticipation of reward, spike output of the OFC was phase-locked to theta oscillations, while no such locked spiking was found after reversal of action-outcome contingencies. Phase-locking returned with relearning. These studies indicate that theta oscillations seem to be involved in learning processes and retrieval of stimulus-outcome associations; processes mainly implicated in goal-directed behavior (Womelsdorf et al., 2010).

Recently higher frequency local field oscillations (>20 Hz) have been studied in the ventromedial striatum during learning on a T-maze (Howe et al., 2011), showing changes in both the spatial and temporal structure of firing activity in the ventromedial striatum during habit formation. This was reflected by changes in gamma and beta frequency ranges. A study by Kimchi et al. (2009) found increased theta power in the DLS compared to the DMS after presentation of a stimulus signaling reward availability throughout training on a simple nose-poke task for reward, using a random interval schedule. In addition, significant spike-phase coherence was found in the delta band (<5 Hz), which was greater in the DLS compared to the DMS. These findings are in contrast with evidence suggesting a main role

of theta oscillations in goal-directed processes involving the DMS.

In light of these contrasting findings, we assessed local field potentials (LFPs) in the DMS and DLS of rats during habit formation. If theta oscillations are mainly involved in goal-directed processes, we expect to find increased low power oscillations in the DMS compared to the DLS during the first sessions of the task, in which goal-directed action is assumed to be prominent, in comparison with later sessions, in which low frequency power is expected to diminish in the DMS compared to the DLS. If theta oscillations only occur in goal-directed action, no increase in theta power in the DLS compared to the DMS is expected when behavior is habitual.

The role of the dopamine D1 receptor in synaptic plasticity in the dorsal striatum

The dorsal striatum consists for 90% of GABAergic medium spiny neurons (MSNs) and interneurons. The MSNs receive dopaminergic input from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) as well as glutamatergic input from the cortex and intralaminar thalamus (Lovinger, 2010). Instrumental learning requires long- term synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD). Both forms are found in different areas of the striatum, with LTP more prevalent in the dorsomedial striatum and LTD more prevalent in the dorsolateral striatum (Lovinger, 2010).

Next to the NMDA and AMPA receptors, the dopamine D1 receptor seems to play an essential role in striatal LTP (Calabresi et al., 2000; Kerr and Wickens, 2001; Lovinger et al, 2003), though its exact role is not yet clear (Lovinger, 2010). The D1 receptor is mainly expressed in the so called ‘direct pathway’, consisting of the MSNs projecting to the output structures of the basal ganglia, leading to the expression of actions. MSNs of the ‘indirect pathway’, targeting the pallidum and subthalamic nucleus which in turn target the output structures of the basal ganglia, mainly express D2 receptors as well as the adenosine receptor A2A, which is also essential for LTP (Shen et al., 2008). Interaction between the pathways is presumed to cause the gating of one action sequence, while other possible action sequences are inhibited.

Since the D1 receptor has a lower affinity for dopamine than the D2 receptor, it has been hypothesized that the D1 receptor (and its involvement in LTP) only plays a role in the early stages of skill learning, when phasic increases in dopamine are observed (Yin et al., 2009), after which the A2A receptors could take over (Lovinger, 2010). In our study we perfused the dorsal striatum with the D1 receptor antagonist SCH at the end of the habit training phase, which, according to the hypothesis described above, should have no effect on the behavioral performance of the rats, though slight electrophysiological changes might be observed.

MATERIALS AND METHODS

Subjects

Adult male Wistar (Harlan) rats (n=8) were housed in standard type 4 Makrolon cages, weighed and handled daily, and kept under a reversed 12 h light/dark cycle (on: 19:00; off: 7:00). The weight of the subjects was 390-465g at the time of the surgery. All experiments were conducted during their active cycle. Every procedure was in accordance with the National Guidelines for Animal Experimentation, and approved by the Animal Experiment Committee from the Royal Netherlands Academy of Arts and Sciences.

Behavioral shaping

Animals were maintained on 90% of their free-feeding body weight, with water available ad libitum. During a first session, subjects were exposed to a skinner box adapted in size and shape for electrophysiological recordings and they were allowed to freely explore the environment for 15 min. Nose-poke and lever presses were then shaped for two (left and right) behavioral alternatives with equal reward amount in a fixed ratio (FR) program. The ratio was increased along training sessions up to FR4. Training continued until they reached 90% of the required responses in at least 3 continuous sessions. After this initial shaping the subjects were implanted for electrophysiological recordings and reversed microdialysis.

Surgery

Animals were anesthetized with 0.07 ml/100 g Hypnorm i.m. (0.2 mg/ml fentanyl, 10 mg/ml fluanison) and 0.04 ml/100 g of Dormicum i.m. (5 mg/ml midazolam), and then they were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Local anesthesia (10% Xylocaine spray; Astra Hässle AB, Mölndal, Sweden) was used on the skin, in the ears and after the incision, on the skull and the surrounding tissue. Body temperature was maintained at 37.5°C using a heating pad, and eyes were protected with methylcellulose eye drops. After exposure of the cranium, six small holes were drilled into the cranium to accommodate surgical screws; one screw served as ground in the hemisphere contralateral to the implant.

The implant was an in-house custom made drive (the Combidrive) (van Duuren et al., 2007) that consisted of a circular array of 12 tetrodes and 2 reference electrodes with a microdialysis probe (2mm membrane) in the middle (see Fig. 2). A larger hole (3mm diameter) for the Combidrive was then drilled over the dorsal striatum in the right hemisphere with the center at 3mm ML and 0.5mm AP relative to bregma (Paxinos & Watson, 2007). Before implantation, tetrodes were exposed 2 mm, and the microdialysis probe 5 mm. The dura was then opened, and the bundle of the combidrive was lowered onto the cortex inserting the exposed components. Afterwards it was anchored to the screws with dental cement. To protect the brain from the dental cement, the exposed tissue around the bundle was first filled with a silicone elastomer (Kwik-Sil; WPI, Sarasota, FL). During surgery and the immediate recovery period (until the subjects regained movement) 1 ml sterile saline was injected subcutaneously every hour for hydration. After surgery, when rats started regaining consciousness and responded to light pain stimuli, 0.07 ml/100g Temgesic (Schering-Plough BV, United Kingdom) was administrated i.m. to reduce pain. In the course of a few days the tetrodes were lowered gradually until the dorsal

striatum was reached at 4mm DV while the reference electrodes were left at 2mm DV. The tip of the probe was located 5mm DV so the middle of the 2 mm dialysis membrane surface would be at the level of the recording tetrodes. Animals were allowed to recover for at least 1 week before further training and recordings were initiated.

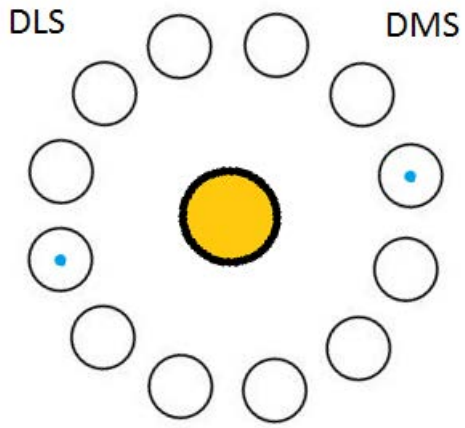

Figure 2. Schematic overview of tetrode and microdialysis probe configuration. Tetrodes were positioned in a circle with the microdialysis probe in the middle (yellow). Circles with a blue dot indicate the locations of tetrodes selected for analysis. Note that this figure is not to scale.

Behavioral task

After surgery, animals were further trained in a concurrent choice program (FR4) with two options: left and right lever, with different reward magnitudes. This task was intended to develop a strong preference for the higher reward alternative, resulting in a more automatic and habitual choice of this lever. The high reward lever (HRL) offered always 4 reward pellets, while the low reward lever (LRL) offered 2. Left and right lever were balanced for HRL and LRL across subjects and the feeder was located in the center of these levers and equipped with an infrared beam to detect nose-pokes (Fig.3A). Each session consisted of 8 blocks with 8 trials each. Within a block, each of the first 6 trials offered only one lever so there was a forced choice (FoCh) in order to allow the subjects to sample the contingency and prevent side bias. The 6 FoCh trials of each block were distributed for equal availability of HRL and LRL (3 trials each) with a random order. The last two trials of each block were free choice (FrCh) since they offered the two levers simultaneously (Fig.3B). To avoid alternative changes after lever choice during FrCh trials, the first press on the selected lever retracted the other alternative. Rats performed one session each day.

The beginning of each trial was signalized with illumination of the feeder. Upon an initial nose-poke in the illuminated feeder (NPini) the feeder light went off. After one second, a light above the presented lever(s) was turned on, and after one second more the lever(s) would show. Four presses would retract the lever and turn on the feeder light. Upon nose-poke of the feeder (NPfin) reward pellets were delivered after one second during which the feeder light remained on. Each trial was followed by an inter trial interval (ITI) of 5, 10 or 15 seconds, distributed randomly along the session (Fig. 3B).

For each session the number of omissions and choices for HRL and LRL was assessed. During each session an infrared motion detector registered the presence/absence of movements in 0.2 sec intervals. Additionally, after the initial nosepoke (see Fig. 3B) during the delay period before signalization and lever availability, approach to the lever location was scored manually trough video recordings. Rats were trained with the same HRL (balanced for side across subjects) for 17 sessions (Fig. 3C). Gray squares illustrate the sessions analyzed for electrophysiology. During each session, artificial cerebrospinal fluid was perfused trough the microdialysis membrane (see below). However, at the end of the training the D1 antagonist SCH23390 (Sigma, Germany) was included in the perfusion in order to evaluate its behavioral and electrophysiological effects. An additional regular training session (session 17) was made to assess potential long term effects of the SCH perfusion.

Reversed microdialysis

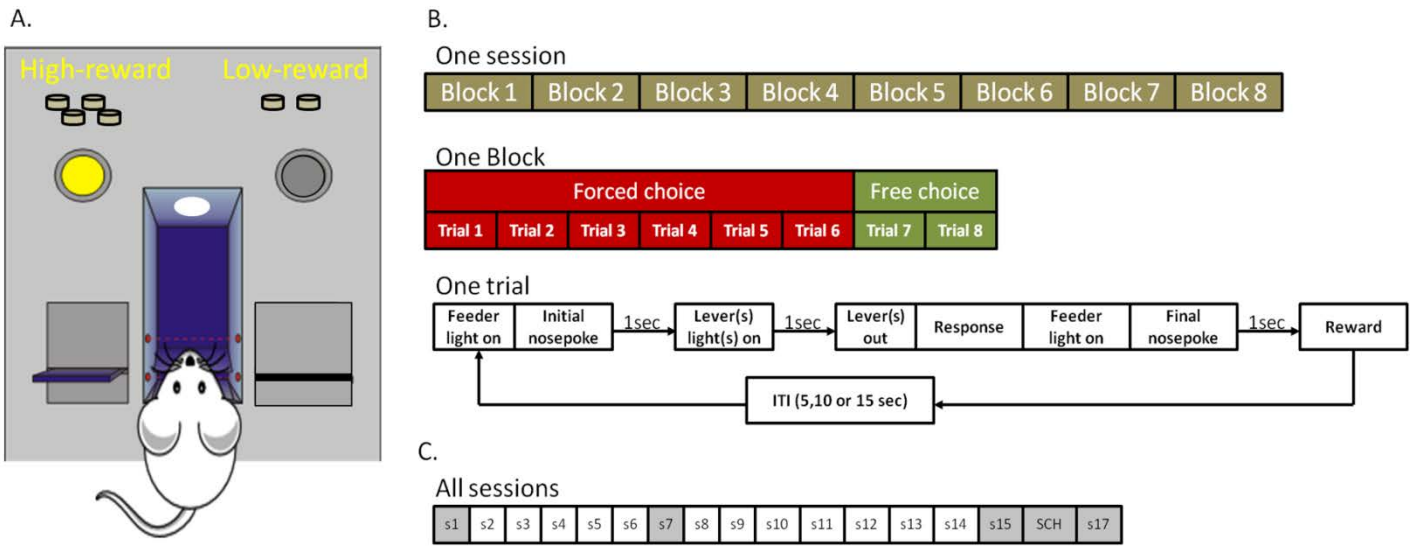

Figure 3. Behavioral task. A. Rats were trained to choose between a high and low alternative, associated always with the same side, with sides balanced across subjects. This task produced a fast preference for the high rewarded lever (HRL). B. Each session included 8 blocks. Whithin each block there were trials with individual presentation of one of the levers (forced choice) and concurrent presentation of the lever (free choice). An initial nosepoke was required to start the trial, and a final nosepoke to collect the reward. After these nosepokes there was always a delay. C. Specific sessions (marked in gray) were selected to analyze electrophysiological changes. Sessions at the beginning middle and end of the training were used to assess the development of the learning process. The final stage of training (session 15) was compared with a session in which SCH was perfused in the dorsal striatum and with an additional regular session (session 17) to check long term effects of the dopamine antagonist.

During session 16 (SCH session), the microdialysis probe in the combidrive was used to perfuse the D1 antagonist SCH23390 (SCH)(10-5 mol/L). SCH was diluted in Mili-Q water, aliquoted in 20µl vials and stored at -80˚C. Before perfusion session, SCH was dissolved in fresh phosphate-buffered artificial cerebrospinal fluid (aCSF) that contained 143 mM NaCl, 1.2 mM CaCl2, 2.7 mM KCl, 1.0 mM MgCl2, 0.26 mM NaH2PO4, and 1.74 mM Na2HPO4, pH 7.4. 2. An Univentor 801 microinfusion syringe pump (Univentor, Zejtun, Malta) was used to pump the solutions through 167-cm-long polyetheretherketone tubing (0.51 mm o.d., 0.13 mm i.d.; Aurora Borealis Control) that ran toward the inlet of the dialysis probe (flow rate, 2µl/min). All connections were made of polyvinylchloride tubing (0.38 mm i.d.). Between sessions all tubing was rinsed with Milli-Q water and methanol.

Electrophysiological recordings

All electrophysiological recordings were performed inside a Faraday cage isolating auditory and electrophysiological noise. All signals were recorded against ground (skull screw) for single unit (analyzed elsewhere) and local field potential (LFP's) using a Cheetah recording system (Neuralynx, Tucson, AZ). The position of the tetrodes was not modified across the experiment. During each session, signals from the individual leads of the tetrodes were passed through a low noise unity-gain field-effect transistor preamplifier, insulated multiwire cables, and a fluid-enabled 72-channel commutator (Dragonfly Inc., Ridgeley, WV) to digitally programmable amplifiers. LFPs recordings were obtained from the signal on a selected electrode in each tetrode at 30.3 KHz. The signal was fed to an amplifier (gain: 1000), filtered between 0.1-200 Hz. The amplifier output was digitized and stored on a Windows XP station. Recordings were downsampled to 606Hz and the electrophysiological responses to reward expectancy (1 second window after final nospoke) and trial start (1 second window after initial nosepoke) were analyzed to compare activity of the DMS and DLS across training sessions and under SCH perfusion (see Fig. 3B).

Lesions and Histology

After the end of the training, rats were anesthetized with 0.3 mL of 50 mg/mL sodium pentobarbital solution (ca. 40-50 mg/kg), and current was passed through each tetrode to make lesions marking the ends of the tetrode tracks (25 µA, 10 sec). Two to three days later, rats were deeply anaesthetized with a lethal dose (0.8-1.0 mL, or ca. 100-145 mg/kg) of sodium pentobarbitol, and brains were fixed by transcardial perfusion with 4% paraformaldehyde in 0.1M KNaPO4 buffer. Brains were post-fixed and cut transversely at 30 µm on a sliding microtome. Sections were processed for Nissl substance and examined microscopically to identify the lesions and tetrode tracks.

Data Analysis

Two subjects were excluded because of blocking of the microdialysis membrane, accuracy of the implant and insufficient quality of the electrophysiological signal. Behavioral data were analyzed with repeated measures anova for 6 rats with sessions and choice as within-subject factors.

For LFPs analysis, two tetrodes, one in the most lateral and one in the most medial location, were selected in each subject as the source of DMS or DLS signal, respectively. The data was re-referenced to the average of implanted brain area in order to attenuate the influence of volume conduction by removing the shared signal from the electrodes. Time-frequency representations (TFRs) were generated for each rat and condition separately. In order to obtain the time-frequency estimates of power all trials were convolved with a family of wavelets, defined as a series of Gaussian-windowed complex sine waves ($e^{i2\pi tf} e^{-[t]^2/(2*\sigma^2)}$), where t is time and f is frequency, which increased from 2 to 50 in 100 logarithmically placed steps. σ is the width of the frequency band, which was defined as $5/2\pi f$ which gives an adequate trade-off between time and frequency resolution. After convolution power was calculated by taking the square of the absolute value of the complex number at each time-frequency point. The resulting time-frequency plots were then baseline-corrected using a relative-change correction from 500 to 100 ms pre-stimulus in order to highlight any change in frequency-power after stimulus onset.

Statistics were performed with parametric trial-based permutation testing, in order to account for the small sample size, differences in rats per condition and differences in the number of trials per rat. Permutation testing as implemented here works by subtracting the two averaged TFRs (DMS-DLS) from two randomly chosen set of trials and building a distribution of TFRs that are expected under the null hypothesis (no difference between conditions). We then performed a first-level z-test to test where the observed average and permuted average was significantly different ($p < 0.01$). Cluster-level statistics were then computed by calculating the probability ($p < 0.05$) of a certain cluster-size appearing under the H0 of no difference between conditions. Any cluster failing to reach this cluster-size was subsequently set to zero and thus rejected.

Local field activity at an initial and final trial cue (regardless of trial type or choice) was analyzed to assess the difference in activation of the DMS and DLS over sessions. As the initial nosepoke precedes choice implementation (i.e. pressing a lever) as well as information about the type of trial (i.e. forced or free choice, indicated by subsequent lever extraction), the time of nose-poking was used as initial trial cue, whereas the final nosepoke before reward delivery was used as final trial cue.

RESULTS

Histological confirmation

Six of the subjects showed accurate localization of the microdialysis probe and the microdrive tetrodes. Figure 4 illustrates these coordinates for each subject.

Choice behavior

Data for choice behavior (only free choice trials) and its development across training sessions were tested for normal

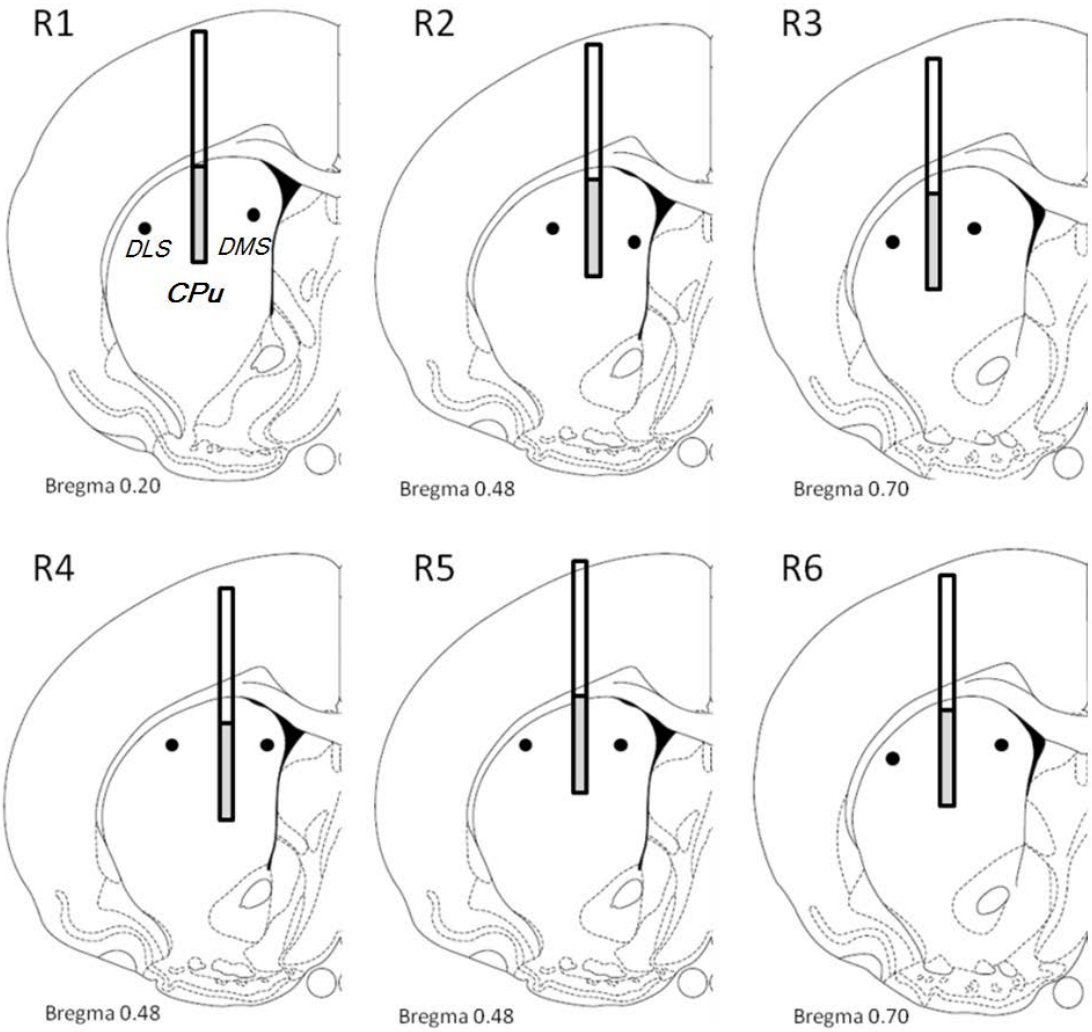

Figure 4. Histological confirmation of electrode and probe localization in 6 subjects. CPu: caudate putamen (striatum), DLS: dorsolateral striatum, DMS: dorsomedial striatum.

distribution and analyzed with repeated measures ANOVA. A two-way repeated measure using sessions and lever choice as within subject factors was conducted to determine if there was a statistical significant difference between the percentage of choices for HRL, LRL and OM across sessions. These percentages were significantly different among each other, as evidenced by the within-subjects effects for the factor choice $F(2,14)=56.41$, $p<0.001$; and variated along training sessions as showed by similar effects in the interaction between the two factors $F(14,98)=3.58$, $p<0.001$)(Fig. 5A). A one-way repeated measures ANOVA for the percentage of HRL choices along sessions showed that selection of HRL increased with training $F(7,49)=3.96$, $p=0.002$. In this way, preference for HRL was rapidly increased and stabilized around an average of 70% (Figure 5A). Within subject contrasts showed that at the beginning of training choices were equally distributed among HRL and LRL, however along training the percentage of choices for HRL became significantly higher after session 5 (Fig. 5A).

In order to analyze changes in choice behavior due to perfusion of SCH (session 15 and 17) or aCSF (session 16), a separated repeated measures comparison of choices in three sessions (before, during and after perfusion of SCH) was made with choice and perfusion as within-subject factors. This comparison showed no significant differences among the distribution in the percentage of choices for HRL, LRL and OM. This suggests that blockade of D1 receptors had no effect on the distribution of choices made by the subjects (Fig 5B). The same statistical analysis was applied to the amount of movement during the sessions, showing that it was not affected by SCH perfusion (Fig. 5C). Taking together these data suggest that the behavioral task quickly developed a clear preference for HRL, that D1 blockade in the dorsal striatum was unable to modify.

Pre-approach behavior

Along the training sessions, the subjects developed an early approach towards HRL immediately after the initial nose-poke, even before the lever(s) light(s) indicated which alternative(s) would be available, including both, free and forced choice trials (see figure 3B). In this pre-approach (PA) behavior, the subjects would start the trial by nose-poking in the central feeder and immediately move towards the side associated with the high reward. At the beginning of the training, this happened very scarcely for both HRL and LRL, but with more experience, it clearly developed into a fast, automatic response with higher frequency for HRL (Fig. 6A).

The data for PA behavior and its development across training sessions were tested for normal distribution and analyzed with repeated measures ANOVA. A two-way repeated measure using sessions and PA-side as within subject factors was conducted to determine if there was a statistical difference between the

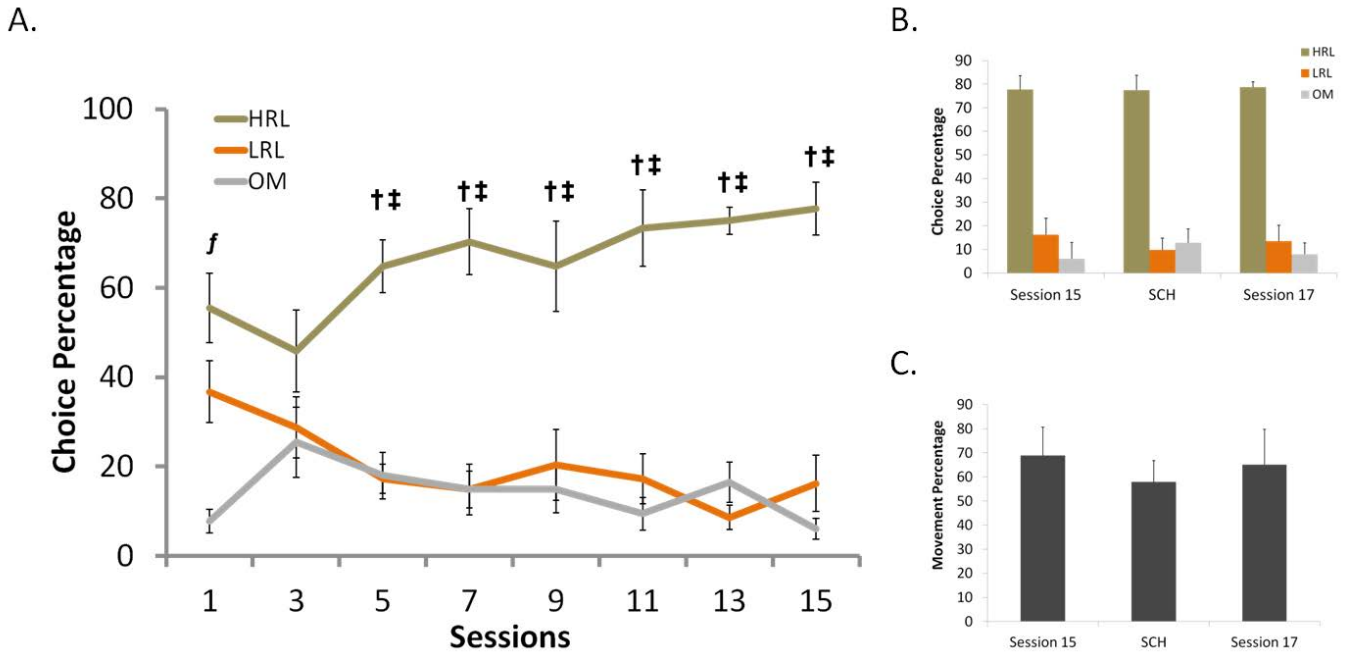

Figure 5. Choice behavior along training. A. Average of choice percentage (n=8). Initial choice between HRL and LRL was equally distributed, but around an initial/intermediate phase of the training (session 5) preference for HRL was already significantly higher. B. SCH did not show a significant impact in the distribution of choices, as shown by the comparison with training sessions before and after. C. The amount of movement was not affected by the D1 receptor blockade. Bars: SEM. represent p<0.005 for HRL vs LRL (†); HRL vs OM (‡);LRL vs OM (ƒ).

percentage of PA for HRL, LRL and OM across sessions. The percentages were significantly different among each other, as evidenced by the within-subjects effects for the factor PA-side $F(2,14)=13.33$, $p=0.001$; and changed along training sessions as showed by similar effects in the interaction between the two factors $F(14,98)=3.41$, $p<0.001$. Additionally, a one-way repeated measures ANOVA for the percentage of PA to HRL along sessions showed that this behavior increased with training $F(7,49)=3.96$, $p=0.02$. Within subject contrasts confirmed that during initial training sessions, PA was very low and equally distributed among HRL and LRL, while omissions were very high. However along training, the percentage of PA for HRL became significantly higher after session 11 (Fig. 6A). These results suggest that PA behavior was developed trough sessions and was clearly higher towards HRL.

Furthermore, in order to explore changes in PA behavior due to perfusion of SCH or aCSF, a separated repeated measures comparison of choices in three sessions (before, during and after perfusion of SCH) was made with session and PA-side as within-subject factors. The percentage of PA-side showed a trend $F(1,7)=4.80$, $p=0.065$, and the interaction of PA-side and sessions was significant $F(1,7)=6.69$, $p=0.036$. Further t test comparisons evidenced that when SCH was perfused the percentage of PA to HRL decreased significantly compared to session 15 ($t=2.58$, $p=0.036$) and 17 ($t=2.65$, $p=0.032$). On the other hand PA to LRL under SCH showed a statistical trend to increase in comparison to session 15 ($t=-2.22$, $p=0.062$) and 17 ($t=-2.17$, $p=0.059$). Omissions remained equal as compared to regular aCSF sessions (Fig. 6B). These results suggest that distribution of PA between HRL and LRL

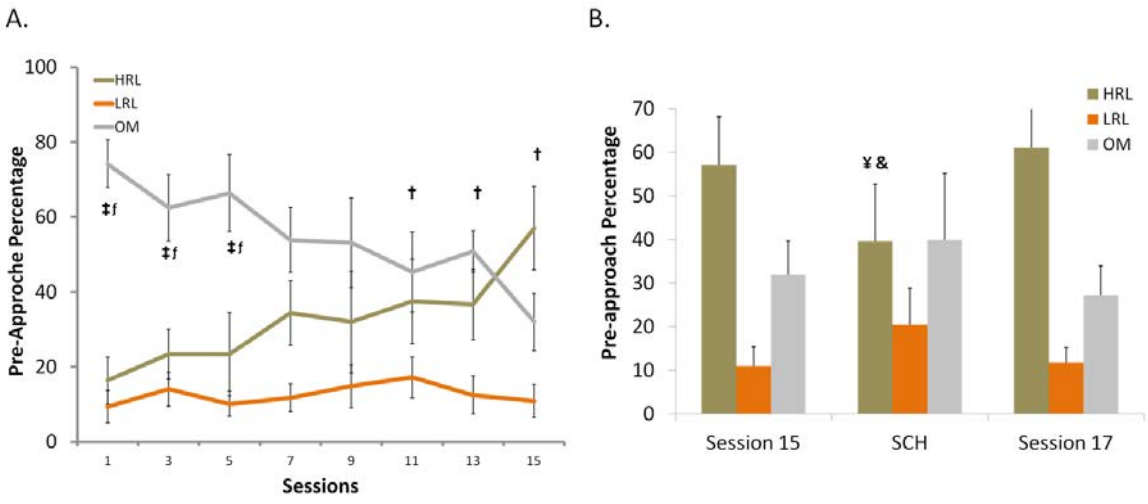

Figure 6. Pre-approach behavior. A. Average of pre-approach percentage (n=8). Trough the learning sessions rats increased the percentage of trials in which they approximated the HRL before the alternatives were signalized or shown. B. D1 blockade decreased pre-approaches to HRL and increased them to LRL. Bars:SEM. Symbols represent p<0.005 for HRL vs LRL (†); HRL vs OM (‡);LRL vs OM (ƒ); Session15 vs SCH (¥); Session 17 vs SCH (&).

was modulated by D1 antagonist SCH in a reversible manner.

Local field potential

The power of frequencies between 1 and 50 Hz was analyzed in response to initial and final trial cues in all trials in order to find differential activity between DMS and DLS along training and during SCH perfusion (Fig. 7). Warm colors indicate higher values in power difference after the subtraction (DMS - DLS), and the significance is indicated by the black line surrounding clusters with $p<0.05$. Regarding the trial-start expectancy after initial nosepoke (Fig. 7A) the initial session of the training (S1), was characterized by a significant higher power of DMS in the low frequency range (1-10 Hz). This difference was less strong in a more intermediate phase of the training (s7) and disappeared by the last session of training (S15). During SCH perfusion, this difference in power was significant again, however it returned to previous levels after SCH was gone (S17). The significant difference between DMS and DLS power and its dynamics across sessions was not observed during reward expectancy after the final nosepoke. These results suggest that DMS and DLS balance in response to trial-start cues changes along choice training sessions and that this activity is modulated by dopamine trough D1 receptors.

DISCUSSION

Our findings demonstrate that rats quickly developed a clear preference for the larger reward indicated by both lever presses

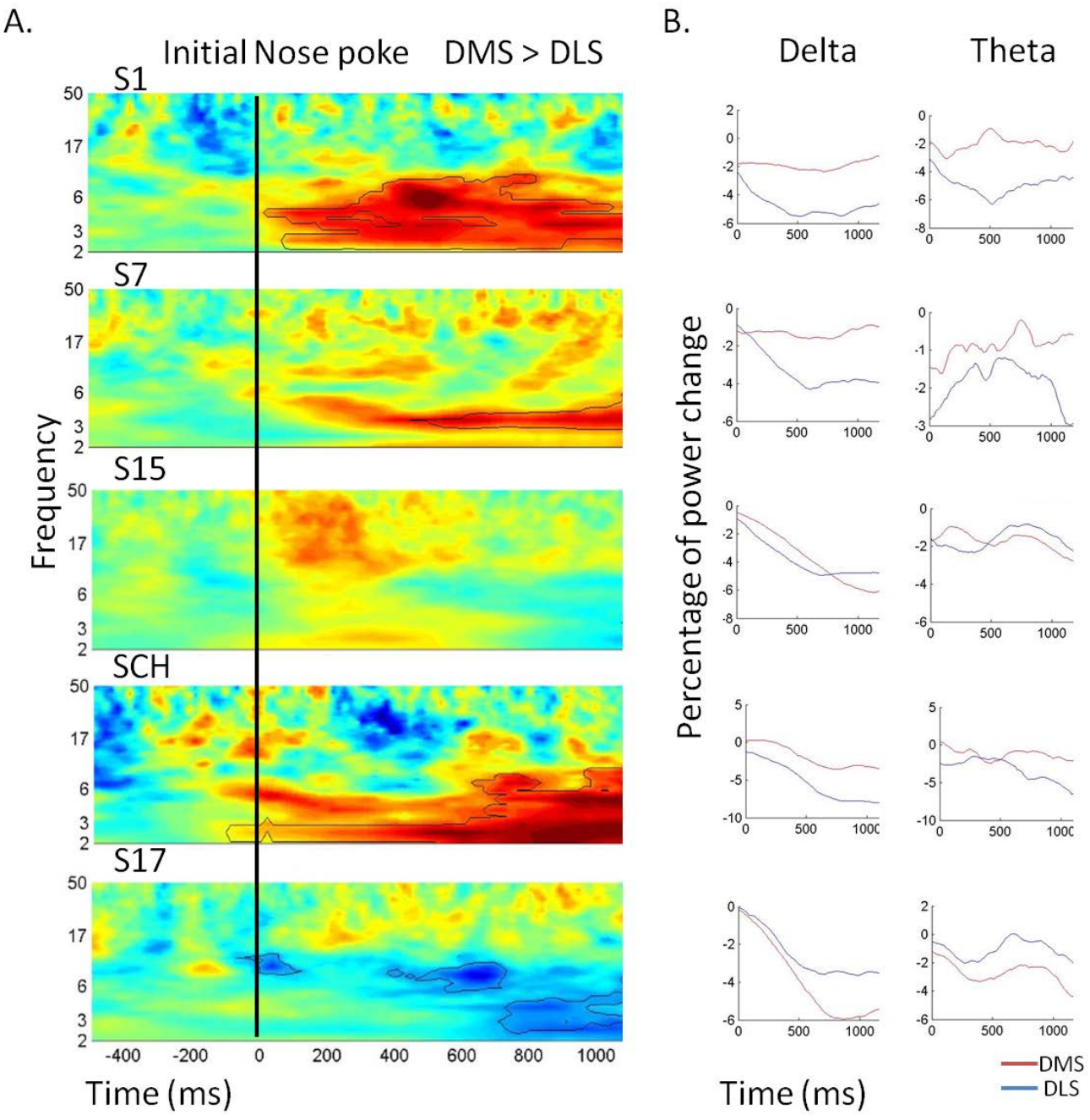

Figure 7. Local field potentials in response to trial start of dorsomedial striatum compared to dorsolateral striatum across learning sessions and under perfusion of D1 antagonist SCH. A. Time-frequency representation of responses to trial start before lever availability. Surrounded areas represent significant differences in clusters. Warm colors are positive values. During these 2 seconds rats developed a pre-approach response described in the behavioral results. It can be observed a DMS significant higher power of low frequencies at the beginning of the training that disappears trough the sessions and is even inverted at the end of the training. Perfusion of SCH re-instated the differences observed in the initial sessions and this effect was reversed in a normal session. B. ERP plots of separated bands. Delta (1-4 Hz),Theta (5-10 Hz).

and pre-approach behavior. Perfusion of the dopamine D1 receptor antagonist SCH during one of the last sessions did not significantly alter choice indicated by lever presses, but did alter pre-approaches of the respective lever sides, decreasing the amount of pre-approaches to the lever associated with the larger reward compared to the previous and subsequent session. Dynamic changes in activity represented by low frequency (1-10 Hz) oscillations in the DMS versus the DMS were observed over sessions, with decreasing activity in the DMS compared to DLS up to session 15. With the introduction of SCH in session 16 the activity was again higher in the DMS, compared to both the session before and after SCH perfusion.

It is possible that blockage of the D1 receptor with the current dose and flow rate slows down the gating of the action to approach the large reward lever by disrupting activity in the direct pathway of the basal ganglia, thereby increasing the inhibition through the indirect pathway of the pre-approaches, but leaving the general choice unaffected. This would be in line with a study assessing the effect of intraperitoneally injected SCH on locomotion activity (Agmo & Soria, 1999). Importantly, a recent study by Eagle and colleagues (2011) showed a decrease in reaction time after a stop signal when the DMS was injected with SCH with no effect on normal go-trials in a stop signal reaction time task, indicating a specific role of the dopamine D1 receptor in action inhibition. The authors also conclude that this increase in reaction time is not caused by a general slowing of responses. In addition, an association of D1 receptor functioning with increased premature or impulsive responding is found after injection of a D1 agonist in the nucleus accumbens (Pezze et al., 2007), while a intraperitoneal injection of SCH is found to reduce premature responding in rats on a 5 choice serial reaction time task (van Gaalen et al., 2006). That infusion of SCH only has an effect on behavior in session 16 and not in the subsequent session is in line with the findings of Agmo & Soria (1999), who only found a dose dependent effect on locomotion up to 24 hours after injection.

In line with findings by van Wingerden and colleagues (2010) and as suggested by Womelsdorf and colleagues (2010) the increased low-frequency power in the DMS compared to the DLS in the early learning phase of the task confirms the involvement of theta oscillations in goal-directed behavior, which is further supported by a decrease in theta as well as delta oscillations in the DMS compared to the DLS over sessions, while behavior was shifting from goal-directed to habitual responding. This change in low-frequency power in the dorsal striatum was found only after the initial nose-poke representing the start of a trial, after which the rats had to press a lever, even though the nose-poke at the end of a trial was similar in every respect, except that reward delivery followed this final nose-poke. Therefore it can be said that the changes in low-frequency power were not the result of reward anticipation per se, but more likely reflected retrieval of action-outcome associations at a decision point (Womelsdorf et al., 2010).

Infusion of SCH seems correlated with increasing low-frequency power in the DMS compared to the DLS. It is possible that an increased inhibition of locomotor behavior caused by SCH enables goal-directed processing to take over habitual responses, by shifting the balance between activity in the DMS and DLS networks in favor of the DMS. A switch to goal-directed processing cannot be distinguished in behavior in our task, as the large reward remains the best option to choose, although the decrease in pre-approaches to the lever associated with the large reward might indicate increased flexibility in behavior. This hypothesis could be tested in a follow-up study with a reversal paradigm in which SCH should enable the transition from habit back to goal-directed behavior after reversal of the contingencies. If this is indeed the case, D1 receptors seem not only necessary for habit or skill acquisition, as was initially hypothesized, but also seem to play an important role when habits are already acquired.

In summary, we found that the transition from goal-directed to habitual behavior goes hand in hand with a relative shift in low-frequency power in the DMS and DLS, confirming the involvement of theta-oscillations in goal-directed responding. Dopamine D1 receptors in the dorsal striatum seem to modulate the input reflected by these oscillations, but has only effect on pre-approach behavior and not on choice. Further analysis of the intermediate sessions as well as comparisons of trials in which the large reward was chosen versus choice for the small reward would provide more insights on the relationship between low-frequency oscillations in the DMS and DLS and habitual choice behavior. Whether activity of the D1 receptor actually mediates habitual behavior requires further research, for example with a contingency reversal paradigm or the use of agonists. In addition, further research could focus on the dynamic shift in input reflected by oscillations in the DMS and DLS during contingency reversals, providing more insight into activity dynamics in a situation when the goal-directed and habit systems are in conflict.

Acknowledgements

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The Influence of tDCS on Learning, Craving and Alcohol Avoidance Training: An EEG Study

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ABSTRACT

Introduction: Heavy drinking is associated with an automatic cognitive bias to approach alcohol cues. Retraining this automatic biases decreases subsequent alcohol consumption. However, the effectiveness of the training differs between individuals. Transcranial Direct Current Stimulation (tDCS) could improve the effectiveness of the cognitive bias training. TDCS is a non-invasive method to modify cortical excitability, which has been shown to have beneficial effects for working memory, reduce craving and amplify effects of cognitive training. Since retraining automatic action tendencies towards alcohol improves treatment outcome in alcoholic patients and tDCS enhances the effects of training, this might be a promising technique to treat alcoholism.

Methods: The effects of tDCS on accuracy in a stimulus response learning task were assessed with a push-pull task. Participants had to learn based on feedback which of the eight presented stimuli had to be pushed or pulled. Half of the participants received tDCS (15 min, 1mA) and the other half received sham stimulation. The effects of alcohol avoidance training and tDCS on alcohol consumption were examined in a 2 (alcohol avoidance training vs. control training) x 2 (tDCS vs. sham stimulation) design. Participants in the alcohol avoidance training condition consistently had to push alcohol stimuli away, whereas participants in the control training pushed stimuli based on whether there was a person in the picture or not. Alcohol consumption was measured by means of the Time Line Follow Back (TLFB), both pre- and post (7 days) training. TDCS was applied prior to both task and EEG was recorded during the tasks to reveal neuronal mechanisms underlying the behavioral effects of tDCS and alcohol avoidance training.

Result: The results showed that tDCS enhanced accuracy in a stimulus response learning task and that tDCS affected neuronal activity during accurate and inaccurate trials differently. Alcohol avoidance training decreased alcohol consumption the week after (TLFB) and the neuronal responsiveness to alcohol stimuli declined relative to control training, but tDCS did not have an additional effect on alcohol consumption nor on the neuronal responsiveness to alcohol stimuli.

Conclusions: This study showed that tDCS also improves accuracy in stimulus-response learning. The effects of tDCS on time-frequency representations indicated that tDCS either amplified ongoing neuronal processes both functional and less function or protected against dysfunctional activity. The alcohol avoidance training declined the neuronal responsiveness to alcohol stimuli, which could imply that alcohol stimuli were processed more similarly to neutral stimuli after alcohol avoidance training. Taken together, the enhanced learning effects of tDCS could further improve the effectiveness of alcohol avoidance training, when applied concurrently.

INTRODUCTION

Alcohol addiction

More than 78.000 people in The Netherlands suffer from alcohol dependence (De Graaf et al., 2010a), in 2008 765 people died as the direct result of alcohol addiction (CBS) and for an additional 1000 people alcohol was the secondary cause of death (Van Laar et al., 2010). The health care costs of alcohol addiction have risen from 400 million euro in 2005 to more than 1 billion euro in 2007 (Slobbe et al., 2011). Hence, alcohol addiction is a significant problem among the Dutch population and costs are still rising.

To begin with, what is addiction? According to the DSM-IV, addiction is a cluster of cognitive, behavioral and physiological symptoms, with a pattern of repeated self-administration that can result in tolerance, withdrawal and compulsive drug taking behavior (American Psychiatric Association, 2000). The cycle of addiction consists of three stages: 1) binge/ intoxication, reflecting the (ab)use of the substance, 2) withdrawal/ negative affect and 3) preoccupation/ anticipation, also known as craving and known to be an important precursor for relapse (Koob & Volkow, 2010). These stages are associated with differential neuronal pathways and neurochemical responses. The drug use targets the reward system, consisting of dopaminergic neurons in the ventral tegmental area and the nucleus accumbens (Pierce & Kumaresan, 2006; Nestler, 2005). Negative affect and withdrawal after drug use are processed by the amygdala (Koob & Volkow, 2010) and are associated with a hyperactive stress response (Nestler, 2005). Craving recruits a widely distributed network including the dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate (Koob & Volkow, 2010; Wilson, Sayette & Fiez, 2004) and hippocampus (Koob & Volkow, 2010).

How does addiction develop? The incentive salience hypothesis postulates that the repeated use of substances changes the sensitivity of these neurocircuits in response to normal rewarding stimuli (Nestler, 2005) and to drug-related cues (Robinson & Berridge, 2003). Chronic drug use decreases the baseline levels of dopamine as a result of which regularly rewarding stimuli become less incentive (Nestler, 2005). At the same time the reward circuitry becomes more sensitive to drug-related cues (Robinson & Berridge, 2008). Stimulus valuation is an important process preceding motivation. In this way, the system that normally regulates this incentive attribution becomes sensitized for drug-related cues as drug abuse is prolonged. This can lead to a pathological motivation to seek drugs (Robinson & Berridge, 2008) and compulsive drug taking (Robinson & Berrigde, 2003). Additionally, these altered dopamine levels persist after the acute effects of the drug and could therefore be a possible mechanism underlying neurochemical tolerance, relapse and craving (Nestler, 2005).

The hypersensitivity of the reward system in response to drug-related cues is thought to result in several automatic cognitive biases that are important for development and maintenance of, in this case alcohol, addiction (Robinson & Berridge, 2003; Stacy & Wiers, 2010; Wiers et al., 2011). First, heavy drinkers but not light drinkers have an attentional bias (Field et al., 2007). Attentional bias refers to involuntary selective attention for substance related cues, resulting from repeated pairing of, for instance, alcohol cues with the effects of alcohol use (Schoenmakers et al, 2010). Second, a memory bias, reflecting the automatic activation of alcohol related associations (Wiers et al., 2011), correlates highly with alcohol consumption in a reciprocal manner (Grenard et al., 2009) and third, an approach bias develops which refers to automatic action tendencies to approach alcohol (Wiers et al., 2007). The alcohol approach bias is related with urge to drink and positive expected outcomes in anticipation of alcohol consumption (Palfai & Ostafin, 2003). These cognitive biases draw attention to alcohol stimuli, make alcohol related memories more salient and can result in alcohol approach behavior, respectively.

Neurophysiological evidence for the attentional bias is provided by Herrmann et al. (2001). They examined the ERP components of heavy and light drinkers in response to alcohol cues and neutral cues. Heavy drinkers showed an increased P3 component in response to alcohol cues compared to neutral stimuli. The amplitude of the P3 component correlated with habitual alcohol consumption. Since the P3 component is thought to reflect attentional load, Herrmann et al. (2001) interpreted the pronounced P3 amplitude as increased attention towards alcohol related stimuli. The difference in amplitude between alcohol and neutral stimuli was not visible for light drinkers.

Moreover, alcohol-related cues and craving also result in differences in the neuronal responsiveness. For example, craving is associated with an increase in EEG arousal that leads to a decrease in alpha oscillatory power (Liu et al., 1998). Analogously, an increase in alpha power could be the result of a decrease in craving (Lee et al., 2009). Lee et al. (2009) argue that the EEG signal is a biological marker for neuronal cue reactivity. This is in line with a study from Kim et al. (2003), who found more activation in general in the frontal frequency domain of alcoholic patients that viewed alcohol pictures, compared to neutral control pictures. This is evidence that craving and alcohol stimuli elicit a more active neuronal response in the time-frequency domain too.

Besides sensitization of the reward systems and the development of automatic cognitive biases, repeated drug use also affects the activity and functionality of frontal regions. Chronic use is associated with a reduced baseline activity in frontal regions (Volkow et al., 2004) and can distort prefrontal functions like inhibition and cognitive regulation (Robinson & Berridge, 2003). These executive functions serve as a protective prerequisite against the development of the automatically activated pathological wanting of the drug (Robinson & Berridge, 2008). For example, Grenard et al. (2008) found that individual differences in working memory capacity predicted the effects of automatic drug-related processes in such a way that higher working memory capacity weakened the impact of automatic processes. This is in agreement with the view that addiction and compulsive drug taking behavior result from an imbalance between relatively reflective controlled processes and relatively automatic appetitive processes (Robinson & Berridge, 2003; Wiers et al., 2007). The reflective system has a regulatory function, whereas

the impulsive system is approach oriented and leads to automatic approach behavior (Wiers et al., 2007). Furthermore, decreased frontal activity can result in increased subcortical activity (Robinson & Berridge, 2003). Drug abuse can lead to an imbalance between these systems, which makes one susceptible to damaging and destructive behavioral cycli (Robinson & Berridge, 2003).

Why does addiction persist despite the harmful consequences for the patient? Besides the reflective-automatic dichotomy a distinction can be made between implicit and explicit cognitions towards addiction (Stacy & Wiers, 2010). Explicit cognitions refer to the conscious knowledge of the patient that the addictive behavior is harmful and should not be continued. However, implicit cognitions do not require conscious and deliberate recollection of previous events and therefore patients are often unaware of their implicit attitude towards substance use (Robinson & Berridge, 2003; Stacy & Wiers, 2010). It is important to note that this explicit-implicit dichotomy differs from the reflective-automatic distinction in such a way that automatic approach tendencies in the reflective-automatic distinction can also exist within the awareness of the patient, for example with craving. The explicit-implicit processes are specifically relevant for maintenance of the addiction (Stacy & Wiers, 2010). If the explicit attitude towards alcohol is negative, while the implicit attitude is still positive, this can result in the contradictory behavior that is often seen in alcoholic patients. In determining which attitude is dominant working memory capacity plays a role (Thush et al., 2008). For participants with a high functioning working memory, alcohol behavior was predicted by their explicit attitude towards alcohol, whereas the alcohol consumption of participants with a lower functioning working memory was predicted by their implicit attitude towards alcohol (Thush et al., 2008). Thus, reflective processes at least partly determine the role of implicit and explicit attitudes. However, if there is an imbalance between reflective an automatic processes, like is the case with addiction (Robinson & Berridge, 2003), the implicit cognitions are dominant in predicting alcohol consumption.

Cognitive Bias Modification

If implicit cognitions play a role in maintenance of the addiction, modification of these biases might help with the treatment of alcoholic patients. First, what implicit cognitions can be measured and how? In a review paper Stacy and Wiers (2010) have summarized the most used measures to assess implicit cognition. One of the measurements to examine the presence and strength of implicit cognitions is the Implicit Association Test (IAT). In this task reaction times are measured between affective stimuli and target stimuli. An attentional bias towards alcohol stimuli results from faster reaction times for alcohol stimuli (target) than for soft drink stimuli (neutral stimuli). Besides an attentional bias a modified version of the IAT is also used to reveal an approach bias. In this version the affective stimuli are replaced with action tendencies (approach vs. avoidance). Another measurement is the Visual Probe Task. In this task two stimuli are presented simultaneously. One of the stimuli is the target stimulus, in this case alcohol, and the other is a neutral control stimulus. After the short presentation of the stimuli a probe appears on the screen to which the participant has to respond. Again shorter latencies for target stimuli reflect an attentional bias. An approach bias can also be measured in a slightly different version of this task (Stacy & Wiers, 2010).

In addition to these measurements Wiers et al. (2009) adapted the regular version the Approach Avoidance Task (Rinck & Beckers, 2007) into an alcohol Approach Avoidance Task. In this task participants have to either push or pull pictures of alcoholic beverages and soft drinks based on their format (landscape or portait). The response is made with a joystick and while the response is made, the pictures zoom either in during a pull response or out during a push response. The advantage of this task relative to the approach-avoidance IAT is that the required processes during this implicit action tendency task do not tap into relatively explicit semantic knowledge that is necessary for the approach avoidance IAT and therefore the AAT is more similar to the implicit tendencies it is thought to reveal (Rinck & Beckers, 2007). By means of this alcohol AAT Wiers and colleagues (2009) showed that specifically male heavy drinkers with the OPRM1 G-allele showed a relative strong alcohol approach bias.

In summary, heavy drinkers show an attentional bias for alcohol related stimuli (Field et al., 2007), which is also visible on a neurophysiological level in terms of an amplified P3 component (Herrmann et al., 2001). Their memory bias for alcohol-associations is thought to both results from repeated alcohol consumption and might be a partial cause for increased alcohol consumption (Grenard et al., 2009). Furthermore, hazardous drinkers show an approach bias for alcohol pictures (Wiers et al., 2007) and this alcohol approach bias is positively correlated with urge to drink and positive expected outcomes in anticipation of alcohol consumption (Palfai & Ostafin, 2003). Taken together, these results indicate that automatic cognitive biases are important in the maintenance of alcohol addiction. Next and more relevant for treatment is whether it would be possible to alter these biases in order to counter the vicious cycle of preoccupation, consumption and withdrawal.

First, Schoenmakers et al. (2007) used a visual probe task not only to assess attentional bias, but also to retrain participant to direct their attention towards soft drinks. In the training condition the probe appeared in most but not all (600/624) trials on the location of the soft drink. This way participants were implicitly trained to direct their attention to soft drinks. In the control condition the probe appeared in half of the trials on the location of a soft drink and in the other half of the trials on the location of an alcoholic beverage. In the last assessment block results showed that the initially present attentional bias towards alcoholic beverages had shifted towards an attention bias for soft drinks in the attentional retraining condition. Although the retraining was effective, this effect did not generalize to new stimuli and a different task and did not decrease craving. Next, Schoenmakers et al. (2010) tested the attentional retraining on alcoholic patients to assess the effectiveness of multiple training sessions on generalization to other stimuli and craving. Results from

this study point out that five sessions of attentional bias modification was effective in generalization to other stimuli up to three months. However no effects on craving were found. Nevertheless seems attentional bias modification effective on a behavioral level (Schoenmakers et al., 2007; Schoenmakers et al., 2010) and the effects of retraining are also present on a neurophysiological level, as found by Eldar and Bar-Haim (2010). They trained anxiety patients to divert their attention away from the threat. EEG data shows that the P2 and P3 amplitudes in response to threatening stimuli were decreased after attentional training. Thus, although not in an alcohol setting, the effects of attentional training are also visible on a neurophysiological level.

Following attentional bias modification, Wiers and colleagues (2010) examined the effects of approach bias modification on alcohol approach tendencies and subsequent alcohol consumption. Consistently pushing or pulling alcohol pictures away in the alcohol AAT should influence the implicit cognition towards alcohol. In this first study 42 heavy drinking male student participants were trained to either push alcohol pictures away or pull alcohol pictures with a joystick. However, they did this without conscious awareness, since they were instructed to respond based on the format of the picture. After the training approach tendencies were tested and alcohol consumption was measured in a taste test. The participants that were trained to push alcohol showed a stronger avoidance tendency for alcohol pictures after the training than before. The effects on alcohol consumption were also congruent with the training condition, implicating that participants in the alcohol pull condition drank more beer in the taste test than participants in the alcohol push condition. However, this effect was only found for participants that showed effectiveness of the training. Nevertheless, action tendency modification has a stronger effect than attentional bias modification, since one session of 440 trials already shows generalization to other stimuli, like words and other sets of pictures (Wiers et al., 2010). This was not the case with one session of 624 trials of attentional bias retraining (Schoenmakers et al., 2007). The strong effects of action tendency training might be related to the idea underlying embodied cognition. That is, perception and cognition do not only lead to action, but neuronal activation of certain motor schemes is also able to influence cognition and perception (Garbanini & Adenzato, 2004). This motor feedback might have a stronger influence that attentional processes (Wiers et al., 2010).

To test the clinical effectiveness of alcohol avoidance training, Wiers et al. (2011) applied the alcohol avoidance training to alcoholic patients. Patients were randomly assigned to one of four conditions. In the first condition patients implicitly pushed alcohol pictures away, based on picture format. In the second condition patients were explicitly instructed to push alcohol pictures away. The third group of patients received control training, in which fifty per cent of the alcohol pictures were pushed and the other half was pulled. Finally, the last group did not receive any training at all. The study consisted of four sessions of training. Both implicit and explicit training conditions showed a change from alcohol approach bias to alcohol avoidance bias and there was no difference in effectiveness between implicit and explicit instructions. These effects also generalized to untrained stimuli and the effects of the alcohol bias were visible on an implicit association task. And, most interestingly, the alcohol avoidance training improved treatment outcome.

In conclusion, both attentional retraining (Schoenmakers et al., 2007; Schoenmakers et al., 2010) and alcohol avoidance training (Wiers et al., 2010; Wiers et al., 2011) are effective in modifying respectively the attentional bias for alcohol-related stimuli and the action tendency to approach alcohol stimuli. Although the alcohol avoidance training yields stronger results than the attentional bias modification in terms of generalization, there is a large variability in the effectiveness of the alcohol avoidance training. From the 42 participants, only eleven in the alcohol approach condition and twelve in the avoidance condition were successfully trained (Wiers et al., 2010). If the training does not alter the approach bias for alcohol stimuli, it is highly improbable that the training affects subsequent alcohol consumption, craving or other processes that play a role in maintenance of alcohol addiction. Therefore Wiers et al. (2010) explored which factors relate to the success of the training. From the four variables, which were weekly alcohol consumption, urge to drink beer, condition and an interaction between condition and weekly alcohol consumption, only urge to drink alcohol prior to the test was negatively related to success of the training. This implicated that, although alcohol avoidance training by itself is already a promising method to counter the vicious cycle of implicit alcohol related cognition, there is room for improvement. A possible technique that could improve the effectiveness of the alcohol avoidance training is transcranial direct current stimulation (tDCS).

tDCS

Transcranial Direct Current Stimulation (tDCS) is a non-invasive method to modify cortical excitability. A weak constant current is delivered through two electrodes, the anode and the cathode, which makes the stimulation polarity specific (Fregni et al., 2005) and can respectively enhance or reduce neuronal communication (Nitsche et al., 2008). tDCS has an effect that persists after stimulation offset (Fregni et al., 2005). The aftereffects of tDCS are thought to be mediated by modulated NMDA receptor density (Liebetanz et al., 2007). However, the mechanisms underlying the aftereffects remain unclear (Ardolino et al., 2005). Nevertheless, it is a promising technique, because tDCS is a non-invasive and painless method that can manipulate rather than passively measure brain activity (Floel & Cohen, 2007). Beneficial effects of anodal stimulation have been found for, among others, depression treatment (Fregni et al., 2006), reduction of craving for alcohol (Boggio et al., 2008) and cigarettes (Fregni et al., 2008a) and enhancement of working memory (Fregni et al., 2005).

Fregni and colleagues (2005) were one of the first to study the influence of tDCS on working memory. They defined working memory as the ability to temporary hold and manipulate information necessary for complex tasks (Fregni et al., 2005). A 3-back task was used to examine working memory. The participants performed the task during the stimulation. The anode was placed on the

dorsolateral prefrontal cortex (DLPFC), whereas the other electrode was placed on the supraorbital area. The DLPFC is associated with working memory (Petrides, 1995). Participants both received sham stimulation, where the stimulation was turned off after 5 seconds, and real stimulation, which was a constant current of 1mA that was delivered for 10 minutes. To prevent carry over effects they started the second stimulation session 60 minutes after the first one had ended. Both accuracy and error rate improved in the real condition. Reaction time, however, did not improve. To check whether the effects of the stimulation were focal and if they were polarity specific, they performed the same experiment as described above six months later. By placing the anode on the primary motor cortex Fregni and colleagues (2005) made sure the effects were due to stimulation of the DLPFC and not due to stimulation in general. In the second control experiment they reversed the anode and cathode and as a result the neuronal excitability of the DLPFC was decreased. In both control experiments no effects were found, indicating that only anodal stimulation of the DLPFC improves working memory.

Whereas Fregni and colleagues (2005) tested the effects of tDCS on a concurrently performed working memory task, Ohn et al. (2008) examined the after effects of the stimulation. They also used a 3-back task for working memory assessment. The experiment consisted of two sessions with two weeks intermediate to prevent carry over effects. All participants received, per session, either sham stimulation (5 seconds of stimulation) or real tDCS (30 minutes) in a counterbalanced order. Participants performed the task before, during and 30 minutes after stimulation. The anode was placed on the left DLPFC and the cathode on the contralateral supraorbital area, similar to the experiment of Fregni et al. (2005), and the intensity was also 1 mA. Accuracy was enhanced after 20 minutes of stimulation and lasted up till 30 minutes after stimulation offset. However, there was no effect of tDCS on error rate and reaction times. An explanation why Ohn et al. (2008) did find effects of DLPFC stimulation on accuracy, but not on error rate might be that accuracy is a result of processes like encoding, maintenance and decision making, which are important functions of the DLPFC, whereas error detection also depends on the anterior cingulate and part of the temporoparietal cortex.

How can these after effects be explained? As described before, anodal tDCS depolarizes the resting membrane potential of neurons. As a result of this neurons need less input to produce an action potential. When an action potential is produced the receptor densities in the cell membrane change (Kandel, 2000). This makes the neuron more susceptible to subsequent input. If the subsequent input consists of a brief period of strong synaptic activation these changes in receptor densities result in a strengthening of synaptic connections (Floel & Cohen, 2007). This mechanism is known as long term potentiation (LTP). LTP could be the mechanism underlying the after effects of tDCS. In line with this explanation it could also be the case that effects of memory training are enhanced when the training is paired with tDCS (Floel & Cohen, 2007).

This enhancement of training via tDCS is what Andrews et al. (2011) tested. She and her colleagues tested whether cognitive training paired with anodal tDCS on the DLPFC improved performance on a subsequent working memory test more than only cognitive training or tDCS. There were three counterbalanced conditions with a week in between to prevent carry over effects in which every participant took part. In the first condition participants only received tDCS at an intensity of 1 mA for ten minutes. In the second condition participant received sham stimulation, which was tDCS at an intensity of 1 mA but for only 30 seconds, while performing a n-back task. In the last condition participants both received real tDCS (1 mA for ten minutes) and performed an n-back task. The assessment task, a digit span working memory task, was administered before and ten minutes after each session. The participants that received real tDCS and working memory training showed improvement over time on the digit span task, contrary to the participants that either received sham tDCS and training or only real tDCS, who did not show any improvement at all. These results are consistent with the explanation given by Floel and Cohen (2007). tDCS combined with cognitive training can amplify the effects of training alone.

tDCS and EEG

If tDCS has beneficial effects for working memory performance, it would be interesting to examine changes in brain activity as a result of tDCS. This could shed a light on the underlying mechanisms of the working memory enhancement. Keeser et al. (2011) examined the effects of tDCS on resting state EEG and event-related potentials during a working memory task. They applied a constant current of 2 mA for 20 minutes on the left DLPFC, with the cathode on the contralateral supraorbital region. After the stimulation EEG was first recorded during an eyes closed resting period, followed by an n-back task. During resting state a decrease in delta power was found in the frontal electrodes and an increase in beta power on electrode Fz. A decrease in slow oscillatory power (delta) and an increase in higher oscillatory power (beta) reflect more neuronal activity (Keeser et al., 2011). This increase in neuronal activity, i.e. a more alert state in the fronto-parietal network, is the result of the excitatory influence of anodal tDCS stimulation. These results are in accordance with a study of Ardolino et al. (2005), who found an increase in delta power after cathodal, as contrary anodal, stimulation.

TDCS also affects the subsequent neuronal activity in the n-back task that followed the stimulation. Keeser et al. (2011) examined the amplitude and latency of the P2 and P3 component. The P2 component is thought to reflect memory load (Klaver et al., 1999) and P3 is related to focal attention during stimulus detection (Polich, 2007), which are both important functions of working memory (Keeser et al., 2011). The amplitudes of both the P2 and P3 component were increased at Fz and both components showed a reduced latency at Cz, compared to sham stimulation. Additionally, a negative correlation between the amplitude of P3 and error rate was found. This indicates that the behavioral improvement of working memory as a result of tDCS was mediated by amplified neuronal responsiveness of two ERP components that are important for

working memory. So tDCS changes the resting oscillatory activity and the amplitudes of working memory-related ERP components.

Besides ERP components, working memory is also associated with changes in oscillatory power and coherence between brain regions. Upper alpha (10-12 Hz) and theta (4-7 Hz) are two frequency bands of interest for working memory research (Klimesch, Schack & Sauseng, 2005). Jensen and Tesche (2002) found that oscillations in the theta frequency band in frontal areas increased with the number of items that were kept in memory during the retention period. Thus, sustained theta activity reflects active maintenance in working memory. Furthermore, Jensen et al. (2002) also found increased alpha power on Cz and Pz during the retention period in the Sternberg task. They interpreted the task related increase of alpha activity as an increased need for task irrelevant inhibition, but kept open the option that alpha might directly be involved in memory maintenance.

The combination of alpha and theta activation in relation to working memory was also examined by Sauseng et al. (2005). They tested how coherence in the fronto-parietal network varied with working memory demands. Participants had to execute a simple retrieval task and a more demanding manipulation task. Sauseng et al. (2005) found an increased connectivity of theta oscillations in the long range fronto-partietal connections and a decrease in anterior short range upper alpha band oscillations for the manipulation task as opposed to the simple retrieval task. They interpreted the decrease in alpha coherence as a task specific reduction of cortical inhibition at the frontal sites.

These results seem contradictory to the memory load dependent increase in alpha power as was found by Jensen et al. (2002). However, the increase of alpha found by Jensen et al. (2002) was firstly found at posterior sites and secondly, coherence is influenced by both changes in power as well as phase coupling. Thus, it might be possible that the decrease in anterior upper alpha was confounded with an increase in alpha power (Sauseng et al., 2005). Although there is no consensus concerning the precise role of upper alpha oscillations, alpha power is related to working memory (Jensen et al., 2002; Sauseng et al., 2005) as is theta power (Jensen & Tesche, 2002; Sauseng et al., 2005).

How does tDCS modulate these oscillatory characteristics of working memory? Zaehle et al. (2011) tested the effects of tDCS on a 2-back task and its underlying neural activity. In two separate sessions they applied anodal and cathodal stimulation with an intensity of 1 mA for 15 minutes on the DLPFC. In both sessions the participants also got sham stimulation (1 mA, 30 seconds) preceding the real stimulation. After both the sham and the real stimulation EEG was recorded during the 2-back task that participants performed. Zaehle et al. (2011) analyzed the frequency bands in a posterior region of interest, the occipito-parietal electrodes, because this region is also involved in working memory tasks (Owen et al., 2005) and found that anodal stimulation increased the event-related oscillatory power in the theta and alpha band, whereas cathodal stimulation decreased the theta and alpha power. These results are in accordance with the study of Jensen et al. (2002) who also found an increase in alpha power on the occipito-parietal sites during a working memory task. Thus, these frequency bands are not only amplified with increasing working memory load, but also as a result of the by tDCS induced working memory enhancement.

In short, tDCS has a beneficial influence on working memory performance and this modulation is also visible in the neuronal activity that is associated with working memory, like an amplified P2 and P3 component and increased oscillatory power in the theta and alpha band. An interesting application of these working memory enhancing effects of tDCS is the treatment of alcohol addiction and craving.

tDCS and craving

Several studies have shown that tDCS on the DLPFC reduces craving (Boggio et al., 2008; Fregni et al., 2008a). Craving is defined as a strong desire (Boggio et al., 2008) elicited by deprivation of the substance or exposure to cues that are associated with the desired substance (Fregni et al., 2008a). Craving increases the activity in the DLPFC (Olbrich et al., 2006; George et al., 2001), the region that is involved in planning of substance use, integration of substance related cues and planning and memory in general (Wilson, Sayette & Fiez, 2004). The DLPFC has connections with the mesolimbic dopamine pathway, further consisting of the ventral tegmental area, nucleus accumbens, amygdala, hippocampus and medial prefrontal areas (Pierce & Kumaresan, 2006). These mesolimbic pathways play an important role in the rewarding aspects of drugs and alcohol addiction (Robinson & Berridge, 2008).

How can stimulation of the DLPFC reduce craving? Although explanations of the results remain highly speculative (Fregni et al., 2008a), some possible mechanisms are proposed. Boggio et al. (2008) suggested that tDCS on the DLPFC could interfere with the activity of the reward pathway, in other words decreases the signal-to-noise ratio of the internally generated neural activity and consequently decreases alcohol craving (Boggio et al., 2008). Nitsche et al. (2006) showed that the after effects of tDCS were diminished when a D2-blocker was administered to subjects, so the effects of tDCS are dependent on dopamine levels. It is therefore plausible that tDCS on the DLPFC modulated activity in the mesolimbic dopamine pathways.

However tDCS enhances cortical excitability, i.e. improves neuronal communication, so the activity in the DLPFC should increase in favor of craving and not interfere with the mesolimbic reward pathway. TDCS has not shown to have disruptive effects on local cortical activity (Fregni et al., 2008a). Thus another mechanism might be involved in the reduction of craving by tDCS. The DLPFC is involved in executive functions and assessment of future consequences and social control. Therefore Fregni et al. (2008a) suggested that the decrease in craving could be due to an increased capability to suppress initial urges, i.e. exhibiting more executive control and thinking more about future consequences. In line with this explanation is a study from Fecteau et al. (2007a), in which they found that stimulation of the DLPFC reduced risk taking

behavior in the BART task.

This is in accordance with the dual-process model as described by Wiers et al. (2007). As pointed out before in this paper, this model proposes that addictive behaviors result from an imbalance between reflective and impulsive processes. The reflective processes have a regulatory controlling function, whereas the impulsive system is approach oriented and leads to automatic approach behavior towards alcohol stimuli (Wiers et al., 2007). Similarly, several studies have shown that working memory capacity moderates substance use and substance related associations (Grenard et al., 2008), that inhibition training, another function of the DLPFC, can reduce alcohol associations (Houben et al., 2011) and even that working memory training can reduce alcohol use (Houben, Wiers & Jansen, 2011). Likewise, if tDCS enhances working memory as aforementioned it can probably mediate automatic alcohol approach behavior and craving by enhancement of executive control. However, this specific combination of increased executive control, decreased craving and decreased alcohol consumption has not been tested so far.

Another way of applying tDCS to reduce alcohol approach behavior is to make use of the increased neuronal plasticity and enhanced learning capacity that tDCS induces. Both attentional bias (Schoenmakers et al, 2007; Schoenmakers et al, 2010) and alcohol approach behavior (Houben, Nederkoorn, Wiers & Jansen, 2011; Wiers et al., 2010; Wiers et al., 2011) can be retrained in order to modulate the associations and decrease subsequent alcohol use. Regarding beneficial effects of tDCS on working memory and learning, this method might enhance the effects of the attentional bias modification and the re-training of automatic action tendencies.

Hypotheses and expected results

The first aim of this study is to examine the effects of transcranial direct current stimulation on learning stimulus response associations and neuronal event related components and oscillatory dynamics related to working memory. Anodal tDCS on the DLPFC compared to sham stimulation would increase accuracy (Ohn et al., 2008; Keeser et al., 2011; Andrews et al., 2011) on the AAT. The increased accuracy is mediated by enhanced amplitude of the P3 component (Keeser et al., 2011) in response to the stimuli, an increase in theta power in the frontoparietal network (Jensen & Tesche, 2002; Sauseng et al., 2005; Zaehle et al., 2011), a decrease in frontal upper alpha (Sauseng et al., 2005) and an increase in alpha power on the posterior sites (Jensen et al., 2002; Zaehle et al., 2011) during the task.

The second aim is to replicate that tDCS reduces craving and to test whether this reduction in craving is related to the improvement of working memory. If tDCS on the DLFPC enhances the reflective system, this could be measured by a delay discounting task. With a delay discounting task the costs to obtain a larger reward in the future as compared to a smaller reward immediately, can be quantified (Kalenscher & Pennartz, 2008). Rational decision making would predict that the costs for the delayed reward would be reduced, approximating zero. The DLPFC is involved in integration of future consequences and action-reward contingencies (Kalenscher & Pennartz, 2008), so it is plausible that stimulating the DLPFC would enhance rational decision making. If the reduction in craving is paired with an increase in rational decision making, that is, reduced costs, this could be the mechanism underlying the reduction in craving.

Finally, this study will test the effects of tDCS on alcohol avoidance training and subsequent alcohol consumption. If tDCS enhances plasticity and the neuronal network becomes more susceptible to learning during tDCS, which is tested in the first aim of this study, the altered alcohol associations induced by the training might become stronger than they would without tDCS. The effectiveness of the alcohol avoidance training compared to the control training will be measured by means of the amount of alcohol consumption the week after the training. First, the effects of training will be examined and second, the interaction with tDCS will be tested. Expectations are that the alcohol avoidance training reduces alcohol consumption in the week after the training (Wiers et al., 2010) and that tDCS amplifies this effect as opposed to sham stimulation (Andrews et al., 2011).

METHODS

Participants

Participants were 61 right handed students of the University of Amsterdam (18 men, 43 women) between 18 and 30 years old, with a mean age of 21,5 (sd = 2.69). They were enrolled in the research participants system of the university and received three research credits or 30 euro for participation. Participants with a history with neurological and/ or psychiatric diseases or used psychoactive medication were excluded from the study. Furthermore, participants were screened for their alcohol use and participants that had no experience with alcohol use at all were also excluded. This study was approved by the local Ethics Committee of the University of Amsterdam. All participants had given written consent. The participants were randomly assigned to either sham or real stimulation and in the last part of the experiment to either the alcohol avoidance training condition or the control condition. For the first two aims of this study, this resulted in two conditions, namely tDCS and sham stimulation, and for the last aim of this study a two-by-two between subject design was used, with equal numbers of participants in every condition. There were no differences in age ($F(3,57) = 0.95$, $p = .424$) and AUDIT score ($F(3,57) = 0.56$, $p = .642$) between the four conditions. Additionally, men and women were equally distributed over the four conditions ($\chi^2(3) = 0.497$, $p = 0.92$).

Materials and instruments

The AUDIT was used to assess the extent to which participants were dependent on alcohol. The Alcohol Use Identification Test (AUDIT) is a screening method for excessive drinking and helps to identify alcohol dependence (Saunders et al, 1993). An AUDIT score between 8 and 15 indicates hazardous drinkers with mild alcohol problems. All AUDIT scores were in the range from 3 to 29, with a mean of 11.21 (sd = 4.95), so the majority of the participants (N = 47) was classified as a hazardous drinker.

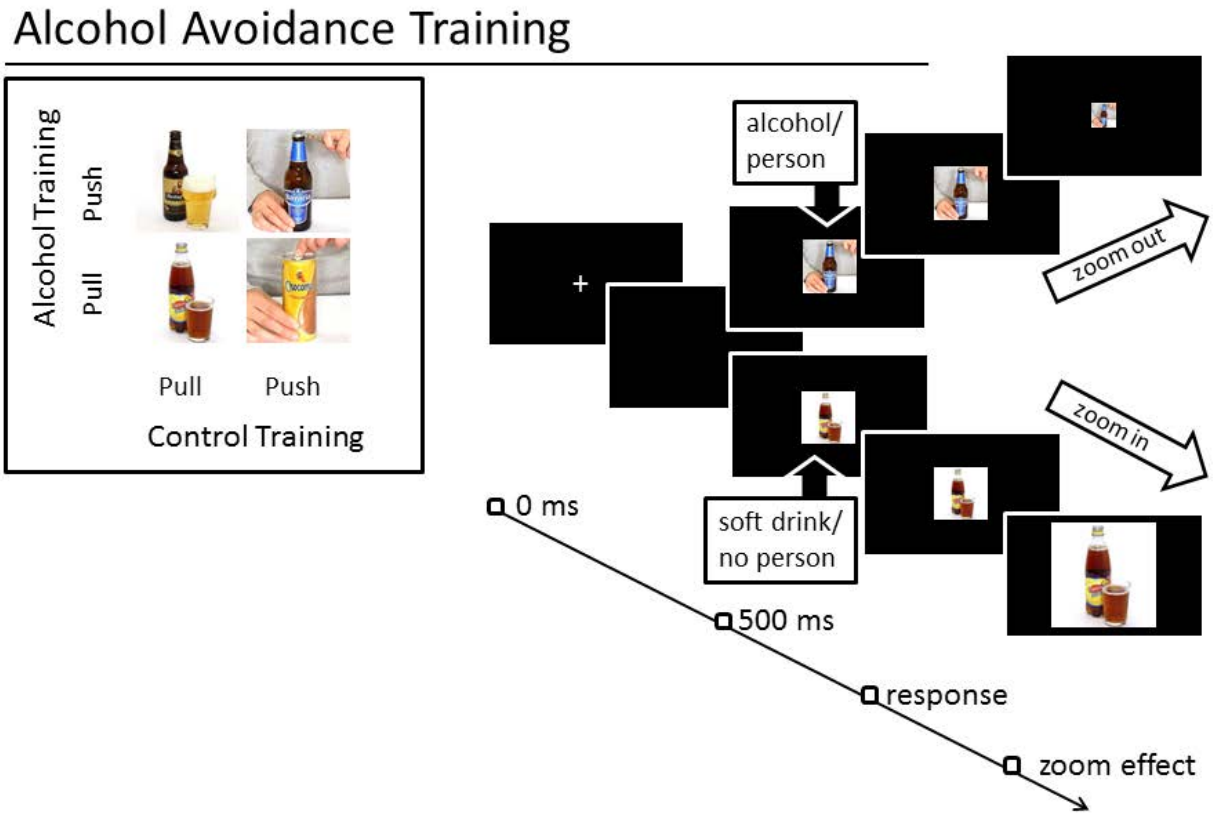


Figure 1. Left: In the alcohol avoidance condition participants push or pull pictures based on whether the drink contains alcohol or not. In the control condition participants push or pull pictures based on whether there is a person in the picture or not. Right: Illustration of the AAT. First a fixation cross appeared (0 ms), then 500 ms after the fixation cross the stimulus was presented. Hereafter the participant could respond, as a result of which the picture either zoomed in (pull) or zoomed out (push). If the response was incorrect, the word "incorrect" appeared on the screen after the picture had disappeared.

The TLFB was used to examine alcohol consumption. The Time Line Follow Back (TLFB) is a retrospective measure of alcohol consumption and is reliable when administered by computer (Sobel et al., 1996). In the version that was used in this study participant were asked to report their daily alcohol consumption for a period of fourteen days back.

Rational, long term thinking was measured by a delay discounting task. This is a measure of how willing participants are to wait a specific time in order to receive a larger reward (Kalenscher & Pennartz, 2008). Participants had to choose between two amounts of money, one of which they would hypothetically receive today and the other, higher amount of money they would receive later in time. If they preferred the lower amount of money now, the amount of money that they would receive later increased. If they preferred the higher amount of money later over the lower amount now, the higher amount of money decreased. This procedure repeated several times until the preference of the participant switched three subsequent times from now to later and back. The difference between money that was offered now and later in the last trial could be viewed of as extra value that was necessary to compensate for the delay period. In this test three delay periods were assessed, namely a day later, a month later and a year later.

Craving for alcohol was measured by eight items from the Alcohol Craving Questionnaire (ACQ; Singleton, Henningfield & Tiffany, 1994). These items loaded on the factors strong desire to drink and no desire to drink (Love, James & Willner, 1998). The participants had to agree or disagree to the sentences on a seven-point Likert scale. Two of the statements were negative. To avoid response bias for either positive or negative tendencies, the values of every positive statement were divided by the total number of positive (N = 6) questions and vice versa for the negative questions (N = 2).

In the stimulus response learning task participants had to push or pull pictures by pressing two corresponding buttons. Every picture had only one correct response and participants had to learn by feedback which picture required which response. If the pull response was made the picture the picture zoomed in towards the participant, i.e. became larger. If the push response was given the picture zoomed out, i.e. became smaller. Feedback ("correct", "incorrect") appeared on the screen after the picture had disappeared. The buttons corresponding to the pull and push response switched every block.

In the next part of the task, the alcohol approach avoidance training (AAT) was used as described by Wiers et al. (2009). However, there were two differences with the alcohol AAT that was used by Wiers et al. (2009). First, the response was made by button presses instead of pushing or pulling a joystick, but the pictures still zoomed in or out after the response was made. Second, since Wiers et al. (2011) found that there is no difference in effectiveness between explicit and implicit instruction, participants in this task did not respond to the format of the pictures (implicit instructions), but were explicitly told to respond based on the content of the picture.

The stimuli consisted of pictures of either alcohol beverages or soft drinks (neutral visually similar stimuli) and they contained either a person holding the glass or opening the bottle or solely a beverage without any person in the picture. Person/ no person was counterbalanced of alcohol and soft drink stimuli. In this task the participants did not have to learn every picture separately, but there was a general explicitly given rule that determined whether the pictures should be pushed or pulled. In the alcohol training condition participants had to push alcoholic stimuli away, whereas in the control condition participants had to either push or pull pictures with a person in it. The AAT consisted of three parts, each part contained six blocks of sixteen trials. In each of the three parts different stimuli were presented.

Procedure

This study consisted of a questionnaire that participants filled in at home, one session in the lab and another questionnaire a week after the session in the lab. A summary of the procedure in chronological order can be found in Figure 2. The pretest contained questions regarding demographic data (age, gender and handedness), the AUDIT and the TLBF. The pretest was filled in one or two days prior to the session in the lab.

The session in the lab consisted of two computer tasks, the delay discounting task and a cue reactivity task, followed by 15 minutes of stimulation during which the participants did these tasks again and finally the EEG was recorded while participants performed a stimulus response learning task and an alcohol approach avoidance task (AAT). In the cue reactivity task participants were presented multiple pictures of alcoholic beverages, while their desire to drink alcohol was monitored before and after the series of pictures by means of the ACQ (Carter & Tiffany, 1999).

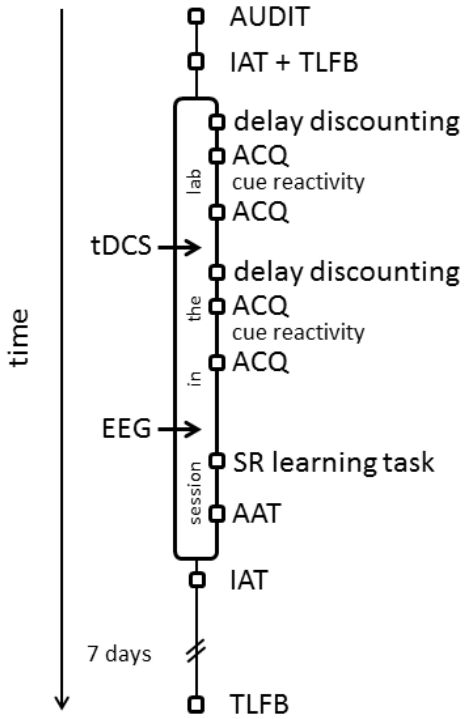


Figure 2. The chronological order of tests that were done in this study.

Next, the tDCS was applied for fifteen minutes. Half of the participants received real stimulation and the other half received sham stimulation. During the first seven to eight minutes participant did not have to do anything. During the last seven to eight minutes participants performed both the delay discounting and the cue reactivity task again. After the stimulation was finished EEG was recorded while they performed the stimulus response learning task and consecutively the alcohol AAT. Finally, a week after the session in the lab, participants filled in the TLFB again in order to examine the effects of tDCS and alcohol training on actual drinking behavior.

tDCS

For the transcranial direct current stimulation a NeuroConn DC-stimulator (neuroConn GmbH, Germany) was used with a maximum output of 4.5 mA. Two saline-soaked sponge electrodes were used with a surface of 35 cm² (Nitsche et al., 2008). In the real tDCS condition the stimulation was applied for 15 minutes at an intensity of 1mA, which has proven to be safe (Iyers et al., 2005), with a ramp of 8 seconds. In the sham condition the same procedure was performed, but the stimulation faded out after 30 seconds, which made it unable for participants to distinguish between real and sham stimulation (Boggio et al., 2008; Gandiga, Hummel & Cohen, 2006). Additionally, analyses of the side effects of the stimulation yielded no differences between sham stimulation and tDCS ($t(59) = .033$, $p = .463$). It can therefore be assumed that with these parameters, there is no difference in perception between sham stimulation and real tDCS. The anode was placed on the DLPFC and the cathode on the supraorbital area (Fregni et al., 2005; Ohn et al., 2008). For localization of the anode the position of the F3 (10-20 system) electrode was used as has proven to be a reliable method for localization of the DLPFC (Herwig et al., 2003).

EEG recordings

The EEG-data was recorded during with a BioSemi 32 electrode EEG cap. Only twelve electrodes of interest (Fpz, Fz, Cz, Pz, POz, Oz, F3, C3, P3, F4, C4 and P4) were used to record the signal. This was in order to reduce the time that was necessary to attach the EEG, because the after effects of tDCS decrease after stimulation offset (Ohn et al., 2008). The recordings started with all participants within 15 minutes after stimulation offset. The electrodes were placed according to the International 10/20 System (Jaspers, 1958). Electrode offsets relative to the grounds was kept below 20 mV. EOG was measured by bipolar leads above and below the eyes and on the outer canthi of the left and right eyes. Linked mastoids were used as reference. EEG was recorded for approximately 25 minutes with a sampling rate of 1024 Hz and stored on disk for offline processing.

Offline the EEG data were re-referenced to the linked mastoids, the data was filtered with a low cutoff of 0.1 Hz and a high cutoff of 70 Hz. The sampling rate was adjusted to 256 Hz and the data were segmented in epochs of -1000 ms to 3500 ms after the fixation

cross. Automatic artifact rejection was applied and bad segments were not included in further analyses. Gratton and Coles ocular correction was applied (Gratton, Coles & Donchin, 1983) with bipolar ocular channels and blink detection by algorithm. Finally, the data were baseline corrected over a period of 250 ms just before the start of the trial (fixation cross). For the ERP analyses the EEG was additionally filtered at 15 Hz, whereas for the oscillatory data the EEG was filtered at 40 Hz.

Analyses

To test whether tDCS enhances learning in the stimulus response learning task, an ANOVA was performed with tDCS condition as between subject factor, block (one to six) as within subject factor and accuracy as dependent variable. Post hoc tests of the contrasts of every block revealed in which block accuracy differed significantly between tDCS and sham stimulation.

For the EEG data the dependent variables were the peak amplitude of the N2 and P3 components. The peak latency was determined by visual inspection of the grand average. The between subject factor was tDCS or sham stimulation and the within subject factor was electrode site (Fz, Cz, Pz). To examine whether stimulation condition and/ or electrode site had an effect on the amplitude of the N2 and P3 components, a repeated measures MANOVA was performed. Furthermore the amplitude of the N2 and P3 component of accurate versus inaccurate responses were analysed using a repeated measures MANOVA. The N2 peak was defined as the peak between 140 and 160 ms after stimulus presentation and the P3 peak was defined as the peak between 350 and 450 ms after stimulus presentation. Peak latency was determined by visual inspection.

To examine the differences in oscillatory power between tDCS and sham stimulation continuous wavelet transform with complex Morlet wavelets (Morlet parameter c = 5) was performed on every trial, separately for each electrode and then averaged per condition (tDCS vs. sham). Next, in order to reduce the multiple comparison problem, the average power value was calculated over all data points in a specific time and frequency window. These average power values were tested with t-tests.

To test the second hypothesis a repeated measures ANOVA was done with stimulation condition as between subject factor, time (before and after stimulation) as within subject factor and craving score, delay costs for a day, delay costs for a month and delay costs for a year as dependent variables. An interaction effect of stimulation condition and time was expected. To determine the direction of the effects simple contrasts were examined. Furthermore to relate craving to the delay costs a Pearson correlation was calculated between on one hand the delay costs per day, month and year and on the other hand the craving scores before tDCS and before cue reactivity, before tDCS and after cue reactivity, after tDCS and before cue reactivity and after tDCS and after cue reactivity.

To examine the effects of alcohol avoidance training first the difference in consumed alcohol before and after the training (TLFB) was calculated for every participant. The post-test of the TLFB was filled in a week after the session in the lab. Therefore only the seven most recent days of the TLFB were taken into account. Next, an ANOVA was performed with training condition (alcohol avoidance and control condition) and stimulation condition (tDCS and sham) as between subject factor. A main effect was expected for training condition and additionally an interaction effect between training and stimulation condition was expected.

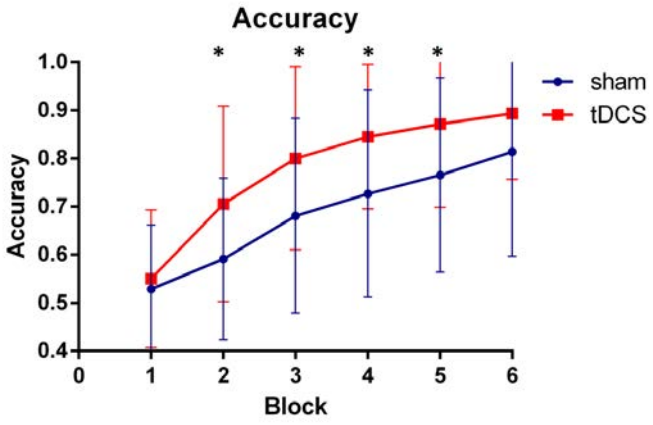
Finally, to reveal a possible mechanism underlying the alcohol training and examine facilitating effects of tDCS on the training, the amplitudes of the N2 and P3 components and oscillatory power spectra in response to alcohol stimuli versus neutral stimuli were tested similarly to the analyses of the stimulus response learning task. Significance levels in all tests were □=.05.

RESULTS

Stimulus Response Learning

Responses with a reaction time slower than five seconds (N = 19) were excluded from analysis. The repeated measures ANOVA revealed a significant main effect of tDCS on accuracy (F(1,59) = 6.92, p = .011). TDCS had a larger accuracy over all blocks (M = .778, sd = .026) than sham stimulation (M = .685, sd = .024). To investigate in which block the difference emerged, independent sample t-tests (two-tailed) were performed. The accuracy on the stimulus response learning task differed significantly between tDCS and sham in block two (t (59) = -2.42, p = .019), three (t (59) = -2.36, p = .022), four (t (59) = -2.45, p = .017) and five (t (59) = -2.19, p = .033). The accuracies of the tDCS group were higher compared to the sham group. Figure 3 displays the mean accuracies per block for tDCS and sham.

Figure 3. Accuracy on the AAT over blocks (1-6). In block two to five tDCS had a higher accuracy than sham stimulation. The error bars represent standard deviation.



To analyze the effects of tDCS on the N2 and P3 component during the learning task, a repeated measures MANOVA was performed. The data of three participants were not included in this analysis (N = 58). No main effects of tDCS were found (F(1,56) = .119, p>.5), nor interaction effects between tDCS and ERP component (F(1,56) = 2.72, p = .105). Furthermore, the difference in peak amplitude

between accurate and inaccurate responses was analyzed. A trend was found for accuracy ($F(1,56) = 3.22$, $p = .078$), indicating that the average peak amplitude of accurate trails was higher ($M = 2.36$, $sd = .565$) than those of inaccurate trials ($M = 1.10$, $sd = .798$).

To investigate the influence of tDCS on oscillatory power, the power spectra of tDCS and sham stimulation were calculated. These are depicted in Figure 4. An increase in beta power (12-25 Hz) on Fz and a decrease in slow delta power (1-4 Hz) were expected, reflecting a more excitatory state of the fronto-parietal network (Keeser et al., 2011). Contrary to the expectation an increase in delta power (3 Hz) was visible on Fz from 234 – 1000 ms. ($t(41) = -2.0866$, $p = .0216$) The average delta power for tDCS was higher ($M = 3.9950$, $sd = 3.1058$) than for sham stimulation ($M = 2.6093$, $sd = 1.7012$). Furthermore, on Fz tDCS elicited a higher ($M = 3.6900$, $sd = 2.5167$) power than sham stimulation ($M = 2.4202$, $sd = 1.2737$) in the theta band (6 Hz; $t(39) = -2.3985$, $p = .0106$). The small increase in theta was also present on Cz ($t(47) = -2.2609$, $p = .0142$). TDCS showed a higher ($M = 2.5527$, $sd = 1.5533$) theta power than sham stimulation did ($M = 1.7545$, $sd = 1.0741$). Moreover, an increase in upper alpha (11-13 Hz) was visible on Pz ($t(38) = -2.6999$, $p = .0051$) before stimulus presentation in tDCS condition ($M = 1.8385$, $sd = 1.1112$) compared to sham stimulation ($M = 1.2128$, $sd = 0.5371$). A small increase in lower alpha (8-9 Hz) was found just before and after stimulus presentation on Pz ($t(43) = -1.7364$, $p = .0448$). The average power of tDCS was 3.4846 µV ($sd = 3.2667$), contrary to the 2.2505 µV ($sd = 1.9292$) in the sham condition.

Although the data so far suggest that tDCS has little effect on oscillatory dynamics in the current task, the interaction between tDCS and accuracy resulted in interesting patterns. This implies that tDCS affects the neuronal activity of accurate and inaccurate trials differently. In Figure 5 these interaction effects are shown, with in the left column the differences between tDCS and sham during inaccurate trials and in the right column the differences between tDCS and sham during accurate trials. First, during accurate trials tDCS enhanced theta (5-6 Hz) between 484 and 734 ms ($t(54) = -1.744$, $p = .0433$). The average theta activity for tDCS was 3.244 µV ($sd = 1.660$), whereas sham stimulation exhibited 2.524 µV ($sd = 1.467$) in this time window. Also an increase in beta power (25-28 Hz) was visible on Fz from 630 to 650 ms ($t(42) = -1.615$, $p = .0568$) for tDCS ($M = 0.974$, $sd = .556$) compared to sham stimulation ($M = 0.781$, $sd = 0.314$). Next, a difference in frontal lower alpha [430 – 610 ms] was present ($t(40) = -2.069$, $p = .022$) between accurate trials with tDCS ($M = 0.271$, $sd = 7.993$) and inaccurate trials with tDCS ($M = -1.377$, $sd = 3.963$). Furthermore, a trend level posterior theta increase was visible between 500 and 812 ms ($t(53) = -1.250$, $p = .108$). TDCS showed a higher ($M = 3.552$, $sd = 1.877$) oscillatory power than sham stimulation did ($M = 2.973$, $sd = 1.627$).

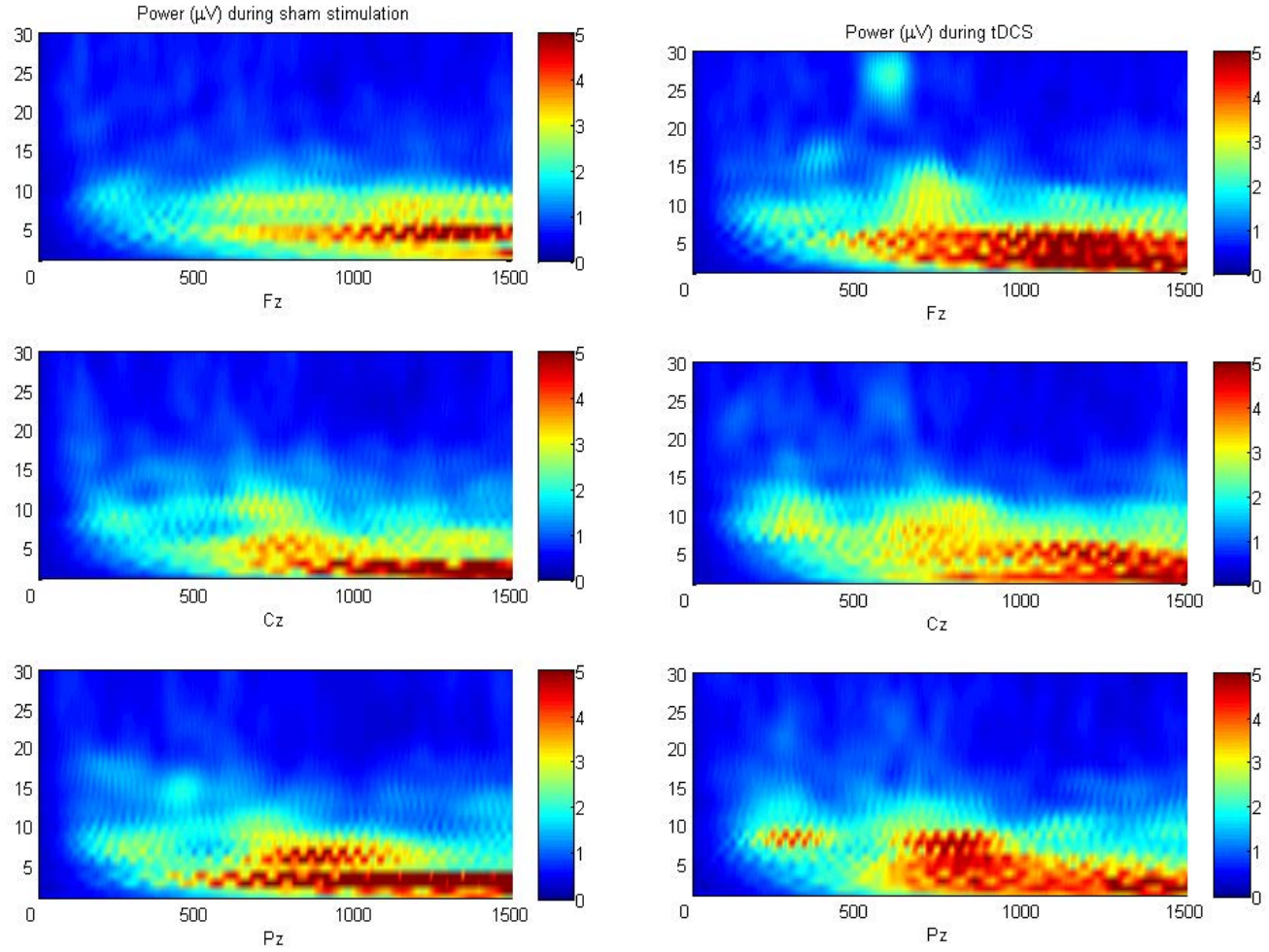


Figure 4. The power spectra (µV) of sham stimulation (left) and tDCS (right).

However, during inaccurate trials rather different patterns were visible. For example, the frontal theta increase was not visible (p = .275). Frontal upper alpha activity [970 – 1100 ms] during inaccurate trials was larger for tDCS (M = 2.770, sd = 2.534) than for sham stimulation (M = 1.936, sd = 1.855), although this difference was on trend level (t(49) = -1.420, p = .080). And frontal delta significantly increased between 390 and 630 ms (t(32) = -2.401, p = .0111) with tDCS (M = 4.182, sd = 3.983) compared to sham stimulation (M = 2.286, sd = 1.287). Finally, on Pz upper alpha [630 – 875 ms] was significantly increased (t(36) = -2.387, p = .0111). TDCS showed an average power of 3.699 µV (sd = 2.964) and sham only 2.248 µV (sd = 1.287). Moreover, accurate trials with tDCS showed a decrease in posterior delta power [400 – 805 ms] (M = -1.747, sd = 7.993) compared to inaccurate trials with tDCS (M = 0.941, sd = 7.272), although this difference was on trend level too (t(57) = 1.363, p = 0.890).

So differences between tDCS and sham stimulation were visible in the power plots of accurate and inaccurate trials, indicating that tDCS affected the neuronal activity that was present during accurate trials and inaccurate trials differently.

Figure 5. The interaction effect of accuracy: the difference in power (µV) between tDCS and sham stimulation in inaccurate trials (left) and accurate trials (right).

Craving and delay discounting

For these analyses the data of three participant were not taken into account, because the task was not completely finished (N = 1) or the data of the delay discounting task contained negative values (N = 2), which was a sign that the participants did not perform the task correctly (i.e. preferred a debt tomorrow over a gain today). The repeated measures ANOVA was done with stimulation condition (tDCS and sham stimulation) as between-subject factor and as within-subject factors craving before tDCS and before cue reactivity, craving before tDCS and after cue reactivity, craving after tDCS and before cue reactivity and craving after tDCS and after cue reactivity. The analysis yielded no significant effects of craving over time, although a trend was visible (F(3,54) = 2.54, p = .067). Further exploratory analyses, a paired t-test, revealed that this trend could be the result of a decrease in craving (t (57) = 2.46, p = .017) between the second (M = -.33, sd = .039) and the third (M = -.38, sd = .042) measurement, respectively before tDCS and after cue reactivity versus after tDCS and before cue reactivity. Moreover, no significant results were found for tDCS (p>.5) and for interaction between tDCS and time (p>.5).

The repeated measures ANOVA of delay costs revealed no significant main effects of tDCS (F(1,55) = 1.35, p = .25), nor an interaction effect of tDCS and time (p>.5). Finally, Pearson correlations (two-tailed) were calculated between craving and delay costs. Since no effects of craving were found, the average craving score was used to calculate the correlation. Outliers (N = 9) were defined as scores higher than two standard deviations above the mean delay costs per day, month and year and were excluded from analysis. Craving did not correlate significantly with the delay costs

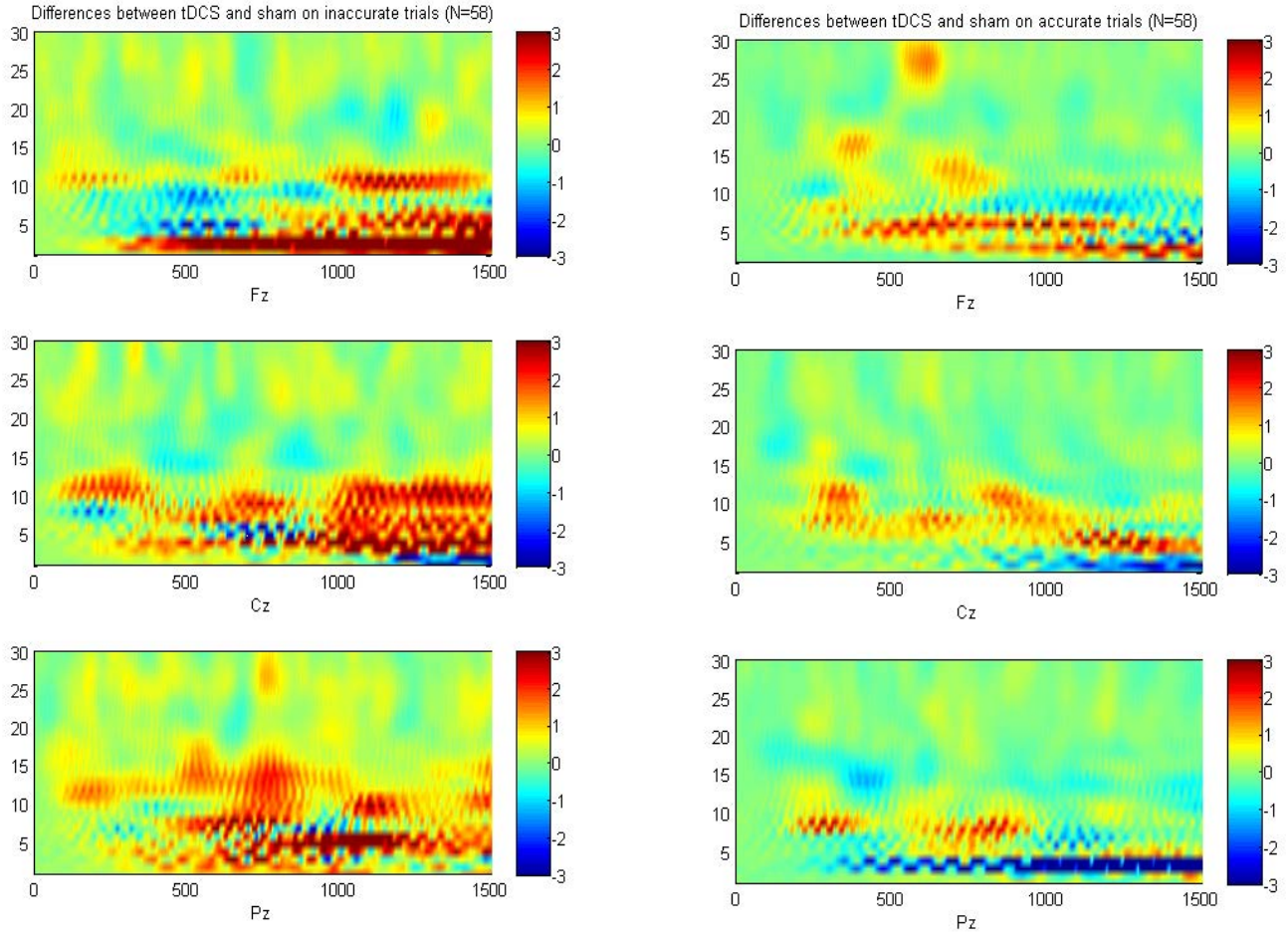


Figure 5. The interaction effect of accuracy: the difference in power (µV) between tDCS and sham stimulation in inaccurate trials (left) and accurate trials (right).

per day and month (p>.5) or per year (p = .31).

Alcohol Avoidance Training

To examine the presence of an initial bias towards pulling alcohol pictures, the IAT data of the pre-test were analyzes (N = 53). The average difference in response time between alcohol and approach words and alcohol and avoidance words did not significantly differ from zero (t(52) = -.838, p = .406), nor did the alcohol avoidance training group differ significantly from the control training group (t(51) = .101, p = .92). Remarkably, after the alcohol avoidance training, the IAT scores still did not differ significantly from zero (t(52) = -.941, p = .351). This could be an indication that the training might not have had the desired effect (Wiers et al., 2010).

Nevertheless, the effectiveness of the alcohol avoidance training was tested. Two outliers, defined as higher than two standard deviations above the mean TLFB score, were excluded from analyses (N = 51). The alcohol avoidance training did have an effect on trend level on drinking behavior (t (49) = 1.975, p = .053). The increase in alcohol consumption the week after the training is depicted in Figure 7. The group that received alcohol avoidance training has increased their alcohol consumption with 1.55 beverages (sd = 1.169), whereas the group that did not receive training increased their drinking behavior with 2.35 (sd = 1.670) alcoholic beverages the week after the training compared to the week before training. However, a smaller increase is no decrease. Additionally, no effect of tDCS on drinking behavior was found (t (-1.01), p = .319).

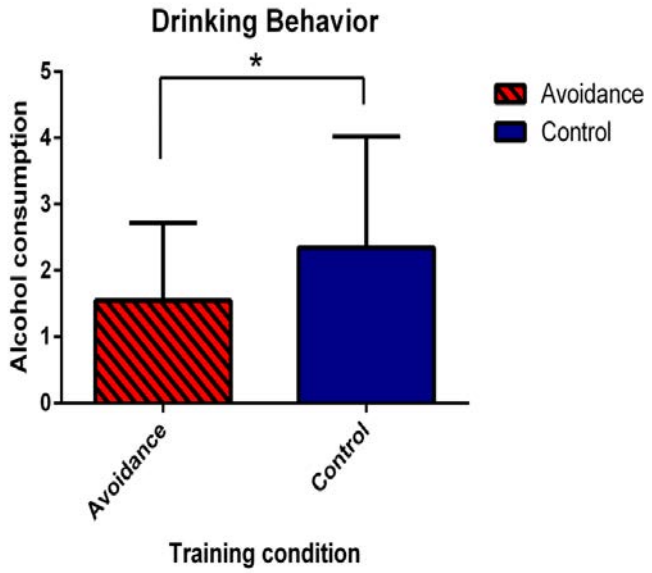


Figure 7. Alcohol consumption the week after training. The increase in alcohol consumption in the avoidance training condition is on trend level (p = .053) smaller than the increase in alcohol consumption in the control condition.

To uncover the neural mechanisms underlying the effect of the avoidance training EEG data was analyzed. For these analyses, the data of 58 participants were analyzed. Alcohol stimuli versus soft drinks was the within-subject factor, training condition and stimulation condition as between-subject factors and the dependent variables were peak amplitudes of the N2 and P3 component. Both alcohol avoidance training (F(2,53) = 0.968, p = .386) as tDCS (F(2,53) = 2.356, p = .105) did not result in differences in N2 and P3 peak amplitude on any of the electrodes. A main effect of alcohol stimuli on the amplitude of P3 component was found (F(1,54) = 8.508, p = .005). The P3 component elicited by alcoholic stimuli had a higher amplitude (M = 2.492 µV, sd = .678) than the P3 related to non-alcoholic stimuli (M = 1.582 µV, sd = .689). The N2 component did not show a significant difference in amplitude (F(1,54) = 1.774, p = .189). The ERPs of alcohol vs. non-alcohol stimuli on electrode Pz are shown in Figure 8. No interaction effect between alcohol and electrode was found (F(8,47) = 1.177, p = .333), so the ERPs of all three electrodes are comparable.

Additionally, analyze whether there is a difference between alcohol avoidance training and control training alcohol on the alcohol event related potentials could shed a light on the neurophysiological mechanisms underlying the beneficial effect of training on alcohol consumption. However, no interaction effect was found for alcohol avoidance training and drink type (F(2,53) = 2.599, p = .084), which implicates that the peak amplitudes of the N2 and P3 component in response to alcohol and soft drink stimuli were not affected differently in the alcohol avoidance training condition compared to the control training. Also no interaction was found for tDCS and drink type (F(2,53) = .778, p = .465).

The wavelet analyses resulted in the time-frequency representations as depicted in Figure 9. The left plot shows the power values elicited by soft drink stimuli, whereas the right plot shows the power values in response to alcohol stimuli. A decrease in alpha power for alcoholic beverages was expected (Lee et al., 2009) and higher power values on frontal sites (Kim et al., 2003). Higher power values for alcohol stimuli compared to soft drink stimuli were found frontally in the delta band from 757 – 1085 ms (t(82) = -1.753, p = .0415), theta band from 144 – 504 ms (t(83) = -1.833, p = .0351) and the upper alpha band 730 - 761 ms (t(70) = -2.577, p = .006). Alcohol stimuli elicited more power (M = 3.830 (3.087), M = 1.891 (1.499) and M = 2.001 (2.178), respectively) than soft drinks (M = 3.040 (1.498), M = 1.488 (0.740) and M = 1.222 (0.749), respectively).

A difference in alpha power (11-13 Hz) was found on Pz (t(68) = -2.1367, p = .0181), but contrary to the expectations alcohol stimuli resulted in an increase in alpha power (M = 2.0475, sd = 2.515) compared to soft drink stimuli (M = 1.307, sd = 0.7996).

Additionally, alcohol stimuli elicited higher power values (M = 1.4465, sd = 1.167) than soft drink stimuli (M = .9906, sd = .5780) in the beta band (12-15 Hz) on Cz (t(83) = -2.6662, p = .0046) from 738 – 839 ms. A decrease of power was found in the delta band (1-4 Hz) on Cz from 750-1000 ms (t(79) = 1.8398, p = .0348). Alcohol stimuli decreased the delta power (M = 2.7889, sd = 1.6799) in comparison to soft drink stimuli (M = 3.76, sd = 3.6895). Thus, in general alcohol cues were associated with a more active neuronal state, i.e. higher beta power and decreased delta power, than soft drink stimuli.

What is the role of alcohol avoidance training on the power spectra of alcohol and soft drink picture? If there are differences between

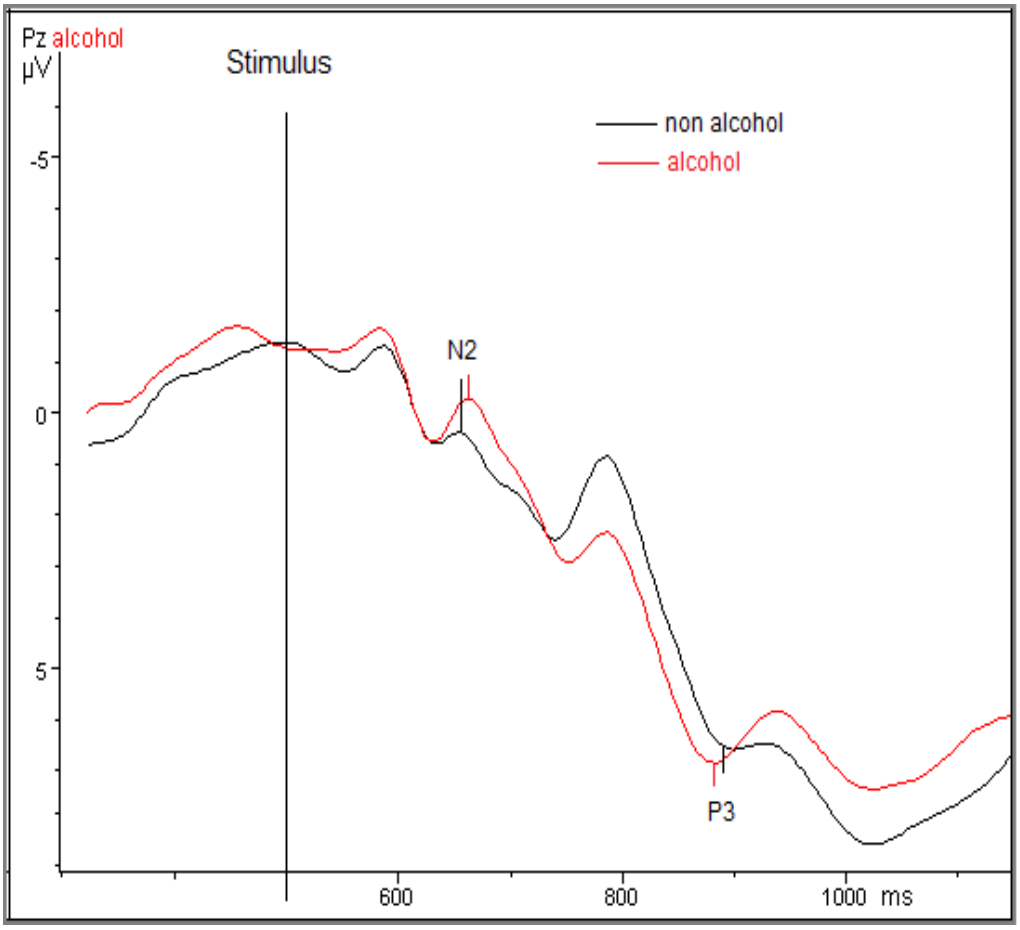


Figure 8. The difference in P3 amplitude between alcoholic stimuli and non-alcoholic stimuli on electrode Pz.

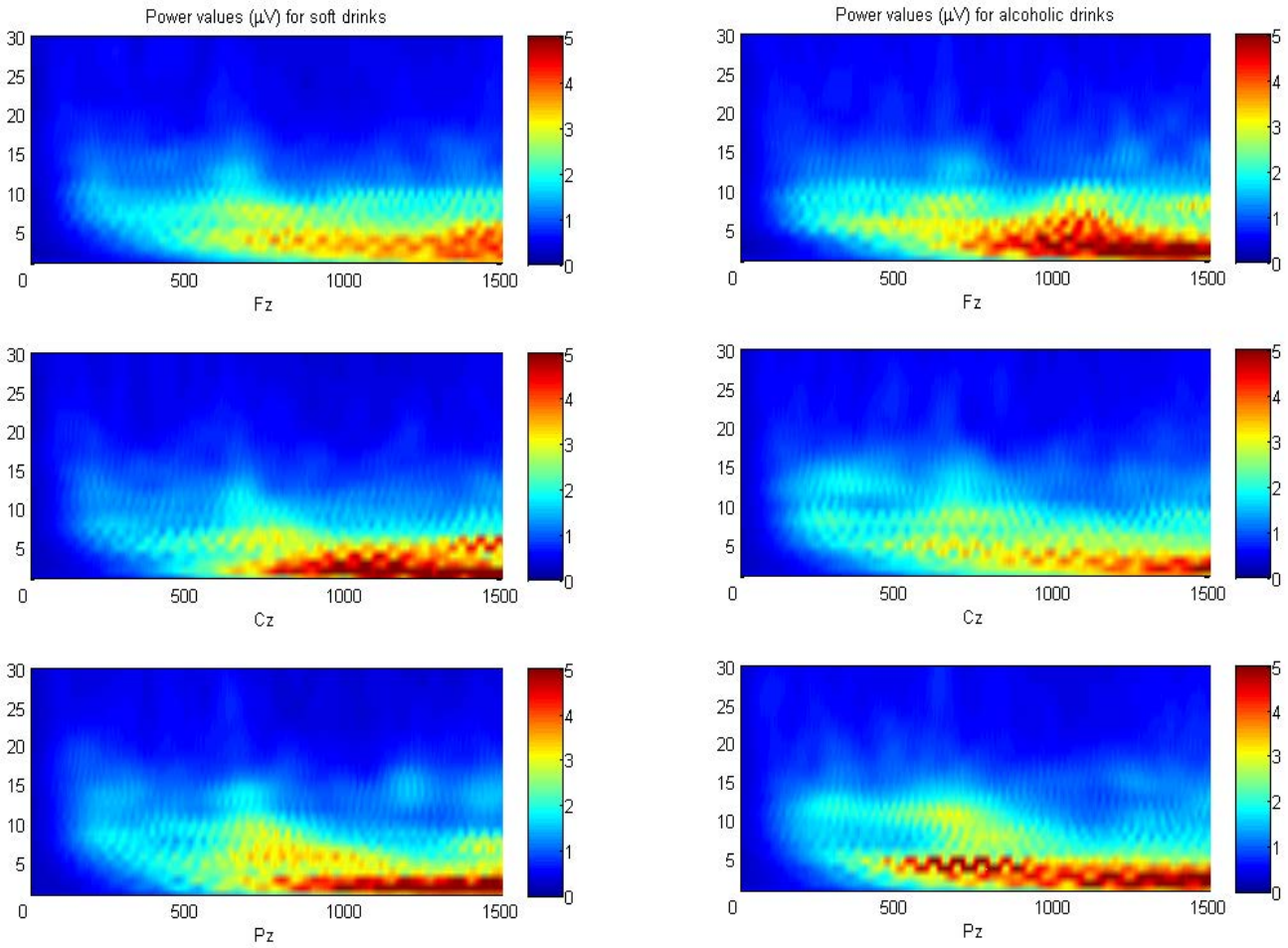


Figure 9. Power values (μV) for trails with soft drinks (left) and alcoholic stimuli (right).

alcohol avoidance training and control training in the power spectra of alcohol stimuli and soft drinks, this could reveal the neuronal mechanism underlying the effectiveness of the training. If the differences in power between alcohol and soft drinks diminish in the alcohol avoidance training condition, that would point to the possibility that the alcohol stimuli are processed as more neutral stimuli, i.e. lose their emotional significance and, hence, a cognitive bias is modified. The power plots (Figure 10) show the difference in power between soft drinks and alcoholic beverages in the control training (left) and the alcohol avoidance training condition (right). Because the stimulus was presented 500 ms after the cue, differences due to alcohol and soft drink stimuli could only have appeared after that. Therefore only differences after 500 ms will be discussed.

On Fz the power did not differ significantly between alcohol avoidance training and control training. However locations Cz and Pz yielded significant differences. A significant difference between alcohol avoidance training and control training could be seen in the delta band on Cz ($t(34) = 1.854$, $p = .0360$). A borderline significant decrease in delta power was visible in the power plot of the control training ($t(29) = -1.662$, $p = .0535$). Alcohol stimuli elicited less delta power ($M = 2.739$, $sd = 1.7189$) than non-alcoholic beverages did ($M = 5.38163$, $sd = 8.233$). This difference was absent in the avoidance training condition ($p = 0.165$).

Furthermore, on Pz in the control condition a significant increase in upper alpha (10-11 Hz) was visible from 500 ms to 625 ms ($t(32) = -1.776$, $p = .0424$). Alcohol trials had a higher power ($M = 2.7606$, $sd = 3.286$) than soft drink trials ($M = 1.600$, $sd = 1.065$). This difference between alcohol and neutral stimuli was not found in the alcohol avoidance condition ($p = .236$). However, later in time (926 – 1027 ms) alcohol stimuli ($M = 1.956$, $sd = 1.377$) elicited more alpha power than soft drink stimuli did ($M = 1.234$, $sd = 0.592$) in the alcohol avoidance training ($t(55) = -2.534$, $p = .0063$). This difference was absent in the control training ($p = .165$).

Finally, a difference in theta power between avoidance training and control training was visible on Pz from 400 – 625 ms ($t(31) = 2.0019$, $p = .0269$). In the control training alcohol stimuli elicited more theta power ($M = 2.7445$, $sd = 8.0267$) than in the alcohol avoidance condition ($M = -0.424$, $sd = 2.4774$). At 851 - 1093 ms this effect was reversed ($t(52) = -2.6422$, $p = .0054$). Alcohol stimuli in the control condition showed a decrease in theta power ($M = -1.7861$, $sd = 2.6604$), relative to soft drink stimuli ($M = 3.816$, $sd = 2.480$) and in comparison to alcohol avoidance training ($M = -0.084$, $sd = 2.2057$).

Figure 10 shows that there was a general power decreasing effect of alcohol avoidance training. This implies that the differences in power between alcoholic stimuli and soft drinks decreased in the alcohol avoidance condition. Next, what is the effect of tDCS on these differences? If tDCS would further decrease these differences, that could point to an amplified effect on training. However no additional effects of tDCS on the alcohol avoidance training condition were

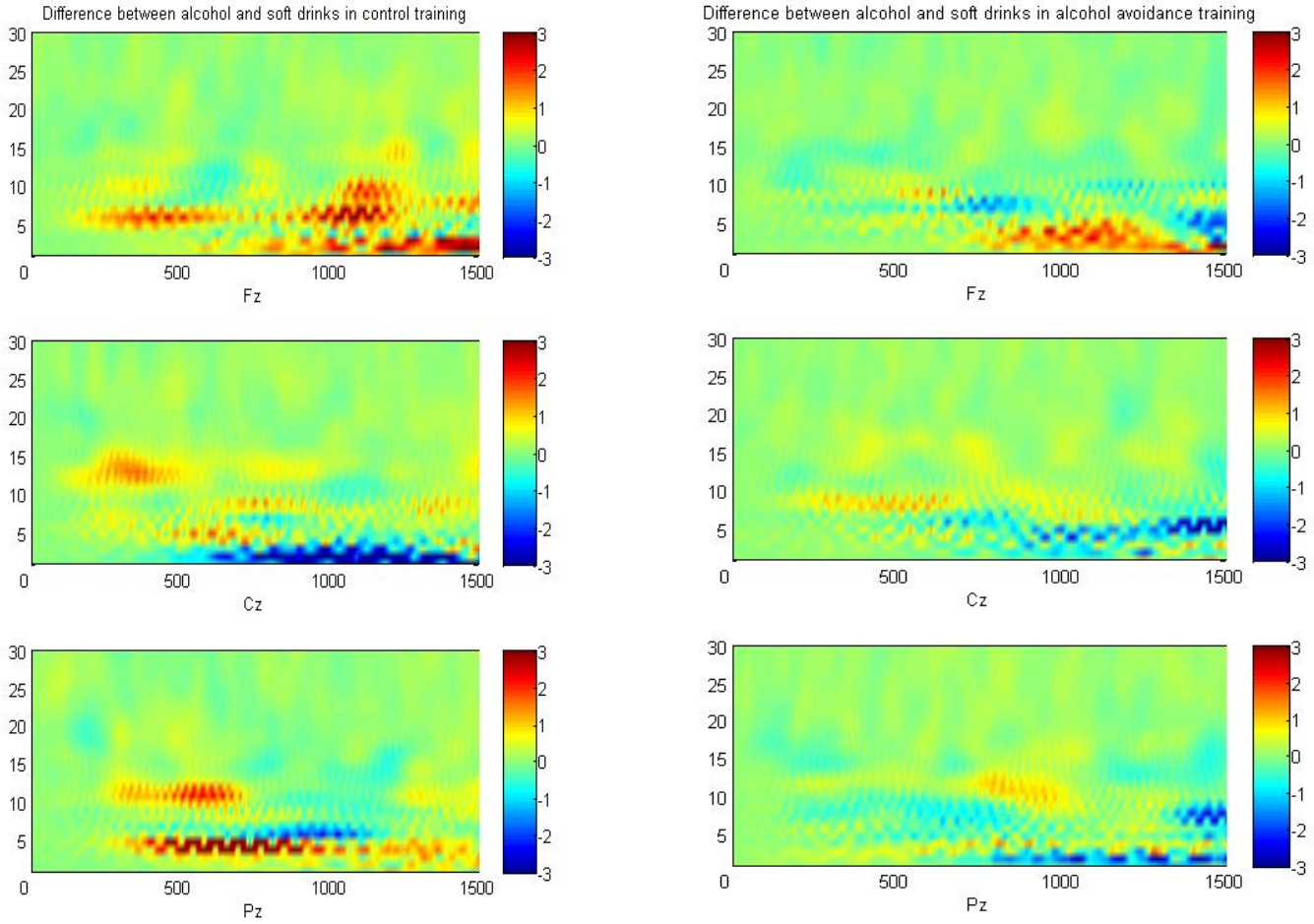


Figure 10. Difference in power (μV) between soft drink pictures and alcoholic beverages in the control training (left) and in the alcohol avoidance training condition (right).

found. This is consistent with the absence of an additional effect of tDCS on alcohol consumption.

Finally, from the IAT scores, it appeared that the training might not have had the expected effect on the approach bias. In order to examine what factors might have determined success of the training a linear regression was performed with alcohol dependency (AUDIT) , desire to drink (ACQ), tDCS condition and presence of an initial alcohol avoidance bias (IAT prior to training) as criteria. Results showed that AUDIT (p = .715), ACQ (p = .835) and tDCS condition (p = .157) did not predict successful training. However, initial presence of an alcohol avoidance bias did predict success of the training (t = -3.532, p = .002, □ = -4.62). In further exploratory analyses, the differences in power for successfully and not successfully trained participants were calculated. These differences might reveal neurophysiological components that predicted success of the training and are shown in Figure 11. A difference in upper alpha (11-14 Hz) power from 500 – 757 ms was found on Cz (t(18) = -2.2108, p = .020). Successful trainees had a higher average power in the upper alpha band (M = 1.7995 μV, sd = 0.9559) than unsuccessful trainees (M = 1.1832 μV, sd = 0.4062). Also a decrease in theta power (5-7 Hz; t(17) = 2.1475, p = .0235) was visible at the posterior site of the effective training group (M = 1.4167, sd = 0.5747), compared to the unsuccessful training group (M = 2.2388, sd = 1.2644).

Manipulation Checks

Unfortunately, it appeared that there were significantly less alcohol pictures in the AAT than pictures of soft drinks (F(1,54) = 160.44, p < .001) due to a technical error. The average percentage of alcohol stimuli was 42.8 per cent (sd = .006), whereas the remaining 57.2 per cent (sd = .006) were pictures of soft drinks. However, training condition and stimuli type did not interact (F(1,54) = .926, p = .34), so the amount of alcohol pictures was equal in the training condition and the control condition. Contrary, stimulation condition did interact with type of stimuli on trend level (F(1,54) = 3.77, p = .057). Participants in the sham condition had less alcohol pictures (M = .42, sd = .008) than the participants in the tDCS condition (M = .44, sd = .008).

To make sure the amount of pictures that participants pulled and pushed were equal between conditions another repeated measures ANOVA was performed, with pushed and pulled pictures as within subject factors and stimulation condition and training condition as between subject factors. A main effect or response (push or pull) was found (F(1,54) = 15.00, p < .001). Pull responses were made more often (M = .529, sd = .007) than push responses (M = .471, sd = .007). An interaction effect was found for training condition and response (F(1,54) = 21,35, p < .001). Participants in the training condition pulled significantly more pictures (M = .56, sd = .010) than did the participants in the control condition (M = .49, sd = .011).

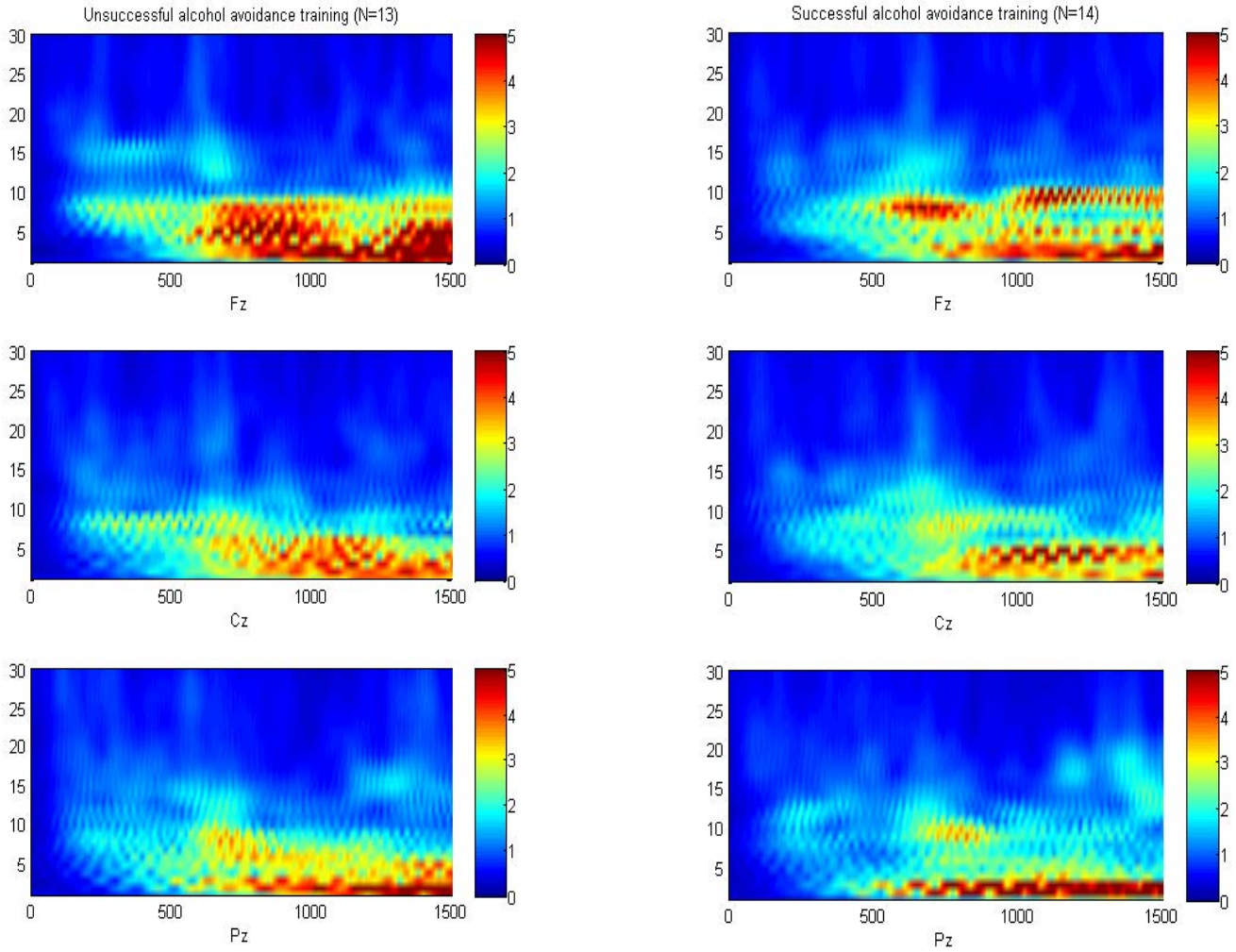


Figure 11. Power values (μV) for participants that were not successfully trained (left) and successfully trained to avoid alcohol (right).

This difference in response resulted from the fact that participants in the training condition always had to push alcohol pictures and since there were less alcohol pictures than soft drink pictures, the participants in the training condition pulled more pictures.

The consequences of this technical error ware that the conclusions with respect to the main effects of alcohol stimuli versus soft drink stimuli should be interpreted with caution, for those results could also have occurred as the result of the amount of a certain type stimulus. However, the conclusions regarding the effect of alcohol avoidance training remain intact, because both conditions (alcohol avoidance training and control training) were similar concerning the amount of alcohol and soft drink pictures.

DISCUSSION

In this study, the presumed beneficial effects of tDCS were further examined. First, it was shown that tDCS as opposed to sham stimulation enhanced accuracy in a stimulus response learning task. Both behavioral data and neurophysiological data were analyzed. An increase in accuracy was expected (Ohn et al., 2008) and found, but the neurophysiological results were ambiguous. Second, it was expected that tDCS reduced craving (Boggio et al., 2008) and if so, this might be explained by an imbalance between emotional and rational thinking in favor of more long-term thinking, which was measured by a delay discounting task. However, no effect of tDCS was found on either craving or delay discounting, nor was there a correlation between these two measures. Finally, an effect of alcohol avoidance training (Wiers et al., 2011) on subsequent drinking behavior was expected and found, although on trend level. Additionally, since tDCS was thought to enhance effects of training (Andrews et al., 2011), an amplified effects of tDCS on the training effect was expected, but not found. Furthermore, in search of a neurophysiological explanation for the training effect, ERP components and oscillatory dynamics were examined. However, again the neurophysiological results were equivocal.

Stimulus Response Learning

The finding that accuracy increases as a result of the after effects of tDCS confirms the results of Ohn and colleagues (2008). In the first block there was no difference in accuracy yet between the two stimulation conditions. The difference developed over the following blocks and disappeared again in the last block. This could have two causes. First, it is possible that tDCS has a ceiling effect on facilitating task performance. For example, Mulquiney et al (2011) found no effects of tDCS on accuracy during a Sternberg task. They explained this lack of effect with a behavioral ceiling-effect, namely that everyone performed well on the task, as a result of which no performance enhancing effects of tDCS were visible. This could also be the case in the last block of the stimulus response learning task. The participants with tDCS learned the stimulus-response associations faster, as was pointed out by the differences in accuracy during block two to five. However, both conditions ended the last block with an accuracy around eighty per cent. It is possible that tDCS had a positive influence on the acquisition of the stimulus response associations, but that the participants in the sham condition eventually reached a similar accuracy in the last block. Therefore this effect could disappear in the last block. More evidence that effectiveness of tDCS can reach a ceiling can be found in a study of Costa et al. (2012). They found in a color discrimination task that the adult visual cortex is adapted so well that the enhanced cortical excitability by tDCS has very little beneficial additive effect. This too is an indication that the subtle effects of tDCS are limited when the cortical effectiveness is high itself.

A second possible explanation is that the strength of the after effects of tDCS had decreased in the last block. This block took place approximately 20 minutes after stimulation offset, taken into account the time that was necessary for attaching the EEG electrodes (15 min) and the stimulus response learning task itself (6 minutes). Ohn et al. (2008) found after effects up until 30 minutes after stimulation offset, but they used 30 minutes of stimulation. However, in this study only 15 minutes of stimulation was used. Nitsche et al. (2007) found that the neuronal excitability decreases after stimulation offset. They measured excitability by the relative amplitude of the motor evoked potential (MEP). Directly after stimulation the amplitude of the MEP was 1.4 times as large as in the baseline period, whereas after twenty minutes the amplitude of the MEP had decreased to a factor 1.2 relative to the baseline. This decrease in after effect could also explain the lack of difference in the last block. Nevertheless, these results pointed out that tDCS has a facilitating effect on accuracy in a stimulus response learning task.

Since tDCS is thought to enhance cortical excitability (Nitsche et al., 2008), this should be visible in in the neuropsychological data. Enhanced excitability should lead to a larger P3 amplitude. This was also found by Keeser et al. (2011) on electrode Fz. However, the data of this study did not show this effect on P3 amplitude. What was found though was an increased amplitude for accurate trials versus inaccurate trials. With the P3 amplitude reflecting focal attention during stimulus detection (Polich, 2007), an increased P3 component could reflect an increased focal attention. Enhanced attention as a prerequisite for accurate responses could explain the association between enlarged P3 components and accurate responses.

Taken together, tDCS increased accuracy and accurate responses were associated with an enlarged P3 amplitude, but tDCS itself had no effect on the P3 amplitude. It is possible that tDCS had only partly an effect on accuracy, namely in the blocks two to five. For the analyses of the P3 amplitude the EEG data of all six blocks were used. It is possible that the blocks in which no effect of tDCS on accuracy was found cancel out an hypothetical effect of tDCS in the blocks where the difference appeared. If the responses are separated in accurate and inaccurate responses chances are still that a larger amount of accurate responses derive from the tDCS condition than from the sham condition. However, in this case the effect of accuracy on the P3 amplitude could be less disguised than when the effect of tDCS on the P3 amplitude. Therefore is it possible that no effect of tDCS on the P3 amplitude was found, while

this effect was found for accuracy.

Furthermore, in search of the neuronal mechanism underlying tDCS, time-frequency analyses were done to reveal induced neuronal activity. An increase in frontal beta and a decrease in frontal delta were expected (Keeser et all., 2011). This would reflect a more active neuronal state as the result of the enhanced excitability due to tDCS. However, both components were not found. This could be explained by the difference in design between this study and the study of Keeser et al. (2011). They recorded the EEG during resting state, while in this study EEG was recorded during a stimulus response learning task. It is possible that tDCS influences ongoing neuronal activation. If the ongoing neuronal activation during resting state differs from the neuronal activation during a learning task, it is plausible that the effects of tDCS on this neuronal activity are different too. This is in line with the results that were found, namely a small increase in frontal theta and a small increase in posterior lower alpha as the result of tDCS. Both frontal theta (Sauseng et al., 2002; Jensen & Tesche, 2002) and posterior lower alpha (Klimesch, 1997; Jensen et al., 2002) have an important role in working memory. The fact that tDCS enhanced the power in both frequency bands could point to a possible mechanism underlying the behavioral enhancement in accuracy as a result of tDCS. However, these effects remain rather small and are therefore not more than solely indications.

More interesting is the interaction between tDCS and accuracy. From these results it appeared that tDCS affected accurate and inaccurate trials differently than sham stimulation did. During accurate trials tDCS enhanced both frontal and posterior theta power, relative to sham stimulation. This pattern was not visible during inaccurate trials. Like aforementioned is frontal theta power an important component of working memory (Jensen & Tesche, 2002) and the by tDCS induced increase in frontal theta power could point towards a neurophysiological mechanism underlying the performance enhancing effect of tDCS. However this increase in theta was only present during accurate trials. This implicates that tDCS did not boost theta synchronization in general, but that the effectiveness of tDCS interacted with ongoing neuronal processes in such a way that it amplified the ongoing neuronal processes, rather than increased oscillatory power of frequencies that are related to working memory in general. According to Jensen and Tesche (2002) frontal theta power increases with working memory demand, but during inaccurate trials the stimulus might not be kept in memory and therefore coincide with a absence in theta power.

A similar conclusion can be drawn from the by tDCS induced posterior theta increase during accurate trials. Zaehle et al. (2011) found a pronounced increase in posterior theta as the result of tDCS during a working memory task and interpreted this theta activity as relevant for encoding and retrieval. Although the task that was used in this study was not a working memory task, but a stimulus response learning task, the same tDCS induced posterior theta enhancement was found. Since the stimulus response learning task in this study also recruited processes like encoding and retrieval, similar results could be expected. However, the increased posterior theta was only found during accurate trials, not during inaccurate trials. This could imply that during inaccurate trials tDCS did not enhance oscillatory components related to retrieval and encoding. Additionally, Zaehle et al. (2011) did not distinguish between accurate and inaccurate trials. Nevertheless are the results from this study and the results of Zaehle et al. (2011) compatible. For example, it could be the case that the accurate trials dominated in their study and that therefore the differential effects of tDCS were biased towards the effect of tDCS on accurate trials, namely an increase in posterior theta. The implication of this enhanced posterior theta is that tDCS relative to sham stimulation enhances the oscillatory activity that plays a role during encoding and retrieval and consequently may underlie the performance enhancing effects of tDCS on a behavioral level (Zaehle et al., 2011).

Together with theta activity upper alpha activity plays a role in memory tasks. Frontal theta is associated with maintenance of items in working memory (Jensen & Tesche, 2002), whereas posterior theta is merely related to encoding and retrieval (Zaehle et al., 2011). Together with posterior theta, upper alpha activity is also related to information retrieval from long term memory (Klimesch, Schack & Sauseng, 2005). However, theta triggers changes in power in the upper alpha band (Sauseng et al., 2002) and synchronizes activity during working memory tasks, whereas upper alpha desynchronizes during retrieval (Klimesch, Schack & Sauseng, 2005). As noted before, the stimulus response learning task that was used in this study recruits retrieval. Therefore a decrease in upper alpha was expected. Upper alpha activity in this study was also affected differently as a result of tDCS during accurate trials and during accurate trials. During accurate trials the upper alpha did not differ between tDCS and sham stimulation. However, during inaccurate trials, upper alpha increased both frontally and posteriorly. If upper alpha during the stimulus response learning task is expected to decrease (Klimesch, Schack & Sauseng, 2005), an increase in upper alpha might be viewed of as less functional activation for this particular task, since correct retrieval of stimuli is a prerequisite for an accurate response. Hence, dysfunctional activation can result in an inaccurate response. The increased upper alpha synchronization that was present in the tDCS condition on during inaccurate trials can be interpreted two ways.

First, an explanation for the increased upper alpha during inaccurate trials could be that tDCS not only amplifies functional activity and consequently leads to performance improvement, but also amplifies relatively dysfunctional ongoing activity that is present during inaccurate responses. No studies that examine the influence of tDCS on accuracy and on neurophysiological components have distinguished between accurate and inaccurate trials before, so it is a possibility that this result has not been found before. What has been found is that the effects of brain stimulation methods like TMS and tDCS are susceptible to the initial neuronal state that is present (Silvanto, Muggleton & Walsh, 2008). For example, the stimulation intensity of TMS that is required to induce phosphenes depends on the strength of occipital alpha power that is present

(Romei et al., 2008). Additionally, state dependency can be used to target specific neuronal populations (Silvanto, Muggleton & Walsh, 2008). By activation of neurons preceding stimulation, these neurons are specifically facilitated by TMS (Cattaneo & Silvanto, 2008). Analogously, if the initially present neuronal activity during tDCS was less functional with regard to the task, it is plausible that tDCS amplified this less functional activity too. In contrast, if tDCS enhanced neuronal memory components, like theta and upper alpha, and these neurophysiological alternations are related to an increased performance on the task, the amplified dysfunctional activity should also impact behavioral performance, but in a negative way.

The second explanation, more in line with other research regarding the beneficial effects of tDCS (Fregni et al., 2005; Nitsche et al., 2008; Ohn et al., 2008; Andrews et al., 2011) is that tDCS protects performance against dysfunctional activation. The results in Figure 7 showed that there was more upper alpha synchronization present during inaccurate trials in the tDCS condition than during inaccurate trials in the sham condition. Assuming that, like aforementioned, upper alpha synchronization impairs retrieval, it is possible that participants in the sham condition made a response error while exhibiting a certain amount of upper alpha synchronization. However, the difference in upper alpha synchronization between tDCS and sham could reflect the difference in dysfunctional activity that both conditions need in order to make an inaccurate response. From this point of view, participants in the tDCS condition only made an error when they had more upper alpha power, i.e. were less susceptible to dysfunctional activity. This could imply that tDCS protects behavioral performance against initially present less functional neuronal activity in a stimulus response learning task.

Finally, delta activity showed a similar interaction pattern between accuracy and tDCS. Delta power decreased posteriorly during accurate trials under influence of tDCS, but not under influence of sham stimulation. However, during inaccurate trials tDCS enhanced frontal delta power. In resting state EEG a frontal decrease of delta power as the result of tDCS was found by Keeser et al. (2011). They interpreted this decline in power as a more alert state of the fronto-parietal network. The decrease in delta during accurate trials indeed confirms that tDCS decreased delta power at a posterior location, which is part of the fronto-parietal network. Nevertheless, the decrease in delta power was only found for accurate trials under influence of tDCS. Inaccurate trials combined with tDCS resulted in an increase in frontal delta power, which would reflect according to Keeser et al. (2011) a less active neuronal state. If a more active neuronal state and a less active neuronal state can be viewed of as respectively more functional and less functional activity during a learning task, then the aforementioned two explanations apply here too. Either tDCS amplified the initially present dysfunctional increase in delta that leads to the inaccurate response or more delta power is necessary for participants to make an error.

However, Keeser et al. (2011) recorded the EEG during resting state, so whether decreased delta is functional for a learning task cannot be determined from their study. Nevertheless is there evidence that a more active fronto-parietal network is related to working memory tasks (Sauseng et al., 2005), that slow delta oscillations are functionally related to theta oscillations (Ba□ar et al., 2001) and indirectly to working memory tasks too (Sauseng et al., 2002). Thus, it is possible that the delta power decrease during accurate trials and the delta power increase during inaccurate trials were not solely the result of the enhanced cortical excitability due to tDCS, but also had a supporting function during the task.

In summary, tDCS seems to affects neuronal task relevant activation in a selective manner. The effects of tDCS on oscillatory patterns differed for accurate and inaccurate trials. During accurate trials tDCS was associated with an increases in frontal and posterior theta reflecting maintenance of items in working memory (Jensen & Tesche, 2002) and retrieval (Zaehle et al., 2011), respectively and with a decrease in delta power, reflecting either a more active neuronal state (Keeser et al., 2011) or functionally to working memory related oscillatory activity (Sauseng et al., 2002; Ba□ar et al., 2001). In contrast, during inaccurate trials tDCS showed an increase in frontal and posterior upper alpha power and an increase in frontal delta power. Given that upper alpha is thought to desynchronize during retrieval (Klimesch, Schack & Sauseng, 2005), the pronounced increase in upper alpha under tDCS could reflect an impaired retrieval under tDCS. The increase in frontal delta under influence of tDCS can be interpreted as a less active neuronal state (Keeser et al., 2011), which might result in inaccurate responses. The difference in activation that tDCS elicited in accurate and in inaccurate trials seems to point towards the view that tDCS influences ongoing neuronal activity, rather than increases oscillatory working memory components and thus performance in general, and that its effects differ depending on the neuronal state (Silvanto, Muggleton & Walsh, 2008). However, from the data of this study it cannot be determined whether tDCS amplifies initially present less functional activity that might lead to inaccurate responses, i.e. upper alpha and synchronization and increases in delta power, or that tDCS has a protective function against less functional neuronal activity, indicating that participants with tDCS require stronger dysfunctional activation patterns in order for their performance to decline.

Craving and delay discounting

In the second part of this study the effects of tDCS on craving and delay discounting were tested. Eight items of the Alcohol Craving Questionnaire (Singleton, Henningfield & Tiffany, 1994) were used that loaded on the factor desire to drink (Love, James & Willner, 1998). Previous studies have shown that tDCS on the DLPFC decreases craving for alcohol (Boggio et al., 2008) and cigarettes (Fregni et al., 2008a). However, no effect of tDCS was found in this study. This could first be due to the method of stimulation. Boggio et al. (2008) and Fregni et al. (2008a; 2008b) used bilateral stimulation on the left and the right DLFPC, contrary to the unilateral stimulation as was used in this study. According to Fregni et al. (2008b) the activity in the right DLFPC is crucial for reduction of craving for food

and risk taking, so cathodal stimulation on the right DLPFC could decrease craving. However, the exact mechanisms remain highly speculative (Fregni et al., 2008b). Since this region was unaffected by unilateral tDCS on the left DLFPC, it is plausible that no craving is reduced. Additionally, Boggio et al. (2008) used a higher intensity (2mA) and a longer stimulation time (20 minutes), contrary to 1mA for 15 minutes as was applied in the current study.

A second explanation for the absence of effect in this study could be that craving was not induced in the first place. The results of Boggio et al. (2008) show that craving is not decreased compared to baseline, but that an increase of cue induced craving is limited by tDCS. In order for tDCS to limit the ability to induce craving, a cue induced craving is essential. Boggio et al. (2008) have shown that pictures of alcoholic beverages effectively can do this. However, in the current study no difference in craving was found between the two measurements before tDCS. If craving was induced by the alcoholic beverages, a difference in craving would have been found between the first measurement (before cue reactivity and before tDCS) and the second measurement (after cue reactivity and before tDCS). This was not the case. According to Boggio et al. (2008) the enhanced activation in the DLPFC interferes with the reward pathways activated by craving, through a decrease of the signal-to-noise-ratio of the craving signal as a result of which drug-seeking processes are distorted. Logically following, if no drug seeking processes are induced, no reward pathway signal can be distorted, hence no craving can be reduced.

Next, the effect of tDCS on long term thinking, as measured by a delay discounting task, was examined. The DLFPC is involved in decision making and processes future consequences and reward contingencies (Kalenscher & Pennartz, 2008) and DC stimulation of the DLPFC decreases risk taking behavior in a BART task (Fecteau et al., 2007a), so a shift towards more long term thinking as the result of tDCS was expected. However, results showed no difference in delay costs between tDCS and sham stimulation. It is plausible that, as with craving, the parameters of stimulation cause the absence of effect. Fecteau et al. (2007b) found that only bilateral stimulation with the anode on the right DLPFC decreases risk taking decision making. They postulated that the decrease in risk taking decision making was the result of an altered interhemispheric balance. Other evidence regarding lateralization of risk related behaviors comes from Fregni et al. (2008b), who found risk aversive behavior after stimulation of the right DLPFC. If the right DLPFC (Fregni et al., 2008b) and the interhemispheric balance (Fecteau et al., 2007b) are important for risk taking decision making, this might explain why unilateral left DLFPC stimulation does not reduce delay costs as found in this study.

Finally, an association between craving and long term thinking was expected for several reasons. First, the neuronal circuits that are related to reward processing are altered in alcohol addicts (Robinson & Berridge, 2003) and the delay discounting task reflects the processing of reward contingencies (Kalenscher & Pennartz, 2008). Therefore the delay discounting task could reveal some craving related alterations. Second, alcohol addiction results from an imbalance between reflective and impulsive processes (Wiers et al., 2007), craving is viewed of as one of the automatic impulsive processes that lead to alcohol related behavior (Robinson & Berridge, 2003) and the delay discounting task depends on rational, reflective systems (Kalenscher & Pennartz, 2008). Thus, the size of the delay cost (the amount of money necessary to wait a certain amount of time) could be increased with the amount of craving that is present. Evidence for this line of thought comes from Field et al. (2007), who found that heavy drinkers showed a more pronounced discount of delayed rewards. They interpreted the increased delay costs as an indicative of short-term thinking. The pronounced discounting in heavy drinkers was correlated with alcohol craving, alcohol consumption and the presence of an attentional bias for alcohol related stimuli (Field et al., 2007).

However, in this study no such correlation was found between craving and pronounced discounting. The initial absence of cue induced craving and the tDCS parameters that were not optimal to target risk taking behavior might have contributed to this expected but not found result.

Alcohol avoidance training

In the last part of this study the effects of alcohol avoidance training on drinking behavior and neurophysiological components were examined. Alcohol avoidance training was expected to decrease alcohol consumption, which is mediated by an altered approach bias towards alcohol-related stimuli (Wiers et al., 2010). The results of the TLFB showed that the alcohol avoidance training group consumed less alcoholic beverages in the week after the training than the control training group did. However, both groups increased their alcohol consumption relative to the week before the training and the effect was only minor. Nevertheless, alcohol avoidance training did have an effect on alcohol consumption. This is in line with results from Wiers et al. (2010), who found a decrease in beer consumption during a taste test after alcohol avoidance training.

In order to reveal the neuronal mechanisms underlying the alcohol avoidance training EEG was analyzed. The amplitude of the P3 component in response to alcohol stimuli was larger than for soft drinks. This confirms the results found by Herrmann et al (2001). Since the P3 component is thought to reflect focal attentional during stimulus detection (Polich, 2007) an enhanced P3 component in response to alcohol pictures could point towards the presence of an attentional bias for alcohol stimuli. If the alcohol avoidance training would have an effect on the amplitude of the P3 component, this could indicate neuronal mechanism underlying the training. As for example was the case in the study of Eldar and Bar-Haim (2010). They retrained anxiety patients to divert their attention away from threat. As a result of the training the amplitude of the P2 and P3 components decreased, reflecting a successful attentional retraining. However, alcohol avoidance training in the current study did not show such a decreased P3 amplitude in response to alcohol stimuli. This could have two causes. First, the attentional retraining was only effective in anxiety patients contrary to not anxious control

participant (Eldar & Bar-Haim, 2010). Analogously, the alcohol avoidance training might only affect heavy drinking participants. Still, the majority of participant (N = 47) in this study was classified as heavy drinking, so that could not explain the effects. The second explanation is that Eldar and Bar-Haim (2010) retrained attentional bias, whereas the current study retrained the behavioral approach bias. Although a pronounced alcohol related P3 component was present in the data, the cognitive bias training targeted automatic action tendencies. The attention related P3 component was not affected by approach bias modification. This indicates that these biases are represented differently in the neuronal signal, or at least that the approach bias is not represented by the P3 component contrary to the attentional bias.

Next, the oscillatory patterns in response to alcohol and alcohol avoidance training were analyzed. According to Kim et al. (2003) alcohol stimuli elicit a more power frontally in general, reflecting a cue reactive state of the brain. Additionally, a decrease in alpha power could be found, representing an increased craving (Liu et al., 1998; Lee et al., 2009). Indeed, alcohol stimuli elicited more delta power, more theta power and more upper alpha power than soft drink stimuli did. Furthermore, on Cz a decrease in delta and an increase in beta were seen. This specific combination of oscillatory components was also found by Keeser et al. (2011). Although they did not measure EEG activity in relation to alcohol, their interpretation could be relevant for these results. They interpreted the decrease in slow wave oscillations (delta) and the increase in relatively fast oscillations (beta) as a more active neuronal state. In their study this more active neuronal state was the results of tDCS on resting state EEG (Keeser et al., 2011), but in the context of alcohol stimuli this pattern could also represent a more active neuronal state. Moreover, this is consist with the frontally increased neuronal responsiveness to alcohol-related stimuli (Kim et al., 2003). Thus, alcohol stimuli seem to elicit an increased neuronal activation compared to soft drink stimuli.

Now what happens during the alcohol avoidance training with the oscillatory reactivity to alcohol stimuli? If the difference in power between alcohol and soft drink stimuli would decrease as a result of alcohol avoidance training, that would point to a possible neuronal mechanism underlying the effectiveness of the alcohol avoidance training. This way, alcohol stimuli would elicit neuronal activity that is similar to neutral soft drink stimuli, which would reflect modification in how alcohol stimuli are processed. In the control training several oscillatory components were found in response to alcohol stimuli, among which less delta power on Cz, an increase in upper alpha and an increase followed by a decrease in theta on Pz. In contrast, all these oscillatory components were not present in the data of the alcohol avoidance training group. As pointed out before this could be an indication that alcohol avoidance training alters the neuronal activation that is associated with the processing of alcohol stimuli, in such a way that initially more responsive activation towards alcohol is decreased and consequently alcohol pictures are processed similarly to soft drink pictures. In terms of cognitive biases, these results indicate that the alcohol avoidance training not only decreases subsequent alcohol consumption (Wiers et al., 2010) by modifying the alcohol approach bias (Wiers et al., 2011), but that modification of this bias is established or at least visible on a level of neuronal activity.

Finally, although Wiers et al. (2009) found in a similar task that heavy drinkers showed an approach bias and that this bias could be altered by alcohol approach or avoidance training, Wiers et al. (2010) also found that alcohol avoidance or approach training is not effective for everyone. Approximately half of the participants is susceptible to the alcohol training (Wiers et al., 2010). They found that urge to drink beer before the training negatively predicted the outcome of the training. Alcohol consumption was not related to success of the training. Because the effect of the alcohol avoidance training in the current study was relatively small and did not reflect a decrease but only a smaller increase in alcohol consumption relative to the control training group, success of the training was examined. Similar to the results of Wiers et al. (2010) only approximately half of the participants in the training condition were trained successfully. Contrary to the results of Wiers et al. (2010) success was not related to desire to drink alcohol prior to the training. Also alcohol dependence, measured by the AUDIT, and tDCS condition did not predict a successful alcohol avoidance training. The oscillatory patterns of successfully and unsuccessfully trained differed from each other. Effective training was associated with an increase in upper alpha was found on Cz and a decrease in theta on Pz. This specific pattern of activation is related to working memory (Klimesch, Schack & Sauseng, 2005), although reversed in direction. Theta increases reflect maintenance of items in memory (Jensen & Tesche, 2002), whereas upper alpha desynchronization reflects retrieval from long term memory (Klimesch, Schak & Sauseng, 2005). The data suggested that successful training requires less theta and more upper alpha can be exhibited. So generalized that would indicate that less working memory was recruited for execution of the task. If so, it is plausible that the task is performed in an automatic manner, suggesting that the automatic contingencies between alcohol stimuli and the push movement were already established or present in these participants. If so, that should be visible in the IAT scores prior to the training, which indeed did predict success of the training. This finding would suggest that participant that already have an alcohol avoidance bias prior to the training are more successfully trained to avoid alcohol than participants that have an approach bias towards alcohol.

However, these oscillatory patterns could also be interpreted differently, namely these components (decreased theta and increased upper alpha) were also found in the data of the control group as reflecting the difference in activation between alcohol and soft drink stimuli. However if these components are related to the neuronal response to alcohol stimuli compared to soft drink stimuli, it would be expected that the unsuccessfully trained group would show this pattern of activation, since the response to alcohol stimuli might not be trained in both the control group and the unsuccessful group. Both ways, the factors and oscillatory components that predict or determine success of the training should be studied more

extensively in order to improve the effectiveness of the alcohol avoidance training.

Interventions and Future Research

This study has shown that anodal DLPFC stimulation increased accuracy on a stimulus response learning task. TDCS seemed to have a different effect on accurate and inaccurate trials. However, whether this was because tDCS amplified any ongoing neuronal activation and thus also less functional activation or that tDCS served a protective function against less functional neuronal activity could not be determined from this data and should be studied further. Furthermore, the results of this study have shown that alcohol avoidance training can affect alcohol consumption. Alcohol avoidance training did not modulate the attentional P3 component, but it affected the neuronal response to alcohol stimuli. The data suggested that during alcohol avoidance training the enhanced neuronal reactivity to alcohol stimuli was decreased towards a more neutral neuronal activation, similar to the activation elicited by neutral soft drink stimuli. However, it remains unclear what exactly the oscillatory components reflect in response to alcohol stimuli. This should also be studied more extensively in future research.

What are the application of these results with respect to alcohol addiction research and treatment? Because tDCS enhanced stimulus response learning, it could improve the effectiveness of the alcohol avoidance training. Although in this study no effect of tDCS on alcohol avoidance training was found due to probably the diminished after effects during the alcohol avoidance training, concurrent tDCS with the alcohol avoidance training should improve the training effects. Since retraining approach biases has beneficial effects for treatment outcome in alcoholic patients (Wiers et al., 2011), tDCS could enhance these positive treatment effects and consequently assist alcoholic patients to prevent them from relapse.

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An EEG study into mechanisms of cognitive control: Visual entrainment of alpha and theta oscillations

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ABSTRACT

Cognitive control supports the capacity for adaptive and flexible behavior during circumstances in which conflict arises during information or response processing. This conflict is reliably captured within speeded response tasks such as the Eriksen flanker paradigm. In this study we investigated the notion that cognitive control is embedded within a pre-frontal network of regions supported by oscillatory activity in the theta frequency (4-8Hz) activity. We also considered the role of alpha (8-12Hz) activity over posterior regions, previously associated with suppression of task-irrelevant stimuli and considered an outcome of top-down control processes. We used visual flicker at 6 Hz and 10 Hz to investigate whether entrainment could propagate from visual regions to frontal structures involved in cognitive control, and in turn modulate these two systems. We report evidence of entrainment within occipital regions, as well as the behavioral impact of visual flicker on task performance.

INTRODUCTION

Effective processing of information is fundamental to cognition, supporting adaptive and flexible behaviour across several contexts. Although several factors contribute to the efficiency of information processing, the monitoring, detection and resolution of so-called conflict is particularly important (Botvinick et al., 2001; Botvinick, Cohen, & Carter, 2004). In this context, conflict during the processing of information can arise when two or more sources of information compete, or interfere, with one another to influence the outcome of a response or selection (van Veen & Carter, 2006). Upon detection, conflict resolution may be taken to refer to reactive processes seen to occur as executive functions attempt to adapt to, and overcome, the presence of conflict. A good example of such a reactive process is represented in neurophysiological suppression of task irrelevant sensory information, while another is slowing of responses to facilitate accurate processing of conflicting information. It is important to note that conflict can occur during processing at the perceptual, response, or semantic level. Accordingly, cognitive control systems operate at on perceptual, motor, and cognitive levels. For the remainder of this paper, the term cognitive control is taken to refer to a set of processes contributing toward the capacity of executive systems to monitor for, detect, and respond appropriately to the presence of conflict.

Convergent neurophysiological evidence suggests that cognitive control is attributed to neural activity spread over subdivisions of the prefrontal cortex. (Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004; Ridderinkhof, Forstmann, Wylie, Burle, & van den Wildenberg, in press). In this regard, the medial prefrontal cortex (mPFC), including the anterior cingulate cortex (ACC), and the lateral prefrontal cortex (lPFC) have distinct roles within a cognitive control network (Ridderinkhof, Wildenberg, Segalowitz, & Carter 2004). Accounts of MFC functioning highlight its role in coordinating processing of task-relevant information, and maintaining vigilance for the occurrence of conflict (Botvinick, Cohen, & Carter, 2004). In part this function is maintained by signaling the presence of conflict and alerting subsequent components of the cognitive control network to the need for adaptive alteration to either processing itself, or behavioural responses, through, for instance, motor control. Further,

oscillatory activity between the lPFC and mPFC provides plausible basis for communication necessary for the implementation of adaptive changes (Cavanagh, Cohen & Allen, 2009). Neuroimaging and electrophysiological studies provide a great deal of support for the cognitive control network, often adopting paradigms that purposefully present individuals with one or more sources of conflict.

This study aims to demonstrate the neurophysiological and behavioural correlates of cognitive control expressed in performance during a conflict-inducing paradigm. Neurophysiological support for the conflict control network will be highlighted by showing how oscillatory dynamics of multiple frontal mechanisms are modulated by detection of conflict and errors. We then intend to relate these electrophysiological mechanisms to their down-stream outcomes, specifically motor-related adaptations geared at altering behaviour in response to conflict.

Cognitive control literature

A number of processes are engaged in order to preside over task-related information processing: these monitor for, detect, and respond appropriately to the presence of conflict by exerting control over response selection mechanisms. Collectively these processes may be grouped under the term cognitive control processes, and are thought to form an embedded network within frontal structures. Cognitive neuroscience research into cognitive control is a broad field; but to provide an appropriate context for this study, we can review key findings across three lines of research: firstly, event-related-potential (ERP) studies into error detection (Gehring, Goss, Coles, Meyer, & Donchin, 1993; Luu & Tucker, 2001); second, neuroimaging and behavioral findings from interference paradigms provide an anatomical perspective to accounts of the cognitive control network (Kerns et al., 2004). Lastly recent work into how cognitive control has been characterised in terms of neural oscillatory dynamics that accompany it. The latter case provides the foundations for the present study. Nonetheless, the convergent nature of these findings should be taken into account.

On speeded-response tasks such as the Stroop or Eriksen flanker paradigms, subjects balance competing demands of speed and accuracy under conditions in which stimulus or response properties induce cognitive conflict. Successful performance therefore relies upon activation of cognitive control processes that optimise information processing (and behaviour) related to the particular objective or goal. For this reason these tasks are used to investigate cognitive control and its neural underpinnings. Several neural and behavioural correlates of the activation of the cognitive control network have since been determined (Kerns et al., 2004; Cavanagh, Cohen & Allen, 2009).

Consider the flanker task, in which participants must identify and respond to a central target embedded within a row of distracting items (which may be the same as, or different from, the target; therefore either facilitating the task, or inducing conflict on a perceptual level). On incongruent trials, those containing distractors that are different from the target and thus create conflict, subjects' responses are typically delayed following adaptive modification to motor processing. By slowing responses, more time is granted for information processing, assuring a greater likelihood that conflict is taken into account and a correct response follows. Similarly, on trials following commission of errors, responses are typically slower also. Both post-error slowing, and delayed responses to the presence of conflict, are considered the result of coordinated changes made to a response-related network subsequent to the engagement of cognitive control systems. Both conflict and errors have been shown to elicit the error-related negativity (ERN) – an average of potentials occurring ~50ms to 150ms post response (Nigburg et al., 2010, Luu & Tucker, 2001). These studies have reported the source of the ERN to be within medial frontal regions, and that it arises from ongoing oscillatory activity of the MFC. The ERN is therefore often used an electrophysiological marker for the activation of the cognitive control network; while its origins hint at shared MFC-based neural circuitry for conflict and error detection.

Although providing a coarse neural marker for engagement of cognitive control the ERN does not fully capture the extent to which several frontal structures contribute toward the neural activity underlying control functions. Neuroimaging and electrophysiological studies reveal that successful control stems from coordinated activity of multiple prefrontal structures, including the MFC (Ridderinkhof et al., 2004; 2010), the lateral PFC (Cavanagh, Cohen & Allen, 2009) and the ACC (Kerns et al., 2004).

The series of control operations carried out by the control network are arguably rooted within ACC activity – which is increased upon detection of errors and during high conflict conditions, serving to alert the system as a whole to resolve conflict (Carter & van Veen, 2007). For instance, ACC activity on incongruent trials of a Flanker task is greater when the previous trial was congruent, than when it was incongruent. In this situation, interference is reduced on a trial (n) if it occurs after an incongruent trial (n-1); since trial n-1 engaged cognitive control systems, the impact of conflict can be attenuated through prepatory response systems (Gratton et al., 1992). This suggests ACC to contribute to performance monitoring through active conflict detection in a reactionary capacity, since it responds to rapid and ongoing changes in information (Carter at al., 2008).

Correlations made between ACC activity on conflict/error trials, and PFC activity on subsequent trials, are suggestive these two components interact within a conflict-control loop (Kerns et al., 2004). After being signaled by the ACC, information processing may subsequently be enhanced or suppressed in accordance with task directives and previous behavioural outcomes. This top-down control is exercised through the PFC, which may re-orient processing of posterior sensory information in favour of appropriate responses. Electrophysiological studies provide evidence that adaptive changes of this sort are supported by interactions between the lateral PFC and medial PFC; providing a neural basis of an action-monitoring system (Cavanagh, Cohen & Allen, 2009). These findings have contributed to the strong notion that MFC (in particular ACC) is responsible for conflict monitoring, registering commission of errors, and signaling to the lateral prefrontal cortex (a secondary

control system) to exert higher cognitive control to resolve conflict (Botvinick et al., 2001; van Veen & Carter, 2006).

Broadly speaking, it is widely agreed upon that prefrontal structures rarely perform a singular function. Instead, many are multi-modal in that they perform a functional role across a range of executive processes. The PFC for instance is implicated in many forms of higher-order cognition, including working memory (for a review see, Miller & Cohen, 2001), and its activation across a range of tasks makes attributing a singular function to the PFC difficult. What seems consistent is the notion that complex executive systems and functions emerge through distributed patterns of neural (oscillatory) activity. In this manner "cognitive control functions [are not] supported by specific dedicated systems or neural circuits; they are better conceived of as emergent properties, being established by the configuration and tailoring of existing subordinate processes in such a fashion that 'new', unique functions emerge" (Ridderinkhof et al., 2010).

Neuronal oscillations

Oscillations can be described across two parameters: amplitude and phase. Amplitude (or power) of an oscillation at the site of an electrode corresponds to a measure of synchrony between local (cortical pyramidal) neuronal assemblies; the same is true for larger neural regions when applied across a spread of electrodes (Nunez, 2000). Phase coherence across electrodes is taken as a measure of synchrony between distributed neuronal assemblies (Fries, 2005). In this manner, oscillatory activity reflects rhythmic changes in excitability of neuronal populations across a variety of both spatial and temporal scales (Buzsáki, 2006).

Neuronal oscillations also hold a functional significance for neural systems. Synchronised neuronal oscillations (or neuronal coherence) between groups or subsets of neurons renders communication more effective, arguably allowing for large-scale integration across multiple processing pathways and neural systems within the brain (Womelsdorf & Fries, 2006). Convergent evidence from multiple cognitive domains, including memory, attention, perception and decision-making support this notion (Engel, Fries & Singer, 2001; Fries, 2005; Ward, 2003). For instance, sensory-motor integration, an operation requiring considerable coordination between independent streams of information, is associated with synchronisation within frontal and parietal cortices (Caplan et al., 2003). Within this field of research, distinct frequency bands of neuronal coherence have been attributed distinct cognitive and physical properties. For instance, neuronal coherence within the gamma frequency range (30-80 Hz) is thought to be responsible for visual binding of information (Muller et al., 1997), as well as support attention (see Engel, Fries & Singer 2001 for a review). Taken together, oscillations provide a general mechanism that is conducive to efficient information processing whilst allowing for emergence of characteristics such as suppression that serve important cognitive functions.

The role of theta activity in cognitive control

Studies into the electrophysiological dynamics surrounding working memory (Jensen, 2006), and cognitive control networks (Cavanagh, Cohen, & Allen, 2009; Cohen et al., 2009), have shown that these operations occur against a background of oscillatory coherence in the theta (4-7 Hz) frequency range. The theta dynamics of these processes are typically detected over lateral and midline frontal regions, consistent with the purported role of the MFC in these processes (Trujillo and Allen, 2007; Cohen et al., 2008; Cavanagh, Cohen, & Allen, 2009). Luu & Tucker report that lateral and midline theta oscillations over the prefrontal cortex index rapid evaluative decisions, including errors, in relation to speeded-response demands (Luu and Tucker, 2001). Meanwhile, the ERN has been shown to arise from perturbations to ongoing theta oscillations within the centromedial frontal cortex, and lateral sensorimotor areas. Taken together evidence indicates that action monitoring, conflict and error detection processes and cognitive control in general, are supported by neural coherence within the theta range.

More specifically, theta activity predicts trial-to-trial activity of cognitive control systems. For instance, high conflict or error trials induce theta power increases localised to the lateral prefrontal cortex and sensory-motor areas (Cohen & Cavanagh, 2011), as well as the ACC (Wang et al., 2005; Tsujimoto et al., 2006). In line with action-monitoring account (Botvinick, Cohen, & Carter, 2004), theta power and synchrony between the lateral and medial prefrontal cortex is seen to decline on trials preceding errors, and to increase upon commission of errors (Cavanagh, Cohen, & Allen, 2009; Cohen & Cavanagh, 2011). Collectively, these findings indicate that frontal theta within the lateral and medial prefrontal cortex, (in conjunction with sensory-motor areas) facilitates adaptive behavioral changes after detection of errors or conflict.

Functional alpha activity

Neural systems must rely an effective filtering system to efficiently parse out relevant from irrelevant information. Neural suppression represents a mechanism by which the processing capacity of a region may be reduced, rendering its output less salient – in turn supporting task relevant and efficient processing, as well as response inhibition (Jensen & Mazaheri, 2010). Despite early misconceptions surrounding cortical idling, endogenous oscillations within the alpha range (8-13 Hz) have since been attributed a functional significant role within inhibition and attentional processes (Klimesch et al., 1998 & 2007; Thut et al., 2006; Palva & Palva 2007; Meeuwissen et al., 2010). Prefrontal structures are thought to coordinate posterior-occipital interactions resulting in sensory inhibition aimed at marginalising irrelevant information which otherwise results in conflict (Klimesch Sauseng & Hanslmayr, 2007; Jensen & Mazaheri, 2010). Suppression may support cognitive control through rendering sensory representations less accessible to, among others, response-capture systems (von Stein et al., 2000).

Recent studies demonstrate that amplitudes of ongoing alpha activity within sensory areas can have significant impact on perceptual performance (Hanslmayr et al., 2005; Romei et al.,

2008). Hanslmayr et al., report a negative relationship between pre-stimulus alpha power and perceptual performance: lower pre-stimulus alpha power was predictive of superior performance, while poor performance was associated with high alpha power prior to stimulus (Hanslmayr et al., 2005). Pre-stimulus alpha power has also been shown to predict conscious perception of near-threshold stimuli (Matthewson, et al., 2009, 2010; Romei, Gross, & Thut 2010). Furthermore, alpha power increases contralateral to the hemifield of a to-be-ignored visual stimulus – again indicating a functional role for alpha during task performance (Kelly et al., 2006).

These findings have been interpreted as showing that increases in alpha phase coupling represent a top-down shift of attention towards a relevant stimulus (Jensen & Mazaheri, 2010; von Stein et al., 2000). Some studies support a reciprocal and functional relationship between alpha and gamma bands in which alpha oscillations reflect internal representations of a stimulus (subject to top-down modulation or processing), while gamma oscillations reflect the bottom-up processing of a stimulus (Sauseng et al., 2005; Jensen & Mazaheri, 2010). The interactions between alpha and gamma activity may therefore allow for controlled processing of sensory information, where pre-stimulus alpha power, determined by executive or top-down control, can facilitate improved perceptual and cognitive performance (Palva & Palva, 2007).

Functional inhibition can also relate to the suppression of inappropriate motor responses, also seen during conflict resolution (Ridderinkhof et al., 2010; Aron et al., 2004). Response inhibition of this sort is typically studied using Go/No-Go and Stop-Signal (countermanding) tasks where subjects are unexpectedly required to suppress a previously learnt, or prepotent, behavioural response. This type of inhibition has been associated with dorsal lateral and ventral lateral subdivisions of the PFC (Mishkin, 1964). More recently response inhibition has been associated with the supplementary motor cortex (SMC), particularly the pre-SMA, responsible for selecting and preparing actions, and stimulus-response associations (Mofstofsky & Simmonds, 2008; Brass & Haggard, 2007). In all these instances, functional inhibition not only supports effective information processing in general, but also contributes directly to reducing cognitive interference.

Neural Entrainment

Oscillations within EEG can be classified in terms of their relationship to stimulation: endogenous (or background activity), evoked, or induced (Herrmann 2001). Endogenous oscillations are not related to stimulation, and as outlined earlier, in some cases reflect functional activity. Evoked oscillations appear in response to stimulation, and are phase-locked to a stimulus (or entrainer). Lastly, induced oscillations occur post-stimulation but may not be phase-locked to the stimulus. A considerable amount of psychophysiological EEG experiments demonstrate that neurons within the human visual cortex can synchronise their firing to the phase of a flickering visual stimulus – producing entrainment of a neural region (Regan 1989; Silberstein 1995; Herrmann 2001).

Typical entrainment stimuli involve the use of visual flashes or auditory tones. With both visual and auditory entrainment, stimulation can be delivered to both hemispheres concurrently, or toward a specific hemisphere through lateralised stimulus presentation (i.e. monaurally, or, in the case of vision, to individual visual fields). Shortly after the seminal discovery of alpha EEG by Berger in 1929, Adrian & Matthews demonstrated that the alpha rhythm could be amplified through presentation of alpha frequency visual stimulation (1934). Neural entrainment has also been researched in a variety of clinical contexts including surgery, alleviation of stress, headaches, and anxiety (reviewed in Huang & Charyton, 2008).

Entrainment of the occipital visual cortex is typically observed through EEG responses that resonate at the same frequency as the source of stimulation; a so-called steady-state visual evoked potential (SSVEP). Herrmann reports SSVEPs within the visual cortex as flicker frequency was increased in 1 Hz steps from 1 Hz to 100 Hz (Herrmann, 2001). SSVEPs present within the visual cortex, were shown to be driven by the flickering visual stimulus, and not endogenous processes. The evoked fundamental response was stronger for some frequencies than others- evidence of selective frequency preferences within the 10-, 20-, 40-, and 80-Hz frequency range (Herrmann, 2001).

In view of this literature, it remains an interesting research proposition to test whether visual entrainment may be used to induce, or strengthen existing, networks operating at specific frequencies, specifically those lying beyond visual regions. In relation to the current study, whether theta frequency visual flicker may induce a theta frequency SSVEP that propagates to frontal regions and thereby supports cognitive control networks that are known to operate within the theta frequency band. Visual flicker is an ideal method of entrainment in this context, as it can be readily combined with a conflict-inducing paradigm, such as the Eriksen flanker task outlined above. Visual entrainment is typically short in duration, and may therefore be introduced just prior to target stimuli of the flanker task. This protocol would mean that each trial has its own entrainment period, plausibly increasing the likelihood of successful entrainment, compared to, for instance, pre-block entrainment in the hope that frontal entrainment effects last a sufficient enough duration that they modulate behavioural across all trials of that block.

Against this backdrop of literature, we investigated whether visual flicker at alpha (10 Hz) and theta (6 Hz) frequencies could be used to modulate endogenous oscillatory activity within occipital and anterior regions respectively. In so doing we asked if entrainment could enhance cognitive response related networks. We predicted that both would facilitate improved performance on a modified version of the Flanker task – a robust interference paradigm. A control condition using an irregular but visually matched flicker should demonstrate if any behavioural differences related to visual flicker are the result of cognitive mechanisms.

We predicted that theta (6 Hz) and alpha (10 Hz) flicker frequency would bring about improved performance through posterior and anterior mechanisms in two independent manners: (1) 6 Hz flicker to strengthen theta based cognitive control networks within the

MFC and related frontal regions; and (2) 10 Hz flicker to facilitate task performance in a similar manner to how endogenous alpha activity has been shown to do so, through suppression of automatic responses processed in occipital regions that may otherwise produce conflict and lead to errors. In the case of alpha flicker, we therefore expected slower, but more accurate performance.

Concurrent to this we predicted to reproduce findings that midline frontal theta-band activity is associated with the activation of the cognitive control network. This would be successfully demonstrated through evidence of increased activity of this sort being correlated with the detection of conflict (i.e. incongruent trials) and consequences of the presence of conflict (i.e. post-error slowing).

Our behavioural dependent measures were (1) accuracy (calculated from the number of errors versus correct responses) and (2) response speed (calculated as a response latency from stimulus presentation to response) – increases in accuracy would constitute improved performance, while increased response speed would also provided accuracy was not degraded as a result. Our neurophysiological dependent measures were power and phase of oscillatory activity within theta (4-7 Hz) and alpha (10-13 Hz) frequency bands. Trial comparisons of interest included congruent versus incongruent trials – over which conflict effects (behavioural and neurophysiological) were predicted to be found on incongruent trials. Comparisons of visual flicker conditions are outlined in detail in Materials and Methods.

MATERIALS AND METHODS

Participants

A within subjects design of 21 participants (16 female) with normal or corrected to normal vision. Subjects were recruited through the University of Amsterdam participant recruitment system (https://www.test.uva.nl/sdpms/). Prior consent was asked of each subject before the experiment began. Subjects read an information form containing task instructions, they were reminded of the exclusion criteria and that could withdraw at any time. Other than this information, subjects were naive to the purposes of the experiment. All experiments were carried out under the ethical guidelines set out by the University of Amsterdam Ethical Committee (http://ce.psy-uva.nl/).

Task

A modified version of the Eriksen flanker task was used – see figure 1 for a graphical illustration of the paradigm. The subjects' primary task was indicate the central letter that was present in a string of five characters as quickly as possible using a thumb-response box – making this a two choice task. On each trial of this task, the target character may be either the same (congruent) or a different (incongruent) than flanking characters. This paradigm lends itself to research designs concerned with the study of response conflict (see Discussion).

Given that strings are constructed using target and distractor letters that are perceptually similar (e.g. X-Y or E-F), when strings contain both letters from a pair, interference or conflict occurs in the trial. Conflict engages cognitive control systems in response to the presence of incongruent target/distractor letter strings. In contrast, congruent trials are defined by letter strings made up of a single character; a target which is considerably easier to detect and respond to.

Stimuli

Flankers

Strings were always made up of five characters: two flanker (distractor) letters either side of a central letter (target). Each block contained 200 trials based on a pair of letters: X-Y, E-F, B-R, or M-N. In each block, congruency was randomised, with 50% incongruent. Block order was counter-balanced across subjects in order to avoid any training effects being reflected within a particular character pair (one may conceivably be harder than another for instance). Response-mapping was held constant within and between subjects so that within each string the earliest letter in the alphabet (X, E, B, M) was associated with a left thumb response, and the remaining letter with a right thumb response. Characters were printed in Arial font and a single space was left between letters.

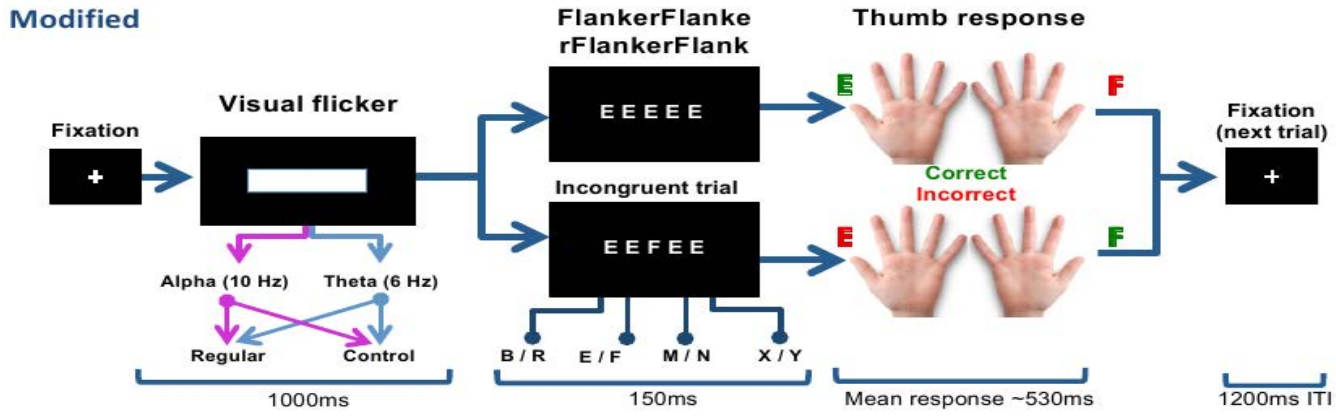

Figure 1: Schematic illustration of the modified Flanker paradigm. A single trial is depicted from left to right beginning with a fixation point. The entrainment period lasted 1000ms; flicker parameters were randomized such that within a block, flicker was either regular or control flicker; counter-balanced blocks contained either alpha (10 Hz) or theta (6 Hz) flicker. On the flanker task, trials were either congruent or incongruent; counter-balanced blocks contained 200 trials using a pair of letters to form five-character strings. Subjects responded to identify the central target letter in the string with a button press using their thumbs. Responses were followed by a fixation cross and an interval before the next trial began. Durations of visual flicker, flanker stimuli presentation, and inter-trial interval (ITI) are shown along the bottom.

Visual Flicker Entrainment

Preceding each character string on a trial, a solid black bar flickering at 6Hz (theta) or 10Hz (alpha) appeared at the center of the screen for a period of 1000ms. Subjects were cued to attend to the position of the flicker with a fixation point that remained at the center of the screen before and during the flicker. The visual angle of entrainment stimuli can be calculated using the formula θ = 2*arctan (H/2D): where, θ is visual angle; H is a stimulus dimension of height or width (150 x 50 pixels; 40 x 13 mm); and D is the distance between the eye and the stimulus (maintained at 90 cm); resulting in a visual angle of 2.54o x 0.83o. The monitor refresh rate was set to 60 Hz, equal to 60 monitor refreshes per 1000ms of entrainment.

To control for visual effects of flicker, such as it serving as a cue rather than an entrainer, each flicker frequency had an irregular counterpart. This control flicker was composed of visual stimuli of the same dimensions as regular flicker, however the interval between successive flashes was randomised. By offsetting the temporal nature of flicker, the rhythmic nature of genuine flicker was abolished, rendering it incapable of evoking resonant oscillations (or entrainment). This control flicker was randomly interleaved with regular flicker, and present in 50% of trials in each block. Additionally, we counter-balanced the pairing of strings (X-Y, E-F, B-R, or M-N) to flicker condition (6Hz or 10Hz).

Procedure

Subjects were first provided with an Information Brochure and asked to provide their consent to participating in the study. They were then fitted with EEG. Subjects were made aware of the effects of blinking and overt facial or muscle movements and asked to avoid making these and other movements during trials. Finally, subjects were fitted with EMG sensors to their thumbs and instructed on appropriate ways to respond using the response-box (located on arm-rests of a chair).

All experiments were conducted in a dimly lit room. Each of the four blocks began with 10 practice trials under the same conditions as that block. Rest breaks were offered after every 50 trials, at which point subjects were presented with on-screen feedback regarding their behavioural performance (percentage correct and average reaction time).

At the beginning of each trial subjects were cued to central fixation point. After 1200ms fixation, the flicker appeared over the cross for a duration of 1000ms, this was followed by the flanker stimuli which was presented for 50ms. The five-letter string of characters contained a central target letter either the same (congruent) or different (incongruent) than its flanking pairs. Subjects were required to manually identify the target letter using thumb-response boxes beneath their thumbs. After a response was made there was an inter-trial interval of 1200ms after which the fixation point reappeared and the next trial began (see Figure 1 for a illustration of the paradigm).

Blocks were randomised with either 6 or 10 Hz flicker. Regular and irregular flicker were randomised across trials within each block. Each subject completed two such blocks with alpha frequency flicker, and two with theta frequency flicker. Altogether, testing lasted approximately 45 minutes.

Recording and Preprocessing of EEG & EMG data

Electrophysiological activity was measured from 64 cranial electrodes, acquired at a sampling rate of 512Hz, amplified 500X, with impedances <20 KΩ (Biosemi EEG system). Additionally, two horizontal electro-oculogram electrodes were used to record eye movements, and two electrodes were attached to the earlobes (used as a reference). All electrode sites were grounded through CSR/DRL electrodes.

Electromyographic (EMG) recordings were taken from the flexor pollicis brevis muscle of each thumb using a pair of surface electrodes, placed on a subject-by-subject basis roughly 5mm apart on the thenar eminence.

Trials containing blink, muscle and other non-cognitive artifacts were removed after visual inspection. Four subjects' data were excluded from analysis due to levels of noise. Eye-blinks occurring during regular flicker were rejected on account of the notion that a single blink would disturb the periodicity of the flicker - rendering them almost equivocal to our control, and contrast condition, of irregular flicker. Independent Component Analysis (ICA) was applied prior to time-frequency analysis to assist in the removal of blink and non-cognitive artifacts. Error trials, post-error trials, and partial error trials were all removed, on the notion that additional EEG dynamics that accompany these events would disturb flicker effects that would otherwise be present.

Processing of EEG data

All EEG processing was conducted using Matlab (The MathWorks). Offline, raw data was high-pass filtered at 0.5 Hz and epoched between -1.5s and +3s around each trial. Two datasets were created, the first time locked to response triggers and the second to flicker onset. For the purposes of clarification, in all analyses and plots, we state whether data presented is time-locked to response (RT-locked; where time 0 corresponds to button press), or time-locked to stimulus onset (stimulus-locked; where time 0 corresponds to flanker stimulus onset).

Current Source Density

A current source density (CSD) was applied to all EEG data. The CSD method computes the second spatial derivative of voltage between neighbouring electrodes, producing a signal with heightened spatial resolution. The CSD transformation provides a more informed view of local area neural dynamics, by marginalising the contributions of distal neural activity that is otherwise conveyed through volume conduction.

EEG analyses time-frequency decomposition

Time-frequency analysis was carried out with using customised scripts, running in Matlab (MathWorks). The EEG time series in each epoch was convolved with a set of complex Morlet wavelets,

defined as a Gaussian-windowed complex sine wave: e2 i ϖ f t e-t^2 / (2*σ2) Here t is time, f is frequency, where the sine wave (f) increased from 1Hz to 30 Hz in 20 logarithmically spaced steps. σ defines the width (or cycles) of each frequency band, set at 4/2ϖf. The width of frequencies was logarithmic, such that it provided a suitable trade-off between temporal and frequency resolution: with greater frequency resolution at lower frequencies, and greater temporal resolution at higher frequencies.

To allow for comparison of power effects across frequency bands, EEG data was normalised using a decibel (dB) transform: dB power =110 x log 10[power/baseline]. Here, the baseline for dB conversion was established as average power at each frequency band in a 200ms window, -1300 to -1000 ms prior to stimulus onset.

Conditions

Trials were sorted one of eight conditions: theta regular congruent (TRC); theta regular noncongruent (TRN); theta irregular congruent (TIC); theta irregular noncongruent (TIN); alpha regular congruent (ARC); alpha regular noncongruent (ARN); alpha irregular congruent (AIC); alpha irregular noncongruent (AIN).

RESULTS

Conflict effects

Average time for response following stimulus presentation was 515ms on congruent trials, and 548ms on incongruent trials (see table 1). This difference of 33ms, a congruency effect, was significant [F(1,19) = 23.901; p<0.001] (see table 2). As well as being faster to respond, accuracy was 7.7% higher on congruent trials (mean 91%) compared to incongruent trials (83%: see table 1). The effect of congruency on accuracy was significant at the α<0.05 level [F(1,19) = 19.598; p = 0.000] (see table 2). These findings represent typical conflict effects in line with literature surrounding the impact of interference on performance.

Flicker effects on behavior

A 2 (frequency) x 2 (regularity) x 2 (congruency) ANOVA was applied to both reaction time and accuracy datasets. All results were evaluated against the α = 0.05 level for significance. The main effect of flicker frequency (alpha vs. theta) on reaction time was significant [F(1,19) = 5.241; p = 0.034] (see table 2). A subsequent one-sided paired samples t-test supported this finding and revealed that response times following theta frequency flicker (mean 524.192ms) were indeed significantly faster than those following alpha frequency flicker (mean 538.949ms), t(79 = -3.663; p < 0.001) (but see figure 3).

Additionally a main effect of flicker frequency (alpha vs. theta) on accuracy was significant, [F(1,19) = 7.196; p = 0.015] (see table 2). A follow-up one-sided paired-samples t-test showed that accuracy after theta frequency flicker (90%) was significantly higher, t(79 = 4.345; p < 0.001), than accuracy after alpha frequency flicker (85%). In figure 3 we can see the effects of visual flicker condition on both accuracy and reaction time.

Furthermore this ANOVA showed that reaction times on trials following regular flicker (at either alpha or theta frequency) were not significantly different from those on trials following irregular flicker, [F(1,19) = .437, p = 0.517]. There were no significant differences in accuracy following regular and irregular flicker either [F(1,19) = 3.154, p = 0.92] (see table 2). This result would seem to undermine an apparent flicker effect between alpha and theta since it implies that regular flicker had no greater (or lesser) effect than control flicker in terms of modulating performance.

A marginally significant second order interaction between flicker frequency, regularity, and congruency was also found, [F(1,19)=845.866; p = 0.058] (see table 2). Further analysis showed this being driven by a congruency x flicker interaction within alpha flicker conditions. This interaction was not found within the accuracy

1a. Flanker task reaction times per condition (ms)

(Condition)	Mean* (ms)	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Theta	524.192	18.335	485.817	562.566
Alpha	538.949	18.444	500.345	577.552
Congruent	515.032	20.167	472.822	557.243
Incongruent	548.108	16.484	513.606	582.610

1b. Flanker task accuracy per condition (% correct)

(Condition)	Mean* (%)	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Theta	89.5	.018	85.7	93.3
Alpha	84.8	.028	78.9	90.6
Congruent	91.0	.021	86.6	95.3
Incongruent	83.3	.026	77.8	88.7

*Table 1 |Behavioural results from Flanker task performance. (a) The upper table shows mean reaction time data; (b) the lower table shows accuracy data. Theta and alpha conditions are averaged over both congruency conditions and over regular and control flicker conditions. Congruent and Incongruent conditions are averaged over all flicker conditions. * Reaction times reflect latency between stimulus presentation until manual response (in ms).*

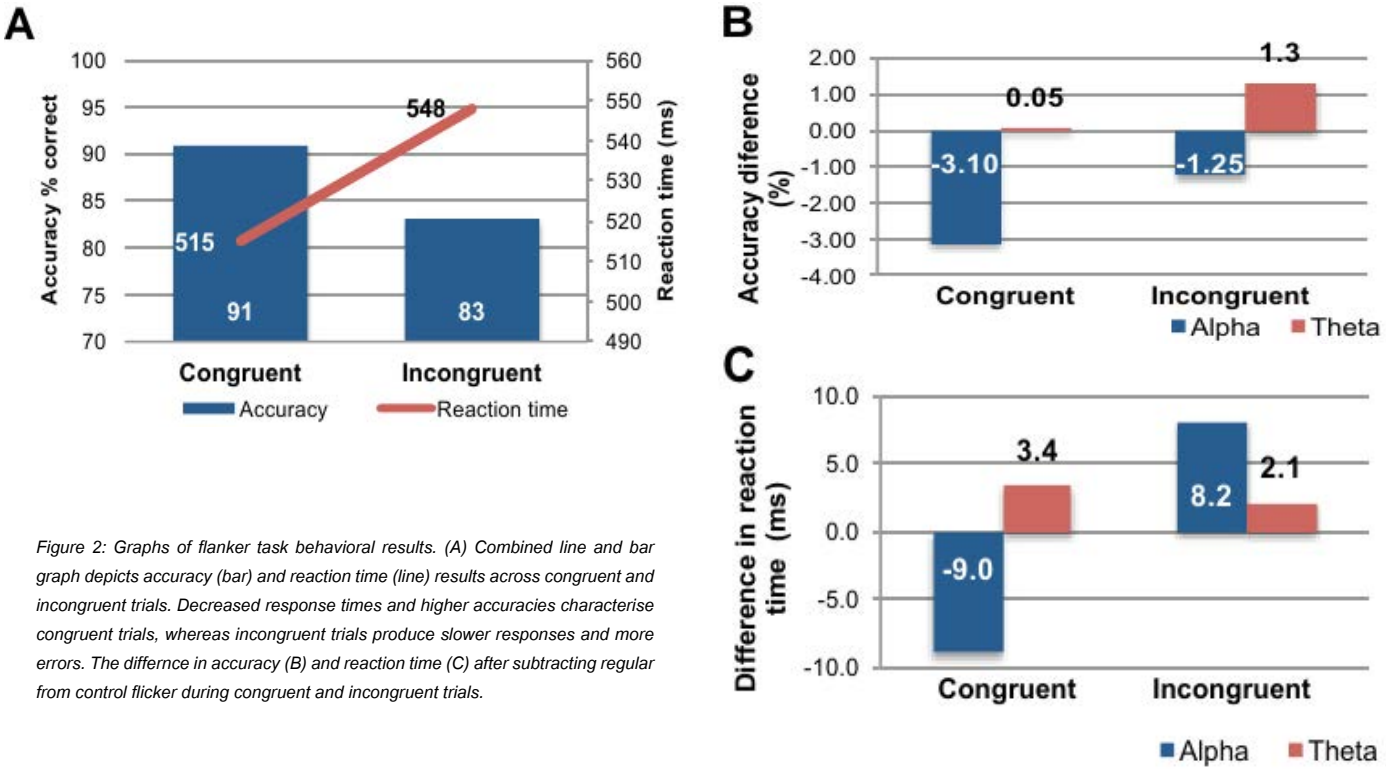

Figure 2: Graphs of flanker task behavioral results. (A) Combined line and bar graph depicts accuracy (bar) and reaction time (line) results across congruent and incongruent trials. Decreased response times and higher accuracies characterise congruent trials, whereas incongruent trials produce slower responses and more errors. The differnce in accuracy (B) and reaction time (C) after subtracting regular from control flicker during congruent and incongruent trials.

data set. No further interactions were found with reaction time or accuracy datasets.

EEG Results

Analysis of EEG data was performed after all procedures described in Methods were completed. Two datasets were created, one time locked to stimulus presentation, and one to response. For the purposes of clarification: stimulus-locked is time-locked to flanker stimulus onset, response-locked is time-locked to manual response. These data sets allow us to consider neural activity corresponding to entrainment, and to conflict processing or resolution most readily. Finally, frequency windows for analysis of entrainment were chosen according to visual flicker frequency.

Conflict related frequency dynamics

A significant increase in theta power (4-7 Hz) over MFC was found on incongruent compared to congruent trials occurring between -100ms and 150ms around the time of response [$F(1,17) = 8.582$; $p = 0.009$] (see figure 4 and 9 & table 4). These power increases were not accompanied by increases in theta phase over MFC during the same time window. Electrode FCz was chosen for these analyses, as it is considered to best reflect MFC activity. We did not include error trials or trials following error trials in these analyses.

Visual cortex effects of entrainment

Alpha entrainment induced alpha frequency (10-13 Hz) electrophysiological activity within occipital visual regions during visual flicker period, a time window of -800ms to flanker stimuli onset (see table 5 and figure 6). This effect of alpha frequency visual flicker was significant [$F(1,17) = 53.401$; $p < 0.001$] (see table 6). Power activity was accompanied by significant increases in alpha phase values over Oz during the same time-frequency window, and same conditions [$F(1,17) = 84.630$; $p < 0.001$] (see tables 5 & 6, and figure 7).

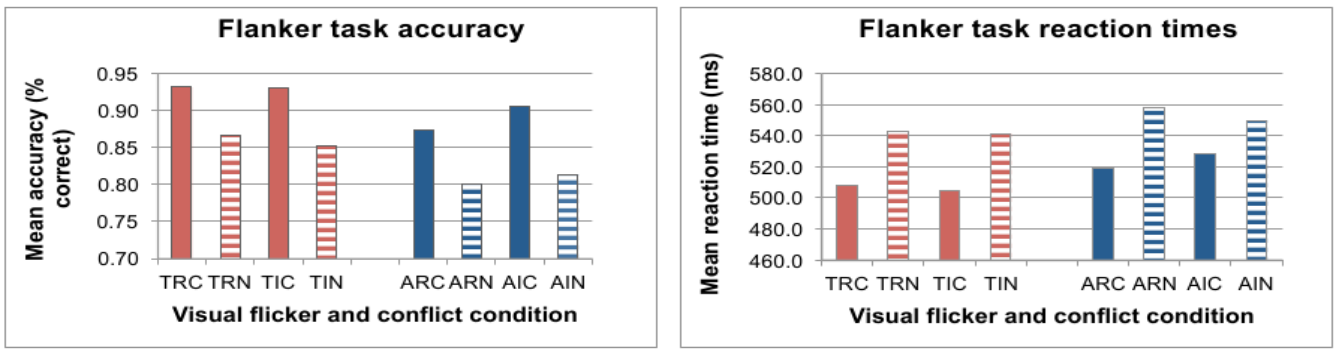

Figure 3: Conflict and flicker effects in accuarcy and reaction times. The data presented above clearly demonstrate the conflict effect: reduced accuracy, and slower response times during incongruent (striped bars) compared to congruent (solid bars) conditions. Condition differences between theta (red) and alpha (blue) visual flicker can also be observed: theta conditions led to greater accuracy and faster responses on average than alpha conditions. The assosiation between alpha visual flicker and slower response times is noteworthy, as it is line with literature surrounding the inhibitory role of alpha band oscillations. Note, however, that there was no significant difference between regular and irregulat flicker at either alpha or theta frequency (when comparing within the same conflict contition i.e. congruent or inconguent). Condition labels: theta regular congruent (TRC); theta regular noncongruent (TRN); theta irregular congruent (TIC); theta irregular noncongruent (TIN); alpha regular congruent (ARC); alpha regular noncongruent (ARN); alpha irregular congruent (AIC); alpha irregular noncongruent (AIN).

2a: Effects and interactions within reaction time dataset from a 2 (frequency) x 2 (regularity) x 2 (congruency) ANOVA.

Source	df	F	Sig.
Frequency	1	5.241	.034
Regular	1	.134	.719
Congruency	1	23.901	.000
Frequency * regular	1	.437	.517
Frequency * congruency	1	.656	.428
Regular * congruency	1	2.697	.117
Frequency * regular * congruency	1	4.061	.058

2b: Effects and interactions within accuracy dataset from a 2 (frequency) x 2 (regularity) x 2 (congruency) ANOVA.

Source	df	F	Sig.
Frequency	1	7.196	.015
Regular	1	.429	.520
Congruency	1	19.598	.000
Frequency * regular	1	3.154	.092
Frequency * congruency	1	.328	.573
Regular * congruency	1	2.385	.139
Frequency * regular * congruency	1	.085	.774

Table 2: Results of two 2 (frequency) x 2 (regularity) x 2 (congruency) repeated measures ANOVAs with (a), reaction time and (b), accuracy datasets. Interactions and effects indicate main effects of congruency and frequency.

Theta entrainment induced theta frequency (4-7 Hz) power and phase activity within visual regions during the same time window (-800ms to flanker stimuli onset; see table 5 and figure 6). Increases in theta frequency power [F (1,17) = 53.401; p < 0.001], and phase [F (1,17) = 84.630; p < 0.001], values were significant over the occipital region when averaging over electrode Oz (see tables 5 & 6; figure 6 & 7). For both alpha and theta entrainment analyses EEG data was averaged according to flicker conditions, and over congruency (i.e. ARC & ARN vs. AIC & AIN; TRC & TRN vs. TIC & TIN).

Anterior effects of entrainment

Beyond physiological effects of visual entrainment within occipital visual processing regions, one of our principle research questions surrounded the neurophysiological effects of visual entrainment at a higher cognitive level. Specifically we asked whether a visual flicker at theta frequency would be able to enhance endogenous theta power (and phase) within MFC.

We used a series of 2(regularity) x 2(congruency) ANOVAs to test for anterior entrainment of power and phase at three frontal

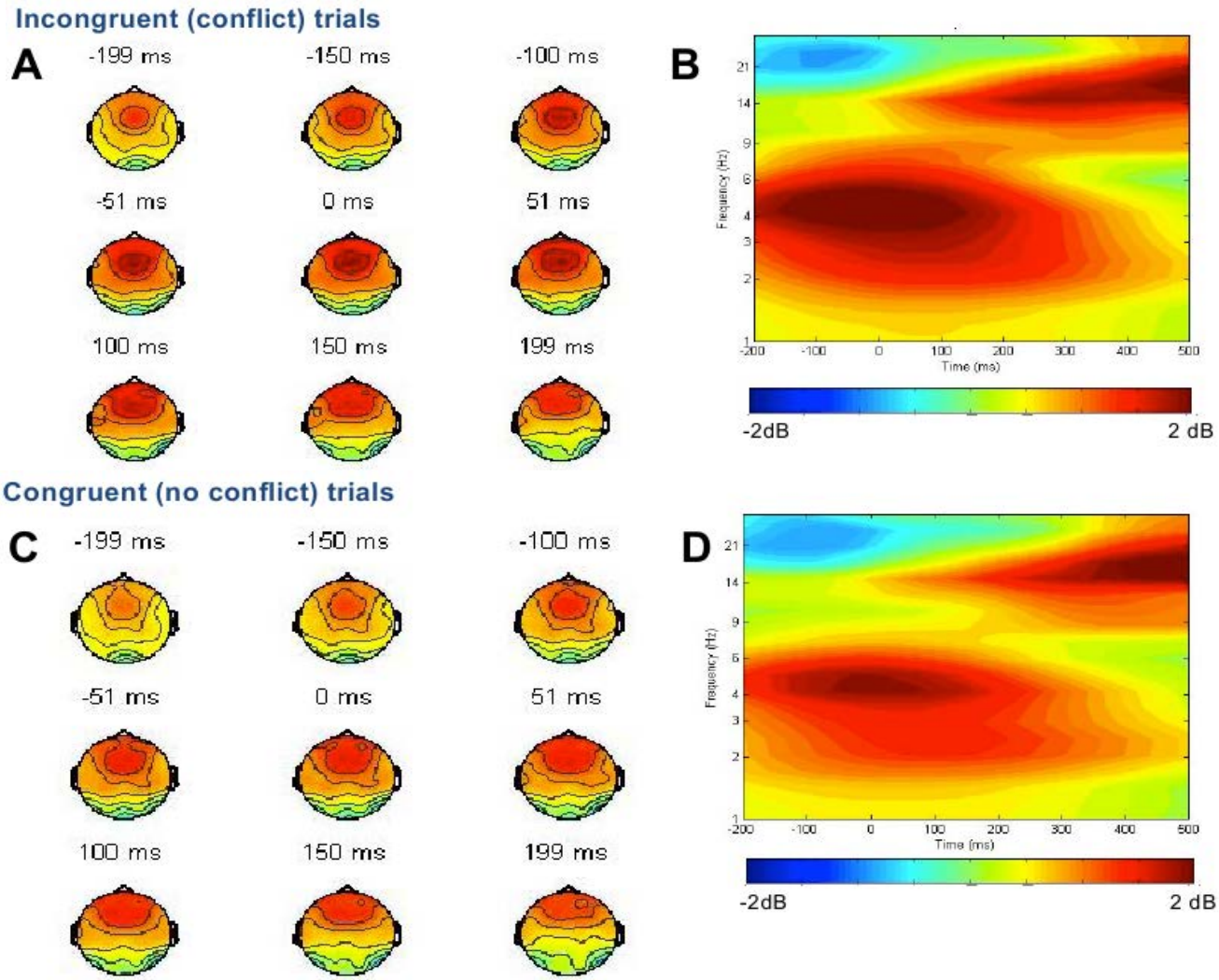

Figure 4: Conflict effects over Medial Frontal Cortex. Upper panel shows topograhical (A) and time-frequency (B) plots of theta (4-7 Hz) power (dB) activity around the time of response (0 ms) during incongruent trials. The smae EEG data can be seen for congruent trials in the lower plots (C,D). A more prominent build-up of activity can be seen during incongruent (high conflict) trials compared to congruent trials. Time-frequency plots are of activity at electrode FCz. All data is response-locked.

4a: Conflict effect over FCz in theta (4-7 Hz) frequency range.

Congruency	Mean power*	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Congruent	1.438	.170	1.080	1.796
Incongruent	1.785	.200	1.363	2.207

4b: Tests of within-subjects conflict effect over FCz in theta (4-7 Hz) frequency range.

		Type III Sum of Squares	df	Mean Square	F	Sig.
Congruency	Sphericity Assumed	1.084	1	1.084	8.582	.009
Error (congruency)	"	2.147	17	.126		

*Table 4: Theta frequency band conflict effects over MFC. (4a) The upper table provides mean power values around the time of response; incongruent trials produce increased power at electrode site FCz, as compared to congruent trials. (4b) The lower table demonstrates this conflict effect was significant. *EEG data is response-locked, 100 ms pre- and 150 ma post-response.*

regions: the MFC (electrode FCz), the lateral prefrontal cortex (electrodes F5 and F6) and the pre-SMA (electrodes C3 and C4) after theta visual flicker. For these analyses, we used stimulus-locked data, windowed -200 and 200ms around the onset of flanker stimuli and a frequency window of 6-8 Hz; the data was averaged over the respective electrode/s, and over congruency conditions. The analyses revealed no significant effects of entrainment (refer to figure 8 for plots of MFC activity).

For these analyses we chose a time window similar to that which we used to assess conflict effects at MFC, -200ms to 200ms around the time of flanker onset (0ms). This time window reflects the period where visual effects of entrainment were present (-200ms to flanker onset) and therefore where any anterior effects of entrainment should be observed. It also includes a period of cognitive processing and preparation (up to 200ms post flanker onset) where subjects are actively processing the task stimuli, and discerning the target identity) and therefore a time when (putatively) any beneficial or enhancing effects of entrainment would have to occur. Theoretical basis for this notion is grounded in findings that theta dynamics immediately prior to a response predict upcoming errors. Specifically, disruption to on-going theta activity (perhaps through lack of attention or stochastic endogenous processes) is strongly associated with the later commission of errors. On the basis of this, if theta entrainment was capable of restoring or strengthening this on-going (endogenous) theta activity, then performance may consequently be enhanced as well.

Furthermore, no significant effects of entrainment were found after changes to the parameters outlined above. For instance, removing congruent trials, to assess if an entrainment effect existed when trials contained endogenous (conflict-driven) theta, did not yield significant results either. This analysis was conducted since it may have been the case that rather than (or was well as) producing an effect within MFC, theta entrainment could have enhanced theta power within motor regions associated with task performance – however, we did not find evidence to support this prediction.

DISCUSSION

We set about to understand the neural and behavioural effects of

5a: Alpha flicker entrainment effects in power and phase (10-13 Hz) over Oz

Condition	Mean*	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Power Regular	2.187	.799	.500	3.873
Irregular	.974	.736	-.579	2.528
Phase Regular	.624	.053	.512	.735
Irregular	.123	.005	.112	.134

5b: Theta flicker entrainment effects in power and phase (4-7 Hz) over Oz

Condition	Mean*	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Power Regular	2.646	.676	1.220	4.073
Irregular	1.704	.609	.420	2.988
Phase Regular	.612	.041	.525	.700
Irregular	.145	.005	.134	.156

*Table 5: Power and phase entrainment effects after (a), alpha and (b), theta visual flicker. * Stimulus-locked EEG data, windowed between -800 ms and flanker stimuli onset.*

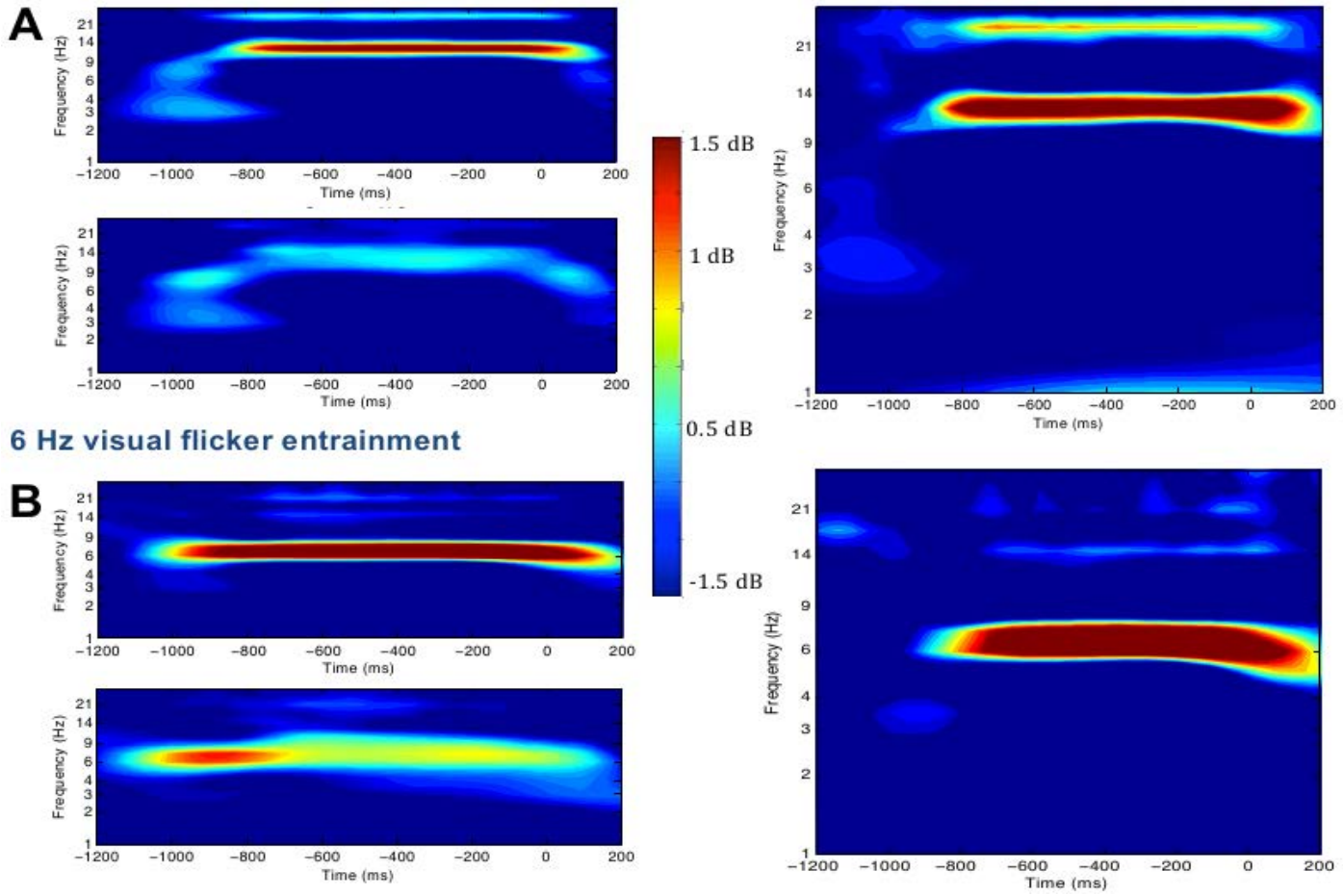

Figure 6: Visual flicker induced power effects within the visual cortex. Time-frequency plots of power (dB)activity at electrode Oz. Panel A left shows alpha (10 Hz) regular (upper) and irregular (lower) visual flicker effects between -1200 ms and 200 ms post flankerflankerflankerflankerflankerflankerflanker stimulus onset. The right plot shows the differnce in EEG signature between these two flicker conditions. Panel B shows the same physiological effects within the theta band range. Clear increases in power at the frequency of entrainment are evident. These increases in power provide to be significant.

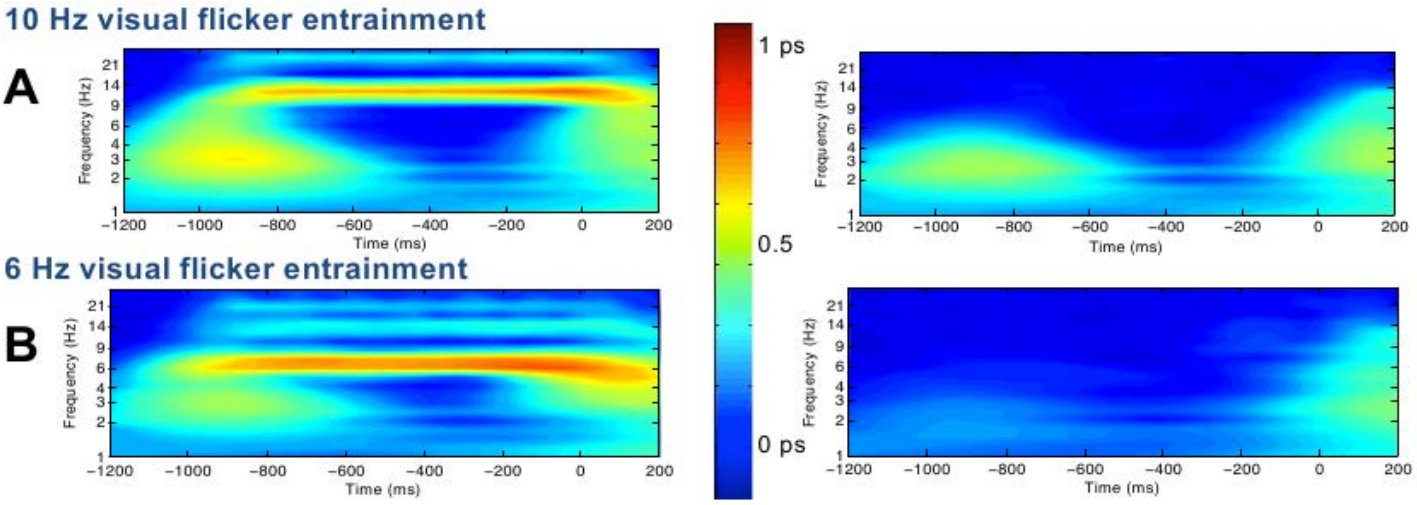

Figure 7: Flicker induced phase activity over visual cortex. Time-frequency plots of phase activity at electrode Oz. Top row shows alpha (8-12 Hz) regular (left) and irregular (right) visual flicker effects between -1200 ms and flankerflankerflankerflankerflankerflankerflanker stimulus onset. Bottom row shows theta (4-7 Hz) regular (left) and irregular (right) visual flicker effects within the same time window. Blue bars show dlicker onset; purple bars show flankerflankerflankerflankerflankerflankerflanker stimulus onset. Clear increases in phase are evident in both regular flicker conditions, resonating the visual flicker frequency. These increases in phase proved to be significant.

Table 6: Effects of alpha (10-13 Hz) and theta (4-7 Hz) frequency entrainment over Oz.

Condition		Type III Sum of Squares	df	Mean Square	F	Sig.
Alpha entrainment (power)	Sphericity Assumed	13.222	1	13.222	53.401	0.000
Error	"	4.209	17	0.248		
Alpha entrainment (phase)	Sphericity Assumed	2.254	1	2.254	84.63	0.000
Error	"	0.453	17	0.027		
Theta entrainment (power)	Sphericity Assumed	7.983	1	7.983	43.157	0.000
Error	"	3.145	17	0.185		
Theta entrainment (phase)	Sphericity Assumed	1.964	1	1.964	140.227	0.000
Error	"	0.238	17	0.014		

Table 6: Power and phase entrainment at alpha and theta frequencies. EEG data is stimulus-locked, between -800 ms and flanker onset.

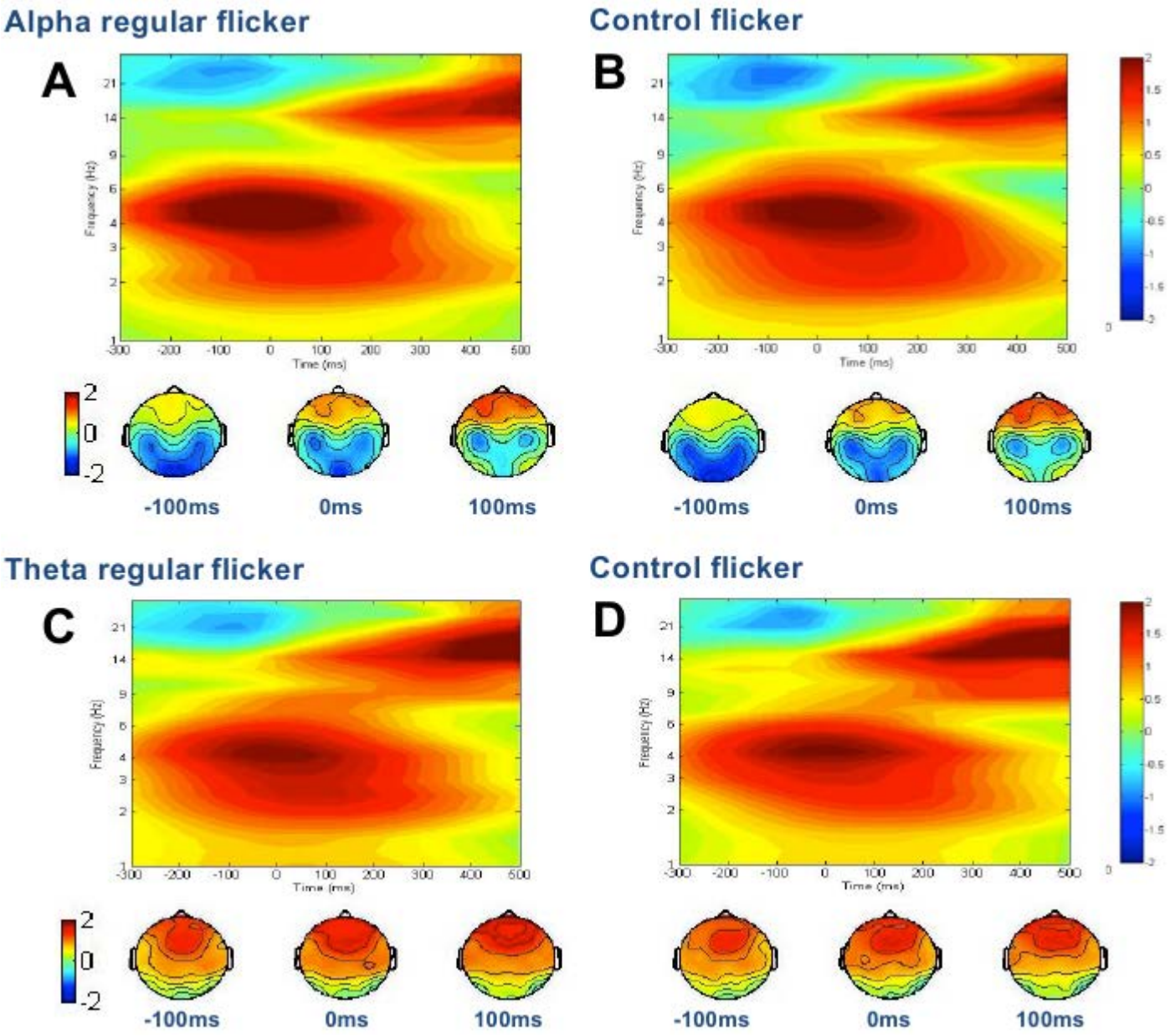

Figure 8: Power effects over MFC and topographical maps of alpha / theta band activity following each visual flicker condition. (A) Time-frequency plot of alpha (8-13 Hz) power (dB) effects at FCz within a 800 ms window, locked to response time (0ms) following regular flicker. (B) Same as (A) but following control alpha flicker. Below each time-frequency plot is the corresponding topograhic representation of the same EEG data, within a 200 ms time window. In the lower panels (C,D), theta (4-7 Hz) power effects are shown across the same parameters. Data were averaged over flicker condition (e.g. ARC/ARN vs. AIC/AIN).

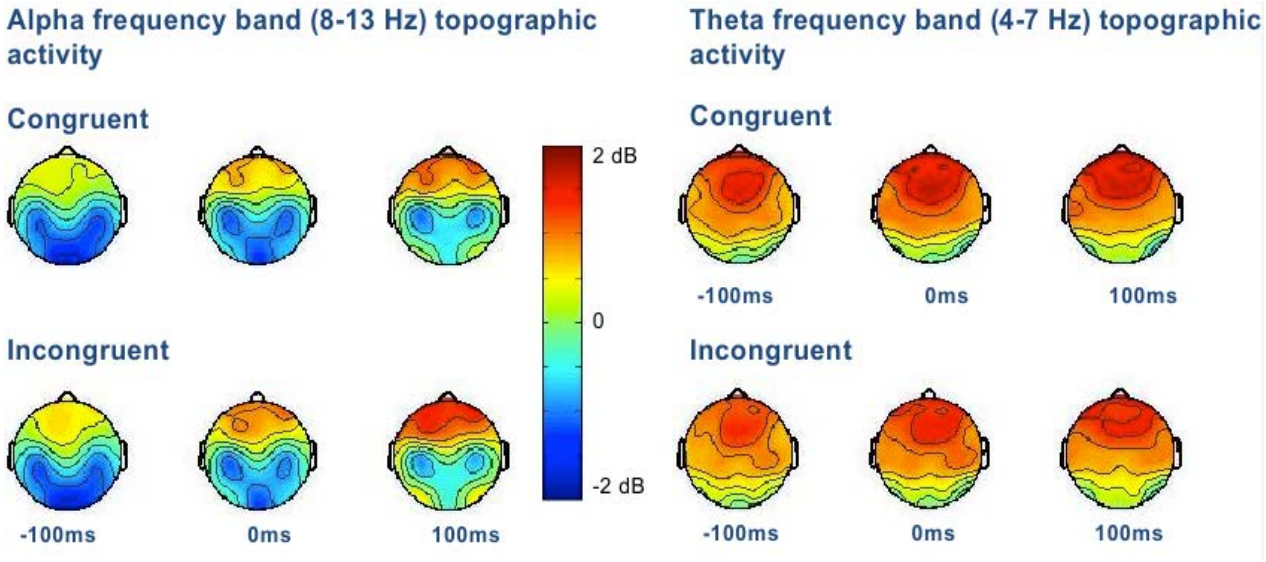

Figure 5: Topography of response related activity. Power effects within alpha (left: 8-13 Hz) and theta (right: 4-7 Hz) frequency bands during congruent trials (top bar), and incongruent trials (lower bar). EEG data is response locked, averaged over flicker condition. Note, no differences appear between regular and control flicker, the data presented here are averaged over flicker condition (i.e. ARC/AIC vs. ARN/AIN). Alpha and theta frequency ranges show considerably different patterns of topograhic activity over the same time course around response time.

visual flicker entrainment during performance on a Flanker task. More specifically we investigated whether entrainment is able to modulate oscillatory synchronisation of posterior and anterior regions. In turn, we investigated whether such modulations would be reflected in the behavioural performance of subjects (as measured through speed and accuracy). We used a modified version of the Eriksen Flanker task that featured pre-trial visual flicker at alpha (10 Hz) or theta (6 Hz). EEG activity was subject to trial rejection and forwarded to time-frequency analysis procedures.

Occipital and frontal entrainment to flicker

Our immediate hypotheses concerned the physiological effects of entrainment at a visual level, within occipital processing regions of the visual cortex. Time-frequency analysis of EEG data from this region confirmed our predictions: visual flicker at both 6 Hz and 10 Hz resulted in entrainment activity within occipital regions at a frequency that resonated with visual flicker. We found that 6 Hz (mid-theta band) flicker entrained both power and phase of oscillations within the occipital cortex to frequencies within the 4-7 Hz range. Occipital resonance of visual flicker was evident after 10 Hz flicker (mid-alpha band) as well; here, entrainment of oscillatory power and phase occurred between 10-13 Hz. Crucially, irregular flicker did not produce entrainment effects at either frequency indicating that our control flicker worked as intended; providing a suitable visual replacement for regular flicker without any entrainment effects.

The entrainment effects we report here were immediately visible upon inspection of time-frequency data. Both power and phase values increased shortly after flicker onset; entrainment was seen within 200ms after visual flicker began. This latency reflects the time taken for stimulus information to reach the visual cortex. Entrainment effects were then sustained throughout the period of visual flicker, persisting for 200ms after visual flicker cessation. These findings demonstrate similar findings to visual physiological research into steady state visual evoked potentials (SSVEPs: Herrmann, 2001). Hermann has shown how visual stimulation can produce fundamental, sub-harmonic and harmonic oscillations within the visual cortex; interestingly, a harmonic of 10 Hz flicker was also demonstrated in the present study (Herrmann, 2001).

Having found evidence of entrainment within the visual cortex, we then sought to investigate whether entrainment of occipital areas propagated or was able to feed-forward to frontal regions. For these analyses, we considered neural activity of the MFC, the lateral prefrontal cortex (putatively responsible for redistributing attentional resources and initiating changes to motor thresholds), and motor regions (involved in coordination of motor responses). Theoretical basis for our hypotheses surrounding the effects of theta flicker within these areas was grounded in converging evidence from studies illustrating that EEG dynamics within the theta band support the detection processes, as well as the engagement of cognitive control (Trujillo and Allen, 2007; Cohen et al., 2008; Cavanagh, Cohen, & Allen, 2009). Specifically, disruption to on-going theta activity (perhaps through lack of attention or stochastic endogenous processes) is observed prior to commission of an errors. Meanwhile errors themselves serve as triggers for the re-engagement of control, a process reflected in increased theta band synchronisation on error trials between the MFC and the lateral prefrontal cortex (Cavanagh, Cohen, & Allen, 2009). With this in mind we anticipated that if occipital entrainment was able to feed-forward and entrain frontal structures, then theta frequency entrainment effects may restore, or strengthen, endogenous theta activity within cognitive control structures. In turn performance may be enhanced to the extent that fewer errors are made and responses are faster.

Contrary to our predictions, visual flicker at 6 Hz (theta) did not produce any significant differences in theta power or phase within frontal structures compared to its irregular control counterpart. This would suggest that this method of visual entrainment is not

capable of modulating oscillatory activity beyond the visual cortex. Alternatively, our results may suggest that visual entrainment in general cannot be used to strengthen on-going amplitude of theta-based networks. The first case is most certainly possible; however, speculation as to precisely why entrainment did not propagate (or what putative mechanisms may support propagation) is difficult with the current data, and remains outside the scope of the study. In the second case, even if visual flicker were able to entrain frontal regions, it may be that the oscillatory dynamics of endogenous theta-based networks are too finely tuned to benefit in any way from such coarse entrainment. Consider the temporal nature of endogenous theta -based error or conflict detection during interference and speeded-response paradigms: here, it would seem, entrainment to a frequency of 6 Hz may disrupt the ability of neurons to rapidly switch between different states in a manner that supports conflict resolution and flexible behaviour. Visual flicker therefore, may undermine the flexibility that is typically expressed within electrophysiological activity of frontal structures.

Finally, it is worth considering that further analyses with the current data sets obtained from this study may yet reveal entrainment effects at a neural level. For instance, despite failing to entrain frontal structures an outstanding questions surround whether or not theta flicker served to increase synchrony between frontal structures such as the lateral prefrontal and medial prefrontal cortex. Synchrony between these two regions in the theta band has previously been demonstrated to fluctuate in response to the activity of the cognitive control system – increasing upon commission of errors (Cavanagh, Cohen & Allen, 2009). Unfortunately, even if entrainment induced modulation of MFC $\rightarrow$ lPFC synchrony is found in future, it will not be associated with any significant behavioural effects since regular and irregular flicker produced no significant differences in performance.

At the same time, directed synchrony between the MFC and occipital regions may also have been subject to modulation through entrainment effects. Earlier, we reported a topographical spread of alpha activity from posterior areas towards the parietal regions, which failed to produce significant entrainment effects within the frontal structures of interest. However, previous studies have reported increased directed synchrony between the MFC and OCC in response to conscious and unconscious errors – interpreted as evidence of a long-range mechanism involved in post-error adaptation (Cohen, van Gaal, Ridderinkhof & Lamme, 2009). The reported increases in MFC $\rightarrow$ OCC synchrony occurred within a 2-12Hz range, it is possible therefore that flicker-induced entrainment at either alpha or theta frequencies (both within a 2-12 Hz range) would affect the strength of the synchronous oscillations between MFC and OCC regions. This being the case, the behavioural results we reported would seem cast doubt over the likelihood that entrainment affected MFC $\rightarrow$ OCC synchrony: the literature to date suggest MFC $\rightarrow$ OCC synchrony provides a top-down control mechanism; suppressing, enhancing or otherwise interfering with the frequency dynamics of this mechanism (through entrainment) should therefore lead to a modulation of performance, but yet no significant behavioural differences were observed between the experimental and control flicker. If therefore an entrainment effect was found at this level, then it is interesting that it did not result in any significant behavioural changes.

Conflict effects and mid-frontal theta oscillations

We investigated how conflict affected power and phase of theta activity across the MFC and the lateral prefrontal cortex. These regions have previously been associated with phasic increases in theta band activity during error and conflict trials (Cohen et al., 2009; Cohen & Cavanagh, 2011). In particular, as outlined in the Introduction, studies have shown how medial frontal theta coherence supports interactions between the MFC and lateral prefrontal cortex, and coordinates cognitive control network activity. Firstly, we showed typical increases in theta power, but not phase, over the MFC around the time of flanker stimulus onset, increasing toward the time of response. This medial frontal theta effect provides a neural marker of the engagement of cognitive control systems, which monitor task performance and evaluate the need for increased control.

Secondly, we found that increases in theta power were pronounced during incongruent trials and attenuated during congruent ones; reflecting the relatively greater engagement of control in view of increased conflict. We chose electrode FCz for these analyses because it had previously been shown to provide greatest resolution of the MFC activity, in particular the ACC. The conflict effects we report in this study are in line with those predicted by conflict and action monitoring accounts of medial frontal activity during interference tasks (Gratton et al., 1992; Botvinick et al., 2001; Botvinick, Cohen, & Carter, 2004).

Visual entrainment effects on performance

Our primary hypotheses concerned how entrainment may modulate performance on the flanker task. Whilst we did not find evidence of entrainment within frontal regions, occipital entrainment may have modulated performance nonetheless. In spite of this, and contrary to our hypotheses, our results would suggest that visual flicker at 6 Hz and 10 Hz does not produce any significant differences in performance. There was a tendency toward slower response times and reduced accuracy following alpha (10 Hz) flicker, compared to theta flicker conditions. Note that the slowest response times occurred after alpha regular flicker, on incongruent trials – this block also produced the lowest accuracy on the task. However caution is required in interpreting this effect. Although we report a significant difference between performance on trials following alpha compared to those following theta visual flicker it is important to note that no significant differences were found between regular and irregular visual flicker within alpha or theta conditions. This second result indicates that the differences in performance between trials occurring after alpha conditions and after theta conditions could not have been driven by an entrainment effect. Since irregular flicker was shown not to entrain occipital or frontal regions, the differences

between alpha and theta visual flicker cannot be attributed a functional significance. That is to say that while a difference was found, it was not driven by a functional event such as entrainment to a specific frequency.

For a number of reasons, the results of alpha visual flicker are more surprising than the behavioural results related to theta frequency flicker. Firstly, there are strong grounds on which to base the prediction that visual flicker within the alpha range would suppress visual processing. Alpha synchronisation actively diminishes the processing capacity of a specific region, and by routing information between regions in accordance with task-relevance, whilst suppressing task-irrelevant pathways establishes effective task-oriented information processing (Jensen & Mazaheri, 2010; Klimesch, Sauseng & Hanslmayr, 2007). Secondly, given that flanker stimuli are visually processed, and alpha entrainment was observed within V1 immediately prior to responses, it is reasonable to expect that processing of visual information around this time would have been slowed, and any responses made upon the information attenuated. Such entrainment effects, however, were not found within the behavioural data – at least not at a significant level. A possible interpretation of these results may be that alpha frequency flicker cannot serve to replicate (or enhance) the effects of top-down alpha suppression; and hence despite physiological entrainment of occipital sensory processing regions, there could be no subsequent behavioural modulations of performance.

Potential individual differences

It is possible that visual flicker may not affect all individuals in the same physiological manner, indeed some may be more susceptible to entrainment than others. Significant differences have previously been reported in precise frequencies of tonic (endogenous) activity (Cohen & Cavanagh, 2011). In essence, this means that differences exist across the population in the frequency at which theta-based networks may emerge; some may exhibit greater oscillatory amplitude at 4 Hz, others at 5 Hz or 6 Hz. Equally, functional-alpha activity may be expressed within sensory cortices at different frequencies within the 8-13 Hz range. For this reason, perhaps it is the case that visual flicker may have been more effective in eliciting a cognitive response if its frequency was calculated on a subject-by-subject basis. In this way, subject-specific visual flicker within the alpha and theta bands would match the endogenous (or actual) frequency exhibited during alpha suppression and theta-based network activity. Such a method may prove more effective in modulating performance than that of a more generic frequency flicker.

At the same time, investigating if theta entrainment within anterior regions occurred at subject-specific frequencies could provide a valid extension to analysis of the data. The analysis thus far cannot account for the variance in the [exact] frequency at which subjects' exhibit conflict related theta activity. Peak power in the theta band has been considered functionally relevant. It is possible that, while we found no significant entrainment of anterior structures within a 4-7Hz window, entrainment effects within frontal structures may have occurred at subject-specific frequencies.

These questions warrant further analyses on the EEG data collected during this study, however interpretation must be made in light of behavioural effects if they are to hint at the causal role of alpha and theta band oscillations during cognitive control operations.

Future research

Alongside statistical, and analytic procedures that may yet be conducted on the present data set, several changes to the design of the study may in future work prove to reveal further aspects of cognitive control.

Firstly, there are a considerable number of ways in which to present entrainment stimuli, some of which are discussed in the Introduction. This study used black and white flickering bar masking the area that flanker stimuli would subsequently occupy. Flicker occurred at either 6 Hz or 10Hz and lasted 1000ms. These parameters evoked strong increases in power and phase within the primary visual cortex, resonating at the same frequency as flicker. However, we did not see any evidence of the propagation of posterior oscillatory activity towards anterior structures. In modifications to the task, higher intensity flicker, or peripheral flicker may produce an alternate effect on cortical areas and/or cognitive processing. Equally the timing of flicker could be adjusted to coincide with the actual processing of flanker stimuli (i.e. visual masking of flanker stimuli) rather than occurring in the period immediately prior to flanker stimuli onset.

Finally, flicker could be incorporated into flanking and target stimuli in one of two ways: firstly, both target and distracting flanker letters themselves could flicker at a particular frequency (e.g. theta, alpha or gamma) – an approach similar to the one taken by this study. Alternatively, and perhaps more elaborately, distracting flanking items could be presented at alpha frequency, whilst targets could be shown at gamma or theta frequency. This method could yield behavioural results that support dissociable mechanisms involved in target enhancement (theta and gamma based) and distractor suppression (alpha based). Either design could reveal behavioural effects that could not be seen within the present study.

The methods suggested above are found within frequency-tagging protocols, in which stimuli are presented at different frequencies in order to quantify changes in the amplitude of SSVEPs as a measure of attention directed toward each stimulus-component representation. In a typical SSVEP paradigm a task relevant stimuli is tagged with a specific frequency at the time of presentation, the SSVEP of which can be tracked through EEG measurement. Scherbaum and colleagues have report use of a frequency tagging approach in a study of conflict resolution: they tagged distractor and target stimuli in a Flanker task to observe conflict adaptation effects within trials, which they conclude may contribute to trial-to-trial effects (Scherbaum, Fischer, Dshemuchadse, & Goschke, 2010). In another instance, attended stimuli were shown to elicit increased SSVEP amplitudes compared irrelevant stimuli. Changes in amplitude of SSVEP may reflect the deployment of aforementioned

alpha suppression. In this way, frequency tagging allows for mechanisms of top-down attention to be quantified in terms of the SSVEP modulation of stimulus representations (Toffanin, de Jong, Johnson & Martens, 2009).

Lastly, we may ask why is theta band activity related to cognitive control? More precisely, by what physiological mechanism does 4-7 Hz neural coherence promote cognitive functioning? The same question may be extended to alpha frequency oscillations, and their reported role in cognitive suppression. Why it is that alpha or theta band oscillations emerge to support dynamic cognitive operations remains an important question to be answered by cognitive neuroscience.

CONCLUSION

This study has illustrated neurophysiological and behavioural aspects of cognitive control expressed during performance in an interference paradigm. Our main findings are in line with work showing that multiple frontal mechanisms are engaged by detection of conflict and errors. Conflict evokes transient engagement of a cognitive control system, reflected in coordinated oscillatory activity across the MFC and lateral PFC. Medial frontal oscillatory coherence within the theta frequency band appears to mediate communication across these regions - supporting cognitive control functions responsible for adaptive adjustments in performance in the process.

Beyond reproducing typical conflict effects, a modified version of the Eriksen flanker task featuring a visual entrainment paradigm provided results into the behavioural, and neurophysiological effects of visual flicker. We report power and phase entrainment of occipital areas after regular flicker at alpha (10 Hz) and theta (6 Hz). Through additionally demonstrating that task performance following the control condition of irregular flicker did not significantly differ from the regular flicker, we may interpret the results to indicate that visual flicker does not, through entrainment of frontal regions, significantly modulate behaviour.

The relationships currently being established between oscillatory dynamics and cognitive processes provide extraordinary insight into how neural systems may operate. In the future, a more precise understanding of these mechanisms and their physiological basis may give rise to a comprehensive and holistic understanding of how the brain processes information, responds to its environment, and allows for cognitive processes to unfold seamlessly.

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Feedback-guided learning in a virtual navigation task using spatial and non-spatial cues

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ABSTRACT

An increasing body of evidence associates theta-band oscillatory activity over the medial frontal cortex with the reception of negative feedback. A model developed by Cohen et al. describes other oscillatory mechanisms that can possibly be linked to different stages of feedback-guided learning. In the current study we focused on stimulus-response association learning. We recorded high-density EEG activity during a virtual navigation task in which participants had to learn where in a maze a reward was located. Learning was based on either spatial or non-spatial cues that were supposed to be processed differently and therefore to activate a different network for stimulus processing. Consistent with previous reports, theta-band activity over medial frontal areas increased following negative feedback. Furthermore, learning based on spatial cues was associated with increased theta-phase synchrony between left temporal areas and both medial frontal and motor-response areas. Additionally, non-spatial cue learning was linked to increased theta-gamma coupling between occipital areas and both medial frontal and motor-response areas. Thus, differences in patterns of functional connectivity between medial frontal, stimulus-processing and motor-response areas allowed the dissociation of networks involved in learning based on different types of stimuli.

INTRODUCTION

Adapting one's behavior based on feedback is an essential aspect of learning. Feedback-guided learning has long been associated with the feedback-related-negativity, an event-related brain potential generated when human participants commit errors (C. B. Holroyd & Coles, 2002). An increasing body of evidence has found increases in theta-band (4-8 Hz) activity over the medial frontal cortex (MFC) to be associated with learning from negative feedback (Luu, Tucker, & Makeig, 2004; Trujillo, 2007; van de Vijver, Ridderinkhof, & Cohen, 2011). Furthermore, in an experiment by van de Vijver et al. (van de Vijver et al., 2011) it was shown that the strength of this theta-band activity was predictive of the performance on the consecutive trial.

A model developed by Cohen et al. (Cohen, Wilmes, & van de Vijver, 2011) describes other oscillatory mechanisms that can possibly be linked to different stages of feedback-guided learning. The model's core predictions include that learning stimulus-response associations is accompanied by an increase in synchrony between stimulus-processing and/or motor-response areas. Additionally, these changes in synchronization are driven by a top-down influence of prefrontal cortical regions in a frequency band specific manner: theta-band specific medial frontal, and beta-band specific ventromedial oscillations accompanying learning from negative and positive feedback, respectively. Furthermore, these changes in inter-area synchronization between prefrontal, motor-response and/or stimulus-processing areas are reflected within subjects (changes during learning) and across subjects (individuals who learn better show stronger synchronization). In the current study, we focused on within subject changes in inter-area functional connectivity between medial frontal, stimulus-processing and motor-response areas following negative feedback.

According to the model described above (Cohen et al., 2011), learning stimulus-response associations can be measured by

increased functional connectivity between stimulus-processing and/or motor-response areas. Subsequently, it should be possible to distinguish between patterns of activity accompanying learning based on information derived from different kinds of stimuli. Learning to navigate through an environment can depend on egocentric and allocentric navigational strategies. An egocentric strategy depends on a response associated with environmental features (landmarks), whereas allocentric strategies are based on a cognitive map that incorporates the spatial relations among different features (Livingstone-Lee et al., 2011). Where the latter method makes use of spatial properties of the scene, the former method can be based upon simple association learning (e.g. turn right upon viewing a certain stimulus). In the current paradigm, we dissociated between learning based on allocentric and egocentric cues by using spatial and non-spatial stimuli, respectively.

In humans (Cornwell, Johnson, Holroyd, Carver, & Grillon, 2008), as well as animals (Benchenane et al., 2010), goal-directed navigation (in an allocentric setting) has been associated with theta-band activity in medial-temporal structures, as opposed to aimless movement through the environment. When 'navigation' (stimulus-response learning resembling egocentric navigation) depends on non-spatial (e.g. color) cues, visual areas in the occipital cortex are expected to show the strongest response (PLENDL et al., 1993; Zeki & Marini, 1998). Visual stimulus processing has moreover previously been associated with gamma-band activity in the primary visual areas (Eckhorn, Frien, Bauer, Woelbern, & Kehr, 1993; Martinovic & Busch, 2011). Therefore, we expected to be able to dissociate between patterns of activity accompanying feedback learning based on spatial (allocentric strategy) and color (egocentric strategy) cues. We furthermore expected this dissociation likely both between brain regions processing the different stimuli and between frequency-band specific activity. Whereas navigation through a virtual environment has been associated with theta-band activity over both left (Cornwell et al., 2008; Ekstrom et al., 2005; Epstein, 2008; Hassabis et al., 2009; Marsh et al., 2010) and right (Baker & Holroyd, 2009; Ekstrom et al., 2005; Epstein, 2008; Hassabis et al., 2009; Jacobs et al., 2010; Marsh et al., 2010) temporal cortices, we expected gamma-band activity (Eckhorn et al., 1993; Martinovic & Busch, 2011) over occipital areas (PLENDL et al., 1993; Zeki & Marini, 1998) during color processing.

In the current study, subjects navigated through several virtual mazes based on information derived either from spatial or colored cues while their neural activity was recorded using high-density EEG measurements. Both navigational approaches can be described as a form of reinforcement learning, altering the decision which way to turn after negative feedback. Therefore, the learning of stimulus-response associations should be influenced by medial frontal feedback mechanisms. The learning of these associations may be indicated by an increase of synchronous activity between stimulus and motor areas, and stimulus-processing and motor-response areas could furthermore be influenced in a top-down fashion by prefrontal regions.

In this experiment the first goal was to replicate previous findings showing increases in theta-band activity over medial frontal areas after negative feedback (Luu et al., 2004; Trujillo, 2007; van de Vijver et al., 2011). Additionally we expected the magnitude of this activity to be predictive of the performance on the consecutive trial (van de Vijver et al., 2011). Further we wanted to test the following hypotheses: first, we predicted that learning stimulus-response associations goes together with increases in synchrony between stimulus-processing and motor-response areas. To investigate this hypothesis, we first determined the areas that are most responsive to the different stimuli, and subsequently measured the synchronous activity with motor channels. Second, as learning from feedback seems to be regulated in a top-down fashion by prefrontal regions (Cohen, 2009; Cohen, Ridderinkhof, Haupt, Elger, & Fell, 2008; Nigbur, Cohen, Ridderinkhof, & Stürmer, 2011), we expected that the medial frontal cortex executes top-down influence on both stimulus-processing and motor-response areas. Measuring functional connectivity, using inter-site phase synchrony and cross-frequency coupling, between the MFC, stimulus-processing and motor-response areas, tested this hypothesis.

METHODS

Participants

Eighteen right-handed subjects (2 men; mean age = 21.8, range = 17-23 years) with normal or corrected to normal vision participated in this experiment either for course credits or a monetary reward (€ 21). They signed informed consent documents that were approved by the local ethics committee before participation. Data from six subjects were excluded from analysis. One subject did not give enough incorrect responses, this left too few trials in specific conditions to analyze (one condition only counted 15 trials). The removed data from the five remaining excluded subjects showed excessive artifacts, either due to movement or technical difficulties.

Task

Participants performed a reinforcement-learning task, in which they had to navigate through different virtual mazes (figure 1). The task consisted of 14 blocks of each 80 trials. Within each maze a colored square was shown. Subjects had to focus their attention on one type of stimulus (maze/color) as indicated at the beginning of a block of trials. In every block, the subjects had to learn to associate 4 of the (relevant) stimuli with a left-handed and the other 4 with a right-handed response, corresponding to the left and right direction in the maze.

At the start of a trial, the subjects were placed at the beginning of a virtual maze. Participants had 1200ms to give a left or right response with their left and right index finger, respectively. After a 500ms delay they automatically navigated forward and subsequently a left or right turn was made (739ms). After another 500ms delay either positive (+) or negative (×) feedback was presented for 1000ms. If the participant did not respond within 1200ms, the colored square turned red, indicating the need for a faster response. After an inter-trial-interval of 800ms, the next trial started.

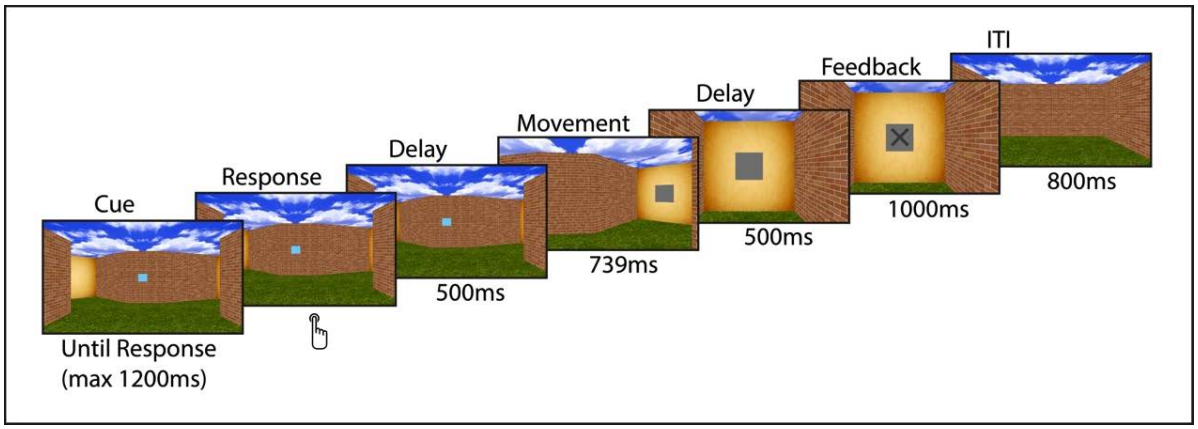


Figure 1. Sequence of evens during one example trial. Subjects had to attend to either the color of the square or to the spatial layout of the maze. Participants responded either with their left or right index finger, corresponding to the left and right direction in the maze.

The task consisted of 1120 trials. Self paced rest breaks were given after each block. Participants performed at least 2 training blocks, one with maze and one with color as the relevant stimulus dimension, to familiarize them with the task. Altogether, including setup, the experiment lasted approximately 3 hours.

Stimuli

For every block, 8 different mazes and 8 different colors were pseudorandomly chosen out of a total of 24 mazes and 24 colors. All mazes and colors (each 24) were randomly divided over every 3 subsequent blocks of trials. All mazes were variations on the standard T-maze, both the left and right arm of the maze could have one of 4 angles (0, 33.3, 66.6 or 90˚) with reference to the starting position. The colors were all shades of blue and green.

A pseudorandom combination of a maze and a color were used in each trial, the presentation of the stimuli was restricted in that every one of the stimuli of the relevant and irrelevant dimension was presented before the same stimulus was shown again. This ensured that every possible combination of stimuli occurred at least once and at most twice during a block of trials.

Signal recording and processing

EEG data were recorded at 1000Hz using a high density EGI amplifier from 256 electrodes. Further preprocessing and analysis were conducted using the EEGLAB toolbox (Delorme & Makeig, 2004) in Matlab (The MathWorks, Natick, MA). Offline, the data were downsampled to 250Hz, high-pass filtered at 0.5Hz, and a bandpass filter from 49 to 51Hz was applied to decrease line-noise. Subsequently, the data were epoched from -4.5 to 3 seconds surrounding the feedback. All trials were visually inspected and those containing artifacts not related to blinks were removed from the data.

All data were re-referenced to the average of the combined signal from all the electrodes. After independent component analysis, the components containing either blinks or noise were manually selected and removed from the data. Subsequently, the data were then Current Source Density (CSD) transformed (Kayser & Tenke, 2006) to reduce the effects of volume conduction and increase spatial selectivity.

Experimental Conditions and trial selection

In order to analyze the effects that post-feedback activity patterns might have on learning stimulus-response associations, trials were separated into eight different conditions. Both the success (correct/ incorrect) on the current trial and the success on the consecutive trial were of interest. This amounts to four conditions per type of stimulus (maze/color): correct trials followed by correct trials (CC), correct trials followed by incorrect trials (CI), incorrect trials followed by correct trials (IC) and incorrect trials followed by incorrect trials (II). To correct for any effects that a different number of trials across conditions can have, the same number of trials was used for every condition. The minimum number of trials in any condition determined the number of trials used in all condition. Trials from the remaining conditions were selected by matching their reaction times to those of the trials from the smallest condition. This was done by first calculating the Z-value for the reaction time of every trial. Next we matched the Z-values of the trials in the smallest condition to the Z-values of the trials in the remaining conditions. If the minimal amount of trials in any condition was under 40, remaining conditions were left with 40 trials. The remaining trials used were those with the smallest Z-values, those that deviate as little as possible from the mean of the distribution.

After exclusion of trials with artifacts, the number of trials (SD) per condition (maze/color) was 51.25/51.25 (13.31/13.31) for correct followed by correct trials, 50.33/48.83 (14.22/15.54) for correct followed by incorrect trials, 51.25/51.25 (13.31/13.31) for incorrect followed by correct trials, and 51.08/50.25 (13.46/14.51) for incorrect followed by incorrect trials. The minimal amount of trials for any subject in any condition was 28.

Time-frequency analysis

All data analysis were performed using custom written Matlab scripts (Cavanagh, Cohen, & Allen, 2009; Cohen et al., 2008). Oscillatory dynamics were extracted by multiplying the power spectrum (obtained from the fast Fourier transform) of single-trial feedback-locked data with a family of complex Morlet wavelets, defined as:

$$e^{-2i\pi ft} \cdot e^{\frac{-t^2}{2\sigma^2}}$$

where *t* is time and *f* is frequency, which increased from 2 to 90 Hz in 50 logarithmically spaced steps, and then taking the inverse fast Fourier transform. σ defines the width of each frequency band and was set according to $x/2\varpi f$, where x increased from 3 to 12 in the same number of steps.

From the resulting complex signal, we extracted estimates of frequency-band specific power and phase angles values. Power is defined as the modulus of the resulting complex signal $Z(t)$ $[real(Z(t)^2 + imag(Z(t)^2]$. Power values were baseline corrected and converted to a logarithmic scale by a decibel transformation: $power_{dB} = 10 . \log_{10} [Z(t) / Z(baseline)]$, with a cue-locked -500 to -200ms pre-cue baseline. This corrects for the 'power law' stating that power at low frequencies is intrinsically higher than higher frequencies, and therefore allows one to make valid comparisons across frequency bands. Phase angle values were used to compute inter-trial phase coherence (ITPC) and inter-site phase synchrony (ISPS), and is defined as ϕ_t=arctan (imag[z(*t*)] / real[z(*t*)]). ITPC reflects the consistency of phase values and is defined as:

$$ITPC = \left| \frac{1}{n} \cdot \sum_{t=1}^{n} e^{i\phi\, t} \right|$$

where *j* and *k* are two different electrodes. Cue-locked -500 to -200ms pre-cue, condition specific baselines were subtracted from the inter-trial phase coherence and inter-site phase synchrony. This removes any tonic differences in the signal between conditions, which allows for a valid comparison of phasic differences. Both measures vary between 0 and 1, where 0 means no phase consistency and 1 reflects perfect consistency of phase values. Note that for ISPS, the phase values do not have to be equal; there can be a phase lag between two electrodes as long as the latency is consistent over trials.

Using cross-frequency coupling (CFC), we determined whether fluctuations in power of higher frequencies were nonuniformly distributed over the phase of slower oscillations (Canolty et al., 2006; Cohen & van Gaal, 2012). Specifically, we calculated the phase values of medial frontal areas (channel 8) in the theta-band (defined as a wavelet with a center frequency of 6 Hz) and power values ranging from 40 to 90 Hz (20 logarithmically spaced frequencies). Subsequently, we determined whether higher power values were nonuniformly distributed over lower frequency phase:

$$\sum_t \left| \frac{a_t e^{-i\theta t}}{n} \right|$$

where *a* are power values, θ are phase values, and *t* are time points. We computed CFC-values from a 700ms temporal window surrounding one time point, 400ms post feedback (surrounding the maximal error-response). A nonuniform distribution of power over phase can occur in the absence of CFC. To control for this, a non-parametric step is required in order to test the likelihood of each time-frequency cross-frequency coupling value occurring due to chance. For every condition and every time-frequency-electrode value we performed permutation testing with 500 iterations in which the power time series was cut randomly and the second part of the time series was placed before the first part, and the modulation was recomputed. In this procedure the power and phase time series were preserved, but shuffled with respect to each other. Permutation testing leaves a distribution of (permuted) CFC values, the actual CFC value was recomputed as the normalized distance away from this distribution. This procedure results in Z-values, which can be used in regular parametric statistics. For a more detailed account on the method see (Cohen & van Gaal, 2012).

Statistical analysis

To ensure participants indeed learned the stimulus-response associations we divided the blocks of 80 trials into 10 bins of 8 trials. We compared the average amount of correct responses in the first bin to those in the last bin for both maze and color blocks. For this comparison we defined a 2 × 2 repeated measures ANOVA as (Type of stimulus) × (Bin number).

For the first goal of this experiment, we focused on the role of medial frontal theta oscillations in learning. Here stimulus specific patterns of activity are not of interest yet. Therefore, conditions were averaged over maze and color blocks, leaving us with conditions CC, CI, IC and II. Statistical analyses were performed on theta-band power and ITPC over medial frontal areas (channel 8).

To analyze patterns of activity accompanying learning stimulus-specific associations we decided that we would separate trials into four conditions: current correct and incorrect trials for both maze and color blocks. This decision was made because the predictive effects of MFC theta magnitude after errors on the performance on consecutive trials that were found by van de Vijver et al. (2011) could not be replicated in the current study. Furthermore, the next-success effects that were found over frontopolar and lateral prefrontal areas showed contradicting results. ITPC did show learning effects in line with previous results (van de Vijver et al., 2011), whereas power was greater on next-incorrect trials, challenging these learning effects. For these stimulus specific learning patterns we made additional use of ISPS and CFC measures.

For power, ITPC and ISPS the signal was averaged in the theta-band (4-8 Hz). Subject-specific condition averaged peaks were detected in a 100-700ms time window following feedback. This peak-finding procedure allows for individual specific patterns of brain activity. Average peak values from a 100ms time window (± 50ms from the peak) were entered into 2 × 2 repeated measures ANOVAs. To analyze the effect of theta magnitude on learning the ANOVAs were defined as (Current Success) × (Next-trial success), for stimulus specific learning effects the ANOVAs were defined as (Type of stimulus) × (Current Success).

In order to be able to analyze response related learning effects, we created two extra channels, one for contralateral and one for ipsilateral motor activity, named Comot and Ipmot, respectively. We selected channels 65 and 182 to represent response related

sensorimotor activity. Motor channel activity for trials on which the response was given with the contra-/ipsilateral hand was selected and concatenated to form Comot/Ipmot. The repeated measures ANOVAs were defined as (Current Success) × (Motor channel) to analyze theta-magnitude over motor channels and ISPS with medial frontal areas. To analyze stimulus-specific motor activity a 2 × 2 × 2 repeated measures ANOVA was defined as (Type of stimulus) × (Current Success) × (Motor channel).

All channels used in the analyses, other than channel 8 (MFC) and 65 and 182 (motor channels), were selected based on visual inspection of the topographical activity maps. Statistical analyses were only applied after selection of the channels.

RESULTS

Behavioral results

Participants showed an increase in the average amount of correct responses per 8 stimuli over the time course of blocks of trials (figure 2). The number of correct responses was significantly higher in the last bin of 8 trials compared to the first bin ($F(1,11) = 109.147$, $p<0.001$). Learning effects did not differ between maze and color blocks ($F(1,11) = 1.793$, $p = 0.210$). These results demonstrated a learning effect over the time course of blocks of trials, and additionally that there were no differences in the learning of stimuli between maze and color blocks.

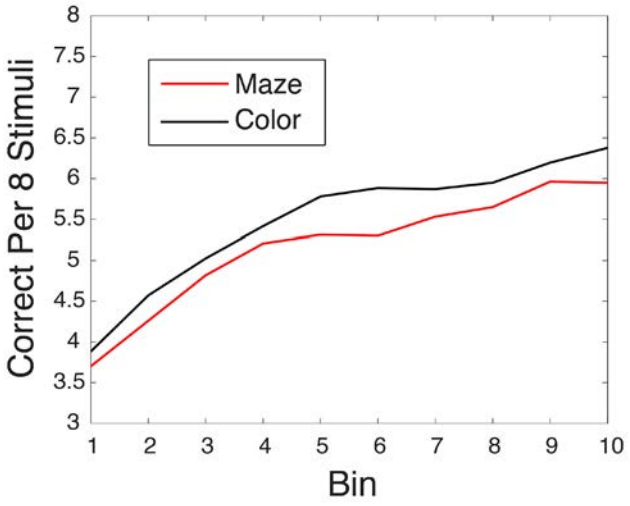


Figure 2. Average behavioral performance. Per block of 80 trials subjects were required to learn the stimulus-response relationship for 8 different stimuli. The y-axis represents the average amount of correct responses per 8 stimuli. The x-axis represents the time course of the block, the number of the 8-trial bin.

Theta-band oscillations at medial prefrontal scalp sites

Both medial frontal (channel 8) theta power ($F(1,11) = 9.069$, $p = 0.012$) and ITPC ($F(1,11) = 9.830$, $p = 0,009$) were larger for incorrect compared to correct trials (figure 3). There were no power ($F(1,11) = 0.123$, $p = 0.732$) or ITPC ($F(1,11) = 1.538$, $p = 0.241$) differences for next-success, nor did we find any interaction effects for power ($F(1,11) = 0.007$, $p = 0.934$) or ITPC ($F(1,11) = 1.670$, $p = 0.223$). In further exploratory analysis based on the topographical maps in figure 3 and 4, we examined the theta activity patterns of frontopolar (channel 21), lateral prefrontal (channel 30, 40, 41 and 42) and right temporal (channel 74, 75, 83 and 84) regions.

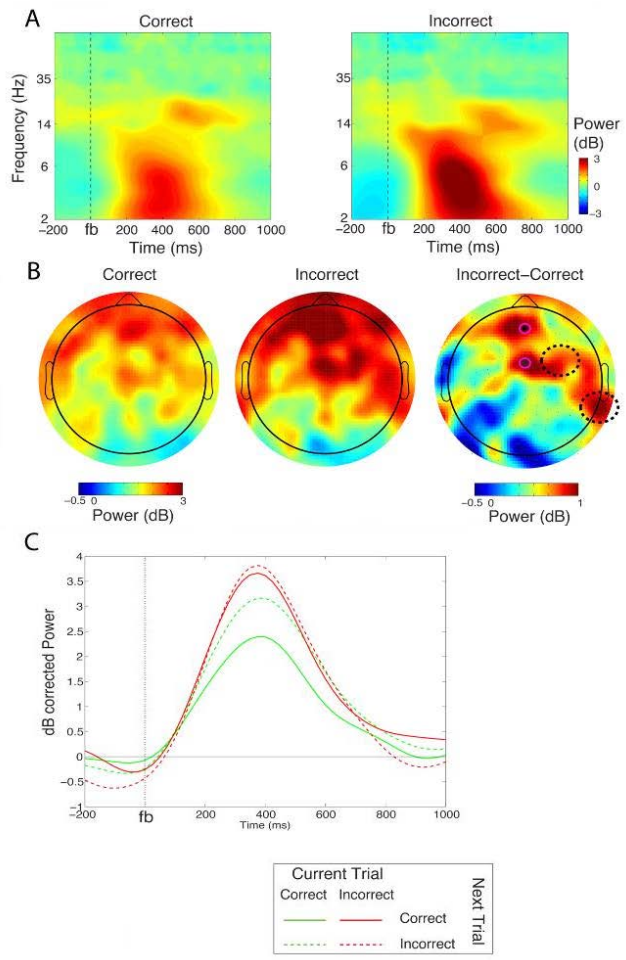


Figure 3. Post-feedback theta (4-8 Hz) power. (A) Time-frequency representations of post-feedback (fb) power at channel 8 (MFC) for correct and incorrect trials. (B) Topographical plots of theta power 200-400ms post-feedback. From left to right: correct trials, incorrect trials and their difference. In the difference plot several channels are highlighted: 2 single channels (purple highlight): channel 8 (medial frontal) and 21 (frontopolar), and 2 groups of channels (dashed circle): channels 30, 40, 41 and 42 (lateral prefrontal) and channels 74, 75, 83 and 84 (right temporal). (C) Averaged theta power for current and next incorrect and correct trials over channel 21 (frontal).

First we found that frontopolar areas showed higher theta power ($F(1,11) = 11.957$, $p = 0.005$) and higher ITPC ($F(1,11) = 34.679$, $p<0.001$) for incorrect versus correct trials. Power was also higher for next-incorrect compared to next-correct trials ($F(1,11) = 8.958$, $p = 0.012$), whereas ITPC showed a significant interaction effect ($F(1,11) = 5.512$, $p = 0.039$). Follow-up t tests revealed that theta ITPC increased more for next-correct compared to next-incorrect trials on current incorrect trials. This was demonstrated by executing a t test comparing the difference score between IC and CC with the difference score between II and CI, IC-CC compared to II-CI. Second, lateral prefrontal areas showed greater theta power on incorrect versus correct trials ($F(1,11) = 7.433$, $p = 0.020$), and power was also larger for next-incorrect compared to next-correct trials ($F(1,11) = 6.175$, $p = 0.030$). Furthermore, lateral prefrontal ITPC was marginally higher for current incorrect ($F(1,11) = 3.356$, $p = 0.094$) and next incorrect ($F(1,11) = 3.572$, $p = 0.084$) trials. Finally, right temporal areas showed greater power for incorrect versus correct trials ($F(1,11) = 6.783$, $p = 0.024$). ITPC measures over these areas were only marginally greater on current incorrect

trials (F(1,11) = 3.609, p = 0.084), and there were no effects of next-success. Additionally, ISPS between MFC and frontopolar, lateral prefrontal and right temporal areas did not differ with current or next-trial accuracy.

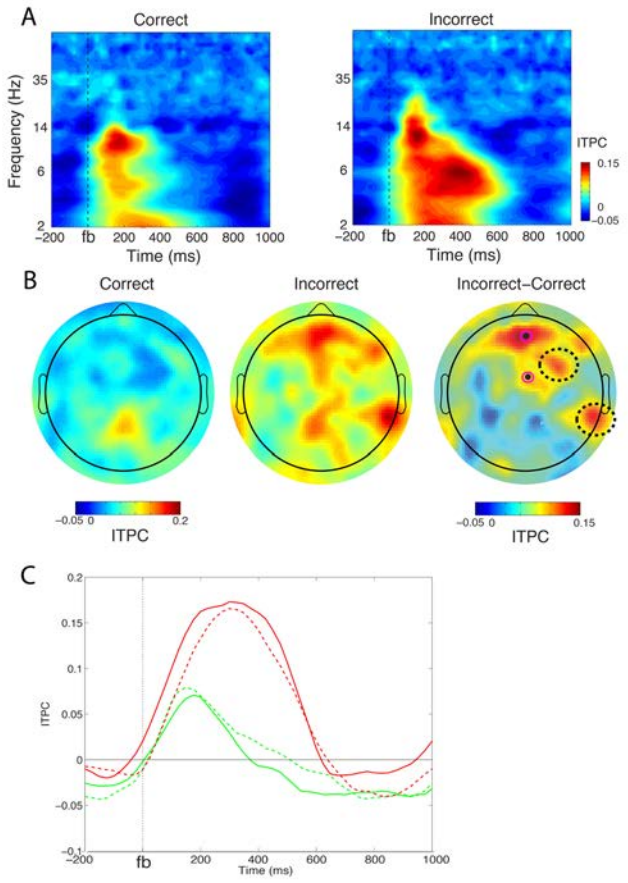


Figure 4. Post-feedback theta (4-8 Hz) ITPC. (A) Time-frequency representations of post-feedback (fb) ITPC at channel 8 (MFC) for correct and incorrect trials. (B) Topographical plots of theta ITPC 200-400ms post-feedback. From left to right: correct trials, incorrect trials and their difference. In the difference plot several channels are highlighted: 2 single channels (purple highlight): channel 8 (medial frontal) and 21 (frontopolar), and 2 groups of channels (dashed circle): channels 30, 40, 41 and 42 (lateral prefrontal) and channels 74, 75, 83 and 84 (right temporal). (C) Averaged theta ITPC for current and next incorrect and correct trials over channel 21 (frontal).

Thus post-feedback theta power was greater on incorrect compared to correct trials over medial frontal, frontopolar, lateral prefrontal and right temporal areas. This same effect was found for medial frontal and frontopolar ITPC. Furthermore, frontopolar areas showed a greater increase in theta ITPC for next-correct compared to next-incorrect trials on current incorrect trials, whereas frontopolar power was greater on next-incorrect trials. Lateral prefrontal areas additionally showed greater power and stronger ITPC for next incorrect trials.

Revealing stimulus-processing areas

In order to locate the brain areas that represented the different kinds of visual stimuli, we compared topographical maps of maze and color conditions for power in both the theta (4-8 Hz) and the gamma band (40-90 Hz), and for ISPS in the theta band using motor and medial frontal areas as seeds (Figure 5).

Visual inspection of these maps led us to further analyze several areas of interest. Two pools of occipital channels (channels 106, 114 and 115, and channels 124, 125, 137, 138, 149 and 150) seemed to show greater theta power on maze and color trials, respectively, these differences were however not significant (figure 5A). Subsequently, examination of gamma-band topographical maps (figure 5B) did not reveal any specific stimulus-processing areas.

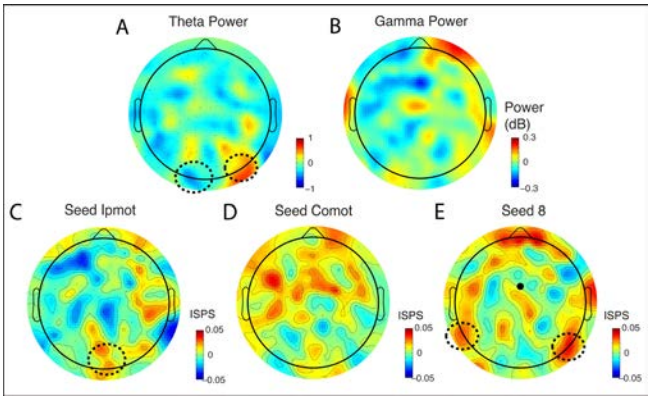


Figure 5. Post-feedback topographical maps of the differences between maze and color (maze>color) trials in (A) Theta power (4-8 Hz), the right highlighted occipital area covers channels 106, 114 and 115 ; whereas the left covers channels 124, 125, 137, 138, 149 and 150 (B) Gamma power (40-90 Hz), (C) ISPS with seed channel Ipmot, the occipital highlighted area covers channels 125, 136 and 137 (D) ISPS with seed channel Comot, and (E) ISPS with seed channel 8, the right highlighted occipital area covers channels 95, 96 and 115; whereas the left temporal area covers channels 170, 171, 178 and 179.

Learning stimulus-response mapping should be associated with increases in synchrony between stimulus-processing and motor areas. Therefore, areas involved in the processing of the stimuli could be defined based on topographical maps with motor seed channels (figure 5C and 5D). Only one occipital area covered by channels 125, 136 and 137 seemed to show greater ISPS on maze trials with channel Ipmot, yet this did not turn out significant.

Stimulus-processing areas could show functional connectivity with medial frontal areas after feedback, therefore we additionally examined topographical ISPS (figure 5E) and CFC (figure 7A) maps with a medial frontal seed channel. Maze shows marginally stronger ISPS on a right occipito-temporal pool of channels (95, 96 and 115) compared to color (F(1,11) = 4.132, p = 0.067). ISPS at this location is furthermore stronger on incorrect compared to correct trials (F(1,11) = 5.543, p = 0.038). There was no interaction between stimulus type and accuracy. A similar effect is seen over a left temporal pool of channels (170, 171, 178 and 179). This area shows marginally stronger ISPS for maze (F(1,11) = 4.189, p = 0.065) compared to color, and ISPS was stronger for incorrect compared to correct trials (F(1,11) = 5.514, p = 0.039) (figure 6A). Again there was no interaction effect. Based on this topographical map (figure 5E), no clear areas show stronger ISPS activity for color compared to maze conditions.

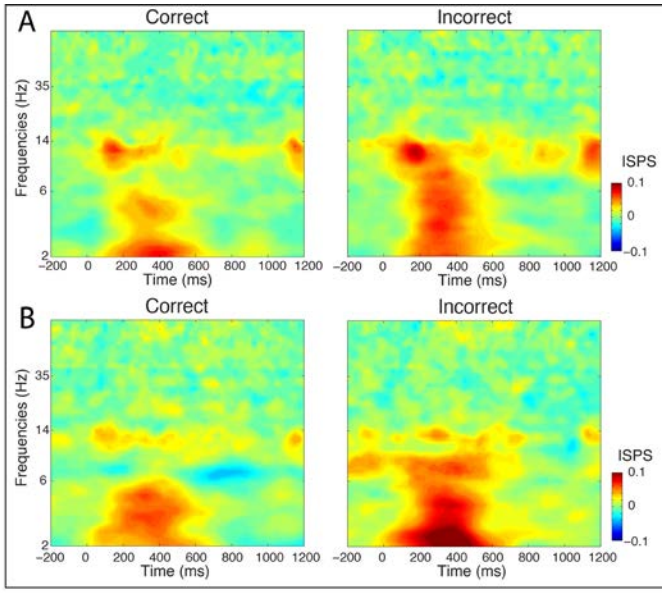
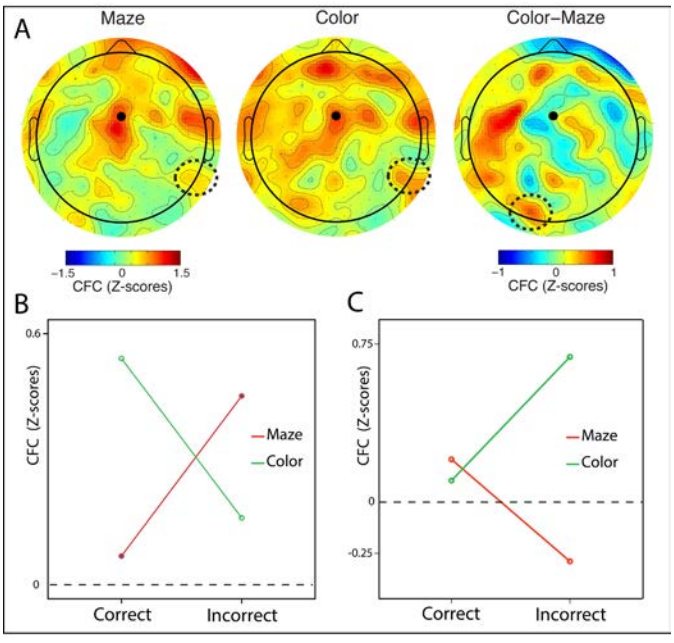


Figure 6. Time-frequency representations of intersite phase synchrony between a pool of channels 170, 171, 178 and 179 and (A) channel 8 for correct and incorrect trials, and (B) channel Comot for correct and incorrect trials.

Based on the topographical maps of differences in CFC between color and maze conditions (figure 7A), we further examined the CFC patterns for an occipital group of channels (channel 138, 149, 150). This group of channels showed greater CFC on color compared to maze trials ($F(1,11) = 5.300$, $p = 0.042$). A significant interaction effect ($F(1,11) = 16.500$, $p = 0.002$) indicated that CFC increased only for incorrect trials in the color condition (figure 7C). Follow up t tests confirmed that CFC values differed significantly between maze and color for incorrect trials ($t(1,11) = -3.442$, $p = 0.006$), but not for correct trials ($t(1,11) = 0.612$, $p = 0.553$). A right temporal area (figure 7A, channel 74, 83 and 85) seems to show CFC on both maze and color trials, furthermore an overlapping area also showed involvement in error processing (figure 3B and 4B). Examining the CFC condition differences more closely (figure 7B) revealed an interaction effect for stimulus type and accuracy ($F(1,11) = 6.290$, $p = 0.029$). Follow up t tests confirmed that the CFC increased on incorrect compared to correct trials for maze stimuli whereas it decreased on incorrect compared to correct trials for color stimuli.



In summary, two (occipito-) temporal areas demonstrated more functional connectivity with the MFC for maze compared to color stimuli (figure 5E), and one occipital area showed more connectivity with MFC for color compared to maze stimuli (figure 7A). Connectivity between MFC and the (occipito-) temporal areas was also larger on incorrect compared to correct trials (figure 6A), whereas connectivity between the MFC and the occipital area was only larger for color than maze stimuli on incorrect trials (figure 7C). An additional right temporal area showed stronger connectivity for color compared to maze stimuli on correct trials while the connectivity was stronger for maze compared to color stimuli on incorrect trials.

Learning indicated by synchrony with motor-response areas

Theta band ISPS between medial frontal areas and motor channels increased marginally on incorrect versus correct trials ($F(1,11) = 3.692$, $p = 0.081$). There were however no differences between channels (Ipmot and Comot) nor an interaction effect.

Based on the topographical maps of ISPS with motor channels (figure 5C and 5D) no clear stimulus-processing areas were revealed. However, now that we have found stimulus-processing areas that seem involved in forming stimulus-response associations, we can test for connectivity between motor and stimulus-processing areas. Of interest are a left temporal and a right occipito-temporal area for maze stimulus processing, an occipital area for color stimulus processing and a right temporal area which showed an interaction effect for stimulus type and accuracy.

Theta-band ISPS between motor channels and the left temporal pool of channels was larger on incorrect compared to correct trials ($F(1,11) = 5.906$, $p = .033$), ISPS was also larger for Comot compared to Ipmot ($F(1,11) = 6.106$, $p = 0.031$) (figure 6B). This was however not specific for the maze conditions as there were no interaction effects. The same analyses for both the right occipito-tempral pool of channels associated with maze stimuli processing and the right temporal pool of channels yielded no significant differences. ISPS with the occipital pool of channels revealed an interaction effect of type of stimulus × current success × motor channel ($F(1,11) = 5.796$, $p = 0.035$). Follow up t tests revealed that ISPS was larger for channel Ipmot on correct trials for color compared to maze stimuli. Thus the left temporal areas associated with processing maze stimuli, and the occipital areas associated with learning from color stimuli also show functional connectivity with motor areas.

Figure 7. Overview of cross-frequency coupling (CFC) data. The non-uniform distribution of gamma power (40-90 Hz) over medial frontal (channel 8) theta-phase (6 Hz) (A) Topographical maps of CFC, highlighted in all figures are medial frontal areas (channel 8). From left to right: maze, color, and their difference. The right temporal areas highlighted in the maze and color maps are covered by channels 74, 83 and 84 (see figure 7B). The occipital areas highlighted in the difference map, are covered by channels 138, 149 and 150 (see figure 3C). (B) Average CFC between channel 8 and channels 74, 83 and 84 (highlighted in maze and color topographical maps). The y-axis represents normalized CFC in Z-scores. The x-axis shows correct and incorrect conditions. (C) Average CFC between channel 8 and channels 138, 149 and 150 (highlighted in the topographical maps of the difference between color and maze). The y-axis represents normalized CFC in Z-scores. The x-axis shows correct and incorrect conditions.

DISCUSSION

In the current study we investigated the role of medial frontal, sensorimotor and stimulus-processing areas in a feedback learning paradigm. Whereas we did find increases in theta-magnitude following negative feedback, the predictive effects of MFC theta magnitude on the performance on consecutive trials that were found by van de Vijver et al. (2011) could not be replicated in the current study. As hypothesized, specific stimulus-processing areas show involvement in learning stimulus-response associations. Two (occipito-) temporal areas show greater functional connectivity with the MFC for maze compared to color stimuli, and one occipital area showed more connectivity with MFC for color compared to maze stimuli. Of these areas the left temporal area associated with maze stimulus processing and the occipital area associated with color processing also show functional connectivity with motor areas.

Theta-band oscillations after negative feedback

Consistent with previous findings (Cavanagh et al., 2009; Cohen et al., 2008; Luu et al., 2004; Trujillo, 2007), we demonstrated medial frontal increases in theta-band oscillatory activity after negative feedback. These dynamics seem to signal undesirable outcomes and possibly the need for a behavioral adjustment. Additionally, frontopolar, lateral prefrontal and right temporal regions also showed increased theta-band activity after negative feedback. Whereas lateral prefrontal areas are more often associated with the signaling of increased need for cognitive control (Cavanagh et al., 2009; van de Vijver et al., 2011), frontopolar and right temporal areas are less frequently associated with error processing.

Frontopolar theta-band activity has previously been linked to uncertainty-driven exploration (Cavanagh, Figueroa, Cohen, & Frank, 2011; Daw, O'Doherty, Dayan, Seymour, & Dolan, 2006), with a direct relationship between increasing uncertainty of the chosen option and EEG power (Cavanagh et al., 2011). Others coupled frontopolar activity to top-down response-selection processes between multiple conflicting representations (Koechlin & Summerfield, 2007). In the current paradigm, task demands were high as stimuli only showed subtle differences. With increasing perceptual demands, active top-down processing of the stimuli was required in order to perform on the task. Increases in frontopolar activity on incorrect compared to correct trials can be interpreted as an increase in task demands when we assume that, in general, the differentiation between stimuli is more difficult (more uncertain) on incorrect trials.

Right temporal lobe activity has previously been associated with memory processes (Anderson, Rajagovindan, Ghacibeh, Meador, & Ding, 2010), attention and working memory processes (Raghavachari et al., 2001), and navigation through virtual environments (Cornwell et al., 2008; Ekstrom et al., 2005). Here, power effects were not related to spatial or non-spatial stimuli, but to the current succes on the trial. This effect is thus most likely associated with (working) memory processes.

Van de Vijver et al. (2011) demonstrated that medial frontal theta dynamics do not only signal the need for behavioral adjustments, its magnitude is also predictive of the performance on consecutive trials. Over the MFC we did not find the same results, yet we did find next-succes effects over frontopolar and lateral prefrontal areas. These results are however inconsistent. Frontopolar and lateral prefrontal theta power is stronger on next-incorrect compared to next-correct trials. This contradicts possible learning effects found by van de Vijver et al. (2011). Furthermore, frontopolar areas showed a greater ITPC increase on current incorrect trials for next-correct compared to next-incorrect trials. In light of reinforcement learning, this could indicate a learning effect on current incorrect trials. However, because of the inconsistent results, no definite conclusions can be drawn about the importance of the magnitude of frontopolar and lateral prefrontal theta-band activity for feedback based learning in the current paradigm.

Top-down influence on stimulus-processing and/or motor-response areas

In this experiment, we investigated specific predictions proposed by Cohen et al. (2011). We examined if learning stimulus-response associations is paired with increases in synchronous activity between stimulus-processing and motor-response areas. Additionally we tested if learning stimulus-response associations is accompanied by increases in functional connectivity between stimulus-processing, motor-response and medial frontal areas. We demonstrated increases in functional connectivity between the MFC and stimulus-processing areas after negative feedback. Furhtermore, we can dissociate between patterns of activity corresponding to learning based on spatial (maze) or non-spatial (color) cues (figure 8).

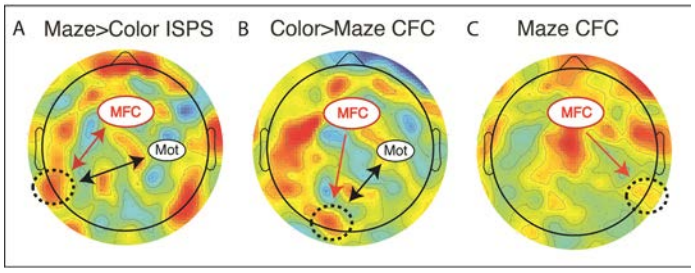


Figure 8. Overview of topographical maps of functional connectivity between medial frontal (MFC), stimulus-processing (dashed circle) and motor-response (mot) areas. Functional connectivity is indicated by arrows between areas. Note that CFC is indicated by a unidirectional arrow, assuming directional connectivity(A) theta-band ISPS with medial frontal seed channel for maze - color stimuli. (B) theta-gamma coupling with medial frontal seed channel for color – maze stimuli, (C) theta-gamma coupling with medial frontal seed channel for maze stimuli.

Learning based on maze stimuli

Human spatial navigation has previously been associated with both left (Cornwell et al., 2008; Ekstrom et al., 2005; Epstein, 2008; Hassabis et al., 2009; Marsh et al., 2010) and right (Baker & Holroyd, 2009; Ekstrom et al., 2005; Epstein, 2008; Hassabis et al., 2009; Jacobs et al., 2010; Marsh et al., 2010) lateralized activity, assumed to be located over (para)hippocampal areas. The precise localization of these areas differs over studies, task demands and measurement techniques.

We found two areas (left-temporal and right occipito-temporal)

that showed marginally greater ISPS with the MFC when participants attended to maze compared to color stimuli. Consistent with previous accounts (Benchenane et al., 2010; Cornwell et al., 2008) these condition differences were found in the theta-band. ISPS for both these areas also showed increases on incorrect compared to correct trials. However, only left temporal areas did subsequently show an effect in ISPS with contralateral motor areas on incorrect compared to correct trials. The left temporal area thus seems to be involved in top-down mediated stimulus-processing and stimulus-response associations (figure 8A). Considering the marginally significant effects, it has to be kept in mind that these results are based on the data of twelve subjects. After testing more particiapants it will become clear whether these areas are in fact important in maze stimulus-processing and stimulus-response learning.

Left lateralized actvity in navigation tasks has been related to context-dependent episodic memory (Burgess, Maguire, & O'Keefe, 2002), goal directed navigation, particularly in learning a new environment's spatial layout (Cornwell et al., 2008), and to the use of verbal strategies(Frings, Wagner, Quiske, et al., 2006a). Moreover, in a spatial memory task, women showed left lateralized hippocampal activity compared to a right lateralization in men, additionally woman reported the use of verbal strategies (Frings, Wagner, Unterrainer, et al., 2006b). In the current study, of the 12 participants included in the analyses, 10 were women. One explanation for finding left lateralized effects is thus either the participation of mostly female subjects and/or the use of verbal strategies. In line with this train of thought, it is possible that instead of 'navigating', subjects remembered a maze stimulus (an episodic memory, a shape or a verbalisation). Consistently, patients with lesions in the left hippocampal formation showed deficits in both episodic memory as well as verbal tasks, whereas the right hippocampus was related to visuo-spatial processing (Bohbot et al., 1998).

Whether the left temporal areas were recruited when using a verbal strategy or for navigational purposes, we demonstrated increases in synchronous activity with medial frontal areas on maze compared to color trials that were also higher on incorrect compared to correct trials. Note that we cannot infer a top-down recruitment of stimulus-processing areas by the MFC, for that we would additionally need to compute a directional measurement of functional connectivity (e.g. granger causality). These areas furthermore showed increased synchrony with contralateral motor areas on incorrect compared to correct trials. These patterns of activity sugest underlying mechanisms for top-down mediated stimulus-repsonse learning.

Learning based on color stimuli

Gamma band activity has been associated with a wide variety of cognitive processes (Herrmann, Fründ, & Lenz, 2010), for instance visual stimulus processing (Eckhorn et al., 1993; Martinovic & Busch, 2011), visual attention (Martinovic & Busch, 2011; Taylor, 2004) and visual short term memory (Tallon-Baudry, Bertrand, Peronnet, & Pernier, 1998). Attending to the color, more than the shape or velocity of a stimulus, evokes enhanced activity in specific extrastriate regions of the visual cortex, with a larger left lateralized effect (Corbetta, Miezin, Dobmeyer, Shulman, & Petersen, 1990).

In the current study we found a left lateralized occipital area showing stronger theta-gamma coupling with medial frontal areas for color compared to maze stimuli (figure 7). This effect was furthermore only stronger on incorrect compared to correct trials. As hypothesized, processing color stimuli elicits gamma-band activity over occipital areas. Furhtermore, theta-gamma coupling may be related to amongst others working memory processes (Lisman, 2005; Lisman & Buzsaki, 2008). In the current study, the theta-phase is determined top-down by medial frontal areas, mediating gamma activity in stimulus-processing areas. Thus, theta-gamma coupling wherein the MFC executes top-down influence on visual areas may thus be the mechanism by which learning color stimulus associations takes place. Additionally, this area showed larger ISPS with ipsilateral motor areas for color compared to maze stimuli on correct trials, suggesting that this connection is important in stimulus-response learning (figure 8B).

Learning based on maze/color stimuli

A right temporal areas was also found to show increases in functional connectivity with the MFC on incorrect compared to correct trials for maze stimuli, whereas for color stimuli a decrease was found. As mentioned above, this area has previously been associated with (working) memory processes (Anderson et al., 2010; Raghavachari et al., 2001). In animal research theta-gamma coupling involving the hippocampus has been found strongest at the moment of decision making in a T-maze task (Tort et al., 2008). In humans, theta and gamma activity, as well as theta-gamma coupling, over medial temporal structures has been associated with visuo-motor learning (Perfetti et al., 2011). In navigation tasks, the co-occurrence of theta and gamma-band activity over temporal and parietal areas has been interpreted in light of coupling between areas previously associated with allocentric and egocentric navigation (White, Congedo, Ciorciari, & Silberstein, 2011). Altough we established theta-gamma coupling between the MFC and temporal areas (figure 8C), this might be part of a larger network associated with egocentric and allocentric navigation. This could explain the dissociation between the strength of the functional connectivity in learning based on color (egocentric) and maze (allocentric) cues.

Increased functional connectivity and learning from negative feedback

After negative feedback, prefrontal areas, particularly medial frontal areas, show the need for behavioral adjustments. In order to optimize behavior, the representation of the correct action, upon the presentation of a specific stimulus, has to be established and updated. Cohen et al. (2011) proposed that synchronous activity between medial frontal areas, stimulus-processing and/or motor-response areas is fundamental to stimulus-response learning. We demonstrated that the MFC recruits specific stimulus-processing areas after negative feedback through theta-band ISPS and theta-

gamma coupling for spatial and color cues, respectively. These findings indeed support the above-mentioned proposed mechanism for top-down influence on stimulus-processing during feedback-guided learning (Cohen et al., 2011) as well as the idea that memory processes depend on synchrony between brain areas (Fell & Axmacher, 2011).

Increases in functional connectivity between stimulus-processing and motor-response araes upon learning were however less clear. One of the model's core predictions include that learning stimulus-response associations is accompanied by an increase in synchrony between stimulus-processing and motor-response areas. Whereas we did find increases in inter-site phase synchrony with motor areas for both color and maze stimulus-processing areas, these did not show a clear pattern for learning stimulus-response associations, as synchrony was only found after negative feedback. Our focusing on theta-band asscociations between these areas might explain the lack of finding this dissociation. For future analysis/experiments looking into functional connectivity between stimulus-processing and motor-response areas, it might prove fruitful to focus on a wider range of frequency-bands.

CONCLUSION

In the current study we found learning-related increases in functional connectivity between medial frontal and stimulus-processing areas. We demonstrated this for two types of stimuli that activate different networks for stimulus processing. Medial frontal areas seem to execute top-down influence on these stimulus-processing networks after negative feedback, signalling the need for behavioral adjustments.

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Automatic learning of morphology-based associations in Dutch

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Word processing necessarily involves the use of acoustic or orthographic information together with the activation of semantic representations of the observed word. Psycholinguistic studies also point to the existence of phonological and morphological levels of processing, although the boundaries of these levels have not been well defined. The focus of this research is to evaluate a task that probes the capacity of Dutch native speakers to process information specifically at the morphological level in an automatic and unconscious way. Crucially, only morphological, but not semantic or orthographic information, must be used in the task in order to learn the association. Results show that indeed subjects were able to learn the association between morphological information and appropriate response. Furthermore, the time at which stimuli were presented indicates that the processing of morphological information must have occurred early in word processing, and probably did so automatically and unconsciously. Possible future research is proposed to continue perfecting the method to assess morphological processing.