

Exploring the temporal dynamics of the spatial working memory *n*-back task using steady state visual evoked potentials (SSVEP)

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The neural networks associated with spatial working memory (SWM) are well established. However, the temporal dynamics of SWM-related brain activity are less clear. This study examined changes in temporal neurophysiology during the spatial *n*-back task using steady state probe topography (SSPT) to record cortical steady state visual evoked potentials (SSVEPs) at 64 scalp locations. Twenty healthy male volunteers participated in the study. The findings identified three different time periods of significance during the spatial *n*-back task—an early perceptual/encoding period (approximately 0–500 ms), an early delay period just following the stimulus disappearing from view (approximately 850–1400 ms), and a late period lasting the final second of the delay and anticipation of the new stimulus (approximately 2500–3500 ms). The delay period was associated with increases in frontal and occipital region amplitude, consistent with previous findings in more basic working memory tasks. The two different SSVEP components during the delay appear reflective of the additional “executive” demands associated with the *n*-back and may suggest variable roles for the PFC during different stages of the delay. All three *n*-back levels demonstrated a relative consistent electrophysiological profile, indicating that this pattern is specific to the spatial *n*-back task. Nevertheless, these findings supported the hypothesis that memory load modulates activity within the networks identified, consistent with previous neuroimaging studies. The current findings may offer a framework in which to further investigate the temporal aspects of SWM.

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Introduction

Working memory refers to the short-term maintenance and manipulation of an active representation of information for future processing or recall (Baddeley, 1992). It is a critical building block for higher cognitive functions such as language, planning, and problem solving. Working memory has become one of the most studied concepts within cognitive neuroscience, due largely to the fact that working memory deficits are cardinal in a number of psychiatric disorders including schizophrenia, in which such deficits are commonly associated with poor functional outcomes (Green et al., 2000; Manoach, 2003).

Neuroimaging studies have been useful in uncovering the neural networks associated with working memory. In non-human primates, electrophysiological single cell recordings have identified individual neurons within the dorsolateral PFC (dlPFC) which show elevated persistent and tuned activity (so called “memory fields”) during the spatial working memory delayed-response (DR) task, a task requiring retention of information over a delay followed by simple recall (for a review, see Goldman-Rakic, 1996). Similarly in humans, neuroimaging studies have revealed activation of the lateral PFC in addition to more posterior brain regions during the DR task (for reviews, see Courtney et al., 1998; Wager and Smith, 2003). Human neuroimaging studies have also examined the neural networks associated with the more complex *n*-back task, which includes additional demands such as manipulation, sustained attention and constant updating of working memory and is also designed so memory load can be parametrically manipulated whilst keeping other task demands constant (Gevins and Cutillo, 1993; McEvoy et al., 1998). Studies using PET and fMRI have provided evidence that the spatial *n*-back task activates a distributed network of regions, including the dlPFC, posterior parietal cortex, and anterior cingulate (Owen et al., 2005). Evidence has further demonstrated that working memory

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load results in increased activation generally within the working memory networks (Braver et al., 1997; Cohen et al., 1997).

Neuroimaging studies have also attempted to delineate the neural networks associated with sub-processes of working memory. For example, fMRI studies have been able to separate the encoding, maintenance, and response periods of the DR task, demonstrating that the PFC and a number of posterior brain regions are activated during all three periods albeit to different degrees (i.e. Courtney et al., 1998; Haxby et al., 2000). Similar examination of the *n*-back task is made more complex as there is no clear dissociation between different sub-processes of the task, in addition to the fact that the *n*-back task requires global task-related processes due to its continuous nature (such as sustained attention and retention of goals). Nevertheless, studies have provided evidence to suggest that the dorsolateral PFC is more likely recruited by “executive functions” involved in complex working memory tasks (i.e. the *n*-back), with the ventrolateral PFC more important for simple storage tasks (for reviews, see Courtney et al., 1998; D’Esposito et al., 1998; Owen et al., 1999; Owen, 2000; Wager and Smith, 2003).

While fMRI and PET studies have given insight into the possible differentiation of working memory sub-processes, particularly in the PFC, they are limited by their temporal resolution to detect dynamic sub-second changes in neurophysiology. Electrophysiological (EEG) recording remains the highest temporal resolution neuroimaging technique. The steady state visual evoked potential (SSVEP), elicited by a task-irrelevant 13 Hz visual flicker, is a particularly useful evoked potential technique for studying cognitive processes. The SSVEP has high temporal resolution (in the order of hundreds of milliseconds), enhanced signal to noise ratio above that of standard EEG techniques, and has previously demonstrated reliable and specific topographic changes during cognitive tasks (i.e. Silberstein et al., 1990, 1995, 1998, 2000a,b, 2001, 2003; Kemp et al., 2002; Gray et al., 2003; Perlstein et al., 2003; Kemp et al., 2004). SSVEP signals are characterised by changes in amplitude and phase (latency) components which are reflective of the neuronal activity within pyramidal cells of the neocortex (Silberstein et al., 1995, 2001). The amplitude of SSVEP is a function of the number of pyramidal cells firing in synchrony with the visual 13 Hz flicker. SSVEP phase reflects changes in latency between the SSVEP signal and the 13 Hz flicker and is indicative of the physiological delay between stimulus and response waveforms. These latency changes have been suggested to reflect summed changes in synaptic transmission time related to post-synaptic excitation or inhibition processes (Silberstein et al., 1995, 2000b). Whilst PET and/or fMRI yield important information related to the location of neural activity via changes in rCBF or BOLD signal (the assumed haemodynamic correlate of neural activity) (i.e. see Arthurs and Boniface, 2002), it is unclear whether these changes index increased excitation, inhibition, or both. The ability to observe rapid changes in excitatory and inhibitory processes as measured by SSVEP latency is a significant advantage of the SSVEP technique, specifically in light of evidence in non-human primates that inhibition of neurons within the frontal cortex is important in establishing the “memory fields” used to hold information online (for a discussion, see Goldman-Rakic, 1996).

The SSVEP associated with the delayed-response (DR) task has been examined by two separate laboratories (with differing SSVEP methodology). These studies demonstrated that the delay of a DR task is associated with increases in SSVEP amplitude within the

PFC (Silberstein et al., 2001; Perlstein et al., 2003) and occipital–parietal sites (Silberstein et al., 2001), in addition to a reduction in latency in these same regions (Silberstein et al., 2001). SSVEP data have also been observed to distinguish between the delay and encoding component of a DR task. Whilst the delay period was associated with amplitude increases, early perceptual processes (or “encoding”) were associated with SSVEP amplitude *reductions* at prefrontal sites (Silberstein et al., 2001). These amplitude reductions appear analogous to the transient reduction in spontaneous alpha activity event-related desynchronisation associated with increased vigilance (Pfurtscheller and Aranibar, 1977; Pfurtscheller and Klimesch, 1990).

To date, there has been no study of the temporal characteristics of the *n*-back task using SSVEP. Therefore, the current study used SSVEP to examine the electrophysiological profile of the spatial *n*-back task, with 3 levels of difficulty (1-, 2-, and 3-back version). The first hypothesis was that, consistent with the DR task, the *n*-back task would reveal a different SSVEP profile in the early perceptual/encoding component and the delay component. We further hypothesised that the delay period would reveal amplitude increases consistent with previous findings in the DR task. However, the main aim of this study was to examine the temporal characteristics of the delay period of the spatial *n*-back task. Finally, we hypothesised that amplitude and latency changes within the PFC and parietal cortex would show memory load effects, consistent with previous neuroimaging studies (Braver et al., 1997; Cohen et al., 1997).

Methods

Participants

Twenty young healthy males (mean age \pm standard deviation = 22.9 ± 6.4 years) participated in the study. All participants were right-handed, as assessed by the Edinburgh Inventory (Oldfield, 1971), nonsmokers, and medication-free for at least 1 month prior to testing. Medical and psychiatric screening comprised an initial telephone screening (including administration of the Prime-MD, Pfizer, 1996) and a consequent semi-structured clinical assessment with a physician. Exclusion criteria included history of neurological or psychiatric disorders (including history of depression or anxiety disorders in first degree relatives), chronic physical illness, medication and/or drug use, or excessive alcohol consumption. The human research ethics committee, Swinburne University of Technology approved the study. Participants were recruited via advertisements on university notice boards and websites, and all participants gave written informed consent.

Procedure

All participants were asked to refrain from alcohol for 24 h prior to the study. On the day of testing, participants were asked to refrain from consuming caffeine, to consume a small breakfast before 10:30 am, and to arrive at the laboratory at 12:30 pm. All participants were provided a standardised lunch of two slices of toast with jam and one small glass of orange juice on arrival, given approximately 1.5 h prior to testing.

Participants were seated approximately 2.5 m from a computer monitor in a dimly lit soundproofed room and were fitted with a lycra electrode cap comprising of 64 monopolar leads, positioned

according to the international 10/20 system. Nose and linked ear electrodes were used for ground, and impedance on all electrodes was generally below 5 k Ω . Participants were then fitted with a set of modified half-mirrored goggles, which superimposed the 13 Hz white light flicker over the visual field to elicit the SSVEP. The visual flicker subtended a horizontal angle of 160° and a vertical angle of 90° and had a modulation depth of 45% when viewed against the background. Recorded brain electrical activity was band-pass-filtered from 0.74 to 74 Hz and digitised at a rate of 500 Hz with 16-bit accuracy, consistent with previous studies (i.e. Silberstein et al., 1998, 2001, 2004; Kemp et al., 2002, 2004; Gray et al., 2003).

Participants had attended a pre-study training session on a previous occasion to familiarise themselves with the task. On the day of testing, participants completed subsequent practice following electrode cap setup, completing each task twice under SSVEP conditions to familiarise themselves with the flicker. During testing, task *n*-back order (visuo-motor control, 1, 2, or 3 back) was quasi-randomised. Before each *n*-back was performed, participants completed a small (1 min) practice of that *n*-back to ensure that they were completing the correct task. Following this small practice, the subject completed 2 sets of 40 trials per *n*-back level (i.e. a total of 80 trials per *n*-back), with a small break in between. This was completed for each *n*-back (control, 1, 2, or 3 back), and testing was completed within 1 h.

Spatial n-back task

The *n*-back task is a measure of SWM with a sustained attention component (Gevins and Cutillo, 1993; McEvoy et al., 1998). This version of the *n*-back task was developed for this experiment using Pipscript software (which provides millisecond accuracy in stimulus presentation and response recording) and has also been administered in other studies (e.g. Green et al., 2005). The task involved the presentation of a series of white dots on a black background (with a central white fixation cross). Each stimulus comprised of one dot, presented for 500 ms with inter-stimulus intervals of 3000 ms. Each dot could appear in any one of 60 locations (taken from a square matrix of 8 dots across \times 8 dots down, with the four middle dots missing to allow for the central fixation cross). Participants were required to indicate whether each dot was in the same location as the dot “*n*-back” (either 1-back, 2-back, or 3-back, depending on task instructions). For each *n*-back level, 80 responses were elicited. Versions of the task were created for each *n*-back level and control task. Each *n*-back level had the same proportion of “matching” responses (40%) and “non-matches” (60%). Of the non-matching responses, 10% were “false alarm” incorrect matches (i.e. 2-back in a 1-back task), 20% were “close non-matches” (defined as a non-match within a 4-dot proximity of the stimulus), and 30% were “far non-matches” (defined as a non-match at least 4 dots away from the stimulus in all directions). Response was forced choice and made with a button box with thumbs resting on right and left buttons; a “yes” (right button response) was required for matches and “no” (left button response) was required for non-matches. The control task was designed to match motor and visual aspects of the 1-, 2-, and 3-back task and was used as a visuo-motor control. The control task comprised of a visual equivalent (but alternative) version of the 1-, 2-, and 3-back task presentation with 80 stimuli, in which participants alternated responses between the right button (“yes” response) and left button (“no” response) regardless of the

position of the stimulus, which closely approximated the motor responses of the 1-, 2-, and 3-back tasks (i.e. *n*-back tasks: 40% “yes” response/60% “no” response). Memory load analysis was conducted by comparing the 1-, 2-, and 3-back tasks directly.

Steady state probe topography (SSPT) signal processing

The principal features of SSPT signal processing have been described previously (Silberstein et al., 1990, 1995). Briefly, Fourier analysis was employed to extract the SSVEP from the brain electrical data for each electrode by calculating the 13 Hz Fourier coefficients (FC) for each stimulus cycle. The FC time series was smoothed by averaging overlapping blocks of 10 FCs. Each electrode within each task was checked individually for artefact, as described previously (Silberstein et al., 1995). Previous research has demonstrated the SSVEP to be resistant to electro-myographic (EMG) noise contamination (Gray et al., 2003).

SSVEP data analysis

Epochs of 3.5 s (1 epoch = 1 trial = stimulus display + delay) were extracted from the SSVEP for each task. The amplitude and phase (inverse of latency) of the SSVEP were normalised for each participant by subtracting the average amplitude or phase (as appropriate) for all electrodes from each electrode time series. Following normalisation, individual participants’ epochs were averaged to create a cross-subject epoch (of each electrode) for each task condition (control, 1-, 2-, and 3-back). The subtraction design used in this study involved subtracting the *mean* SSVEP amplitude and latency recorded during the control task from the *time series* SSVEP amplitude and latency recorded during each activation task (1-, 2-, and 3-back), at each electrode, for all correct trials. Due to the faster button-press response times (and associated temporal changes in SSVEP) during the control task, the mean amplitude and latency of the control task are used in the subtraction to avoid confounding results. SSVEP phase variations are presented in millisecond (ms) latencies: (change in phase/ $2\times\pi$) \times (1000/13).

Presentation of SSVEP data

For each subtraction, cluster maps of Hotellings *T* statistics, amplitude difference, and latency difference were generated for the entire epoch (*x* axis) and displaying all electrodes (*y* axis), as described previously (Gray et al., 2003; Kemp et al., 2004). This is done to reduce the probability of type 1 error as a number of randomly distributed type 1 error would be expected within point-wise *t* tests within a 3.5-s epoch, whilst examination of cluster plots increases the likelihood of a robust effects through identification of consecutive statistical spatiotemporal clusters (Guthrie and Buchwald, 1991; Murray et al., 2002; Gray et al., 2003; Kemp et al., 2004). Electrodes (presented on the *y* axis of the cluster plots) are compartmentalised into regions approximately associated with frontal (electrodes 0–20, including Fp1, Fp2, F7, F3, Fz, F4, and F8), centro-parieto-temporal (electrodes 21–52, including T3, C3, Cz, C4, T4, T5, P3, Pz, P4, and T6), and occipital (electrodes 53–63, including O1, Oz, and O2) regions. Based on these cluster plots, time periods of significance were selected for subsequent generation of topographical Hotellings *T* statistical maps and difference maps.

Statistical analysis

Based on evidence from spatial principal components analysis, the SSVEP forms 5 independent factors, and therefore adjustment of Hotellings T statistic P values (2-tailed) by a division of 5 is employed to correct for multiple comparisons (Silberstein et al., 1995). A threshold of $P < 0.05$ corrected for multiple comparisons was employed for all primary analyses. Memory load effects for electrodes within the defined working memory region were afforded a less stringent uncorrected threshold of $P < 0.05$. Statistics are reported as corrected unless otherwise stated in text.

Behavioural data were analysed using repeated measures ANOVA within the statistical package for the social sciences (SPSS Inc., Chicago, IL). Examination of the relationships between change in SSVEP (between control and each n -back level) and corresponding change in performance was examined using Pearson's product moment correlation coefficient.

Results

Behavioural data

Accuracy and reaction time (latency) data are shown in Fig. 1. As expected, repeated measures analysis of variance (ANOVA) revealed that memory load significantly affected both accuracy [$F(1.5,28.5) = 79.31$, $P < 0.001$, Greenhouse–Geisser adjusted] and reaction time [$F(1.7,31.4) = 83.78$, $P < 0.001$, Greenhouse–Geisser adjusted]. Planned comparisons revealed a significant increase in reaction time and decrease in accuracy between each increment in memory load [control vs. 1-back: accuracy $F(1,19) =$

25.6, $P < 0.01$, reaction time $F(1,19) = 89.2$, $P < 0.01$; 1- vs. 2-back: accuracy $F(1,19) = 71.1$, $P < 0.01$, reaction time $F(1,19) = 82.1$, $P < 0.01$; 2- vs. 3-back: accuracy $F(1,19) = 32.8$, $P < 0.01$, reaction time $F(1,19) = 9.1$, $P < 0.01$].

SSVEP data

Task-related differences in amplitude and latency were calculated by subtracting the mean activation of the control task from the time series of each n -back (1-, 2-, and 3-back tasks). The resulting time series differences are presented in Fig. 2 as cluster plots [time (epoch of 3.5 s) \times electrodes] for the amplitude, latency, and associated Hotellings T statistics of each n -back. Warmer colours indicate SSVEP amplitude and latency reductions relative to baseline, with cooler colours representing relative SSVEP increases.

Fig. 2 shows an overall consistency between the 3 n -back contrasts, with 3 major clusters of significance corresponding to changes in both amplitude and latency. Consistent with previous research (Silberstein et al., 2001), an early component was observed whilst the stimulus was displayed (0–539 ms), which differentiated from the delay component. Two further clusters were observed during the delay period; an early, shorter cluster (847–1386 ms) and a later more sustained cluster (2541 ms–3500 ms). In order to examine the activations within these 3 clusters, we generated mean topographical maps for all three time clusters/epochs, shown in Fig. 3.

Fig. 3a displays the first epoch (0–539 ms). This time period encompasses the perceptual encoding aspect of the task in which the stimulus is still visible. The most prominent significant difference was observed within frontal electrodes, which was associated with bilateral reduction in both amplitude and latency. Significant changes were also evident in temporal regions (with a bias to the left hemisphere), which appear driven by latency reductions. However, these temporal changes in latency did not significantly differ between hemispheres ($P > 0.05$). Changes in amplitude in parieto-occipital electrodes were evident but only reached statistical significance in the 3-back task (relative to control).

Fig. 3b displays the second epoch (847–1386 ms), encompassing the early delay period. The most dominant visual feature of this epoch was amplitude increases in the frontal region, significant at prefrontal regions in all n -back conditions (relative to control). A pattern of increased latency was observed within fronto-temporal regions, with an associated reduction in latency in more posterior temporo-parieto-occipital regions, which was dominant in the left hemisphere. This is most statistically robust in the 2-back condition, although it reached significance at temporal sites in the 1-back and in parieto-occipital electrodes in the 3-back task. Significant changes were also observed in the left temporal region, with both amplitude and latency reductions evident.

Fig. 3c displays the third epoch (2500 ms–3500 ms), encompassing the last second of the delay. This final epoch revealed a similar SSVEP pattern to that observed within the first epoch (0–539 ms), with latency and amplitude reductions associated with significant changes in frontal electrodes. Occipital amplitude increases were also evident but only reached significance at the 3-back level.

Memory load analysis

To examine the effect of increasing memory load on SSVEP amplitude and latency, a direct examination of the differences

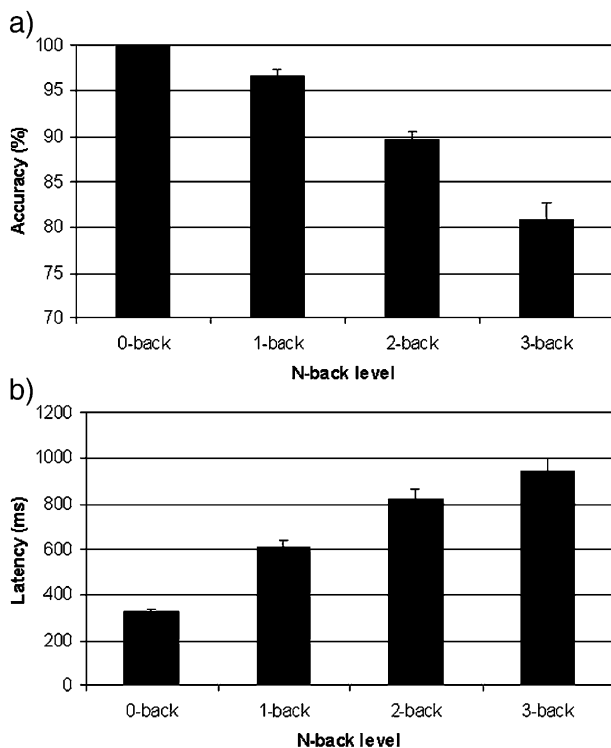


Fig. 1. (a) Mean accuracy (percentages correct) and (b) and mean reaction time for all trials (latency) for all levels of the spatial n -back. Error bars represent the standard error of the means.

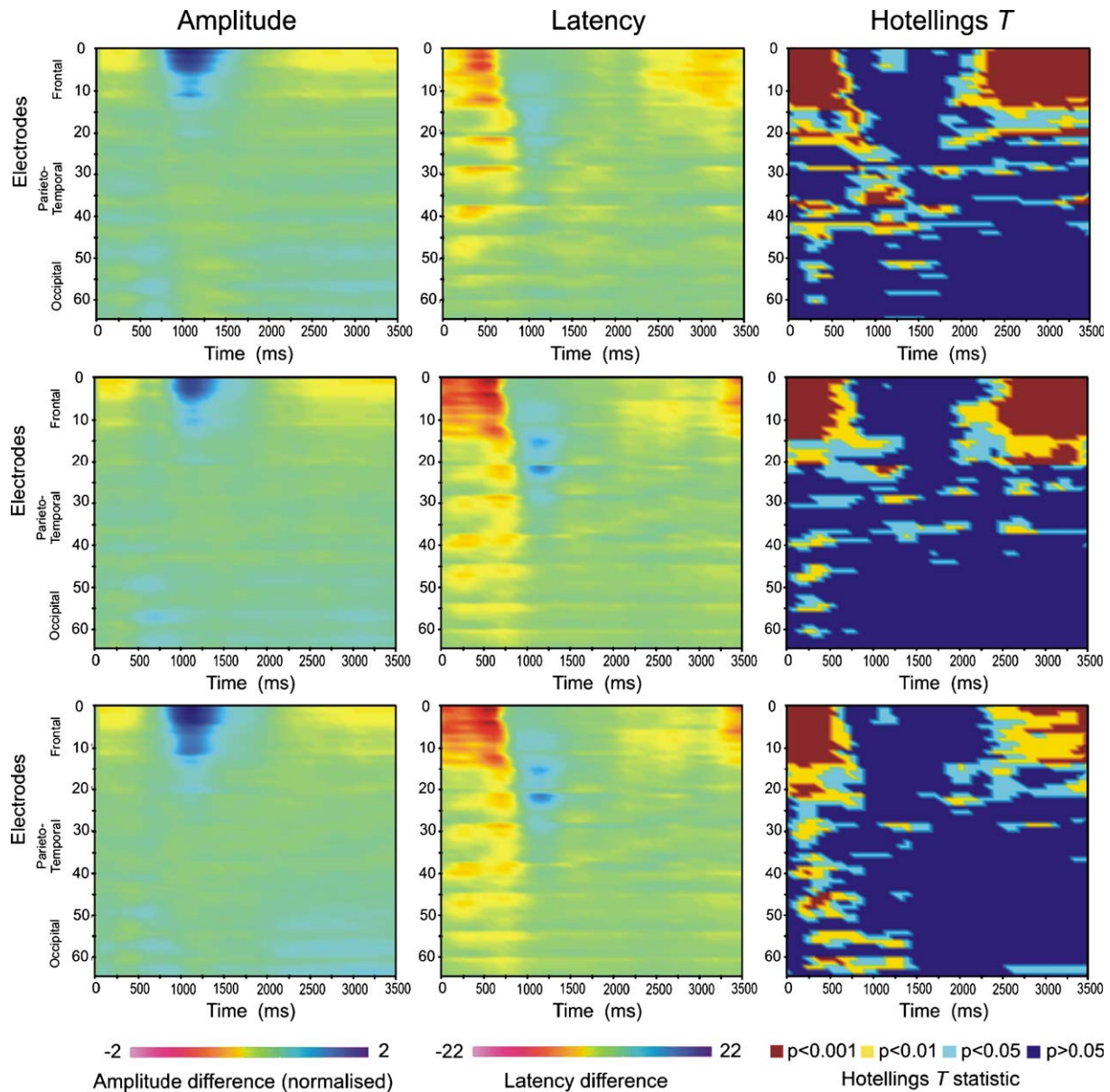


Fig. 2. Cluster plots of amplitude, latency, and Hotellings T values for the 1-back (top row), 2-back (middle row), and 3-back (bottom row) for all electrodes (y axis) over time (x axis). Warmer colours represent both reductions in amplitude and latency relative to control task. Hotellings T values are corrected for multiple comparisons (see scale for corresponding P values).

between the 1- and 2-back, and 2- and 3-back task was conducted. Fig. 4 shows the electrodes which significantly differed with memory load increases, for the domain (latency or amplitude) which primarily contributed to the significant difference. With consideration of the probability of type 1 error over 64-point wise t tests (i.e. 64 electrodes) per comparison (i.e. 2 comparisons: 1-back vs. 2-back, and 2-back vs. 3-back), effects were reported for regions in which a cluster of electrodes reached significance (Guthrie and Buchwald, 1991; Murray et al., 2002; Gray et al., 2003; Kemp et al., 2004). Within the first epoch, there was evidence of a U-shaped pattern of activation between n -backs in latency effects within the frontal area. The 2-back task showed a greater reduction in amplitude than both the 1- or 3-back task, although this only reached significance for the 2-back vs. 1-back difference (electrodes 6 and 10, $P < 0.05$ uncorrected). Similarly,

memory load had a non-linear effect on occipital amplitude, with a lower amplitude in the 2-back compared to the 1-back task (electrode 61, $P < 0.01$ uncorrected, electrode 62, $P < 0.05$ uncorrected), and higher amplitude in the 2-back compared to the 3-back task (electrodes 56, 57, 62, 63, $P < 0.05$ uncorrected).

In contrast to the first (and indeed the third) epoch, memory-load-related effects in the second epoch appear driven primarily by SSVEP latency changes. In the frontal region, there was a greater reduction in latency in the 3-back task compared to the 2-back (electrodes 0, 1, 5, $P < 0.01$ uncorrected, electrodes 2, 3, 4, 6, $P < 0.05$ uncorrected). In the left temporal region, there was an apparent inverted U effect in the magnitude of latency reduction. The 2-back task showed significantly greater latency increase than the 1-back (electrodes 21, 22, $P < 0.05$ uncorrected) and 3-back task (electrodes 21, 29, $P < 0.01$ uncorrected; 22, 28 corrected, $P <$

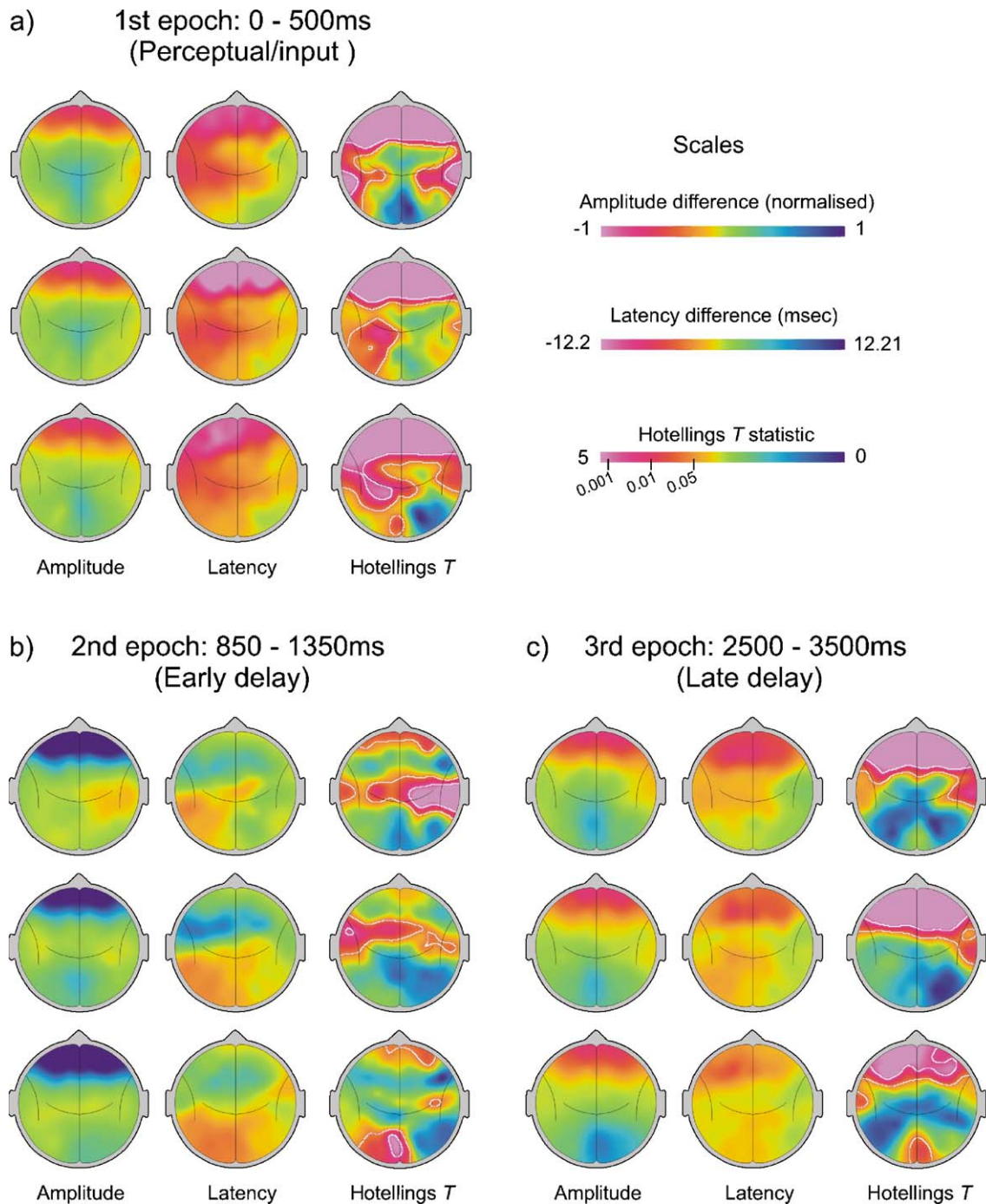


Fig. 3. Topographical SSVEP maps of amplitude, latency, and Hotellings T values for the 1-back (top row), 2-back (middle row), and 3-back (bottom row), relative to the control task. Three significant time periods (epochs) are presented: (a) period whilst the stimulus is present (epoch 1: 0–500 ms), (b) early in the delay period (epoch 2: 850–1350 ms), and (c) last second of the delay period (epoch 3: 2500–3500 ms). Warmer colours represent both reductions in amplitude and latency relative to control task and larger T values in the Hotellings maps (see scale for corresponding P values).

0.05 uncorrected). However, in the right occipital region, latency reduction increased linearly with increased memory load, although this only reached significance between the 2- and 3-back tasks (electrodes 61, 62, $P < 0.05$ uncorrected).

In the third epoch, significant differences were only observed between the 2- and 3-back tasks. There was a significant attenuation of latency reduction frontally between the 2- and 3-back task (electrodes 0, 3, 4, 5, 6, 10, $P < 0.05$ uncorrected), in

addition to evidence of load related increases in amplitude in the right occipital region (electrodes 57, 63, $P < 0.01$ uncorrected, electrodes 58, 61, $P < 0.05$ uncorrected).

Correlation between SSVEP and performance

The possible correlation between SSVEP and performance was examined over the entire epoch (46 time points of 77 ms duration =

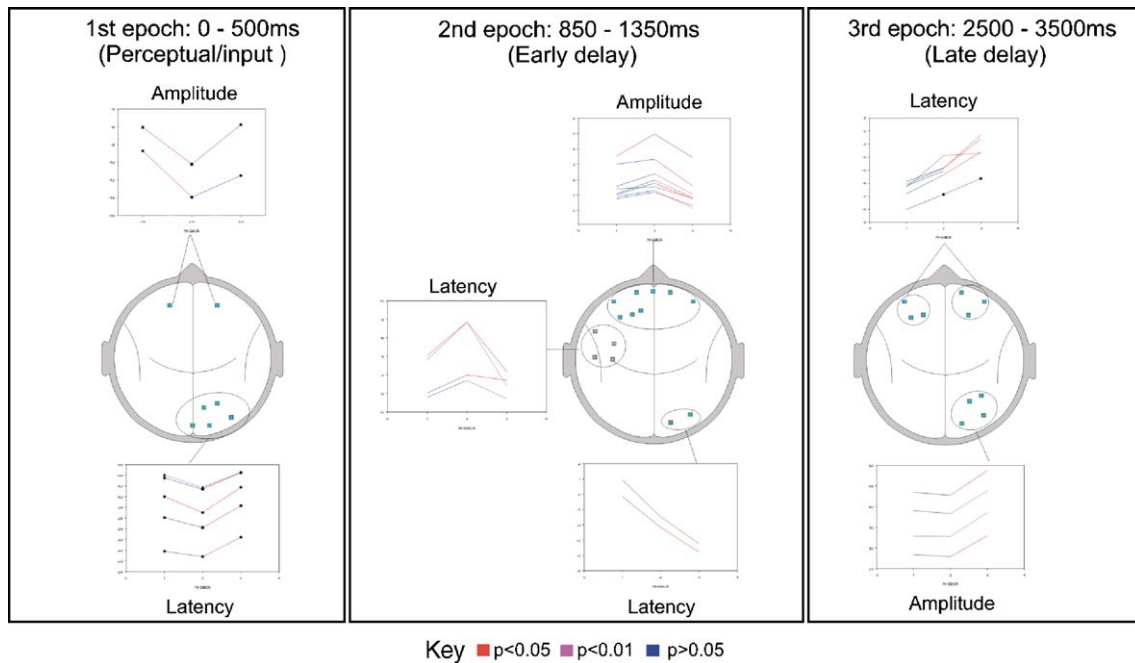


Fig. 4. Memory load differences between the 1-, 2-, and 3-back task.

3.5 s) for all 64 electrodes, for each n -back level, using Pearson's product moment correlation coefficient. Due to the large number of multiple comparisons, correlations were only considered if they: (1) occurred in clusters of at least 3 successive time points (i.e. lasting above 200 ms); (2) were present in at least 3 electrodes within the same region; and (3) were significant in at least 2 of the n -back levels. These analyses revealed no significant correlations between either SSVEP amplitude or latency and accuracy or reaction time which satisfied the 3 criteria above at the significance level of $P < 0.05$.

Due to the finding that the SSVEP of the n -back clusters into 3 significant time periods, we examined whether any of these time periods were significantly related to performance. For each of the three smaller epochs, changes in amplitude and latency were calculated (between the control and each n -back level) for the 4 regions most significantly activated by the task: frontal region (average of electrodes 0, 1, 2, 3, 4), left fronto-temporal region (average of electrodes 12, 13, 14, 21, 22), right fronto-temporal region (average of electrodes 11, 19, 20, 26, 27) and the occipital region (average of electrodes 55, 56, 60, 61, 62). A correlation analysis was conducted between SSVEP (amplitude and latency) and changes in behavioural responses (accuracy and reaction time changes between control and each n -back level). There were no significant correlations between either SSVEP amplitude or latency and accuracy or reaction time at any of the regions tested (frontal, left fronto-temporal, right fronto-temporal, occipital), in any of the 3 epochs identified (all $P > 0.05$).

Discussion

The current study examined the temporal dynamics of the SWM n -back task using SSVEP. Supporting our first hypothesis, the findings demonstrated a different SSVEP amplitude pattern during the perceptual/encoding and delay components of the task, consistent with findings during the DR task (Silberstein et al.,

2001). Consistent with our main hypothesis, the delay period exhibited increases in amplitude in both frontal and occipital regions, also consistent with findings during the DR performance (Silberstein et al., 2001; Perlstein et al., 2003). However, the main finding of this study is that the delay period was associated with two relatively distinct electrophysiological stages. Specifically, early in the delay (just following the stimulus disappearing from view), amplitude increases in frontal regions were observed, in addition to latency increases in the fronto-temporal regions and latency reductions more posteriorly. In contrast, late in the delay (the last second of the delay), a reduction in prefrontal amplitude and latency was observed, in addition to an increase in occipital amplitude. Importantly, all three n -back levels demonstrated a relative consistent electrophysiological profile, suggesting that this pattern is specific to the spatial n -back task. Nevertheless, these findings supported the hypothesis that memory load would modulate SSVEP within the network identified, consistent with previous neuroimaging studies (Braver et al., 1997; Cohen et al., 1997).

In terms of cortical topography, the present findings are consistent with the extensive literature outlining a distributed network associated with the spatial n -back task (Owen et al., 2005). Furthermore, the majority of SSVEP changes across the task were within the frontal regions consistent with electrophysiological evidence in non-human primates and humans (for reviews, see Goldman-Rakic, 1996; Wager and Smith, 2003). One difference observed in the current study was that latency reductions during the delay appeared biased to the left hemisphere (although hemispheric differences were not significant). This left hemispheric bias contradicts evidence suggesting that SWM activates a primarily right hemisphere network (Wager and Smith, 2003). However, whilst the stimulus had no overt verbal content, this apparent left hemisphere bias may be related to verbalisation of the position of the stimulus in the display field as tasks of verbal working memory activate a left hemisphere dominant network (Smith et al., 1996; Clark et al., 2001).

Consistent with previous research, changes in memory load were related to changes in task-related brain activity (Braver et al., 1997; Cohen et al., 1997; Callicott et al., 1999; Linden et al., 2003; Owen et al., 2005). Indeed, memory-load-related changes in both SSVEP amplitude and latency were observed within the working memory network during each of the time periods identified. During the early delay, frontal cortex changes exhibited an inverted U response; that is, both fronto-temporal latency and frontal amplitude increases were greater in the 2-back task than either the 1- or 3-back task. U-shaped memory load responses have previously been observed in frontal regions during the *n*-back task using fMRI (Callicott et al., 1999; Linden et al., 2003), and it has been suggested that this reflects a capacity-constrained response in which activation increases within a region until capacity is reached (i.e. 2-back task), at which point activation declines (i.e. 3-back task) (Callicott et al., 1999). In the later delay, there is evidence of linear memory-load-related effects in both the frontal and parieto-occipital regions, particularly between the 2- and 3-back tasks when working memory is more heavily stressed by load. Specifically, as memory load increased, latency increased within the frontal cortex and amplitude increased within the parieto-occipital cortex. The findings of linear changes in activation within frontal and parietal regions are consistent with previous studies of the *n*-back task (Braver et al., 1997; Cohen et al., 1997) and indicate that increased load was related to incremental recruitment of these regions during this time period in a manner that did not exceed capacity constraints. Overall, the difference in the pattern of memory load effects observed in the early delay (generally non-linear) and late delay (generally linear) suggests that capacity increased with delay. The nature of this effect is unclear, but it could be speculated that this reflects more efficient storage of working memory in the later delay, which may be related to greater involvement of “executive functions” (and less pure memory maintenance) during this later delay period.

Memory load was related to a decrease in behavioural performance as expected. Analysis of the possible relationship between changes in SSVEP and changes in performance revealed no significant correlations; however, this is not entirely surprising. As the *n*-back task is a complex series of separate and potentially overlapping sub-processes, behavioural performance is likely to reflect the sum of these sub-processes rather than individual time periods. These findings suggest that memory load increases result in changes in SSVEP amplitude and latency within previously activated regions and behavioural performance is not highly related to any specific time period but more likely to the sum of sub-processes comprising the task.

Encoding vs. delay period

A primary aim of the current study was to replicate the differing SSVEP amplitude profile between the early perceptual/encoding and delay components. The current findings supported this distinction. During perceptual encoding (when the stimulus was still present), SSVEP amplitude was reduced over frontal, temporal, and parietal regions, consistent with both previous SWM findings (Silberstein et al., 2001), and with reductions in parieto-occipital SSVEP amplitude observed during visual vigilance tasks (Silberstein et al., 1990; Nield et al., 1998). Such amplitude reductions have previously been interpreted as excitation of these regions related to increased visual vigilance (Silberstein et al., 1990; Nield et al., 1998).

In contrast, amplitude increased in both frontal and occipital regions during the delay. These findings support our primary hypothesis of amplitude increases during the delay and are consistent with previous SSVEP findings during the DR task (Silberstein et al., 2001; Perlstein et al., 2003). Increased amplitude within the PFC is likely to reflect neuronal activity within the pyramidal cells (Silberstein et al., 2001) and is consistent with electrophysiological studies in non-human primates which have established that pyramidal neurons in the PFC consistently fire during the hold period of delayed-response working memory tasks and that these “memory fields” hold information online in an active representation (Goldman-Rakic, 1996).

Amplitude of the SSVEP signal is assumed analogous to alpha activity within the brain (Silberstein et al., 1995, 2001; Gray et al., 2003; Kemp et al., 2004). Whilst reductions in alpha activity have traditionally been interpreted as reflecting increased “activity” or mental processing, the current findings fit well with a developing understanding of amplitude and cognition, in which amplitude (or alpha) changes may be related to the “type” of cognitive process (see Silberstein et al., 2001). Specifically, for intake tasks (involving attention is paid to the external environment), findings have been associated with reductions in alpha, whereas internal tasks (involving active rejection of the external environment and focus on internal content, as occurs in working memory) have been associated with *increases* in alpha (Ray and Cole, 1985). More recent findings have also demonstrated increases in alpha activity during mental imagery (Tesche et al., 1995) and increases in upper alpha range activity (10–13 Hz) at frontal and temporal sites during episodic memory (Klimesch et al., 1999).

A neurophysiological model proposed by Silberstein et al. (1995, 1998, 2001) suggests that rhythmic activity in the cortico-cortico loops is an important generator of the SSVEP and that reticulation of neural activity within these loops may provide a mechanism for holding information “online”. Therefore, during the SWM delay, the observed increase in amplitude could be interpreted as reflecting an increase in the efficiency or “loop gain” of these cortico-cortico loops (Silberstein et al., 2001). In contrast, the observed reduction of amplitude is likely to reflect a desynchronisation of these loops (Silberstein et al., 1995), and in more sensory tasks (i.e. visual vigilance or encoding), this may reflect a reduced efficiency of these loops as a direct result of enhancing sensory inputs to layer 4 and consequent inhibition of layer 1 (for a full discussion of cortico-cortico loops, see Silberstein et al., 1995).

Delay-related activity

Amplitude

The main finding of this study is that the delay period was associated with two relatively distinct electrophysiological stages. The early delay component (approximately 850–1400 ms) was associated with amplitude increases in the frontal region, whilst the later delay component (approximately 2500–3500 ms) was associated with amplitude *reductions* in the PFC region and associated amplitude increases in the occipital region. These findings may reflect a dual role of the PFC within a complex SWM task. Whilst the importance of the PFC in holding working memory online is well established, there is similarly evidence indicating PFC involvement in other (more “executive”) aspects of

the working memory process, such as organisation and control of the working memory “content”, implementation of strategies to facilitate memory, and updating of working memory content (Halgren et al., 2002; Bor et al., 2003). Although the functional significance of the frontal amplitude reduction in the late delay period is unknown, prefrontal amplitude reductions have previously been associated with cognitive set changes during the Wisconsin card sort test, a well-known test of executive function (Silberstein et al., 1995), and therefore the current reductions possibly reflect PFC reallocation to executive (non-maintenance) aspects of the SWM process.

The amplitude increases observed in the current study (i.e. early delay component: frontal increases, late delay component: occipital increases) may indicate a shift in “holding information” from frontal to occipital regions over the duration of the delay. This suggestion is in line with previous findings from a study in patients with partial epilepsy (Halgren et al., 2002). Halgren et al. (2002) observed sustained co-activation of the occipital cortex with fronto-centro-parietal cortices during a working memory task. These authors inferred a directionality of relationships between cortical areas based on phase lag measures, which indicated that later in the task the flow of information was from the frontal region to the occipital region. It was suggested that such findings are highly consistent with the use of the occipital cortex as a visuo-spatial sketch pad, potentially controlled by executive functions within the frontal cortex (Halgren et al., 2002), and the current findings fit well with such a model.

Latency

Variations in SSVEP latency have been interpreted as reflecting variation in neural information processing speed (Silberstein et al., 2001; Gray et al., 2003; Kemp et al., 2004). An increase in latency is indicative of an increase in inhibitory processes within the cortico-cortico loops, whilst a reduction in latency is suggestive of an increase in excitation (or reduction of inhibition) (Silberstein et al., 1995, 2001). Three major latency-related changes were observed during the spatial n -back task. During the perceptual/encoding period, SSVEP latency reductions were observed bilaterally in the frontal cortex (with a general reduction in more posterior regions). These findings are consistent with latency reduction during the encoding/perceptual period of a DR task (although in the Silberstein et al. (2001) study these effects failed to reach significance) and with latency reductions during visual vigilance tasks (the A-X Continuous Performance task) (Silberstein et al., 2000a,b).

Latency changes were also evident during the early and late delay periods. During the early delay period, SSVEP latency was relatively *increased* within fronto-temporal regions and *reduced* in more posterior temporo-parieto-occipital regions. In contrast, during the late delay stage (2500–3500 ms), latency was *reduced* within frontal regions. These findings suggest that early in the delay there is an increase in inhibitory processes within the fronto-temporal region. In contrast, in the later delay, we observed latency reductions in frontal regions which are suggestive of increased excitation within the prefrontal cortex during the late delay period.

Proposed model of spatial n -back-related SSVEP

While the exact mechanisms underlying the SSVEP changes are unknown, based on current understanding of SSVEP, it is suggested

that, early in the delay, frontal amplitude increases are likely to be involved in “holding” information online, and the fronto-temporal latency increases may be involved in facilitating this online maintenance through inhibition of adjacent neurons. In contrast, during the last second of the delay, both amplitude and latency are reduced in the frontal cortex (likely reflecting overall excitatory processes), and occipital amplitude is increased. It is suggested that the frontal cortex is reallocated to executive aspects of the task (which may include manipulation of information, response preparation, and anticipation of the new stimulus), with a shift in the “holding” of the working memory content to the occipital region.

The memory-load-related effects observed in this study support such a proposition. Based on the above model, it would be expected that memory load increases would result in incremental changes in the frontal cortex during the early delay (associated with holding information online), and similar changes in both frontal and posterior regions would be observed during the later delay. As discussed above, the memory-load-related findings of this study demonstrated that, during the early delay, both frontal amplitude and fronto-temporal latency followed an inverted U response pattern as memory load increased. In line with the interpretation of U-shaped memory load response patterns suggested by Callicott et al. (1999), these findings indicate that both amplitude and latency increased as the load on memory increases, until capacity was reached (2-back task), at which time the increase diminishes with performance. In the later delay, there is evidence of linear memory-load-related effects in latency in frontal regions and amplitude in parieto-occipital regions, specifically between the 2- and 3-back tasks when working memory is more heavily stressed by load. Whilst it is unclear why the memory-load-related effects change from non-linear to linear as the delay lengthens, it could be speculated that this reflects an increased efficiency in working memory storage in the later delay due to the involvement of non-mnemonic “executive functions” during this period.

The complexity of the n -back task, which comprises a number of separate and potentially overlapping sub-processes, along with the probability that different participants execute the n -back task using different strategies, means that even with the high temporal resolution of the SSVEP we cannot delineate each working memory sub-process. However, whilst the above model is speculative, the pattern of SSVEP activations does appear specific to the processes involved in the n -back task per se, with an overall consistency in the pattern of cortical effects noted between each n -back task level. Despite the complexity of the task, the n -back exhibits a consistent temporal profile regardless of memory load.

Summary

In summary, the current findings demonstrate that the SWM n -back task exhibits three distinct time periods: an early perceptual/encoding component (consistent with previous research) in addition to two distinct time periods during the delay. The different SSVEP amplitude pattern identified between the perceptual and mnemonic components of the task is consistent with previous research (Silberstein et al., 2001) and fits well into a developing understanding of amplitude in reference to the “type” of cognitive process. The two different SSVEP components during the delay appear reflective of the complexity of the task and the additional demands associated with the n -back, which appear evident specifically later in the delay. Consistent with previous fMRI and

PET findings, all three time periods exhibited memory-load-related effects on the SSVEP response. It is suggested that these findings offer a framework in which to further investigate the temporal aspects of working memory.

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