Original research paper

The effect of a single dose of multivitamin and mineral combinations with and without guaraná on functional brain activity during a continuous performance task

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Objectives: Relatively few studies have explored the possibility of acute cognitive effects of multivitamin ingestion. This report explores the acute brain electrophysiological changes associated with multivitamin and mineral supplementation, with and without guaraná, using the steady-state visually evoked potential (SSVEP).

Methods: Based on the known SSVEP correlates of A-X continuous performance task (CPT) performance, and sensitivity to acute psychopharmacological manipulations, the A-X CPT was adopted as a task paradigm to explore treatment-related neurophysiological changes in attentional processing. Twenty healthy non-smoking adults aged 21–39 years (mean age = 28.35 years, SD = 5.52) took part in this double-blind, placebo-controlled, randomized, balanced crossover design study.

Results: The study demonstrated both transient and tonic changes in the SSVEP response during completion of the A-X CPT following multivitamin and mineral treatment both with and without guaraná. Transient changes in SSVEP response in prefrontal regions were observed after a single dose of a multivitamin and mineral preparation indicative of enhanced activity within brain regions engaged by the attentional demands of the task. This pattern of change in frontal regions was correlated with improved behavioural performance after treatment with the multivitamin and mineral combination. Where tonic shifts in SSVEP response were investigated, multivitamin and mineral treatment was associated with a pattern of increased inhibition across posterior regions, with enhanced excitatory processing in prefrontal regions. In contrast, multivitamin and mineral treatment with additional guaraná showed a tonic shift towards greater excitatory processes after a single treatment, consistent with the caffeine content of this treatment.

Discussion: While preliminary in nature, these findings suggest a single multivitamin/mineral dose is sufficient to impact on functional brain activity in task-related brain regions.

Keywords: Multivitamin, Guaraná, Attention, Steady-state visually evoked potential (SSVEP), Neuroimaging, Acute effects, Continuous performance test

Introduction

Although the effects of chronic supplementation with multivitamin and mineral preparations on cognition have been the subject of considerable research (for reviews see Grima *et al.*[1] and Kennedy and Haskell[2]), relatively few studies have explored the possibility of acute cognitive effects of multivitamin ingestion. Investigating multivitamin and mineral supplementation in children aged 8–14 years, Haskell *et al.*[3] provided support for possible acute effects in cognitive assessments 3 hours after administration of the first treatment. Specifically, improvements in both accuracy and response time on a selective attention task, in addition to improved accuracy on a paired associates test after this single dose, were reported. The mechanisms underlying acute cognitive effects of vitamin administration are unknown, but specific vitamins act as co-factors for systemic and central metabolic processes, including those involved in neurotransmitter pathways.[2] Kennedy *et al.*[4] also demonstrated acute cognitive effects associated with multivitamin and mineral treatment in an adult population; however, the treatment utilized in this study also contained guaraná. Beginning 30-minute post-dose, assessment on a cognitive demand battery (CDB) comprising repeated assessments of serial subtraction, which require repeated subtraction of three or

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seven starting from a random three-digit number, and a rapid visual information processing (RVIP) tasks revealed significantly greater accuracy and reduced response time on the RVIP task after treatment with the multivitamin and mineral combination when compared to placebo.[4] As the active treatment in the study by Kennedy *et al.*[4] also contained guaraná extract, further research is required in order to properly differentiate the potential acute effects of single-dose multivitamin and mineral administration from that of guaraná. Guaraná (*Paullinia cupana*), found in the central Amazon, contains caffeine and other purine alkaloids,[5] saponins, tannins, and catechins.[6] Given these numerous potentially nootropic constituents, it is perhaps unsurprising that guaraná in isolation has been found to have acute effects on cognition.[7,8]

To this end, a randomized, double-blind, placebo-controlled, three-arm balanced crossover study was designed to explore the acute effects of multivitamin and mineral supplementation, both with and without guaraná. Assessments included measures of mood and cognitive function, in addition to both haemodynamic and electrophysiological measures of functional brain activity using functional magnetic resonance imaging (fMRI) and steady-state visually evoked potential (SSVEP). Behavioural and fMRI outcomes from this study have been reported elsewhere.[9] Briefly, treatment with multivitamin plus guaraná was associated with improved accuracy and response time on the serial three subtraction task as part of the CDB, in addition to benefits in self-rated contentment. No benefits to mood or cognitive task performance were observed for the multivitamin and mineral combination in isolation. Analysis of functional brain activation during RVIP task completion revealed a pattern of greater activation in task-related brain regions after both multivitamin and mineral treatments compared to placebo. Specifically, multivitamin and mineral treatment alone was associated with significantly elevated activity in regions linked with sustained attention and RVIP task performance,[10] including bilateral cerebellar regions. In contrast, combination of multivitamin, mineral and guaraná treatment revealed a different pattern of brain activity, with greater activations in the medial frontal and parietal regions. Bilateral parietal activity during the RVIP task has previously been linked with behavioural performance on the task, which is thought to involve working memory demands by supporting the online holding and updating of incoming digits.[10,11] Activation in frontal and supplementary motor areas has also been linked with RVIP performance, moreso with response speed than accuracy.[10] While complex interactions between the cerebellar regions and the frontal and parietal cortical regions during working memory[12] and sustained attention[13] suggest an important role for the cerebellum in these processes. As such, single doses of multivitamin and mineral treatments, both with and without guaraná, may influence a complex network of brain regions linked with attentional and working memory processes. The present report describes the acute brain electrophysiological changes associated with multivitamin and mineral supplementation using SSVEP.

The SSVEP has found a range of applications in cognitive neuroscience (for a review, see Vialatte *et al.*[14]). Steady-state topography (SST) explores fluctuations in the amplitude and phase of the SSVEP response during cognitive engagement using a diffuse task-irrelevant 13 Hz visual flicker.[15] As typical electroencephalogram (EEG) artefacts such as muscle activity, eye movements or blinks have relatively broadband spectra, the narrow-band nature of the SSVEP has important advantages over traditional EEG methods, including reduced susceptibility to noise contamination.[16,17] While different cognitive processes demonstrate variable relationships with the SSVEP amplitude,[18] interpretation of task-related fluctuations in SSVEP amplitude has been considered similar to traditional interpretations of event-related desynchronization and event-related synchronization of alpha oscillatory activity.[19] For example, while reduced amplitude is observed during tasks requiring ongoing processing of sensory information, the maintenance phase of an object working memory task has been linked with increase in SSVEP amplitude,[18] similar to increased alpha activity observed during working memory maintenance.[20–22] The SSVEP phase, often discussed in terms of latency, is thought to reflect the loop transmission time of cortico-cortical loops, and is thus linked with neural information processing speed such that phase advance (or latency reduction) reflects an increase in excitatory processes (and/or a reduction in inhibitory processes), with phase lag (increased latency) reflecting a decrease in excitatory processes (and/or an increase in inhibitory processes).[16,23] Used in this way, fluctuations in the SSVEP have been linked with a range of cognitive processes,[18,24–26] in clinical populations,[23,27] and to probe the neurocognitive effects of psychopharmacological manipulations.[28–31] Of particular relevance to the present investigation, Macpherson *et al.*[29] used the SSVEP in our laboratory to investigate the effects of 16 weeks multivitamin supplementation on neurocognitive function in elderly women. Significant increases in SSVEP latency were observed for participants receiving multivitamin treatment in comparison to placebo during completion of a spatial working memory task, with behavioural performance improvements found to correlate with the latency increases over central midline sites in the multivitamin

treatment group.[29] While this study utilized a chronic dosing regimen, it nonetheless demonstrates the high degree of sensitivity with which changes in neurocognitive function associated with nutrient supplementation may be captured with the use of SSVEP technique.

One of the cognitive domains with which SSVEP changes according to task demands have been most reliably measured is attention. Early work with the SSVEP found evidence of decreased amplitude in central and parietal electrode sites where task demands were increased for visual vigilance.[15] It has been proposed that tasks which require ongoing processing of sensory information, such as the A-X continuous performance test (CPT), will be associated with reduced SSVEP amplitude. As the sensory inputs to cortical layer IV are enhanced during such processing, the re-entrant cortico-cortical loop transmission efficiency through cortical layer I, which is thought to be responsible for increased SSVEP amplitude, is inhibited.[16,18] Subsequently, the CPT has been used to explore the SSVEP correlates of attentional processes in clinical populations and healthy controls. The A-X version of the CPT involves presentation of a sequence of single letters and requires a response upon presentation of a target stimulus ('X') where this is preceded by a cue stimulus ('A') among a pseudo-random sequence of letters to prime attentional resources. A reference task, which requires a response upon presentation of a target within a predictable sequence of letters, is contrasted as a control condition for the basic sensorimotor components of the task. The presentation of both the cue and target letters in the active version of the task has been associated with amplitude and latency reductions in frontal electrode sites, which is indicative of frontal activity during cue and target processing.[32] The latency of the SSVEP response was again found to be reduced (i.e. phase advance) in frontal electrode sites upon presentation of both the cue and target letters in healthy adults as part of an investigation of attention deficits in schizophrenia.[23] This investigation also found that frontal SSVEP latency after target presentation was correlated with response times, such that greater latency reductions were associated with better performance. Silberstein *et al.*[23] also reported this relationship between frontal SSVEP latency and response time in a sample of schizophrenic patients; however, comparing this patient group with healthy controls found that latency in the frontal regions was significantly greater in the patient sample, suggesting reduced frontal activity in the patient sample. This pattern of SSVEP latency reduction (phase advance) in the frontal regions during the A-X CPT has also been observed in healthy children, while children with attention-deficit hyperactivity disorder (ADHD) fail to show the same reduction.[27] In summary, previous research using the SSVEP response to probe the neural correlates of attention has typically reported reduced amplitude and latency (or phase advance) in the frontal brain regions during performance of attention tasks. Importantly, these latency reductions in the frontal regions are associated with better performance. Finally, this frontal latency reduction is not evident in populations known to demonstrate impaired attentional processing.

Continuous performance tests have formed an important part of assessing both the deficits and impact of pharmacotherapy in ADHD.[33] These tasks have also been adopted as part of functional MRI investigations of frontal lobe function in schizophrenia[34] and ADHD,[35] and have proven to be sensitive to acute manipulations of brain biochemistry in healthy adults. For example, neurocognitive changes associated with a single dose of an α_{2A} adrenoreceptor agonist (guanfacine) have been demonstrated during the A-X CPT in healthy adults, using a placebo-controlled crossover design.[36] As part of this study, greater blood-oxygen-level dependent (BOLD) signal activation was observed upon presentation of the cue ('A') across a network, including dorsolateral prefrontal cortex regardless of treatment using fMRI to monitor brain haemodynamic activity. In addition, this study found further increased BOLD activation during the cue period in left prefrontal cortex after guanfacine treatment. Studies manipulating central serotonin levels via acute tryptophan depletion have also demonstrated the sensitivity of the CPT to acute psychopharmacological investigations. On exploring behavioural performance in healthy adults and a group diagnosed with ADHD using a variant of the A-X CPT, it was found that tryptophan depletion was associated with increased response times in healthy adults, while reduced response times were observed for the ADHD group.[37] While acute tryptophan depletion may impact on mechanisms beyond serotonin synthesis and subsequent availability,[38] this nonetheless further demonstrates the sensitivity of A-X CPT variants to acute pharmacological manipulations. Based on the known SSVEP correlates of A-X CPT performance, in addition to this proven sensitivity to acute psychopharmacological manipulations, the A-X CPT was selected as an ideal task paradigm for exploring the neurocognitive acute effects of a single dose of multivitamin/mineral combinations on attentional processes.

Methods

This study adopted a randomized, double-blind, placebo-controlled, three-arm balanced crossover design.

Participants

Twenty healthy, non-smoking adults aged 21–39 years (mean age = 28.35 years, SD = 5.52) were recruited to take part in behavioural and brain electrophysiological assessments across the three treatment visits, with a minimum 1 week washout period between visits. Participants were required to abstain from consuming alcohol and caffeine in the 12 hours prior to each treatment visit. All participants were regular caffeine consumers, right-handed, not currently taking medications, vitamins or herbal extracts, with no history of psychiatric or neurological illness. The results of the behavioural assessment, as well as an additional fMRI investigation, have been reported previously.[9] In order to achieve a fully balanced sample across treatment sequences, 18 participants were considered for the present exploration of functional brain activity assessed using the SSVEP. The last participants to receive the two over-represented treatment sequences (in terms of completion date) were those participants excluded from analyses. The study was conducted in accordance with the Declaration of Helsinki, and had approval by the Swinburne University Human Research Ethics Committee (Ref SUHREC 2010/300). The trial was registered with the Australia New Zealand Clinical Trials Registry: ACTRN12612001116819.

Treatments

Two multivitamin and mineral preparations, one with and one without guaraná, formed the active treatments under investigation, while the placebo was an effervescent drink with similar colouring and flavouring. These active treatments, hereon referred to as MV1 and MV2, are available over the counter as Berocca® Performance and Berocca® Boost, respectively. These treatments are detailed in Table 1 below. MV1 contains higher doses of B-group vitamins and vitamin C, while MV2 contains lower doses of these vitamins but the added guaraná component. All treatments were prepared by a disinterested third party, and were provided in 330 ml of solution from bottles in identical opaque sleeves.

Procedure

Participants completed three testing visits, as well as an initial screening and familiarization visit. During the initial visit, written informed consent was obtained, participants were screened for eligibility, demographic, and morphometric information obtained, and the requirements of the full testing days explained. Following the preliminary visit, full testing days all followed an identical procedure, consuming a different treatment at each visit using a randomized, double-blind, balanced crossover design. Behavioural assessment was conducted at each visit both before and 30-minute post-dose, after which participants were prepared for brain electrophysiological recordings. Details and outcomes from the behavioural assessment have been described previously.[9] The SSVEP assessment took place approximately 90 minutes after treatment ingestion.

SSVEP recordings

The brain's electrical activity was recorded using an electrode cap, with 60 scalp electrodes positioned according to the extended 10–10 system (Quik-Caps; Neuroscan, Inc., Abbotsford, Victoria, Australia). On-line recordings were referenced to an electrode positioned between Cz and Cpz, with a ground electrode between Fz and Fpz. Recordings were also taken from both left and right mastoids to facilitate a linked mastoid reference in off-line analysis. Electrode impedances at all sites were maintained below 8 kΩ. EEG was amplified and filtered (from DC to 70 Hz) prior to digitization to 12-bit accuracy at a rate of 1000 Hz using Synamps^2 Amplifiers and Scan 4.3 Software (NeuroScan, Inc.). The stimulus used to evoke the SSVEP is a diffuse 13 Hz sinusoidal flicker superimposed on the visual field by a pair of goggles (for further details on the SSVEP technique, see Vialatte *et al.*[14]). The goggles comprised two

Table 1 Contents of multivitamin and mineral treatments, both with and without guaraná

	MV1 Multivitamin and mineral treatment (Berocca® Performance)	MV2 Multivitamin and mineral plus guaraná (Berocca® Boost)
Vitamin B1 (mg)	15	1.40
Riboflavin (vitamin B2) (mg)	15	1.60
Nicotinamide (B3/niacin) (mg)	50	18
Vitamin B5 (mg)	23	6
Vitamin B6 (mg)	10	2
Folic acid (vitamin B9) (μg)	400 μg	200 μg
Vitamin B12 (μg)	10 μg	1 μg
Biotin (vitamin B7) (μg)	150 μg	150 μg
Vitamin C (mg)	500	60
Calcium (mg)	100	100
Magnesium (mg)	100	100
Zinc (mg)	10	9.5
Guaraná	–	222.2 mg (40 mg caffeine)

half-silvered mirrors that reflect two light-emitting diode arrays over the participant's visual field, subtending a horizontal angle of 160° and a vertical angle of 90°. The maximum intensity at the peak of the stimulus waveform was 5.0 cd/m^2, and the modulation depth was 45%.

Cognitive tasks – A-X CPT

The A-X CPT task was presented on a 17-inch LCD monitor, viewed from a distance of approximately 60 cm using the Gentask module of STIM2 software (NeuroScan, Inc.). The active task consisted of pseudo-random presentation of a sequence of letters. Each letter was presented for 267 ms, separated by a blank screen appearing for 1500 ms duration. Participants were required to respond by right-hand button press using a microswitch when the target letter ('X') appeared, but only when immediately preceded by the cue letter ('A'). The proportion of cue–target pairs was 20% of all stimuli. The reference version of the task presented a predictable sequence of letters (A, B, C, D, and E), with participants required to press the microswitch on each occurrence of the letter 'E'. A total of two blocks of 100 stimuli (each containing 20 cue–target pairs) were presented for both the A-X CPT and the reference task, with a duration of approximately 3 minutes each.

SSVEP processing

Raw data were visually inspected for artefact, with problematic electrodes replaced with a weighted average of surrounding electrodes. Signal processing utilized the BrainSci Software to extract the SSVEP signal using established processing routines (for details, see Silberstein *et al.*[15]), while statistical analysis and mapping used custom MATLAB scripts (MATLAB 2011a, The Mathworks Inc., Natick, MA, USA). First, a band-pass filter between 0.1 and 70 Hz was applied to all raw EEG data. For each task, the SSVEP was extracted from the raw EEG by calculating Fourier coefficients over a 20 stimulus cycle period, using a cosine window centred at the 13 Hz stimulus frequency, yielding a temporal resolution of 0.77 seconds. The 20-cycle evaluation period was then shifted one stimulus cycle, and the coefficients were recalculated. This process was applied for the entire duration of EEG data for each task, for each of the 60 electrodes.

The SSVEP time series was then epoched with a 6-second window from 2 seconds prior to the onset of the cue stimulus ('A' for active task and 'D' for reference task). Only trials where the target followed the cue and a correct response was received were included for the present analysis. All correct trials were then averaged for each participant at each visit. In order to control for the inter-individual and inter-session differences in the amplitude and phase of the SSVEP response, both amplitude and phase were normalized to mean activity of the reference task at that visit. The SSVEP amplitude was normalized to the mean amplitude for the reference task across all timepoints and electrodes for the reference task. Similarly, mean SSVEP phase across the 6-second epoch within the reference task at each electrode site was subtracted from the corresponding electrode site for each timepoint of the active task. For those comparisons in which the focus was on SSVEP response changes directly linked to attentional processing, the normalization of amplitude and phase was done separately to the reference task within each treatment visit in order to control for non-specific effects of treatment on the SSVEP. In addition, analyses, regarding the tonic effects of the two treatments on general cortical excitability, normalization of *all* placebo, and multivitamin/mineral treatment groups were conducted using the reference task from the placebo visit.

Cross-subject averaging was then conducted for all participants and tasks during each treatment visit. Three segments of interest were then calculated using the average of the amplitude and phase SSVEP time series. These segments were as follows: the cue ('A' for active task, 'D' for reference task, 250 ms window from cue onset), the target ('X' for active task, 'E' for reference task, 1000 ms from target onset), and the hold period (corresponding to the 1500 ms period between disappearance of the cue and onset of the target).

The statistical strength of SSVEP differences was examined using the bivariate Hotelling's T^2 parameter. A bivariate paired *t*-test was used, because the SSVEP amplitude and phase data comprise complex numbers expressed as real and imaginary components. Significant statistical results from these analyses indicate evidence that the mean vector of these real and imaginary components, in Cartesian coordinates, differs between conditions. These statistically significant differences can then be probed using the difference maps for amplitude and phase. The application of a strict Bonferroni correction to account for comparisons at each electrode site would have inflated the possibility of Type 2 error, as it does not take into account the high degree of inter-correlation between electrode sites.[39,40] Previous research using spatial principal components analysis of the scalp SSVEP has found no more than five independent factors are required to account for 95% of spatial variance.[24,39] For this reason, the alpha level was adjusted to 1% (i.e. $P < 0.01$, Bonferroni corrected $P = 0.05/5$). The amplitude and phase differences, along with the *P*-values arising from Hotelling's T^2 contrasts, were mapped topographically using adapted scripts from the EEGLAB toolbox.[41] Consistent with previous

SST reports, amplitude reductions and phase advance (latency reduction) are plotted using warmer colours, as these changes are typically interpreted in terms of activation or excitatory processes.

SSVEP comparisons

Statistical comparisons assessed three separate research questions concerning the SSVEP response during A-X CPT performance. (1) Contrasts were calculated between active and reference tasks at the placebo visit in order to first characterize the SSVEP activity associated with task performance in the absence of treatment effects. (2) Sections of the active task were then contrasted between each active treatment condition and the placebo visit, as well as direct comparison between the two active treatments, in order to quantify transient changes in SSVEP response during attentional processing associated with acute doses of multivitamin and mineral preparations with and without guaraná. (3) Tonic changes in cortical excitability were probed by analysing a single averaging window comprising the entire task segment from cue, hold, and target, with all treatment visits normalized to the placebo visit.

While the SSVEP has been used to track transient fluctuations in functional brain activity with a temporal resolution in the tens of milliseconds, averaging the SSVEP response across a larger temporal window may offer insights into more stable patterns of change in tonic cortical excitability. The SSVEP phase is thought to reflect the transmission time of cortico-cortical loops, and is thus linked with neural information processing speed such that phase advance reflects an increase in excitatory processes (or a reduction in inhibitory processes), with phase lag reflecting the opposite.[16,23] Changes in the SSVEP phase have typically been probed on the millisecond scale afforded by the SST method in response to cognitive task completion. As an additional analysis (research question (3) above), we explored tonic changes in cortical excitability associated with treatment by averaging the SSVEP response across a large task segment, and changing the normalization strategy. While treatment effects during processing of the A-X CPT targeted activity unique to attentional processes, and were thus normalized to reference task of the same treatment sessions, this additional analysis probed all changes in SSVEP response associated with treatment and thus the normalization procedure was based on the reference task at the placebo visit for all treatments. In essence, normalizing all treatments to the placebo visit and averaging across the entire task period explored more tonic neurophysiological shifts in cortical excitability, whereas the former analysis (research question (2) above) controlled for non-specific shifts in SSVEP response, facilitating analysis of treatment effects unique to the attentional networks transiently engaged during CPT performance at each treatment visit.

Results

Behavioural performance

No significant treatment effects were observed for accuracy, as the percentage of correct detections of targets, or response time on the A-X CPT. A large number of participants performed near ceiling for accuracy data, suggesting task difficulty may not have been sufficient to permit detection of behavioural effects of the treatment (averaged across all treatment visits, mean accuracy = 94.49%, SD = 5.40). The behavioural outcomes of the CDB completed prior to SSVEP recordings have been reported elsewhere.[9]

SSVEP task effects: A-X CPT

Topographic maps displaying the amplitude and phase differences between active and reference task at the placebo visit, along with the results of Hotelling's bivariate T^2 comparisons are shown in Fig. 1. The SSVEP phase lag, indicative of reduced excitatory or greater inhibitory activity, was apparent in the right temporal regions across the entire task (maximal at electrode T8). In contrast, a pattern of SSVEP amplitude reduction was observed in the frontal and parieto-occipital regions across all task segments. The cue period was characterized by significant amplitude reductions in the left frontal regions, which increased over the hold and target segments to extend over greater frontal regions. Though largely non-significant, amplitude reductions were also observed across posterior regions during the cue period. This was accompanied by prefrontal SSVEP phase advance during the target period, further corroborating previous findings during the target period of the CPT,[23,27] where amplitude reductions and prefrontal phase advance (or latency reduction) have been reported for task-related SSVEP activity during A-X CPT performance.[23,27]

SSVEP treatment effects during A-X CPT

The SSVEP response during the active CPT for both active treatments was contrasted against the active task at the placebo visit, with all SSVEP data normalized to the corresponding reference task for each treatment visit. Topographic maps displaying the SSVEP amplitude and phase differences between MV2 and placebo post-dose recordings using the active (A-X CPT) tasks referenced to their corresponding control tasks at are displayed in Fig. 2, together with the results of Hotelling's bivariate T^2 tests. No significant differences in the SSVEP response were observed during A-X CPT performance for MV2. Trends towards amplitude reductions and phase advance

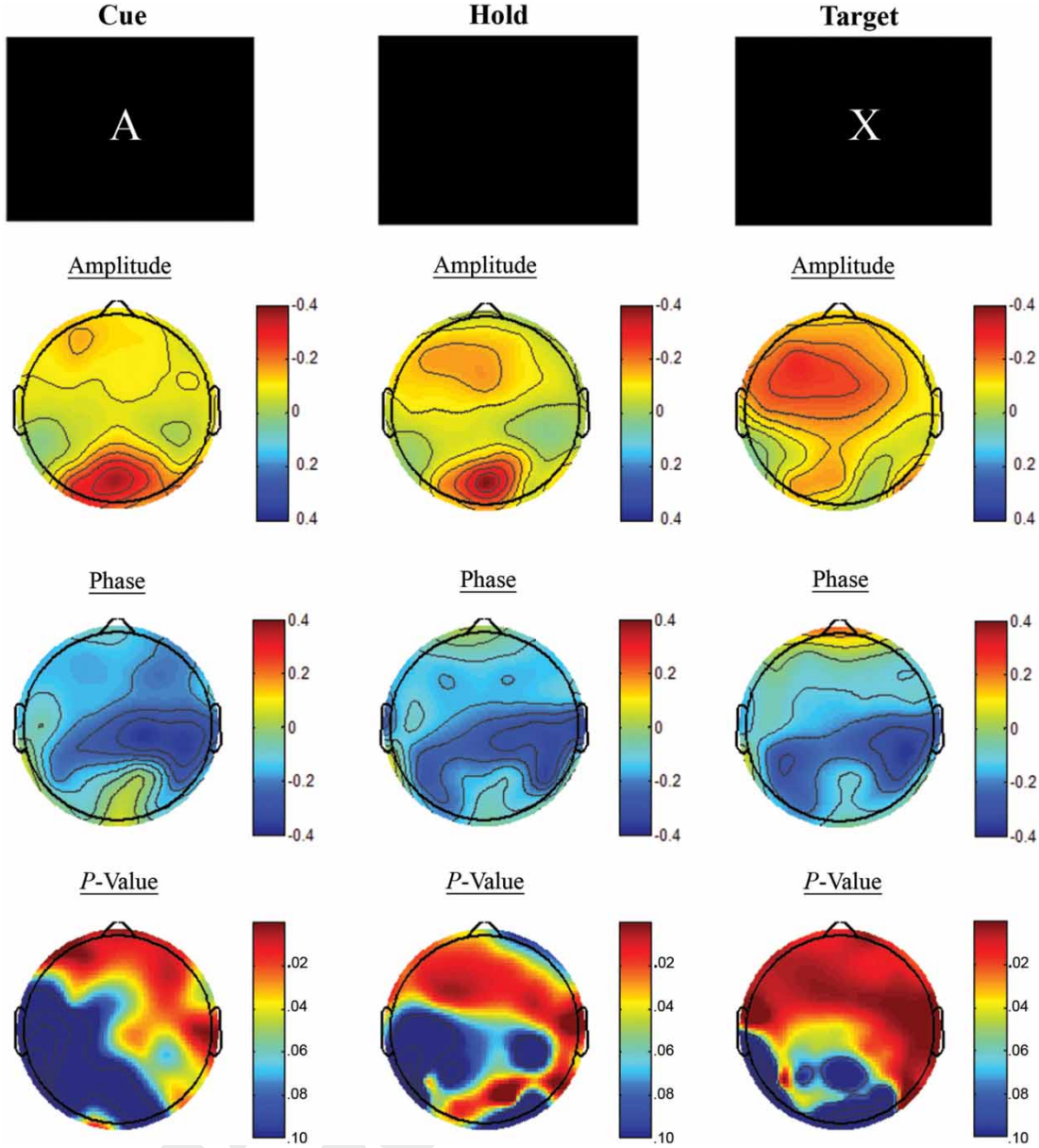

Figure 1 Task-related activations for the A-X CPT at the placebo treatment visit. Each column shows topographic maps of SSVEP amplitude and phase differences between A-X CPT and the reference task, under which the *P*-values resulting from the Hotelling's T^2 contrast of the active and reference versions of the task are mapped. Red colours represent amplitude and latency reductions (phase advance) and lower *P*-values, respectively. Cool colours represent amplitude increases and latency increases (phase lag).

were observed, particularly in posterior regions during the cue and hold periods, and within frontal regions during the target period.

Topographic maps displaying the SSVEP amplitude and phase differences between MV1 treatment and placebo post-dose recordings using the active (A-X CPT) tasks referenced to their corresponding control tasks at are displayed in Fig. 3, together with the results of Hotelling's bivariate T^2 tests. Contrasting MV1 treatment with placebo revealed significant differences in SSVEP responses during both the hold and target segments of the task. A pattern of further frontal amplitude reduction and phase advance (latency reduction) was apparent at the MV1 treatment visit during the hold segment of the A-X CPT, reaching significance ($P < 0.01$) in four frontal electrodes. A significant phase lag (latency increase) in a single fronto-temporal site showing significant differences between MV1 and placebo visits was also observed in the hold period between the disappearance of the 'A' and the appearance of the 'X'. This pattern of frontal SSVEP amplitude reduction and prefrontal SSVEP phase advance persisted into the target segment of the task, with much of the region marginally significant ($P < 0.05$); however, only a single right frontal electrode site (F4) exceeding the adjusted threshold for significance. While greater prefrontal phase advance and amplitude reduction was observed,

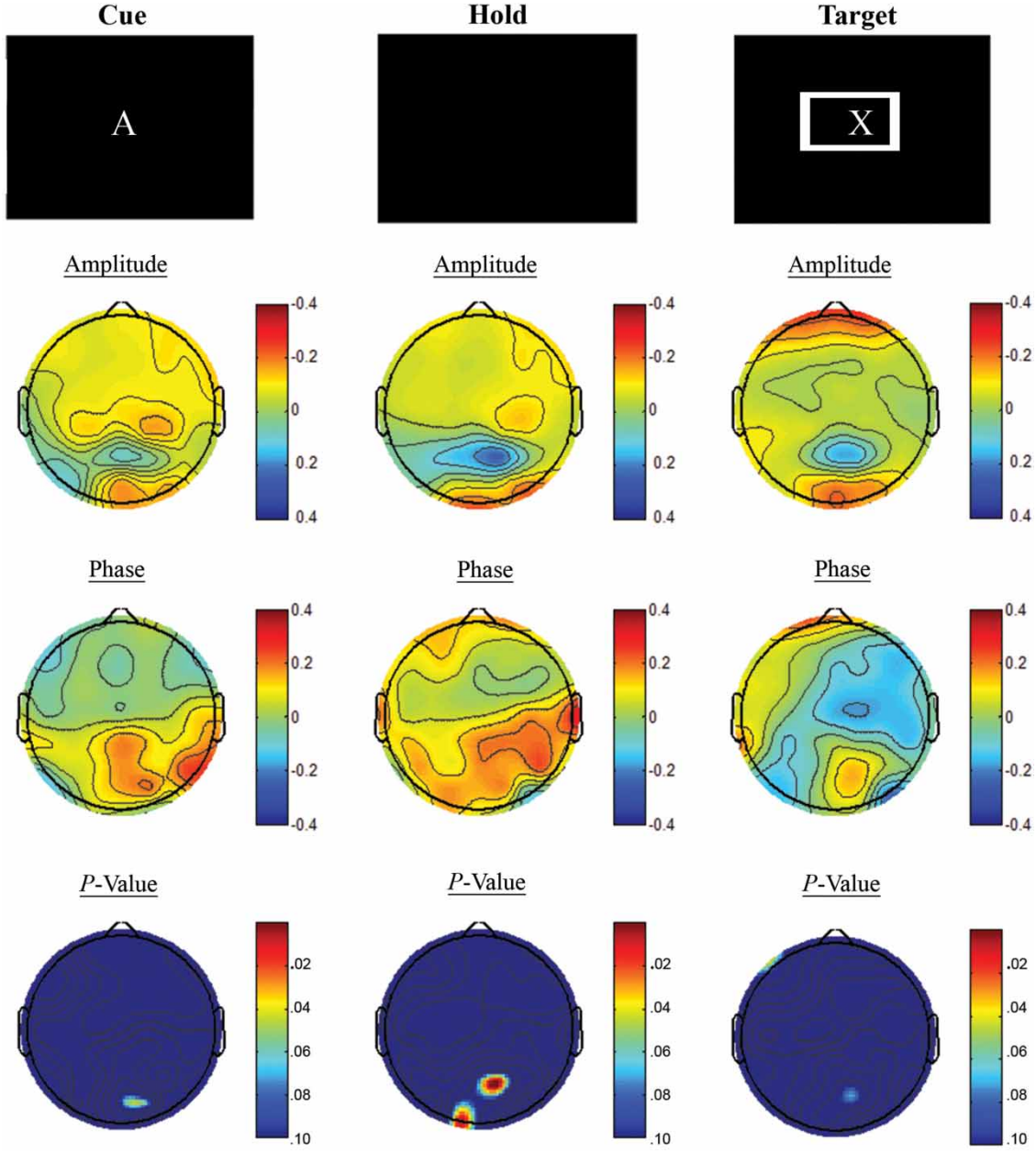

Figure 2 Topographic maps of differences in SSVEP amplitude and phase for the A-X CPT at the multivitamin/mineral plus guaraná treatment visit (MV2) and the placebo visit. Each column shows topographic maps of SSVEP amplitude and phase differences between for A-X CPT at active versus placebo, with the bottom map showing the *P*-values resulting from the Hotelling's T^2 contrast of the two treatments (comparisons revealed no electrode sites $P < 0.01$). Warm colours indicate amplitude and latency reductions (phase advance) and lower *P*-values, respectively. Cool colours represent amplitude increases and latency increases (phase lag).

the finding of significant SSVEP phase lag (or relatively less phase advance) in neighbouring right fronto-temporal regions suggests a more focal change in task-relevant prefrontal regions, and relatively less diffuse activity across other frontal regions during this period. Fig. 4 shows the direct contrast between the two active treatment conditions, where MV1 treatment was subtracted from MV2. This comparison showed that MV2 was associated with greater phase advance during the cue period in the prefrontal regions, shifting to fronto-central regions during the hold and target periods. This confirms the observation that MV1 treatment alone resulted in patterns of prefrontal amplitude reduction and phase advance in a focal manner, where other fronto-central regions showed relatively less phase advance when compared to the MV2 treatment.

Tonic SSVEP treatment effects

Topographic maps displaying the tonic SSVEP amplitude and phase differences (averaged across all A-X CPT segments) between each active treatment and placebo, as well as direct comparison of the two active treatments, are displayed in Fig. 5 together with the results of Hotelling's bivariate T^2 tests. Analysis of tonic changes in SSVEP response revealed

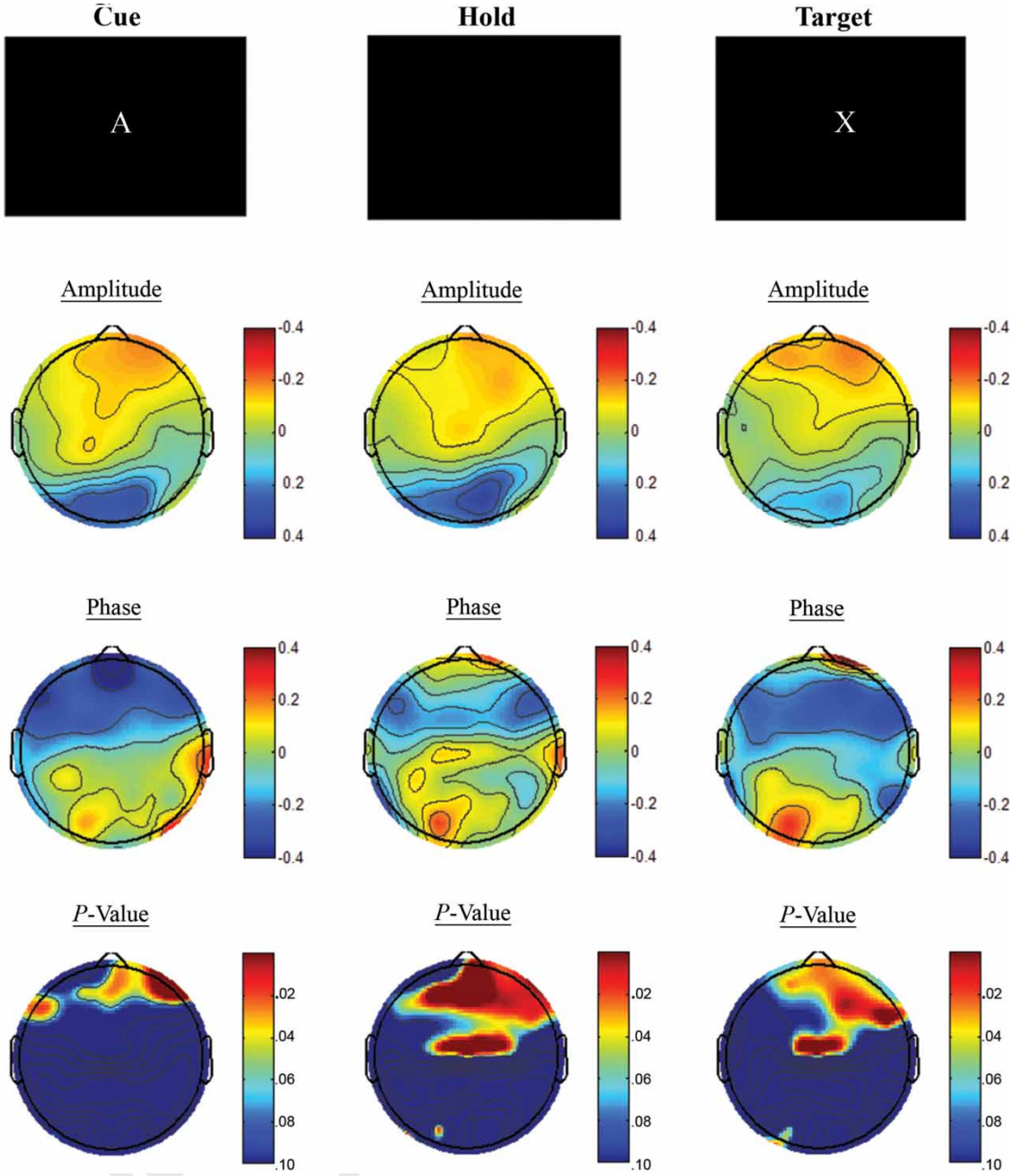

Figure 3 Topographic maps of differences in SSVEP amplitude and phase for the A-X CPT at the multivitamin/mineral treatment visit (MV1) and the placebo visit. Each column shows topographic maps of SSVEP amplitude and phase differences between for A-X CPT at active versus placebo, with the bottom map showing the *P*-values resulting from the Hotelling's T^2 contrast of the two treatments. Warm colours indicate amplitude and latency reductions (phase advance) and lower *P*-values, respectively. Cool colours represent amplitude increases and latency increases (phase lag).

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strikingly opposing patterns across the two treatment conditions when compared to placebo. MV2 (Berocca Boost) was associated with a pattern of phase advance (latency reduction) across most electrode sites, reaching statistical significance in the left and right parieto-temporal regions. The occipital regions also showed trends ($P < 0.05$) towards greater phase advance and reduced amplitude. These findings are indicative of increased excitatory processes and/or decreased inhibitory processes across distributed cortical regions. In contrast, multivitamin and mineral treatment in the absence of guaraná (MV1; Berocca Performance) was predominantly associated with significant phase lag across parietal, temporal, and fronto-central regions. Importantly, this was with the exception of right prefrontal regions which displayed significant phase advance and reduced amplitude – which is consistent with the analysis of transient changes displayed in Fig. 3. Direct comparison of the two active treatments further emphasized the greater phase advance across distributed brain regions, with the exception of the right prefrontal sites, in the MV2 condition. This pattern of results after MV1 treatment is indicative

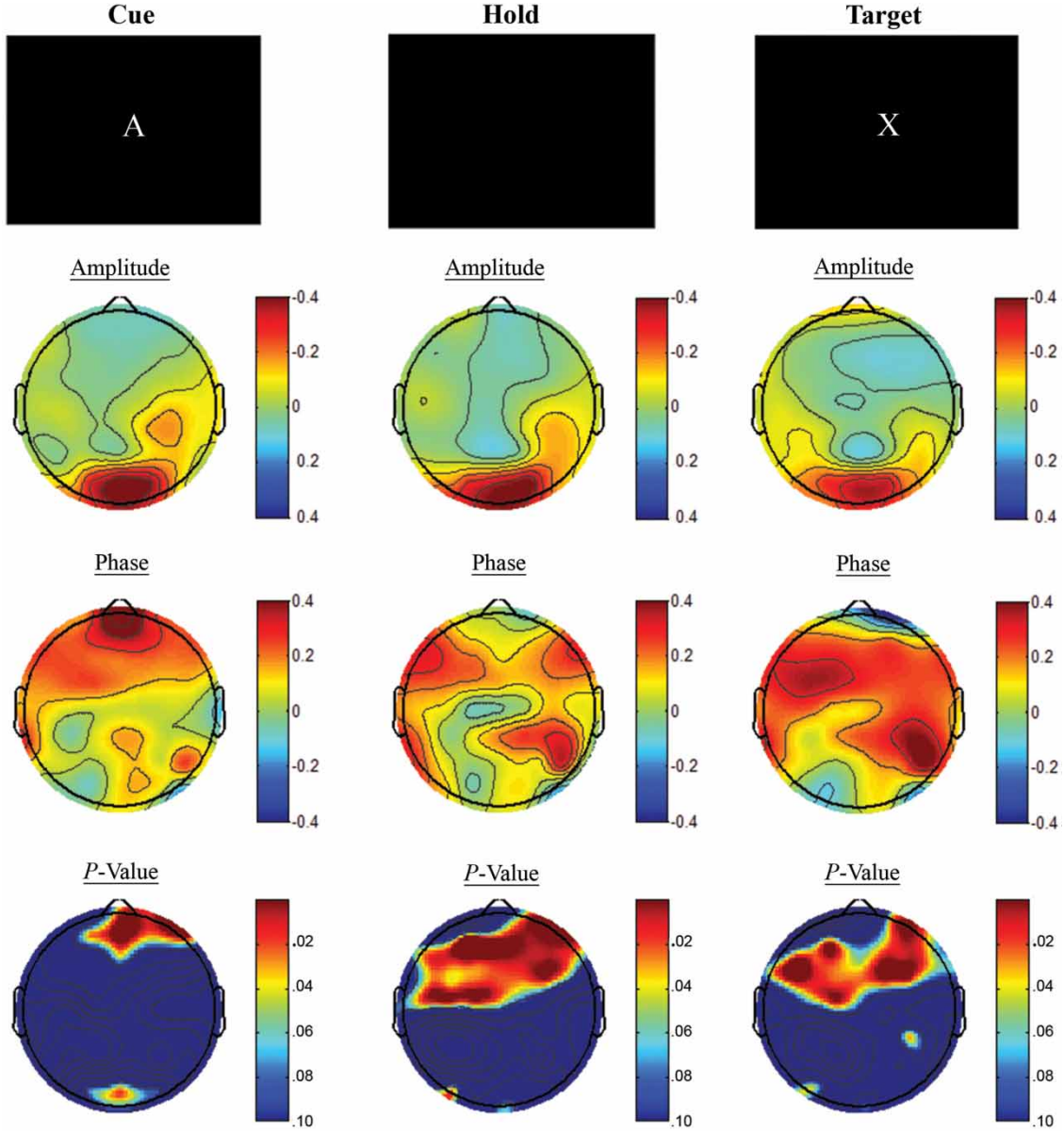

Figure 4 Topographic maps of differences in SSVEP amplitude and phase for the A-X CPT between the two active treatments (MV2 minus MV1). Each column shows topographic maps of SSVEP amplitude and phase differences between for A-X CPT at MV2 versus MV1, with the bottom map showing the *P*-values resulting from the Hotelling's T^2 contrast of the two treatments. Warm colours indicate amplitude and latency reductions (phase advance) in the MV2 treatment and lower *P*-values, respectively. Cool colours represent amplitude and latency reductions (phase advance) in the MV1 treatment.

of greater inhibition (or reduction of excitation) across much of the cortex, with a focal increase in excitatory processes in right prefrontal foci.

Exploratory analysis correlating SSVEP treatment effects with changes in task performance

Exploratory follow-up analyses were conducted, which explored the extent to which the observed treatment-related changes in SSVEP response were associated with task performance by correlating change in performance (the mean response time difference between treatments) with changes in SSVEP response. Previous research to demonstrate changes in SSVEP response after multivitamin treatment used this strategy to demonstrate correlations between SSVEP response and that of task performance.[29] In addition to using amplitude and phase changes in isolation, a composite measure of SSVEP change incorporating both amplitude reductions and phase advance was calculated. As the major treatment and task-related changes during AX-CPT performance involved both amplitude reduction and phase advance, this composite measure was calculated in order to further probe the relevance of this combined pattern of activity. The composite SSVEP change measure was calculated by standardizing the change in SSVEP response to a *z*-score, separately for amplitude and phase. As the direction of change was inversely related for amplitude (negative change) and phase (positive change),

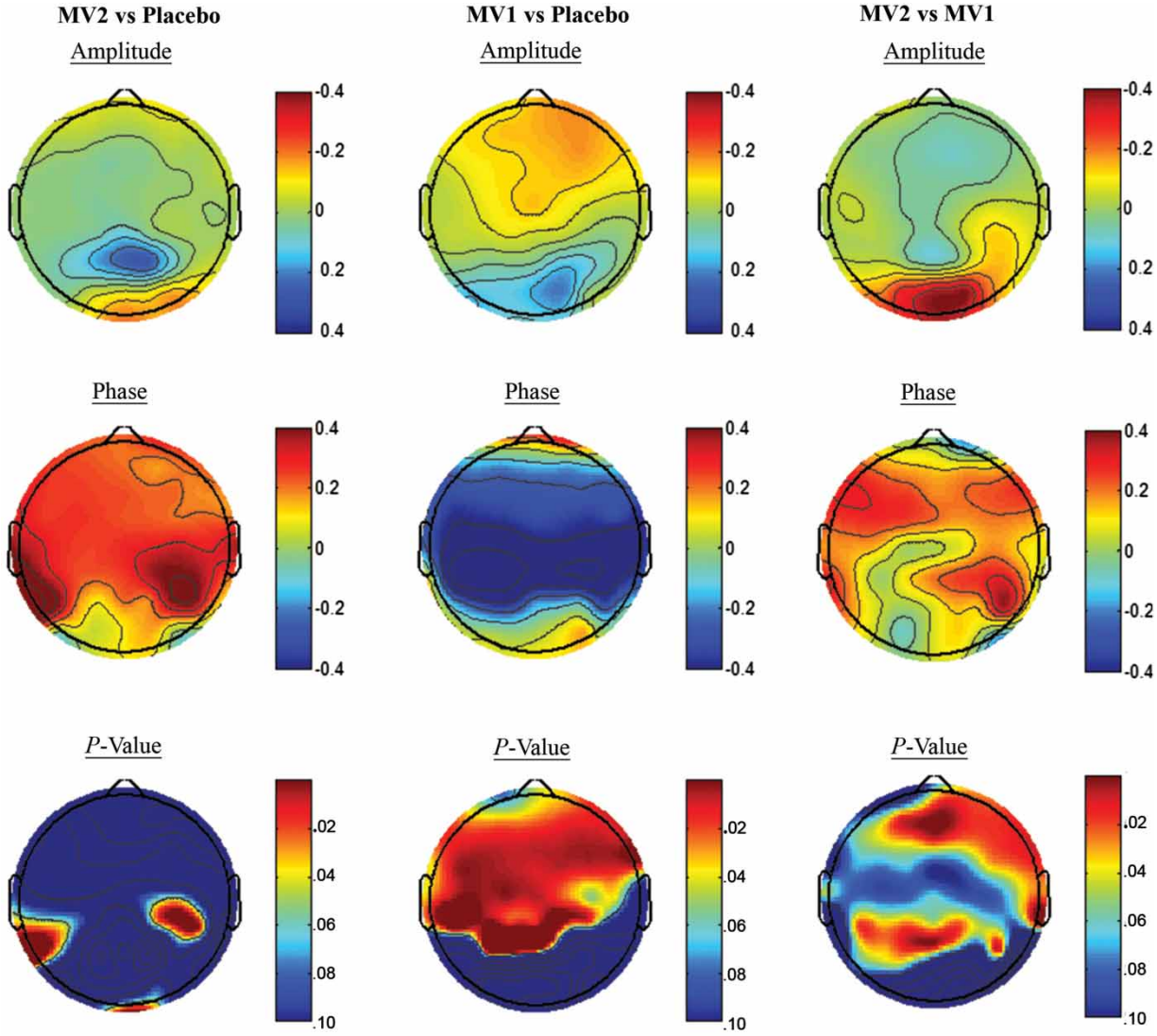

Figure 5 Tonic changes in SSVEP response associated with treatment, averaged across A-X task performance. Left column shows contrasts for MV2, MV1 combination is shown in the middle, while the direct comparison of the two active treatments is shown on the right. Topographic maps (from top) show amplitude and phase differences between treatment and placebo, with *P*-values plotted for corresponding Hotelling's T^2 test. Warm colours indicate amplitude and latency reductions (phase advance) and lower *P*-values, respectively. Cool colours represent amplitude increases and latency increases (phase lag).

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amplitude change scores were first inverted such that positive scores indicated greater reductions in amplitude in order to facilitate combining the measures. These standardized scores for amplitude and phase were then added to provide a composite measure of SSVEP change, where more positive scores indicated a greater pattern of amplitude reduction and phase advance. Previous work has applied normalization to a range of EEG time–frequency measures (see e.g. Grandchamp and Delorme[42]), where it has also been used as a method which enables analysis of spectral measures whose magnitudes inherently differ, ensuring such measures provide similar weight in subsequent analysis.[43]

These exploratory follow-up analyses were confined to the major treatment-related SSVEP effects observed when contrasting the active treatments and placebo. Specifically, as part of the transient task-related SSVEP findings for the MV1 treatment, mean change in SSVEP phase and amplitude was extracted across the four frontal electrodes which significantly differed during the hold period with respect to placebo, in addition to change in the single, right frontal electrode found to significantly differ during the target period. During the hold period, neither change in amplitude ($r = 0.219$, $P = 0.384$) nor phase ($r = -0.309$, $P = 0.211$) was significantly correlated with performance changes. However, a significant correlation was observed between the combined SSVEP change (encompassing amplitude reduction and phase advance) with change in response time for the hold component ($r = 0.481$, $P = 0.043$). Fig. 6 shows this relationship, where those participants showing a shift towards reduced amplitude and phase advance at the MV1 treatment visit also tended to show greater improvement in response time on the A-X

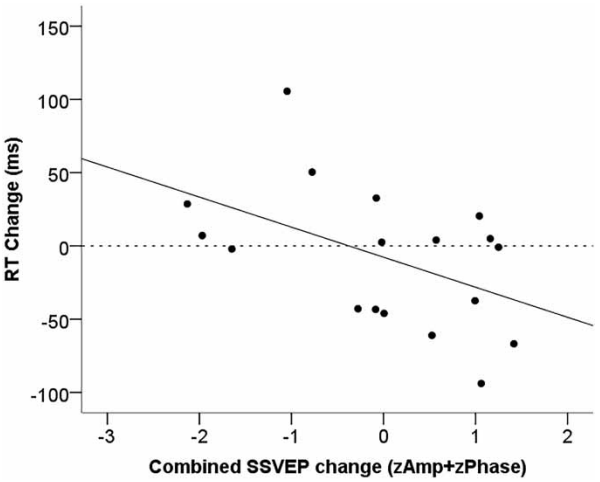

Figure 6 Scatterplot showing association between change in response time on A-X CPT (MV1 – placebo) and composite score of change in SSVEP activity (greater amplitude reduction and phase advance at MV1 treatment associated with more positive scores).

CPT (in the frontal regions where this pattern of change was significant at the group level when contrasting MV1 and placebo). No association was observed between SSVEP changes at the single, right frontal site explored during the target period and performance.

Discussion

The present study explored the acute neurocognitive effects of single-dose multivitamin and mineral combinations on attentional processes. The study demonstrated changes in the SSVEP response during completion of the A-X CPT in the period preceding and during detection of a target stimulus after multivitamin and mineral treatment. These changes, specifically reduced SSVEP amplitude and phase advance (latency reduction) in prefrontal regions, suggest that a single dose of a multivitamin and mineral preparation may enhance the activity within brain regions already engaged by the attentional demands of the task during preparation for, and detection of, target stimuli within a sustained attention task. Importantly, the multivitamin and mineral preparation which contained lower doses of B-vitamins but added guaraná (MV2; Berocca Boost) was not found to be associated with transient changes in SSVEP response during this task when compared to the response following placebo treatment. Direct comparisons of the two active treatments showed relatively greater phase advance in fronto-central regions in the MV2 treatment; however, this did not extend to prefrontal regions found to show greater amplitude reduction and phase advance after treatment with MV1 alone. The follow-up exploratory analyses found that the changes in SSVEP response after treatment with MV1 were found to correlate with improvements in response time, suggesting these differences in SSVEP response at the MV1 treatment were associated with better performance. Where tonic shifts in SSVEP response were investigated, multivitamin and mineral treatment (MV1; Berocca Performance) was found to be associated with a clear shift towards greater phase lag across posterior regions, with SSVEP phase advance observed in only a very specific prefrontal region, suggesting a pattern of increased inhibition across posterior regions, with enhanced excitatory processes in prefrontal regions. Multivitamin and mineral treatment with the addition of guaraná (MV2) was found to be associated with a tonic shift towards greater SSVEP phase advance across distributed brain regions, suggesting a general shift towards greater excitatory processes after a single treatment with this multivitamin, mineral, and guaraná combination.

SSVEP task effects

While the primary focus of the present study was on the change in SSVEP response associated with single-dose multivitamin supplementation, it is noted that task-related changes in SSVEP activity are in line with previous research. In particular, studying the active task versus the reference task at the placebo visit revealed decreased SSVEP amplitude in the frontal and posterior regions, and the SSVEP phase advance after presentation of the target stimulus in the prefrontal regions. These patterns of SSVEP response have been observed previously during similar attention tasks,[15,23,27,32] and have been demonstrated to be compromised in children with ADHD[27] and patients suffering from schizophrenia.[23] That we observed these patterns of activity in the presence of repeated exposure to the task (analysis of task effects at the placebo visit includes six participants each on their first, second, and third exposure to the task) demonstrates the robust networks and brain regions engaged by the task.

Multivitamin and mineral treatment alone: changes in SSVEP response

Administration of a single dose of a multivitamin and mineral combination (MV1), in the absence of guaraná, resulted in transiently reduced amplitude and phase advance in the SSVEP response in the frontal brain regions during completion of a cued attention task. This pattern of response suggests greater activation of task-relevant frontal brain regions during both the orienting and allocation of attentional resources which follow the presentation of the cue, and also upon detection of a target stimulus. Interestingly, while the present study did not find a significant effect of treatment on response times at the group level, an exploratory follow-up analysis

found a significant association between change in response time and the observed changes in amplitude and phase within these frontal regions during the anticipatory hold component of the task. While these findings are exploratory, these results suggest that the pattern of change found to be significant at the group level after MV1 treatment was associated with improved performance on the task. Further, this pattern of greater SSVEP phase advance in prefrontal regions has been correlated with faster response times in healthy adults previously.[23] These findings further corroborate the previously reported increases in functional brain activity associated with a multivitamin and mineral treatment during RVIP task completion using fMRI.[9] While the present study observed increased activity in the frontal regions during the A-X CPT, the fMRI findings suggested increased activity in cerebellar regions. Cerebellar regions have been found to interact with cortical brain regions engaged by visual attention tasks,[13] however, cerebellar regions are unlikely to contribute significantly to scalp recorded SSVEP responses. Thus, the difference in brain regions, found to be altered by multivitamin and mineral treatment, is likely to be explained by the differing demands of the tasks as well as the fundamental differences in the methods used to record brain neurophysiology.

Analysis of tonic changes in SSVEP response associated with the multivitamin and mineral treatment (MV1) revealed a pattern of strong SSVEP phase lag across much of the cortex, with right prefrontal regions exhibiting phase advance and amplitude reduction. These findings suggest greater inhibition, or relatively less excitation, was apparent across distributed posterior brain regions, with the converse of more prominent excitatory activity, or relatively less inhibition, in the prefrontal regions. This pattern of results may reflect greater neural efficiency[44] with which focal task-relevant brain regions are engaged, as a result of greater inhibition across distributed cortical regions not functionally linked to ongoing attentional processing. Macpherson *et al.*[29] also observed SSVEP phase lag (increased latency) after multivitamin supplementation in older females. Similarly, the findings of this chronic supplementation study were interpreted as evidence of enhanced inhibitory processes after multivitamin treatment; however, direct comparisons are difficult given the different cognitive tasks (CPT versus spatial working memory), the treatment regimen (single dose versus 16-week supplementation), and the different treatments (the chronic study treatment included a number of herbal extracts such as *Bacopa monniera* and *Ginkgo biloba*).

Multivitamin and mineral treatment with guaraná: changes in SSVEP response

Somewhat surprisingly, the transient changes in the SSVEP response during A-X CPT completion did not significantly differ between placebo and multivitamin, mineral plus guaraná treatment (MV2). Direct comparisons between the two active treatments suggested relatively greater phase advance across fronto-central regions in the MV2 treatment condition; however, this did not extend to the prefrontal regions during hold or target components of the task (where the major transient task-related effects were observed for the MV1 treatment). Our previous fMRI study[9] reported that this treatment was associated with benefits to mood and cognition, as well as increased functional brain activation over-and-above that observed in the multivitamin and mineral without guaraná using fMRI. While this treatment contained lower doses of B-vitamins, acute behavioural effects have been previously demonstrated for guaraná in isolation[7,8] and in combination with multivitamin and mineral supplements.[4] One possible explanation for the lack of transient changes in SSVEP response after this treatment, when compared to placebo, is the normalization procedure applied to the raw SSVEP amplitude and phase. Normalization of both amplitude and phase data is commonly adopted in order to reduce inter-individual differences in the SSVEP response. As part of this investigation, normalization of both SSVEP amplitude and phase was conducted for each treatment separately, such that the reference task at each visit formed the basis for normalization. As the focus of the investigation was transient changes in the SSVEP response related to attentional processing, this normalization procedure was deemed most appropriate. Thus, these findings do not contradict the effects of treatment on neurocognition, but suggest that a single dose of multivitamin and mineral treatment with guaraná does not uniquely influence the transient task-related SSVEP shifts related to cued attention.

The analysis of more tonic shifts in SSVEP response associated with multivitamin and mineral plus guaraná treatment revealed phase advance across distributed brain regions, consistent with the presence of greater excitatory processes. This pattern of results is consistent with the observation of greater phase advance seen in the transient task-related analyses, and further corroborates the findings of our previous fMRI investigation.[9] Further, these findings are consistent with the known central actions of caffeine, of which the MV2 treatment contained 40 mg derived from the guaraná. Caffeine in moderate doses has demonstrated benefits to mood and aspects of cognitive function,[45,46] and is known to exert (largely

opposing) central effects as an antagonist of two adenosine receptor types (for a review, see Fredholm *et al.*[47]). While A_{2A} receptors are primarily linked with dopaminergic neurons of the striatum and nucleus accumbens, A_1 receptors are distributed across cortical, hippocampal, and cerebellar regions. Caffeine is thought to increase cortical excitation through blockade of these A_1 receptors, which results in presynaptic release of glutamate. It has been proposed that the SSVEP phase reflects the transmission time of cortico-cortical loops such that the phase advance reflects an increase in excitatory processes, with phase lag reflecting the opposite.[16,23] As guaraná contains a number of potentially bioactive constituents beyond caffeine, including saponins, tannins, and catechins,[6] further research contrasting guaraná with caffeine treatment alone would be required to further explore the contribution of these constituents to the neurocognitive effects observed. Finally, in addition to probing the transient task-related changes, these findings suggest that the SSVEP technique may also be sensitive to more tonic shifts in cortical neurophysiology associated with psychopharmacological investigations.

This exploration of the neurocognitive effects of single-dose multivitamin/mineral supplementation is preliminary in nature and a number of limitations must be considered. It is important to note that these neurophysiological changes were observed in the absence of parallel behavioural differences on the A-X CPT. The lack of observed behavioural effects is potentially explained by the evidence of ceiling effects in the task, suggesting task difficulty was insufficient to detect subtle changes brought about by treatment. Alternatively, it may be that the observed changes in brain activity are sub-threshold for impacting behavioural performance on such tasks. This investigation adopted the A-X CPT to probe attentional processes based on the known SSVEP correlates of this variant of the task.[23,27,32] As such, while alternative A-X CPT variants have shown sensitivity to acute psychopharmacological manipulations,[36,37] the primary purpose of this variant of the A-X CPT was to serve as an activation task. While treatment-related changes in SSVEP amplitude and phase were found to be correlated with changes in behavioural performance in the MV1 treatment condition, future research may employ modifications to the A-X CPT in order to further assess the potential acute neurocognitive effects of multivitamin/mineral preparations (see e.g. Mette *et al.*[37]).

Summary

Dietary intake of vitamins and minerals is known to be essential for healthy neurocognitive functioning, with a range of deficits linked to micronutrient deficiencies.[48–52] While these micronutrients are important cofactors in energy metabolism and neurotransmitter metabolism,[53–58] the psychopharmacological impact of a single dose of a multivitamin and mineral preparation remains relatively unexplored. The findings from the present study provide preliminary evidence to suggest that single doses of multivitamin and mineral preparations, both with and without guaraná, influence functional brain activity in healthy younger adults. Consistent with the known actions of caffeine, multivitamin and mineral treatment with the addition of guaraná produced tonic changes in the SSVEP response consistent with greater cortical excitation. Multivitamin and mineral treatment alone was found to enhance transient shifts towards greater excitation in the prefrontal regions during completion of the CPT. Exploratory analyses suggested this pattern of change was correlated with changes in behavioural performance on the task, such that SSVEP amplitude reduction and phase advance were associated with improved response times after treatment with multivitamin and mineral treatment. These findings are preliminary in nature and require further exploration, particularly in the absence of acute behavioural effects after multivitamin/mineral treatment observed at the group level. However, these findings converge with our previously reported changes observed using fMRI,[9] suggesting a single multivitamin/mineral dose is sufficient to impact on functional brain activity in task-related brain regions.

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