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Smith, Anika Kathryn, Wade, Alexander Robert Patrick orcid.org/0000-0003-4871-2747, Penkman, Kirsty Elizabeth Helena orcid.org/0000-0002-6226-9799 et al. (1 more author) (2017) Dietary modulation of cortical excitation and inhibition. Journal of Psychopharmacology. pp. 632-637. ISSN: 1461-7285

https://doi.org/10.1177/0269881117699613

Smith, Wade, Penkman & Baker (2017) *Journal of Psychopharmacology*
doi: 10.1177/0269881117699613

Dietary modulation of cortical excitation and inhibition

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Abstract

The balance of excitatory and inhibitory neurotransmitters in the brain affects both neural responses and behaviour in humans and animals. Here we investigated whether dietary intervention aimed at increasing levels of the inhibitory neurotransmitter GABA can influence neural responses to basic sensory stimuli. Using a steady-state EEG paradigm, we found that the neural response to visual patterns was reduced in individuals who consumed a yeast-extract product rich in substances associated with the production of GABA (glutamate and B vitamins), but not in a control group who consumed a placebo substance (N=14 per group). This demonstrates that the balance of excitation and inhibition in the brain can be influenced by dietary interventions, suggesting possible clinical benefits in conditions (e.g. epilepsy) where inhibition is abnormal.

Keywords: steady-state EEG, inhibition, GABA, diet, precursors

1 Introduction

The healthy brain depends on a delicate balance of excitation and inhibition governed by neurotransmitters such as gamma-aminobutyric acid (GABA). Atypical levels of GABA have been associated with disorders such as epilepsy (Petroff et al., 1996), autism (Robertson et al., 2016), anxiety (Nemeroff, 2003) and depression (Honig et al., 1988), suggesting potential therapeutic benefits to modulating its concentration. Although dietary supplements containing GABA are available commercially, their efficacy is limited due to low GABA permeability across the blood/brain barrier (Kakee et al., 2008). Inspired by work showing that serotonin levels are increased by consumption of serotonin precursors (i.e. tryptophan (Shabbir et al., 2013)), we asked whether consuming substances associated with the production of GABA could modulate the balance of inhibition and excitation in the brain.

GABA functions as the primary inhibitory neurotransmitter in the mammalian nervous system. It is produced in the brain as a part of the GABA-glutamate cycle, with pyridoxine (vitamin B6) as a co-factor in this synthesis, and other substances such as vitamin B12 having a modulatory effect on its concentration (Ikeda et al., 1997). It is well established in neurophysiological work that evoked responses in early visual cortex are strongly modulated by GABA-ergic processes (Katzner et al., 2011; Morrone et al., 1987). Infusing a GABA antagonist (gabazine) into the cortex of anaesthetized cats has been shown to increase responsiveness by up to 300% (Katzner et al., 2011). This 'response gain' effect should provide a clear index of GABA availability in cortex, in that increasing GABA concentration should reduce the neural response evoked by visual stimuli to below normal levels.

We measured steady-state visual evoked potentials (Norcia et al., 2015) using electroencephalography (EEG) as a baseline index of neural excitability. In this paradigm, flickering visual stimuli are displayed at different contrast levels, and a frequency-locked response is recorded from posterior regions of the scalp over visual cortex (see Figure 1 for details). Participants then consumed either a yeast-extract substance rich in glutamate and B-vitamins, or a control substance (peanut butter), each day for one month (while also continuing their normal diet), followed by a repeat of the EEG measurements. We determined that the active substance contained around 116 times more vitamin B12, 3 times more vitamin B6, and 1.85 times more glutamate than the control substance (see Table 1 for further details). Any change in neural response as a consequence of the treatment is therefore likely to be due to these substances promoting increased GABA availability.

Table 1: Concentration of glutamate and B-vitamins in the two substances used in the experiment. Inhomogeneity of the peanut butter led to greater variability between analyses for the glutamate analysis (2 analytical replicates; 3 subsamples, error term represents coefficient of variance).

Nutrient	Yeast-extract	Peanut butter	Ratio
Vitamin B6 (Pyridoxine)	0.57 mg / 100 g	0.19 mg / 100 g	3.0
Vitamin B12 (cyanocobalamin)	29 µg / 100 g	0.25 µg / 100 g	116.0
Glutamate (as glutamic acid)	2.8 g / 100 g (±12%)	1.5 g / 100 g (±43%)	1.85

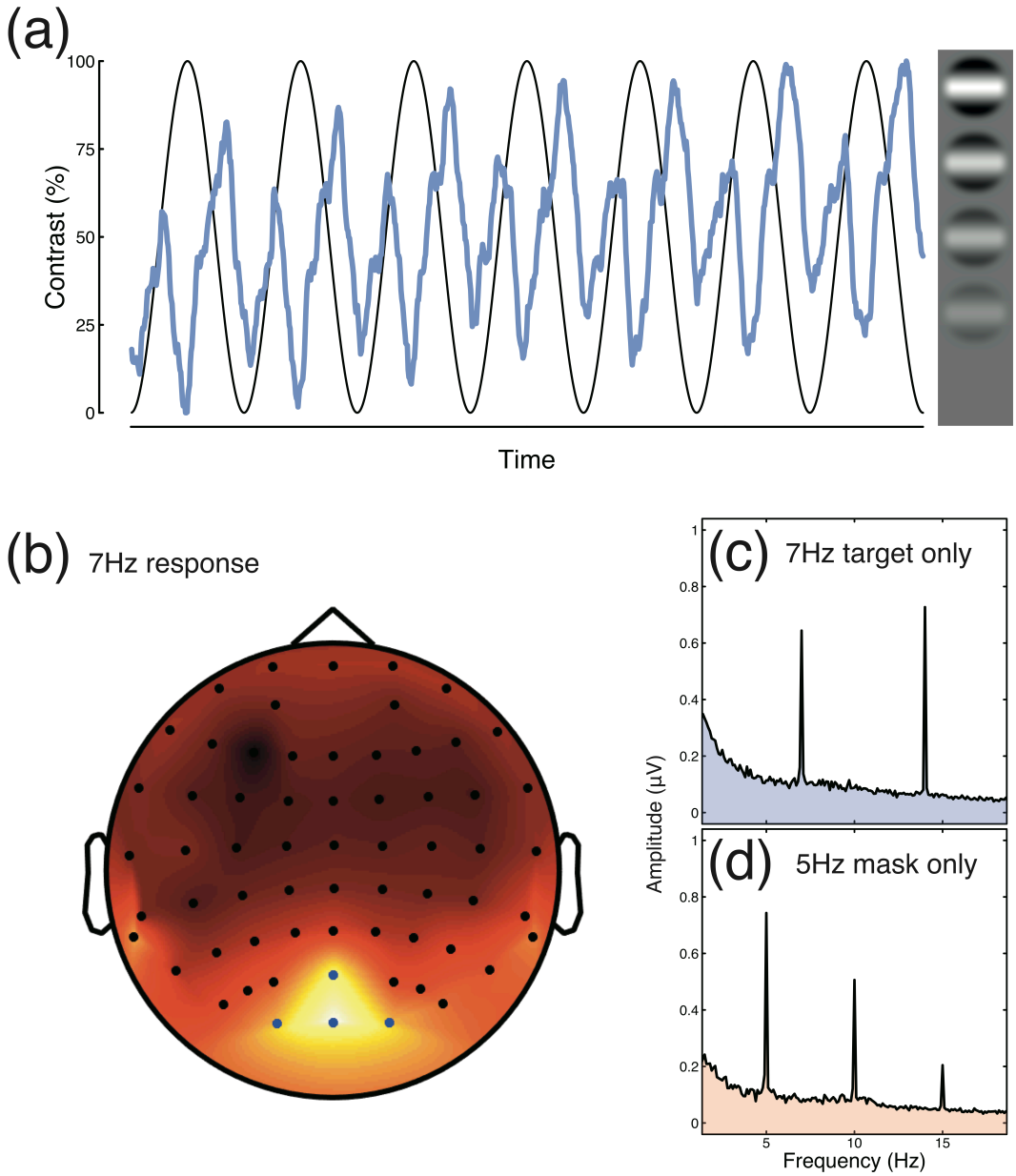

Figure 1: Illustration of the steady-state visual evoked potential paradigm. A visual stimulus flickering in contrast at a defined temporal frequency (smooth black curve in panel a) will produce a modulation of the EEG waveform (blue trace in panel a) over posterior scalp regions (panel b) at the target frequency and its harmonics. The dependent variable is the amplitude in the Fourier spectrum at the flicker frequency, which shows excellent isolation from adjacent frequencies, implying high signal-to-noise ratios (panel c,d). The data used to produce this figure were averaged over 28 observers in the baseline condition (pre-treatment) at the highest target contrast (panels a-c) and for a condition in which a mask was shown in isolation (panel d).

2 Methods

2.1 Participants

A group of 28 adult volunteers (10 male, mean age 22) completed the study after providing written informed consent. They were assigned randomly to either the control or treatment group, and were not informed of the experimental hypotheses. Because the experiment involved viewing flickering stimuli, we ensured that participants did not have a history of epilepsy. We also confirmed that participants did not smoke, suffer from nut allergies, or use controlled substances recreationally. Procedures were approved by the research ethics committee of the Department of Psychology at the University of York.

2.2 Apparatus and stimuli

Stimuli were displayed using a gamma corrected ViewPixx display (VPixx Technologies Ltd., Montreal, Canada) running at 120 Hz. We recorded EEG signals at 1 kHz from 64 electrodes across the scalp (see Figure 1b for electrode locations) using a Waveguard cap and the ASAlab system (ANT Neuroscan, Netherlands). Stimulus onset and condition was recorded on the EEG trace using a low-latency digital trigger. EEG signals were stored on disk for offline analysis.

Stimuli were small patches of sine-wave grating, spatially curtailed by a raised cosine envelope (see Figure 1a for examples). Each patch had a spatial frequency of 0.5c/deg, and a diameter of 3deg. We tiled the patches across the display in a regular 17x9 grid separated by 3 degrees of visual angle. The central element was removed to leave a space for an attentional control task, described below. To minimise adaptation, we randomised the orientation of the elements on each trial. Stimuli were presented using Matlab (The MathWorks Inc., Massachusetts, USA) and the Psychophysics Toolbox extensions, running on an Apple Macintosh computer.

The yeast-extract substance was a commercially available spread (*Marmite*; Unilever Plc., London, UK) that is fortified with B-vitamins. The control substance was smooth peanut butter containing peanuts (97%), palm oil and salt. We tested 3 subsamples of both substances for glutamate content (following 24 hour acid treatment to hydrolyse any peptide-bound glutamic acid) using reverse-phase high pressure liquid chromatography (Penkman et al., 2008). During hydrolysis, glutamine undergoes rapid irreversible deamination to glutamic acid (Hill, 1965), so as it is not possible to distinguish these, they are reported as Glx. Since similar processes would likely occur during metabolisation, this was not a major concern. We also had the B-vitamin content assessed at an ISO17025 accredited laboratory in the UK. Detailed results of these analyses are presented in Table 1.

2.3 Procedures

Participants first completed four blocks of the EEG experiment. In each block, trials of 11 s duration were presented. Trials were presented in random order, with a 3 s inter-trial interval. There were 7 target contrast levels from 0% to 64%, and 2 mask contrasts (0% and 32%). The target stimuli were cosine-phase sine-wave gratings that flickered sinusoidally in contrast between 0% and their nominal maximum at 7 Hz. The mask stimuli were superimposed sine-phase gratings of the same spatial frequency but orthogonal orientation to the targets, that flickered at 5 Hz. The effect of the orthogonally-presented mask stimuli is to suppress neural responses to the target stimuli, with higher GABA levels being thought to result in greater suppression (Morrone et al., 1987). Each condition was repeated twice per block, resulting in 8 repetitions per observer. Next, participants were given a jar of either the active or placebo substance and instructed to consume 5 mL (one teaspoon full) daily for one month. Their diets were otherwise unchanged. At the end of this time they returned to the lab and repeated the same EEG experiment. Participants also completed an adherence questionnaire, which indicated that no participants forgot to take more than 4 doses across the four weeks, and when they did so typically (in all but one case) took a double dose the following day. A subset of 5 participants in the treatment group repeated the experiment a third time three months after the initial testing session.

During all blocks of the experiment, participants completed a contrast discrimination task in the centre of the screen that was intended to maintain fixation and attention. The stimuli were the same sine-wave gratings that were used as a stimulus in the EEG experiment, but always with a horizontal orientation. The duration of each trial was 1400ms total, in which pairs of stimuli with different contrasts were presented sequentially for 500 ms (inter-

stimulus-interval of 400 ms), flickering at 6 Hz. The task was to report which of the stimuli appeared higher in contrast. In one interval the contrast was drawn from a normal distribution with a mean of 0% and a standard deviation of 2%. In the other interval the mean was 2% and the standard deviation was 2%. Negative contrasts constituted a phase reversal.

We discarded the first 1000 ms of each EEG trial, and took the Fourier transform of the remaining 10000 ms. The amplitudes at the first and second harmonic of the target and mask frequencies were taken as dependent variables. We averaged amplitudes coherently across trials and across the four posterior electrode locations that gave the strongest signal (electrodes Oz, O1, O2 and POz, highlighted blue in Figure 1b). We then took the absolute value (discarding phase information) to calculate averages across observers and to perform statistical testing. Because the results of the treatment at the target frequency were similar in the 0% and 32% mask conditions (see Figure S1a,b), we ran a 4-way mixed ANOVA on these data (see supplementary table S1). At the mask frequency, there was no modulation in response when the mask contrast was 0%, but a substantial modulation when the mask contrast was 32% (see Figure S1c,d). We therefore ran two separate 3-way mixed ANOVAs for these conditions (see supplementary tables S2 & S3). Raw data are available online at: https://dx.doi.org/10.6084/m9.figshare.3507410.v1

3 Results

The steady-state paradigm produced monotonic contrast response functions that were locked to the target frequency of 7 Hz (see Figures 1c and 2a,b). When a second high-contrast component (which we term the 'mask') was added flickering at a different frequency (5 Hz), it produced a strong response locked to its frequency (see Figures 1d and 2c,d). The mask response was suppressed by high target contrasts (downward trend in Figure 2c,d), consistent with results using similar stimulus arrangements in both humans and animals (Busse et al., 2009; Heeger, 1992).

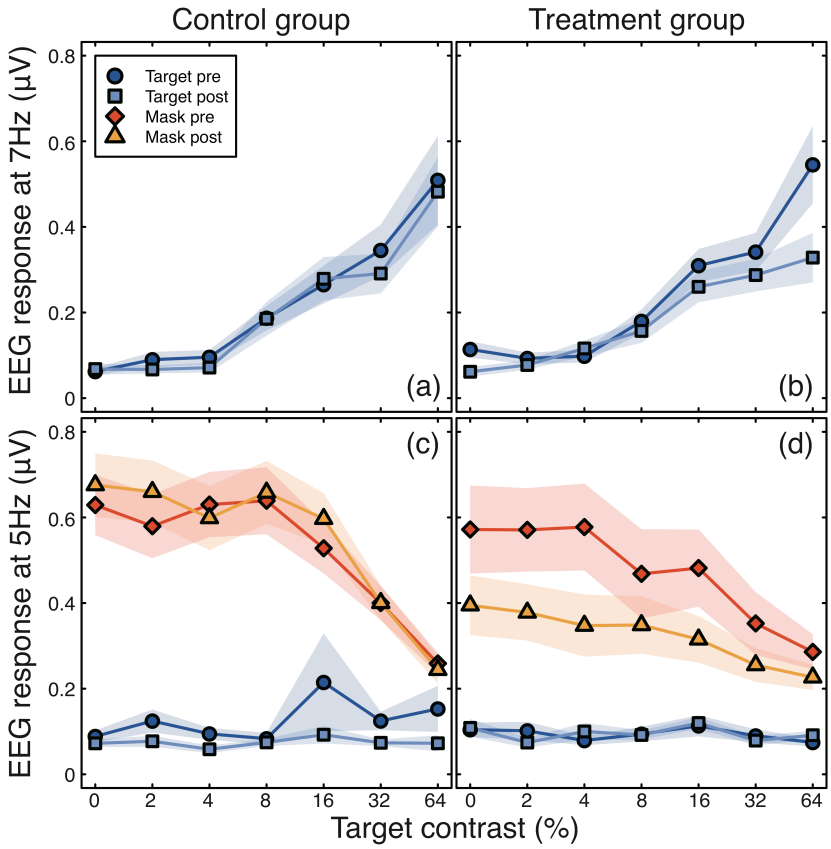

Figure 2: Steady-state EEG responses at the target (panel a,b) and mask (panel c,d) frequencies in both the placebo control group (left) and the treatment group (right). Each data point is the average of 14 participants, with shaded regions indicating ±1SE of the mean.

At the target temporal frequency (and at its second harmonic – see Figure S2 in supplementary information), evoked responses at high contrasts (> 8%) were reduced following consumption of yeast-extract, but not peanut butter (Figure 2a,b). This is supported by a significant interaction ($F_{2.53,65.73} = 4.10$, $p < 0.05$, partial $\eta^2 = 0.14$) between substance (active vs. control), time (baseline vs. post-consumption) and target contrast (collapsing across mask contrast levels, which were similarly affected, see table S1 in supplementary information). The involvement of target contrast in the interaction is critical, as it demonstrates that only stimulus-evoked responses were affected – the baseline 'noise' levels when viewing a blank screen remained constant, ruling out nuisance variables such as practise effects and equipment changes. There was also no modulation of responses at the mask frequency when the target was presented alone (blue circles and squares in Figure 2c,d, see table S2).

We replicated the critical effects at the mask temporal frequency (5 Hz), where a consistent reduction in amplitudes (average of 30%) was observed at all target contrast levels following treatment (Figure 2d), but not placebo (Figure 2c). Interactions between substance and time ($F_{1,26} = 8.46$, $p < 0.01$, partial $\eta^2 = 0.246$) and between substance, time and contrast ($F_{4.52,117.54} = 3.06$, $p = 0.015$, partial $\eta^2 = 0.105$) were significant at the mask frequency (see supplementary table S3).

Since visual responses are modulated by attention (Verghese et al., 2012), we considered whether subjects in the active group might have paid less attention to the display during their second testing session. Throughout all experiments, participants performed a challenging contrast discrimination task in the centre of the display to maintain fixation and attentional focus. We confirmed that performance remained constant across testing sessions for both the active (pre/post averages of 68% and 69% correct, $p = 0.59$) and control (pre/post averages of 67% and 66% correct, $p = 0.69$) participants, ruling out changes in attention as a confounding factor in this study. We also assessed how long the effects might last by recalling a subset of participants from our treatment group and retesting them two months after they had ceased consuming the active substance. Response levels were still reduced, but no longer significantly different from baseline in this group at either temporal frequency, suggesting that the effects begin to wear off after resuming a normal diet (see Figure 3).

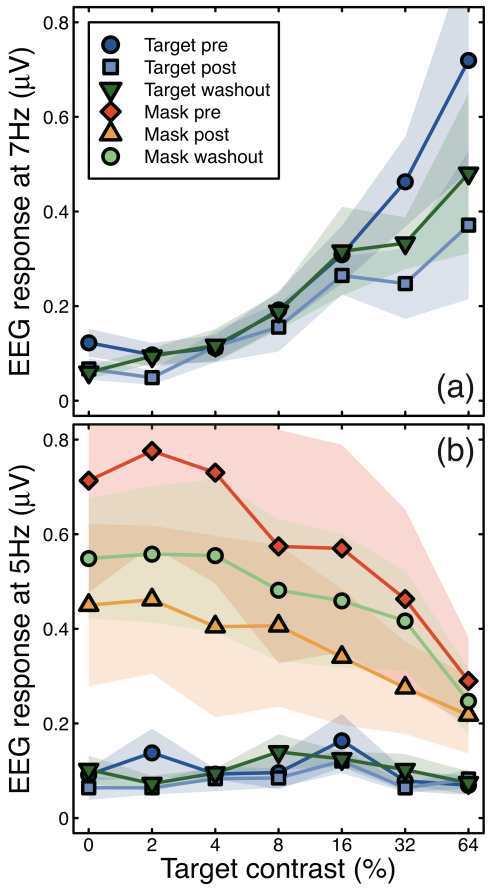

Figure 3: Data from a subset of 5 participants in the treatment group who returned after a further two months during which they did not consume the active substance. The effect of the treatment after one month is still apparent for this subgroup (compare circles and squares at high contrast in panel a). At 7 Hz there was a main effect of time ($F_{1,4} = 8.16$, $p < 0.05$, partial $\eta^2 = 0.671$) and time*contrast interaction ($F_{6,24} = 4.58$, $p < 0.01$, partial $\eta^2 = 0.534$). However, following the washout period of 2 months, responses had begun to return towards baseline (triangles in panel a, green circles in panel b), and were no longer significant ($p>0.05$ for main effect of time and time*contrast interactions). These results suggest that the effects might be relatively long lasting, in the order of weeks or months.

4 Discussion

Using a steady-state EEG paradigm, we found that a dietary intervention had a significant effect on the brain's response to visual stimuli, compared with consumption of a placebo. This was unlikely to be a consequence of attentional lapses, and the effects were reduced after two months of resuming a normal diet. These findings are consistent with an increase in the availability of GABA in visual areas of the brain that inhibits the excitability of neurons responsive to the stimulus (Katzner et al., 2011). Although we did not observe clear

changes in suppression between stimuli (i.e. the reduction in response to the target caused by presentation of the mask did not substantially increase following treatment, as shown in Figure S1b), we also anticipate that dietary modulation of neural inhibition will prove important for understanding basic suppressive processes in the healthy brain (Morrone et al., 1987).

Patients with epilepsy show increased visual responses using a similar steady-state paradigm to that used here (Porciatti et al., 2000; Tsai et al., 2011). This raises the possibility that dietary interventions geared towards increasing GABA concentration might reduce excitability to normal levels, and potentially alleviate some symptoms of the disorder such as seizure frequency (particularly for photosensitive epileptics). This might be of particular utility in treating patients who either do not respond to traditional medication, or who cannot take it for other reasons (e.g. pregnancy, or interactions with other drugs). The apparent involvement of GABA in other neurological and mental health conditions (Honig et al., 1988; Nemeroff, 2003; Robertson et al., 2016) suggests further potential for deployment of dietary interventions.

Could some alternative pathway be responsible for our results? One possibility is that vitamin B12 is involved as a co-factor (Briani et al., 2013) in the production of myelin (the fatty sheath that insulates axons and improves nerve conductance). Increased myelin should speed up transmission of signals through the nervous system, yet an additional analysis of the phase component of our steady-state data indicated no significant change in the response lag at the mask frequency following treatment (t-test for mask alone, t=-1.55, df =13, p=0.15; the mask frequency was chosen because it was slower than the target frequency (5Hz vs 7Hz) so will have fewer wraparound artefacts). Changes in myelin could conceivably influence signal amplitudes, though this would presumably be through increased signal fidelity (reduced noise). This account would predict increased amplitudes following supplementation, yet the effect of the treatment was to reduce amplitudes (see Figure 2b,d). We intend to investigate this possibility further in future studies using structural MRI. It is also conceivable that other micronutrients present in yeast-extract (such as tyrosine, tryptophan, phenylanaline, niacin, folic acid and riboflavin) could have affected our results. However, we are not aware of any plausible pathway by which these nutrients could affect excitation and inhibition, and their concentration in the active substance is not dramatically higher than in a typical diet.

The findings of this study suggest that dietary intervention may modulate cortical excitation and inhibition, which may be due to increased GABA concentration. If this is the case, although previous studies have shown that drugs such as lorazepam can transiently affect both GABA concentration and perception (van Loon et al., 2013), this is the first demonstration (to our knowledge) of dietary modulation of the same pathways. We anticipate that the processes involved will operate over a longer time-scale, and perhaps be more stable than drug treatments. Since our active substance contained several ingredients, we hope to determine from future work which substance(s) are key to modulating levels of this neurotransmitter in the cortex. Though it is possible that the effects may be due to substances present in the yeast extract other than those highlighted here, the dramatically high concentrations of vitamin B12 in our active substance (see Table 1), as well as potentially high levels of marginal deficiency in the population (Allen, 2009), make this nutrient the most likely candidate for driving our effects.

5 Author Contributions

AKS: designed the experiment, ran the experiment, analysed the data, wrote the paper
ARW: designed the experiment, wrote the paper
KEHP: ran the experiment, analysed the data, wrote the paper
DHB: designed the experiment, ran the experiment, analysed the data, wrote the paper

6 Funding

Supported in part by the Wellcome Trust (ref: 105624) through the Centre for Chronic Diseases and Disorders (C2D2) at the University of York. Also supported by the Leverhulme Trust.

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8 Supplementary Material

Table S1: Full ANOVA table for the 7 Hz response, corresponding to the data in Figure S1a,b. The model was a 4-way mixed ANOVA, with between subjects factor of substance (treatment vs control) and within subjects factors of time (pre and post treatment), mask contrast (0 and 32%) and target contrast (7 levels). Significant effects are highlighted in bold. Non-integer degrees of freedom indicate Greenhouse-Geisser corrected values.

	F-ratio	Num df	Denom df	*p*	Partial η^2
Substance	0.22	1	26	0.882	0.001
Time	**4.37**	**1**	**26**	**0.046**	**0.139**
Mask contrast	**4.21**	**1**	**26**	**0.050**	**0.144**
Target contrast	**46.61**	**1.4**	**36.53**	**<0.001**	**0.642**
Substance * time	1.89	1	26	0.182	0.068
Substance * mask	0.03	1	26	0.873	0.001
Substance * target	0.35	1.4	36.53	0.630	0.013
Time * mask	3.94	1	26	0.058	0.132
Time * target	**4.88**	**2.53**	**65.73**	**0.006**	**0.158**
Mask * target	**8.77**	**2.55**	**66.27**	**<0.001**	**0.252**
Substance * time * mask	0.27	1	26	0.608	0.010
Substance * time * target	**4.10**	**2.53**	**65.73**	**0.014**	**0.136**
Substance * mask * target	0.15	2.55	66.27	0.903	0.006
Time * mask * target	0.59	3.45	89.70	0.645	0.022
Substance * time * mask * target	1.53	3.45	89.70	0.207	0.056

Table S2: Full ANOVA table for the 5 Hz response in the 0% mask contrast condition, corresponding to the blue data points in Figure S1c,d. The model was a 3-way mixed ANOVA, with between subjects factor of substance (treatment vs control) and within subjects factors of time (pre and post treatment) and target contrast (7 levels). No effects reached significance. Non-integer degrees of freedom indicate Greenhouse-Geisser corrected values.

	F-ratio	Num df	Denom df	*p*	Partial η^2
Substance	0.14	1	26	0.714	0.005
Time	3.04	1	26	0.093	0.105
Target contrast	2.02	1.78	46.30	0.149	0.072
Substance * time	3.31	1	26	0.080	0.113
Substance * target	0.96	1.78	46.30	0.457	0.035
Time * target	0.54	1.71	44.32	0.559	0.020
Substance * time * target	0.70	1.71	44.32	0.482	0.026

Table S3: Full ANOVA table for the 5Hz response in the 32% mask contrast condition, corresponding to the red/orange data points in Figure S1c,d. The model was a 3-way mixed ANOVA, with between subjects factor of substance (treatment vs control) and within subjects factors of time (pre and post treatment) and target contrast (7 levels). Significant effects are highlighted in bold. Non-integer degrees of freedom indicate Greenhouse-Geisser corrected values.

	F-ratio	Num df	Denom df	*p*	Partial η^2
Substance	2.79	1	26	0.107	0.097
Time	**4.39**	**1**	**26**	**0.046**	**0.145**
Target contrast	**29.50**	**2.08**	**53.99**	**<0.001**	**0.532**
Substance * time	**8.46**	**1**	**26**	**0.007**	**0.246**
Substance * target	3.01	2.08	53.99	0.056	0.104
Time * target	1.78	6	156	0.107	0.064
Substance * time * target	**3.06**	**4.52**	**117.54**	**0.015**	**0.105**

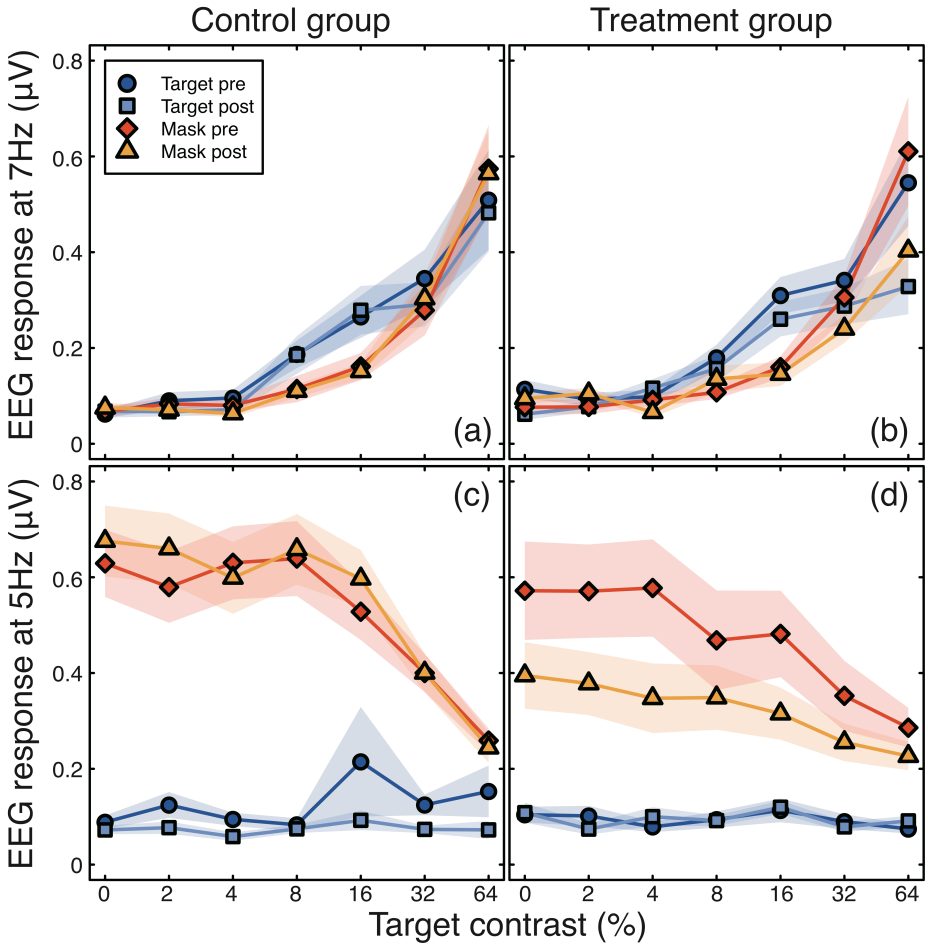

Figure S1: Reproduction of Figure 2 with data added at the target frequency for the 32% mask condition (red diamonds and orange triangles in panels a,b). These data were removed from the main figure to reduce clutter, but show the same pattern of reduced amplitudes at high target contrasts following treatment for the treatment group (panel b), but not the control group (panel a).

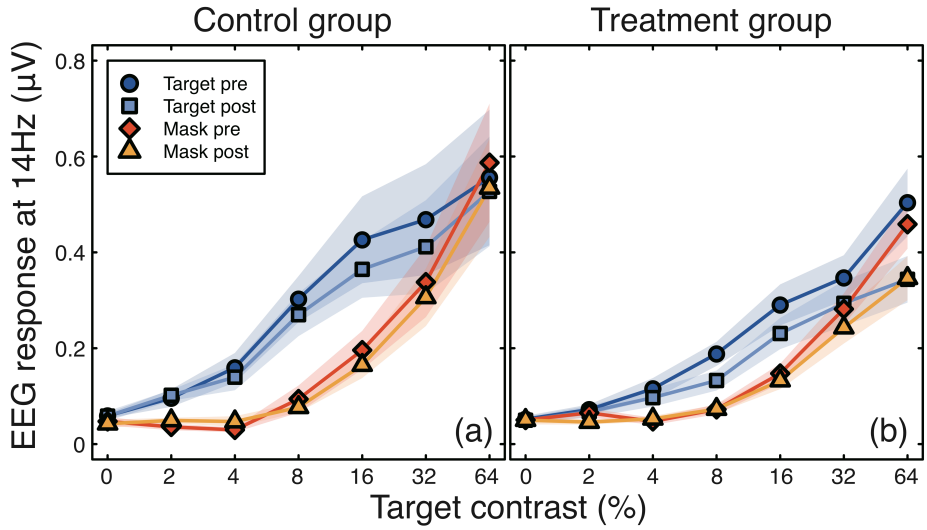

Figure S2: Steady-state responses at the second harmonic of the target frequency (14 Hz), in the same format as Figure S1a,b. There was an amplitude reduction in the treatment group at high contrasts for the post-consumption phase (squares and triangles). Response at the second harmonic of the mask frequency (10 Hz) fell within the alpha band and were therefore noisier and less reliable (not shown).