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Acute gabapentin administration in healthy adults. A double-blind placebo-controlled study using transcranial magnetic stimulation and 7T ^{1}H-MRS

Katherine Dyke [a,*], Sophia E. Pépés [a], Ben Babourina-Brooks [b], Chen Chen [b], Soyoung Kim [a,c], Hilmar P. Sigurdsson [a], Amelia Draper [a], Masud Husain [d], Parashkev Nachev [e], Penny Gowland [b], Peter G. Morris [b], Stephen R. Jackson [a,b,c]

[a] School of Psychology, University of Nottingham, UK
[b] Sir Peter Mansfield Imaging Centre, University of Nottingham, UK
[c] Institute of Mental Health, School of Medicine, University of Nottingham, UK
[d] Nuffield Department of Clinical Neuroscience, University of Oxford, UK
[e] Institute of Cognitive Neuroscience, University College London, UK

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ABSTRACT

Gamma-aminobutyric acid (GABA) and glutamate are the primary neurotransmitters responsible for modulating excitatory and inhibitory signalling within the human brain. Dysfunctional GABAergic and glutamatergic signalling has been identified as a key factor in a range of neuropsychiatric conditions; hence measurement and modulation of these neurometabolites is important for improving our understanding of neuropsychiatric conditions and treatment options. Gabapentin (GBP) is one of several drugs developed to increase GABA levels and is routinely prescribed for conditions such as epilepsy and neuralgia. While animal and human studies indicate that GBP can elevate GABA levels, its exact mechanisms of action are not fully understood, although animal studies indicate that GBP does not have a direct effect upon GABAergic receptors.

To investigate the impact of acute GBP administration in the human motor system we used two complimentary approaches – transcranial magnetic stimulation (TMS) and magnetic resonance spectroscopy (MRS). MRS and TMS measures of GABA have repeatedly been found to be uncorrelated and are likely to reflect different pools of synaptic and extra synaptic GABA, hence, measuring both within the same participants allows for an in-depth assessment of GBP effects.

Despite significantly increased ratings of fatigue and tiredness within the GBP group, we failed to find any statistically significant changes in our MRS or TMS measures of GABA. Measures of MRS Glutamate (glu) and glutamine (gln) were also not affected by the administration of GBP. These findings are important as they run counter to previous work, and suggest that the effect of an acute dose of GBP is likely to be subject to substantial individual variation, with timing of measures particularly likely to impact observed effects. These findings have implications for the use of acute GBP dosing as a means to explore GABAergic function in health and disease.

1. Introduction

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the human brain. It is estimated to be present in 25–50% of synapses and plays a critical role in maintaining neural excitability within a functional range. GABA is synthesised from glutamate, which in turn is derived from glutamine; all three form part of a metabolic cycle which can be drawn upon for neurotransmission (Rae, 2014).

Multimodal neuroimaging approaches have provided compelling evidence that alterations in GABAergic signalling are prevalent in a range of neuropsychiatric and neurodevelopmental conditions; including, Tourette syndrome (Orth, 2009; Draper et al., 2014; Puts et al., 2015 Lerner et al., 2012); obsessive compulsive disorder (OCD) (Simpson et al., 2012); autism spectrum disorder (Puts et al., 2017 Sapey-Triomphe et al., 2019); mood and anxiety disorders (Luscher et al., 2011) and schizophrenia (Shaw et al., 2020). Consequently, approaches which

* Corresponding author.
E-mail address: katherine.dyke@nottingham.ac.uk (K. Dyke).

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modulate GABAergic signalling are appealing as potential therapeutic interventions.

Several drugs have been developed which have notable effects in increasing GABA levels, one of which is Gabapentin (1-(aminomethyl) cyclohexaneacetic acid [Neurontin®]) (GBP). GBP is a widely prescribed drug, typically used to treat conditions such as epilepsy and neuralgia, and was originally synthesised as an analogue of GABA. However, experiments in animals found no evidence that GBP has a direct effect on GABAergic receptors (Taylor et al., 1998 Ziemann et al., 2015). Thus, GBP does not appear to interact directly with GABA-A or GABA-B receptors (Goa and Sorkin, 1993; Sills, 2006 Taylor et al., 1998), nor does it appear to be a GABA-A (Kondo et al., 1991) or GABA-B agonist (Lanneau et al., 2001). Instead, it is thought that GBP acts via α2δ voltage-dependent calcium channel subunits (Ziemann et al., 2015), and that its overall effect is to increase GABA synthesis and turnover (Loscher et al., 1991). It should be noted that much of the research into the effects of GBP has been conducted in rats, and it is significant that a study comparing the effects of GBP in human and rat neocortical slices revealed substantial differences (Errante et al., 2002). Caution is therefore warranted when extrapolating from rodent to humans.

MRS and TMS are non-invasive approaches, which can be used to measure concentrations and synaptic activity relating to several neurometabolites including GABA and glutamate, within localised areas of brain tissue. Consequently, these techniques have been applied to study the underlying mechanisms of drugs such as GBP in the human brain. Interestingly, while MRS and TMS measures of GABA appear to be largely uncorrelated, and likely reflect different pools of GABA with distinctive underlying mechanisms (Dyke et al., 2017; Stagg et al., 2011; Stagg et al., 2011 Tremblay et al., 2013), both have been reported previously to be modulated by GBP administration (Cai et al., 2012; Kuzniecky et al., 2002; Petroff et al., 1996; Rizzo et al., 2001 Ziemann et al., 1996).

To our knowledge, two studies have explicitly used TMS to assess changes in motor cortical excitability following acute administration of GBP. These studies assessed the impact of GBP on various single and paired pulse TMS measures, including short interval intra-cortical inhibition (SICI), short interval intra-cortical facilitation (ICF) and motor threshold (MT). SICI is a paired pulse TMS approach, during which a subthreshold conditioning stimulus (CS) is delivered to the motor cortex 1–5 ms prior to a supra threshold test stimulus (TS) (Hanajima and Ugawa, 2008). It is widely thought that the CS results in a short-lasting inhibitory postsynaptic potential in corticospinal neurons via the activation of a low-threshold cortical inhibitory circuit. The engagement of this circuit is then thought to inhibit the action potentials generated in the same pool of corticospinal neurons in response to the subsequent TS (see Ziemann (2013) for a review). When measured in the motor cortex, the result of the CS-TS paring is a smaller motor evoked potential (MEP) than that obtained from the TS alone. Animal models and pharmacological studies have suggested that the effects of SICI are strongly mediated by the activity of GABA-A receptors (Hanajima and Ugawa, 2008 Ziemann, 2013); with a lesser contribution from GABA-B receptor activity (McDonnell et al., 2006). Interestingly, while the general consensus is that GBP does not directly impact GABA-A receptors (Goa and Sorkin, 1993; Sills, 2006 Taylor et al., 1998); in humans 800 mg and 1200 mg doses of GBP have been reported to increase SICI 3 h after administration (Rizzo et al., 2001 Ziemann et al., 1996). These findings may suggest indirect mechanisms of action through which the overall balance of excitation/inhibition is altered.

In contrast to SICI, ICF measures excitatory neuronal circuits within the motor system. ICF is also measured using a paired pulse TMS approach, however, the interval between pulses is set at 7–20 ms (Kujirai et al., 1993), and although the facilitation of motor excitability is the reverse of SICI effects, the underpinnings of these approaches appear to be distinct (Ziemann, 2013). Mechanistically, both N-methyl-D-aspartate (NMDA) receptor antagonists and benzodiazepines which positively modulate GABA-A receptors have been found to reduce ICF (Ziemann, 2013). This suggests that ICF is in part modulated via processes that involve glutamatergic and GABAergic mechanisms. In both previous studies, GBP was found to reduce ICF (Rizzo et al., 2001 Ziemann et al., 1996).

Unlike SICI and ICF, MT has not been reported to be altered by GBP (Rizzo et al., 2001 Ziemann et al., 1996). MT is measured using single TMS pulses to identify the lowest stimulator output required to yield a MEP approximately 50% of the time. MT is known to be effected by voltage-gated sodium channel blockers such as carbamazepine (Menzler et al., 2014 Ziemann et al., 1996) and is generally considered to reflect cortico-cortical axons and their excitatory contacts with corticospinal neurons (see Ziemann et al. (2015) for review). Hence, the lack of change in this measure following GBP suggests that the drug does not cause widespread change in motor cortical excitability through widespread changes, which act on calcium and sodium channels. Rather, TMS studies suggest that the effect of GBP is to modulate cortical excitability within the motor cortex through indirect, non-synaptic, modulation of GABA and (potentially) glutamate levels. However, neither of the TMS studies reported here were placebo controlled, and there appears to have been no subsequent attempts to establish the effects of GBP using TMS.

Evidence from MRS studies support the hypothesis that GBP influences GABA. Three notable studies have been documented, the first of which was conducted by Petroff et al. (1996), in which elevated levels of GABA in the occipital cortex were reported for patients with epilepsy who were medicated with GBP compared with individuals who were taking antiepileptic medications such as carbamazepine. These results were dose specific, with those taking higher doses showing higher GABA levels. In a more recent 7T MRS study conducted with healthy participants Cai et al. (2012), found that a single 900 mg dose of GBP significantly elevated levels of GABA within the visual cortex. This elevation was far greater than that seen in a control condition in which participants were scanned twice within the same day, without drug or placebo administration. Hence, the effects are unlikely to be accounted for by natural GABA fluctuations over time. Finally, Kuzniecky et al. (2002) studied the effects of acute and chronic doses of GBP in healthy participants using MRS at a field strength of 4.2 T. A single acute dose of the drug (adjusted to participant's weight (17 mg/kg)), caused significant increases in GABA measures 6 but not 3 h after ingestion. Significantly elevated levels of GABA were also measurable after a 4-week course of the drug, although not after 2 weeks when compared to baseline levels.

Despite the common use of GBP, few double-blind, placebo-controlled studies have been conducted in humans, and none have been conducted using complimentary approaches within the same group of subjects. This study aims to extend the existing literature using a double-blind cross-over design, in which a range of TMS approaches, and MRS measures acquired at ultra-high field (7T) were used to assess change in cortical excitability and neuro-metabolites.

In addition to measurement of MT, SICI and ICF, we assessed changes in input-output (IO curves) and long interval intra-cortical inhibition (LICI), as the effects of GBP on these measures has yet to be studied. We also make a distinction between 1 ms and 3 ms SICI, as the mechanisms underlying the two appear to be different (Cengiz et al., 2013; Fisher et al., 2002; Roshan et al., 2003 Vucic et al., 2009) and some (Stagg et al., 2011), but not all (Dyke et al., 2017) have found a relationship between 1 ms but not 3 ms SICI and MRS GABA. IO curves are measured by recording MEP amplitudes evoked from various intensities of TMS, they allow the measurement of activity from neurons which are spatially further away from the centre of activation and can be used as an index of excitability within a wider region of the cortex. In particular, they are thought to reflect the strength of corticospinal projections (Chen, 2000) and are regulated by glutamatergic and GABAergic mechanisms in addition to neurotransmitters including serotonin and noradrenaline (Ziemann et al., 2015). Whereas LICI (Claus et al., 1992; Di Lazzaro et al., 2002 Valls-Sole et al., 1992) is a paired pulse TMS approach thought to be largely underpinned by the engagement of circuits linked to GABA-B receptor activity (McDonnell et al., 2006).

In this study an ultra-high field (7T) scanner was used to acquire MRS.

When compared with lower field strengths, this has the advantage of producing increased signal-to-noise ratio (SNR) and greater chemical shift dispersion (Tkác et al., 2009). The increased SNR improves the detection sensitivity and efficiency of metabolites, especially those with low concentration such as GABA. Greater chemical shift dispersion increases the separation of signals with similar resonant frequencies, allowing a more accurate identification and quantification of each metabolite. For instance, due to spectral overlapping the differentiation of GABA, Glu and Gln signals are difficult in ^{1}H spectra at field strengths of 3 T or less (Puts and Edden, 2012), and Glx (a composite measure of Glu + Gln) is reported instead. By contrast, GABA, Glu and Gln become separable at field strengths of 7 T or above.

2. Method

2.1. Participants

29 healthy, right-handed adults (age range 19–27) participated in the study. All participants were free from neurological or psychiatric illness and any contra-indications for MR scanning or TMS. Of the 29 participants tested, 14 were assigned to the placebo condition and 15 were assigned to the GBP condition. During assignment efforts were made to ensure that groups were approximately balanced for age, sex and BMI. Due to the exclusion of one data set due to poor quality TMS in the placebo group, and one data set from the GBP group due to poor quality MRS data, there were slight variations between sample sizes. Participant demographics for the different conditions can be seen in Table 1. All participants completed MRI and TMS sessions before and after receiving GBP/placebo (See Fig. 2).

2.2. MR acquisition

MRI data were acquired using an ultra-high field 7T Philips Achieva system (Philips Healthcare Best, Netherlands) with a 32-channel radio frequency head coil at the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham. Participants were placed supine and head-first into the scanner. Foam pads were inserted between the participant's head and the coil to minimise and control head movement; a pair of prism glasses was provided to allow participants to view a screen outside the magnet bore.

At the start of each imaging session ^{1}H image localiser and B0 maps were acquired followed by BOLD-fMRI T_2*-weighted images, which were acquired to guide placement of the left primary motor cortex (M1) spectroscopy voxel in the following MRS scans. The BOLD-fMRI used a single shot EPI sequence (TR/TE = 1999/25 ms, FOV = 208 × 192 mm3, matrix = 112×112, 30 slices, slice thickness = 4 mm, no slice gap, 160 dynamics). During the fMRI scan, eight blocks of bimanual finger-to-thumb opposition tapping were performed in a blocked-trial paradigm as follows. The words 'TAP' and 'REST' were alternately displayed for 8s and 32s, respectively. Participants were asked to tap their thumbs to each finger with both hands simultaneously and continuously during the 'TAP' phase, and to rest (withhold movement) during the 'REST' phase. Maximum activation of the left M1 was found by analysing the BOLD response on-line using Philips IViewBOLD software.

T_1-weighted anatomical images were then acquired with a MPRAGE sequence (TR/TE/TI = 7.3/3.4/998 ms, FA = 8°, FOV = 224×224 × 120 mm^3, isotropic resolution = 1 mm^3) for tissue segmentation. Anatomical landmarks from these images were also used to assist in the placement of the left M1 voxel for MRS.

In vivo ^{1}H MRS data were acquired from a voxel of interest (VOI = 20×20 × 20 mm^3) placed over the hand area of the left M1 (Fig. 1A) using a STEAM sequence (TE/TM/TR = 17/17/2000 ms, sample size = 4096, spectral bandwidth = 4000Hz, phase cycling = 8, 288 averages, 9.6mins). Water suppression was performed using multiply optimised insensitive suppression train (MOIST) (Murdoch, 1993). Prior to this, a non-suppressed water reference spectrum (16 averages) from the same VOI was acquired for eddy current correction and quantification. B0 shimming of the VOI was performed automatically by the Philips pencil beam (PB) algorithm (Gruetter, 1993) in order to increase B0 field homogeneity.

Table 1
Participant demographics for analysis of drug manipulation data. Data are presented as mean value ± sd.

	N	Sex (m/f)	Age	BMI
Placebo MRS	14	8F, 6M	22.3 ± 2.4	21.8 ± 3.6
Placebo TMS	13	7F, 6M	23 ± 2.5	22.1 ± 3.7
Gabapentin TMS	15	7F, 8M	23.6 ± 2.6	21.3 ± 2.6
Gabapentin MRS	14	7F, 7M	23.6 ± 2.4	21.9 ± 2.6

2.3. TMS measurements and EMG recording

TMS was delivered using a Magstim Bistim system (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (70 mm diameter of each winding). The coil was held tangentially to the scalp and positioned 45° from the midline, resulting in a posterior to anterior current flow. Neuro-navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used in conjunction with individual T_1-weighted anatomical images to aid initial coil placement over the hand area of left M1. Coil location was then refined using a hot spot approach, in which the cortical location which consistently yielded the largest motor evoked potential (MEP) amplitudes from the first dorsal interosseous (FDI) muscle was identified as the optimal stimulation site. Neuro-navigation was used throughout to ensure highly accurate coil placement during and between the two TMS sessions.

MEPs were recorded using disposable Ag-AgCl surface electrodes attached to the right FDI muscle in a belly-tendon montage. The signals were amplified and bandpass filtered (10 Hz- 2 kHz, sampling rate 5 kHz) then digitalized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany).

All trials were controlled using an in-house program (written using Matlab: Mathworks, MA, USA), with an inter-trial interval (ITI) of 5s occurring between each single/pair of pulses. Intra-cortical inhibition and facilitation were investigated using a range of TMS paired pulse protocols with various CS-TS pairings and ISIs of 1, 3, 10 and 12 ms (Kujirai et al., 1993) and 100 ms (Claus et al., 1992 Valls-Sole et al., 1992). IO curves were collected first with the order of intensities tested full randomized. Immediately after paired pulse and unconditioned trials were collected, also in a randomized fashion.

2.4. Threshold determination

Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP with a peak-to-peak amplitude of >50 μV in the relaxed FDI muscle in a minimum of 5 of 10 trials. A 1 mV (SI 1 mV) threshold was also determined by calculating the lowest intensity needed to evoke an MEP of 1 mV in 5 of 10 consecutive trials.

2.5. Input output curves

TMS intensities at 100, 110, 120, 130, 140 and 150% of RMT were used. 10 pulses at each of the 6 intensities were delivered in a randomized order.

2.6. Unconditioned trials

A total of 30 unconditioned trials were measured at SI 1 mV.

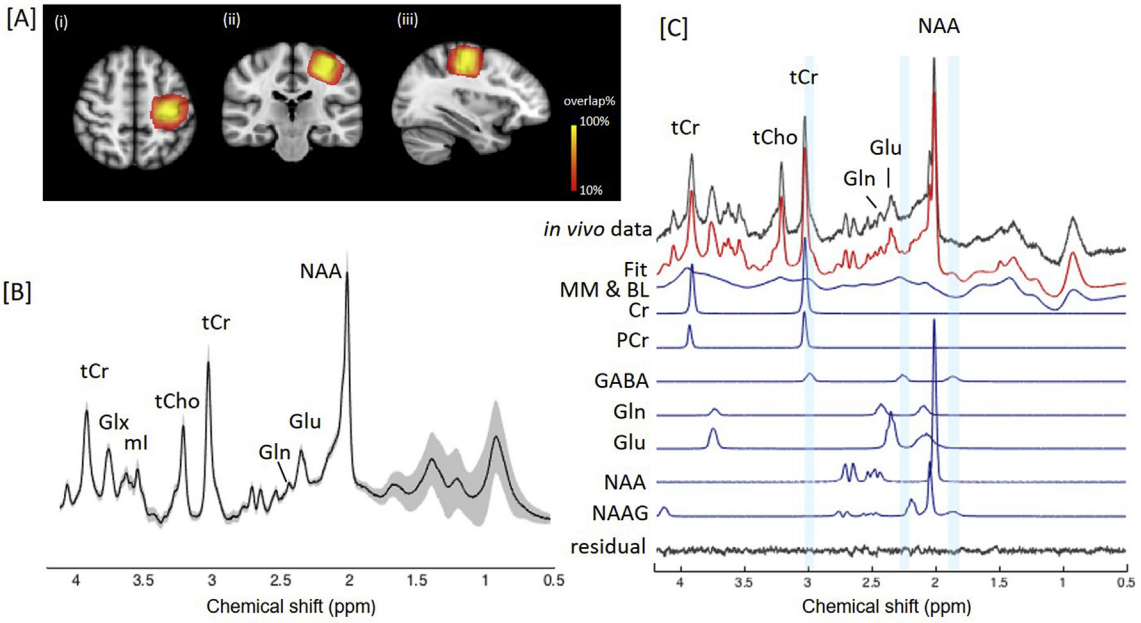

Fig. 1. [A] Position of the voxel of interest (VOI = 20×20 × 20mm3) located over the left-hand area of M1 shown in (i) sagittal, (ii) axial (iii) and coronal views. [B] Standard deviations (shaded area) overlying the group mean in vivo spectrum acquired from the VOI obtained with STEAM sequence (TS/M = 17/17 ms) at 7T are shown (N = 27). [C] A representative in vivo spectrum obtained from the M1 VOI is shown, together with its LCModel fit. Residual and fitted signals for metabolites of interest and macromolecules (MM) and baseline (BL) are shown.

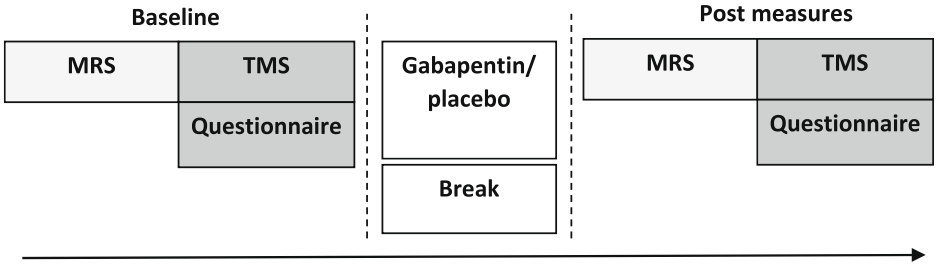

Fig. 2. Schematic showing study timeline. On average the second MRS measures were collected 2 h after drug/placebo intake. TMS measures were collected an average of 3 h post intake.

2.7. Short interval intracortical inhibition (SICI)

SICI was measured using 1 and 3 ms ISIs. The selection of conditioning stimuli (CS) intensities was informed by previous work (Dyke et al., 2018 Fisher et al., 2002) which revealed 1 ms SICI to have a lower threshold than that of 3 ms SICI. CS intensities of 45,50,55 and 60% RMT were used to measure 1 ms SICI, whereas 60, 65, 70 and 75% RMT were used to measure 3 ms SICI. Each CS was followed by a supra-threshold test stimulus (TS) of SI 1 mV delivered to the same location. Ten trials were measured for each CS-TS pairing for both 1 and 3 ms ISIs.

2.8. Long interval intra-cortical inhibition (LICI)

A single ISI of 100 ms was tested using a supra-threshold CS of 110% RMT and a TS delivered at SI 1 mV. A total of 20 trials were measured.

2.9. Intracortical facilitation (ICF)

10 and 12 ms ISIs were measured using a CS at 75% RMT followed by a SI 1 mV TS 20 trials were measured for each ISI.

The testing sessions commenced at the same time each morning for all participants and lasted approximately 6 h in total. Baseline MRI measures (T_1 weighted anatomical and MRS) were collected first, approximately 30 min after this baseline TMS measures were collected, followed by a simple bespoke questionnaire to indicate their perceived level of fatigue and tiredness on a scale of 1 (absent) to 10 (severe). Participants were then given a drink containing 900 mg of GBP or with nothing added. A strong sugar free blackcurrant flavoured drink was used to successfully mask the taste of the drug. The researcher who administered the drink and assigned participants to experimental conditions played no role in the data analyses. All other researchers were kept blind to experimental condition until processing of the data was completed.

After receiving the drug or control drink, participants ate a light lunch which typically involved a sandwich, a low sugar snack such as crisps and a low sugar drink (most typically water). During the break participants were closely monitored and engaged in restful activities such as watching films or reading. Participants were re-scanned on average approximately 2 h after consuming the control drink (117 ± 18.5 min) or GBP containing drink (122 ± 12 min). The second set of TMS measurements were then performed. On average these took place approximately 3 h following control drink (167 ± 16.5 min)or GBP intake (179 ± 18.5). If necessary, thresholds (RMT/SI 1 mV) were adjusted during the second measurement. Finally, participants were asked to rate their level of fatigue and tiredness again on the questionnaire. Participant comfort was closely monitored throughout the experiment.

2.10. Analyses of MRS data

The MRS data were processed using a MATLAB (The MathWorks Inc., Natick,MA, USA) routine developed in-house, prior to metabolite quantification. Spectra from individual acquisitions and from all 32 receivers were acquired from the scanner separately. These raw signals were combined using the scheme reported by Hall et al. (2014), then phase corrected and frequency aligned. *In vivo* ^{1}H spectra were then fitted and quantified with the LCModel software package (Provencher, 1993). The basis set used for quantification included an experimentally acquired macromolecule spectrum and model spectra for 20 metabolites. The LCModel analysis was performed within the chemical shift range 0.2–4.2 ppm. Water scaling was applied using the non-suppressed water reference. The LCmodel control parameters were based on previously published parameters (Tkác et al., 2009). Metabolites with Cramer-Rao lower bound (CRLB) > 20% were rejected from further analysis. The linewidth of in vivo spectra for the data collected at baseline was 10.35 ± 1.99 Hz and 10.22 ± 1.29 for data collected in the second session. Total Cr (tCr, i.e., PCr+Cr) was used as the internal reference for quantification in accordance with previous recommendations (Danielsen and Ross, 1999; Stagg and Rothman, 2014) and MRS-GBP studies (Cai et al., 2012; Kuzniecky et al., 2002 Petroff et al., 1996). The data were also analysed without this correction (not shown but available upon request), and revealed no additional statistical significant differences when assessing change in metabolites following GBP or Placebo administration.

Tissue fractions within the MRS voxel were calculated using the T_1 weighted anatomical data, segmented using SPM 12 (Ashburner and Friston, 2005 Penny et al., 2006). Intraclass correlation coefficients (ICC) and mean coefficients of variation (CV) calculated across individuals for pre and post measures indicate good agreement between pre/post measures of grey matter (GM) (mean CV = 16.3%, ICC(2,1)=.78); white matter (WM) (mean CV = 7%, ICC(2,1)=.81); cerebrospinal fluid (CSF) (mean CV = 18.8%; ICC(2,1)=.81); and total tissue (mean CV = 17.0%, ICC(2,1)=.81) within the MRS voxel. Although voxel placement was similar, we applied the corrections below for tissue fraction to limit the impact of subtle differences in voxel composition. All results were also run without this correction, and revealed no additional statistically significant differences.

(GABA concentration ratio / (GM%+WM%))*100

(Glu concentration ratio / (GM%+WM%))*100

(Gln concentration ration /(GM%+WM%))*100

2.11. Analysis of TMS data

All trials were carefully inspected visually and trials in which there was evidence of pre-contraction of the FDI muscle within the period 500 ms prior to an MEP were excluded. The mean amount of data excluded on this basis for IO curves was below 2% for pre (1.73 ± 3.32) and post (0.096 ± 2.22) measures. For paired pulse and unconditioned MEPs this was less than 2.5% for pre (1.05 ± 1.74) and post (2.25 ± 4.29) measures. For the remaining data, peak-to-peak MEP amplitudes were measured using in-house software (programmed using Matlab, The Mathworks, MA, USA). When analysing individual participant data, median values were calculated to indicate average MEP amplitude in response to a particular stimulator output.

2.12. TMS recruitment (IO) curves

Single pulse TMS IO curves were measured by calculating for each individual the median MEP amplitude for each given TMS intensity (100–150% RMT). For two participants 150% was particularly uncomfortable, for these participants MEPs evoked from TMS intensities of 100–140%RMT only were measured. The resultant median MEP values for each intensity were fitted using linear slopes. R^2 values for each time point (pre/post) and group (GBP/Placebo) indicate good-excellent fits to the data (mean ± Sd of R^2 values for pre placebo: 0.926 ± 0.046; pre GBP: 0.934 ± 0.040; Post placebo: 0.919 ± 0.062 and post GBP: 0.901 ± 0.959). For completeness, sigmoidal curves were also fitted to the data, the results of which can be found in supplementary material. Fitting with sigmoidal curves did not alter the conclusions in any material way.

2.13. Paired pulse data

Paired pulse trials were analysed by calculating for each individual the median MEP amplitude for each CS intensity at each ISI. These values were then divided by the median MEP amplitude for unconditioned trials to create a ratio measure. For 1 ms and 3 ms SICI, numerous CS intensities were measured. The ratios for each CS intensity assessed by calculating the median of these ratios to indicate the average level of inhibition. Further analysis of individual CS intensities for 1 and 3 ms SICI can be found in Supplementary Material, including assessment of the data when CS intensities were fitted with a linear slope. Baseline data showing effective increase/decrease in MEP amplitudes as a result of the CS-TS protocols for 1 ms SICI, 3 ms SICI and ICF can also be found in Supplementary Material.

Although LICI was measured, we chose not to analyse the data further. In the Placebo condition 7/13 data sets revealed total elimination of MEP during LICI at baseline, for participants in the GBP condition this was apparent in 1/15. The abolishment of any MEP at baseline in so many data sets makes pre/post comparisons problematic, particularly for assessment of the placebo group as for many subjects an increase in LICI is an impossibility.

2.14. Statistical analysis of TMS and MRS data

Statistical analysis were completed using JASP version 0.12.1.0 (JASP-Team, 2020) with the exception of intraclass correlation coefficients (ICC) calculations which were computed using SPSS version 25 (IBM-Corp, 2017). Assumptions for the use of parametric statistics were assessed using relevant plots and statistical tests (QQ plot, Box-M, Shapiro-Wilk and Levene's tests). Significant outliers were identified using Grubbs test and removed prior to further analysis, these are detailed in relevant sections. Effect sizes for parametric data are reported as partial eta squared (η^2_p) for ANOVA and Cohens d (d) for *t*-test. For Mann-Whitney U tests, effect sizes are reported as rank biserial corrections (r_{rb}), for Wilcoxon Ranked Sign, matched rank biserial correlation values (r_{mrb}) are shown.

Initial statistical analysis compared the two groups at baseline to identify whether any systematic differences were present prior to the administration of placebo/GBP. When relevant assumptions were met, subsequent analysis using mixed models ANOVAs (within subjects factor: time (pre/post); between subjects factor: group (placebo/GBP) were conducted. For 12 ms ICF data, Wilcoxon ranked sign tests were used to compare pre/post differences for each group as the assumptions required for ANOVA were not met. Additional between-group comparisons were conducted using change ratios (post/pre values) and independent samples t-tests/Man-Whitney U tests. Bayesian statistics are reported alongside analyses of pre/post and ratio analyses. For mixed models ANOVAS these were calculated in JASP with the order set to compare against data modelled for the null hypothesis. Bayes factors are presented as BF_{01}, meaning values above 1 are suggestive of support for the null hypothesis with larger values suggestive stronger support in favour of this over the alternative. All Bayesian analyses were run with the alternative hypothesis set as non-directional (akin to the two-tailed approach taken with parametric measures).Cauchy prior values were kept at the default value set in JASP of 0.707. For Bayesian Mann-Whitney U-tests the number of samples was set at 1000.

Relationships between MRS and TMS measures were previously

assessed and can be found in (Dyke et al., 2017).

3. Results: perceptions of tiredness and fatigue

Questionnaire data was obtained for 12/14 participants in the placebo groups and for 14/15 participants in the GBP group. There were no statistically significant changes in ratings of fatigue ($t(10) = -0.77$, $p = .459$, $d = -.0232$) or tiredness ($t(10) = -0.412$, $p = .689$, $d = -.124$) in the placebo group. In both instances Bayesian paired samples t-tests suggest anecdotal ($BF_{01} = 2.617$) and moderate ($BF_{01} = 3.124$) evidence in favour of the null. However, for the GBP group ratings of tiredness ($t(13) = -3.078$, $p = .009$, $d = -.823$) and fatigue (*Z=-3.483, p=.005, r_{mrb}=-1.0)* significantly increased. Bayesian paired samples t-tests for tiredness ($BF_{01} = 0.083$) suggest strong support in favour of the experimental hypothesis. Relevant data are shown in Fig. 3.

4. Results: acute effects of GBP on TMS measures

4.1. Threshold values (RMT/SI1mv) and baseline differences

Independent samples t-tests were used to ensure that the TMS parameters used for IO curves and paired pulse measures were equivalent between groups. These revealed no statistically significant differences between RMT expressed as %Maximum stimulator output (MSO) for placebo ($M = 46$, $SD = 7.756$) and GBP ($M = 45.866$, $SD = 4.223$) at baseline ($t(17.94)$=.055, $p = .955$, d=.022). Minor adjustments to RMT in the post session occurred for 2 participants in the placebo group and 1 in the GBP group, no statistically significant differences between post placebo/GBP measures were found ($t(26) = -0.163$, $p = .872$, $d = -.062$). Bayesian analyses revealed anecdotal support in favour of the null hypothesis for differences between groups in pre ($BF_{01} = 2.823$) and post ($BF_{01} = 2.798$) measures.

To ensure the MEP amplitudes did not differ between conditions, we compared the median MEP amplitudes from the 10 pulses collected at 100%RMT during IO curve measurement. Mann Whitney U tests revealed no statistically significant differences between groups at baseline ($U = 90$, $p = .747$, $r_{rb} = -.077$) or post ($U = 119$, $p = .339$, r_{rb}= .221). Bayesian Mann-Whitney U-Tests revealed anecdotal support in favour of the null hypothesis for differences between groups in pre ($BF_{01} = 2.607$) and post ($BF_{01} = 2.052$) measures.

No statistically significant differences were found between SI1mV values expressed as %MSO for placebo ($M = 54.846$, $SD = 7.701$) and GBP ($M = 56.466$, $SD = 6.446$) at baseline ($t(26) = .-606$, $p = .55$, $d = -.230$). As with RMT, minor adjustments were made to S1mV in the post session. A total of four minor adjustments occurred for the placebo group and three for the GBP group, no statistically significant differences were found between the two groups post placebo/GBP ($t(26) = -0.892$, $p = .381$, $d = -.338$). Bayesian analyses revealed anecdotal support in favour of the null hypothesis for differences between groups in pre ($BF_{01} = 2.462$) and post ($BF_{01} = 2.1$) measures.

Assessment of the median amplitude of 30 pulses collected at S1mV during paired pulse data collection using Man-Whitney U tests confirmed that there were no statistical differences in MEP amplitudes produced by SI1mV in the baseline condition (U= 130, $p = .142$, r_{rb}= .333). However, MEP amplitudes were significantly higher in the post placebo condition (*Mean* = 1609, *SD* = 657) in comparison for the post GBP condition (*Mean* = 10991, *SD* = 613.772), $U = 145$, $p = .029$, r_{rb}= .487. Bayesian Man-Whitney U tests suggest anecdotal support for the null hypothesis for the existence of group differences at baseline ($BF_{01} = 1.505$); and anecdotal evidence in favour of rejecting the null ($BF_{01} = 0.638$) when exploring group differences in the post measures. Further inspection of the data and analysis of ratio data (post/pre) detailed below, suggest this is due largely driven by slightly higher MEPs overall in the post placebo condition (see Fig. 4).

Independent samples t-tests confirmed that there were no baseline differences between placebo and GBP groups for 1 ms SICI median inhibition ($t(26) = -0.101$, $p = 920$, $d = -.038$), 3 ms SICI median inhibition ($t(26) = -1.348$, $p = .189$, $d = -.511$) or 10 ms ICF ($t(26)$=.226, $p = .823$, d=.086). Mann-Whitney U tests also confirmed no statistically significant differences between IO curve slope ($U = 107$, $p = .683$, r_{rb}= .097) or 12 ms ICF ($U = 81$, $p = .467$, $r_{rb} = -.169$) at baseline. Equivalent tests run using Bayesian approaches all provided anecdotal support in favour of the null hypothesis and are as follows: 1 ms SICI ($BF_{01} = 2.815$); 3 ms SICI ($BF_{01} = 1.445$); 10 ms ICF ($BF_{01} = 2.772$); 12 ms ICF ($BF_{01} = 2.426$); Linear IO slope ($BF_{01} = 2.634$).

4.2. Impact of GBP/Placebo on TMS measures

Having established that no baseline differences exist between the two groups, addition statistical analyses were conducted to explore any change in measures across time points (pre/post) between groups (GBP/placebo).

4.3. SI1mV MEP amplitude

A mixed models ANOVA revealed no significant main effect of time ($f(1,26)$=.005, $p = .942$, $\eta^2_p = .000$), or interaction ($f(1,26) = 2.317$, $p = .140$, $\eta^2_p = .016$) for MEP amplitudes. Bayesian repeated measures ANOVA revealed moderate support in favour of the null for time ($BF_{01} = 3.74$) and the time* group interaction ($BF_{01} = 3.392$).

Analysis of change ratios using an independent samples *t*-test revealed no statistically significant differences between groups ($t(26) = 1.710$, $p = .099$, d=.648); however, Bayesian independent samples *t*-test suggested very weak anecdotal evidence in favour of the experimental hypothesis ($BF_{01} = 0.974$). The placebo group showed a slight unexpected increase in MEP amplitude whereas the GBP group showed more of a tendency towards decreased MEPS (see Fig. 4).

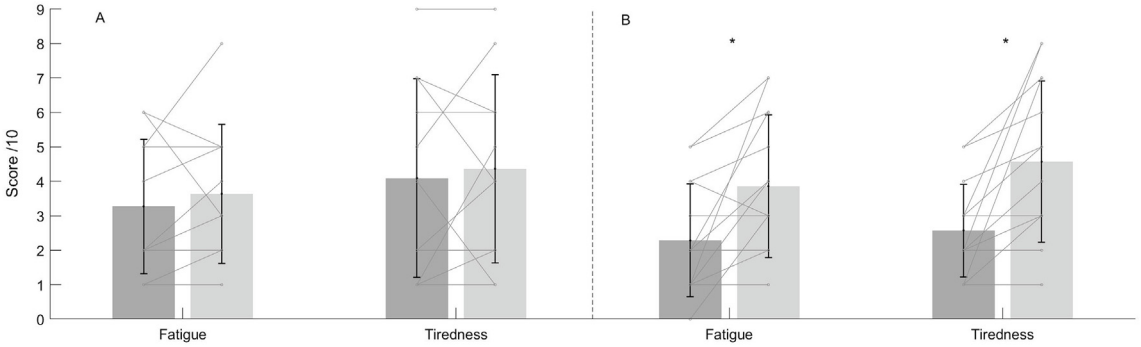

Fig. 3. Mean ± SD self-report ratings of tiredness and fatigue shown A: pre (dark grey) and post (light grey) placebo intake and B: pre (dark grey) and post (light grey) GBP intake. Individual data points also shown in light grey.

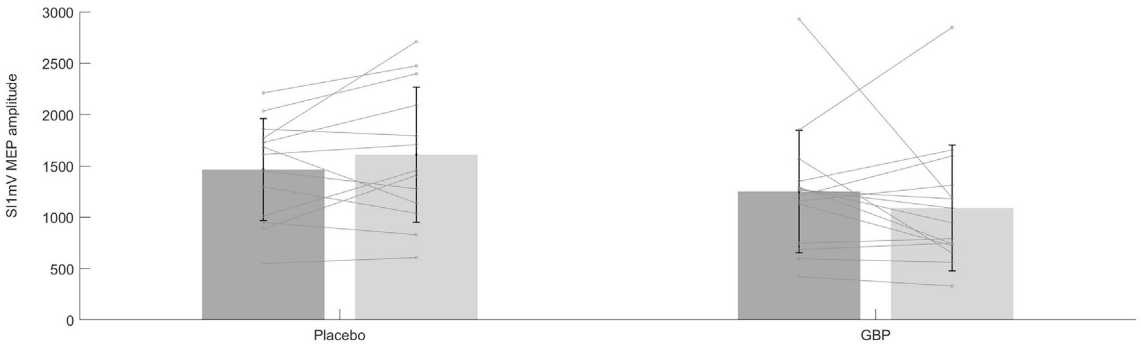

Fig. 4. Mean ± SD of MEP amplitudes obtained for SI1mV measures for pre (darker grey) and post (lighter grey) conditions in placebo and GBP groups. Individual data points are shown in light grey.

4.4. IO curve

A mixed models ANOVA revealed no significant main effect of time ($f(1,26)$=.077, p = .783, η^2_p = .003), or interaction ($f(1,26)$=.993, p = .328, η^2_p = .037) for IO curve slope. Bayesian repeated measures ANOVA revealed moderate support in favour of the null for time (BF_{01} = 3.426) and substantial support for a null effect for the time* group interaction (BF_{01} = 9.577).

Comparison of the change ratio data between the two groups using a Mann-Whitney U test also failed to reveal any statistically significant differences (U = 111, p = .555, r_{rb}=.138). A Bayesian Mann-Whitney U test revealed anecdotal support in favour of the null (BF_{01} = 2.206). Relevant data are shown in Fig. 5.

4.5. SICI

Relevant data are shown in Fig. 6A and Fig. 6C. No significant main effects of time (pre/post) ($f(1,26)$=.047, p = .829, η^2_p = .002) or interaction between condition and time ($f(1,26)$=.473,p = .498, η^2_p = .018) were found for median inhibition measured using 1 ms SICI. Bayesian repeated measures ANOVA revealed moderate support for time (BF_{01} = 3.761) and strong support for time* group interaction (BF_{01} = 15.851) in favour of the null hypothesis.

There were also no significant main effect of time ($f(1,26)$ = 1.89, p = .181, η^2_p = .068) or interaction ($f(1,26)$ = 1.227, p = .278, η^2_p = .045) for median inhibition as measured by the 3 ms SICI protocol. Bayesian repeated measures ANOVA revealed anecdotal support for time (BF_{01} = 1.876) and moderate support for time* group interaction (BF_{01} = 4.837) in favour of the null. Additional statistical analysis performed using each individual CS intensity also failed to reveal any statistically significant results for either 1 or 3 ms SICI conditions (see Supplementary Material).

Mann-Whitney U tests revealed no statistically significant differences between ratio of change in inhibition caused by 1 ms SICI protocols in the placebo group vs GBP group (U=75, p = .316, r_{rb} = -.231). Nor were there any statistically significant differences in change ratios were found for inhibition caused by 3 ms SICI (U = 125, p = .217, r_{rb}=.282). Bayesian hypothesis testing revealed anecdotal support in favour of the null, for differences between groups in 1 ms SICI (BF_{01} = 1.745) and 3 ms SICI (BF_{01} = 1.804) ratio measures.

4.6. ICF

Relevant data are shown in Fig. 6B and Fig. 6D. There was no significant main effects of time ($f(1,26)$=.224, p = .640, η^2_p = .009) or interaction between condition and time ($f(1,26)$=.116, p = .736, η^2_p = .004) for 10 ms ICF. Bayesian repeated measures ANOVA revealed moderate support for time (BF_{01} = 3.354) and strong support for the time* group interaction (BF_{01} = 21.175) in favour of the null hypothesis.

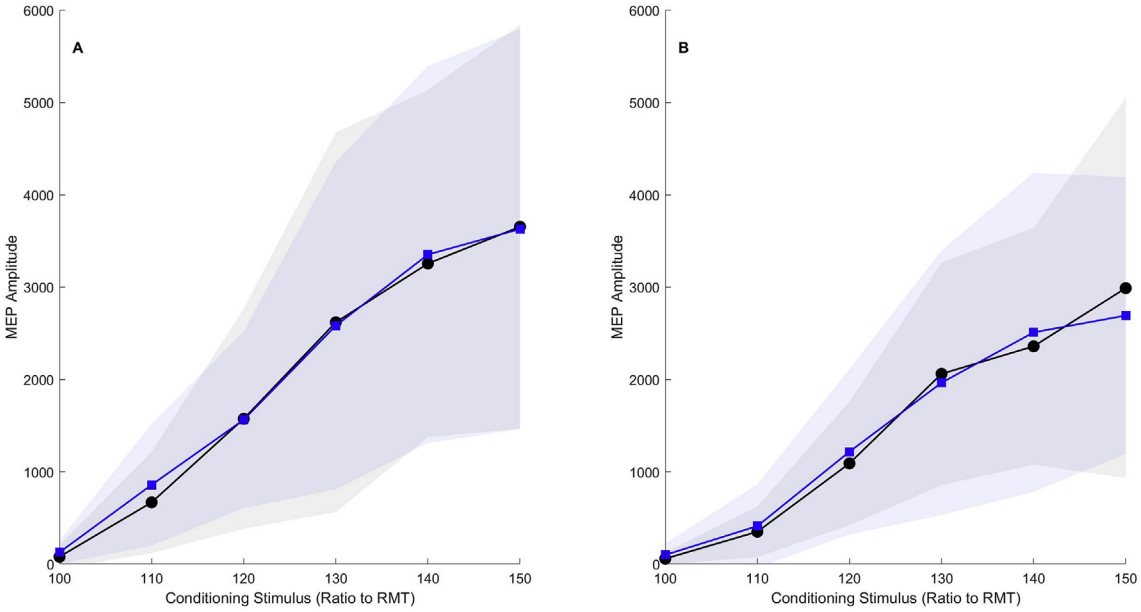

Fig. 5. Mean ± SD of MEP amplitudes for pre (black circles) and post (blue squares) conditions in placebo (A) or GBP (B) groups.

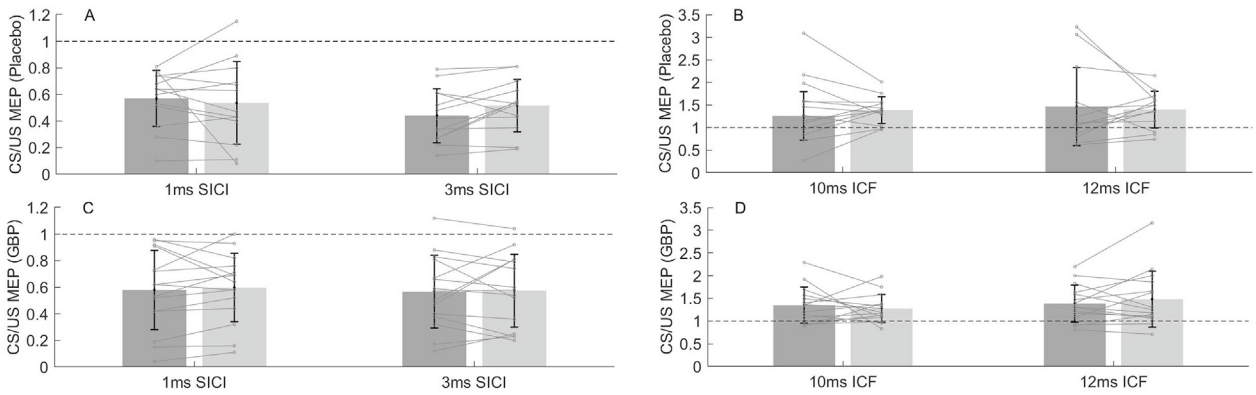

Fig. 6. Mean ± SD values showing average inhibition/excitation calculated by dividing the median amplitude of MEP values for each CS by the amplitude of MEP amplitudes obtained from unconditioned TS. A & B show before (dark grey) and after (light grey) placebo. C & D show before (dark grey) and after (light grey) GBP. Dashed line indicates no change from unconditioned test pulses. Values above indicate facilitation, below indicate inhibition. Individual data are shown in light grey.

Man-Whitney U tests also failed to reveal any statistically significant difference between change ratios of 10 ms ICF for placebo and GBP conditions ($U = 95$, $p = .829$, r_{rb}=.056), this analysis was performed with one outlier removed from the ratio data in the placebo condition. Bayesian Mann-Whitney *U* test using the same data showed anecdotal evidence in favour of the null hypothesis (BF_{01}=2.760).

The assumption of equality of variance was violated for post measures, therefore, separate Wilcoxon Ranked sign tests were calculated in place of ANOVA; these revealed no significant differences between pre and post 12 ms ICF measured in in placebo condition (Z = -.40, p = .735, r_{mrb} = -.121) nor in the GBP condition (Z = 50 p = .599, r_{mrb} = -.167). Bayesian paired samples t-tests revealed moderate support in favour of the null (BF_{01} = 3.427) for the placebo group and anecdotal support in favour of the null hypothesis for GBP ($BF_{01=}$ 2.71).

An independent samples *t*-test for 12 ms ICF also revealed no statistically significant differences between the two groups when assessing change ratios (t(26)=.5, p = .621, d= .218). Bayesian independent samples *t*-test using the same data suggests anecdotal evidence in favour of the null hypothesis ($BF_{01=}$2.57).

5. Results: acute effects of GBP on MRS measures

5.1. Baseline

Independent samples t-tests revealed no statistically significant differences between the two groups for baseline measures of Glu/tCR (t(26) = −590, p = 0.560, d = -.223); Gln/tCR (t(26) = -1.309, p = 0.202, d = -.495) or GABA/tCR (t(26) = 0.214, p = 0.832, d = 0.081). Bayesian independent samples *t*-test using the same data suggests anecdotal evidence in favour of the null hypothesis for Glu/tCR (BF_{01} = 2.484); Gln/tCR(BF_{01} = 1.503) and GABA/tCR(BF_{01} = 2.783).

5.2. Impact of GBP/Placebo on MRS measures

5.2.1. Glu

A mixed models ANOVA found a significant effect of time (Pre/Post) f(1,26) = 9.067, p = 0.006, η^2_p = .259. But no significant interaction between group and time f(1,26) = 0.398, p = .533, η^2_p = .015 for Glu/tCR measures. Bayesian repeated measures ANOVA revealed substantial support for the alternative hypothesis regarding time (BF_{01} = 0.1275) and anecdotal support of the alternative hypothesis for the time* group interaction (BF_{01} = 0.59) ..

Follow-up paired samples t-tests revealed that this effect was largely driven by a statistically significant *decrease* in Glu/tCR in the GBP group (t(13) = 2.716, p = .018, d=.726), but not in the placebo group (t(13) = 1.604, p = .133, d=.429). Bayesian paired samples t-tests suggest there is substantial evidence for a pre/post difference in the GBP group (BF_{01} = 0.28) and anecdotal support for the null (BF_{01} = 1.312) for placebo group. However, considering the lack of significant interaction, this should be treated with caution, particularly as this decrease is apparent in both conditions (Fig. 7).

An Independent samples *t*-test revealed no statistically significant differences between change ratios for Glu/tCR following GBP or Placebo (t(26)=.587, p = .562, d=.222). Bayesian independent samples *t*-test using the same data suggests anecdotal evidence in favour of the null hypothesis ($BF_{01=}$2.488).

5.3. Gln

Grubs test revealed a significant outlier for one participant in the placebo condition, this data point was consequently removed prior to further analysis. A mixed model ANOVA found a significant effect of time (Pre/Post) (f(1,25) = 5.982, p = .022, η^2_p = .193) but no interaction between time and groups (f(1,25)=.154, p = .698, η^2_p = .005). Bayesian repeated measures ANOVA revealed substantial support for the alternative hypothesis regarding time (BF_{01} = 0.310) and anecdotal support of the null hypothesis for the time* group interaction (BF_{01} = 1.017).

Both groups showed a decrease in Glu in the second measurement (Fig. 7). Post hoc paired samples t-tests revealed this to be non-significant for the Placebo group (t(12) = 1.883, p = .084, d=.522) and GBP group (t(13) = 1.733, p = .107, d=.463) when assessed individually. Bayesian paired samples t-tests suggest there is anecdotal support for the alternative hypothesis (BF_{01} = 0.918) for placebo, and anecdotal support for the null in the GBP group (BF_{01} = 1.123).

An Independent samples *t*-test revealed no statistically significant differences between change in Gln following GBP or Placebo (t(26)=.628, p = .535, d=.237). Bayesian independent samples *t*-test using the same data suggests anecdotal evidence in favour of the null hypothesis ($BF_{01=}$2.441).

5.4. GABA

A mixed models ANOVA found no statistically significant effect for time (pre/post) (f(1,26)=.599, p = .446, η^2_p = .023), and no significant interaction (f(1,26)=.000, p = .986, η^2_p = <.000). Bayesian repeated measures ANOVA revealed anecdotal support for the null hypothesis regarding time (BF_{01} = 2.751) and strong evidence in support of the null hypothesis for the time* group interaction (BF_{01} = 23.591).

Assessment of ratio data using an independent samples *t*-test also failed to show any statistically significant difference between GABA/tCR change following GBP in comparison to placebo (t(26)=.191, p = .850, d=.072). Bayesian independent samples *t*-test using the same data suggests anecdotal evidence in favour of the null hypothesis ($BF_{01=}$2.797).

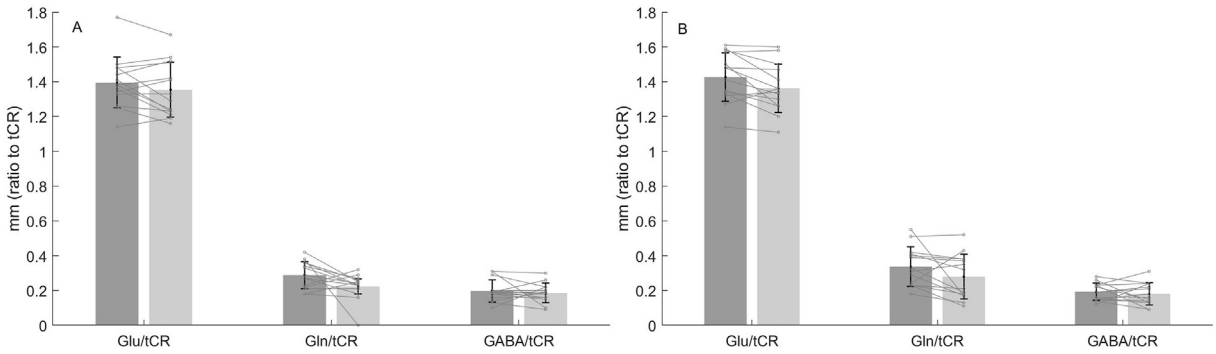

Fig. 7. Mean ± SD values showing CSF corrected measures of GABA, Glu and Gln as ratios to tCR. A: before (black) and after (grey) placebo. B: before (black) and after (grey) GBP.

6. Discussion

Altered GABAergic function is a hallmark of many neurological and psychiatric disorders. Therefore, the study of methods that can modulate GABA through pharmacological means are of interest, as they may hold therapeutic promise for several conditions. However, to maximize the potential of these approaches it is important that mechanisms of action are well explored and defined. In the current double-blind, placebo-controlled study, we set out to investigate the effects of GBP on GABA-mediated TMS measurements and MRS measures of targeted neuro-metabolites (i.e., GABA, Glu, Gln). Specifically, we assess changes in MRS measures of GABA, Glu and Gln, alongside TMS measures known to be underpinned by GABAergic and glutamatergic mechanisms. While previous studies have looked at the effects of GBP on MRS and TMS separately, this study represents the first to do so in the same group of participants within the same session. The main results of our study are summarized below.

6.1. Key findings

1 There was a significant effect of ingesting a single 900 mg dose of GBP on perceptions of fatigue and tiredness. Participants in the GBP group reported statistically higher levels in comparison to the placebo group.
2 However, the 900 mg dose of GBP did not significantly alter any of the TMS measures taken approximately 3 h after ingestion.
3 MRS measures taken approximately 2 h following GBP ingestion were also not significantly altered when compared to the placebo group. Specifically, there was no change in GABA.
4 Glu/tCR was significantly decreased following GBP administration, but not placebo. While noteworthy, this should be treated with caution as comparisons between groups failed to support a statistically significant effect.

6.2. Effects of GBP on perceived tiredness and fatigue

Participants in the GBP condition reported statistically higher levels of fatigue and tiredness in self-report questionnaires, which were taken approximately 3.30–4 h following GBP uptake. Researchers who were blind to experimental condition also noticed higher levels of fatigue in these participants while coming out of the scanner (approximately 2.3 h post ingestion) and during TMS. While tiredness and fatigue are well documented side effects of GBP, the previous work exploring effects on TMS/MRS measures did not systematically report this for each participant; although tiredness (Ziemann et al., 1996) and being mildly sleepy (Cai et al., 2012) were listed as reported GBP side effects.

Interestingly, the relationship between tiredness/fatigue/wakefulness and cortical excitability in healthy adults is rather unclear. Some studies measuring SICI, have reported this to be reduced following prolonged periods of wakefulness (Kreuzer et al., 2011), however, others have found increases (Manganotti et al., 2001) or no significant change in the measure (Badawy et al., 2006 Manganotti et al., 2006). Increased excitability within frontal regions from morning-evening and following sleep deprivation has been reported using an EEG-TMS approach (Huber et al., 2013); however, studies of morning-evening variation in the motor cortex have typically failed to show any systematic change as a consequence of time awake (Doeltgen and Ridding, 2010; Strutton et al., 2003; ter Braack et al., 2019).

There is less available research assessing tiredness/fatigue/wakefulness and MRS measured GABA; however, one study which examined GABA change within the sensorimotor cortex over five time points from early morning to early evening failed to find any significant systematic change over time (Evans et al., 2010). This suggests that normal circadian rhythms have limited impact on MRS-GABA levels.

Although we cannot fully equate our ratings of fatigue/tiredness to the above studies of sleep deprivation/morning-evening change, we feel that on balance it is unlikely that increased fatigue/tiredness masked any real effects of the drug manipulation in our measures. Subsequent studies with larger sample sizes may wish to systematically record and assess this.

6.3. Effects of GBP on TMS measures

GBP was not found to significantly alter any of the TMS measures when compared with the placebo group. This was somewhat surprising, as based on the previous literature (Rizzo et al., 2001 Ziemann et al., 1996) it was expected that GBP would significantly increase SICI and reduce ICF. To our knowledge, this study is the first to use a double blind, placebo-controlled method to investigate the effects of GBP on TMS measures. This study also appears to be the first not to show a clear effect of GBP. It is not entirely clear why the present study failed to replicate previous findings, although it is possible that subtle differences between experimental designs may have contributed.

We employed a standardized dosing approach in which all participants in the GBP condition received a 900 mg dosage of the drug. We did not tailor the dose to weight, however, post hoc calculation of this reveals our dosage ranges from 10 to 18 mg per kg (mean = 14.4, SD = 2.7). We cannot rule out that this dosage was too low, however it should be noted that while the 900 mg dose used was lower than that use by Ziemann et al. (1996) (1200 mg) it was nonetheless higher than the 800 mg used by Rizzo et al. (2001). We explored the effect of weight/dose on subsequent change on TMS/MRS measures using correlational analysis, but failed to find evidence of any significant relationships (data not shown but available on request; largest correlation found between weight/dose and change in 10 ms ICF ($r(13)=.372$, $p = .173$).

Another possible reason for the lack of replication may be the timing between drug uptake and TMS measurement. On average the second TMS measurements were taken 3 h after receiving GBP, this ranged from 2.36

to 3.25 h between participants. In their study Rizzo et al. (2001) found increased SICI and decreased ICF 3 h after participants received an 800 mg dose of GBP. This may suggest that in our study the duration between measures was too short for some individuals. However Ziemann et al. (1996), found significant effects of SICI and ICF after just 2 h. Unfortunately, as different doses and timings were used in all studies it is not possible to draw strong conclusions about the lack of effects seen here. If a similar study were to be conducted it would be informative to measure the effects over a prolonged period in order to map the time course of GBP and its peak effects.

6.4. Effects of GBP on MRS measures

Contrary to previous findings (Cai et al., 2012; Kuzniecky et al., 2002 Petroff et al., 1996), GBP did not significantly alter levels of MRS-GABA. Nor did it have a significant impact on measures of Glu/tCR or Gln/tCR. The only significant effect seen for the MRS analysis was a significant reduction in Glu/tCR levels following GBP but not placebo. This should be treated with caution, as the interaction between time and group was not significant and comparison of change ratios (post/pre) for each group failed to show statistical differences. Yet, subsequent correlational analysis to assess any relationship between change in MRS measures and GBP dose/weight revealed a significant association r(12)= .594, p = .025), in which higher dose-weigh ratio was associated with higher levels of Glu, this was not significant for GABA/tCR or Gln/tCR (analysis not shown but available on request). One previous study reported measuring Glu, and failed to find any change approximately 2.5 h after GBP administration (Cai et al., 2012). Given the known interactions between Glu and GABA, this finding is noteworthy and warrants further attention if a similar study into GBP were conducted.

There are several methodological reasons which may explain why our results differ to those reported previously. The first of which is the interval between drug uptake and the second MRS scan. In our study, approximately 2 h elapsed between drug intake and the second MRS scan (range of 1.45–2.23 h). This is a little short of the 2.5 h interval reported by Cai et al. (2012). Detailed information regarding GBP uptake and impact on MRS-GABA is not readily available, however, it is of interest that while Cai et al. (2012) found significantly elevated GABA levels 2.5 h after drug uptake, similar effects were only found 6, but not 3 h after intake by Kuzniecky et al. (2002), suggesting additional factors such as dosage may also impact. An additional notable difference between our MRS approach and that of the three previous studies (Cai et al., 2012; Kuzniecky et al., 2002 Petroff et al., 1996), is that we placed our MRS voxel over the primary motor opposed to occipital cortex. Although GABA levels have been found to differ between regions (Greenhouse et al., 2016), they have also been reported to be stable within subjects in the motor cortex (Ferland et al., 2019 Yasen et al., 2017). It is not clear why GBP would impact more on visual than motor regions, so while this is notable for comparing methodologies it does not seem a likely explanation for our lack of effects.

A further difference between this study and those previously conducted, is that due to the length of this study participants were able to eat immediately following drug consumption. Although this may seem problematic, there is little evidence that gabapentin absorption is negatively influenced by food. In fact, some studies have even found that foods with higher levels of protein such as a milk based breakfast option (Gidal et al., 1996) and chocolate pudding (Gidal et al., 1998) have been found to moderately enhance absorption.

Finally, although our sample size is larger than past work exploring effects of a single dose of GBP using TMS (N = 6 (Ziemann et al., 1996); N = 11 (Rizzo et al., 2001)) and MRS(N = 6 (Kuzniecky et al., 2002); N = 11 (Cai et al., 2012), we acknowledge that it is still relatively small and in some cases our analyses may be underpowered. Future studies should carefully consider sample size with reference to previous work including the data presented here.

7. Summary

It is not clear why, despite evidence of altered physiological state (fatigue/tiredness ratings) following GBP we observed no clear differences in either our TMS or MRS measures. It is possible that the MRS measures were conducted a little too early to allow for enough change to be measured, however TMS data were conducted at a similar interval post drug ingestion to those in which significant effects were reported. While our findings should not be taken as evidence that GBP does not cause measurable GABA change, they do suggest that these effects may be subject to substantial individual variation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynirp.2021.100003.

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