A preliminary study of dynamic neurochemical changes in the dorsolateral prefrontal cortex during working memory

Hyerin Oh[1], Adam Berrington[1], Dorothee Auer[1], Ben Babourina-Brooks[1], Henryk Faas[1], and Jeyoung Jung[1]

[1]University of Nottingham

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Abstract

Working memory (WM) is one of the fundamental cognitive functions associated with the dorsolateral prefrontal cortex (DLPFC). However, we still know little about the neurochemical mechanisms of WM in the DLPFC. Here, we investigated WM-related dynamic neurometabolite and hemodynamic responses in the DLPFC. We measured Glx (glutamate+glutamine) and GABA alterations as well as blood-oxygen-level-dependent (BOLD) signal changes during a WM task combining functional magnetic resonance spectroscopy and functional magnetic resonance imaging (fMRI). In the DLPFC, we found that a 2-back task increased Glx concentrations and larger BOLD signal changes, and that these task-modulated Glx increases were positively correlated with task-induced regional activity. Importantly, task induced Glx changes in the DLPFC were associated with individual WM performance. Higher Glx increases were associated with increased DLPFC activation and lower WM task performance in the individuals. There were no changes in DLPFC GABA levels during WM processing. Our findings suggest that glutamatergic modulation in the DLPFC may play a critical role in WM processing and its performance.

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Running Title: Neurochemical changes in the DLPFC during working memory

Hyerin Oh[1,3], Adam Berrington[2], Dorothee P Auer[1,3,4], Ben Babourina-Brooks[1,3,4], Henryk Faas[1,3], and JeYoung Jung[5] *

[1]Mental Health & Clinical Neurosciences, School of Medicine, University of Nottingham, Nottingham, United Kingdom

[2]Sir Peter Mansfield Imaging Centre, School of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom

[3]Sir Peter Mansfield Imaging Centre, School of Medicine, University of Nottingham, Nottingham, United Kingdom

[4]NIHR Nottingham Biomedical Research Centre, Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom

[5]School of Psychology, University of Nottingham, Nottingham, United Kingdom

Corresponding author:

Dr. JeYoung Jung

School of Psychology, University of Nottingham, University Park, Nottingham NG7 2RD UK

Tel: +44(0) 115 846 7241

Email: jeyoung.jung@nottingham.ac.uk

ORCID number for the first author: https://orcid.org/

0000-0001-5179-2480

ORCID number for the corresponding author: https://orcid.org/

0000-0003-3739-7331

Abstract

Working memory (WM) is one of the fundamental cognitive functions associated with the dorsolateral prefrontal cortex (DLPFC). However, we still know little about the neurochemical mechanisms of WM in the DLPFC. Here, we investigated WM-related dynamic neurometabolite and hemodynamic responses in the DLPFC. We measured Glx (glutamate+glutamine) and GABA alterations as well as blood-oxygen-level-dependent (BOLD) signal changes during a WM task combining functional magnetic resonance spectroscopy and functional magnetic resonance imaging (fMRI). In the DLPFC, we found that a 2-back task increased Glx concentrations and larger BOLD signal changes, and that these task-modulated Glx increases were positively correlated with task-induced regional activity. Importantly, task induced Glx changes in the DLPFC were associated with individual WM performance. Higher Glx increases were associated with increased DLPFC activation and lower WM task performance in the individuals. There were no changes in DLPFC GABA levels during WM processing. Our findings suggest that glutamatergic modulation in the DLPFC may play a critical role in WM processing and its performance.

Keywords : working memory, DLPFC, functional magnetic resonance spectroscopy, glutamate, GABA, fMRI

Introduction

Working memory (WM) is a central cognitive ability that temporarily stores received information and manipulates that information for other cognitive functions (Baddeley, 1992). It plays an important role in reasoning, comprehension, planning, problem-solving, and the guidance of decision-making and is supported by the frontoparietal brain regions (Linden, 2007; Salazar et al., 2014). Although WM is one of the most studied cognitive functions, with rich evidence of the involvement of the dorsolateral prefrontal cortex (DLPFC) in brain (Barbey et al., 2013; D'Esposito and Bradley, 2015), fundamental questions remain regarding the neurochemical mechanisms of DLPFC activation that underpins WM in the human brain.

Electrophysiological studies with primates have demonstrated that WM is generated by the recurrent excitation of pyramidal cell microcircuits in the cortical layer III of the DLPFC (for a review, see Goldman-Raki, 1995). Pharmacological studies have reported that glutamate binding post-synaptic N-methyl-d-aspartate (NMDA) receptors and $GABA_A$ receptors in the DLPFC play a crucial role in WM in animals and humans (Honey et al., 2004; Krystal et al., 2005; Moghaddam and Adams, 1998; Wang et al., 2013; Sawaguchi et al., 1988; Rao et al., 2000; Auger & Floresco., 2015). Findings from these studies suggest that glutamatergic and GABAergic activity in the DLPFC is engaged in generating WM processing and performance.

Neurometabolites such as glutamate and GABA can be measured non-invasively *in vivo* using magnetic resonance spectroscopy (MRS). To date, four functional MRS (fMRS) studies in humans have investigated dynamic changes in glutamate and GABA in the DLPFC during WM processing at 3T. These studies reported glutamate increases between 2.7% and 3.9% compared to baseline . The only previous GABA-edited (MEGA-PRESS) study (Michels et al., 2012) failed to show glutamate modulation in the DLPFC. Conversely, they demonstrated a transient DLPFC GABA increase with subsequent decreases with a trend association of GABA decrease and performance. These findings provide some clues for the role of neurometabolites in WM processing but the association with task performance and hemodynamic BOLD changes remain poorly understood, with further uncertainty resulting from the lack of adequate control conditions.

Here, we aimed to expand and replicate previous findings in a more controlled experimental design by combining functional MRS in the DLPFC with functional magnetic resonance imaging (fMRI) and performance assessment during a WM task. Importantly, previous functional MRS studies compared the WM task with baseline (fixation or resting) to detect task-modulated changes in neurochemicals In fMRI, the need for a carefully chosen task 'control' condition is well-established as uncontrolled resting baseline is known to be inadequate to assess task-specific modulation in BOLD signal changes (Amaro & Barker., 2006). Typically, a 0-back task is used as a control condition for a 2-back task as a 0-back requires attention but no WM demand (Miller et al., 2009). Accordingly, we tested three conditions, including 2-back, 0-back (control), and baseline (fixation), in this study to evaluate task-specific modulations in neurometabolites and BOLD signal changes in the DLPFC. Consistent with the previous studies, we hypothesised that a 2-back task would increase Glx levels compared to the resting and control conditions in this preliminary study. Recent neuroimaging studies combining functional MRS and fMRI have demonstrated that stimulus-induced glutamate changes in visual cortex were positively correlated with stimulus-induced BOLD responses in the visual cortex (Ip et al., 2017, 2019) and in the DLPFC . Therefore, we hypothesised that glutamate changes in the DLPFC would be positively associated with task-induced BOLD signal changes during a 2-back task. In addition, we expected that GABA levels in the DLPFC would be decreased during the 2-back task relative to baseline. Furthermore, we explored the relationship between glutamate and GABA changes in the DLPFC and WM performance.

Materials and Methods

Participants

Twelve healthy young participants were recruited (9 females, mean age: 25 ± 2 years). All participants had normal or corrected-to-normal vision. All gave informed consent written form, approved by the University of Nottingham ethics committee.

Magnetic resonance image (MRI) acquisition

All images and spectra were acquired using GE 3.0T MR scanner (Discovery MR750, GE Healthcare, Milwaukee, Wisconsin) with a 32-channel head coil. Anatomical images were acquired using a T1-weighted inversion recovery spoiled gradient-echo sequence (BRAVO on the GE platform) with 1mm isotropic voxel size (repetition time (TR) = 7.3ms, echo time (TE) = 3.0ms, in-plane resolution 1 × 1 mm^2, slice thickness = 1mm, field of view (FOV) = 256 x 256 mm^2, matrix = 256 x 256, flip angle = 12°). For the fMRS scan, a 40 × 25 × 30 mm^3 voxel of interest (VOI) was positioned in the left DLPFC. Chemical Shift Selective saturation (CHESS) was used for water suppression, and non-edited 16 water unsuppressed reference acquisitions were acquired. GABA-edited MEGA-PRESS spectra were acquired with an editing pulse applied at 1.9 ppm to acquire GABA signal for 12 mins (TR/TE = 2000/68ms, 160 edited spectra and 160 non-edited spectra). Functional images were acquired using an echo planar imaging sequence (EPI, TR/TE=2000/20ms, in-plane resolution 3 × 3 mm^2, slice thickness = 3mm, FOV = 192 x 192 mm^2, matrix = 64 x 64, 40 slices, flip angle = 77°). In total, 240 imaging volumes were acquired.

Task and procedure

An n -back working memory task was used to activate the DLPFC, where the MRS VOI was placed (Fig. 1a). There were three task conditions in fMRS: resting, 0-back, and 2-back (Fig. 1b). Participants were asked to stare at a cross (a fixation) at the resting session. In the 0-back task, participants were asked to press the given button when the letter 'X' was presented. During the 2-back task, participants were instructed to press the given button if the letter was matched the letter presented two trials before. For each task, the fMRS scan consisted of seven blocks interleaved with 8s of fixation. Each block had 46 trials and each letter was displayed for 1500ms followed by 500ms of blank screen. The fMRI session consisted of 6 blocks of 0-back and 6 blocks of 2-back task, interleaved. Between task conditions there were fixation blocks for 4s. A task block had 18 trials of each task condition. A trial started with 500ms fixation followed by the letter presented for 1500ms. PsychoPy software (Peirce et al., 2019) was used to display stimuli and to record responses.

N-back performance

We quantified working memory performance on the 2-back task using d Prime (d ') scores. d ' is calculated using the formula: d ' = norminv (hits/total target trials) – (false alarm/total non-target trials) (Macmillan & Creelman., 1990). Norminv represents the inverse of the standard normal cumulative distribution, false alarm represents the number of non-target trials with a button response. Reaction time (RT) to hits was also calculated. Two participants were excluded due to software malfunction during data acquisition.

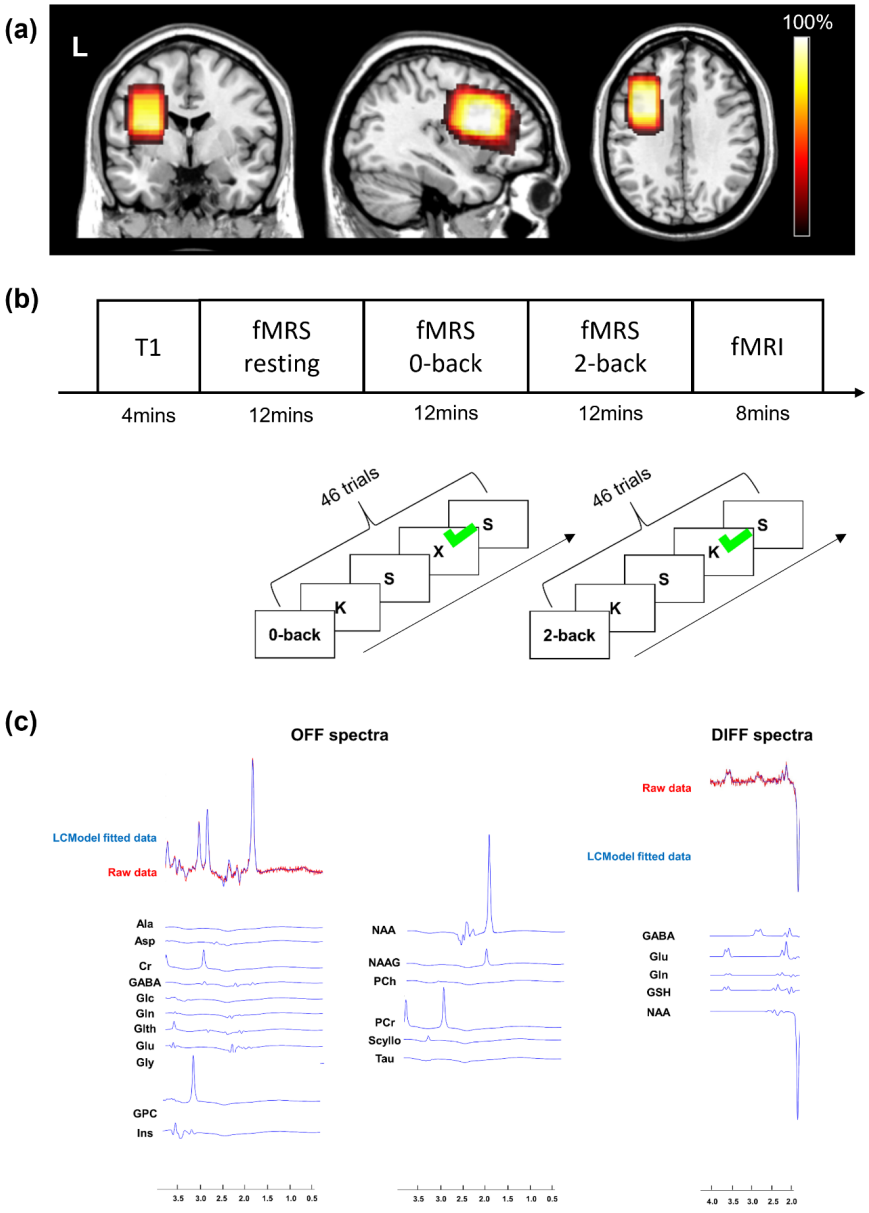

Figure 1. (a) MRS VOI in the left DLPFC. Colour bar represents the overlapping DLPFC VOIs across the participants. (b) Experimental procedure and task. (c) An example MRS spectrum and fitted spectra for each metabolite from DLPFC. Raw data is represented in red colour. Fitted spectra by LCModel are shown

in blue colour.

MRS processing and quantification

Prior to fitting, MRS data were pre-processed using an in-house pipeline written in MATLAB (The MathWorks Inc, Natick, MA, USA). Raw data (in GE .p file format) were coil-combined and eddy current corrected using the unsuppressed water reference. Frequency and phase correction was performed using spectral registration , aligning individual ON and OFF spectra to the mean OFF spectrum. To remove corrupted transients from subject motion etc. individual spectra were rejected as outliers if the mean square error over the choline (Cho) peak was $> \pm 3$ standard deviations from the mean OFF spectrum . Aligned spectra were averaged and subtracted to create difference (DIFF) spectra. Spectra were averaged over blocks of 8 transients [32 seconds] for both dynamic DIFF and OFF spectra to examine metabolite changes across conditions. The rationale for this approach was to perform exploratory analysis on dynamic changes in each metabolite during the scanning session, consistent with prior studies (Woodcock et al., 2018; Michels et al., 2012). Spectral quality characteristics are summarised in Table S1. The processed spectra were quantified by LCModel . GABA was measured in DIFF spectra (ON-OFF), and Glx, total N-acetylaspartate (tNAA), and total creatine (tCr) were measured in non-edited (OFF) spectra. Metabolites were quantified by referencing to unsuppressed water signal. Glx and GABA measurements less than 50% in Cramer-Rao Lower Bound (CRLB) were included. The lenient CRLB criterion was applied to avoid biasing low concentration estimation (Kreis., 2016).

Tissue segmentation was performed to examine partial volume effects over the MRS VOI. T1-weighted images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using SPM12. The DLPFC VOI consisted of 34±6 (mean±standard deviation) % of GM, 62±7% of WM, and 4±2 % CSF on average. As neurochemical concentrations including Glx and GABA are substantially higher in the GM compared to WM , we used GM volume as a covariate in following analyses.

fMRI pre-processing and analysis

All anatomical and functional images were pre-processed by the BRC pipeline (version 1.5.5) . The preprocessing included slice timing correction, brain extraction, motion correction, normalisation, and spatial smoothing with 8mm full width at half maximum (FWHM). Linear registration using FLIRT was employed to the T1 anatomical image for boundary-based registration and to Montreal Neurological Institute (MNI) standard space. Motion correction was performed based on FLIRT and six motion parameters were generated. The segmentation of WM and CSF were computed by FMRIB's Automated Segmentation Tool (FAST) from T1 anatomical images, and time series data were measured from WM and CSF to control physiological signal noise. One participant was excluded due to errors in the file.

General Linear Model (GLM) analysis was performed using SPM12. For individuals, a design matrix was created by modelling 2-back, 0-back, and fixation with six motion parameters as regressors. T-contrast images were generated between the 2-back and 0-back (2-back $>$ 0-back). Group analysis was conducted using a random effect model (one-sample t-test). A statistical threshold was set at $p < 0.001$ at a voxel level and $p < 0.05$ at a cluster level with at least 50 contiguous voxels after family-wise error (FWE) correction.

Region of Interest (ROI) analysis was performed by Marsbar toolbox (http://marsbar.sourceforge.net/). The DLPFC ROI was defined by combining Brodmann area (BA9 and BA46) and the DLPFC MRS VOI. The mean BOLD signal changes from the DLPFC ROI were extracted for each condition (fixation, 0-back, and 2-back).

Statistical Analysis

The performance (accuracy and reaction time, RT) of each task was computed and compared through a paired t-test.

To explore the dynamics of Glx and GABA, we performed a repeated measures ANOVA with condition (baseline, 0-back, 2-back) and time (8 time points) as within-subject factors, accounting for GM volume, the

order of the session, sex, and age. We conducted a linear mixed model analysis with the condition as the main factor as well as GM volume, the order of the session, sex, and age as covariates in Glx and GABA to examine neurochemical changes in the DLPFC across task conditions (resting, 0-back and 2-back). *Post-hoc* paired t-tests were performed for the comparisons between task conditions. False-discovery rate (FDR) correction was applied for multiple comparisons.

Partial correlation analysis was used to examine the relationship between task-induced neurochemical changes found in the linear mixed model, fMRI BOLD signal changes and working memory task performance. GM volume in the voxel, sex, and age were included as covariates. FDR correction was applied for multiple comparisons. We reported the results thresholded at $p_{\text{FDR-corrected}} < 0.05$.

Results

Behavioural results

Participants' performance on the WM task was compared between 0-back and 2-back conditions. The planned paired t-test revealed that participants performed better at the 0-back task condition than the 2-back task condition in both accuracy (0-back: 99.69% +- 0.30, 2-back: 89.91% +- 6.40, t = 4.773, p = 0.001) and RT (0-back: 0.41s +- 0.04, 2-back: 0.60s +- 0.06, t = -9.785, $p < 0.001$).

Task modulated neurochemical changes in DLPFC

To investigate task-modulated effects in Glx and GABA, we conducted a linear mixed model with the task condition (resting, 0-back, and 2-back) as a main factor, accounting for GM volume, the order of session, sex, and age as covariates. Glx showed a significant condition effect ($F_{(2,191)} = 6.226$, p = 0.002) (Fig. 2a). *Post-hoc*paired t-tests revealed that 2-back task significantly increased Glx concentrations in the DLPFC compared to the resting (p = 0.001). 0-back task also significantly increased Glx levels in the DLPFC relative to resting (p = 0.037). There was no significant difference in Glx level between the 0-back and the 2-back. Unlike Glx, we did not observe the effect of condition in GABA ($F_{(2,194)} = 0.871$, p = 0.420) (Fig. 2b). It should be noted that the ANOVA with task condition and time demonstrated a significant main effect of task condition in Glx ($F_{1,10} = 4.995$, p = 0.049) only. There was no significant effect of the time and interaction between them. GABA did not show any significant effects of the condition and time.

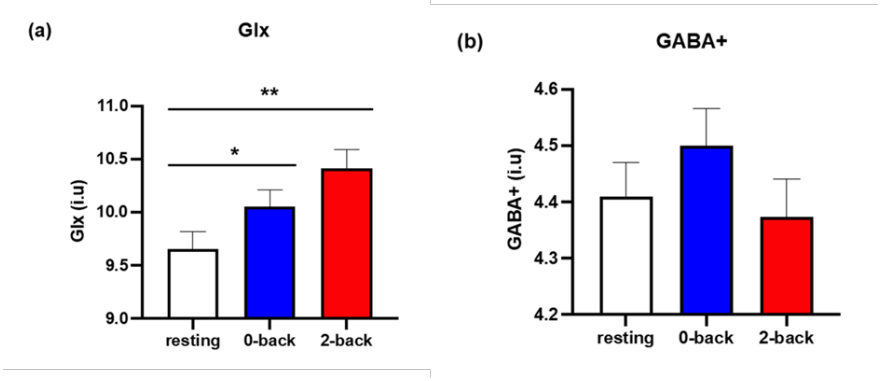

Figure 2. Task modulation effects in DLPFC Glx and GABA. (a) Mean Glx levels for each task condition (b) Mean GABA levels for each task condition. Error bars represent standard errors. **: $p < 0.01$, *: $p < 0.05$. Institutional unit = i.u.

Glx and fMRI BOLD signal change

2-back task-induced significant activation in the bilateral DLPFC, left inferior frontal gyrus, supplementary motor area, superior parietal cortex, intraparietal sulcus, and left inferior temporal gyrus (Fig. 3a, Table

S2). ROI analysis revealed that the 2-back task significantly increased BOLD signal in the DLPFC compared to the fixation (t = -1.98, p = 0.0378) (Fig. 3b).

To examine the relationship between task-modulated metabolites and DLPFC BOLD signal changes, we performed a partial correlation analysis accounting for GM volume, sex, and age. This analysis showed that there was a significant linear relationship between Glx and BOLD responses during 2-back task (r = 0.897, $p_{FDR\text{-}corrected}$ = 0.031) (Fig. 3c). To investigate further the relationship between ΔGlx and the BOLD signal changes, a confirmatory partial correlation was conducted. The ΔGlx (2-back – resting) was significantly and positively correlated with BOLD signal changes from 2-back to fixation (r = 0.895, p = 0.003) (Fig. 3d). Any meaningful relationship between ΔGlx (2-back – 0-back) and BOLD signal change (2-back – 0-back) was not found. GABA did not show any significant relationship with the DLPFC BOLD signal changes.

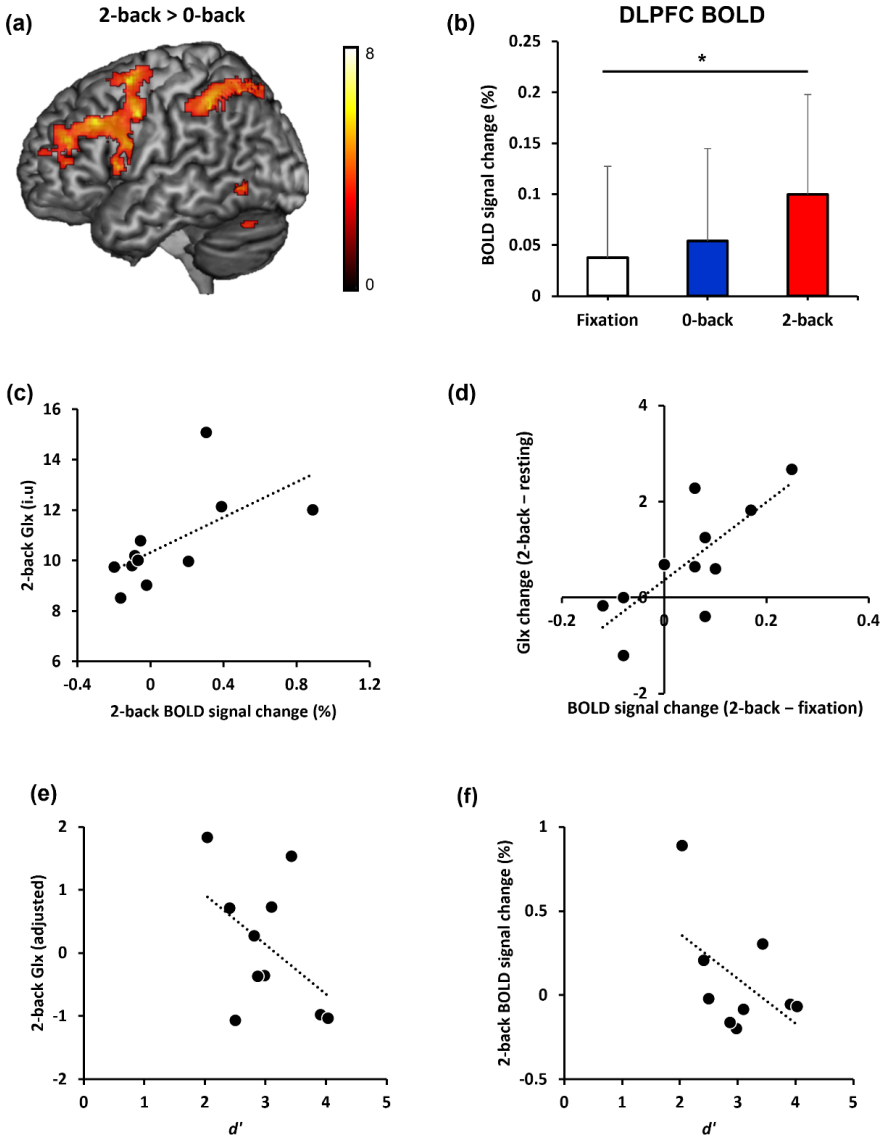

Figure 3. (a) fMRI results of the contrast of interest (2-back > 0-back). Colour bar represents Z score. (b) BOLD signal changes in the DLPFC across task conditions. (c) Scatterplot showing the positive correlation between Glx levels and fMRI BOLD signal changes in the DLPFC during 2-back task condition. (d) Scat-

terplot showing the positive correlation between ΔGlx (2-back – resting) and DLPFC BOLD signal changes (2-back – fixation). (e) Scatterplot showing the negative correlation between DLPFC Glx levels and *d'* . The plotted individual Glx values were accounted for GM value, sex, and age. (f) Scatterplot showing the negative correlation between DLPFC BOLD signal changes and *d'* . Error bars indicate the standard errors. * $p < 0.05$

Glx, BOLD signal changes, and working memory performance

A partial correlation analysis investigated the relationship between Glx and *d'* (2-back task performance), regressing out GM volume, sex, and age. There was a significant and negative correlation between Glx levels and *d'* (r = - 0.866, $p_{\text{FDR-corrected}} = 0.031$) (Fig. 3e). To investigate the relationship between task-induced BOLD signal changes and *d'* , a partial correlation analysis was performed, accounting for GM volume, sex, and age and demonstrated a significant correlation (r = - 0.859, $p_{\text{FDR-corrected}} = 0.031$) (Fig. 3f). GABA did not show any significant relationship with the task performance.

Discussion

Converging evidence has implicated the DLPFC contributions to human working memory (Barbey et al., 2013; D'Esposito and Bradley, 2015), but the underlying neurochemical mechanisms of the DLPFC remain unclear. Here, we investigated task-modulated neurometabolite changes in the DLPFC in relation to BOLD signal changes and performance during working memory processing. Our combined functional MRS with fMRI approach confirmed that the 2-back task increased Glx concentrations and BOLD signal changes in the DLPFC. Task-modulated Glx changes were linked to task-induced regional haemodynamic response in the DLPFC. Importantly, the task modulated Glx increase in the DLFPC was associated with individual task performance and BOLD activation during working memory processing. Our findings, while preliminary due to the small sample size and partially exploratory nature of our study, suggest that task modulation of DLPFC Glx may critically underpin working memory processing and performance and highlight that state-of-the-art fMRS at 3T offers a robust noninvasive, neurometabolic window to brain function.

To our best knowledge, this is the first study demonstrated that task modulated Glx changes in the DLPFC was associated with haemodynamic responses to DLPFC activation. This is well in line with cumulative evidence from ultrahighfield MRS/MRI studies establishing tight dynamic coupling of glutamate and BOLD increase in visual and motor activation (Ip et al., 2017; Martínez-Maestro et al., 2019; Koush et al., 2021; Kurcyus et al., 2018; Bednařík et al., 2015; Schaller et al., 2014). Glutamate is the major excitatory neurotransmitter in the brain, but MRS detectable glutamate can be more closely linked to energy metabolism as glutamate is a key metabolite in the tricarboxylic acid cycle and the metabolic Glu pool is substantially larger than the neurotransmitter pool (Hyder et al., 2006; Rothman et al., 2003. In the healthy brain, metabolism and neuronal processes are tightly coupled, evidenced by upregulation of glucose consumption and glutamate-glutamine cycling during task-driven neuronal activation . There is also a known tight coupling between haemodynamic responses and TCA activity supporting the notion that the observed coupled Glx/BOLD changes reflect coupling of task modulated regional energy metabolism and vasodilation. . Therefore, the increased glutamate/Glx levels measured by functional MRS reflect increased glucose metabolism while the extent of possible contribution from excitatory neurotransmission remains unclear.

Glutamate is present in neuronal, glial, and metabolic pools in the brain (Rae, 2014). As MRS measured glutamate are the overall tissue content of glutamate, it is challenging to distinguish whether dynamic changes in glutamate reflect neurotransmitter change or metabolic processes. Previous studies have shown that the glutamate changes related to the metabolic process are slow, about 18mins (Maddock et al., 2016), whereas glutamatergic neuronal transmissions occur in milliseconds and can be measured about a few seconds at 7T functional MRS (Ip et al., 2017; 2019). Our paradigm is more compatible with the metabolic processes - glucose consumption and glutamate-glutamine cycling (Hyder et al., 2006; Rothman et al., 2003). However, primate studies have repeatedly demonstrated that spatial working memory is associated with increased neural spiking activity in the DLPFC (for a review, see Arnsten, 2009). Our results may reflect increase in excitatory neural activity along with energy metabolism in the DLPFC during working memory processing

(Lea-Carnall et al., 2023). Future studies will be needed to elucidate this issue with event-related functional MRS design at 7T.

Interestingly, we observed an inverse correlation between working memory performance and 2-back task modulated Glx levels as well as BOLD signal changes during the 2-back task. Individuals with lower Glx concentrations along with less task-induced BOLD responses during working memory processing showed better task performance (Fig. 3e-f). These results seem to be contrasted with the involvement of the DLPFC in working memory such that DLPFC activation increases are associated with performance improvements (for a review, Just & Carpenter., 1992). However, Rypma and D'Esposito (1999) demonstrated the inverse relationship between DLPFC activation and working memory task performance, showing that the increased DLFPC activity was related with poorer task performance (slower reaction time). They explained their results with a model of neural efficiency – individuals with better processing efficiency show lower brain activation (Neubauer & Fink., 2009). Haier et al (1992) have reported that individuals with higher score on Raven's Advanced Progressive Matrices showed reduced glucose metabolic rate in the DLPFC compared to individuals with lower score. Consistent with these findings, our results provide better understandings of the individual variability in task performance and their neurochemistry in the DLPFC during working memory processing, supporting the neural efficiency hypothesis (Neubauer & Fink., 2009; Nussbaumer et al., 2015). Subject with lower working memory efficiency could require more resources in the DLPFC leading to an increase in Glx levels along with BOLD signal changes.

Although we observed task-related Glx increase during the 2-back task relative to the baseline, by replicating previous findings (Woodcock et al., 2018; 2019), we did not find a significant increase of Glx during 2-back task compared to 0-back task, but a minor significant increase of Glx during the 0-back task vs resting (fixation). In this study, we had the 0-back task as a control task for working memory processing to detect a task-specific modulation in neurochemicals. Previous work used the continuous visual fixation cross as the baseline and demonstrated that the fixation generated less variability and lower Glx levels compared to other control conditions such as eyes closed, flashing checkerboard, and finger tapping (Lynn et al., 2018). However, these results were not properly compatible with the results of the DLPFC BOLD signal changes, which were estimated by comparison with a control condition. In our data, there was a significant increase of BOLD response during the 2-back task compared with the baseline (fixation) but no difference in BOLD between the 2-back task and 0-back task. It might explain the no difference of Glx levels between the 0-back and 2-back conditions.

Contrary to our hypothesis, we found no evidence of GABA changes in the DLPFC during working memory processing. Few functional MRS studies have reported task-modulated GABA changes in the motor cortex at 7T and the DLPFC at 3T . These studies showed a reduction of GABA over 20 mins during cognitive processing (Michels et al., 2012; Kolasinski et al., 2019). In this study, GABA levels were measured for 12 min, which might not be sufficient to detect GABA changes in a cortical region. A recent study failed to observe GABA changes in the motor cortex with a 6 min motor learning task . Further studies to detect GABA changes during a cognitive condition may require a relatively longer functional MRS session or several repetitive sessions with a task.

This study has several limitations. First, our sample size was relatively small. Due to the COVID, there were many barriers to recruit participants and then we replaced the scanner after re-opening the MRI centre. However, we have replicated previous findings by demonstrating the task modulated Glx changes in the DLPFC (Woodcock et al., 2018; 2019) and a significant relationship between the Glx changes and BOLD signal changes during a task and cognitive processing (Koush et al., 2021; Kurcyus et al., 2018; Ip et al., 2017, 2019; Bednařík et al., 2015; Martínez-Maestro et al., 2019; Schaller et al., 2014). Second, the order of task was fixed in functional MRS sessions. Although we included the order of task as a covariate in the analysis, we could not exclude the possibility that they might affect our findings. Third, we employed the GABA-edited MEGA-PRESS sequence to measure GABA changes. Although the editing is crucial for the GABA measurements, long echo times make the quantification of metabolite concentrations susceptible to low SNR and confounds of different relaxation times (Choi et al., 2020; Ramadan et al., 2014).

Conclusion

The present study reports first preliminary findings that suggest a neurometabolic mechanism for working memory performance. We confirm that a prolonged block-design working memory task increases Glx in the DLPFC and show that the neurochemical responses were positively coupled with hemodynamic responses as shown by regional BOLD increases. Moreover, the extent of both Glx and BOLD signal increases was inversely associated with working memory performance, in keeping with the neural efficiency hypothesis. Our results suggest a critical role for task-modulated glutamate in working memory performance as reflected in BOLD signal changes.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgements

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, J.J.

Author contribution

The original design was co-created by JJ, BF, BBB and HO. AB and BBB contributed to MRS analysis. HF, JJ, and DPA contributed to advice on analysis design/plan and data interpretation. The draft was written by HO and JJ. All authors reviewed the final version of the draft.

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Figure legends

Figure 1. (a) MRS VOI in the left DLPFC. Colour bar represents the overlapping DLPFC VOIs across the participants. (b) Experimental procedure and task. (c) An example MRS spectrum and fitted spectra for each metabolite from DLPFC. Raw data is represented in red colour. Fitted spectra by LCModel are shown in blue colour.

Figure 2. Task modulation effects in DLPFC Glx and GABA. (a) Mean Glx levels for each task condition (b) Mean GABA levels for each task condition. Error bars represent standard errors. **: $p < 0.01$, *: $p < 0.05$. Institutional unit = i.u.

Figure 3. (a) fMRI results of the contrast of interest (2-back > 0-back). Colour bar represents Z score. (b) BOLD signal changes in the DLPFC across task conditions. (c) Scatterplot showing the positive correlation between Glx levels and fMRI BOLD signal changes in the DLPFC during 2-back task condition. (d) Scatterplot showing the positive correlation between ΔGlx (2-back – resting) and DLPFC BOLD signal changes (2-back – fixation). (e) Scatterplot showing the negative correlation between DLPFC Glx levels and *d'* . The plotted individual Glx values were accounted for GM value, sex, and age. (f) Scatterplot showing the negative correlation between DLPFC BOLD signal changes and *d'* . Error bars indicate the standard errors. * $p < 0.05$