

# Increased glutamate levels observed upon functional activation in the anterior cingulate cortex using the Stroop Task and functional spectroscopy

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It has been shown in recent studies that it is possible to detect changes in the main excitatory neurotransmitter, glutamate, upon functional activation with visual and motor paradigms using a 7 T MRI and functional magnetic resonance spectroscopy. A cognitive task would be desirable for this technique because it could then be used to examine psychiatric disorders that have cognitive deficiencies. The aim of the work presented here was to use functional magnetic resonance spectroscopy with a 7 T MRI to show that increases in glutamate can be observed within the anterior cingulate cortex using the Stroop Task as the activation paradigm in healthy controls. Significant glutamate increases ( $0.24 \pm 0.09 \mu\text{mol/g}$ ,  $P < 0.025$ ), comparable with what has been reported in the studies of the occipital cortex and motor cortex, were observed when the participants ( $n = 7$ ) performed the task, followed by a trend toward returning to baseline in the post-task recovery period ( $-0.23 \pm 0.13 \mu\text{mol/g}$ ). This method would be ideal for the study of neuropsychiatric disorders that have been

shown to have abnormal resting glutamate levels and cognitive deficiencies in the anterior cingulate cortex, such as schizophrenia. This exploratory study is the first to demonstrate functional magnetic resonance spectroscopy in the anterior cingulate with a cognitive task using a 7 T MRI. *NeuroReport* 26:107–112 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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## Introduction

Proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) is a useful tool for the noninvasive study of the brain's chemistry. <sup>1</sup>H-MRS has an inherently low signal-to-noise ratio (SNR); thus, traditional studies have assumed constant metabolite concentrations during the long scan durations required to acquire a spectrum with sufficient SNR for accurate quantification. Advances in MRI technology such as higher magnetic field strengths, improvements in radiofrequency head coils, pre-amplification, and signal detection systems have considerably increased the available SNR for <sup>1</sup>H-MRS. This has made it feasible to examine the brain in a dynamic state using functional <sup>1</sup>H-MRS (fMRS). It has been shown previously with fMRS that alterations in the local neurochemistry occur when a particular area of the brain becomes engaged in task transaction [1–8]. One metabolite of interest is glutamate (Glu), the main excitatory neurotransmitter in the brain, which has been shown to be related directly to the brain's consumption of glucose for the production of energy [9] and has been implicated

in the pathophysiology of psychiatric disorders, such as schizophrenia [10,11].

Few studies have used fMRS to date. Three studies have shown small (2–4%) but significant increases in the Glu concentration in the occipital cortex upon functional activation with a visual stimulus, such as a flickering checkerboard [1,2,5]. Recent results showed a 2% increase in the motor cortex upon functional activation with a finger-tapping paradigm [6]. Two other studies have used pain as a stimulus and have identified 9.3 and 18.1% increases in the mean Glu concentration in the anterior cingulate cortex (ACC) and the anterior insular cortex, respectively, when the participants are exposed to the pain [3,4].

Cognitive tasks are generally less sensitive for detection when assessed through standard BOLD fMRI than visual (flickering checkerboard) or motor (finger tapping) tasks [12]. To the best of our knowledge, there has been only one study to date that has used fMRS to examine metabolite changes using a cognitive task as the activation paradigm [8]. This study did not find any changes in Glx [Glu + glutamine (Gln)], but they did find that GABA increased in concentration upon functional stimulation of the left dorsolateral prefrontal cortex and

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then decreased in subsequent runs of the task. Although Glx, in this case, combines Glu with Gln, it would be ideal to use a cognitive task to examine the response of Glu independent of Gln as both are crucial to brain function. As Glu abnormalities have been found in neuropsychiatric disorders in the ACC [10,11], a cognitive task that robustly activates the ACC is desirable for the investigation of abnormalities in the dynamic regulation of Glu levels in this area.

In addition to influencing our perception of pain, the ACC is implicated in many other cognitive functions, including selective encoding of stimulus properties [13], general attention [14], and organization of conflicting stimuli [15]. The aim of this exploratory study is to show that with fMRS of the ACC, it is feasible to detect Glu concentration changes using a cognitive task as the activation paradigm. The Colour Stroop Task is a common psychological task that involves differentiating potentially conflicting word and color stimuli under congruent (i.e. the word 'RED' written in red ink) and incongruent (i.e. the word 'BLUE' written in red ink) conditions [16]. The Stroop Task was chosen for this study because it activates the ACC and has been implicated previously in schizophrenia [15], a potential application of this technique.

It has been proposed that Glu concentrations will increase upon functional activation of the ACC with a cognitive task because of the increase in the cycling rate of neuronal Glu with Gln with an approximate 1:1 stoichiometry to the neuronal glucose oxidation rate, which is directly related to functional MRI signal changes upon stimulation [9]. No other specific hypotheses have been made in terms of other metabolites.

## Methods

Seven healthy control individuals (age  $39.8 \pm 3.8$  years) provided informed written consent according to the guidelines of the Review Board for Health Sciences Research Involving Human Subjects at Western University. The participants included five men and two women.

In this study, the Stroop Task involved four conditions, incongruent, congruent, word only (the name of the color written in white), and color only (written as 'XXXX'), with each condition represented in 25% of the trials. Four colors were chosen for the task: red, green, blue, and yellow. The participants were asked to respond as quickly and accurately as possible on a four-button keypad. They were allotted 2 s to respond, and stimuli were separated by 1 s of cross fixation. The task was explained to each participant and rehearsed outside of the scanner until it could be performed consistently with 80% accuracy or greater. An angled mirror attached to the head coil allowed the participants to visualize the words of the Stroop Task projected on the screen in the scanner

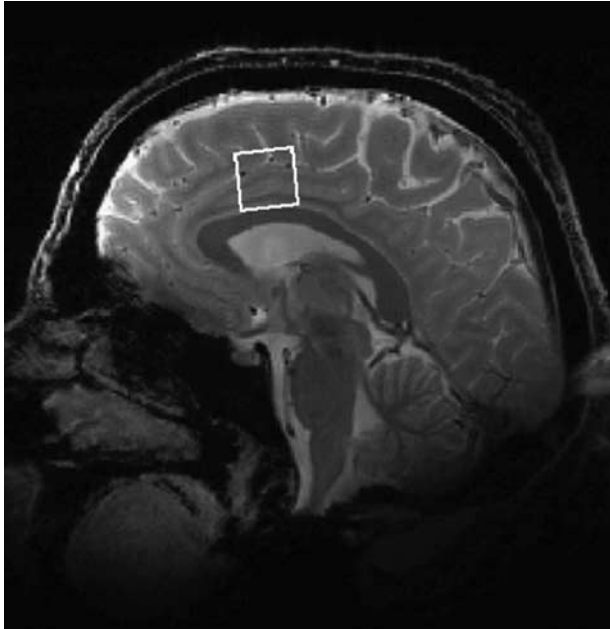
synchronously with the fMRS acquisition. Participants were initially positioned in the scanner with the button press keypad in their hands ready to perform the task. This minimized additional movement at the onset of the Stroop Task. The fMRS acquisition began with a 4-min resting period in which the participants were asked to fixate on a cross in the center of the projection screen. The final 3 s of the resting period were used to prompt the participant that the Stroop Task was about to start with the word 'Ready' projected onto the screen. The Stroop paradigm then lasted for 4 min and was followed by a 4-min recovery period. The procedure was written using PsychoPy [17]. The accuracy and response times were recorded to ensure participants' compliance.

All measurements were acquired on a 7.0 T Agilent/Magnex head-only MRI (Agilent, Inc, Walnut Creek, California, USA) with a Siemens AC84 head gradient coil (Siemens, Erlangen, Germany), located at the Center for Functional and Metabolic Mapping at Western University. A transmit-only receive-only head coil with 15 transmitters and 23 receivers [18] was used for all scans. A map of the transmit field for each transmitter was acquired at the beginning of the session to facilitate optimized homogeneity correction of the transmit field for each scan using a B1-shimming approach developed in-house [19]. The magnetic field uniformity (B0-shim) was adjusted automatically over the field of view with first-order and second-order shims using RASTAMAP [20] before all acquisitions.

The MRS voxels were  $2.0 \times 2.0 \times 2.0$  cm ( $8 \text{ cm}^3$ ) in size. In every individual, a voxel was centered medially and encompassed the bilateral ACC using two fast low-angle shot 2D anatomical imaging sequences in the sagittal [45 slices, repetition time (TR)=950 ms, echo time (TE)=5.23 ms, flip-angle ( $\alpha$ )=30°, gap between slices=1 mm, thickness=2 mm, field of view (FOV)  $220 \times 220$  mm, and matrix size of  $220 \times 200$ ] and axial (20 slices, TR=500 ms, TE=5.23 ms,  $\alpha$ =30°, gap between slices=1 mm, thickness=2 mm, FOV= $220 \times 220$  mm, and matrix size  $220 \times 220$ ) directions, both using lipid saturation. Voxel positions were prescribed by the scanner operator (R.T.) using anatomical landmarks as trained by a neuroanatomist (N.R.) to ensure consistent voxel placement (Fig. 1).

MRS spectra were acquired individually during the performance of the Stroop paradigm using an ultra-short echo time stimulated echo acquisition mode sequence with outer volume suppression [21] [TR=3 s, TE=10 ms, mixing time (TM)=32 ms, 4000 complex pairs, four steady state scans, 1 s acquisition time, eight-step phase cycle] with 16 water unsuppressed spectra and 240 water suppressed spectra, 80 spectra for each 4 min section of the Stroop paradigm (Rest, Stroop, Recovery). An eight-pulse VAPOR preparation sequence, with an additional water suppression pulse during the TM period

Fig. 1



A sagittal cross-section of the brain with the MRS voxel placed in the anterior cingulate cortex. MRS, magnetic resonance spectroscopy.

[21], provided efficient water suppression. A separate metabolite suppressed spectrum was acquired to assess the macromolecule content [22]. Each acquisition outputted 23 spectra, one for each receiver, which required channel combination before use [23]. After the channels had been combined, each spectrum was frequency and phase corrected before being averaged together. Quality Eddy Current Correction [24] reduced linewidth distortions before spectral fitting. Spectra were quantified using fitMAN, a time-domain fitting algorithm [25]. All spectra were inspected visually for quality.

Because of our directional hypotheses for Glu concentrations, a three-level repeated-measures analysis of variance (rmANOVA) design using the metabolite concentrations at each 4 min (80 spectral averages) section of the functional paradigm was examined using SPSS v.20 (IBM Corp, Armonk, New York, USA) to determine significant variations over time for Glu ( $\alpha=0.025$ , one tailed with Bonferroni correction). Because of the exploratory nature of this paper, the remaining metabolites were also tested for significance using a repeated-measures multivariate analysis of variance (rmMANOVA) ( $\alpha=0.025$ , two tailed with Bonferroni correction). Only metabolites with Cramer–Rao lower bounds less than 20% were included in the analysis.

## Results

The average linewidths of the unsuppressed water signal were  $12.8 \pm 2.0$  Hz after first-order and second-order shim

adjustments. The average metabolite linewidth in the resting condition was  $8.6 \pm 0.8$  Hz and no significant narrowing of the linewidths was observed in the activated condition compared with the resting condition. The water signal was suppressed sufficiently as the residual water signal was less than the height of the NAA peak in every spectrum.

A total of 15 metabolites were quantified consistently with Cramer–Rao lower bounds less than 20% for each of the resting, activated, and recovery spectra (Table 1). The time course of group-averaged Glu activation in 1 min increments is shown in Fig. 2 (all stats were performed on the 4 min, 80 averaged spectra). The concentration appears to increase upon onset of the Stroop Task, plateau, and then decrease over 2 min toward the baseline. When the Glu concentrations between the resting, Stroop, and recovery periods were compared, there was a significant  $2.6 \pm 1.0\%$  ( $0.24 \pm 0.09$   $\mu\text{mol/g}$ ) increase in the activated state relative to the initial resting period ( $P=0.02$ , one tailed), followed by a nonsignificant  $-2.4 \pm 1.2\%$  ( $-0.23 \pm 0.13$   $\mu\text{mol/g}$ ) trend for the Glu signal to return to baseline after the activation period had ended ( $P=0.06$ , one tailed). However, when Glu concentrations were normalized to their baseline value in the resting period, the relative decrease in the recovery period became significant before Bonferroni corrections ( $0.025 < P < 0.05$ ). Six of the seven participants showed this increase in Glu upon activation (1.0, 1.3, 5.3,  $-0.4$ , 6.0, 0.3, and 5.0%) and six of the seven participants

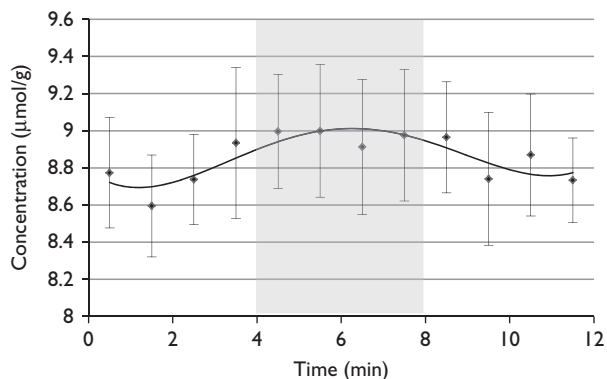
**Table 1** Quantified metabolites with their resting concentrations ( $\mu\text{mol/g}$ ) and relative changes (%) during the task completion and the recovery period presented as mean  $\pm$  SE

	Resting concentration ( $\mu\text{mol/g}$ )	Concentration change (task–rest)		Concentration change (recovery–task)	
		%	<i>P</i>	%	<i>P</i>
GLN	$2.4 \pm 0.3$	$-0.2 \pm 5.6$	0.738	$6.8 \pm 4.7$	0.179
GLU	$8.9 \pm 0.3$	$2.6 \pm 1.0$	*0.021	$-2.4 \pm 1.2$	0.046
TAU	$2.4 \pm 0.3$	$5.1 \pm 7.3$	0.509	$-1.4 \pm 5.6$	0.539
ASP	$2.5 \pm 0.6$	$0.3 \pm 5.9$	0.853	$-1.7 \pm 3.5$	0.404
NAAG	$1.1 \pm 0.3$	$2.6 \pm 7.2$	0.730	$-4.6 \pm 7.6$	0.505
MYO	$7.9 \pm 0.5$	$0.9 \pm 1.5$	0.567	$1.7 \pm 1.4$	0.261
SER	$2.0 \pm 0.3$	$7.5 \pm 6.7$	0.237	$-2.4 \pm 6.3$	0.482
GLC	$0.8 \pm 0.2$	$-6.4 \pm 10.2$	0.955	$14.9 \pm 5.3$	0.061
NAA	$7.9 \pm 0.3$	$2.6 \pm 1.8$	0.199	$-1.1 \pm 0.5$	0.309
LAC	$0.5 \pm 0.1$	$22.9 \pm 20.3$	0.351	$35.4 \pm 13.1$	0.155
GLY	$0.7 \pm 0.1$	$2.1 \pm 1.5$	0.200	$-12.2 \pm 7.0$	0.130
SCY	$0.4 \pm 0.0$	$3.3 \pm 2.8$	0.279	$1.5 \pm 3.7$	0.663
ASC	$1.0 \pm 0.1$	$-11.5 \pm 11.6$	0.764	$33.1 \pm 17.5$	0.030
TCHO	$2.1 \pm 0.1$	$2.2 \pm 1.2$	0.111	$-0.6 \pm 0.6$	0.399
TCR	$7.7 \pm 0.3$	$2.2 \pm 1.2$	0.122	$-0.0 \pm 0.6$	0.920
GLX	$11.3 \pm 0.5$	$2.0 \pm 1.7$	0.302	$-1.1 \pm 1.1$	0.324

ASC, ascorbate; ASP, aspartate; GLC, glucose; GLN, glutamine; GLU, glutamate; GLY, glycine; GLX, glutamate + glutamine; LAC, lactate; MYO, myo-inositol; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; SCY, scyllo-inositol; SER, serine; TAU, taurine; TCHO (cho+PC+GPC), total choline; TCR (CR+PCR), total creatine.

\* $P < 0.025$  significance (Bonferroni adjusted) for a two-tailed comparison, with the exception of Glu, which is one tailed because of it being a planned directional comparison.

Fig. 2



A time course of the average glutamatergic response to the Stroop Task (shaded area) in the ACC. Each data point represents the temporal average of 20 spectra (1 min) per participant combined and quantified and subsequently averaged over all participants ( $n=7$ ). Error bars indicate the interindividual SEM at each point. The trend line is a fourth-order polynomial fit to the data. The shaded area indicates the 4 min of Stroop activation. ACC, anterior cingulate cortex.

showed the subsequent decrease (1.2, -2.0, -8.8, -1.5, -1.1, -2.9, and -1.6%).

Other than Glu, the remaining metabolites were not significant in the rmMANOVA. It should be noted, though, that in the univariate pair-wise comparisons, ascorbate (Asc) increased  $33.1 \pm 17.5\%$  ( $0.20 \pm 0.06 \mu\text{mol/g}$ ) during the 4-min recovery period relative to the activation period ( $P=0.03$ , two tailed). However, this is only a trend because of the Bonferroni correction.

All participants recorded at least 90% correct responses. Group-averaged response times were  $725 \pm 278$  ms for the congruent condition,  $976 \pm 261$  ms for the incongruent condition,  $750 \pm 270$  ms for the word-only condition, and  $721 \pm 230$  ms for the color-only condition.

## Discussion

Using a Stroop Task as the functional paradigm for an fMRS examination of the dynamic metabolic response to activation in the ACC, an observed significant increase of  $2.6 \pm 1.0\%$  was found in Glu upon onset of the task, followed by a trend to return to the baseline in a small sample size of healthy controls ( $n=7$ ). The high percentage of correct responses shows that there was high compliance with the Stroop Task among the participants.

The increase in Glu is comparable with recent fMRS studies of the occipital cortex [1,2,5] and the motor cortex [6], which found Glu increases from 2 to 4% over the course of minutes, with a subsequent decrease upon cessation of the stimuli. Figure 2 indicates that the concentration increased to its new steady state within the first minute of activation. This is relatively faster than that in the above studies, which show that it takes

1–2 min before the concentration reaches its new state. One possible explanation is that because of the involvement of ACC in attention networks [14], and, specifically, selective encoding of stimulus properties [13] the ACC had already been in a state of minor activation. This is consistent with the final data point of the first resting condition in Fig. 2 that is seemingly already ascending toward the peak concentration. The resting Glu concentration is slightly lower than what was observed in other fMRS studies at 7 T [1,2,5,6]; however, different scanners, techniques, and brain areas studied led to natural variation in the observed values between studies.

One departure from previous fMRS studies at 7 T [1,2,5,6] is that a significant difference in lactate or glucose was not observed. These are difficult metabolites to quantify reliably; thus, perhaps more participants would be needed to observe significant differences.

It is conceivable that the ACC accumulates Glu faster than other brain regions upon stimulation as can be extrapolated from a study of the ACC using a painful stimulus [3]. Mullins and colleagues used event-related fMRS at 3 T, in which one resting spectrum and one activated spectrum are acquired per bolus of pain. After many boluses of pain, there are enough resting and activated spectra that can be averaged to detect a rapid metabolic response. A 9.3% increase in Glu and an 11.4% increase in Glx (Glu + Gln) were observed. The Glu increases observed by Mullins *et al.* [3] are markedly higher than the response detected in the present study. This could be because of the nature of the experimental design (event-related design vs. block design). It is possible that with an event-related Stroop Task, larger similar increases in Glu in the ACC could be observed. However, with the current block design, the magnitude of Glu changes agrees more with the previous work in the visual cortex [1,2,5] and the motor cortex [6].

One study at 3 T presented GABA changes with a working memory task in the dorsolateral prefrontal cortex, but did not detect any changes in Glx (Glu + Gln) [8]. The study used a dedicated pulse sequence to detect GABA specifically, which can otherwise be a difficult metabolite to quantify as it was not consistently quantified reliably in this study. They observed an initial increase in GABA concentrations, but subsequent decreases with repeated runs of the task. In a future study, it would be interesting to observe how Glu would respond to repeated runs of the Stroop Task.

Another study that was carried out at 3 T has used an fMRS technique to study the ACC using visually evoked sexual arousal [7] and found significant 10–20% increases in Glx with activation. At lower field strength, it is often necessary to measure a combination of Glu and surrounding metabolites that can include Gln, glutathione, GABA, and sometimes macromolecules together as Glx.

It is not clear which metabolites were included in their Glx definition, making it harder to compare these results. A common definition of Glx is simply Glu plus its precursor Gln, as was used in the pain study [3] and the working memory study [8] mentioned above. Using this definition, Glx was examined in this study, but no significant differences were observed.

The increase in Asc in the recovery period, although not significant after Bonferroni correction or at the multivariate level, is intriguing. It has been reported previously by Wilson *et al.* [26] that Glu triggers the release of Asc from astrocytes and increases the extracellular concentration of Asc. Asc is an antioxidant. This is important because Glu neurotransmission leads to increased mitochondrial activity and as a result increased free radicals [27]. Wilson *et al.* [26] showed that this effect takes some time to observe; thus, it is possible that the increase in Glu during the activated phase led to the delayed increase in Asc concentration that was observed in the recovery phase.

Glu is the main excitatory neurotransmitter in the brain. It is synthesized from glucose in the mitochondria, released into the synaptic cleft through exocytosis, and then binds with the postsynaptic membrane to induce neurotransmission [28]. It is then taken up into the adjacent glial cell, where it is converted into Gln before being transferred back into the neuron and converted back into Glu [28]. There is an increase in glucose consumption during functional activation that specifically supports the increased turnover rate of the Glu/Gln cycle [9]. Therefore, the increased number of Glu molecules flowing within the Glu/Gln cycle during the functional activation of the ACC with the Stroop Task could explain the increased Glu observed in bulk brain tissue using MRS. The lack of change in Gln concentrations may indicate that the conversion of Gln back into Glu in the neuron is not a rate-limiting step in the Glu/Gln cycle.

This proof-of-concept study shows that the Stroop Task is a robust task that can be used for fMRS studies in the ACC. This introduces a new method to study the abnormalities of Glu modulation in brain disorders. Schizophrenia research may particularly benefit from this method as it is a population that has been shown to have abnormal resting levels of glutamatergic metabolites in the ACC compared with healthy controls [10,11] and impaired ACC activation during performance of the Stroop Task [15]. Dysfunction of glutamatergic metabolism may manifest itself as an inability to dynamically upregulate Glu cycling or excessive upregulation of Glu, or even Gln, concentrations during performance of the Stroop Task. The limitations of the study include its small sample size and the possibility that the Glu changes observed are because of movement-induced phase shifts from the participant responding to the task, although it is unlikely that a phase shift would induce consistent

increases in Glu rather than decreases. Future studies should have a larger sample size and focus on fMRS in neuropsychiatric disorders.

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## Conflicts of interest

There are no conflicts of interest.

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