MAGNETIC RESONANCE SPECTROSCOPY (MRS) TO DOCUMENT CHANGES IN NEUROCHEMISTRY.

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Statement of Originality

I hereby certify that the work embodies in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Thesis by Publication

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by Assistant Dean of Research Training, attesting to contribution to the joint publications (as shown in the Appendix).

Dr Scott Quadrelli

Acknowledgments

I dedicate this thesis to my daughters, Isobel and Mia.

My PhD has been a challenging and a rewarding experience, thank you to all those who have helped along the way.

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Publications arising from this thesis

Chapter 2 – Published.

Mountford, C., **S. Quadrelli**, A. Lin and S. Ramadan (2015). "Six fucose-alpha(1-2) sugars and alpha-fucose assigned in the human brain using in vivo two-dimensional MRS." NMR in Biomedicine **28**(3): 291-296.

Chapter 3 – Published.

Quadrelli, S., C. Mountford and S. Ramadan (2016). "Hitchhiker's Guide to Voxel Segmentation for Partial Volume Correction of In Vivo Magnetic Resonance Spectroscopy." Magnetic Resonance Insights **9**: 1-8.

Chapter 4 – Accepted.

Quadrelli, S., C. Mountford and S. Ramadan (2018). "Systematic review of *in-vivo* neuro Magnetic Resonance Spectroscopy for the assessment of Posttraumatic Stress Disorder" Psychiatric Research: Neuroimaging.

Chapter 4 – Submitted and under review.

Quadrelli, S., N. Tosh, A. Urquhart, K. Tricky, R. Tremewan, G. Galloway, L. Rich, R. Lea, P. Malycha, and C. Mountford. "Posttraumatic Stress Disorder Affects Fucose-α(1–2)-glycans in the Human Brain: Preliminary Findings of Neuro Deregulation using In Vivo Two Dimensional Neuro MR Spectroscopy" Translational Psychiatry. Submitted 15/5/18.

Chapter 5 – Submitted

Quadrelli, S., K. Ribbons, J.Arm, O. Al-iedani, , J. Lechner-Scott, R. Lea and S. Ramadan (2018). "2D *in-vivo* L-COSY spectroscopy identifies neurometabolite alterations in multiple sclerosis" Radiology.

Abstract

Magnetic resonance spectroscopy (MRS) is a non-invasive technique that can be used to determine the chemical composition of biological tissues in a conventional MRI scanner. Due to its non-invasive nature, *in vivo* proton MRS has been referred to as a 'virtual biopsy' and allows a unique metabolic fingerprint of certain pathologies to be determined. There are a variety of technologies available to perform MRS, including one-dimensional (1D) and two-dimensional (2D) spectroscopy. 1D spectroscopy has several limitations, including difficulty separating peaks secondary to peak overlap. In contrast, 2D spectroscopy separates the resonances in a second magnetic frequency, allowing unambiguous assignment of metabolites and new molecules to be assigned that were previously not visible in the 1D spectrum.

The first contribution to arise from this thesis was to apply the novel spectroscopy technique, 2D MRS, specifically 2D-Localised COrrelated SpectroscopY (2D L-COSY), to assign and identify fucosylated glycans in the brain *in vivo*. Using 2D L-COSY up to six fucose-α (1-2) – galactose species were able to be successfully assigned. These species have previously been shown to be important in learning and memory. This is the first time in decades that a new metabolic assignment has been made in the brain *in vivo* using MRS.

Post processing is an important step in spectroscopy data analysis, ensuring accurate and reproducible results. In the second contribution to arise from the thesis, methods to perform partial volume correction for spectroscopy studies were developed and evaluated. Additionally, methods to extract metrics of interest, such as the number of white matter lesions, from a MRS voxel were determined and published. This allowed these metrics of interest to be correlated with the metabolic differences found using spectroscopy. These techniques were then applied in some of the clinical studies described below.

In this thesis, 2D L-COSY was applied to several clinical conditions, specifically Posttraumatic Stress Disorder (PTSD) and Multiple Sclerosis (MS) as well as the healthy brain. PTSD is a debilitating trauma and stressor related disorder that results in complex somatic, cognitive, affective and behavioural effects after an individual is exposed to a traumatic event. In the process of the study, a systematic review of the literature characterising metabolite differences in PTSD, assessed using MRS, was undertaken. Currently, there is no objective imaging diagnostic tool for PTSD, and there is a need for further imaging tools to diagnose and monitor the condition. Unlike PTSD, MS is a chronic autoimmune demyelinating disease that has typical imaging findings on MRI. Despite this, there is a disconnect

between the MRI imaging findings and the patient's clinical disease severity. There is a great need for further imaging bio-markers in MS. Given the emergence of multiple new biological disease modifying agents, further imaging tools are needed to determine the efficacy of new treatments.

Using 1D MRS and 2D L-COSY in PTSD, multiple metabolic differences were identified that separated patients from healthy controls. Using 1D spectroscopy, there was a reduction in absolute inositol, inositol: Cr in the posterior cingulate cortex, and an increase in (Glu): tCr and Glx: tCr in the posterior cingulate cortex. Reduced inositol in the posterior cingulate cortex has not been previously described using 1D MRS and may be secondary to apoptosis of astrocytes. Using 2D MRS additional metabolic differences in PTSD were identified, not previously described using 1D MRS, such as a reduction in total fucose and the fucosylated glycans fuc IV and VI in the posterior cingulate cortex. Fucosylated glycans are thought to be contained within synapsin proteins in the brain, and this may be the first *in vivo* evidence of dysregulation of synapsin in PTSD. The only metabolite (IMI-1) that correlated with clinical symptoms was found using 2D L-COSY.

Multiple differences in chemical signatures were again found using 2D L-COSY to quantify neurochemical changes in the brains of patients suffering from relapsing and remitting multiple sclerosis (RRMS). Specifically, a significant reduction in multiple N-acetylaspartate (NAA) signatures, GABA and Glx, was identified in the posterior cingulate cortex in RRMS when compared to healthy controls. Of the clinical symptoms measured, visual spatial function and attention were most correlated with metabolites in the brain. Here the first *in vivo* evidence has been provided that 2D L-COSY has the potential to detect metabolic alterations in the normal appearing brain in multiple sclerosis and PTSD. Metabolic variability associated with clinical symptoms was detected despite only examining a localised region in both conditions.

This research has shown that 2D L-COSY is a useful additional spectroscopy technique, which can be used to identify additional neurochemical changes in the brain, when compared to conventional 1D spectroscopy. Although technological advances are required, this technique may one day provide clinicians with much needed imaging biomarkers for conditions that have no conventional imaging or limited clinically relevant imaging findings.

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